Mujeebur Rahman Khan *Editor*

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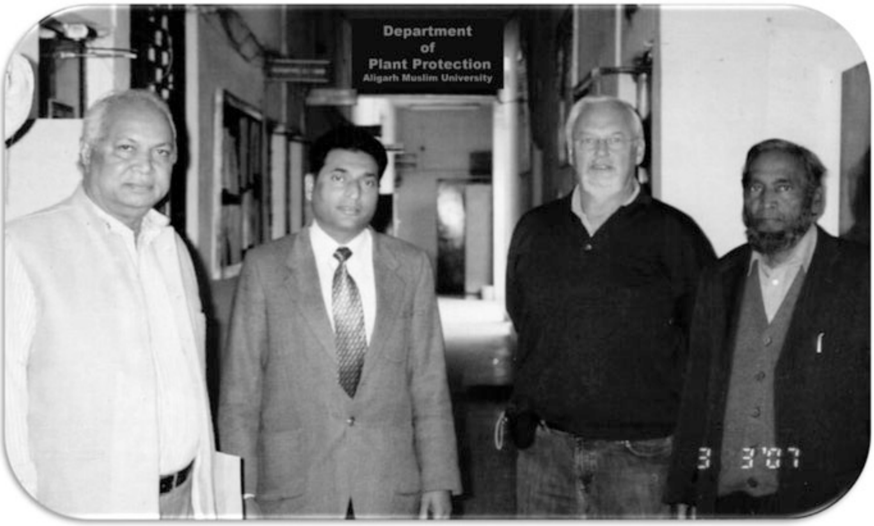
Dedicated to
Prof. Mohammad Shamim Jairajpuri
(Aligarh Muslim University, India)
Prof. Richard A. Sikora
(University of Boon, Germany)
Dr. Mohammad Rafiq Siddiqi
(CABI Institute of Parasitology, UK)
for their invaluable and extensive
contributions to nematology



A rare picture having above legendary nematologists together, who on my request visited the Department of Plant Protection, Aligarh Muslim University, India, in 2007. (L to R: M. S. Jairajpuri, M. R. Khan, R. A. Sikora, and M. R. Siddiqi)

Foreword I

Biotic stresses are serious constraints that impact productivity of crops. Nematodes, an important cause of biotic stress are responsible for around 15-20% crop damage. Generally, nematodes attack underground parts of a plant and do not incite specific symptom on its foliage. As a result, the damage does not become obvious. In order to control nematode infestation, farmers apply pesticides, which cause various adverse effects on the environment besides being costly. Under this situation, the novel biological and biotechnological methods may play an important role in protecting agricultural crops from nematode infestations.

I am glad that Prof. Mujeebur Rahman Khan from Aligarh Muslim University has brought out a book, ***"Novel Biological and Biotechnological Applications in Plant Nematode Management"***, which covers important aspects of nematode management in crops. The book embodies twenty three chapters contributed by expert nematologists from different countries. Contents of the book are divided into two sections. The first section contains chapters on different novel methods of nematode management, whereas the second section includes chapters describing specific nematode problems in agricultural crops, highlighting the economic importance, symptoms, and novel methods of nematode management. I hope that this book shall serve as an important reference for the students, academicians and research professionals concerned with crop protection from nematodes world over.

I congratulate Prof. M. R. Khan, the editor of the book and the contributors of the chapters for their efforts in bringing out this useful publication.

Trilochan Mohapatra

DARE
New Delhi, India

ICAR
New Delhi, India

National Academy of Agricultural Sciences
New Delhi, India
10 May 2023

Foreword II

This new book is devoted to novel developments in biological and biotechnological techniques and their potential integration into practical plant parasitic nematode management. It is a timely and important addition to our knowledge of how these technologies are linked to the process of reducing nematode impact on crop losses. The future success of nematode management in both low and high input agriculture, undoubtedly, will be determined by the development of novel technologies for incorporation into strategies that reduce losses caused by nematode pests.

Agriculture is now confronted with complex interacting constraints that limit food production. These constraints include soil degradation, increased heat and drought, declining soil fertility, unstable markets, and often overlooked expanding root health problems. These complex interacting factors will lead to decreases in crop productivity and thereby an increase in food insecurity on a global scale. Therefore, a major shift in how we manage crop health, and more importantly root health, is urgently needed. It has been estimated that one-third of global agricultural production is lost to pests and diseases, with an estimated 10% of those losses due to the activity of plant parasitic nematodes. With the ever increasing global human population and expanding problems with food security, there is a dire need for development of novel technologies for pest management.

The book contains 23 chapters, each chapter beginning with an extensive literature review, followed by visions of new and improved avenues of management. Also important is the fact that of the 43 authors and co-authors, the majority come from institutions in subtropical or tropical climatic zones where nematode losses are most severe. This book takes a new look at what can be done to offset losses caused by plant parasitic nematodes. The chapters add significantly to our knowledge of biological and biotechnical approaches to nematode management. Furthermore, the knowledge presented in these chapters will aid scientists in their efforts to develop new and appropriate management strategies in both low- and high-input agriculture.

The book is divided into two parts: (1) Novel methods of nematode management and (2) Nematode problems and their management. The first part includes chapters dealing with the global status of nematode pests, limitations and challenges facing management, host resistance, biological and biotechnological methods, microbial consortia, omics, transgenics, nano-material and formulation, and nano-sensors for diagnosis. The chapters in the second part review the present state of the art of management of economically important plant parasitic nematodes across a wide spectrum of agricultural crops. These chapters also discuss the integration of novel biological and biotechnological technologies into present management approaches. The information and concepts presented in this book will give the reader an up-to-date view of biological and biotechnological approaches and how they might improve nematode management in the future.

I congratulate the editor, Prof. M. R. Khan, and all the authors for their efforts in producing an important book that will be valuable to scientists, extension experts, teachers, and students.

Richard A. Sikora

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25 May 2023

Preface

Nematodes are important pests of agricultural crops and inflict economic loss to crops worth billions of dollars. Nematodes in general are soil inhabitants and attack underground parts of the plant, as a result the crop damage remains unrecognized to farmers unless it becomes severe. There are different methods of nematode management, but farmers largely rely on chemical control despite serious issues of contamination of food and the environment associated with pesticides. However, because of growing concern for pesticidal contamination and consequent public preference to organic foods, eco-friendly methods of nematode management are in high demand. With these concerns, this book titled *Novel Biological and Biotechnological Applications in Plant Nematode Management* was conceptualized and is finally brought out. The book presents up-to-date information on applied aspects of novel biocontrol and biotechnological nematode management methods and offers practical solutions to nematode infestations in economically important agricultural crops. The book contains 23 chapters, which are grouped into two parts. The first part contains ten chapters on different novel methods of nematode management, whereas the second part embodies 13 chapters describing specific nematode problems in agricultural crops highlighting nematode distribution, economic importance, symptoms, life cycle, and important biocontrol and biotechnological management measures. This book shall serve as an important reference source to UG/PG students, academia, professionals, scientists, researchers, and extension personnel dealing with plant nematodes in universities, institutes, bureaus, directorates, research stations, etc.

I am extremely grateful to the nematologists for contributing valuable chapters and sparing time for this academic endeavour. I am highly indebted to Dr. Trilochan Mohapatra and Dr. Richard A. Sikora for contributing valuable *Foreword* for this book. I would like to thank Springer Nature Singapore Pte Ltd. for accepting our proposal, and thereafter for offering best help and cooperation in resolving the issues arose during the publication of this volume with a special mention to Ms. Mahalakshmi Shankar, Project Coordinator (Books). I earnestly appreciate the untiring, exhausting, and selfless help offered by my students, Dr. Ziaul Haque, Dr. Faheem Ahamad, Dr. Tanveer F. Rizvi, Mr. M. Akram Khan, Mr. Rahul K. Sharma, Mr. Irfan Ahmad, Mr. M. Haniph Shah, and Mr. M. Shahid A. Ansari, throughout the publication process of this book.

Aligarh, Uttar Pradesh, India
15 August 2023

Mujeebur Rahman Khan

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Editor and Contributors

About the Editor

Mujeebur Rahman Khan is a Professor and Chairperson at the Department of Plant Protection, Aligarh Muslim University (AMU), India, and has served as the Dean, Faculty of Agricultural Sciences, AMU. He obtained Ph.D. in Botany (Plant Pathology & Nematology) from AMU, and worked as postdoc at the North Carolina State University, California Department of Food and Agriculture, USA, and the Commonwealth Institute of Parasitology, UK. He has to his credit more than 300 research publications and review articles, 18 books, 2 patents on biopesticides (USA and India), and 10 major research projects. He has guided 14 PhD and 52 MSc students. Dr. Khan is a Fellow of the National Academy of Agricultural Sciences (India), Indian Phytopathological Society, etc., and Editor of *Indian Phytopathology*. He is a recipient of various awards: Dr. K. C. Mehta Award (NAAS), B. B. Mundukar Award (IPS), Outstanding Scientist Award (AMU), Prof. H. L. Chakraborti Award (ISCA), Outstanding Scientist Award (SPSS), Prof. H. M. Shah Memorial (NSI), and Best Research Project Award (AMU). His research interest includes plant disease management, impact of climate change and environmental contamination on plant disease development, and biosynthesis of nanoparticles and their effects on plants and microbes.

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Part I

Novel Methods of Nematode Management

Nematode Pests of Agricultural Crops, a Global Overview

1

Mujeebur Rahman Khan

Abstract

Plant nematodes constitute a major group of phytopathogens, being most ubiquitous, and account for 80–90% of the multicellular soil fauna. Nematodes are distributed in different agro-ecosystems at a varying population densities. All agricultural crops are attacked by plant nematodes and exhibit 5–20% yield loss valuing to a net monetary loss of around USD 175.0 billions. However, the yield losses may reach to USD 200 billion, if noncommercial crops being grown in developing and under-developed countries are surveyed and included in the estemates. The nematode damage to crops generally remains unrecognized to farmers because of microscopic size and absence of specific symptoms. Plant nematodes also contribute in aggravating the infection incited by soil-borne plant pathogenic fungi and bacteria causing the diseases of complex etiology. Nematodes also act as vector for plant pathogens. Hence, adequate extension service may prove much effective in helping the growers to realize the economic significance of nematodes in crop production as well as the need of adopting nematode management strategies, and the resulting economic returns in terms of yield enhancements. In view of relative economic importance, the management activities should focus on *Meloidogyne, Pratylenchus, Ditylenchus, Heterodera, Aphelenchoides, Radopholus, Globodera, Rotylenchulus,* etc. because of their greater economic significance, wide distribution, and host range. The present chapter offers an overview on the nematode infestation in agricultural crops and its management.

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Keywords

Plant nematodes · Crop damages · Disease management · Cultural practices · Biocontrol · Nematicides · IPM

1.1 Introduction

Nematodes are invertebrate animals with thread-like body which is thin and flexible, tapers at anterior and posterior ends, measuring 3–11 mm length, unsegmented, pseudocoelomic, and bilaterally symmetrical (Khan et al. 2018). Nematodes are most ubiquitous in terrestrial habitats, constituting 80–90% of the multicellular fauna. Nematodes are aquatic animals but have adapted to the soil environment (Khan et al. 2021). Nematodes have around 1.04–1.14 specific gravity which helps them to suspend in water for a longer period (Oostendorp et al. 1991). All terrestrial nematodes do not parasitize plants, rather only a small proportion is phytoparasitic, whereas the rests are free-living (Khan 2008). All nematodes remain vermiform throughout their entire life span or at least for some period (Fig. 1.1a). Further, the shape of nematode body is highly variable, from a thin straight and thread like (*Polenchus*) to saccate with globular shape as in *Globodera* (Fig. 1.1c–m). Within vermiform nematodes, the shape varies from straight, slightly curved, C-shaped to spiral (Fig. 1.1c–g). Similarly, the saccate nematodes also show great diversity from posterior enlargement to entire globular body (Fig. 1.1h–k). All plant parasitic nematodes, either root, stem, or leaf parasites, have a sharp and pointed stylet (spear) at their anterior ends (Fig. 1.1b), which they use to puncture the epidermis and cell wall or to displace the cells while moving inter-cellularly or to feed on host cells (Khan 2008). During feeding and causing mechanical damage to plant tissue, nematodes secrete saliva from the oesophageal glands which induces cell wall dissolution and/or extracorporeal digestion.

Plant parasitism by nematodes is confined to three orders, Dorylaimida (Class: Adenophorea), Tylenchida and Aphelenchida (Class: Secernentea) (Siddique 2005). From the order Dorylaimida, two families, viz., Trichodoridae and Longidoridae, contain major plant parasitic nematodes which differ considerably in the parasitism. The phytonematodes of this order are migratory ectoparasites of underground parts and nibble the surface of young roots, e.g. *Trichodorus* and *Paratrichodorus*, whereas *Longidorus* and *Xiphinema* attach to the root surface to feed for several minutes to hours generally on the root tip causing terminal galls. In addition to direct damage, dorylaim nematodes vector several important plant viruses. For example, *X. index* and *L. elongatus* transmit arabis mosaic virus and tomato black ring virus (nepoviruses), respectively. Whereas, *Trichodorus* and *Paratrichodorus* species act a vector for tobra group such as tobacco rattle virus, pea early browning virus, and pepper ring spot virus.

Majority of the plant nematodes belong to the order Tylenchida and Aphelenchida. The ectoparasitic nematodes constitute the majority, and feed on the root surface, e.g. *Tylenchorhynchus, Paratylenchus, Rotylenchus,*

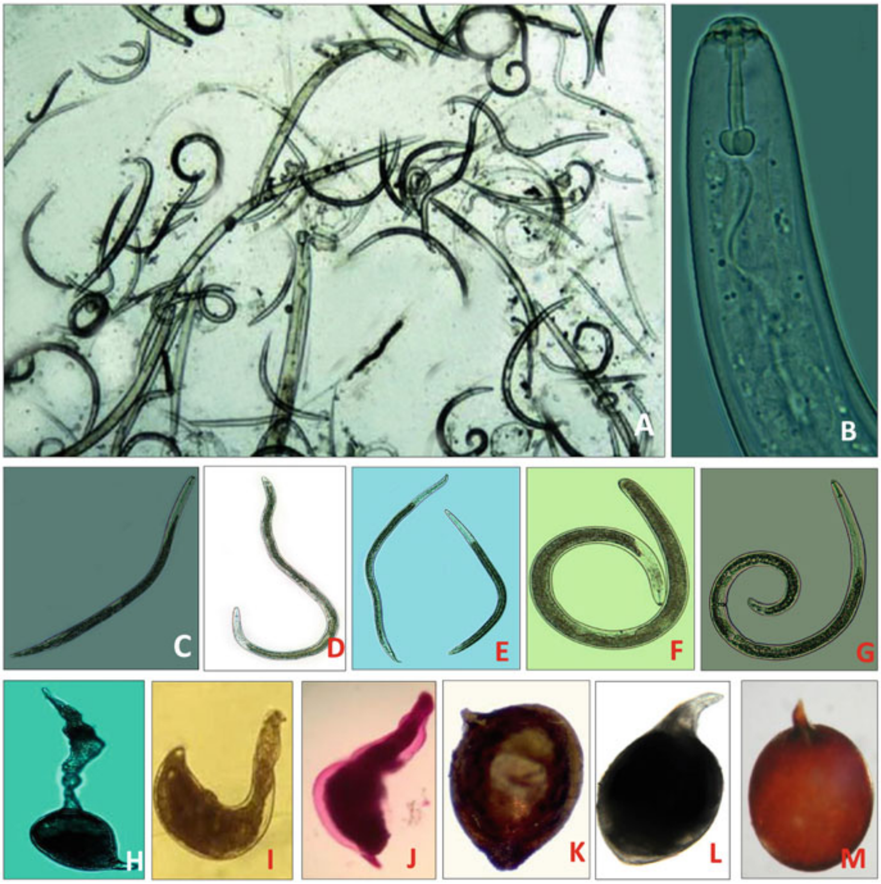


Fig. 1.1 Plant parasitic nematodes in the water suspension isolated from soil under stereomicroscope (**a**); close-up of the anterior end of a nematode showing lips, stylet, oesophagus, etc. (**b**); variation in the body shape of vermiform (**c**–**g**) and saccate nematodes (**h**–**m**). (**c**) *Pratylenchus*, (**d**) *Rotylenchus*, (**e**) *Tylenchorhynchus*, (**f**) *Hoplolaimus*, (**g**) *Helicotylenchus*, (**h**) *Tylenchulus*, (**i**) *Rotylenchulus*, (**j**) *Nacobbus*, (**k**) *Heterodera*, (**l**) *Meloidogyne*, (**m**) *Globodera*

Helicotylenchus, Hoplolaimus, Belonolaimus, Hemicycliophora, etc. Next to ectoparasitic nematodes are endoparasites which fully enter into the host tissue (Khan and Sharma 2020). Among the endoparasitic nematodes, some genera such as *Meloidogyne, Heterodera, Globodera,* etc. become immobile (sedentary) after getting a suitable feeding site, whereas *Pratylenchus, Radopholous*, and *Hirschmannella* remain migratory throughout the life. The *Tylenchulus semipenetrans* (citrus nematode) or *Rotylenchulus reniformis* (reniform nematode) are semi-endoparasites; their body partially enters into the host tissue (Siddique 2005). Some nematodes, *Anguina tritici*, *Ditylenchus dipsaci,* etc., on the advent of adverse conditions enter into quiescent state of anhydrobiosis or cryptobiosis to survive under extreme dry or cool condition. The important nematodes from the order Aphelenchida are stem and bud nematode (*Aphelenchoides* spp.), fig nematode (*Schistonchus* spp.), eucalyptus nematode (*Furgusobia* spp.), wilt nematode (*Bursaphalenchus xylophilus*), red ring nematode (*Bursaphelenchus* = *Rhadinaphelenchus cocophilus.*), etc. (Hunt 1993). All these nematodes except *Aphelenchoides* spp. are transmitted by insect vectors. *Schistonchus caprifici* and *F. tumifaciens* cause galls in the leaves/flowers of fig and eucaplyptus with the aid of insect vectors, *Blastophaga psenes* and *Fergusonina* sp., respectively. *Bursaphelenchus xylophilus* and *B. cocophilus,* after being transmitted by *Monochamus* spp. and *Rhynchophorus palmarum*, feed on stem parenchyma and cause wilt in pine and red-ring in coconut, respectively.

Agricultural importance of plant parasitic nematodes ranks next to fungi, bacteria, and viruses. Around 5–20% yield losses to agricultural crops have been attributed to nematode infestations, which vary with respect to inoculum level, host species, and environmental conditions. The severe infection in a field or area may lead to as high as 80–90% crop damage. Sometimes, a crop fails to give yield of any market value, or foreseeing the crop status, plough the fields without harvesting. On average basis, Nicol et al. (2011) estimated 14.6% agricultural losses due to nematode infestation. The monitory value of annual yield loss inflicted by plant nematodes to the world agriculture may account over to USD 100 billion annually (Sikora et al. 2018), USD 125 billion (Chitwood 2003), or USD 175 billion (Tóthné Bogdányi et al. 2021). However, it is believed that yield losses may account to USD 200 billion, if noncommercial crops being grown in developing and under-developed countries are surveyed and included in the estemates. High yield losses to crops due to nematodes have been estimated in under-developed and developing countries because of having un-planned agricultural practices and low awareness of crop growers to plant nematodes. However, despite of taking planned management practices, nematodes inflict considerable crop losses in developed countries, for example crop losses valuing USD 60 billion occur in USA alone (Nicol et al. 2011). The average yield loss inflicted by nematodes to major agricultural groups of crops on world-wide basis is summarized in Table 1.1.

To protect the crops from nematode attack, farmer's approach towards plant protection in general and nematode management in particular is needed to be changed, and economic consequences of nematode infestation in agricultural crops have to be realized by them so as to undertake proper management measures. Special attention of the farmers should be attracted towards economically important selected nematode problems (genera) as presented in Table 1.2. The crop problems resulting due to infestation with these nematodes have been elaborated and their management options have been offered as under.

1.2 Root-Knot Nematode, *Meloidogyne* spp.

Meloidogyne is a most important nematode genus, and its species are extensively distributed world over (Sasser et al. 1983; Mohiddin and Khan 2014; Fig. 1.2). All kinds of agricultural crops, especially vegetables, pulses, fruits, beverage, fibre, oil

Table. 1.1 Yield losses to agricultural crops inflicted by plant nematodes

Crop	Yield loss (%)	Important nematodes
Cereals	7–77	*Anguina, Heterodera, Ditylenchus, Meloidogyne, Pratylenchus, Aphelenchoides, Hoplolaimus,* etc.
Pulses	6–23	*Heterodera, Meloidogyne, Radopholous, Pratylenchus, Rotylenchulus, Helicotylenchus, Xiphinema,* etc.
Vegetables	5–43	*Meloidogyne, Pratylenchus, Globodera, Radopholus, Rotylenchulus, Tylenchorhynchus, Belonolaimus, Trichodorus, Paratrichodorus, Ditylenchus, Helicotylenchus, Nacobbus,* etc.
Fruit crops	5–80	*Pratylenchus*, *Meloidogyne*, *Radopholus, Xiphinema, Longidorus, Tylenchulus, Rotylenchulus, Trichodorus*, *Paratrichodorus, Belonolaimus, Aphelenchoides, Bursaphelenchus,* etc.
Leafy vegetables	9–20	*Meloidogyne, Pratylenchus, Heterodera, Rotylenchulus Tylenchorhynchus, Belonolaimus, Trichodorus*, *Longidorus,* etc.
Mushrooms	5–23	*Aphelenchoides, Ditylenchus*
Oil yielding crops	7–27	*Heterodera, Meloidogyne, Radopholous, Ditylenchus, Rotylenchulus, Helicotylenchus, Paratrichodorus, Tylencorynchus,* etc.
Beverage crops	4–42	*Pratylenchus, Meloidogyne, Radopholous, Tylenchorynchus, Xiphinema, Belonolaimus,* etc.
Ornamental crops	5–36	*Meloidogyne*, *Aphelenchoides, Ditylenchus*, *Pratylenchus*, *Belonolaimus*, *Trichodorus*, *Xiphinema, Helicotylenchus, Tylenchorhynchus, Rotylenchulus, Radopholus,* etc.
Medicinal plants	Up to 30	*Meloidogyne*, *Pratylenchus*, *Radopholus, Rotylenchulus, Trichodorus*, *Tylenchorhynchus,* etc.
Spice crops	38–64	*Meloidogyne, Radopholus, Pratylenchus, Heterodera, Ditylenchus, Globodera, Tylenchulus, Xiphinema, Rotylenchulus, Helicotylenchus,* etc.
Fibre crops	5–28	*Meloidogyne, Radopholous, Rotylenchulus, Belenolaimous, Helicotylenchus*, *Hoplolaimus, Tylenchorhynchus*, *Nacobbus, Longidorus,* etc.

seeds, ornamentals, cereals, etc., are highly susceptible to the infection of *Meloidogyne* spp. (Table 1.3; Khan 1997). Besides directly damaging the plants, root-knot nematodes possess great potential to synergize bacterial and fungal plant pathogens resulting in the diseases of complex etiology (Khan 1993). The infection with root-knot nematodes may break resistance of cultivars against wilts caused by *Fusarium, Xanthomonas, Pseudomonas,* etc. (Francl and Wheeler 1993; Khan and Sharma 2020).

Root-knot nematodes incite characteristic galls on the roots of infected plants (Khan 2007; Fig. 1.3a–i), but the aboveground symptoms are nonspecific, and the plants show stunted growth with leaf yellowing, loss of vigour, small leaf size, and premature drop (Khan 1997). The absorption and conduction of water and minerals by the root system are impaired, as a result plants experience wilting during day time

Table 1.2 Important nematode genera associated with economically important crops

S. No.	Nematode genera	Susceptible crops
1.	Root-knot nematode, *Meloidogyne* spp.	All crops
2.	Root lesion nematode, *Pratylenchus* spp.	Vegetables, pulses, ornamentals, tobacco, sugarcane, tea, bamboo, maize, barley, coffee, medicinal crops, forage crops
3.	Cyst nematode, *Heterodera* spp.	Cereals, pulses, sugar beet, soybean, etc.
4.	Stem and bulb nematode, *Ditylenchus* spp.	Rice, oat, rye, potato, sugar beet, onion, cucumber, pumpkin, tomato, broad bean, lentil, garden pea, groundnut, coconut, soya bean, strawberry, orange, aster, hyacinths, iris, narcissus, tulips, tea, coffee, sugarcane, mentha, and maize
5.	Potato cyst nematode, *Globodera* spp.	Potato, tomato, egg plant
6.	Citrus nematode, *Tylenchulus* spp.	Citrus family
7.	Dagger nematode, *Xiphinema* spp.	Vegetables, fruits, cotton, tobacco, sugarcane, spices, groundnut, forage crops, medicinal crops
8.	Burrowing nematode, *Radopholus* spp.	Palms, spices, coffee, vegetables
9.	Spiral nematode, *Helicotylenchus* spp.	Vegetables, fruits, tobacco, sugarcane, jute, forage crops, cotton
10.	Reniform nematode, *Rotylenchulus* spp.	Vegetables, pulses, tobacco, groundnut, soybean, coffee, cotton, jute, forage crops
11.	Stunt nematode, *Tylenchorhynchus* spp.	Vegetables, fruits crops, cotton, groundnut, brassicas, sugarcane, forage crops, tobacco, jute, medicinal crops
12.	Lance nematode, *Hoplolaimus* spp.	Vegetables, pulses, forage crops, sugarcane, jute, cotton, tobacco, rice
13.	Foliar nematodes, *Aphelenchoides, Anguina, Bursaphelenchus,* etc.	Wheat, rice, oat, barley, pearl millet, onion, garlic, chili, groundnuts, strawberry, Anthurium, Asiatic lily, Aster, balsam, begonia, carnation, crossandra, chrysanthemum, China aster, gerbera, gladiolus, hyacinths, hollyhock, hibiscus, iris, jasmine, kochia, narcissua, petunia, poppy, rose, tuberose bulb, tulip, Lilly, Zinnia, conifers, fig, eucalypts

(Khan and Khan 1987). Generally, plants in patches show such symptoms. In case of heavy infection, yellowing and other deficiency symptoms and wilting are evenly distributed throughout the entire field. The presence of galls or knots is a most characteristic symptom of the nematode infection. Size and shape of the galls vary with the plant and *Meloidogyne* species, being more dependent on the former. In most of solanaceous vegetables such as egg plant and tomato, galls are large and firm, whereas, on cereals (monocots) such as rice and wheat, terminal hook like root

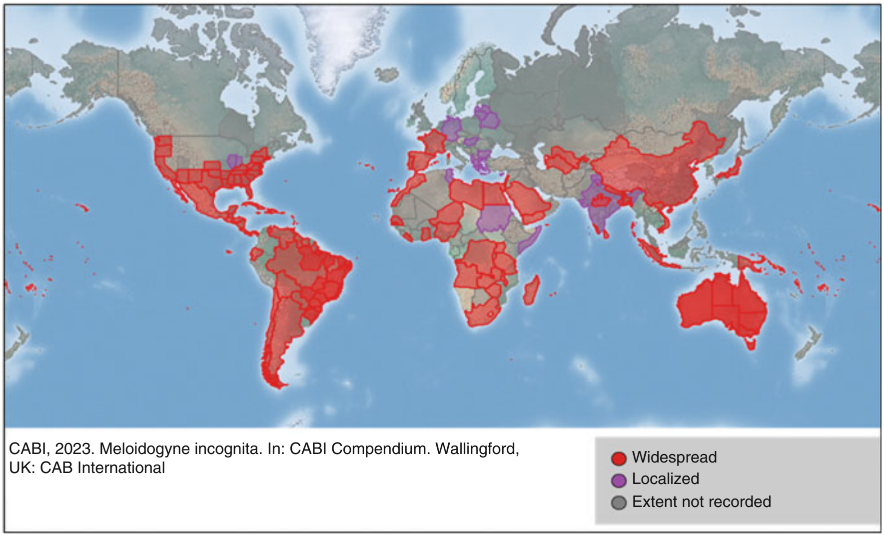

CABI, 2023. Meloidogyne incognita. In: CABI Compendium. Wallingford, UK: CAB International

Fig. 1.2 Global distribution of Root-knot nematode. (CABI 2023)

galls are formed. The galling also affects the nodule formation in pulse crops (Khan et al. 2016).

Root-knot nematodes are sedentary endoparasite and strictly obligate in behaviour. Marked sexual dimorphism is present, females after second moult become obese, whereas males remain vermiform throughout the life except J_3 and J_4. The infective stage is second stage juvenile which emerges from egg. First moulting takes place within the egg. The J_2 penetrates the roots and migrates intercellularly in the cortex and becomes immobile upon finding a suitable site for feeding in the developing stelar tissue, where it feeds on the specialized nurse cells called giant cells throughout the life (Khan 2008). The giant cells (nurse cells) are formed due to repeated endomitosis (nuclear division) without cytokinesis. Concurrent with the formation of nurse cells, the surrounding cortical cells undergo hyperplasia and hypertrophy leading to formation of galls (Bird 1975). At maturity, the adult female lays eggs in gelatinous material called egg mass (200–500 eggs/egg mass) which is secreted by rectal cells. The life cycle completes in 3–4 weeks, depending upon species and environment factors, and generally 2–3 generations complete within a cropping season. The nematode is highly damaging to all kinds of agricultural crops and inflicts high yield losses as summarized in Table 1.3.

1.3 Root Lesion Nematode, *Pratylenchus* spp.

Lesion nematode, also called meadow nematode, is a most important nematode next to *Meloidogyne* spp. with regard to host range and distribution. *Pratylenchus* spp. are widely distributed in subtropical, tropical, and temperate regions (Fig. 1.4). The

Table 1.3 Major species of root-knot nematode, *Meloidogyne,* and yield losses caused to important crops

Crop groups	Major species	Yield loss	Crops
Cereals	*M. incognita* *M. graminicola* *M. oryzae* *M. triticoryzae* *M. nassi* *M. artiellia* *M. salsi*	12–28%	Rice, wheat, oat, barley, pearl millet
Vegetables	*M. incognita* *M. javanica* *M. arenaria* *M. enterolobii* *M. hapla*	5–43%	Potato, eggplant, tomato chili, pepper, cabbage, Chinese cabbage, lettuce, spinach, fenugreek, bathua, broccoli turnip, radish, cucumber, bottle guard, bitter gourd, sponge gourd, pumpkin, and pointed gourd
Legumes	*M. incognita* *M. javanica* *M. arenaria* *M. hapla* *M. artiellia*	20–50%	Broad bean, common bean, chickpeas, soybean, pigeon pea, peanuts, lentils, cowpea, urd bean, and mung bean
Oil seeds	*M. incognita* *M. javanica* *M. arenaria* *M. hapla*	6–12%	Groundnut, rapeseed and mustard, soybean, sunflower, sesame, safflower
Fruits	*M. incognita* *M. javanica* *M. enterolobii* *M. hapla*	15–30%	Avocado, kiwi, fig, guava, papaya, olive, pineapple, date palm, coconut
Ornamentals	*M. incognita* *M. javanica* *M. arenaria* *M. hapla*	15–85%	Anthurium, Asiatic lily, Aster, balsam, begonia, carnation, crossandra, chrysanthemum, China aster, gerbera, gladiolus, hyacinths, hollyhock, hibiscus, iris, jasmine, kochia, narcissua, petunia, popy, rose, tuberose bulb, tulip, Lilly, Zinnia
Beverage crop	*M. incognita* *M. javanica* *M. arenaria* *M. hapla* *M. brevicauda* *M. exigua* *M. african* *M. coffeicola* *M. decalineata*	15–60%	Tea, coffee, sugarcane, tobacco
Fibre crops	*M. incognita* *M. javanica* *M. arenaria*	18–22%	Cotton, jute, mesta ramie, flax, sun, roselle
Medicinal plants	*M. incognita* *M. javanica*	Up to 70%	Henbanes, ashwagandha, brahmi, Safed musli, coleus, and mint

(continued)

Table 1.3 (continued)

Crop groups	Major species	Yield loss	Crops
	M. arenaria *M. brevicaudata*		
Spice crops	*M. incognita* *M. javanica* *M. arenaria* *M. hapla* *M. thailandica*	32–47%	Black pepper, cardamom, ginger, turmeric
Forage crops	*M. incognita* *M. javanica* *M. arenaria* *M. hapla*	8–58%	Berseem, sunhemp, cowpea, lucerne, field pea, mothbean, clitoria, beans, cluster bean, rice bean, oat, barley, sorghum, pearl millet, and maize

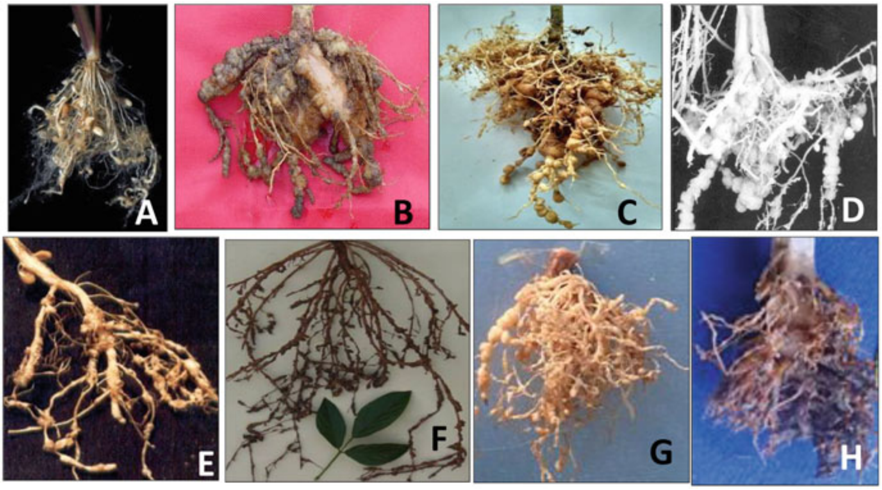
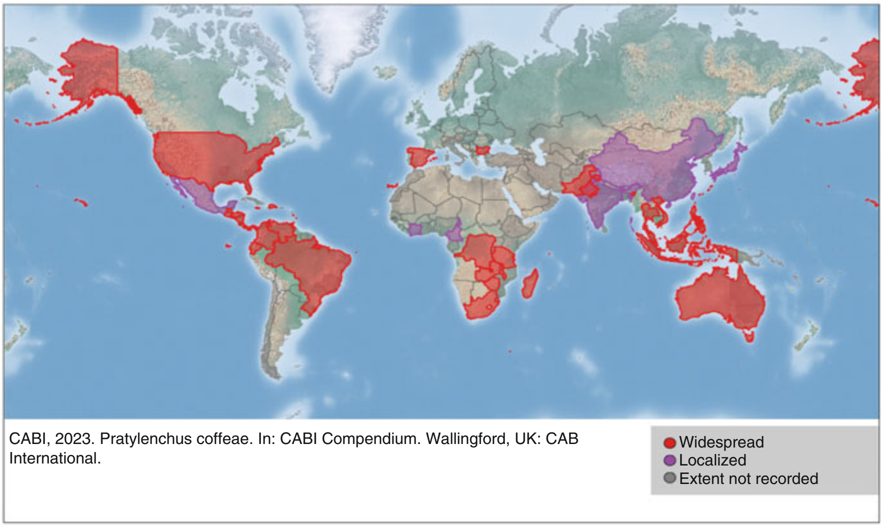


Fig. 1.3 Root galls caused by root-knot nematode, *Meloidogyne* species, on rice (**a**), eggplant (**b**), tomato (**c**), bottle gourd (**d**), chickpea (**e**), pigeon pea (**f**) Egyptian henbane (**g**) and balsam (**h**). (Source: (**a**, **e** and **f**) M. R. Khan, Aligarh Muslim University, (**b** and **c**) Z. Haque, Aligarh Muslim University; (**d**) M. W. Khan, Aligarh Muslim University, (**g** and **h**) R. Pandey, NASI, India)

nematode frequently co-occurs with *Meloidogyne,* but overemphasis on the root-knot nematode has limited our knowledge and research on economic significance of the lesion nematode in agricultural crops. The lesion nematodes are frequently involved in the disease complexes with soil-borne fungi and bacteria (Khan 1993). *Pratylenchus penetrans*, *P. brachyurus, P. coffeae, P. delattrei, P. loosi, P. histeriae, P. neglectus, P. thornei, P. zeae,* etc. are important species which attack plants from all groups of crops and inflict heavy losses (Castillo and Vovlas 2007).



CABI, 2023. Pratylenchus coffeae. In: CABI Compendium. Wallingford, UK: CAB International.

Fig. 1.4 Global distribution of root lesion nematode, *Pratylenchus* spp. (Source: CABI 2023)

Fig. 1.5 Root lesion nematode, *Pratylenchus* spp., infesting maize field (**a**), Sugarcane roots showing necrosis caused by *Pratylenchus zeae* (**b&c**). (Source: (**a**) link.springer.com; (**b**) C. Sankranarayanan, NASI (**c**) D. Prasad, NASI, India)

The infestation with the lesion nematode often remains unrecognized unless plants are uprooted, and the root system is examined for rotting (Fig. 1.5b). The above-ground symptoms are also non-specific and resemble with water and nutrition stress (Fig. 1.5a). Further, these symptoms are not discernible at a moderate population of *Pratylenchus* species. However, a high population of the nematode may cause discernible stunting, leaf yellowing, wilting, and eventual death of young plants. These symptoms generally appear in patches of plants with thin stand and stunted growth. On underground parts, the nematode infection causes characteristic root necrosis in the form of dark lesions of necrotic tissue on the surface and the cortex of the root. Initially, the lesions appear as water-soaked areas on the root surface which later turn reddish-brown to black. Due to continuous endoparasitic migration and feeding of the nematode, the lesions coalesce forming large necrotic areas which may girdle the root (Fig. 1.5b, c). The necrotic tissue often slough off leaving behind the vascular cylinder.

The genus *Pratylenchus* shows migratory endoparasitism. All juvenile stages and adult of lesion nematodes can infect plants. Male individuals are numerous in amphimictic species such as (*P. penetrans*), whereas rare in parthenogenetic species like *P. brachyurus*. After penetrating the underground parts (roots, tubers, rhizomes, etc.), the nematode larvae move in the cortex and feed on the parenchymatous cells. The nematodes can penetrate the roots anywhere, but preferably in the region near the root hair zone, and migrate intra or inter-cellularly, but once inside, move intracellularly. The nematode completes life cycle in 4–8 weeks, depending on the temperature, moisture, host species, etc. (Table 1.4).

1.4 Cyst Nematode, *Heterodera* spp.

Sugar beet cyst nematode *Heterodera schachtii*, cereal cyst nematode (*H. avenae*), soybean cyst nematode (*H. glycine*), maize cyst nematode (*H. zeae*), rice cyst nematode (*H. oryzecola*), etc. are the major species of *Heterodera,* and are distributed world over (Fig. 1.6). The beet cyst is a most economically important nematode in U.S.A., Canada, Australia, Spain, Finland, Ireland, Bulgaria, Turkey, Israel, South Africa, etc. The *H. schachtii* is also a major limiting factor in spinach cultivation. The *H. avenae* is an important nematode on cereals in temperate and other regions (Rivoal and Cook 1993) and is widely distributed in major wheat growing countries worldover (Kort 1972). The soyabean cyst nematode, *H. glycine,* is another major species which inflicts heavy loss to soybean in Americas and Europe in particular (Sikora et al. 2018). The leaves of soybean plants turn chlorotic with suppressed plant growth and pod development. Cabbage cyst nematode, *H. cruciferae* (Skarbilovich 1959), attacks exclusively the brassicas causing significant damage to vegetables and oil yielding brassica crops.

H. avenae is primarily a pest of wheat and barley causing molya disease" in south east Asia (Fig. 1.7c). The sorghum cyst nematode, *H. sorghi,* is a widely occurring species in the millet cultivation in Asia and Africa (Walia and Bajaj 1986; Kaushal et al. 2007). Maize or corn cyst nematode, *H. zeae,* is a pest of maize in India (Koshy et al. 1970), Pakistan (Maqbool 1981), Egypt (Aboul-Eid and Ghorab 1981), USA (Ringer et al. 1987; Sardanelli et al. 1981), Portugal (Chinnasri et al. 1995), Thailand (Correia and Abrantes 2005), and Nepal (Sharma et al. 2001). Similarly, rice cyst nematode, *H. oryzicola,* is reported as a common infestant in paddy (Venkitesan and Charles 1985). The *H. cajani* is widespread in pigeonpea cultivation in India and other major countries growing the crop (Koshy 1967).

Primary symptom of *Heterodera* infestation is reductions in the root growth (Owen et al. 2023). Infected roots often appear bearded as feeding by nematodes promotes the formation of excessive lateral growth. However, presence of young and adult females and cysts throughout the growing season is a characteristic symptom (Fig. 1.7a–d). The cysts at the initial stage are white (females), while at later stage turn brown to black (Yadav and Verma 1971; Srivastava and Sethi 1984). Due to

Table 1.4 Major species of root-lesion nematode, *Pratylenchus,* and the yield losses caused to important crops

Crop groups	Major species	Yield loss	Crops
Cereals	*P. thornei* *P. neglectus* *P. penetrans* *P. crenatus* *P. brachyurus* *P. zeae*	30–74%	Wheat, oat, barley, rice, maize, etc.
Vegetables	*P. penetrans* *P. coffee* *P. brachyurus* *P. lossi* *P. barkati* *P. scribneri*	40–80%	African spinach, brinjal, calabash, carrot, kale, melon, okra, onion, potato, pumpkin, radish, squash, tomato, watermelon, etc.
Legumes	*P. thornei* *P. neglectus* *P. mediterraneus*	4–40%	Broad bean, cowpea, common bean, peas, pigeon pea, peanuts, lentil, soybean, black gram, green gram, etc.
Oil seeds	*P. penetrans* *P. brachyurus* *P. coffeae* *P. crenatus*	30–50%	Soybeans, groundnut, coconut, olive, etc.
Fruits	*P. penetrans* *P. brachyurus* *P. coffeae* *P. vulnus*	20–50%	Apple, avocado, pear, peach, fig, guava, mango, papaya, pineapple, banana, etc.
Ornamental	*P. penetrans* *P. chrysanthus* *P. coffeae* *P. vulnus*	5–8%	Chrysanthemum, rose, asiatic lily, carnation, anthurium crossandra, hibiscus, gardenia, gerbera, gladiolus, lilly, etc.
Beverage crop	*P. brachyurus* *P. coffeae* *P. lossi* *P. pratensis* *P. zeae* *P. besoekianus*	24–78%	Tea, coffee, sugarcane, tobacco, etc.
Fibre crops	*P. brachyurus* *P. sudanensis*	7–28%	Cotton, jute, etc.
Medicinal crops	*P. thornei* *P. brachyurus* *P. coffeae* *P. vulnus* *P. pratensis* *P. indcus*	25–30%	Henbanes, ashwagandha, brahmi, Safed musli, coleus, mint, etc.
Spice crop	*P. coffeae* *P. brachyurus* *P. pratensis* *P. indcus* *P. zeae*	46.6%	Cardamom, clove, ginger, black pepper, turmeric, etc.

(continued)

Table 1.4 (continued)

Crop groups	Major species	Yield loss	Crops
Forage crops	*P. brachyurus* *P. coffeae* *P. vulnus* *P. pratensis*	23–30%	Barseem, alfalfa, oat, Guinea grass, oat, barley, sorghum, pearl millets, maize, etc.

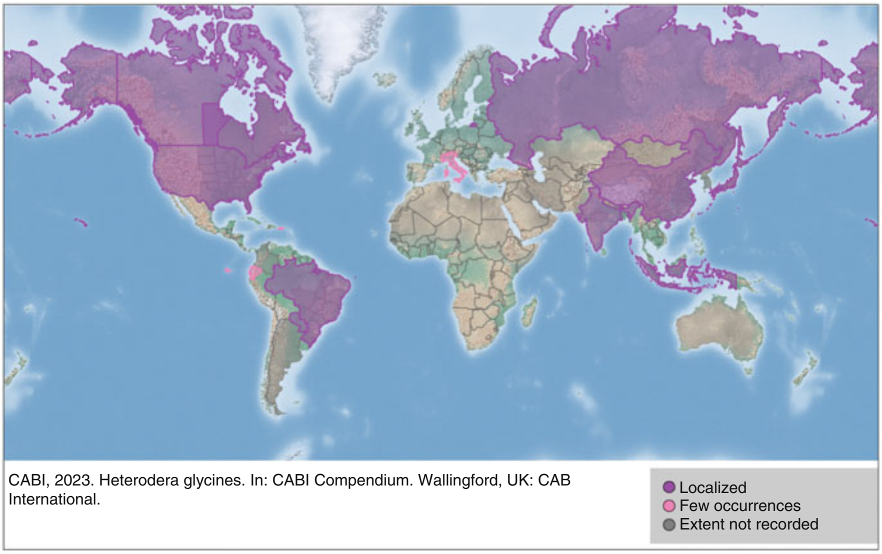


Fig. 1.6 Global distribution of cyst nematode, *Heterodera* spp. (Source: CABI)

internal damage to roots, absorption of water and minerals gets impaired and the infected plants can easily be pulled out. The wilt sets in the infected plants during hotter period of the day, but regain at night when transpiration rate gets slow. The symptoms often appear on plants in patches where nematode population densities are high.

Cysts forming nematodes are sedentary endoparasites. Sexual dimorphism exists and males remain vermiform (except J_3), while females assume obesity inside the root tissue. Reproduction is parthenogenetic. The eggs remain within the female's body, and upon death it becomes a cyst with hard body wall that protects eggs for several years (200–500 eggs/cyst). Second-stage juvenile is infective stage. The J_2 penetrates the host roots and induce the formation of syncytia (nurse cells) in stelar tissue. The female feeds on the syncytia throughout the life. Cyst forming nematodes are important pests of a number of economically important crops and inflict severe yield losses to them as summarized in Table 1.5.

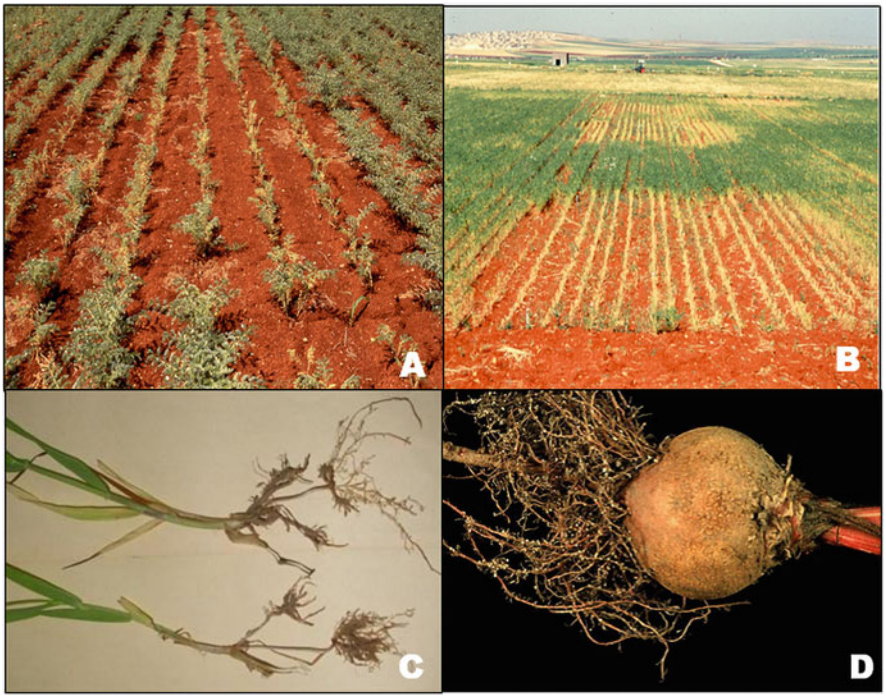


Fig. 1.7 A field of chickpea infested with *Heterodera ciceri* (**a** and **b**), symptoms of *Heterodera avenae* infection in wheat (**c**), sugar beet roots showing cysts (**d**). (Source: (**a** and **b**) Nicola Greco; (**c**) Scott Bauer, USDA Agricultural Research Service, Bugwood.org; (**d**) Mactode Publications, Bugwood.org)

1.5 Stem and Bulb Nematode, *Ditylenchus* spp.

Ditylenchus is an aerial nematode parasite of stem, petiole, leaf, and pods, but also attacks underground parts and ranks fourth important nematode pest of agricultural crops in temperate and subtropical climates (Sasser 1989). It is distributed widely in Asia, America, and Europe (Fig. 1.8). Rice, pearl millet, ground nut, alfalfa, rose, pomegranate, mango, guava, maize, tobacco, sugarcane, bulb crops, mushroom and several other crops are severely attacked by this nematode (Table 1.6).

Infected plants show swelling and deformation of stem with reddish brown lesions. The necrotic lesions on leaf, petiole, and pods turn dark brown (Bernard et al. 2023; Fig. 1.9a–c). The seeds become smaller and show spots. Under heavy infection, shoot dies and secondary branching is initiated. Nematodes are found below testa on either side of radical. About 10,000 juveniles may be found in one seed ectoparasitically. The infective stage is fourth stage juveniles (J_4), which penetrate stem of a young seedling just below the soil surface. The nematode is

Table 1.5 Major species of cyst forming nematode, *Heterodera,* and the yield losses inflected to important crops

Crop groups	Major species	Yield loss	Crops
Cereals	*H. avenae* *H. filipjevi* *H. zeae* *H. sturhani* *H. oryzicola* *H. sorghi* *H. ustinovi*	15–90%	Wheat, barley, rye, maize, rice, sorghum
Vegetables	*H. schachtti* *H. cruciferae*	24–50%	Sugarbeet, cabbage, spinach, broccoli, cauliflower, tomato, carrot
Legumes	*H. ciceri* *H. cajani* *H. goettingiana*	30–80%	Broad bean, cowpea, common bean, garden pea, field pea, soybean, lentil, chickpea, pigeonpea, black gram, green gram, and grass pea
Oil seeds	*H. glycines*	20–30%	Soyabean
Fruits	*H. fici* *H. oryzae* *H. schachtti*	Up to 100%	Fig, banana, pine apple
Ornamentals	*H. mothi*	Up to 8%	Chrysanthemum
Beverage crop	*H. sacchari* *H. tabacum*	3–5%	Sugarcane, tobacco
Forage crops	*H. sturhani* *H. zeae* *H. sorghi* *H. avenae*	17–70%	Cowpea, field bean, mothbean, cluster bean, oat, barley, sorghum, pearl millet, and maize

disseminated through seeds. The nematode thrives well in cooler climate, while at higher temperature, the nematode development is arrested. The infective stage can also survive desiccation up to 8 years by clumping together to form nemic wool (eel wool). Races are reported to exist in *Ditylenchus* populations, for example, oat race and giant race in *D. dipsaci* (Bernard et al. 2023).

1.6 Potato Cyst Nematode, *Globodera* spp.

The potato cyst nematodes, *Globodera rostocheinsis* and *G. pallida,* are important pests of potato, and occur in several countries of EPPO region, Asia, Africa, North America, South America, and Oceania (Fig. 1.10a, b). The infested potato plants show leaf yellowing and foliar wilting which resemble to that caused by soil-borne root rot causing pathogens. The severely infested plants become stunted and show wilting during hotter part of the day. The symptoms occur in patches. The nematode infestation can be confirmed by examining the roots and tubers for presence of white or golden yellow cysts (Kolombia and Fabiyi 2023). The two species can be

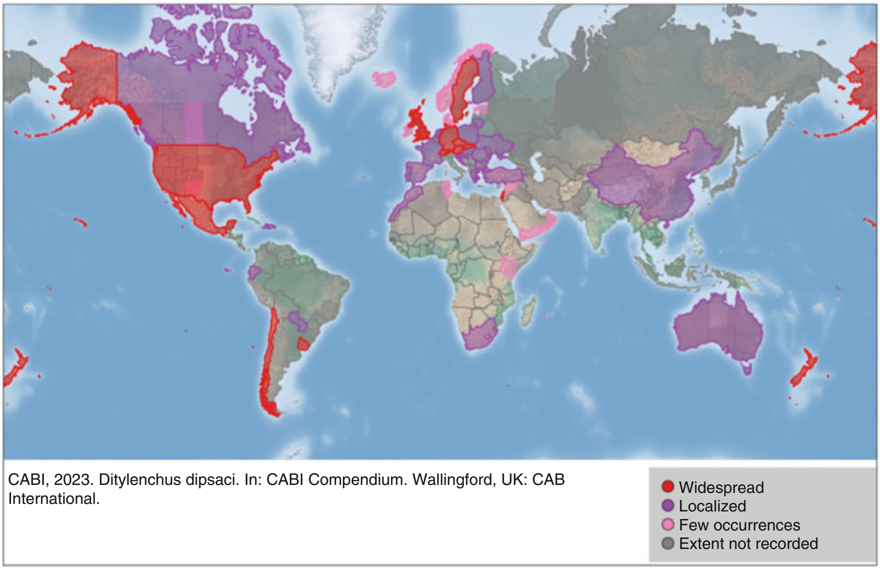

CABI, 2023. Ditylenchus dipsaci. In: CABI Compendium. Wallingford, UK: CAB International.

Fig. 1.8 Global distribution of stem and bulb nematode, *Ditylenchus* spp. (Source: CABI)

Table 1.6 Major species of stem and bulb nematode, *Ditylenchus* species, and the yield losses caused to important crops

Crop groups	Major species	Yield loss	Crops
Cereals	*D. angustus* *D. dipsaci*	20–90%	Rice, wheat, maize, barley, and oat
Vegetables	*D. destructor* *D. dipsaci*	10–90%	Potato, sugarbeet, onion, garlic, cucumber, pumpkin, tomato, cucurbits, carrot, reddish, leafy vegetables
Mushroom	*D. myceliophagus*	Up to 100%	White button mushroom, etc.
Legumes	*D. dipsaci*	20–50%	Broad bean, lentil, pea
Oil seeds	*D. africanus* *D. destructor*	Up to 25%	Groundnut, soybean, peanut
Ornamentals	*D. dipsaci*	7–12%	Gladiolus, lilies, narcissus and other bulbs, ornamental plants
Beverage crop	*D. dipsaci* *D. procerus* *D. africanus* *D. destructor*	Up to 15%	Coffee, sugarcane, tobacco, tea, etc.
Forage crops	*D. dipsaci*	Up to 8%	Maize, barley, sorghum, berseem, etc.

Fig. 1.9 Symptoms of *Ditylenchus angustus* on rice, showing patches on the leaf bases, partial emergence of panicles with twisted leaf (**a** and **b**), Potato tuber showing rotting caused by *Ditylenchus destructor* (**c**). (Source: (**a** and **b**) Bora and Rahman (2010); NASI, India; (**c**) Bonsak Hammeraas, NIBIO-The Norwegian Institute of Bioeconomy Research, Bugwood.org)

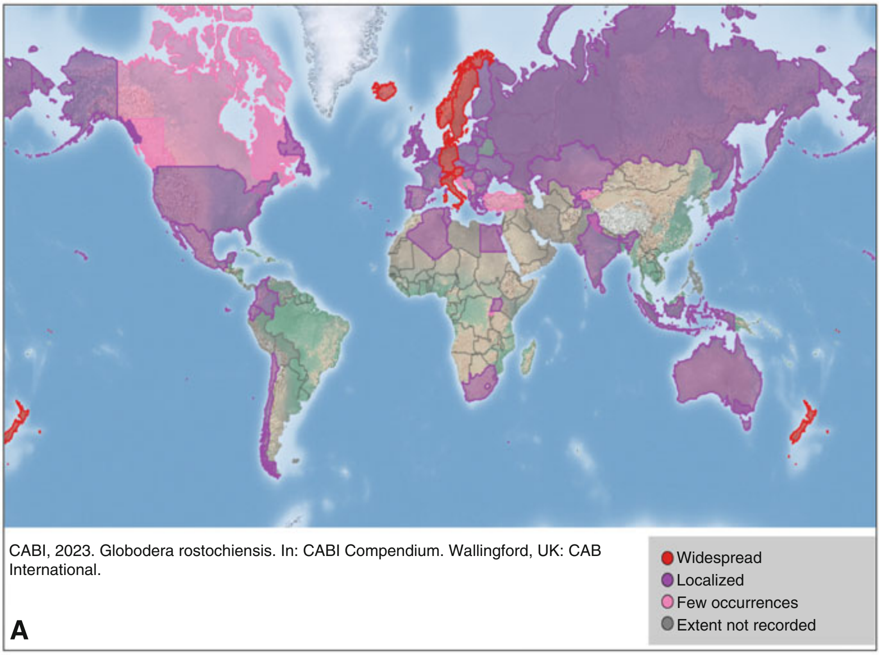
identified on the basis of change in the colour of female at the maturing stage. The cysts of *G. rostochiensis* turn white to yellow, while the cysts of *G. rostochensis* become creamy but no yellow.

The infective stage is second stage juvenile (J_2) which emerges from the egg. The J_2 enters the root and moves towards the vascular cylinder through intra and intercellular migration and becomes sedentary after inducing the formation of syncytia in phloem tissue (Devarajan et al. 2012). At maturity, the sedentary females become globular. The cortex ruptures due to body enlargement and the posterior portion emerges on the root surface. The vermiform males present in the soil mate with the females emerged on the root surface. The females die at maturity containing eggs inside the body called cyst. The cyst is the only stage of development easily visible without magnification (Fig. 1.11). Generally, one generation is completed during a crop season. Both the *Globodera* species contain pathotypes, which are subgroups distinguished on reproduction ability on *Solanum* clones (Canto Saenz and de Scurrah 1977).

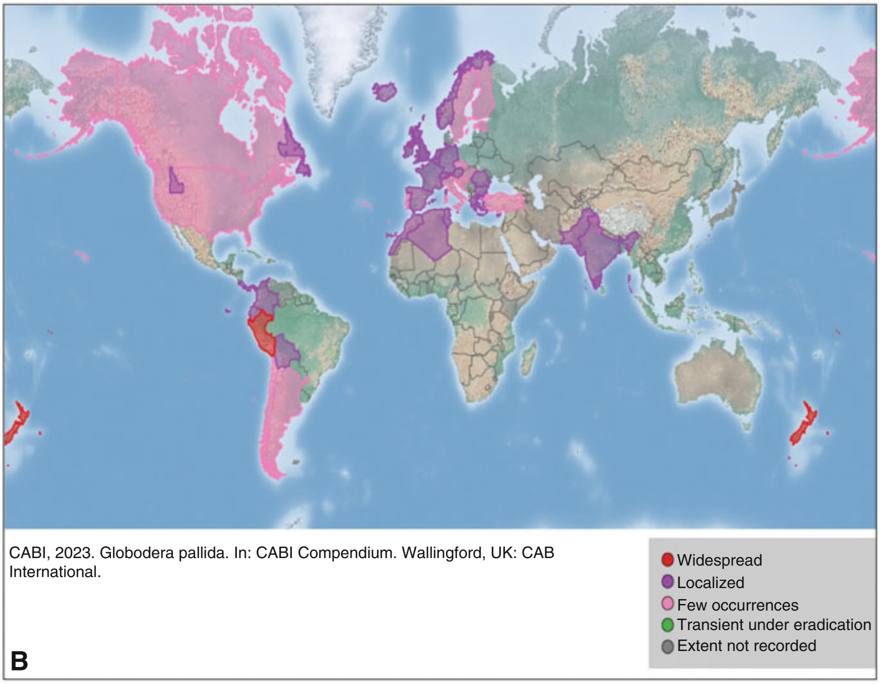
Potato cyst nematodes may significantly reduce the tuber production as well as its quality (Kolombia and Fabiyi 2023). The yield losses up to 80% are reported in the hills of the tropics under high infestation level and continuous potato culture (Prasad 1992). However, on a large-scale assessment, 9% yields loss in potato may occur annually due to PCN (Evans and Brodie 1980).

1.7 Citrus Nematode, *Tylenchulus* spp.

The nematode is worldwide in distribution, especially in citrus cultivation regions (Fig. 1.12). The tropical and subtropical climate and soil type are key determinants for its distribution besides the host factor. The global surveys in citrus growing regions have revealed up to 90% infestation with *Tylenchulus semipenetrans* in USA (Heald and O'Bannon 1987), 89% soils in Iran (Tanha Maafi and Damadzadeh 2008), 94.7% groves in China (Zhu et al. 1992), 77% orchards in northeastern Spain



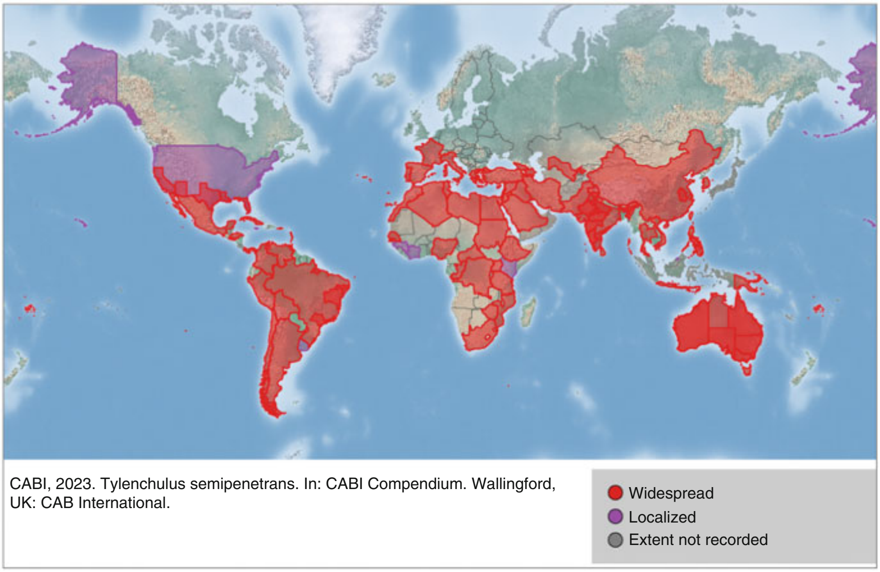
CABI, 2023. Globodera rostochiensis. In: CABI Compendium. Wallingford, UK: CAB International.



CABI, 2023. Globodera pallida. In: CABI Compendium. Wallingford, UK: CAB International.

Fig. 1.10 Global distribution of *Globodera rostochiensis* (**a**) and *G. pallida* (**b**). (Source: CABI)

Fig. 1.11 Potato roots showing cysts of *Globodera* spp. (Source: Central Science Laboratory, Harpenden, British Crown, Bugwood.org)



CABI, 2023. Tylenchulus semipenetrans. In: CABI Compendium. Wallingford, UK: CAB International.

Fig. 1.12 Global distribution of citrus nematode, *Tylenchulus* spp. (Source: CABI)

Table 1.7 Major species of dagger nematode, *Xiphinema* species, and the yield losses caused to important crops

Crop groups	Major species	Yield loss	Crops
Cereals	*X. bergeri* *X. ifacolum*	Up to 11%	Rice, etc.
Vegetables	*X. americanum* *X. diversicaudatum* *X. index*	Up to 32%	Tomato, chilli and bell pepper, onion, garlic, cucumber, carrot, reddish leafy vegetables
Oil seeds	*X. americanum* *X. diversicaudatum*	7–17%	Soyabean, groundnut
Fruits	*X. brevicolle* *X. index* *X. elogatum* *X. diversicaudatum*	5–21%	Fig, guava, lychee, Banana, citrus, olive, pine apple
Ornamentals	*X. diversicaudatum* *X. americanum*	5–12%	Rose, gladiolus, turf
Beverage crop	*X. diversicaudatum* *X. index* *X. americanum*	3–12%	Coffee, sugarcane, tobacco
Fibre crops	*X. diversicaudatum* *X. index* *X. americanum*	7–8%	Cotton, jute

(Sorribas et al. 2008), 58.2% in India (Tiwari and Vadhera 1999), and 98.4% in citrus orchards in Pakistan (Ahmed and Khan 1999). The citrus nematode has a narrow host range and is an important constraint in citrus production causing up to 15–50% yield loss (Table 1.7).

The symptoms induced by citrus nematode are non-specific and difficult to diagnose. There is slow but continuous reduction in terminal growth, followed by reduced vigour of plants (Mokrini et al. 2023). The foliage is sparse, often dull grey-green or bronze-green with reduced leaf size and branches with die-back and slow decline symptoms (Duncan and Cohn 1990). The premature exposure of terminal branches can be recognized meters away from the infected citrus trees (Mokrini et al. 2023). The debilitating effect on the plant health as a whole is referred to as ‘slow decline’, which quite obvious in citrus. The number and size of fruits per plant are reduced. The feeder roots of heavily infected plants are fewer, shortened, and thicker giving dirty or encrusted appearance due to the soil/organic matter particles that adhere to the gelatinous egg mass matrix (Fig. 1.13a, b; Heald and O’Bannon 1987). The feeder roots decay faster.

The citrus nematode is a semi-endoparasite, but its J_2 starts feeding ectoparasitically on root epidermis and undergoes three moults to become immature females, which insert the anterior part deep into cortex within a week and induce a nurse cells system in the cortex to provide required food to the sedentary female feeds during the entire life span. The females reproduce parthenogenetically without mating and lay eggs in the gelatinous matrix called egg mass. The life cycle (egg to

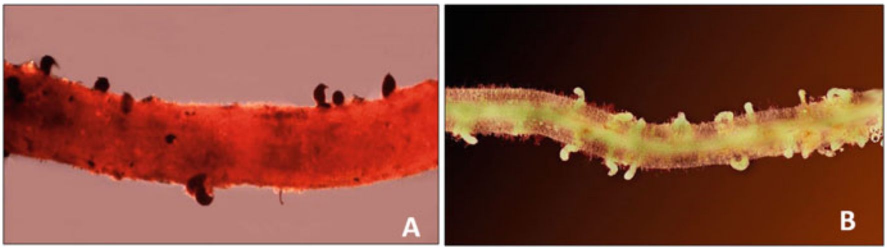


Fig. 1.13 Infestation with *Tylenchulus semipenetrans* on citrus roots (**a**) and *Rotylenchus reniformis* on tomato root (**b**). (Source: (**a**) Mactode Publications, Mactode Publications, Bugwood.org, (**b**) Jonathan D. Eisenback, Virginia Polytechnic Institute and State University, Bugwood.org)

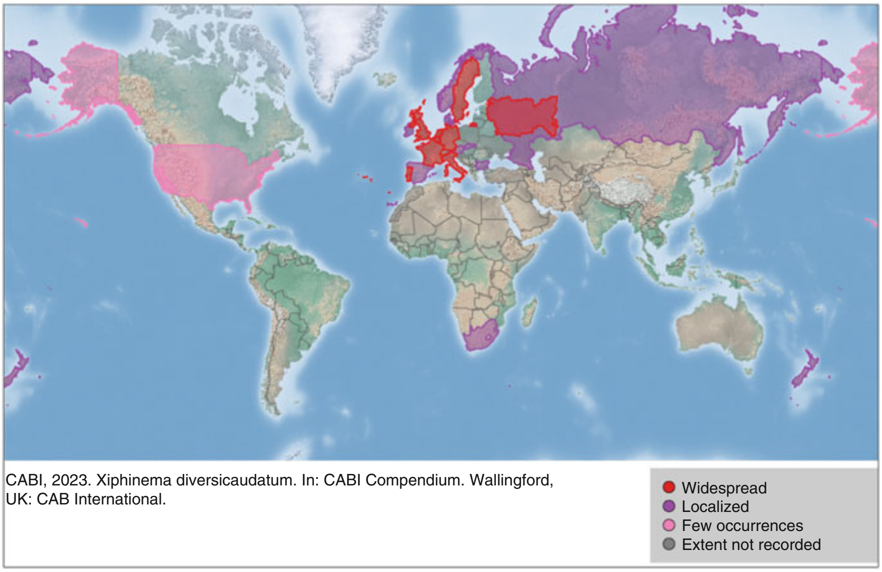
egg) completes within 6–8 weeks at 24–26 °C (Van Gundy 1958). The nematode damage to citrus on world basis has been estimated to the tune of 8.7–12.2% in terms of reduction in fruit yield (Heald and O'Bannon 1987). However, the losses may approach to 50% or even more at the advanced stages of citrus decline.

1.8 Dagger Nematodes, *Xiphinema* spp.

The *Xiphinema* species are ectoparasites of roots of diverse crops and commonly called as dagger nematodes for having dagger shape stylet. *Xiphinema* spp. have been placed in two groups: *Xiphinema americanum* group (61 species, generally virus vectors) and non-*americanum* group (over 215 species) (Gozel et al. 2006; Haque and Khan 2021a). The *Xiphinema* spp. can severely damage the roots of a number of plant species which may eventually lead to the host death.

Xiphinema spp. are distributed widely in temperate and tropical regions in Americas, Europe, Asia, Oceania, and Africa (Fig. 1.14). The nematode attacks a number of vegetables, fruits, turf grasses, etc., causing severe damage to them (Table 1.7; Ye et al. 2012; Crow et al. 2023). Important hosts of *Xiphinema* spp. are tomato, grape, oak, sea grape, pines, hackberry, Brazilian citrus (Gozel et al. 2006; Mokrini et al. 2014), sorghum, cotton, pearl millet, turfgrasses (Table 1.7; Wick 2012; Ye et al. 2012), legumes, sugarcane, chili, pepper, banana, sugar beet, corn (Melton and Shurtleff 1980), cassava, weeds, turf grasses, etc. (Renubala et al. 1991).

Although the dagger nematode is ectoparasite, its damage differs from other ectoparasitic nematodes (Khan 2008). The nematode larvae feed preferably on root-tips for several minutes to an hour causing terminal galls, especially in woody fruit plants (Shokoohi et al. 2023). Due to damage to root system, plants show stunted growth in patches. Some important viruses, cherry rasp leaf virus, tomato ringspot virus, grapevine fanleaf virus, etc., are transmitted by *Xiphinema* spp. (Van Zyl et al. 2012). The symptoms of the virus infections appear more prominently on woody plants than grasses (Palomares-Rius et al. 2012). However, the nematode is



CABI, 2023. Xiphinema diversicaudatum. In: CABI Compendium. Wallingford, UK: CAB International.

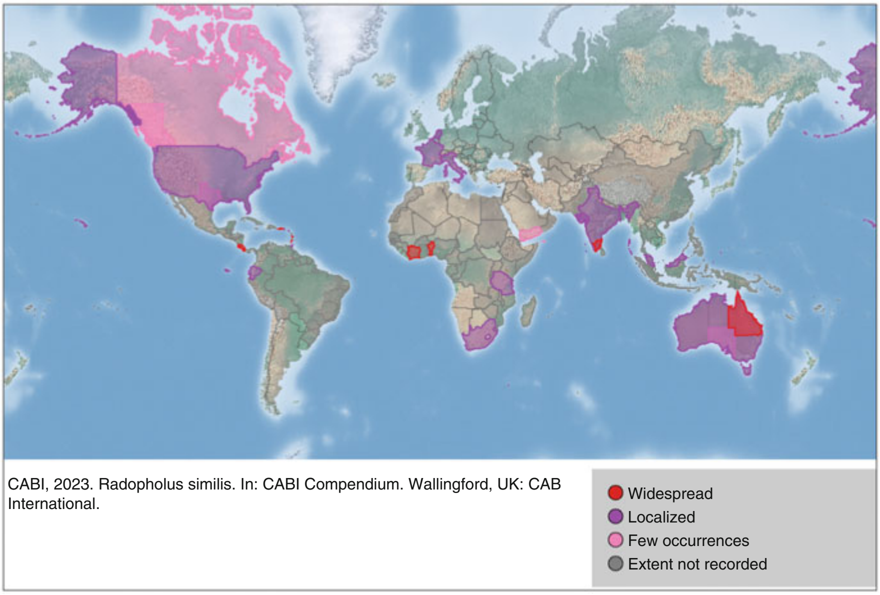
Fig. 1.14 Global distribution of dagger nematode, *Xiphinema* spp. (Source: CABI)

highly damaging to turf grasses (Crow et al. 2023), In the landscape, *Xiphinema* population may reach up to 5000 larvae/litre soil, but the direct attack on roots leads to only moderate damage to susceptible turf grasses such as bentgrass (Ye et al. 2012).

1.9 Borrowing Nematode, *Radopholus similis*

This is an important nematode pest and is widely distributed under tropical and subtropical climates (Fig. 1.15). It causes severe damage to a number of fruits, spices, and ornamentals (Table 1.8). *Radopholus similis* presents major problem in commercial cultivation of palms, citrus, spices, etc. and affects their market value also (Kanzaki et al. 2023). The *R. similis* infestation in banana is commonly known as “toppling disease”, in spice vines as leaf whitening, in citrus as spreading decline, etc. (Fig. 1.15).

The infected plants show general decline symptoms, stunted growth, premature drying/falling of leaves, unthriftiness, and formation of smaller fruits. However, more specific symptoms appear on corms and roots in the form of small sunken lesions on young roots and longitudinal brown to black lesions with surface cracks on older roots (Fig. 1.16a, b). At high population density of *R. similis,* the roots are severely destroyed, resulting in disruption in the absorption of water and nutrients from the soil. The root decay weakens the anchorage of root system to soil, and the plant topples down due to overload of fruits or high winds (Khan 2008, 2023).



CABI, 2023. Radopholus similis. In: CABI Compendium. Wallingford, UK: CAB International.

Fig. 1.15 Global distribution of borrowing nematode, *Radopholous similis*. (Source: CABI)

Table 1.8 Major host crops of borrowing nematode, *Radopholus similis,* and the resulting yield loss

Crop groups	Major species	Yield loss	Crops
Vegetables	*R. similis*	10–44%	Radish, tomato, chilli, bell paper, kale, brinjal, okra, carrot, african spinach, etc.
Oil seeds	*R. similis*	7–12%	Groundnut, soybean, coconut, palm, etc.
Fruits	*R. similis* *R. citrophilus*	7–70%	Banana, citrus, pineapple, grapes, watermelon, pumpkin, squash, etc.
Beverage crop	*R. similis*	Up to 32%	Tobacco, tea, coffee, etc.
Medicinal crops	*R. similis*	Up to 35%	Ginger, turmeric
Spice crops	*R. similis*	38–64%	Black pepper, cardamom, clove, ginger, turmeric, etc.

The burrowing nematode is a migratory endoparasite. All stages are infective. The larvae penetrate the root and move intercellularly in the cortex. Due to movement and feeding, the cortical cells are damaged which appear as necrotic lesions (Sheela and Sundararaju 2012). The larval movements lead to formation of cavities in the cortex which house all stages of the nematodes. The nematode also invades corm and rhizome. The reproduction is amphimictic and life cycle completes in 20–25 days (Sosamma and Koshy 2010). The burrowing nematode may inflict upto

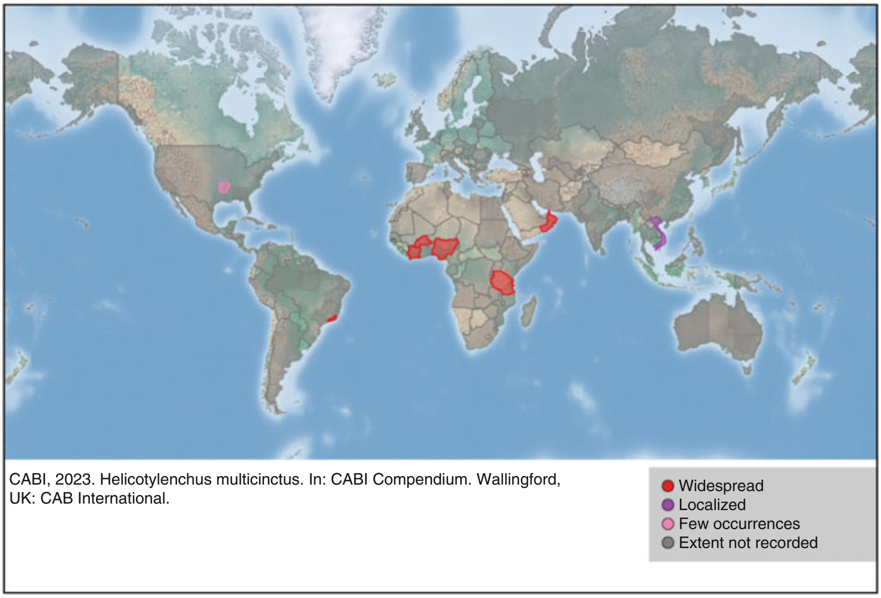
Fig. 1.16 Toppling of banana trees caused by *Radopholus similis* (**a**) and the resulting root necrosis (**b**). (Source: (**a**) Sheela and Sudararaju, NASI, India, (**b**) V. K. Sosamma and P. K. Koshi, NASI India)

10–80% yield loss in several economically crops, depending on the crop, population density, and climatic condition (Table 1.8).

1.10 Spiral Nematode, *Helicotylenchus* spp.

The spiral nematodes, *Helicotylenchus,* is a polyphagous species which is distributed widely in tropical, subtropical and temperate climates of Asia, Europe, Africa, Americas, and Oceania (Fig. 1.17). Important species of *Helicotylenchus* are *H. multicinctus, H. dihystera, H. africanus, H. erythriane,* etc., which may inflict economic losses to a number of crops. The *H. multicinctus* is next to *R. similis* under all types of bananas (Gowen et al. 2005). In temperate climatic zones, *H. multicinctus* survives only under glasshouse condition (Table 1.9).

Spiral nematode feeds ecto or endoparasitically on the outer cortical cells, causing small wounds and lesions (Haque and Khan 2022). The injuries facilitate the infection by soil bacteria and fungi (Khan and Sharma 2020). The external and internal damages to the root system obstruct uptake of water and nutrients leading to weakening, stunting and wilting of plants with suppressed yields (Khan 2023). The internal feeding and migration cause root necrosis, and under severe infection, plants during reproductive phase topple down as happens with banana due to *R. similis* (Sikora and Fernandez 2005). The nematode incites relatively shallow and indiscrete necrotic lesions on banana roots (Gowen et al. 2005). Generally, the nematode larvae feed superficially in the cortex causing severe root and rhizome damage (Speijer 1999). All stages of *H. multicinctus* an be found within roots or soil (Karakas 2007). The nematode reproduces amphimictically, and females lay eggs in cortex, which hatch soon to give rise to second stage juveniles. The juveniles feed within the root or migrate out and invade a fresh root. The nematode may cause up to 55% yield loss



CABI, 2023. Helicotylenchus multicinctus. In: CABI Compendium. Wallingford, UK: CAB International.

Fig. 1.17 Global distribution of spiral nematode, *Helicotylenchus* spp. (Source: CABI)

depending on the host cultivar, nematode population, and climatic conditions (Table 1.9).

1.11 Reniform Nematode, *Rotylenchulus* sp.

The reniform nematode, *Rotylenchulus reniformis,* is a polyphagous species attacking several crops especially vegetable, fruit, and fibre crops (Sikora et al. 2018; Table 1.10). The nematode is considered as an important constraint in vegetable cultivation, but overlooked often as it usually occurs with root-knot nematodes (Hallmann and Meressa 2018). The nematode is worldwide in distribution, occurring in several countries in Asia, Africa, Americas, and Europe (Fig. 1.18).

The infection with reniform nematode results in the stunting of plant growth, and leaves may show curling. Further, above-ground symptoms resemble with water and nutrition stress. Leaf chlorosis can also be seen (Bridge 1983). On underground parts, females along with attached egg masses may be seen under stereomicroscope (Fig. 1.19). The roots invaded by reniform nematode appear bulgy due to protruding posterior part of the female nematode and attached egg mass and look dirty due to soil particles and organic matter that adhere to the egg mass matrix (Khan 2008).

Reniform nematode is a sedentary semi-endoparasitic nematode, and adult female is infective stage. The juvenile stages are non-feeding; it quickly undergoes three superimposed moults to become a young female which inserts the anterior half in the

Table 1.9 Major host plants of spiral nematode, *Helicotylenchus* species, and the resulting yield losses in different crops

Crop groups	Major species	Yield loss	Crops
Cereals	*H. multicinctus* *H. mucronatus* *H. dihystera*	3–12%	Rice, maize, sorghum, etc.
Vegetables	*H. indicus* *H. dihystera* *H. multicinctus* *H. microlobus*	2–33%	Eggplant, okra, cucurbits, carrot, reddish, etc.
Legumes	*H. dihystera* *H. multicinctus* *H. indicus*	6–17%	Chickpea, lentil, mung bean, pigeon pea, etc.
Oil seeds	*H. dihystera* *H. multicinctus* *H. indicus*	10–40%	Soybean, groundnut, oil palms etc.
Fruits	*H. oleae* *H. dihystera* *H. multicinctus* *H. pseudorobustus* *H. varicaudatus*	7–55%	Banana, citrus, guava, pineapple, fig, etc.
Ornamentals	*H. nannus* *H. dihystera* *H. multicinctus* *H. indicus*	3–18%	Rose, turf, gladiolus, tulip, tuberose, etc.
Spice crops	*Helicotylenchus* spp.		Cardamom, ginger, turmeric, black pepper
Beverage crop	*H. dihystera* *H. erythrinae* *H. multicinctus* *H. indicus*	Up to 23%	Tea, coffee, sugarcane, tobacco
Forage crops	*H. dihystera* *H. indicus*	Up to 10%	Berseem, cowpea, Lucerne, oat, barley, sorghum, pearl millet, maize

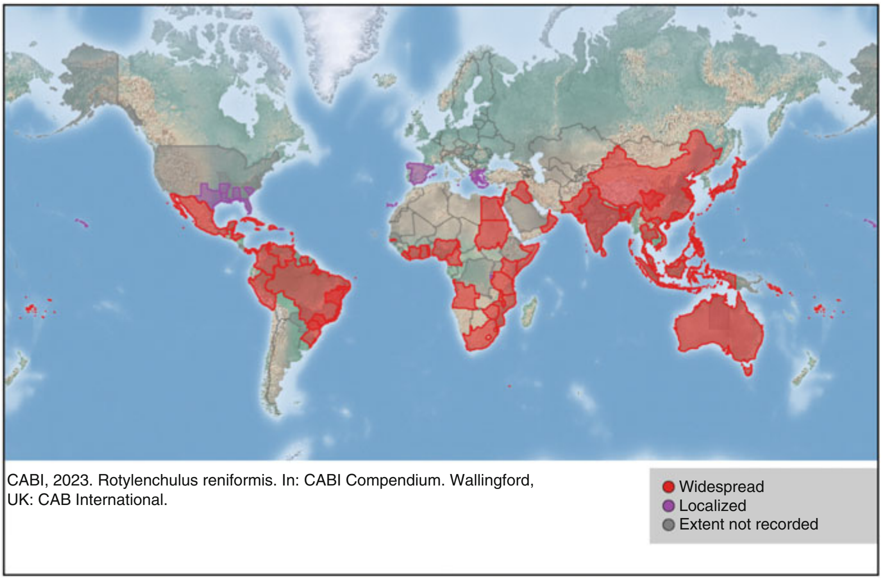
root up to pericycle and induces syncytia around the head region (Haque and Khan 2021a). The life cycle is completed on okra in around 4–5 weeks. The nematode has been found to cause significant crop damage and yield decline to a number of vegetable crops (Table 1.10). A population of 6500 larvae/kg soil caused 33% yield reduction in vegetable crops. Tomato, egg plant, okra, and pepper are highly susceptible to *R. reniformis* and may exhibit 5–16% yield loss (Singh and Khera 1978).

Table 1.10 Major host crops of reniform nematode, *Rotylenchulus* species, and the resulting yield loss in important crops

Crop groups	Major species	Yield loss	Crops
Cereals	*R. reniformis*	12%	Sorghum, pearl millet, etc.
Vegetables	*R. reniformis* *R. parvus*	5–16%	Tomato, okra, eggplant, cucurbits, carrot reddish, leafy vegetables, etc.
Legumes	*R. reniformis*	Up to 15%	Broad bean, cowpea, common bean, chickpea, pigeonpea, urdbean, mung bean, rajmah, lentil, pea, etc.
Oil seed	*R. parvus* *R. reniformis*	Up to 17%	Soybean, ground nut, etc.
Fruits	*R. reniformis* *R. macrosoma*	Up to 25%	Papaya, pine apple, guava, mango, banana, citrus, olive, etc.
Ornamentals	*R. reniformis*	Up to 9%	Chrysanthemum, tuberose, etc.
Spice crops	*R. reniformis*	Up to 46%	Cardamom, ginger, turmeric, etc.
Beverage crop	*R. parvus* *R. reniformis*	Up to 10%	Coffee, tobacco, etc.
Fibre crops	*R. reniformis*	Up to 20%	Cotton, jute, etc.

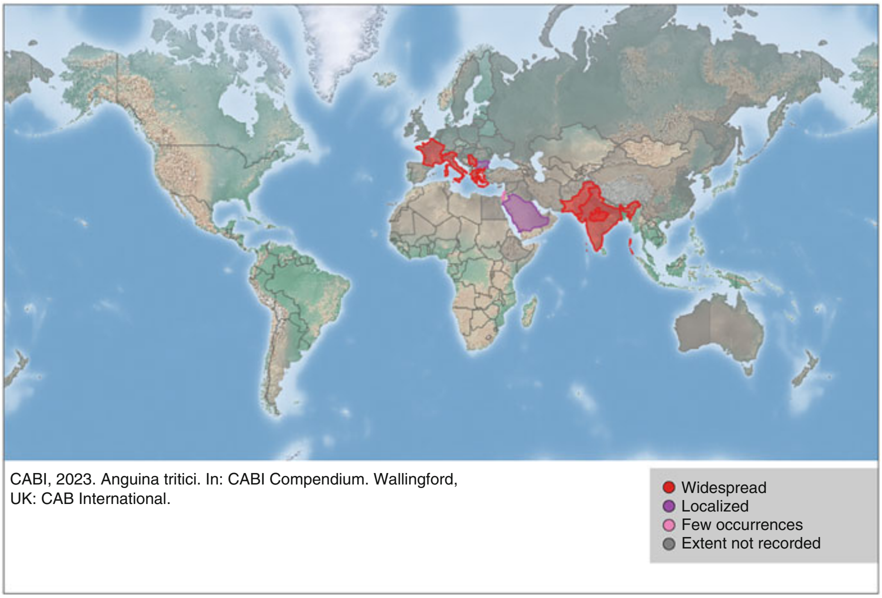
1.12 Foliar Nematodes, *Anguina, Aphelenchoides, Bursaphelenchus,* Etc.

The nematodes which attack stem, leaf, bud, flower, or seeds are known as foliar nematodes. These nematodes are generally active under cool and humid conditions because wetness on the plant surface is essentially required especially for *Anguina, Aphelenchoides, Ditylenchus,* etc. to enable their movement on the surface of stem or leaf (Khan 2008). Majority of the foliar nematodes belong to the order Aphelenchida, but *Anguina* and *Ditylenchus* from the order Tylenchida also have foliar parasitism. The genus *Anguina* includes the species which are seed-borne and are migratory ectoparasites during vegetative growth of the host plant, but become endoparasitic during reproductive phase of the plant growth (Owen et al. 2023). The first nematode in the history of nematology was *Anguina tritici* (seed gall nematode) which was discovered in 1743 by T. Needham infecting the wheat. The seed-gall nematode had been a major problem in Americas and Europe, but have been successfully eradicated by using seed cleaning techniques (Mandal et al. 2010). However, this nematode still assumes significances in the rural and tribal areas in some countries in Asia and Africa (Fig. 1.19) where uncertified seeds from older lots are used to grow wheat and barley. The nematode survives in a quiescent state (J_2) inside the infected grain (Khan et al. 2023) and becomes active when comes in contact with soil moisture. The J_2 migrates from the cockle and feeds ectoparasitically on growing points of leaves of young plants between the compact



CABI, 2023. Rotylenchulus reniformis. In: CABI Compendium. Wallingford, UK: CAB International.

Fig. 1.18 Global distribution of reinform nematode, *Rotylenchulus reniformis*. (Source: CABI)



CABI, 2023. Anguina tritici. In: CABI Compendium. Wallingford, UK: CAB International.

Fig. 1.19 Global distribution of seed gall nematode, *Anguina*. (Source: CABI)

Fig. 1.20 Distribution map of *A. tritici*. Symptoms of infection with *Anguina tritici* (**a** and **b**), *Aphelenchoides besseyi* white tip symptoms on leaves (**c**), and spikelets showing chaffiness (**d**), and *Aphelenchoides besseyi* infested tuberose cv. Calcutta double (**e**). (Source: (**a** and **b**) M. R. Khan, Aligarh Muslim University, (**c** and **d**) D. Prasad, NASI, India; (**e** and **f**) Matiyar R. Khan et al. (2012), NASI, India)

leaf sheath. When flower primordia are initiated, the larvae invade them and feed endoparasitically (McDonald and Nicol 2005). This leads to formation of cockles or seed galls in place of healthy grains. Inside a gall, thousands of nematode larvae in quiescent state (J_2) may survive for decades. The ectoparasitic feeding of J_2 causes crinkling and twisting of leaves, whereas the infected ear heads become short and wide (Fig. 1.20a), containing brown to black small and irregular grains (cockles; Fig. 1.20b) in place of few or all the normal grains. Nematode completes one generation within a season and may inflict 15–65% yield loss (Owen et al. 2023).

The leaf and bud nematodes, *Aphelenchoides* spp., are mycophagous in nature and feed ecto and endo-parasitically on leaves and buds (Hunt 1993). Most of the *Aphelenchoides* spp. are widely distributed in Europe, Americas, Oceania, and temperate areas in Asia (Fig. 1.21). The temperate species attack berries, ferns, ornamental plants, etc. However, *A. besseyi* is a species of tropical and subtropical climate and attacks rice, strawberry, ornamentals, etc. *A. besseyi* is a seed-borne ectoparasite, and predominantly adult females in quiescent state of anhydrobiosis survive on seeds between glumes and grains and cause whitening of leaf tips and chaffiness of the panicle (Fig. 1.20c, d; DeLiang et al. 2018). The quiescent state

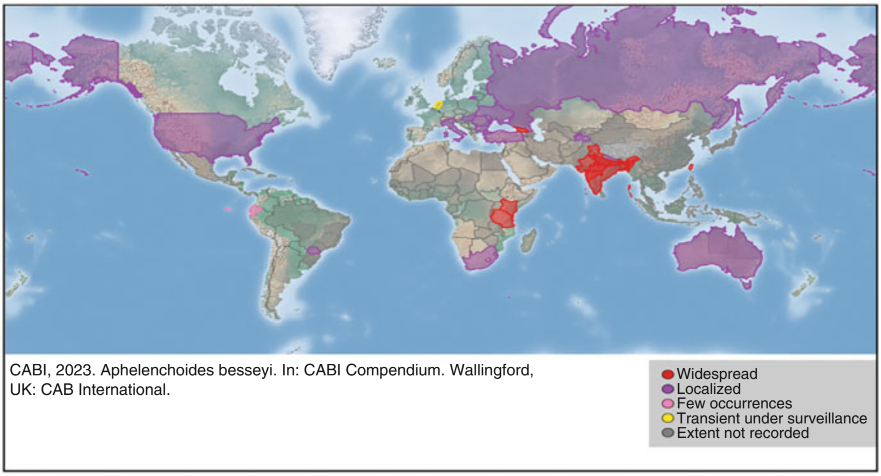


Fig. 1.21 Global distribution of *Aphelenchoides* species. (Source: CABI)

terminates when infested seeds come in contact with water and the nematodes move on plant surface through a film of water to feed upon leaf tips and their margins (Bridge et al. 2005). At the time of flowering, the larvae migrate to apical portion and feed ectoparasitically on ovary, stamens, lodicules, and embryo. *A. ritzemabosi* and *A. fragariae* are migratory endoparasites, but also feed ectoparasitically (Khan et al. 2021). The nematodes move on plant surface and feed ectoparasitically in bud axil or enter the leaves through stomata and feed on mesophyll cells (Khan 2008). During the movement and feeding, the larvae secrete various enzymes. On the advent of adverse conditions, predominantly adult females enter into quiescent state of cryptobiosis to survive under extreme low temperature. Both the species cause characteristic interveinal leaf necrosis in ferns and chrysanthemum, strawberry, tuberose (Fig. 1.20e, f), etc. The berries are also dishaped and the petioles become twisted. Besides infecting plant species, *A. composticola* and other species attack mushroom compost and severely affect the mushroom production. The *Aphelenchoides* spp. are reported to inflict 5–58% yield loss in different crops (Table 1.11).

Some other important foliar nematodes from the order Aphelenchida are fig nematode (*Schistonchus* spp.), eucalyptus nematode (*Furgusobia* spp.), wilt nematode (*Bursaphalenchus xylophilius*), and red ring nematode (*Bursaphalenchus cocophilus* = *Rhadinaphelenchus cocophilus*) (Khan 2023). All these nematodes are of commercial significance and are transmitted by insect vectors (Khan 2008). The nematodes, *S. caprifici* and *F. tumifaciens,* cause galls in the leaves/flowers of fig and eucaplyptus with the aid of insect vectors, *Blastophaga psenes* and *Fergusonina* sp., respectively (Pimentel et al. 2023). Similarly, *B. xylophilus* and *B. cocophilus* are transmitted by *Monochamus* spp. (pine swayer beetle) and *Rhynchophorus palmarum* (black palm weevil), respectively. The larvae of *B. xylophilus* feed on stem parenchyma and cause wilt in conifers, in particular the

Table 1.11 Major host crops of leaf and bud nematodes, *Aphelenchoides* spp., and resulting yield loss to important crops

Crop groups	Major species	Yield loss	Crops
Cereals	*A. besseyi*	10–60%	Rice
Vegetables	*A. fragariae* *A. ritzemabosi* *A. besseyi*	Up to 10%	Tomato, onion, garlic, etc.
Mushroom	*A. composticola*	Up to 100%	White button mushroom
Oil seeds	*A. arachidis*	9%	Groundnuts
Fruits	*A. besseyi, A. fragariae* *A. ritzemabosi*	60%	Strawberry
Ornamentals	*A. ritzemabosi* *A. fragariae*	Up to 38%	Chrysanthemum, tuberose, ferns, etc.

pines, whereas *B. cocophilus* causes red ring in palms, particularly in coconut (Khan et al. 2021). Pine wilt is a very serious disease of coniferous forest in the northern hemisphere and is responsible for death of millions of pine trees each year (Pimentel et al. 2023). Similarly, the red ring is a serious problem in palms in Caribbean islands to Latin American countries (Haque and Khan 2021a). The nematode can inflict up to 80% mortality to coconut palms (Kanzaki et al. 2023).

1.13 Interaction with Other Organisms and Disease Complex

Plant parasitic nematodes, besides causing direct damage, have been often found to synergize plant pathogens in aggravating the severity of the disease caused by fungi (Powell 1979; Khan et al. 2005) and bacteria (Khan 1993; Sitaramaiah and Pathak 1993). The nematodes may also alter the host reaction of a cultivar to the pathogen (Khan and Sharma 2020). A number of cultivars of potato, tomato, chilly, chickpea, pigeonpea, cotton, tobacco, etc. resistant to fungi/ bacteria are reported to become susceptible due to invasion of plant nematodes (Khan et al. 2005). The diseases resulting due to interactions between fungi/bacteria and nematodes are commonly called disease complex (Khan 1993). The disease complexes involving wilt inducing fungi (Francl and Wheeler 1993; Khan and Akram 2000; Akram and Khan 2006) and root-rot causing fungi (Evans and Haydock 1993; Khan and Haque 2013) are of common occurrence as well as of economic significance (Khan et al. 2019, 2023).

Although plant nematodes generally favor soil-born fungi/bacteria in some way, but endoparasitic nematodes have been found to be of greater significance in enhancing the disease severity. *Globodera* sp. and *Rhizoctonia solani* occurring concomitantly caused great loss to potato. The nematode also interacted with *Verticillium dahliae* and enhanced the severity of the wilt disease. *G. pallida* is reported to also have a positive association with *Ralstonia* (=*Pseudomonas*) *solanacearum*. The wilt disease complex in tomato, chilli, egg plant, okra, etc. caused by *Fusarium* and *Meloidogyne* species, has been found to be highly damaging to the respective crops (Evans and Haydock 1993; Khan and Sharma 2020).

Synergistic interaction between *Fusarium pallidoroseum* and phytonematodes has also been observed on maize (Darras 2012). The wilt of maize caused by *Cephalosaparium maydis* also became severe in the presence of *H. zeae* (Singh and Siradhana 1988). The banana wilt caused by *F. oxyspoum* f. sp. *cubense* and *R. solanacearum* got aggravated in co-occurrence of the spiral nematode, *H. multicinctus*. Meagher (1977) recorded that the disease complex caused by *H. avenae* and *Rhizoctonia solani* resulted to greater reduction in the plant growth of wheat than infestation with either pathogen.

1.14 Management of Plant Nematodes

In view of significance of nematodes in crop production with regard to suppression in the plant growth and yield, various management methods are available which may check the nematode build up in the soil as well as in the host tissue. However, effectiveness of these methods varies with nematode population, host species, time of application, etc. Generally, a combination of methods proves more effective than a single method. However, under a given situation, a specific method gives a better control. Hence, selection of a single or combination of methods and the time of application are important aspects of nematode management strategy. Some of the important methods of nematode management are briefly described under:

1.14.1 Cultural Practices

These methods generally help plants to avoid contact with the nematode inoculum and to eradicate or reduce the infestation level in a plant or population density in a field or area. In transplantable crops like tomato, chillies, and brinjal, the nematode management should commence right from the time of raising the nursery. Hence, it is essential to select the nursery site free from the nematode. In the main field, summer ploughing, fallowing, use of nematode free planting material, growing nematode resistant cultivars, and crop rotation are the best cultural practices for nematode management. Some cultural practices effective against plant nematodes are summarized in the following paragraphs.

Exclusion and Prevention of Nematode Spread Nematodes need various means, agencies, and vectors for their spread from one place to other, as their own movement is confined hardly to a meter in a year. The plant materials are very potential means of nematode spread. Hence, there should be certain restrictions on the movement of planting material from the areas having record of nematode infestation. The other way of control on nematode spread at farm level is to prevent the movement of nematode-infected soil adhering to boots, farm implements, etc. by cleaning these items before using in an area or field.

Summer Ploughing and Stubble Pulling Two to three summer ploughings, pulling of stubbles along with root system of previous left-over crops, and their destruction are effective means to reduce the nematode population in the field. Summer ploughing not only leads to disturbing the nematode community structure in the soil, but also causes mortality by exposing them to direct solar light and desiccation.

Selection of Healthy Planting Material A number of crops are propagated vegetatively or by using seedlings raised in conventional nursery beds or by using soil-free medium in trays. Hence, it would be very effective and handy to eliminate the nematode infestation by selecting uninfected corms, seedlings, or root stock for propagation. The risk of spread of endo and semi-endo parasitic nematodes can be easily eliminated by this practice.

Host Resistance Use of nematode-resistant cultivars is the most handy and economically viable approach of nematode management. But, restricted availability of resistant/tolerant cultivars is a phenomenal limitation to this approach for many crops. However, resistant cultivars of a number of crops are available which are quite effective against important plant nematodes.

Crop Rotation Crop rotation is an oldest, most proven, handy, eco-friendly, and cost-effective practice that offers an effective option for nematode management. This practice aims to keep the nematode population under check by manipulating the cropping sequence to minimize the impact of plant nematodes on the plant growth and productivity. The growing non-host crops in a field significantly suppress soil population of nematodes and subsequently reduce nematode infestation in the susceptible crop to follow. Crops such as sorghum, pear millet, maize, wheat, cabbage, cauliflower, mustard, onion, garlic, sesame, sunhemp, sesbania, marigold, etc. are poor host, non-host or antagonistic crops for root-knot nematodes and can be used for crop rotation. Depending upon the preference and need of farmer or time available between two susceptible crops, a crop species is selected which could fit into the cropping sequence. Cultivation of paddy or mung bean after banana reduced the populations of *R. similis*, *P. coffeae,* and *H. multicintus* (Rajendran et al. 1979). Crop rotation with crops like wheat, strawberry, cabbage, cauliflower, mustard, maize, and beans may decrease the population of *Globodera* in potato fields.

Trap Crops There are two types of trap crops which can be used against plant parasitic nematodes. In one type, nematode susceptible crops are planted at high densities and are ploughed back into the soil before the nematode completes its life cycle. In other type, certain plant species attract the nematode just like susceptible ones, but prevent its development and multiplication while inside the host, e.g., *Solanum nigrum* inhibits the development of *Globodera* spp. (Varandas et al. 2020). Both types of trap crops are quite effective against semi-endoparasitic and endoparasitic nematodes. Periwinkle (*Catharanthes roseus*) can act as a trap crop for *Meloidogyne* and *Rotylenchulus* species (Khan et al. 2005). Cowpea can also be used as a trap crop, as it is highly susceptible to the above nematodes, and, after 3–4 weeks, the crop is ploughed breaking the life cycle of the nematode.

Fallowing Maintaining a field without a crop as well as the weeds for a period of 6–8 weeks leads to the starvation of nematodes present in the soil. As a result, a significant proportion of the nematode population is decreased. But absence of nematode susceptible weeds is also to be ensured in the fallow land, otherwise the entire exercise shall go in vain. Though bare fallow ensures significant reduction in the nematode population, its non-productivity nature is an economic concern to the farmers. To further improve effectiveness of the exercise, the field should be deep ploughed. This would enhance the desiccation rate and minimize the growth of intermediate hosts, weeds, etc.

1.14.2 Physical Control

Physical methods are aimed to protect plants from the nematode inoculum that is likely to arrive and to cure the infestation already in progress. Heat or energy in the form of sun light, hot water, or irradiation can suppress the activity of plant nematodes. But it is generally used at pre-planting stage for disinfesting the soil or planting material. Important physical methods of nematode control are discussed in the following paragraphs.

Soil Solarization Soil solarization is a natural method of suppressing the soil-born pathogens, being highly effective against plant nematodes. Besides the pathogens, it also suppresses weed growth. The soil solarization is particularly economic for nematode management in nursery of crops like tomato, chillies, brinjal, rice, etc. Well-prepared and pre-irrigation fields or nursery beds with moisture levels around field capacity are covered by a clear transparent polythene sheet and left undisturbed for 4–6 weeks. Application of organic amendments in combination with soil solarization hastens the decomposition of organics resulting in better nematode control. Soil solarization with 100-gauge transparent poly-sheets in hot summer month for 15 days in tomato and melon gave 92% reduction in the nematode population and 90–116% increase in the plant yield (Candido et al. 2008).

Steaming Soil disinfestation with steam is a practice that has been used in heated glasshouses for almost a century. In recent times, steam is blown under a plastic sheet of 0.25 mm thick. The sheet edges are anchored by ship chains or sand bags and left for 8 h to enable its penetration into the soil. A permanent steaming system consisting of perforated flexible drainpipes wrapped in polypropylene fibre (to prevent silting of perforations) is buried at a depth of about 30–40 cm and about 80 cm apart in which steam is blown. With this system, the temperature in deeper soil layer is raised to much higher level than the under-sheet steaming, providing high degree of nematode control in the soil.

Rabbing It is yet another traditional soil heating technique used against soil-borne pathogens and nematodes. It is the simple technique of burning the crop residues such as stubble and husk of paddy, ragi, pear millet, wheat, etc. over the soil surface. Rabbing of nematode-infested nursery in splitted furrow (9 in. apart and 6 in. deep) with either rice seed husk or wheat straw @ 7 kg/m^2 significantly suppressed the *Meloidogyne* population and weeds (Patel et al. 1983). Rabbing with paddy husk @ 7 kg/m^2 was also found effective in managing *Meloidogyne* spp. in tobacco nursery (Hussaini 1985) and turmeric field (Patel and Patel 2001).

Hot Water Treatment This technique is based on the principle that plant tissue is around 3–5 °C less sensitive to lethal temperatures than the animal tissue. It is an effective treatment in disinfesting the planting materials such as bulbs, tubers, seedlings, seeds, etc. from the endoparasitic nematodes. Treating potato tubers with water at 45 °C for 48 h caused 98.9% mortality to root-knot nematodes without affecting its viability (Nirula and Bassi 1965). Khan et al. (2006) reported good control of floral malady disease in tuberose (*Aphelenchoides besseyi*) by treating the overnight-soaked bulbs with hot water (50 °C) for 20 min. The hot water (51–58 °C) is required to disinfest the unsoaked bulbs. Pre-soaking of seeds in water for 15 h to activate the dormant nematode followed by 52 °C hot water treatment for 15 min proved highly suppressive to *A. besseyi*. Paring and hot water treatments of banana corms at 55 °C for 20 min, followed by neem cake application (1 kg/ha), are effective for reducing *R. similis* and *P. coffeae* population in the corns.

Microwave Irradiation The most effective way of converting energy into heat is by electromagnetic irradiation at 2450 MHz. The microwave irradiation is absorbed by only water molecules, resulting in the resonance of the molecules that leads to the temperature increase. The larvae of *M. incognita* were killed in soil at 5 cm depth with exposure to 2450 MHz radiation at 1250 W for 30 s. Similarly, complete killing of *R. reniformis, H. schachtii,* and *Anguina tritici* has also been obtained at 2450 MHz radiation (Khan et al. 2012). All the nematodes associated with the mushrooms are killed with the same treatment. But for field treatment, microwave irradiation is still not practicable because of its limited penetration into soil.

1.14.3 Biological Control

Biocontrol is aimed to achieve a reduction in the nematode population through the action of microbial antagonists which occur naturally, by modifying the soil and environment suitable to their activity or through their introduction (Stirling 1991; Mohiddin and Khan 2013; Khan et al. 2022). With the recent public awareness for health risk and environmental contamination involved in the use of synthetic chemicals in agriculture, there is increasing demand for food materials through organic farming and biopesticide application. A number of microorganisms such as *Arthrobotrys, Dactylaria, Purpureocellium, Pochonia, Bacillus, Pseudomonas, Trichoderma,* etc. occur in natural soils under different climates (Stirling 1991; Khan

2007; Shahid and Khan 2016, 2019) and may affect the fitness, activity, and parasitizing ability of plant nematodes (Khan et al. 2016). Application of biocontrol agents alone or along with oil or neem cakes at the time of planting is getting popularity in achieving sustainable nematode management in agricultural crops (Stirling 1991; Khan 2007; Khan and Anwer 2011; Sikora et al. 2018; Khan et al. 2021). The nematode antagonists such as *Pochonia chlamydosporia, Purpureocelium lilacinum, Aspergillus niger, Pasturia penetrans,* etc. (Jatala 1986; Stirling 1991; Kerry 2000; Khan et al. 2016) may prove effective in suppressing soil populations of nematodes if applied timely. Similarly, phosphate solubilizing microorganisms such as *Pseudomonas, Bacillus*, *Penicillium,* and *Aspergillus* species/strains (Khan et al. 2009, 2016) may also greatly contribute in protecting the young plants, if applied at the time of planting. In recent decades, *Trichoderma* has emerged as an important antagonist of plant pathogens, especially fungi and nematodes (Mohiddin et al. 2010; Khan and Mohiddin 2018). The biopesticides based on *Trichoderma* spp., *Pseudomonas florescence*, *Purpurocillium lilacinum*, *Bacillus subtilis,* etc., are available (Khan et al. 2009, 2011), which have been found highly effective against the plant diseases caused by fungi and nematodes when applied on the planting material (Sikora et al. 2018; Mohammed and Khan 2021; Haque and Khan 2021b, 2022).

1.14.4 Chemical Control

A concerned grower primarily relies on nematicides to control the disease problem. When infestation level in soil is high, it becomes essential to grow a non-host crop, otherwise soil has to be disinfested with fumigants such as methyl bromide, metham sodium, etc. Since application of most of the fumigants has been banned or it involves soil covering, etc., granular nematicides such as carbofuran, phorate, thionazin, etc. at a dose of 4–5 kg ai/ha can provide satisfactory decline in the nematode population. These nematicides are relatively safer, hence can also be applied at post-planting stage. In case of transplanting crops, it is always advisable to disinfest the planting materials by root-dip treatment with 50–100 ppm carbofuran solutions. When plants are small and nematode infestation has been detected, a foliar spray with phenamiphos or oxamyl @ 5 L/ha can effectively decrease the level of soil infestation. Khan et al. (2014) reported satisfactory control of root-knot nematode in rice by applying phorate through root dip and soil application. In recent year, some new molecules/chemicals such as flupyrum and fluensulphone have been found effective against soil nematodes (Haque and Khan 2022). The fluensulphone (Nimitz™) has a novel mode of action by disrupting the nematode feeding and causing paralysis that cumulatively leads to their death. Similarly, Flupyram selectively blocks cellular energy production in nematodes by inhibiting complex-II system. However, before their wide use, the impact on soil microbial community is needed to be essentially examined. There are other nematicides which can also suppress nematodes. Application of nematicides, however, should be restricted to serious or endemic situations.

1.15 Integrated Nematode Management (INM)

The INM is a systematic approach to reduce the nematode population to a level tolerated by crops without exhibiting economic yield loss. This can be achieved by using different methods, including predators and parasites, genetically resistant hosts, nematicides, cultural practices, solarization, etc. The concept of INM is based to include all classes of pests (pathogens, insects, nematodes, weeds, etc.) with the application of both methodological and disciplinary integration. Hence, INM is a component of IPM which is again a component of agricultural production system. The INM consists of various strategies (Dasgupta 1998), such as: giving maximum reliance to nature, i.e., no overt action coupled with or without planned biological control; employing all principles of control, regulation, prevention, avoidance, eradication, protection, therapy and resistance; selecting control tactics on the parameters of efficacy, economics, and ecology, integrating them in a compatible manner as well as fitting into the agricultural system; and applying the principles of systems and management in all decision-making processes, operations, optimization, and analysis. Hence, INM modules should be developed starting from selecting a site to produce healthy planting material or nursery, reducing the nematode population in field by using solarization, growing trap/antagonistic crops, selecting resistant crops, and applying biopesticides.

1.16 Conclusion and Future Perspectives

The nematode infestation in agricultural crops, in global perspective, has a significant socio-economic impact on the farming community in particular vis-a-vis the entire human population and animal husbandry. The nematode infestations alone or the resulting disease complexes create situations where crop failures or serious crop losses are of common occurrence in different crop production systems. Generally, the small farmers are the bigger sufferers as they are not aware of the consequences of nematode infestation in crops or do not recognize their economic significance. Hence, they do not take any management measure against nematodes, although a number of management options are available to the growers. Nematode management through chemicals is quite handy and effective, but the application often leads to environmental pollution and other hazardous consequences. Hence, emphasis should be given for developing and optimizing eco-friendly management options such as physical, cultural, and biological methods, and to rationally integrating them with other well-proven nematode management practices. Development of such sound management tactics/modules against plant parasitic nematodes would help in improving productivity and quality of crop produce. This shall help tremendously to fight against hunger world over.

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2 Nematode Management in Crops; Limitations and Challenges to Meet Future Food Demands

Oluwatoyin Adenike Fabiyi and Tesleem Taye Bello

Abstract

Plant feeding nematodes have the propensity to individually inflict severe damage or interact with other soil microbial organisms to form a disease complex situation and significant yield losses on many crops worldwide. While the kind of injury inflicted on the plant depends on the species of nematode, severity of infestation largely depends on their population density in the soil and other inherent plant factors which govern resistance or susceptibility to the invading nematode species. Therefore, most nematode management strategies are usually employed to reduce the population density of the pest below the damage potential threshold. Except for the underdeveloped nations of sub-Saharan Africa and some parts of Asia, nematode management worldwide witnessed a drastic change within the last decade. This change is largely attributed to the increased health and environmental concerns associated with heavy reliance on chemical pesticides in pest management. Present day nematode management options have progressed from the traditional total nematode eradication from the soil using nematicides and blunt crop rotation to a broader and more complex management programmes that can ensure sustainable nematode control by bringing down and keeping nematode pest population densities at levels where they can cause minimum damage to crops. This way, growers are able to attain the

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maximum potential yields from their crops at a reasonable cost. This chapter discusses the available nematode management options in crops with their limitations and challenges associated with them.

Keywords

Nematodes · Control · Resistance · Crops · Microbes

2.1 Introduction

It is interesting to know that almost every method currently used in managing nematode pests of crops has undergone some moderate changes in the past decade. Furthermore, most recent researches have extensively studied the management of agriculturally important plant parasitic nematodes (PPNs) within the context of biology, biochemistry, physiology, and molecular biology with the aim of reducing nematode pest population densities either through improving host plant resistance, causing an interruption in the lifecycle of target PPNs, or through creating an environment not conducive for notorious nematodes to thrive in farmers' field. Also, the increasing environmental concern associated with the use of chemicals in modern agricultural practices has also helped to shape the current direction of most research in nematode management (Khan 2023). However, the choice of any nematode management option may depend on grower's goal knowledge of control options and production system which ranged from subsistence or small-scale, conventional to mechanized commercial scale, all of which are influenced by resource availability (Khan 2016). The nematode management strategies are usually employed to reduce their populations in the soil. At the present state of technology, pesticides are most efficient means of management (Khan et al. 2009, 2014), but their application leads to several serious consequences of food and environmental toxicity (Khan 2005, 2007). Under this situation the nematode management strategies are needed to be shifted towards traditional cultural methods coupled with the integration of modern biotechnological approaches to achieve ecofriendly and sustainable nematode management (Khan et al. 2021). This chapter discusses the available nematode management options in crops with their limitations and challenges associated with them.

2.2 Preventive Approach

Exclusion which is mainly achieved through diagnosis and quarantine has proved to be a highly effective and cost-effective way of achieving nematode damage on crops since it prevents the introduction of nematode pests into new areas. This approach is an age-long pest management strategy that has prevented the spread of some economically important nematode pests like the virulent *Meloidogyne enterolobii* from getting into countries where they have not been recorded.

Till date, EPPO publishes periodically maps to show worldwide distribution of nematodes species considered as being of economic or quarantine importance (Ornat et al. 2001). Effectiveness of quarantine has resulted in the prevention of introduction and spread of major nematode pests at local or international level which has helped to achieve significant savings in agricultural crop production worldwide (Haque and Khan 2021). Other preventive strategies such as escape cropping usually achieved by delayed planting to utilize host-free period have proved effective in managing some nematode pests of crops (Ornat et al. 2001).

2.3 Physical and Cultural Management

Cultural management methods can be in the form of several crop-based approaches involving simple direct means like crop rotation, fallowing, cover crops, disease-free materials for planting, maintaining good farm hygiene or may be achieved by combining several methods which require information on nematode pest host range, threshold densities, and cost analysis of using multiple inputs (Bridge 1996). Physical means like flooding, solarization, and soil manipulation through tillage have been used to manage nematode pests.

2.4 Crop Rotation

Crop rotation, though not originally evolved with nematode control in mind, has in due course become important having proved a viable and cost-effective means of managing nematode pests on several crops worldwide (Siddiqui 2003). It is believed that rotation of susceptible with resistant, nonhosts, trap crops, and allelopathic plants would disengage the pest from host for a considerable duration causing starvation and natural weakening or death of the pest, thereby reducing the inoculum level between susceptible hosts (Westerdahl 2009; Zasada et al. 2010). For example, rotations involving resistant and tolerant host crops with some highly susceptible vegetable crops are impressive in the management of *Meloidogyne* species. Furthermore, inclusion of Sunn hemp (*Crotalaria juncea*) in a cotton-based rotation sequence was used to manage root-knot disease in cotton fields (Van Biljon et al. 2015).

In view of the fact that some nematode pests such as *Heterodera*, *Globodera,* and *Xiphinema* species can pull through long periods devoid of host makes it important to know that factors like nematode survivability over time in the absence of the nonhost and length of rotation are important being crucial determinants of the success of the rotation scheme. However, a major limitation of using crop rotation in nematode pest management is finding a poor or nonhost crop having similar gainful value as the vulnerable crop to be employed and utilized as cover crops mainly when nematode pest of concern has a wide host range (Zasada et al. 2010).

2.5 Trap Crop

A trap crop refers to any plant that is an excellent host of a particular insect, nematode pest, or any other organisms of interest which can be used to protect a target crop from attack by the pest (Hokkanen 1991). A potential trap crop is usually cheap to produce and adapts easily to the farmer's cultural practices. A trap crop is usually planted to permit penetration of the infective usually juvenile stage of the nematode pest into its roots where the nematode after continuous feeding assumes the saccate or rotund shape and hence gets trapped within the root system. Nematode control is achieved by timely destruction of the trap crop prior to nematodes laying eggs. Trap crops have been used effectively, especially for managing nematodes belonging to the sedentary endo parasite group such as *Meloidogyne* and *Heterodera* species (Westerdahl 2018). A major limitation for trap cropping is in the mass cultivation of nematode susceptible hosts weeds which often require irrigation to grow and germinate. Furthermore, the cost of trap crop seeds is also a major concern in this regard.

2.6 Flooding

Anaerobic conditions arising from prolonged flooding periods usually cause death of nematodes within the flooded soil (Okada et al. 2011; Cesarz et al. 2017). Most paddy rice fields are usually found to be nematode free in the following dry season after a prolonged flooding periods of the wet season (Liu et al. 2008; Cesarz et al. 2017). This method has been deployed in several paddy rice fields to effectively manage nematode pests of the crop. However, the time-scale of flooding for each nematode pest species needs to be purposeful for effective control to be achieved using flooding. Also, flooding may not be the best method for managing nematodes on some crops as a result of the consequential agronomic effect of lack of soil oxygen due to the anaerobic condition created by flooding on soil structure.

2.7 Solarization

Solarization or heat treatment of the soil was first delineated by Katan et al. (1976) as a preplanting soil treatment method for managing soil-borne weeds and pathogens. This method which involves the process of trapping solar radiation to achieve transformation and preservation of heat by the use of pellucid plastic films which are set down directly on soil has been widely investigated for its effectiveness in soil nematode management (Zasada et al. 2010; Candido et al. 2008). This method which is usually combined with other cultural methods was applied in order to increase the soil temperature by 2–15 °C so as to achieve effective nematode pest control. The efficacy of this method has been reported in the screen house control of RKNs on some common vegetables (Védie et al. 2014; Mashela et al. 2017; Candido et al. 2008). Since efficacy of solarization most of time is determined by the capacity of the soil to be able to retain the heat generated over a long duration of time for nematode control to be achieved, failure of this method has been reported due to factors like resilience of some eggs of nematode to heat, unfavorable climatic conditions, and given that soil can be contaminated immediately following solarization (Chellemi 2002).

2.8 Soil Tillage

Careful moulding up of the thin top layer of the soil in the form of tillage achieved by repeated deep ploughing at regular intervals for up to 30 days during hot and dry season has been effective in eliminating alternative weed hosts and also in bringing down population levels of agriculturally important nematodes pests of crops in the soil (D'Hose et al. 2018).

2.9 Organic Amendments

Organic amendments refer to organic materials derived from several sources such as from agricultural, processing, and industrial waste products. Traditionally, organic products in the form of fresh or composted livestock manure, crop, and forest residues (straw, legumes, chaff, wood chips, sludge's, and shavings) are incorporated into the soil for improving chemical and physical properties of the soil and also to improve the nutrient levels of soil for improved crop production (Larney and Angers 2012; Fabiyi et al. 2018a). Inclusion of these organic modifications to the soil has been known to reduce population densities of most plant parasitic nematodes (Fabiyi 2022a). Implementation of soil amendments from different organic materials to manage nematode pests has been well documented (Oka 2010; Renčo 2013; Onkendi et al. 2014; Rosskopf et al. 2020).

There exists a wide range of organic substances in the sort of soil amendments which are employed in managing agriculturally important nematode pests of manor crops. Nematode control that results from application of organic materials to the soil provides a basic justification of interplay amid the preexisting soil microbes (D'Hose et al. 2018; Rosskopf et al. 2020) which is largely impacted by components such as toxic actions of organic acids, metabolites, other composites of nitrogen identical to ammonia, amino acids, fatty acids, hydrogen sulphide, aldehydes, phenolic composite, and carbohydrates released during the microbial decomposition coupled also with the increase in other beneficial nematodes, insects, and other soil microbes (Thoden et al. 2011). Organic amendments have also been combined with other biocontrol organisms to achieve a reduction in the population of some economically important plant parasitic nematodes of crops (Rosskopf et al. 2020). The major constraint identified in the use of organic amendments continuous availability due to the fact that it is often required in large quantities.

2.10 Resistance

Resistance with respect to plant-nematode interaction is defined as the inherent ability of a plant to avoid or recover from attacks by an invading pest species (Karssen et al. 2013). Resistance may either occur naturally or transferred to a crop cultivar from wild-type breeding lines using conventional or genetic engineering tools (Thomas and Cottage 2006). The application of plant resistance in pest management is considered eco-friendly and cheap, its application not requiring any additional input or technology for the farmer to adopt (Djian-Caporalino et al. 2011; Kamunya et al. 2008; Khan et al. 2010a). One of the most appealing benefits derivable from using host plant resistance is its ability to protect the genetic potential of crops due to the fact that resistance naturally results in improved yields for growers planting in nematode-infested fields having nematode population densities exceeding the damage threshold.

Resistance has been used in managing many economically important plant parasitic nematodes of many crops with significant successes being recorded worldwide (Djian-Caporalino et al. 2011; Kamunya et al. 2008; Ali et al. 2017). Also, resistant rootstocks in some perennial crops like citrus and peach have been developed and used for several decades (Claverie et al. 2004). Furthermore, some root stocks from resistant cultivars have been grafted onto susceptible scions; this practice has been extensively used to manage nematode pests of many food crops vegetables (Thies 2021). Although several food and vegetables are available for which high-yielding resistant cultivars have been developed, a major concern plaguing resistance in PPN pest reduction is the issue of resistance breakdown which, when experienced, might render this method ineffective. For example, *Meloidogyne enterolobii,* being a virulent sp., of root-knot nematode was discovered to break down two genes (Mi-1 and Tabasco) responsible for conferring resistance in pepper and tomato, respectively, to three thermophilic RKNs (*Meloidogyne arenaria, M. incognita, and M, javanica*) (Brito et al. 2007; Thies et al. 2008; Nzeako et al. 2013).

Host plant resistance may not be a universal solution to nematode pest problem for the reason that resistance is often tremendously distinct being effective against only a single species, of nematodes (Williamson and Kumar 2006; Regmi and Desaeger 2020). Also, the benefits of resistance on yield potential mentioned in the earlier paragraph are relative, especially where the nematode population is less than the damage threshold, a resistant variety may have lesser yield potential than the high-yielding susceptible ones. Furthermore, continuous repeated use of a particular resistant host may cause a shift in the species composition of the infested field with species against which the resistance is not effective gaining dominance (Barbary et al. 2015). However, resistance, when found in a huge yielding cultivar, can be the base upon which other techniques can rest (Djian-Caporalino et al. 2011; Kamunya et al. 2008; Ali et al. 2017; Thies 2021). Finally, resistant to several nematodes is currently available only in limited number of crops, which makes continuous development of resistant crop varieties to more nematodes in many more crops a necessity.

2.11 Biological Control

Biological control has to do with use of either an organism, or either its product in combating pest. Biocontrol methods have been reported as having great potential for managing nematode pests (Migunova and Sasanelli 2021). Application of biocontrol principles presents a practicable substitute to toxic chemicals in the reduction of PPN (Mohiddin and Khan 2013; Shahid and Khan 2019). Hence, a wide range of effective strategies based on the use of soil microbial agents have been described (Collange et al. 2011). This approach has proved to be an effective alternative to chemicals in nematode pest management worldwide (Collange et al. 2011; El-Eslamboly et al. 2019). Biological products employed in pest management, usually called biopesticides, are produced from species coevolution and never chemistry to achieve an eco-friendly effect on the environment. Several biological microbes like actinomycetes, bacteria, fungi, predatory nematodes, and others usually with host-specific characteristics have shown great ability in managing plant nematodes; however, the bacteria and fungi groups being the most prominent in the soil ecosystem are the most employed in control of PPNs (El-Eslamboly et al. 2019; Migunova and Sasanelli 2021). Biopesticides are generally perceived as being effective environmentally sound means of managing pest worldwide. Furthermore, they are seen as being as potent as synthetic nematicides and food produced using biopesticides are fancied more by the populace due to health and ecological concerns associated with those produced using chemicals (Migunova and Sasanelli 2021).

The use of fungi as biocontrol agents in managing PPNs has been extensively discussed in literature over the years (Siddiqui and Mahmood 1996; Abd-Elgawad and Askary 2018; Peiris et al. 2020). Most of the fungi used in the biocontrol of PPNs achieve this either by activation of the host plant defense system producing secondary metabolites and enzymes or by competition for space with the PPNs. While some fungi like the Aburscular Michorizal fungi (AMF) reduce the effect of nematode parasitism through modification of root morphology to alter the rhizosphere interactions and also aid in nutrient and water uptake by the host plant, some others like Trichoderma reduce nematode invasion by paralyzing the nematode through antibiosis reactions which causes modification of root exudates for the production of secondary metabolites.

Several species of bacteria from different genera such as Agrobacterium, Azotobacter, Clostridium, Burkholderia, bacillus, Chromobacterium, Corynebacterium, and Serratia, just to mention a few, have been reported to have great potential in managing PPNs from divergent parts of the world (Mandic-Mulec et al. 2015; Khabbaz et al. 2019). Recent studies also paid attention to the groups of Bacilli and Pseudomonads which are known to occur widely in natural ecosystems, elucidating their potential applications in biocontrol of RKNs (Zhao et al. 2018; Dehghanian et al. 2020). Bacteria are acknowledged to turn out metabolites which can have immediate antagonistic effect on PPNs through competition, antibiosis, or parasitism. This is largely dependent on the ability of the bacteria to colonize plant surface, compete effectively for nutrients and ecological niche, and produce either antimicrobial compounds, toxins, or nematicidal compounds (Burkett-Cadena et al.

2008; Berg et al. 2017). Also, indirectly, bacteria can stimulate what is called induced systemic resistance in the host plant defense mechanism (Raymaekers et al. 2020). Furthermore, the biocontrol abilities through the mode of action of PGPB against root-knot (*Meloidogyne* spp.) and cyst (*Heterodera* spp.) nematodes have been revealed in detail emphasizing the relevance and importance of utilizing these bacteria in the management of PPNs (Xiang et al. 2018; Gamalero and Glick 2020).

2.12 Predatory Nematodes as Biocontrol Agents

Nematodes that feed on other nematode groups are termed predatory nematodes and are found mostly belonging to the orders Aphelenchida, Diplogastrida, Mononchida, and Rhabditida (Khan 2008). They usually possess a strong and well-developed buccal cavity with diverse feeding device and different styles of prey hunting, prey capturing, and feeding technique (Bilgrami et al. 1986; Jairajpuri and Bilgrami 1990; Bilgrami and Brey 2005). Apart from acting as biocontrol agents against PPNs, they also play crucial roles in nutrient recycling in the soil. Although their application as biocontrol agents in managing agriculturally important PPNs has existed since the early twentieth century (Cobb 1917; Cassidy 1931), studies involving the application of predacious nematodes in the management of PPNs received adequate attention just recently and many have shown great potential as biocontrol agents for managing PPNs (Bilgrami 2008; Khan and Kim 2007; Kim 2015; Devi and George 2018). *Odontopharynx longicaudata* was found effective in controlling six species of PPNs in a laboratory experiment (Chitambar and Noffsinger 1989). *Mononchoides gaugleri* was found effective in managing the cyst nematode (*Heterodera oryzae*) and *M. incognita* (Khan and Kim 2007). Also, the predatory nematode *Fictor composticola* was found effective in managing ten species of PPNs in a laboratory study conducted by Bajaj and Kanwar (2015). A recent example was in the management of *M. incognita* on cucumber by using *Fictor composticola* (Sidhu 2019). Despite the large volumes of studies obtainable on the idea of assorted predacious nematodes employed in managing economically important PPNs under in vitro conditions, their potential as biocontrol agents on PPNs is yet to be determined under field conditions. Hence, in order to fully harness the potentials of predatory nematodes in the management of PPNs, more studies are required in the area of survival and predation efficiency of these predatory nematodes relative to other ecological determinants in natural scenario. There are some reports which indicate that entomopathogenic nematodes may also suppress plant parasitic nematodes (Khan et al. 2010b).

Agricultural systems being very complex with different microbial agents having varying roles within the rhizosphere, this would impact differently on the effectiveness of biocontrol agents and this usually makes their success rate difficult to predict. Furthermore, biocontrol-based strategies are slow to implement since they usually take time; therefore, a very important consideration in the use of biocontrol for nematode pest management is the requirement to integrate them into other management programs and strategies. Also, a broad and holistic approach for rhizosphere microbiota-soil interaction, coupled with discerning and outlining of plant reactions, could help in envisioning issues caused by biocontrol agent antagonists in soil and other alterations within rhizosphere that can impact final output; thus providing an avenue for achieving constructive nematode management using biocontrol agents despite the constraints that might be posed by the complex nature of agricultural soil.

2.13 Chemical Control

Chemical control of PPNs involves the use of nematicides in managing agriculturally important PPNs. Chemically derived nematicides despite being the most rapid, effective stand-alone method to bring down nematode population densities over a brief period (Moens et al. 2009; Mohiddin and Khan 2014), its use is being discouraged due to increasing environmental concerns which is occasioned by its deleterious effect on the ecosystem (Radwan et al. 2012; Shahid and Khan 2016). PPNs management with chemical nematicides is a diverse subject that has received attention till date (Duncan 1991; Zasada et al. 2010; Jones 2017). The ways and means by which nematicides are used in managing PPNs have improved considerably within the last decade. Largely due to contamination of underground water, many chemical pesticides are no longer in use as a result of increased deregistration in dissimilar regions of the world. Due to the increased restrictions in the use of chemical nematicides worldwide, recent products are formulated with low potentials for ground water contamination (Charlier et al. 2009). The phaseouts of the effective fumigants such as methyl bromide, 1, 2 dibromo-3-chloro propane (DBCP), and ethylene dibromide and non-fumigants like carbofuran, aldicarb, and fenamiphos used in managing agriculturally important PPNs have left a void in nematode pest management since advent of safer, non-fumigant alternatives have not been able to cope with the absence of older nematicides (Nyczepir and Thomas 2009). Production of pesticides itself poses serious environmental and human health issues (Khan 2001; Khan and Khan 2000).

Nowadays, some chemicals have been formulated with potentials of achieving effective pest control at a single dose of application, thereby reducing chemical movement below the rooting zone. These chemicals when applied using drip irrigation method have proved effective on the field having reduced bioaccumulation in plant tissues and no significant mammal toxicity. Also, seed treatment with systemic nematicides is another way of reducing the risk of ground water contamination since this prevents direct application of the chemical to the soil.

The continuous and indiscriminate use of chemical nematicides, however, has caused several problems such as food contamination and poisoning, pesticide resistance, resurgence of pests, and toxic effect on nontarget and beneficial organisms in the environment (Fabiyi and Olatunji 2021a, b; Khan et al. 1988, 1996). Furthermore, many of the available pesticides are very expensive and almost beyond the reach of resource-poor farmers of the underdeveloped world. In semiarid environments where pest populations vary from year to year and yields are low, there may be no realistic economic threshold for farmers to use chemical control. However, for the foreseeable future, traditional chemical nematicides will hold out for an extensive scope of uses and therefore, management practices should be structured and improved upon so as to adjust to their safe and systematic use.

2.14 Plant Extracts as Phytonematicides

The use of plant extracts for nematode control is an age-long practice. Plant extracts have some advantages over chemical nematicides. Apart from being easily biodegradable, they also contain new compounds that nematodes are yet to inactivate or develop resistance against (Grubišić et al. 2018; Forghani and Hajihassani 2020; Atolani and Fabiyi 2020). These attributes have made them seem like a viable alternative to chemical nematicides. For example, natural products from the neem plant contain Azadirachtin which has proved effective in nematode control (Lynn et al. 2010). Essential oil, a natural product from *Eucalyptus globulus* which contained several active substances, was reported to control *Pratylenchus* spp. on maize (Fabiyi et al. 2020a). Other natural products that have controlled *M. incognita* effectively include isovitexin and isochromane glycoside from *Kigelia pinnata* (Atolani et al. 2014a, b). Powdered materials of leaves *Crotalaria juncea, Ocimum gratismum, Eucalyptus officinalis, Hypis suaveolens, and euphorbia heterophylla* when applied to the soil as admix have been established to be remarkable in reduction of *M. incognita* population on Okro plants (Fabiyi 2021a). In addition, powdered pods of *Prosopis africana* applied as soil amendments had a significant effect on *M. incognita* population reduction in field experiments with a corresponding increase in yield of groundnut plants (Fabiyi 2019). Similarly, organic extracts of *Enantia chloranta, Terminalia glaucescens, Citrullus lanatus Tridax procumbens*, *Sida acuta, Mangifera indica, Adansonia digitata,* and *Khaya senegalensis* are equally viable option in *M. incognita* management (Fabiyi et al. 2019; Fabiyi and Olatunji 2021a, b; Fabiyi 2021b, 2022b, c, d). Extracts of crotalaria plant species were found to contain 1,2-dehydropyrrolizidine alkaloids which confer on it useful nematicidal properties and have been effectively used to kill nematodes (Thoden et al. 2009). Extracts from several species of the marigold plant (Tagetes spp.) are found to exhibit strong nematicidal properties due to existence of polyacetylenes, polythienyls, and some linoleic acids in them and have hence been effectively used to control PPNs, especially the RKN and cyst nematodes in in vitro, lab, and field experiments (Leonetti et al. 2011; Faizi et al. 2011).

Other notable examples include the use of some members of the Mustards (*Brassica napus, B. juncea,* and *Sinapis alba)* which were found to contain thiocyanates and isothiocynates as powdered extracts exhibiting great nematicidal potentials and used to control the root lesion (*Pratylenchus* spp) and the RKNs (Zasada et al. 2010; Vig et al. 2009). Also, extracts from the common poisonous plant (Lantana camara) contain 11-oxo triterpenic acid which resulted in huge mortality of RKN in in vitro experiments (Srivastava et al. 2006). Furthermore, methanol extracts of *Ailanthus altissima* plant found to contain 2.4-Decadienal and furfural showed great nematicidal activity (Caboni et al. 2012). However, Fabiyi (2020, 2021c) equally affirmed the nematicidal action of furfural on nematode pests of groundnuts and sugarcane. Likewise, the use of extracts from *Camellia oleifera* cake which has nematotoxic properties to suppress egg hatching in Meloidogyne sp., was reported by Forghani and Hajihassani (2020). In an in vitro study conducted by Jindapunnapat et al. (2018), plant extracts of vetiver (*Vetiveria zizaniodes*) produced high mortality of *M. incognita*. Also, water extracts of chinaberry fruits (*Melia azedarach*) produced an effective control of RKNs (Ntalli et al. 2018). Nematicidal action of extracts from four saponin-rich Medicago plants (*Medicago hybrida, M. murex, M. truncatula,* and *M. heyniana*) in controlling some PPNs: RKN (*Meloidogyne incognita*), Dagger nematode (*Xiphinema index*), and potato cyst nematode (*Globodera rostochiensis),* was tested in a laboratory experiment and was found with varying degrees of effectiveness in managing these nematode species (D'Addabbo et al. 2020). A list of some common plant species used as extracts for controlling agriculturally important plant parasitic nematodes is provided in Table 2.1.

Plants are known to possess a diversity of chemical compounds which serve as good source for managing plant parasitic nematodes. Nonetheless, securing large-scale use of phytonematicides produced from this available variety of plant species bothers down on issues ranging from availability to cost involved in production and marketing as well as regulatory status. Other issues are adaptation to field conditions of the several plant species, toxicity determination in chemical solvents, availability and determination of the most effective of the available extraction, and concentration and application methods for easy adaptability to new environments. In the US, however, some botanicals are absolved from the accustomed risk assessment data prerequisite for registration regarding their low persistence and low mammalian toxicity; more research is still necessary so as to be able to link the mechanisms governing the effectiveness of each botanical extract especially in terms of their bioactivity to be able to fully exploit the nematicidal potentials of these plant extracts.

2.15 Nanotechnology in Nematode Control

Nanotechnology refers to the study and application of science technology and engineering at the nanoscale (Khan et al. 2020). Nanotechnology is a novel area of research that is endowed with unlimited applications in different areas due to the unique properties of nanoparticles which make them adaptable in critical areas of human life such as medical, pharmaceutical, engineering, and agriculture (Fabiyi et al. 2020b). In agriculture, nanotechnology has been utilized in natural resources conservation, crop production, and effective control of major pests and diseases worldwide (Mishra et al. 2014; Ahmed et al. 2020; Fouda et al. 2020; Fabiyi et al. 2020c; Khan et al. 2019a, b). Albeit few, available reports on the application of

Table 2.1 Some common plant species used as extracts for controlling agriculturally important plant parasitic nematodes

Plant sources	Nematode species controlled	Major nematicidal ingredients	References
(Chinaberry tree) *Melia azedarach*	RKN (*Meloidogyne incognita*)	Acids (butyric, acetic, decanoic, hexanoic), 5-hydromethylfurfural	Ntalli et al. (2018)
(Rattlepod) *Crotalaria* spp.	RKN (*M. incognita*), cyst nematode (*Heterodera schachti*), lesion nematode (*Pratylenchus penetrans*).	Alkaloids, 1,2-dihydropyrolizine	Thoden et al. (2009)
Syrian Rue *Peganum harmala*	RKNs	Alkaloid	El Allagui et al. (2007)
(French marigold) *Tagetes patula* *Acacia gummifdera*	*Meloidogyne* spp.	Flavanoids	
(Yellow Restharrow) *Ononis natrix*	*Meloidogyne* spp.		
(African marigold) *Tagetes* spp	RKNs	Polyethienyls, Polyacetylenes, and flavonoids	Chitwood (2002), El Allagui et al. (2007), Marahatta et al. (2010)
(Bluemink) Agerantim houstonianum Silver Ragwort *Senecio bicolor*	*Meloidogyne hapla*	Alkaloids	Thoden et al. (2009)
Gochnatia barrosii	*Melodogyne* spp.	Glycopyranosides, Kaempherol	Dos Santos et al. (2010)
Khaya ivorensis	*Meloidogyne* spp.		Fabiyi et al. (2016)
(White meadowfoam) *Limnanthes alba*	*M. hapla*	*Methoxybenzyl isothiocyanate*	Zasada et al. (2010)
(Garlic) *Allium sativum*	*Meloidogyne* spp.		Cetintas and Yarba (2010)
(Mustard greens) *Brassica juncea* *Sinapis alba*	Lesion nematode (Pratylenchus spp.) RKNs (*Meloidogyne* spp.)	*Glucosinolates*	Zasada et al. (2009)
Terminalia glaucescens	*Meloidogyne incognita*		Fabiyi (2021b)
Pepper *Piper* spp.	*Meloidogyne* spp.	Capsaisin	Dayan et al. (2009), Neves et al. (2009), Edelson et al. (2002)

(continued)

Table 2.1 (continued)

Plant sources	Nematode species controlled	Major nematicidal ingredients	References
Raphanus sativus (Radish) *Eruca sativa*	*Meloidogyne* spp.		Lazzeri et al. (2009)
Euphorbia heterophylla *Eucalyptus officinalis* *Crotalaria juncea* *Hyptis suaveolens* *Occimum gratisimum*	*Meloidogyne incognita*		Fabiyi (2021a)
(West Indian Lantana) Lantana camara	*M. incognita*	Terpenes, Hydroxybenzoic acids Lantanolic acid, Camarin	Shazaukat et al. (2009), Begum et al. (2008), Srivastava et al. (2006)
T. patula (Dwarf marigold)	*Heterodera zea*	Linoleic, α-Terthienyl, and gallic acids	Faizi et al. (2011)
Alfalfa *Medicago sativa*	*M. incognita, Globodera rostochiensis, Xiphinema index*	Terpenes, glycocides of medicagenic acid	Leonetti et al. (2011)
(Rhone aster) *Aster sedifolius*	*Meloidogyne incognita*	Saponins	Di Vito et al. (2010)
T. patula (Dwarf marigold)	*Meloidogyne* spp.		Buena et al. (2008)
Turpentine tree *Pistacia terebinthus*	*Meloidogyne incognita*		Ntalli et al. (2011)
(Fringed rue) *Ruta chalepensis*	*Meloidogyne incognita, Meloidogyne javanica*	2-Undecanone	
(Yellow box) *Eucalypthus melliodora*	*Meloidogyne incognita*	Benzaldehyde	Ntalli et al. (2011)
(Wild tarragon) *Artemisia dracunculus* (Rock band) *Eruca sativa*	*Meloidogyne javanica*		Klein et al. (2011)
(Tree of heaven) *Ailanthus altissima*	*M. javanica*	2,4-Decadienal and furfural	Caboni et al. (2012)

(continued)

Table 2.1 (continued)

Plant sources	Nematode species controlled	Major nematicidal ingredients	References
Eucalyptus globulus	*Pratylenchus* spp.	Isopulegol, geranial, citronella, myrcene, pinene, cineole	Fabiyi et al. (2020a)
(Peppermint) *Mentha spiruca, Mentha piperita*	*M.incognita* *M. arenaria*	Eugenol. Linalool, Geranoil Klein et al. (2011)	Walker and Merlin (1996)
Enantia chlorantha (awopa)	*Meloidogyne incognita*		Fabiyi et al. (2019)
Cymbopogon citratus	*Meloidogyne incognita*	Terpenes	Fabiyi et al. (2018c)
(Mexican tea) *Chenopodium ambrosioides*	*Meloidogyne incognita*	Ascaridole	Chuan et al. (2011)
Prosopis africana	*Meloidogyne incognita*		Fabiyi (2019)
Alstonia boonei	*Meloidogyne incognita*	Fatty acid esters	Fabiyi et al. (2012a)
Bridelia ferruginea	*Meloidogyne incognita*	Fatty acid esters Beta amyrin	Fabiyi et al. (2012b) Fabiyi et al. (2012c)
(Lemon verberna *Aloysia triphylla*	*Meloidogyne incognita*		Duschatzky et al. (2004)
Hyptis suaveolens	*Heterodera sacchari*	Terpenes	Fabiyi et al. (2015)
(Judean Wormwood) Artemisia jundaica	*Meloidogyne javanica*		Oka et al. (2000)
Kigelia pinnata	*Meloidogyne* spp.	Isovitexin, Isochromane glycoside	Atolani et al. (2014a, b)
(Garland chrysanthemum) *Chrysanthemum coronarium*	*Meloidogyne incognita*		Pérez et al. (2003)
(Common thyme) *Thymus vulgaris*	*Meloidogyne incognita*		Cetintas and Yarba (2010)
(Caraway) *Carum carvi* (Lemon scented gum) *Eucalypthus citridora*	RKNs (*Meloidogyne* spp.)		Pandey (2000)
Tridax procumbens and *Sida acuta*	*Meloidogyne incognita*		Fabiyi (2022d)

nanotechnology in managing nematodes have recorded significant successes (El-Saadony et al. 2021).

The success of nanoparticles in nematode management relies solely on the properties of the NPs and method of preparation (Gatoo et al. 2014). Penetration into PPNs is achieved through the size of NPs (Sondi and Salopek-Sondi 2004; Khan and Akram 2020). The control of *M. incognita* with silver nanoparticles has been widely reported (Maggie et al. 2016; Atef and Nassar 2016). Plant-mediated nanoparticles are of great significance in PPN control and they have continually decreased *M. incognita* population. Materials such as rice husk and guinea corn chaff have been employed as stabilizers in silver NP preparations (Fabiyi et al. 2021). The population of rice cyst nematode was notably reduced at 50 g treatment (Fabiyi et al. 2018b). Similarly, green synthesis of silver nanoparticles with extracts of *Eucalyptus officinalis* promoted rice growth, while cyst nematode was reduced (Fabiyi et al. 2020c). Groundnut plants treated with *Ficus mucoso*-mediated silver nanoparticles were found to be robust at harvest in comparison with the control set (Fabiyi and Olatunji 2018). Likewise, *M. incognita* infestation of carrots plants was remarkably reduced with *Cnidoscolus aconitifolius*-mediated silver nanoparticles. AgNPs applied at 50, 100, and 150 mol effectuated higher vegetative growth of carrot plants with impressive yield regardless of *M. incognita* infestation (Fabiyi 2021d). Primarily, the objective of nanoparticles application in agriculture is to combat pest and pathogens. However, some negative effects like reduction in photosynthetic ability of crops, reduction in biomass, broken root caps, and epidermis have been documented (Goswami et al. 2019; El-Moneim et al. 2021). It is pertinent to comprehend the use of nanoparticles for effective use.

2.16 Integrated Nematode Pest Management

Integrated pest management (IPM) is considered an advanced method and defined as a combination of pest community management methods which exploit all available satisfactory expertness in a reconcilable way to ensure reduction in pest population and keep them below economic injury levels (Smith and Reynolds 1966). The concept of IPM in nematode pest management has been reviewed in detail by several workers (Noel 2008; Flint 2012; Fourie and De Waele 2019). All authors agreed that accurate data on nematode population dynamics and economic impact of damage of individual nematode population were necessary to achieve an effective integrated nematode management scheme.

Furthermore, knowledge about merits and demerits associated with nematicide use (pollution, cost), biological agents used in control, and other cultural control dynamics must be considered. Although information is scarce, significant progress has been recorded in agricultural decision making incorporating nematode management options and several of the current options in nematode management are well suited for use in INM programs.

The choice of integration of control methods would depend on the nematode as well as the value of the crop grown. INM will also vary with crops, pests, economic circumstances of the farmer, and location. The future of crop protection, especially in the developing world, will depend upon the use of INM approaches within the context of holistic development of natural resource production systems. It is therefore necessary for research to progress in a way to integrate available cultural, chemical, and biological nematode management methods and that these integrated options can economically and effectively reduce the continued dependence on chemical nematicides in nematode pest management.

2.17 Conclusion and Future Perspectives

Since severity of infestation largely depends on the population density of nematodes in the soil, most management strategies are usually employed to reduce their populations below the damage potential threshold. At the present state of technology, pesticides are most efficient means of management, but their application leads to several serious concequences of food and environmental toxicity. Under this situation the nematode management strategies are needed to be shifted towards traditional cultural methods coupled with the integration of modern biotechnological approaches to achieve ecofriendly and sustainable nematode management.

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3

Novel Biological and Biotechnological Methods of Nematode Management, an Effective Strategy to Enhance Crop Productivity

Giada d'Errico and Landi Silvia

Abstract

Plant parasitic nematodes represent limiting factors for the production of a wide range of crops. Possible alternatives to synthetic nematicides are based on the use of biological and biotechnological procedures, alone or in combination with other control strategies. Evidences of their beneficial effects are found in improved crop protection and agricultural productivity. For the optimal use of beneficial microorganisms, a depth knowledge of the nematode target is strongly needed. Genetic engineering technologies may be applied to improve their biocontrol activity by synergistically overexpressing quite a few characteristics. The most widely investigated approaches ranging from the traditional biocontrol up to molecular breeding are presented in this chapter.

Keywords

Plant parasitic nematodes · Beneficial microorganism · Plant-derived products

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3.1 Introduction

Plant-Parasitic Nematodes (PPNs) are among the most severe soil-borne pests, which provoke annual economic damages of up to $157 billion, worldwide (Jones et al. 2013). For decades, the traditional and most effective control of PPNs has been achieved by using chemical fumigants and mainly organophosphate and carbamate nematicides (Khan 2008). In spite of their reasonable efficacy, several molecules have been withdrawn from the market for serious toxicological and environmental reasons. Moreover, the use of chemical fumigants may also determine the eradication of beneficial microorganisms and the modification of the natural biological equilibrium, thus favoring the establishment and development of aggressive pathogen and pest populations (Gamliel et al. 2000). Additionally, chemical fumigants can alter the functionality of ecosystem services in which the microorganisms are involved (Mocali et al. 2015). The drastic reduction in the availability of commercial pesticides has led researchers to investigate alternative strategies to manage PPNs. An eco-sustainable agrosystem is the focus of modern-day agriculture and will contribute in underpinning future food security. In this context, biological and biotechnological methods represent important alternatives for plant disease management (Dong and Zhang 2006; Collange et al. 2011; Vinale et al. 2012; Khan 2023), limiting the undesirable impact of chemicals on the environment, biodiversity, and human and animal health (Harman and Kubicek 1998; Avis et al. 2008; Bonanomi et al. 2021). Moreover, these methods may exert multiple beneficial effects on plants, enhancing resistance to biotic/abiotic stresses, promoting plant growth, and nutrient availability or uptake. Nevertheless, as suggested by numerous authors (Atkins et al. 2003; Khan et al. 2018; d’Errico et al. 2019; Mohiddin and Khan 2013), effective management of PPNs requires the integrated combination of different strategies (d’Errico et al. 2017). Ecosystems consist of several organisms, such as bacteria, archaea, protozoa, fungi, and nematodes, interacting with each other and with plants and animals (vertebrate and invertebrate). These direct or indirect interactions occur by different mechanisms (predation, parasitism, mutualism, or competition) (Topalovic and Heuer 2019). Biological and biotechnological methods may have several benefits in plant disease management and agricultural productivity. The principal advantage of these methods is represented by their self-sustainability. Generally, they are environment-friendly and play important roles in safeguard agricultural ecosystems discouraging the resistance development in pathogens and pests. This chapter provides an overview of the most recently investigated biological and biotechnological methods of nematode management and enhancements in the crop productivity.

3.2 Beneficial Fungi

Fungi are involved in decomposing organic substance and in recycling carbon, nitrogen, and other nutrients (Shahid and Khan 2019). Commonly, fungi and nematodes living in the rhizosphere produce significant effects on plants. Although several fungi live in close association with nematodes, a small part of them is considered biocontrol agents (BCA). Application of BCAs at the time of planting or post planting stage is getting popularity in achieving sustainable nematode management in crop production (Stirling 1991; Khan 2007; Khan and Anwer 2011; Sikora and Roberts 2018; Khan et al. 2021). Nematode reproduction can be reduced by fungal parasitism, antagonism or predation (Table 3.1), or through the activity of arbuscolar mycorrhizal fungi (Table 3.2) (Siddiqui and Mahmood 1995; d'Errico et al. 2021a, b). Additionally, fungi are an enormous source of bioactive molecules exhibiting various biological activities (Marra et al. 2019; d'Errico et al. 2021a, b). Exploring the relationship between nematodes and fungi is crucial for understanding their effects on the ecosystem and their potential application in pests or pathogens' managements as well as in improvement of quali-quantity characteristics of food (Sinno et al. 2020; Zhang et al. 2020). If anything, also many fungivorous nematodes as *Aphelenchus*, *Aphelenchoides*, *Ditylenchus*, and *Tylenchus* can feed non-selectively on saprophytic, plant-pathogenic, and mycorrhizae (Lamondia and Timper 2016; Hasna et al. 2007; Wall and Caswell 2003). This activity could have some effects on soil microbiome, and consequently modify soil ecology and crop productivity (Ragozzino and d'Errico 2012; Gioia et al. 2020).

The nematode antagonists such as *Pochonia chlamydosporia, Purpureocillium lilacinum,* and *Aspergillus niger* can suppress soil populations of plant nematodes (Jatala 1986; Stirling 1991; Kerry 2000; Khan 2016). However, the beneficial effects produced by antagonistic fungi as *Gliocladium* and *Trichoderma* species could be reduced because of the action of fungivorous nematodes. Studies show that fungi belonging to the genus *Trichoderma*, mainly the species *T. harzianum* and *T. viride*, act using direct and indirect mechanisms against nematodes. Moreover, they produce enzymes and metabolites, as chitinase, glucanase, and protease, effective against PPNs and involved in plant interactions (Lombardi et al. 2020; Marra et al. 2020); alongside they compete for nutritional resources and the reduction of nematode colonization opportunities. In recent decades, *Trichoderma* spp. have emerged as a hero in plant disease management including nematodes (Mohiddin et al. 2010; Khan and Mohiddin 2018), and commercial formulations of a number of species such as *T. virens, T. harzianum,* and *T. hamatum* are available (Khan et al. 2011), which have been found highly effective against fungal and nematode diseases when applied on the planting material (Sikora and Roberts 2018; Mohammed and Khan 2021). Additionally, *Trichoderma* species are plant growth and development promoters of several crop and ornamental plants; they also induce plant defences *versus* various plant pathogens (Hermosa et al. 2012).

Nematophagous fungi are also able to reduce nematode populations (Soares et al. 2018; Persmark and Jansson 1997). The coevolution of predator–prey interactions is regulated by numerous molecular, organismal, cellular, and ecological mechanisms (Wang et al. 2014; Hsueh et al. 2017; Ji et al. 2020; Yang et al. 2020). *Purpureocillium lilacinum* is one of the most investigated soil fungi in PPNs' management (Atkins et al. 2005). In fact, *P. lilacinum* reduce PPNs populations, mainly through sedentary stages (eggs) infection, and increase crop yields. Chitinases, leucinotoxins, acetic acid, and proteases produced by *P. lilacinus* are implicated in the infection process (Khan et al. 2004; Park et al. 2004).

Table 3.1 Fungal genera showing some effects in association with nematodes

Fungal genera	References
Acremonium	Goswami et al. (2008)
Acrophialophora	Siddiqui and Husain (1991)
Allomyces	Stirling (1991)
Aniriopsis	Van der Laan (1956)
Aphanomyces	Jaffee (1986)
Arthrobotrys	Su et al. (2017)
Aspergillus	Ying et al. (2019)
Botryotrichum	Dos Santos et al. (1993)
Catenaria	Tribe (1977)
Cephalosporium	Willcox and Tribe (1974)
Chaetomium	Kooliyottil et al. (2017)
Chaetopsinea	Grant and Elliott (1984)
Cladosporium	Roessner (1987)
Colletotrichum	Van der Laan (1956)
Coniothyrium	Stirling (1991)
Cylindrocarpon	Goffart (1932)
Cystopage	Stirling (1991)
Dactylaria	Jansson and Nordbring-Hertz (1990)
Dactylella	Morgan-Jones and Rodriguez-Kabana (1987)
Diheterospora	Grant and Elliott (1984)
Drechmeria	Timper and Brodie (1993)
Entomophthora	Baunacke (1922)
Exophiala	Morgan-Jones and Rodriguez-Kabana (1981)
Epicoccum	Meyer et al. (1990)
Fusarium	Zareen et al. (2001)
Gliocladium	Amin (2014)
Harposporium	Li et al. (2005)
Hirsutella	Chen and Liu (2005)
Humicola	Vinduska (1984)
Isaria	Baunacke (1922)
Lagenidium	Raup and Sethi (1986)
Leptolegnia	Jaffee (1986)
Macrobiotophthora	Stirling (1991)
Margarinomyces	Van der Laan (1956)
Meristacrum	Jatala (1986)
Metarhizium	Khosravi et al. (2014)
Microdochium	Kooliyottil et al. (2017)
Monacrosporium	Khan et al. (2006)
Monocillium	Ashrafi et al. (2017)
Monotospora	Van der Laan (1956)
Mortierella	AL-Shammari et al. (2013)
Myzocytium	Jatala (1986)
Nematophthora	Kerry and Crump (1980)

(continued)

Table 3.1 (continued)

Fungal genera	References
Nernatoctonus	Timper and Brodie (1993)
Olpidium	Grant and Elliott (1984)
Paecilomyces	Kiewnick and Sikora (2006) Sivakumar et al. (2020)
Penicillium	Sikandar et al. (2020)
Phialophora	Mastan et al. (2019)
Phoma	Van der Laan (1956)
Phytophthora	Jatala (1986)
Plectosphaerella	Kooliyottil et al. (2017)
Pleurotus	Hibbet and Thorn (1994)
Pochonia	Manzanilla-López et al. (2011)
Preussia spp.	Qadri and Saleh (1990)
Pseudeurotium	Van der Laan (1956)
Pseudopopulospora	Godoy et al. (1983)
Purpureocillium	Kooliyottil et al. (2017)
Rhizopus	Grant and Elliott (1984)
Rhopalomyces	Stirling (1991)
Scytalidium	Meyer et al. (1990)
Stagnospora	Rodriguez-Kabana et al. (1981)
Stylopage	Stirling (1991)
Tarichum	Walia et al. (2021)
Thielavia	Rodriguez-Kabana et al. (1981)
Trichoderma	Grant and Elliott (1984)
Trichosporan	Jatala (1986)
Trichocladium	Hay and Skipp (1993)
Trichotheciurn	Swarup and Gokte (1986)
Ulocladium	Rodriguez-Kabana and Morgan-Jones (1988)
Verticillium	Pathania et al. (2022)

Whereas, *Pochonia chlamydosporia* is the most used biocontrol fungus against PPNs (Escudero and Lopez-Llorca 2012). A serinprotease (VC1), isolated from this organism, was found to be active in eggs penetration. The vegetative hyphae parasitize eggs and cysts through the production of the appressoria. Infection begins with a process mediated by glycoproteins and enzymes (such as serinprotease). Recent studies have also shown that *P. chlamydosporia* has the capability to stimulate plant growth and development. Many fungal genera (i.e., *Catenaria* spp., *Lagenidium* spp., *Arthrobotrys* spp., and *Dactylella* spp.) potentially able to control PPNs populations have not achieved commercial success due to the high production costs (Kumar and Arthurs 2021).

Also, mycorrhizae play a crucial role in crop protection (Yang et al. 2014) and in drought tolerance (Zhao et al. 2015). They promote sustainable agriculture and crop productivity, improving nitrogen, phosphorus, and Zinc contents in plant crops (Baum et al. 2015; Berruti et al. 2016). Mycorrhizae can be grouped in:

Table 3.2 Arbuscolar mycorrhizal fungi (AMF) according to Hol and Cook (2005)

Fungi	References
AMF and ectoparasitic nematodes	
Glomus sp.	Kassab and Taha (1990)
G. mosseae	Hasan and Jain (1987)
G. fasciculatum	Jain et al. (1998)
AMF and cyst nematodes	
G. mosseae	Todd et al. (2001) Deliopoulos et al. (2007) Deliopoulos et al. (2010)
G. fasciculatum	Siddiqui and Mahmood (1995)
G. intraradices	Deliopoulos et al. (2007) Deliopoulos et al. (2010)
G. epigaeus *G. fasiculatum*	Jain and Sethi (1988)
G. etunicatum	Benedetti et al. (2021)
Glomus+Gigaspora	Tylka et al. (1991)
Gigaspora margarita	Siddiqui and Mahmood (1995)
AMF and root-knot nematodes	
G. intraradices	Habte et al. (1999) Jothi and Sundarababu (2000) Calvet et al. (2001)
G. mosseae	Jaizme-Vega et al. (1997) Rao et al. (1998) Habte et al. (1999) Jothi and Sundarababu (2000) Talavera et al. (2001) Elsen et al. (2002) Nehra et al. (2003)
G. fasciculatum	Mishra and Shukla (1997) Ranganatha et al. (1998) Nagesh et al. (1999) Borah and Phukan (2000) Labeena et al. (2002) Rao et al. (2003) Samal et al. (2018)
G. deserticola	Rao et al. (1997) Rao and Gowen (1998)
G. aggregatum	Pandey et al. (1999)
G. etunicatum	Bhagawati et al. (2000) Waceke et al. (2001)
G. fulvum	Jothi and Sundarababu (2000)
Glomus sp. K14	Waceke et al. (2002) Cofcewicz et al. (2001)
G. margarita	Cofcewicz et al. (2001) Labeena et al. (2002)
Glomus sp.	Talavera et al. (2002) d'Errico and Caprio (2004)

(continued)

Table 3.2 (continued)

Fungi	References
G. macrocarpum	Labeena et al. (2002)
G. caledonium	Elsen et al. (2002)
G. coronatum	Diedhiou et al. (2003)
Sclerocystis dussi	Labeena et al. (2002)
Acaulospora laevis	Nagesh et al. (1999)
A. laevis	Labeena et al. (2002)
AMF and migratory endoparasitic nematodes	
Glomus sp.	Talavera et al. (2001)
G. intraradices	Forge et al. (2001) Elsen et al. (2003b)
G. mosseae	Forge et al. (2001) Elsen et al. (2003a)
G. aggregatum	Jaizme-Vega and Pinochet (1997) Forge et al. (2001)
G. clarum	Forge et al. (2001)
G. etunicatum	Forge et al. (2001)
G. versiforme	Forge et al. (2001)

ectomycorrhizal; orchidaceous; vesicular-arbuscular; ericoid. The ubiquitous soil fungi belonging to the *phylum* Glomeromycota are arbuscular mycorrhizae found in association with plant microbiome (Gough et al. 2020). The order Glomerales include Glomeraceae and Claroidoglomeraceae families. In recent times, some *Glomus* species have been moved to *Funneliformis* and *Rhizophagus* genera (Redecker et al. 2013).

3.3 Beneficial Bacteria

Bacteria are the most numerous organisms into soils (Clark 1967). Some of them act as biocontrol agents against PPNs (d'Errico et al. 2019). The nematodes biocontrol can be broadly attributed to parasitic bacteria and non-parasitic rhizobacteria. The bacterium *Pasteuria penetrans* has great potential against a very wide host range, principally *Meloidogyne* spp. (Brown and Nordmeyer 1985). However, *P. nishizawae* can parasitize the mature females of cyst nematodes *Heterodera* spp. and *Globodera* spp. (Sayre et al. 1991). Non-parasitic rhizobacteria, widely known as Plant-Growth-Promoting Rhizobacteria (PGPR), belong to the following genera *Agrobacterium*, *Alcaligenes*, *Bacillus, Clostridium, Desulfovibrio, Pseudomonas, Serratia,* and *Streptomyces*. Generally, they have effects in promoting plant growth and in reducing the establishment of harmful microorganisms in the rhizosphere (Schroth and Hancock 1982). Considering their low environmental impact, some biological preparations based on bacteria are available for the control of the main PPNs. The main strength characterizing these products is represented by the increments in crop yields.

Generally, rhizobacteria have been reported to produce enzymes and metabolites with nematicidal activities, induce systemic resistance in plants, influence nematode behaviour and plant recognition , and promote plant growth (Tian et al. 2007; El-Nagdi and Youssef 2004; Luo et al. 2018). Several PGPR, especially some strains of *Bacillus firmus*, *B. aryabhattai*, *B. cereus*, *Paenibacillus barcinonensis*, and *P. alvei*, are mostly known to reduce root-knot nematodes (Viljoen et al. 2019). Currently, only one formulation based on *Bacillus firmus* I-1582 has been registered on numerous crops in Italy. *B. firmus* I-1582 is an aerobic and gram-positive bacterium commonly found in soils. Several research works highlight the efficacy of *B. firmus* against different nematode species on various crops (d'Errico et al. 2019). Among *Bacillus* species, some potential is reported also for *B. methylotrophichus* (syn. *B. amyloliquefaciens* subsp. *Plantarum*), mainly against *Meloidogyne* spp. (Xiang et al. 2017). Finally, *B. megaterium*, available in Italy only as a fertilizer and probiotic, has shown a nematicidal effect against various PPNs. Studies on other genera such as *Pseudomonas* and *Pasteuria* that initially seemed promising have not led to the registration of commercial products for use in the PPNs control. The most important commercially formulated bacterial biocontrol agents in the world are listed in Table 3.3.

3.4 Microbial-Induced Systemic Resistance

Some plant growth promoters might improve plant health through the stimulation of Induced Systemic resistance (ISR), modulated by microbial elicitors as Volatile Organic Compounds, flagellin siderophores, and lypopolisaccharide. These latter trigger ISR by diverse phytohormones such as auxin, jasmonates, ethylene, nitrogen oxide, etc. (Pieterse et al. 2014). Chemical compounds with signalling function in plant defence responses after biotic stresses (i.e., salicylic acid produced by bacteria in rhizosphere) are widely known (Klessig et al. 2000). These defence mechanisms may activate ISR against various plant pathogens and pests including PPNs. A better understanding of the action mode operated by beneficial microorganisms in controlling PPNs will improve the activity of biocontrol strains useful in developing novel biocontrol practices.

Among bacteria, rhizobacteria are well-known to promote nutrient availability and plant regulators (Turan et al. 2021). PGPR belong to 14 genera: *Arthrobacter*, *Bacillus*, *Bradyrhizobium*, *Enterobacter*, *Erwinia*, *Flavobacterium*, *Frankia*, *Klebsiella*, *Microbacterium*, *Proteus*, *Pseudomonas*, *Rhizobium*, *Serratia*, and *Xanthomonas* (Teymouri et al. 2016). Rhizobacteria can be used as bio-fertilizers, bio-remediators, and disease resistance inducers (Khan et al. 2016a, b; d'Errico et al. 2020). Rhizosphere microorganisms can produce active molecules useful in the promotion of plant growth as well as in the development of nematode resistance (Basu et al. 2021). Among plant growth promoter fungi, known to be involved in ISR activation, the most important genera are: *Aspergillus*, *Fusarium*, *Penicillium*, *Piriformospora*, *Phoma*, and *Trichoderma* (Khan et al. 2009; Hossain et al. 2017).

Table 3.3 Bacterial biocontrol agents present in commercial products and used against plant-parasitic nematodes (PPNs)

Species of bacteria	PPNs Target	References
Pasteuria sp.	*Belonolaimus longicaudatus*	Crow et al. (2011)
Pasteuria sp. Ph3	*Rotylenchulus reniformis*	Akhtar and Siddiqui (2010)
P. penetrans	*B. longicaudatus* *M. incognita* *M. arenaria*	Kokalis-Burelle (2015) Tateishi (1998) Subedi et al. (2020)
P. nishizawae	*Heterodera glycines*	Xiang et al. (2017)
P. usage + *Pasteuria* sp.	*B. longicaudatus*	Akhtar and Siddiqui (2010)
B. amyloliquefaciens	*M. incognita*	Burkett-Cadena et al. (2008)
Burkholderia cepacia	*M. incognita*	Meyer and Roberts (2002), Mhatre et al. (2019)
Bacillus subtilis	*M. arenaria*	Ameen et al. (2016)
B. firmus	*Meloidogyne* spp. *H. glycines* *R reniformis*	Xiang et al. (2017) d'Errico et al. (2019) Susič et al. (2020) Malviya et al. (2020)
B. firmus	*R reniformis*	Castillo et al. (2013)
B. firmus	*Meloidogyne* spp. *M. incognita* *Helicotylenchus* spp. *Heterodera* spp.	Keren-Zur et al. (2000) Terefe et al. (2009) Akhtar and Siddiqui (2010)
B. megaterium	*Meloidogyne* spp. *Tylenchulus semipenetrans*	Raddy et al. (2013) Mostafa et al. (2018) El-Zawahry et al. (2015)
B. methylotrophicus	*M. javanica*	Lopes et al. (2019)
Pseudomonas fluorescens	*Meloidogyne* spp. Cyst nematodes	Abd-Elgawad and Askary (2018)
Serratia marcescens	*M. incognita*	Raddy et al. (2013)
B. amyloliquefaciens *B. subtilis*	*M. incognita*	Burkett-Cadena et al. (2008)
B. licheniformis *B. subtilis*	*Meloidogyne* spp.	Xiang et al. (2017)
B. licheniformis *B. subtilis*	Plant-parasitic nematodes	Abd-Elgawad and Askary (2018)
B. coagulans *B. licheniformis* *B. megaterium* *B. subtilis* *P. fluorescens* *Streptomyces* spp.	Plant-parasitic nematodes	Askary (2015) Berry et al. (2009)
B. chitinosporus *B. laterosporus* *B. licheniformis*	*Meloidogyne* spp.	Lamovšek et al. (2013)

(continued)

Table 3.3 (continued)

Species of bacteria	PPNs Target	References
Azotobacter spp. *Pseudomonas* spp. *Serratia* spp. *B. circulans* *B. thuringiensis*	*M. incognita*	Youssef et al. (2017)

3.5 Agricultural Methods and Biofumigation

Among biological control methods are also included soil tillage practices, crop rotation, variation in sowing and harvest time, non-cultivation (fallowing), cover or catch crops, organic matter, and weed control. Special mention should be given to plant species, mostly belonging to Brassicaceae family, known for their nematicidal activity. The different biocidal mechanisms are depending on the biologically active compounds released as consequence of chemical reactions. In particular, green manures using *Brassica nigra* (L.) Koch, *Eruca sativa* Mill, and *Raphanus sativus* L. can release allelochemicals toxic to nematodes (Chitwood 2002). The effect of this practice, known as biofumigation, is mainly due to the release of hydrolysis products as glucosinolates produced by specific plants. The class of biologically active substances against nematodes includes up to 120 compounds including isothiocyanates. Green manure must be carried out when the highest content in glucosinolate in stems and leaves is reached, which correspond to full bloom stage. Other conditions as plant biomass, neutral pH, high temperature, and humidity are essential for the release of isothiocyanates.

3.6 Plant-Derived Products

Plants possess the ability to regulate various defence reactions, involving molecules activated by biotic or abiotic factors. Among plant extracts, algae are the most investigated (Khan et al. 2015). In particular, seaweed extracts obtained by *Ecklonia maxima* Osbeck and *Ascophyllum nodosum* L. contain elicitor molecules which can enhance defence reactions. ISR process entails elicitor binding to specific receptors on plant membrane. Then, secondary metabolites further activate chemical reactions, which leads to an improvement in biotic resistance to plants. This process involves different steps as the phenylpropanoid pathway, the production of defence signal molecules, and the accumulation of pathogenesis-related biocidals as phytoalexins and proteins (Ramkissoon et al. 2017). Stimuli or plant elicitors are activated by glycoproteins, proteins, peptides, polysaccharides, lipids, etc. (Klarzynski et al. 2000). Defence signal molecules as jasmonic and salicylic acids, and/or ethylene lead plant elicitors to ISR reaction or Systemic Acquired Resistance (SAR) (Kunkel and Brooks 2002; Vlot et al. 2009; Zhang et al. 2011). Seaweed extracts contain several bioactive compounds as alginic acid, antioxidants, laminarin, mannitol, oligosaccharides and polysaccharides, phytohormones, vitamins, and minerals (calcium, phosphorus, potassium, etc.) (Klarzynski et al. 2000). These bioactive compounds promote crop yields, and reduce disease damages (Ali et al. 2016). Soil amendments with *Uva lactuca* L. and *Spatoglossus schroederi* Agardh (Kützing) have also shown an effective nematode control (El-Ansary and Hamouda 2014; Paracer et al. 1987). Actually, commercially available plant-based pesticides comprehend: neem (*Azadirachta indica* A. Juss), pyrethrum (*Tanacetum cinerariifolium* (Trevir.) Sch. Bip.), garlic extract (*Allium sativum* L.), sabadilla (*Schoenocaulon officinale* (Schltdl. and Cham.) A. Gray ex Benth.), tobacco (*Nicotiana tabacum* L.), ryania (*Ryania speciosa* Vahl.), clove (*Syzygium aromaticum* (L.) Merr. and Perry), and etc. (Covarelli et al. 2010).

Recent literature report some effects on PPNs using chitin, chitosan, a carob galactomannan biopolymer, and/or their derivatives, alone or in combination with biocontrol agents, agricultural wastes or plant compounds (Escudero et al. 2017; Liang et al. 2018; d'Errico et al. 2021a, b). In addition, also biochar have been investigated for PPNs suppression capacity that could be rate-dependent (Marra et al. 2018; Domene et al. 2021).

3.7 Biotechnology in Nematode Management and Crop Productivity

Biotechnology include the use of biological systems, living organisms, or its derivatives, to make or modify products/processes. Biotechnologies embrace: DNA characterization of plant tissues, pests and cell cultures, monoclonal antibodies, recombinant DNA, and bioprocess engineering (Gianessi et al. 2003); as well as conventional breeding approaches, bioinformatics, plant physiology, microbiology, biochemistry, and molecular genetics and biology (Duke 2011). Several researchers have investigated the use of biotechnology in crop production (Naseer 2014; Khan et al. 2018). Recent researches have deepen particular features of nematode-plant interaction to set management approaches useful in preventing nematode invasion and reducing nematode reproduction. Novel transgenic approaches and genome editing techniques in nematode control strategies are emerging. In this context, biotechnological research on PPNs helps to investigate the gene pool involved in crop species resistance (Fosu-Nyarko and Jones 2015). Generally, tools used for the identification of PPNs are Isozyme electrophoresis and antibodies, Polymerase chain reaction (PCR) (Caswell-Chen et al. 1993), Randomly amplified polymorphic DNA (RAPD) and Restriction fragment length polymerase (RFLP) (Shah and Mir 2015). In addition, nanobiotechnology may also play important role in the management of PPNs (Khan et al 2019a, b, 2020; Khan and Akram 2020).

In host-pest relationship, the plant response depends on both plant species and PPN population. In fact, a plant resistant to one specific nematode population can be susceptible to another species. A specific gene confers resistance to a single nematode species and sometimes only to one or a small number of nematode races or pathotypes. For example, cultivars resistant to the potato cyst nematodes, *Globodera pallina* and *G. rostochiensis*, are available only for one pathotype but these nematode species are often present into soil as mixed populations with several different pathotypes. The prolonged use of these resistant cultivars leads to an increase in nematode populations to which the plant lacks resistance. In tomato plants, this problem has been overcome by the introduction of a dominant gene *Mi*-1 present in *Lycopersicon peruvianum* (L.) Mill. which confer resistance to the most common root-knot nematodes often found in mixed populations (Landi et al. 2018). The main advantage in using resistant cultivars is the potential to achieve economically sustainable production even in the presence of high PPNs densities. Another important benefit is the reduction in PPN levels towards the end of the crop cycle, that make possible to plant subsequently susceptible crops using shorter rotations. The use of resistant cultivars also reduce the risk of damage caused by other pests or pathogens. Although genetical resistance has several positive aspects; the loss in resistance should be carefully evaluated. The most serious advantage is the development of populations able to survive even on resistant plants (Starr and Roberts 2004).

3.8 Conclusion and Future Perspectives

The use of biological and biotechnological methods in pest management systems has aroused much interest. Generally, these methodologies improve plant growth increasing nutrient uptake in plants and the induction of synthesis of some phytohormones, as well as biotic defence responses. Although there are various limits, there also exist many opportunities for a continuing and expanding role of these strategies. Intensive agriculture improves crop yields, but can also negatively affect the environment and human health. As a consequence, environment friendly and sustainable alternatives are needed in PPN management. In this scenario, biological and biotechnological methods could be promising candidates for the containment of nematode densities below a damage threshold. The auspicious are that researchers will develop practices for enhancing the performance of nematode control strategies and improving plant growth and development without side effects.

In most natural soil ecosystems, the interaction among fungi, bacteria, and nematodes in the rhizosphere can be direct or indirect through chemical signals having significant ecological and economical impacts. Therefore, a comprehensive understanding of these interactions and environmental factors influencing the activity of biocontrol agents is of crucial importance in the safeguard of soil ecosystem.

Biological and biotechnological controls can also be effectively used in combination with other control methods, including, for example, soil solarization (Walker and Wachtel 1988) and low application rates of nematicides (d'Errico et al. 2020). Generally, the combination of control strategies induces synergistic effects both on pest management and crop productivity (d'Errico et al. 2022). Special attention is focused on the use of beneficial microorganisms as well as their active metabolites. The successful biocontrol of beneficial microorganisms depends on their capability to colonize the rhizosphere. Genetical improvement of beneficial microorganisms and host crops to increase their establishment and reproduction is pursued. Application of molecular tools will lead to more effective products. Finally, the most important goal is to strengthen the entire soil microbial community in order to hinder the entry of pathogens and pests as well as stimulate plant growth and development, and plant resistance. Thus, researches are aimed at defining new agronomical, biological, and biotechnological practices for the management of agricultural crops. Soil applications of organic matter as well as the use of adjuvants can favor the soil colonization by these biocontrol agents and the persistence of protection over longer periods of time than those that could be guaranteed from synthetic molecules (Abd-Elgawad and Askary 2018).

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Host Resistance, Current Status, and Emerging Advances

4

Nilton Mashavakure and Gayatri Bandaru

Abstract

Plant parasitic nematodes are among the most important biotic constraints with potential for causing crop yield losses of 8–15% across a wide range of crops in the world. Although chemical fumigants have proved to be most effective in controlling plant nematodes for over one and half centuries, they are costly, environmentally unfriendly, and hazardous to human and animal life. As such, alternative methods for controlling nematodes, preferably non-chemical methods, should be developed and adopted by agricultural producers. The use of host plant resistance (HPR) is one of the options that have been used in the nematode management for many years. This has been particularly successful in the control of nematode genera that exhibit specialized host-parasite interaction for part of their life cycles, for example *Aphelenchoides*, *Ditylenchus*, *Globodera*, *Heterodera*, *Meloidogyne*, *Pratylenchus*, *Radopholus*, *Rotylenchulus,* and *Tylenchulus*. The nematode-resistant cultivars have been developed in numerous cultivated crops including chickpea (*Cicer arietinum*), peach (*Prunus persica*), tobacco (*Nicotiana tabacum*), and tomato (*Solanum lycopersicum*) against *Meloidogyne*. Recent trends in the use of HPR in plant nematology have seen new approaches in genome sequencing and genome editing being used extensively to study the complex genomic/transcriptomic interactions in plants in response to pathogen attacks. The use of spray-induced gene silencing (SIGS),

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RNA interference (RNAi), host-induced gene silencing (HIGS), cross kingdom RNAi, and also, engineering plant susceptibility genes is among some new advances that are currently receiving attention in the development of nematode resistant cultivars. These modern technologies have potential to provide sustainable and cost-effective strategies to manage nematodes to reduce crop yield losses due to these parasites and should thus be a subject for future studies.

Keywords

Effectors · Host plant resistance · Genomic interactions · Plant parasitic nematode · RNAi

4.1 Introduction

Nematodes are a diverse group of animals next to insects and are known to be found in every habitat. Plant parasitic nematodes (PPN) are among the most important biotic constraints that have potential for causing serious destruction and economically high yield losses in numerous crops worldwide. Plant parasitic nematodes are the ones which survive on living plant hosts (obligate biotrophs) with different parasitic (feeding) behaviours. Based on feeding behaviour, PPNs fall into two broad categories, ectoparasites and endoparasites (Holbein et al. 2016; Palomares-Rius et al. 2017). Contrary to ectoparasites, endoparasitic nematodes enter into plant root tissues and feed from inside. Endoparasites are further divided into semi-endoparasites, migratory endoparasites, and sedentary endoparasites (Mathew and Opperman 2020; Palomares-Rius et al. 2017). A semi-endoparasite partially penetrates host plant tissues and usually feeds from a permanent feeding cell. Unlike semi-endoparasites, migratory endoparasites will remain and spend most of their time migrating through root tissues. Meanwhile, sedentary endoparasites establish specialized feeding cells which form nutritional sinks and the parasites will remain in the tissue for the rest of their life cycle.

More than 4300 described species of PPN from 197 genera have been reported to infest crops globally (Decraemer and Hunt 2006), causing yield losses of 8–15% depending on crop, edaphic conditions, and geographic location (Khan 2007, 2016, 2023; Singh et al. 2013, 2015). At the generic level, *Helicotylenchus*, *Meloidogyne*, *Pratylenchus*, *Radopholus,* and *Scutellonema* are the most widespread and economically damaging PPN worldwide with a very broad host range (Sibanda et al. 2016). Root-knot and cyst nematodes (Heteroderidae) are considered the most economically important family of PPNs and are also known to parasitize more than 60 species of cultivated crops (Garcia et al. 2022; Hussey and Janssen 2001; Khan 2008; Khan et al. 2009; Onkendi et al. 2014). Ectoparasitic nematodes spend all their life in the soil and feed on cortical root cells by inserting their stylet into the cells while the body remains outside of the plant tissue (Mathew and Opperman 2020). Host plant damage due to PPN infections is expressed in various forms including root lesion, root knots, stunted growth, leaf necrosis and chlorosis, patchy growth, wilting, and susceptibility to secondary infection by other pathogens. Globally, PPNs cause annual crop losses worth over USD150 billion (Ali et al. 2015a; Coyne et al. 2018; Kantor et al. 2022; Nicol et al. 2011). While the magnitudes of crop losses are well documented in most developing countries, these are difficult to estimate in developing countries such as those in southern Africa where the field of nematology is regarded as generally neglected (Coyne et al. 2018).

For a long time, crop producers have relied on soil fumigants as principal method of controlling PPNs, and to a lesser extent on the non-chemical methods such as crop rotation, biological control, and resistant plants (Muitia et al., 2006). Despite their wide use and undoubted effectiveness, chemical nematicides are not without their own challenges. Apart from their high cost, chemical nematicides are associated with other challenges such as adverse impacts on the environment, health hazards to the public, and development on nematicide-resistant strains especially when they are used repeatedly. Moreover, one of the most widely used soil fumigants, methyl bromide, was banned almost a decade ago and it is inevitable that the remaining list of fumigant and non-fumigant nematicides will soon be withdrawn from the market (Onkendi et al. 2014; Paudel and Wang 2021). It is therefore apparent to use alternative nematode control methods that are cost-effective and sustainable. Host plant resistance (HPR) or tolerance has been exploited in a wide range of cultivated crops with the development of PPN-resistant cultivars (Davies and Elling 2015). Resistant plants possess heritable qualities that cause them to be relatively less damaged by a pathogen, while tolerant plants are able to grow and produce yield regardless of infection and injury by PPN. In other words, if a tolerant plant and a susceptible plant undergo the same stress, the former will be able to endure and withstand or, in other words, recover from the adverse effects of stress. However, in earlier comparisons of tolerance and resistance, Politowski and Browning (1978) suggested that a plant cultivar that exhibits susceptibility traits can be said to be resistant if it shows reduced levels of pathogen development..

Evidence shows that interest in understanding the mechanisms behind the conferment of resistance to PPN by host plants dates back to the period soon after the green revolution in the 1960s (Kaplan and Keen 1980). Although evidence from this early work was riddled with procedural inadequacies making it difficult to draw conclusions, the results suggested that presence of repulsive phytochemicals, barriers to penetration by PPN, nutritional deficiency, and hypersensitivity reactions by the host plants were among the mechanisms of HPR. Numerous plants have been shown to be resistant to PPNs including tomato, tobacco, peas, peanut, common bean, soyabean, among others (Muitia et al. 2006; Wendimu 2021). Understanding the role of molecular processes in plants has been useful in explaining the expression of resistance to pathogens by host plants. Plants have over the years of their association with PPN evolved to develop resistance to the pathogens with cell barrier forming the very first line of defence. Pattern recognition receptors (PRR) are situated at the cell surface of plants and these will recognize molecular signals called pathogen-associated molecular patterns (PAMPs) produced in response to infection by plant pathogens including PPNs (Holbein et al. 2016; Przybylska and Obrępalska-Stęplowska 2020). Similarly, damaged plant tissues also produce what are known as damage-associated molecular pumps (DAMPs). The presence of PAMPs and DAMPs in nematode-infected host plant cells in turn triggers basal resistance (innate immunity). Some pathogens can try to overcome defence response by the host plant by producing the so-called effectors that hold back basal resistance resulting in effector-triggered susceptibility (ETS) in the host plant, concomitantly resulting in effector-triggered immunity (ETI). The development of ETI is achieved through use of intracellular nucleotide binding leucine-rich repeat-receptors (NLR), and this is a much stronger and more intense resistance to pathogens than basal resistance (Holbein et al. 2016; Przybylska and Obrępalska-Stęplowska 2020). Moreover, the presence of disease resistance in the host plant results in fast development of ETI coupled with an amplification of pattern-triggered immunity (PTI). In root-knot nematode (RKN) -resistant plants, for example, this culminates in the death of hypersensitive cells (hypersensitive response, HR), and the latter is activated by the gene-for-gene resistance mechanism. Disease resistance genes in PPN-resistance species mediate defence responses that result in physiological changes which make conditions within the plant unfavourable for development of the pathogen.

Numerous resistance (*R*) genes have been recognized and exploited for the development of PPN-resistant cultivars and the first recorded success in HPR involved *Hs1pro-1* in sugar beet against *Heterodera schachtii* (Cai et al. 1997; Williamson and Kumar 2006). Many other *R* genes have since been identified and used in the development of HPR including *Gpa-2* in potato against *Globodera pallida*, *Mi* gene in many tomato cultivars against three common *Meloidogyne* spp., namely *M. incognita, M. arenaria,* and *M. javanica*, *Hero A* in tomato against *G. rostocheinsis,* and *H1* gene for resistance to *Globodera* in potato (Kaloshian et al. 2011; Karavina and Mandumbu 2012; Williamson and Kumar 2006). While several *R* genes are present in wild tomato and other species, the *Mi-1* gene is undoubtedly the single most characterized and commercially used gene. The use of *R* genes is most successful in sedentary endoparasitic PPN such as *Meloidogyne* and *Globodera*, whose effects are noticed in the form of death of specialized feeding cells of the host, culminating in reduced PPN population. This local death at the nematode's site of penetration inhibits penetration of the second juvenile (J2) and/or prevents further development and reproduction of the parasite. In contrast, resistance to ectoparactic and migratory endoparasitic PPN is difficult and its effects are almost peripheral (Karavina and Mandumbu 2012).

4.2 Current Status of HPR Applications in Management of PPN

4.2.1 Crops and Nematode Genera Involved in HPR

It is noteworthy that despite the significant strides that have been made in development of PPN-resistant crop cultivars, there is a plethora of crop species in which no resistance has been identified (Roberts 1992). For circumstances in which PPN-resistant crop varieties have been registered, these have been mainly against nematode genera that exhibit specialized host-parasite interaction for part of their life cycles. As explained above, sedentary endoparasites (e.g., RKN and cyst nematodes) and migratory endoparasites are most susceptible to *R* genes and HPR has been widely developed for these nematode groups (Bingefors 1982; Cook and Evans 1987). Such genera include *Aphelenchoides*, *Ditylenchus*, *Globodera*, *Heterodera*, *Meloidogyne*, *Pratylenchus*, *Radopholus*, *Rotylenchulus,* and *Tylenchulus*. Meanwhile, minimum progress has been made in identification and development of HPR against ectoparasites with only two genera, *Criconemella* and *Xiphinema,* being involved (Roberts 1992). Successes in HPR to migratory endoparasitic nematodes have been registered for a few nematode genera and crops of economic importance. These include HPR to *Pratylenchus brachyurus* in peanut *(Arachis hypogaea)*, *P. penetrans* in potato, *Radopholus citrophilus* in *Citrus* sp., *Aphelenchoides besseyi* in rice (*Oryza sativa*), and *Meloidogyne* in tobacco.

Chickpea (*Cicer arietinum* L.) is a leguminous crop that is produced in more 55 countries worldwide and is a rich source of protein. The crop is susceptible to various species of plant parasitic nematodes, causing huge yield losses. Several efforts have been deployed to identify nematode-resistant germplasm for chickpea, and generally, little success has been reported in this regard. For example, only 20 cultivars have been found to be moderately resistant to *H. ciceri* in India, while just 10 cultivars were found to be resistant to *M. javanica* in Pakistan (Zwart et al. 2019). Some sources of resistance and reasonable resistance to *M. incognita*, *P. thornei,* and *C. arientium* have also been reported in some Indian chickpea lines. The review by Zwart et al. (2019) reveals that among some of the world's crops of economic importance, chickpea is a good candidate for genetic improvement to introgress genes for resistance to PPNs. Despite being resistant, cultivars' response to PPN may vary with a number of factors including the strain of nematode species involved (Lehman and Cochran 1991). For example, there may be different races of the same PPN species and one variety may not be resistant to all these races.

As mentioned above, HPR to ectoparasitic nematodes has been difficult with just a few cases having been recorded globally. The main success stories of HPR for this group of nematodes have been limited to species that exhibit specialized host-parasite relationships, namely *Xiphinema index* in grapes (*Vitis* sp.) and *Criconemella xenoplax* in *Prunus* sp. (Harris 1983; Hussey et al. 1991; Okie et al. 1987; Roberts 1992). Relative to other ectoparasites, these nematode genera have a more specialized relationship with the host, for example, *Xiphinema* inflict modification of the host cells and may cause hypertrophy (Harris 1990). Roberts (1992) identified some widely used nematode-crop combinations covering those crop genotypes that have not shown susceptibility to nematode infection even after many years of exposure to nematode-infested soils. These crops include alfalfa, barley, common bean, citrus, cowpea, Lima bean, oat, peah, potato, red clover, soybean, tobacco, tomato, and walnut. This wide range of crops suggests that HPR is a phenomenon that is present in most plant species and that there is potential for development of PPN-resistant varieties in the majority of economic crops.

New technology such as the use of RNA interference (RNAi) has been employed in engendering HPR to PPNs by cultivated crops, with the technology having been commercialized in some countries including Canada and the United States of America (Coyne et al. 2018). Here, RNAi is used to suppress PPN genes that are responsible for parasitism, nematode development, and mRNA metabolism (Banerjee et al. 2017).

4.2.2 HPR Across Geographical Regions

Globally, resistant or tolerant cultivars have been developed with varying success in a couple of cultivated crops through either simple selection or breeding. In certain geographic locations, there are very limited applications of nematode-crop combinations due to inadequate development of HPR among crops of economic importance. For example, in Africa, a continent with huge crop losses due to nematode damage that are in the magnitude of up to 50%, only four crops have been reported to have resistance to PPN, namely tobacco, tomato, common bean, and cowpea (Roberts 1992; Sibanda et al. 2016). In fact, despite their damaging effects and the high yield losses that they inflict, limited time and resources have been devoted to nematode research and also addressing their negative impact on crops of economic importance in Africa.

4.2.3 Efficacy of HPR in Nematode Control

For HPR to be desirable, it should be durable, implying that it should continue to be effective against the pathogen over a prolonged period. This is usually achieved when several *R* genes (polygenic) are involved as opposed to where a single gene (monogenic) is involved, although this is not always the case. For example, inclusion of the *H1* in some varieties of Irish potato has provided HPR to *Globodera* for over 60 years despite the fact that only one gene is involved (Jones 1985; Roberts 1992). Resistance to *Meloidogyne* in the peach rootstock Nemaguard and tomato hybrid varieties with the *Mi* gene both involving monogenic *R* has shown high levels of durability having been in use for almost 70 years without any reports of virulence silence (Roberts 1992; Roberts et al. 1990). One of the concerns for the *Mi*-1 gene is that resistance is lost at high temperature. Precisely, the *Mi-1* gene becomes inactive at a soil temperature of 28 °C and loses its expression at 32 °C, and thus the plant becomes sensitive to RKN infection. Interestingly, a heat stable resistance gene (Mi-9) has been identified in *Solanum incanum*, suggesting that durability of resistance may be improved by genetic modification and pyramiding resistance genes in cultivars of cultivated crops (Wubie and Temesgen 2019). Although some nine other RKN resistance genes (*Mi*-2 to *Mi*-9 and *MiHT*) have been recognized in wild species of tomato, these have not been successfully transferred to commercial cultivars of the crop (Przybylska and Obrępalska-Stęplowska 2020). Although natural resistance is a preferred and sustainable strategy for PPN control, it is without its own limitation. For example, the *R* gene, *Hero A*, confers satisfactory resistance (>95%) against *G. rostochiensis,* but fair resistance (>80%) to *G. pallida*.

Furthermore, some *R* genes are specific to a limited range of species of PPN, yet crops are often infected by numerous parasitic species. In some cases, evolution of more virulent races of PPN has been reported and these have a tendency of breaking the existing HPR (Fuller et al. 2008).

4.3 New Trends in Host Plant Resistance in Plant Nematology

Global agriculture is facing tremendous qualitative and quantitative losses due to PPNs and management of this underground enemy is far more difficult than it seems. In the course of evolution, PPNs established complex interactions with their hosts with a cascade of effectors directly targeting the host immunity or modulating the host to shut down their defences and play in favour of invading (PPNs). Understanding plant-nematode interactions and underlying molecular mechanisms is very much needed not only to identify the weak spots in their biology, but also to target them using current molecular tools. In the recent past, new approaches in genome sequencing and genome editing have been used extensively to study the complex genomic/transcriptomic interactions among plants in response to pathogen infection. The use of sRNAs (small interfering RNAs) is another state-of-the-art technology to study target genes/genes with known or unknown functions. In this section, we aimed to discuss the latest advancements in host-pathogen interactions and the diverse approaches used to improve plant resistance against plant parasitic nematodes (Ibrahim et al. 2019).

The majority of the plant nematology studies were focused on endoparasites, particularly on RKNs (*Meloidogyne* spp.) and CNs (*Heterodera* spp.), because of their ability to cause higher crop damage and their survival ability on diverse plant species. They establish distinctive relations with their host by using their oesophageal gland (dorsal and sub ventral) secretions that are injected into the host cells soon after feeding initiation. These effector molecules (EFs) have been shown to play a key role in feeding site development and nematode establishment in host tissues. Even though some nematode secretions act as immuno elicitors like ascarosides (Manosalva et al. 2015; Mendy et al. 2017), the bulk of the effectors are known to hold back host defences and act as immunomodulators manipulating the host physiology for the benefit of parasitic nematodes (Goverse and Smant 2014; Siddique and Grundler 2018; Vieira and Gleason 2019). Comparative genomics help in appreciating the range of effector molecules among different plant parasitic nematode genera and their role in the parasitic process.

4.3.1 Nematode Effector Molecules

Currently, genome sequencing technology offers a huge opportunity to study the genome-level information about 138 different nematode species. Genome sequences of major RKN species (*M. incognito, M. javanica, M. arenaria, M. hapla*, etc.) were available in databases like Worm Base, *Meloidogyne* genomic resource project where the information concerning genes, genomes, transcriptomics, and proteomics is available and this provides useful insights into the gene/gene products involved in complex interactions with the host (Martin et al. 2015). There are various nematode effectors that share familiar characteristics with some secreted animal proteins, including the existence of N-terminal signal peptides for secretion or the lack of transmembrane domains.

4.3.2 SPRYSEC Proteins

SPRY domain (SPRYSEC) family of effector proteins is a diverse gene family specific to CN genomes viz., potato cyst nematodes *Globoderaro stochiensis* and *Globodera pallida* (Cotton et al. 2014) and in transcriptomes of soybean CN (*Heterodera glycines*) and cereal CN *Heterodera avenae* (Diaz-Granados et al. 2016). These SPRYSEC effectors are important in host immunomodulation and downregulating the defence response of the host. Ali et al. (2015b) found that *Gr*SPRYSEC-15 along with five other SPRYSEC proteins inhibited host immune response in *Nicotiana benthamian.* In another instance, SPRYSEC protein GpSPRY-414-2 suppressed *Gpa*-mediated resistance by binding to the potato cytoplasmic linker protein (CLIP)-associated protein (CLASP) associated with microtubule stability/growth (Mei et al. 2018). The study on protein GpSPRY-414-2 plays the role of a SPRYSEC effector molecule in modifying host defence responses.

4.3.3 Peptide Mimic Effectors

CEPs are C-TERMINALLY ENCODED PEPTIDE mimics and are a component of a large group of peptide mimic effectors which resemble the endogenous host peptides. The CEPS were first recognized in RKN and *R. reniformis*, but were absent in other syncytial forming nematodes. It was speculated that the possible role of CEPs was to increase host nitrogen uptake and also regulate the syncytial size (Eves-van den Akker et al. 2014). Other peptide mimic effectors like CLAVATA-like (or CLE peptides) effectors have also been observed in most nematode genera. These are understood to be responsible for modifying host developmental signalling pathways and also helping in formation and maintenance of host feeding sites (Gheysen and Mitchum 2019; Guo et al. 2017). These effectors are present in genomes of CNs, RKNs, and reniform nematodes (Petitot et al. 2016; Wubben et al. 2015). This group of peptides has structural diversity, possibly involved in multiple plant signalling pathways.

In a more recent study, two rapid alkalinization factors RALFs- (i.e., MiRALF1 and MiRALF3) like effectors were identified in *M. incognita* with a high expression of these genes in esophageal glands of parasitic juveniles. These RALF-like effectors bind with the extracellular domain of plant receptor kinase, FERONIA, to modulate host immune response and expand the feeding site of nematodes (Zhang et al. 2020). There are some unique effectors specific to plant parasitic nematodes: HYP effectors occur in *G. pallida, H. glycines, and R. reniformis* (Eves-van den Akker et al. 2014); 30D08 effector protein in *Heterodera glycines* and *H. schachtii* (Verma et al. 2018); 10A07 effector from *H. schachtii* (Hewezi et al. 2015); and CN effector Hs32E03 associated with chromatin modulation of the host (Vijayapalani et al. 2018). All these effectors modulated host gene expression to make the host more susceptible to nematode infection.

4.4 New Advancements in Enhancing Host Resistance

4.4.1 Use of RNAi

RNA interference (RNAi) is a conserved regulatory phenomenon throughout eukaryotes (Baulcombe 2004) and the majority of them have a key role in the regulation of host immunity and host-pathogen interaction (Weiberg et al. 2013). RNAi quietens gene expression by producing small interfering RNAs (siRNAs) generated by Dicer or Dicer-like (DCL) proteins and encumbered into Argonaute (AGO) proteins. These siRNAs target the genes with complementary sequences and knock their expression (Baulcombe 2004). In the recent past, studies revealed the movement of sRNAs from hosts to interacting pest/pathogen inducing RNAi which causes ‘Cross-Kingdom or Cross-Species RNAi’ in the latter (Knip et al. 2014; Weiberg et al. 2013, 2015). Host-induced gene silencing (HIGS) is a kind of Cross-Kingdom RNAi where the host plants are genetically modified to express double-stranded RNAs (dsRNAs) targeting a specific parasitic gene in pathogens or pests interfering in the parasitic process (Nunes and Dean 2012). Recent research has found the ability of some fungal pathogens and nematodes to uptake the sRNAs from the environment which was called environmental RNAi which potentially induces silencing of the target genes (Wang et al. 2016; Whangbo and Hunter 2008). Spray-induced gene silencing (SIGS) is an environmental RNAi, where gene-specific dsRNAs or sRNAs are artificially sprayed directly onto host plants and will then be taken up by pathogens causing post-transcriptional gene silencing in target pathogen (Qiao et al. 2021).

4.4.2 Cross Kingdom RNAi

Lately, studies on sRNAs revealed the bidirectional movement of sRNAs from hosts to interacting pathogen/ pest and vice versa as part of host-pathogen interaction which can be called Cross-kingdom RNAi or Cross-kingdom sRNAs trafficking (Wang et al. 2016). This phenomenon was first observed in a plant-fungal interaction where the grey mould pathogen *Botrytis cinerea* transported a panel of sRNAs (known to be fungal effectors) into plant hosts to seize the host RNAi argonaute protein, AGO1, to silence plant immune response genes. Similarly, the plant host also delivered sRNAs into the fungal pathogen including genes that regulate vesicle trafficking (Bc-VPS51, DTCN1, SACI) to silence the virulence genes and disrupt the pathogen invasion.

Later, similar processes were reported in fungal pathogens, such as *Verticillium dahliae* (Wang et al. 2016). *Puccinia striiformis* (Wang et al. 2017a) and *Fusarium oxysporum* (Ji et al. 2021) have been observed where sRNAs were transported into their plant hosts to silence defence response genes. RNA trafficking has also been acknowledged in the parasitic plant, *Cuscuta campestris*, where miRNAs were transported into its host plants to suppress plant defence genes. A similar observation was made when exosomes containing miRNAs were secreted by a gastrointestinal nematode to alter the immune response of mammalian hosts that had been infected by the parasite (Buck et al. 2014). Recent studies demonstrated that the intercellular transport of sRNAs was facilitated by extracellular vesicles (EVs) (Cai et al. 2018, 2019). Apart from sRNAs, these EVs are loaded with RNA binding proteins which help in stabilizing sRNAs (Cai et al. 2021).

4.4.3 Host-Induced Gene Silencing (HIGS)

This approach entails transforming plants to yield dsRNAs/sRNAs of a particular gene of interest. These dsRNAs are subsequently transferred into the pest or pathogen system upon feeding or infection and successful silencing of targeted genes is achieved (Wang et al. 2017b). This method is studied in different pathogen systems and successful silencing of genes was observed (Koch and Kogel 2014; Yadav et al. 2006). HIGS is a versatile tool, which has the potential to target multiple pathogens simultaneously. Nevertheless, HIGS holds great potential in targeted gene silencing, production of genetically modified plants (GMO) is time-consuming, and regulatory problems make this method practically difficult to implement.

4.4.4 Spray-Induced Gene Silencing (SIGS)

This technique was first demonstrated on *B. cinerea* that had been observed to be able to take up sRNAs from the environment. These environmental sRNAs successfully induced gene silencing in targeted genes. This discovery helped in the development of SIGS which is an eco-friendly, GM-free, RNAi-based plant protection technology (Qiao et al. 2021; Wang et al. 2016). In this method, sRNAs complementary to the target gene sequence are sprayed directly onto plant materials which were taken into the pathogen system directly knocking down the targeted gene. This method has the potential to develop RNA-based pesticides which can be directly applied to target pathogens without any off-target effects as well as residue issues which is the most common problem with chemical pesticides.

In fact, RNAs are highly degradable in the natural environment which is a major hurdle for using this method. The development of efficient carriers is essential for the practical utilization of this method. Recent studies are focusing on the development of inorganic nanocarriers to deliver these sRNAs and nanoparticles as carriers for sRNAs have huge possibility for the application of SIGS. Particular examples of these nanocarriers include guanidine-containing polymers, layered double hydroxides (LDH) clay nanosheets, and liposome complexes (Mitter et al. 2017; Niu et al. 2021). Mitter et al. (2017) developed Bio clay technology by using LDH clay nanosheets for loading dsRNAs for its application in SIGS. These bio clay RNAs showed sustained release of dsRNAs as well as high stability in the plant system demonstrating the potential of nanocarrier technology. Other nanocarrier systems include carbon nanotubes which were successfully utilized in transporting biomolecules into plant cells and have the potential to carry dsRNAs into the plant system. Recently, Schwartz et al. established a novel carrier tool for RNAs by packaging in carbon dots which induced successful gene silencing in plants (Schwartz et al. 2020). In *N. benthamiana* and *S. lycopersicum*, carbon dots were applied and successfully caused strong suppression of GFP (green fluorescent protein) transgenes and two endogenous genes responsible for encoding two subunits of the magnesium chelatase protein of the plants (Schwartz et al. 2020). Mimicking the naturally occurring RNA transport pathways is another strategy for RNA delivery which holds great potential. Plant-secreted EVs containing sRNAs are actively taken by pathogens, suggesting the importance of EVs in RNA transport. Lipid-based nanovesicles and peptide-based nanovesicles can be used to mimic naturally occurring EVs which could efficiently deliver sRNAs to plant pathogens.

SIGS technology has many advantages over HIGS like eco-friendly, less time-consuming for technology development, etc. Despite the advantages, it has specific requirements like the success of this technology depends on the pathogen uptake of sRNAs from the external environment and the carrier medium is essential to deliver sRNAs to target pests/ pathogens as RNAs readily degrade in the environment.

4.4.5 Engineering Plant Susceptibility Genes

Natural Plant resistance is often conferred by a single/few dominant resistance genes which are prone to resistance breakdown upon the emergence of new pathotypes. The exploitation of plant susceptible (*S*) genes is a new avenue for providing plants with recessive resistance which may be broad-spectrum resistance to multiple pests/ pathogens. Host *S* genes warrant the pathogen to invade and disease development and hence their inactivation leads to resistance development in plants. Examples of such gene alleles include the *mlo* allele that confers resistance to powdery mildew; the rice *xa13* allele conferring resistance to *Xanthomonas*; and *eIF*4 conferring resistance to potyvirus. Known plant S genes in coordination with pathogen effectors modulate the host for pathogen establishment, sustenance, and suppression of host immunity for successful disease development (Garcia-Ruiz et al. 2021). *S* genes are also important for establishing symbiotic relations with beneficial microbes which have similar cell/ mycelial structures to the pathogens. When these *S* genes responsible for symbiosis are mutated or knocked out, disease reduction was observed in pathogens. For instance, mutation of the *Medicago API* and *RAD*1 genes also perturbed susceptibility to the root infecting *Phytophthora palmivora* (Gavrin et al. 2020; Rey et al. 2017).

Parasitism by the endoparasitic CNs and RKNs requires nematode-dependent cytokinin signalling mediated by histidine kinase receptors to maintain their hypermetabolic nematode feeding sites in the host system. Arabidopsis histidine kinase receptor (ahk) mutant lines *ahk2/3*, *ahk2/4,* and *ahk3/4* exhibited less susceptibility to CN (*H. schachtii*) and RKNs (*M. incognita*) (Dawadi et al. 2021; Siddique et al. 2015). Most pathogens including some bacteria exploit sugar transporter proteins (SWEET) and nutrient secretion systems in host plants for successful reproduction in plants, and this makes them susceptibility hubs and best targets for gene knockdown. Oliva et al. (2019) posit that multi-editing of 6 TALe binding sites within the promoter enabled the disruption of three major SWEET genes in rice varieties, resulting in successful broad-spectrum resistance against the bacterial blight pathogen, *Xanthomonas oryzae PV. oryzae*.

S genes encoding negative regulators of immunity are another important target as plants use them in fine-tuning defence responses and limiting trade-offs (van Butselaar and Van den Ackerveken 2020). Mutation in these *S* genes can offer resistance to a wide array of pests/pathogens. Mutation of *mlo* (*mildew locus o*) membrane proteins, *mlo2 mlo6 mlo12,* in *Arabidopsis* showed a high accumulation of defence-related proteins when infected with powdery mildew pathogen (Kusch and Panstruga 2017). Another example of such interaction was observed in the DOWNY MILDEW-RESISTANT 6 (DMR6) gene which is an S gene which disrupts the salicylic acid (SA)pathway, thereby downregulating the defence response by the plant (Zeilmaker et al. 2015). CRISPR/Cas9 genome editing of DMR6 in tomatoes showed improved resistance to the bacterial pathogen *Xanthomonas* (Thomazella et al. 2016) and *Phytophthor*a infestans in potatoes which causes late blight disease (Kieu et al. 2021).

Targeting host susceptibility genes with mutation/gene knockdown to enhance host resistance is an emerging line of research and it holds great potential to give broad-spectrum resistance against pests/pathogens. Yet there are some drawbacks to this method as *S* genes have pleiotropic effects and perturbing the function of these genes may have some off-target effects like reduced plant physiological fitness, disturbance in interaction with beneficial microbes, and also the risk of becoming susceptible to other pathogens (Garcia-Ruiz 2018).

To overcome the problem of pleiotropic effects of *S* gene inactivation, conventional breeding can be employed by means of appropriate genotypes and also by selected mild *S* alleles. Recent developments in the field of genome editing during the previous decade opened multiple prospects to engineer transformation in crop genomes and thereby broad exploitation of *S* genes (Tian et al. 2020). Genome editing is an excellent tool to refine *S* gene perturbations to obtain positive resistance characteristics while curtail pleiotropic trade-offs. CRISPR/Cas9 technology is an upcoming high-throughput gene editing method. Recently, this technology was used to modify functional SNPs in the SHMT gene (Serine Hydroxymethyltransferase) which upregulated certain functional R-genes and conferred resistance to nematodes by modifying miRNA target sites in NBS-LRR genes (Ibrahim et al. 2019; Leonetti et al. 2018).

Multiplex/multiple gene editing is another powerful tool which is mainly employed in hybrids and polyploid genomes, where the *S* genes, as well as pleiotropic genes, are inactivated at a time to avoid pleiotropic trade-offs. Other possibilities to reduce the pleiotropic effect are to select hypomorphic alleles which show partial loss of gene function. Engineering S gene promoters is a novel approach which employs tissue-specific loss of function of genes minimizing pleiotropic trade-offs (Garcia-Ruiz et al. 2021).

4.5 Conclusions and Future Perspectives

Due to climate change, agriculture today and in the future must contend with severe disease/pest pressure and newly emerging diseases alongside decreasing the chemical crop protection methods. Natural host resistance in combination with emerging genomic technologies helps researchers to achieve this objective. The evolution of new pathotypes does challenge the current scenario of host plant resistance, but understanding the host-parasite interactions which involve pathogen effectors and pathways they target in the plant system provides new avenues for successful disease or pest management. Many effectors are preserved across pathogen genera, but some are pioneer proteins having exclusive roles at the plant-parasite interface. Present-day available genome sequencing techniques readily sequence the whole genomes of target organisms generating a huge amount of genomic data and helping researchers to explore the genetic information of these effector molecules. Gene silencing via RNA interference (RNAi) is conceivable for such functional research; however, the efficiency of RNAi-mediated gene silencing varies greatly depending on the technique of silencing and the target effector. Genetic transformation using Host-induced RNAi (HIGS) technology has been tried in many cultivated crops, but due to GMO regulations, their usage is currently prohibited in many nations. Recently, environmental RNAi /Spray-induced RNAi is gaining importance as it is a non-GMO method and safe for the environment. The use of nano-carriers for delivery of these sRNAs into the pathogen system greatly improves the efficiency of this method. RNAi technology is not only useful in the silencing of parasitic genes, but also helps in understanding gene function. More pathogen effectors must be functionally characterized as the next essential step. In addition, nanobiotechnology may greatly enhance the efficiency of management strategies (Khan et al. 2019a, b, 2020; Khan and Akram 2020).

Engineering susceptibility genes in the plant system is another strategy to improve plant resistance to a broad range of pathogens including insects and nematodes. More knowledge about the plant susceptibility genes as well as pleiotropic trade-off genes helps researchers to develop plant systems with broad resistance bases as well as zero off-target effects. Advanced genome editing techniques have a wide range of potential applications and the deregulation of GMOs would allow the development of resistant varieties in several resilient crops, which would contribute to sustainable agriculture.

Most of the above-mentioned studies were concentrated in pathogen systems. In the past decade, plant nematology studies were concentrated on the identification and functional characterization of effectors using RNAi (Host-induced RNAi). Nematode effectors of endoparasites, viz., root-knot and cyst nematodes, were well studied in a variety of cultivated crops. More recently, effectors from sting nematode, *Belanolaimus longicaudatus* (ectoparasite), were identified to modify the host metabolome specifically suppressing amino acids in African bermudagrass (Willett et al. 2020). This indicates the distribution of vast diversity of effectors in different plant nematodes and it is highly essential to study and characterize them to understand their interaction with hosts and to develop effective control measures to manage these ubiquitous pests.

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5

Biocontrol Strategies for Nematode Management, an Overview

Mujeebur Rahman Khan and F. A. Mohiddin

Abstract

Biocontrol strategies may work as a valid substitute for toxic chemical nematicides in plant nematode management. The biocontrol strategies include the use of filamentous fungi viz., *Pochonia chlamydosporia*., mycorrhizal fungi, endophytic fungi, and bacteria (*Pasteuria penetrans*, *Pseudomonas,* etc.). These microorganisms have either direct or indirect mechanism of action of suppressing plant nematodes. *Purpureocillium lilacinum, Pochonia chlamydosporia*, *Pasteuria penetrans,* etc. directly parasitize eggs and larvae and also induce mortality through the action of secondary metabolites of lytic enzymes, etc. The AM fungi and plant growth-promoting microorganisms protect plants from pathogens through indirect mechanism by increasing nutrient and water uptake, influencing the rhizospheric interactions, competing with pathogens, colonizing the infection sites, and activating the host defense. The present chapter describes various kinds of biocontrol agents that can be used in the nematode management and critically examines their relative effectiveness as well as the potential for commercial application in nematode management.

Keywords

Nematode management · Biocontrol fungi and bacteria · Commercial application

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5.1 Introduction

Plant parasitic nematodes are multicellular, microscopic, and vermiform animals generally present in the soil and feed on different parts of the plant, especially on roots with the help of protrusible stylet. On the basis of their feeding habits, phytonematodes are grouped as ectoparasites, endoparasites, and semi-endoparasites (Khan 2008). The most destructive crop nematodes belong to the sedentary endoparasitic group such as *Heterodera, Globodera,* and *Meloidogyne* (Jones et al. 2013; Khan et al. 2022). Out of around 5000 species of plant nematodes described till now, only a restricted number of species, falling under 20–30 genera, are considered as major plant parasitic species and reduce the yield of economically important crops to a large extent (Khan et al. 2023). A number of estimates have been made on yield loss inflicted by nematodes on agricultural crops which reveal 7–15% decline in the crop yield. The yield losses in terms of monetary value have been estimated as USD 100 billion (Sikora and Roberts 2018) and USD 175 billion (Tóthné Bogdányi et al. 2021).

In light of rapidly growing human population, there is ever-increasing demand of food. To cope up with this situation, the nematode management in agricultural crops has assumed significance and has been recognized as one of the global issues of concern. The symptoms in crop plants inflicted by phytonematodes often remain nonspecific and difficult to correlate with their parasitism and lead to their underestimation by the growers world over (Khan et al. 2023; Jones et al. 2013). In addition, the nematode parasitism also deteriorates food quality and appearance (Palomares-Rius et al. 2017). In order to deal with the arising food crisis vis a vis pesticidal contamination, efforts are being made to develop biological strategies for nematode control in agricultural systems. However, there also seems a need to extend plant nematode resistance along with the use of lower dose of pesticides, which is in accordance with the EU regulations (EC NO. 1107/2009) as the latter is detrimental to humans in addition to the surrounding environment (Khan et al. 2018; Zhang et al. 2017).

In the present scenario, where people are greatly concerned of the risks in chemicals and when cultural methods including cropping sequence, crop rotation, etc. do not offer a quick and economic control of nematodes, biological control becomes obvious option for the present as well as the future nematode management programmes. Biological control of plant diseases is a broad subject. It has varying meanings to different people, but to plant protectionists, it has a common connotation of maintaining the pest population under economic damage level by the action of living organisms without harming the ecology of the area. As a rule of ecology, each living organism acts as predator or parasite on one organism and prey to other organism (Khan 2016). Hence, in all kinds of climates, at least a few organisms occur which can parasitize or predate on nematodes. Host-parasite or prey-predator relationship that develops in a common habitat is a continuous process of energy flow in an ecosystem, which is responsible for maintaining the natural balance in soil biotic community.

The ability of antagonists (parasite/predator) to suppress nematode population low enough not to cause an economic damage to crops is also governed by certain set of environmental conditions in addition to virulence of the biocontrol agent and susceptibility of the host/prey (Khan et al. 2023). Hence, biological control of plant nematodes in a modern and scientific term is "a reduction of nematode population accomplished through the action of living organism(s), which occur naturally by manipulation of environment or through introduction of antagonists" (Stirling 1991). The organisms, which contend the plant nematodes and affect their ability to survive or invade plants, can be referred as antagonists. The antagonists of nematodes mostly act by killing the host/prey nematodes, but may also interfere in some way with their life processes such as egg hatch, larval movement, host finding, etc. (Jabłonowski et al. 1993). In a simple way, antagonists are naturally occurring enemies of nematodes, which can suppress their populations (Poveda et al. 2020). In addition, application of biocontrol agents has great scope in integrated pest management strategies.

Nematode biocontrol has a long history. Fresenius (1852), Lohde (1874), and Kuhn (1877) proposed that parasites and predators might reduce the populations of nematode in soil. Other important researchers are Zopf (1888), Cobb (1920), Thorne (1927), Linford (1937), and Dollfus (1946) who made initial research to establish biocontrol activity of microorganisms against soil-inhabiting nematodes. However, organized studies on this aspect commenced from mid-twentieth century. In the recent past, a bulk of information in the form of elaborated review articles (Jatala 1986; Sayre and Starr 1985; Morgan-Jones et al. 1984; Kerry 2000; Khan 2007, 2016; Khan et al. 2009) and a few books (Jairajpuri et al. 1990; Stirling 1991; Abd-Elgawad and Askary 2015) have dealt with the feasibility and potential of living organisms in suppressing the plant nematode populations in the soil.

A highly diverse range of microbes/multicellular organisms from fungi, bacteria, viruses, protozoans, predatory nematode, mites, collembolan, and other invertebrates can be utilized as biocontrol agents (BCAs) for the purpose of suppressing soil population of plant nematodes. The BCAs alone or with organic materials or cakes may prove effective, hence getting acceptability among the farming community (Khan 2007; Sikora and Roberts 2018; Khan et al. 2021). Biocontrol agents have also been found compatable with pesticides, hence can be applied in integration (Mohiddin and Khan 2013; Shahid and Khan 2019). The nematode antagonists such as *Pochonia chlamydosporia* (Stirling 1991), *Purpureocellium lilacinum* (Jatala 1986), *Aspergilus niger* (Khan and Anwer 2011), *Trichoderma* app. (Mohiddin et al. 2010; Khan and Mohiddin 2018); *Pasturia penetrans,* etc. (Kerry 2000) and plant growth-promoting microorganisms viz., *Bacillus, Pseudomonas, Penicillium,* and *Aspergillus* species/strains (Khan et al. 2009) may greatly contribute to protecting crop plants. In addition to the antagonistic microorganisms, predatory nematodes can also be utilized for controlling phytonematodes (Devi and George 2018). The predatory nematodes can target plant nematodes, e.g., *Odontopharynx longicaudata* predates upon *Meloidogyne javanica, M. gaugleri,* and *M. incognita,* leading to reduction in the soil population (Khan and Kim 2007). Some important beneficial microorganisms belonging to fungi and bacteria which may prove effective in the management programmes targeted against plant nematodes are elaborated under:

5.2 Biocontrol Fungi

5.2.1 Parasitic Fungi

5.2.1.1 *Pochonia chlamydosporia* (=*Verticillium chlamydosporium*)

The fungus *Pochonia chlamydosporia* belonging to the division Ascomycota, Class Hyphomycetes, Subclass Hyphomycetidae, is a commonly occurring fungus in the soils having infestation with cyst and root-knot nematode all over the world (Zare and Games 2003). *P. chlamydosporia* is a versatile, opportunistic species that may compete for a variety of soil-based resources, including nematodes. Nematode eggs seem to be an important source of nutrition for *P. chlamydosporia* present in natural soils. Soil is the only place where the fungus may be found in all of its phases, hyphae, conidia, and chlamydospores (Stirling 1991).

The fungus is reported to parasitize a significant population of eggs of *Heterodera avenae* in fields of cereal crops and significantly reduced their development and hatching (Manzanilla-Lopez et al. 2013). The fungus does not rely on nematodes alone for nourishment because *P. chlamydosporia* has been reported to parasitize certain other fungi and multiply upon them (Ghahremani et al. 2019) as well as eggs of snails (Barron and Onion 1966). Additionally, *P. chlamydosporia* may also colonize roots and destroy cellulase and chitin (Lopez-Moya et al. 2017). The fungal isolates differ significantly in terms of growth, sporulation, temperature requirements, and the formation of chlamydospores. Virulence of the fungus varies considerably, as some strains are weak parasites, while others are aggressive strains (Yang et al. 2012). Moreover, the population of *P. chlamydosporia* in the rhizosphere may increase considerably when root-knot nematodes were present (Bourne et al. 1996).

The application of *P. chlamydosporia,* has been reported to cause 51–78% drop in the population of phytonematodes (Tahseen et al. 2005). The occurrence of *P. chlamydosporia* var. *chlamydosporia* was the most widespread in Spanish soils with an incidence of 70–100% with severe infection of hyphae on eggs on *M. javanica* (Olivares and Lopez-Llorca 2002). Tomato plants cultivated in containers containing *P. chlamydosporia*-treated peat/sand/compost mixture had less eggs, juveniles, and galls (Silva et al. 2017). According to Cannayane and Rajendran (2001), *M. incognita* may be successfully reduced by using *P. chlamydosporia* with a dosage of 20 g fungus colonized substrate/plot (6×10^7 CFU/g substrates) in combination with *Purpureocillium lilacinum* and neem cake. The treatment also gave 58% enhanced yield of brinjal crop. The treatment comprising *P. chlamydosporia, T. harzianum,* and *G. mosseae* caused significant suppression in the proliferation of *Heterodera cajani* in pigeonpea (Siddiqui and Mahmood 1996). Coosemans (1988) reported that *P. chlamydosporia* had established itself in the field soil. The oat seeds colonized by *P. chlamydosporia*, when applied to the soil, induced significant decline in the root galls under field condition (Godoy et al. 1983). In order to rear the fungus, a range of agricultural/organic waste materials have been explored to identify the best material for the fungus mass-multiplication (Khan et al. 2001). Greater colonization of *P. chlamydosporia* on compost, sawdust, and leaf litter was recorded. Khan et al. (2011a) developed commercial formulation of *P. chlamydosporia* on sawdust-flyash-based medium. The seed treatment with the formulation at 5 g/kg seed suppressed the galling by 17–42% in chickpea and pigeon pea.

Mechanism of Action

Nematode eggs and females are infected by actively developing mycelia (dos Santos et al. 2013). When eggs come in contact with the fungus, the hypha gets stimulated and is involved in the invasion process (Manzanilla-Lopez et al. 2013). Seggers et al. (1996) observed that the appressorium is formed at the hyphal tip which gets attached to the eggshell via mucigens, leading to the formation of the infection peg which pierces the eggshell. The fungus causes the vitelline layer of eggshell to disintegrate as well as partially dissolving the chitin and lipid layers, probably as a result of exoenzyme activity. Seggers et al. (1994) reported that serine proteases are also produced by *P. chlamydosporia.* These extracellular enzymes are produced by the fungus in the presence of nematode eggs. Different strains of *P. chlamydosporia* generate subtilisins that differ significantly from one another (Esteves et al. 2009). Enzymatic changes to the eggshell may enhance permeability and perhaps make it easier for toxins to enter and flow within the egg (Castro et al. 2019), which prevent their hatching (Poveda et al. 2020).

5.2.1.2 *Purpureocillium lilacinum* (=*Paecilomyces lilacinus*)

The hyphomycetous fungus, *Purpureocillium lilacinum,* is another commonly occurring soil fungus, especially under tropical and subtropical climates (Jatala 1986). The fungus has been found to be an efficient parasite of eggs of a number of migratory and sedentary endoparasites as well as semi-endoparasitic nematodes. The fungus is ubiquitous in warmer climates and parasitizes nematode eggs and sedentary females (Jatala et al. 1979).

Khan and Ejaz (1997) made some field trials and compared efficacy of *P. lilacinum* with dry neem leaves and aldicarb application in soil on *M. incognita* infection in okra. The fungus inoculations decreased the galls and egg masses/root system and number of eggs/egg mass, but the effect was less than the aldicarb, but corresponding increase in the yield of okra was greater than the nematicide treatment. They also recorded *P. lilacinum* from the eggs, egg masses, and adult females of *M. incognita* excised from the fungus-treated plants. In another study, *P. lilacinum* in the soil application reduced the *M. incognita* galling and increased the yields of tomato (Khan and Akram 2000). The treatments with *P. lilacinum* have been found suppressive to root-knot on tomato (Khan and Goswami 2000), okra (Simon and Pandey 2010), cowpea (Midha 1985), black pepper (Sosamma and Koshy 1997), seasonal ornamental plants (Khan et al. 2005a), watermelon and banana (Devrajan and Rajendran 2002; Sundraraju and Kiruthika 2009), rice

(Khan et al. 2022), etc. The fungus treatments also suppressed *Rotylenchulus reniformis* (Fazal et al. 2011) and *Tylenchulus semipenetrans* (Maznoor et al. 2002) infecting crops. Field application of *P. lilacinum* at 4–6 kg/acre reduced the *Meloidogyne* spp., population in chrysanthemum root zone, and increased flower yield by 18–24% (Anonymous 2017).

Mechanism of Action

Parasitism by *P. lilacinum* on the eggs of root-knot nematodes was first observed by Lysek (1976). Since then, the fungus has been recorded to parasitize a large number of nematodes throughout the world (Jatala 1986). The fungus infection initiates by the hyphal growth on the egg mass matrix followed by its invasion. However, on eggs the hyphae become spiral or prostrate and penetrate the egg shell (Jatala 1986). Morgan-Jones et al. (1984) observed that the fungal hyphae readily invade/enter eggs of root-knot nematodes. The hyphal penetration mainly occurs through the minute pore in the vitelline layer of eggshell.

5.2.2 Filamentous Fungi

The primary classes of filamentous fungi have been investigated and utilized as biocontrol agents to counter plant nematodes. These fungi may affect nematodes through antibiosis, parasitism, paralysis, synthesis of nematoxic metabolites, lytic enzymes, and inducers of resistance (Poveda et al. 2020; Poveda and Baptista 2021; Medison et al. 2021). In addition, these fungi may improve the nutrient and water absorption capacity of plants, or by converting nutrients into utilizable forms, modify the rhizospheric microbial community or lessen pathogenic pressure on the plant (Sindhu et al. 2014). Furthermore, filamentous fungi can trigger hormone-mediated plant defense mechanisms or induce synthesis of strigolactones, salicylic acid, jasmonic acids, etc. to offer resistance against nematode invasion (Khan and Haque 2013). The generation of secondary metabolites and other enzymes as well as changes in the movement of chemical defense components through the plant can help to improve plant defenses. In light of this, utilizing filamentous fungi as efficient biocontrol agents in agriculture is a potential strategy towards sustainable

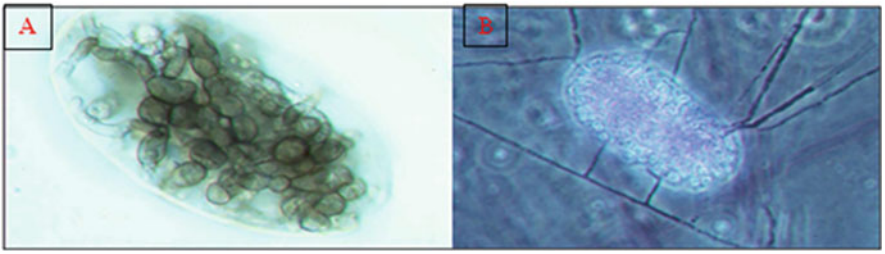

Fig. 5.1 Root-knot nematode eggs parasitized by different opportunistic fungi. (Courtesy photo: (**a**) B. A. Jaffee and (**b**) P. Timper; (http://www.cpes.peachnet.edu))

management of plant nematodes in agriculture crops (Poveda et al. 2020). Among the filamentous biocontrol fungi, *Trichoderma* is the most important and is discussed as under (Fig. 5.1).

5.2.2.1 *Trichoderma* Species

Trichoderma is undisputedly the most promising and thoroughly studied biocontrol agent of plant diseases (Mohiddin et al. 2010). It is an inexpensive, economical, and environmentally beneficial substitute of chemicals (Khan and Mohiddin 2018). The species of *Trichoderma* may colonize both the rhizosphere and the roots of plants, which enable the plant to boost its growth and development in addition to producing several nematode antagonistic toxins (Ansari and Khan 2012a, b; Ansari and Mahmood 2019a, b). It is generally known that some members of this genus can be exploited to manage *Meloidogyne* species (Mohiddin et al. 2010; Samuels et al. 2012; Khan et al. 2022). *Trichoderma* species were found to inhibit galling and egg mass production in chickpea (Khan et al. 2005b), pigeon pea (Khan et al. 2011a, b), rice (Haque et al. 2018), and tomato (Sharon et al. 2007). Naserinasab et al. (2011) observed that culture filtrates of *T. harzianum* strain BI deterred hatching of eggs of *M. javanica* and also caused larval death.

Application of conidial suspensions of *Trichoderma* spp. at pre- and postinoculation of *M. incognita* induced 50% decline in the multiplication of the nematode in cucumber roots (Mascarin et al. 2012). Khattak et al. (2018) examined the genetic diversity among the local isolates of *T. harzianum* and found that a specific strain induced around 76% mortality in *M. javanica* J_2. That isolate M2RT4 of *T. asperellum* in Kenya effectively decreased egg production and root galling in pineapple (Kirigaa et al. 2018). Some *Trichoderma* species, including *T. hamatum, T. harzianum, T. viride,* etc., have been documented to significantly suppress the galling and enhance the plant growth of *M. incognita*-infected tomatoes (Sayed et al. 2019) and cucumber (Mohammed and Khan 2021a, b).

5.2.2.2 Mechanisms of Action

The members of the genus, *Trichoderma,* affect plant pathogens through different mechanisms, such as, direct parasitism, antibiosis, enzymatic hydrolysis, nutrient competition, and induction of resistance which are elaborated under:

1. **Parasitism**: *Trichoderma* spp. produce branched conidiophores which bear conidia. These conidia adhere to the nematode body when they move in soil. Further, coiled hyphae and appressorium-like structures are produced by *Trichoderma* spp. which may aid in making contact of the fungus with nematode and penetrating into the cuticle and eggshell. Spiegel et al. (2005) and Sharon et al. (2009) reported that *T. asperelloides* and *T. harzianum* parasitized juveniles and eggs of root-knot nematodes possibly as a result of peptaibiotics, glycolytic, and chitinolytic enzymes secreted by the fungus. After 48 h of incubation with *T. harzianum*, J_2 of *M. javanica* showed around 84% infection and 95% larvae became immobile (Golzari et al. 2011). Mascarin et al. (2012) noticed that within a few days of inoculation of *T. harzianum,* 64% of the eggs and J_2 of *M. incognita*

had conidia of the fungus attached to them, rendering the larvae immobile. Recently, Khan et al. (2023) recorded 95–100% J_2 mortality of *Anguina tritici*, 2 weeks after treatment of cockles with *T. harzianum.*

2. **Antibiosis**: Antibiosis commonly operates in the suppression of pathogens by *Trichoderma* spp. (Khan and Mohiddin 2018). It is well documented that the fungus synthesizes a number of antibiotics viz., gliovirin, gliotoxin, viridin, viridol, koninginins, pyrones, peptaibols, harzianic acid, sperelines (A & E), and trichotoxins (T5D2, T5E, T5F, T5G, and 1717A) (Harman et al. 2004; Kumar and Khurana 2021). Abd-Elgawad and Askary (2020) observed that *T. viride* produced trichodermin, trichoviridin, dermadin, and sesquiterpene heptalic acid which may suppress the plant nematodes.
   Trichoderma spp. produced toxic metabolites in the liquid culture which had direct role in suppressing root-knot and reniform nematodes (Bokhary 2009). The suppressive impact of the culture filtrates was much greater on the eggs of *M. javanica* than on its juveniles. Kirigaa et al. (2018) reported that *T. asperellum* M2RT4, *T. atroviride* F5S21, and *Trichoderma* sp. MK4 as endophytes successfully colonized the roots of pineapple. Significant reductions in the production of nematode eggs and galling were seen with the above M2RT4 and MK4 isolates. The tested isolates also enhanced the development of root mass of treated plants. *Trichoderma viride* reduced the rate of egg hatching, and its commercial preparations have shown considerable effectiveness against nematodes in tropical greenhouse trails (Akhtar 2000; Hallman et al. 2009).

   Trichoderma species, if applied properly, can effectively prevent root-knot nematodes from developing into their full capacity (Migunova et al. 2018; Herrera-Parra et al. 2018). Al-Hazmia et al. (2019) found notable impact of *T. harzianum* in decreasing egg hatching of *M. javanica*. Similarly, Mukhtar (2018) observed that the number of galls, egg masses/root system, and fecundity rate of *M. incognita* decreased significantly with treatment of *T. viride* and *T. harzianum.* The culture suspension of *T. harzianum, T. hamatum, T. viride, T. virens,* and *T. koningii* and their filtrates suppressed root-knot disease in different crops (Khan 2016). These *Trichoderma* species were highly competent, but *T. harzianum* caused the most significant reduction in the hatching, juvenile mortality, galling, and egg mass production (Khan et al. 2018). Mohammed and Khan (2021a, b) found that *P. fluorescens*, *B. subtilis*, and three *Trichoderma* spp., when applied in soil or seeds, significantly checked the root-knot in cucumber under polyhouse condition. Similarly, the *T. harzianum* and *T. virens* caused significant suppression in the galls and egg masses/root system and root zone population of *M. incognita* (Khan and Rizvi 2013).
3. **Enzyme producers**: The hydrolytic enzymes like chitinases, xylanases, cellulases, glucanases, and proteases which break down the nematode cell wall are produced in ample amount by *Trichoderma* species. These enzymes, which are synthesized in a variety of forms, or isozymes with varying sizes, regulations, and abilities, are typically stable extracellular and low molecular weight compounds (Cheng et al. 2017). The nematode egg shell contains chitin as primary component (Morton et al. 2004). Hence, the chitinases, which are

released by *Trichoderma* spp. as secondary metabolites, are thought to be of utmost significance against plant nematodes.

The cysts and eggs of golden cyst nematode, *Globodera rostochiensis,* perished as a result of enzymatic penetration of *T. harzianum* (Saifullah and Khan 2014). As the chitin coating is broken down by microbial enzymes, *T. harzianum* successfully parasitized *Meloidogyne* and *Globodera* eggs possibly due to the action of chitinases, glucanases, and proteases. Askary and Martinelli (2015) reported that *T. harzianum* invaded the cuticular layer of larvae and eggs multiplied inside and released nematoxic metabolites which led to their death. The cellulolytic activity is also well to occur in *T. viride, T. harzianum*, *T. koningii, T. longibrachiatum,* etc. Similarly, Hussain et al. (2017) commented that producing the lytic enzymes, *Trichoderma* spp. become a potential candidate for bio-management of plant pathogenic fungi and nematodes. For example, lipase enzymes are synthesized by *T. viride* (Kashmiri et al. 2006; Mehta et al. 2017).

4. **Competition**: Because of being aggressive colonizer, *Trichoderma* spp. have potential to compete well for space and nutrients, which may lead to suppression of plant pathogens (Contreras-Cornejo et al. 2016). *Trichoderma* spp. may limit the colonization of pathogens by exerting rhizospheric competition for nutrients and space when applied in the form of conidial suspension (Sivan and Chet 1989a, b). *Trichoderma* spp. are used by a variety of application methods, including seed treatments or soil application. They spread quickly along the root system of the plant after application. However, there are mixed findings about the competition mechanisms involved against nematodes that differ with the strains of *Trichoderma* spp. (Howell 2003; Khan 2016). It appears obvious that colonization of the fungus may exert some discomfort to the nematode juveniles moving in the soil or penetrating/invading the roots (Khan et al. 2023).
5. **Induction of host defense**: Another crucial strategy for controlling phytopathogens is to use biocontrol agents to boost the host defense and resistance (Leonetti et al. 2014). An increase in the activity of phenols, chitinase, peroxidase, etc. can be used to measure the plant response to nematode infestation (Khan and Haque 2013; Khan et al. 2022). Increased concentration of defense enzymes including peroxidase and polyphenol oxidase, as well as some significant defense chemicals like phenols and ortho-dihydric phenol, was found in ground nuts that had been exposed to *T. harzianum* (Sreedevi et al. 2011). Similarly, oil palm trees had significantly greater chitinase activity after having *T. harzianum* treatments, which is a crucial defense enzyme in plants against phytopathogens (Naher et al. 2012; Pusztahelyi 2018). In addition to causing infection to eggs and J_2, application of *T. harzianum* resulted to greater systemic resistance in the host plant, which led to a lower galling in tomato caused by *M. incognita* (Singh et al. 2017). The treated tomato plants had greater concentration of chlorophylls, chitinase, peroxidase, and phenylalanine ammonia-lyase. Greater total phenol and salicylic acid concentrations were found in the marigold leaves, due to inoculation with *M. incognita* at 500, 2500, and 5000 juveniles/kg soil (Khan et al. 2013a, b). Khan et al. (2011a) reported that the five cultivars of

tobacco treated with *T. harzianum* showed an enhanced contents of total phenol and salicylic acid in root-knot nematode-infected plants. In addition, nanobiotechnology may greatly enhance the efficiency of management strategies (Khan et al. 2019a, b, 2021; Khan and Akram 2020).

5.3 Biocontrol Bacteria

5.3.1 *Pasteuria penetrans*

Pasteuria is an endospore-forming bacteria which are strict obligate and mycelia forming parasitizes of nematodes (Sayre and Starr 1985). The genus has stood as an ideal biocontrol agent against nematodes and contains a variety of species that have demonstrated excellent potential of parasitizing plant nematodes (Khan 2007). The *P. penetrans* is most ubiquitous in distribution and is documented to occur in over 50 countries (Siddiqui and Mahmood 1999). The *P. penetrans* is extremely infectious and virulent genus and has been reported infecting over 300 free living and plant parasitic nematode species from around 115 genera (Chen and Dickson 1998). It has been noted that most of the economically important plant nematodes are parasitized by *P. penetrans* (Bird et al. 2003).

5.3.1.1 Mechanisms of Infection

The bacterium, *P. penetrans,* produces endospores which adhere to the cuticular layer of nematode juveniles (Davies et al. 2000). However, not all the nematodes are recognized and adhered by the spores of individual *Pasteuria* populations, possibly because of the narrow host range of spores of each *Pasteuria* species. *Pasteuria nishizawae*, for instance, infects the genera *Heterodera* and *Globodera,* whereas *Meloidogyne* and *Pratylenchus* species are infected by *Pasteuria penetrans* and *P. thornei* (Gives et al. 1999; Atibalentja et al. 2000). In case of root-knot nematodes, the spores germinate after the entry of juveniles into the roots. The juveniles after the root penetration feed normally and galls develop, but reproduction is severally affected. The vegetative microcolonies are formed after the penetration of germ tube into the nematode cuticle. The microcolonies multiply inside the developing female nematode, especially in the reproductive tissue. Eventually, degeneration of female body along with the infected roots takes place that leads to the release of endospores into the soil (Mankau et al. 1976; Sayre and Wergin 1977).

5.3.2 *Pseudomonas, Bacillus,* Etc.

The plant growth-promoting rhizobacterias (PGPR) generally colonized in the rhizosphere or plant roots and enhance the growth and yield of the plant (Mhatre et al. 2019). However, it’s noteworthy to mention that certain PGPRs, especially *Bacillus* and *Pseudomonas* spp., exhibit substantial nematicidal activity against plant nematodes (Khan et al. 2009). The PGPRs have tremendous potential to directly interact with plant pathogens including nematodes while colonizing the rhizosphere or plant roots. One excellent example is the fact that secondary metabolites of *P. fluorescens* CHA0 may destroy J_2 and eggs of root-knot nematodes (Siddiqui and Shaukat 2003). Five bacteria strains, including *B. cereus, B. subtilis, P. putida, P. fluorescens*, and *Serratia proteamaculans*, have demonstrated high suppressive effects against *M. javanica* (Zhao et al. 2018). The PGPRs secrete several growth regulatory chemicals in the rhizosphere which play important role in promoting the plant growth (Gu et al. 2007; Karmani et al. 2011). In a greenhouse experiment, the application of *B. subtilis* culture filtrates applied as a soil drench lowered the nematode parameters in eggplant root (El-Nagdi Wafaa and Abd-El-Khair 2008). Another species, *B. coagulans,* combined with *Glomus aggregatum* or vermicompost, reduced the number of galls and enhanced growth of tomato plants (Serfoji et al. 2010). Application of *P. fluorescens* and *B. subtilis* on seeds or in soil is reported to enhance the plant growth and dry matter production of root-knot nematode-infected tomato (Khan et al. 2018) and cucumber plants (Mohammed and Khan 2021a, b).

Farfour and El-Ansary (2013) observed that application of *B. subtilis* with *T. harzianum* increased the growth characteristics of eggplant and suppressed the galling and *M. incognita* population. *Serratia marcescens, P. fluorescens, B. thuringiensis,* and *B. subtilis* suppressed egg hatching of *M. javanica* and induced J_2 mortality (Mokbel Asmaa and Alharbi Asmaa 2014). Another species, *B. weihenstephanensis,* showed greatest effectiveness in controlling *M. incognita* in infected tomato and brinjal plants (Sarangi et al. 2017; El-Nagdi and Abd-El-Khair 2019). The bacteria significantly decreased the gall index and J_2 population in the soil, roots, and increased tomato fruit yield (Tamalika et al. 2014). *Serratia marcescens, P. fluorescens*, and *B. thuringiensis* (BT14) antagonized *M. incognita* larvae causing considerable decline in their population (Zaghloul et al. 2015). A drastic reduction in the population of *Meloidogyne* $J_{2,}$ in the soil and females, galls, and egg masses in the pea root was recorded with *B. subtilis* or *B. pumilus*. Additionally, the treatments markedly promoted the shoot and pod development (El-Nagdi Wafaa et al. 2018).

5.4 Conclusion and Future Perspectives

There is a vast diversity in the biocontrol agents of plant nematodes. The temperature, nematode species, and plant species have an impact on the geographical distribution of BCAs. However, the most important and prevalent nematode antagonists as revealed from the literature are *Pochonia chlamydosporia, Purpureocelium lilacinum, Trichoderma* spp., *Pseudomonas fluorescens, Pasteuria penetrans,* etc., and they have proven to be extremely efficient against a variety of plant nematodes, especially *Meloidogyne* spp. in different crop pathosystems. However, effectiveness of these biocontrol agent varies widely with the isolate or strain. Sometimes, an effective isolate fails to establish in soil and unable to suppress the nematodes in a new habitat. These circumstances warrant a quest for continuous search of effective native beneficial microbial isolates. Furthermore, using a group of ecologically compatible microorganisms to treat nematode infestations in agricultural crops may be more successful and provide a sustainable remedy of nematodes. In subtropical, tropical, and temperate areas, consortium of *P. lilacinum, Trichoderma* spp., *P. fluorescens* may work better than either microbe in monoculture. Despite the lack of commercially accessible consortium of microbial formulations, the individual formulations should still function well when used in combination. Additionally, quantitative and qualitative standards of bioformulations are needed to be maintained, failing which would result in losing faith of public and farmers in this novel method of management that shall have far-reaching serious consequences. Hence, the state plant protection authorities have to strictly enact the biopesticide regulations on the formulations, manufactures as well the dealers so that quality products become available to the farmers.

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Microbial Consortia: An Approach to Enhance the Effectiveness of Beneficial Soil Microbes

6

Hajar El Hamss, Nabil Radouane, Zineb Belabess, and Rachid Lahlali

Abstract

Chemical inputs likes pesticides and fertilizers are widely used in agriculture today, but they affect soil health and its nutritional value. Since soil microbial consortia are crucial in determining the condition of the soil, their use as a chemical-free substitute is becoming more and more important. This is partly because various microbial consortia may carry out distinct functions in varied soils. Exogenous application of consortia is, therefore, an effective strategy to improve soil nutrients and lower chemical inputs which in turn help maintain soil structure. Microbial consortia collectively acting with different mechanisms of action can have a broader effect and benefit to the soil health. In this chapter, different types of microbial consortia are reviewed. Additionally, when microorganisms function as a consortium, often as combinations of numerous species under various soil conditions, their interaction and methods are discussed. Moreover, the engineering opportunities and biotechnological potential of several microbial consortiums are also highlighted. However, more details are still

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needed about the mechanisms of increasing soil microbiome performance in an ecosystem by the introduction of new microbial strains/consortia.

Keywords

Microbial consortia · Beneficial microorganisms · Soil health · Soil fertility · Plant growth

6.1 Introduction

The soil is considered the staple of life on Earth and the processes that occur underground are mostly powered by rhizosphere microbiome there, sustaining life above ground by driving various ecological and global functions. Because life in the soil is hidden, it frequently goes "out of sight, out of mind". The soil system is incredibly complex and fluctuates dramatically over time and space. Soil consists of three main components: water, an "organic matter" fraction that contained a wide variety of organic silica compounds, and a mixture of trace metals (Jeffery et al. 2010; Nadporozhskaya et al. 2022).

According to a recent microbial study, soil is the fourth most prominent category in bacterial and archaeal community, after host-related, aquatic, and built environmental resources. (Schloss et al. 2016). The important biodiversity on Earth is found in soil, which also provides the physical basis for most human activities. Although soils have been extensively studied and classified with respect to their physical and chemical properties, knowledge about soil biodiversity and function is still limited. Soil organisms contribute to a wide range of different services for the sustainable functioning of a natural ecosystem (Saccá et al. 2017). The soil microbiome is, indeed, extremely diverse, accounting for up to a quarter of all diversity on the earth (Wagg et al. 2019). Bacteria, Archaea, and fungi make up the majority of microbial communities in soil ecosystems. This is because they are extremely numerous and have a large cumulative mass and activity (Saccá et al. 2017). Fungi and bacteria are among microorganisms that inhabit the topsoil and that deserve special attention, since they are the predominant and can affect positively or negatively plants health depending on the species, the plant host, and environmental factors (Jeffery et al. 2010; Lahlali et al. 2021; Odelade and Babalola 2019). The soil microbial population can perform many tasks, including nutrient cycling and carbon (C) sequestration, which help maintain soil fertility and slow down global warming (Hemkemeyer et al. 2021; Lahlali et al. 2021).

Plant development and yield are impacted by soil and related stress conditions in addition to microbial populations and soil ecosystems. Other significant soil pressures include metal contamination, drought, and salinity. It has become crucial to improve soil conditions to increase crop productivity and, therefore, fulfill the rising food demands imposed by rapidly growing world population. There are many ways to solve this issue, one of which is to exploit the microbial potential in the favor of cultivated plants (Panwar et al. 2014). A practical strategy for improving soil quality and ensuring sustainable food production consisted of the promotion of agricultural practices that preserve and strengthen knowledge of the microbial flora of agricultural soils and the microbial consortium contained therein (Aguilar-Paredes et al. 2020). Traditional methods of increasing farming yield through the heavy application of fertilizers and pesticides are usually unsustainable due to high costs, global warming concerns, environmental damage, and safety risks. Therefore, using native soil bacteria to increase yields is a preferred, inexpensive, and reliable alternative to the use of chemical inputs (Reddy and Ramu 2013). Designing an inoculum that combines a variety of beneficial microorganisms with appropriate traits likes N2 fixation, phosphate solubilization, growth stimulation properties, and biocontrol activities is a useful breakthrough in this field. The productivity of several crops, including legumes, cereals, vegetables, and grasses, can be increased with the help of this inoculant. This form of multimicrobial vaccine incorporates a large number of microbes for each main function associated with plant growth and productivity, offering greater constancy and broader application across a range of important crops (Reddy and Ramu 2013). Although several studies have shown that beneficial soil microbes have beneficial effects on crop yield and quality, microbial consortia are still neglected in agriculture (Aguilar-Paredes et al. 2020). Therefore, the many types, interactions, and methods of soil microbial consortiums are discussed in this literature review, as well as their uses in engineering and biotechnology.

6.2 Soil Microorganisms Types

According to taxonomic methods used to estimate the biodiversity, tropical rainforests are home to more than half of the estimated ten million of plant, animal, and insect species in the world. In addition, a single soil sample can contain tens of thousands to millions of different species (Jeffery et al. 2010; Lahlali et al. 2021). In general, a soil substrate can contain 8–15 tons of organisms including bacteria, fungi, protozoa, nematodes, earthworms, and arthropods. The proportional number of microbial species is 10^8–10^9 for bacteria, 10^7–10^8 for actinomycetes, 10^5–10^6 for fungi, 10^4–10^5 for algae, 10^3–10^4 for protozoa, and 10^2–10^3 for nematodes at a depth of 0–6 in. (0–15 cm) (Hoorman and Islam 2010). Therefore, the soil is by far the most biodiverse environment on Earth, with an estimated 1000 billion microbial DNA sequences per gram of soil (Vogel et al. 2009). Although there are numerous bacteria in the soil, their biomass is very minimal due to their small size (40–500 g/m^2) in a depth of 0–6 in. (0–15 cm). Actinomycetes have biomass similar to that of bacteria, but are ten times less numerous and greater in size. Even though the fungus population is less, when the soil is not disturbed, it dominates the biomass (100–1500 g/m^2) in a depth of 0–6 in. (0–15 cm). Fungal and nematode populations are often predominant in cultivated soil, but other microorganisms (bacteria, actinomycetes, and protozoa) are stronger and can tolerate a wide range of soil conditions (Hoorman and Islam 2010). In the rhizosphere, the constrained area next to and surrounding the root, there is a particularly high concentration of bacteria and actinomycetes. Depending on nutrient availability, actinomycetes can account for 10–30% of all microorganisms in the soil rhizosphere. Some plants produce certain types of root exudate to facilitate the development of protective bacteria (Hoorman 2011; White et al. 2018). Microbes are found in large quantities in soil as long as carbon source for energy production is available (Hoorman and Islam 2010). Indeed, the secret to recycling carbon and nitrogen is soil microorganisms (Hoorman 2011; Jacoby et al. 2017; White et al. 2018).

6.2.1 Bacteria

Between 100 million to 1 billion bacteria, on average, are present in a teaspoon of fertile soil (Jacoby et al. 2017). That equates to two cows' worth of mass per cm^2. There could be over a million different types of bacteria and a ton of tiny bacteria active every cm^2. In the rhizosphere, bacteria grow and live in thin water films close to roots and around soil particles (Lahlali et al. 2021). Since they are smaller and more flexible than larger, more complex microorganisms, they may grow and change more quickly in response to shifting environmental conditions. Microorganisms that break down organic matter can be found in most soils (Lahlali et al. 2021). Since the majority of bacteria in the soil must survive periods of starvation or water stress, they have evolved to multiply quickly when these conditions are favorable. In just 30 min, bacterial populations can rapidly double. The structure of bacteria is so basic that it has been compared to a bag of enzymes. Most bacteria are divided into phyla (phylum is a scientific classification of organisms). The following categories can be used to simplify the classification of bacteria: (1) bacteria based on shapes, (2) aerobic and anaerobic bacteria, (3) bacteria that are gram-positive and gram-negative, (4) bacteria that is both autotrophic and heterotrophic, and (5) classification based on phyla. Scientists have divided bacteria into 12 phyla based on their shape, DNA sequencing, environmental requirements, and biochemistry. Additionally, the number of bacterial species and genera matches each phylum. Bacteria that can endure a variety of environments, including those with high temperatures, are included in the taxonomy of bacteria (such as those found in sulfur water springs) and low temperatures (such as those found in Antarctic ice). It also includes bacteria that can withstand different types of environments, such as those that are aerobic or anaerobic, autotrophic or heterotrophic, and those that can withstand extreme acidity or alkalinity (Hoorman 2011).

6.2.2 Fungi

Although they are not necessarily confined to one functional category, soil fungus can be found in a wide variety of them. Many species are parasites that cause a range of economically significant diseases in plants and animals, but the majority of species are saprotrophs, meaning they feed on dead organic matter (Crowther et al. 2012; Lahlali et al. 2021). In addition, many soil fungi can establish a symbiotic association with plants, either by developing mycorrhizas that facilitate nutrient intake or by colonizing plant tissues covertly infected. According to recent results from DNA studies of fungal communities, 1 g of forest soil can have more than 1000 fungi (Lahlali et al. 2021). The parasitic fungus that infects the roots of forest trees is the largest known fungal individual, covering 890 ha and weighing an incredible 80 tons. Due to its huge structure, certain fungal species can live longer times, hundreds or even thousands of years. Estimates of the number of fungal hyphae in soils range from 100 to 700 m/g, or 700 to 900 kg/ha, with forest soils showing the highest values (Jeffery et al. 2010).

6.2.3 Archaea

Archaea are the third type of microorganisms. The only similarities between these organisms and bacteria are that they are both minuscule single-celled organisms without cell nuclei. They may use a wider variety of energy sources than eukaryotes and are similar to bacteria, but archaea have several metabolic pathways and genes closely identical to eukaryotes than bacteria (Gribaldo and Brochier-Armanet 2006). Archaea's cell membranes, on the other hand, differ significantly from those of bacteria and eukaryotes, showing that archaea evolved independently from other prokaryotes and eukaryotes. At first, it was thought that Archaea were extremophiles or organisms that could endure hostile environments like salt lakes and hot springs. Archaea, like other microorganisms, have been discovered practically everywhere, including soils, as a result of increased use of molecular technology, which has helped overcome challenges like lack of cultivability in labs (Gribaldo and Brochier-Armanet 2006; Lahlali et al. 2021). The current biological biomass on Earth may be made up of as much as 20% archaea. Archaea are also recognized as an integral fraction of life on Earth and play a substantial role in the carbon, nitrogen, and sulfur cycles due to the recent expansion of research into these bacteria (Hemkemeyer et al. 2021).

6.3 Type of Microbial Consortia for Use in Soil

Microorganisms live in colonies in natural habitats, and some of them aid plants (Sarma et al. 2015). Many studies have shown that plants can benefit from a single bacterium, but there are additive or synergistic benefits when using microbial consortia (two or more interacting microbes). Table 6.1 provided examples of microbial consortiums that have been applied to various crops in the past. This is because different species can perform different functions in an ecosystem like the rhizosphere (Santoyo et al. 2021). Furthermore, the interactions between a consortium of microorganisms that have been added to the soil and a host plant closely resemble natural soil conditions. As a result, current research is now primarily concerned with figuring out how small microbial consortia might help plants develop and stay healthy in the face of a variety of invasive diseases. This is a

Table 6.1 Examples of consortiums applied in different conditions showing the effect on a given crop

Microbial consortia	Type	Application method	Crop treated	Outcomes	Reference
Azospirillum brasilense, *Azotobacter chroococcum*, *Bacillus polymyxa*, and *Enterobacter cloacae*	Mixture of rhizobacteria	The mixture was inoculated in wheat seeds and then planted under greenhouse conditions	Winter wheat	Increased yield Increased dry weight and total-N	de Freitas (2000)
Bacillus but different strains named: OSU-140, OSU-142, and M-13	A mixture of N2-fixing and P-solubilizing bacteria	The mixture was inoculated to sugar and barley seeds and then grown in field conditions on an experimental farm of the Turkish Sugar factories	Sugar beet Barley	Roots, leaf, and sugar yield increased	Şahin et al. (2004)
Mesorhizobium, Pseudomonas, and Bacillus spp	A mixture of phosphate-solubilizing bacteria with rhizobacteria	The chickpea seeds were dipped in the mixture solution and then sown in soil under greenhouse conditions	Chickpea	Increased nitrogen (N) and phosphorus (P) uptake	Wani et al. (2007)
Pleurotus ostreatus, Phanerochaete chrysoporium and *Hypholoma fasciculare*	A mixture of white-rot fungi	Under laboratory conditions, the mixture was added to PAH-contaminated soil in Sweden	Gasworks plant	Negative interactions occurred as no fungal survived in contaminated soils except *H. fasciculare*	Andersson et al. (2000)
Fusarium oxysporum, Serratia sp, *S. maltophilia* (among other unknown bacteria)	Naturally occurring fungal and rhizobacterial consortium	Isolated from a naturally suppressive soil in Italy to study syntrophic interactions within the consortium	–	Consortium species share nutrients Abiotic stress triggers consortiums to produce toxins to possibly protect themselves	Moretti et al. (2010)

significant departure from the original studies, which focused on a single microorganism (Sarma et al. 2015). The treatments comprising biocontrol agents alone (Stirling 1991; Khan 2007; Khan and Anwer 2011) or along with oil, neem cakes (Sikora and Roberts 2018; Khan et al. 2021) or pesticides (Mohiddin and Khan 2013) are getting popularity in achieving sustainable nematode management in agricultural crops (Khan 2023; Khan et al. 2023). The microbial antagonists, *Aspergilus niger*, *Pochonia chlamydosporia*, *Purpureocellium lilacinum, Pasturia penetrans* etc. (Jatala 1986; Stirling 1991; Kerry 2000; Khan 2016), and phosphate solubilizing microorganisms such as *Aspergillus, Bacillus, Penicillium, Pseudomonas* etc. (Khan et al. 2009, 2016a, b; Sikora and Roberts 2018) may significantly contribute in the sustainable management of plant nematodes. The well known mycoparasitic fungus, *Trichoderma* has also been found effective in suppressing plant nematodes (Mohiddin et al. 2010; Khan and Mohiddin 2018), and numerous formulations of *T. harzianum* and *T. hamatum* of are available in market (Khan et al. 2011), and provide consistently satisfactory control of soil-born pathogens (Mohammed and Khan 2021; Sikora and Roberts 2018; Shahid and Khan 2019).

6.3.1 Bacterial Consortia

Many microbial inoculants have been commercialized as a consequence of extensive research into the effects of various PGPB strains on plants in recent years (de los Santos-Villalobos et al. 2018; Reed and Click 2013). To extend the beneficial biological activities of these bacteria, the creation of a bacterial consortium is attracting attention as a viable technique for sustainable food production. Two or more suitable bacteria from various species that work together in a synergistic or additive manner constitute a bacterial consortium (Panwar et al. 2014; Sarma et al. 2015; Stockwell et al. 2010).

To increase crop development and productivity, beneficial bacteria must interact properly with plants. Some beneficial bacterial isolates *Azospirillium brasilense* Sp7, *Pseudomonas putida* KT2440, *Acinetobacter* sp. EMM02, and *Sphingomonas* sp. OF178A were tested for their ability to interact with six different maize strains in a single bacterial and consortium application. Results underline that the consortium's level of bacterial colonization was higher than that of a single bacterium (Molina-Romero et al. 2021). Similarly, a bacterial consortium's effects, including strains of *Azotobacter chroococcum*, *Azospirillum lipoferum*, and *Pseudomonas putida*, on the control of *Neocosmospora rubicola*, which causes potato rot diseases, have been investigated. In both healthy and *N. rubicola*-infested soil, the bacterial formulation has the potential to boost potato tuber yield (Riaz et al. 2022).

6.3.2 Fungal–Bacterial Consortia

Arbuscular mycorrhiza (AM) is associated not only with plants, but also with endobacterial PGPR in rhizosphere, affecting their activity and producing functional synergies (Bonfante and Anca 2009; Miransari 2010). The interaction between AM fungi and soil bacteria has an impact on how fungal genes are expressed. Examples of interactions include attachment of bacteria to the surfaces of fungal spores, conidia, and hyphae, injection of molecules into spores, dissolution of fungal cell walls, and production of volatile chemicals (Johansson et al. 2004). Consortiums (fungi and bacteria) have multiple uses in sustainable agriculture, such as improving nutrition and managing biological diseases, depending on the agricultural practices that enable them to thrive (Rillig and Mummey 2006).

It has long been known that AM fungi and phosphate solubilizing bacteria (PSB) may have a symbiotic and beneficial interaction (Mahanta et al. 2018; Taktek et al. 2015; Zhang et al. 2014). AM fungus *Rhizophagus irregularis* and PSB *Pseudomonas alcaligenes* can facilitate P mobilization and turnover (Zhang et al. 2014). In addition, the effects of PSB and/or AMF on the mineralization of phytate and its subsequent migration to the host plant (*Medicago sativa*) were investigated. Soil phytate mineralization has been reported to increase as a result of the interaction between AMF and its hyphosphere PSB (Zhang et al. 2014).

6.3.3 Other Types of Microorganisms

The synergistic effects of AMF, PSB, and phosphorylated compost (PC) formed from phosphate sludge and organic waste were explored for plant growth promotion, phosphorus solubilization, and phosphatase activity (alkaline and acidic). When plants were inoculated with consortium and phosphorylated compost (PC + PSB + AMF), a significant increase in growth was observed, consistent with that observed in chemically fertilized plants. Trilaterally infected tomatoes had significantly higher shoot height, shoot and root dry weight, root colonization, and accessible P content than controls. Co-inoculation with PC and AMF significantly increased the rate of alkaline phosphatase activity and mycorrhization rate. It was concluded that the consortium forming by PC, endophytes AMF, and PSB contributed to the successful tripartite inoculation of tomato seedlings and all are cooperatively involved in plant growth and phosphorus solubilization (El Maaloum et al. 2020).

6.4 The Microbial Consortium's Interactions

Microbial consortiums are utilized to maintain soil fertility and increase soil microbial activity without harming the environment. The pressure to increase environmentally friendly agricultural production through pest and disease control has increased the demand for microbial soil inoculants used in agriculture. Microbial inoculants, primarily based on bacteria and fungi, can replace traditional fertilizers (biofertilizers) or perform specific tasks such as bioremediation, improving soil properties and biocontrol of plant enemies (biopesticides) (O'Callaghan et al.

2022). A microbial consortium has more qualities than a single microbial inoculum due to the synergy of the microbes that live there (Aguilar-Paredes et al. 2020).

Numerous times, the action of consortium improves the structural characteristics and physicochemical properties of agriculture soils, such as pH and aeration (Mengual et al. 2014). Understanding how the different microbial consortia members interact and whether they display a cumulative benefit, synergistic, or competitive relationships is essential to achieve this increased fertility in soils. When inoculated into the soil, microbial consortia can create particular interactions at multiple time and spatial scales (physical contact, chemical signaling, and metabolic exchange), resulting in emergent qualities, or properties that are greater than the sum of their parts. In practice, selecting microbial mixtures with high functional diversity may aid in improving the inoculant's chances of success and limiting its rapid decrease in the soil. But how effective, complementary, and synergistic the potential PGPMs are will determine this (Bargaz et al. 2018).

Therefore, it is essential to consider the microbe-microbe interaction while creating a consortium with a competitive edge (Hassani et al. 2018; Niu et al. 2020). When well-selected microbial consortia exert synergistic and/or additive effects, they can display a better efficacy compared to when each microbe is used alone. However, the combination of strains determines this association, and microbial interaction within these consortia can have beneficial or negative effects on the establishment of root system and the rhizosphere composition as well. Synergistic interactions arise when combining multiple microorganisms or several strains of a single microorganism boost the effects of single-strain inoculation. For example, when wheat was inoculated using a combination of bacteria *A. chroococcum*, *A. brasilense*, *Enterobacter cloacae,* and *Bacillus polymyxa*, the yield of winter wheat was higher than when an single bacterium strain was used (de Freitas 2000). Dual inoculation of N2-fixing *Bacillus* bacteria or a mixture of bacteria that fix nitrogen dioxide and release phosphorus enhanced sugar beet root and barley yield more than in a single inoculation with N2-fixing bacteria (Şahin et al. 2004). In comparison to the non-inoculated control, mixte inoculation with *A. chroococcum*, *Mesorhizobium ciceri*, and *Pseudomonas* sp. or *Bacillus sp*. resulted in higher protein content in chickpeas (Wani et al. 2007). However, the success of a microbial consortium might depend on the nutrient status of the soil, especially in nutrient-poor soils compared to consortium supplied in nutrient-rich soils (Burdman et al. 2000), and how much artificial fertilizer has been added.

Mutualism, collaboration, and commensalism are examples of positive interactions that increase the stability of the microbial consortia, whereas negative interactions weaken the community. The resilience and effectiveness of microbial consortium are thus mostly determined by microbial interactions (Che and Men 2019). Negative interactions such as parasitism, predation, and amensalism destroy community structure and disrupt cumulative performance. Biodegradation performance is more frequent in microbiological consortia made up of different bacteria and fungus than in single microbial strains (Mikesková et al. 2012). Therefore, to optimize the performance of a microbial consortium, it is also necessary to understand in advance the nature of microbial interactions (Ghosh et al. 2016). This could only be done by looking at how different microorganisms interact with one another in a mixed culture (Ghosh et al. 2016). Interspecific interactions between microorganisms are, therefore, critical for effective metabolic cooperation in mixed cultures (Seneviratne et al. 2007).

Until far, investigations on how interactions affect microbial consortia have shown them to be context-dependent and related to the microbial strain, the agroecosystem, and the soil type. Microorganisms isolated from one agricultural system usually do not have sufficient potential and efficiency in other nonnative agricultural systems because different zones have different agricultural climatic conditions (Mukherjee et al. 2021). Furthermore, microbial consortia harbor strains that may require distinct growing conditions such as temperature and pH, as well as different nutrients.

Polycyclic aromatic hydrocarbons (PAHs) are well-known water contaminants causing serious health problems in both humans and the environment. For example, under field conditions, the impact of fungus on the level of PAHs and the current bacterial population was examined (Andersson et al. 2000). Only the fungus *Hypholoma fasciculare*, out of two other studied fungal strains, was capable to compete and negatively affect soil bacteria, according to the phospholipid fatty acid analysis (Andersson et al. 2000), highlighting the necessity to further take into account the interactions between and within microbial consortium when introduced and affected by the native soil microflora.

Little is currently known about the interactions within consortium at the molecular level. The entire metaproteome of the consortium has been mapped using metaproteomic techniques combined with matrix-assisted laser desorption/ionization, tandem time-of-flight mass spectrometry (MS), gel electrophoresis, and other methods to uncover fungal and bacterial factors involved in antagonistic or synergistic interactions between consortium members. These findings confirmed the microbial consortium's involvement of *Serratia sp.* and *S. maltophilia*, as well as the presence of other bacteria such as *Yersinia, Pseudomonas*, and *Erwinia*, as determined by 16s rRNA and fluorescent in situ hybridization sequencing. These findings also indicated that the consortium partners shared a lot of nutrients, and pollutants, and generated volatile organic compounds (VOCs) to safeguard them from invaders (Moretti et al. 2010). A potential consortium should, therefore, harbor a variety of microbes from distinct agroecological areas. However, the short-term stability within the artificial microbial consortium makes its long-term effectiveness difficult.

6.5 Microbial Consortia: Mechanisms and Benefits to Enhance the Effectiveness of Soil Microbes

Various reviews discuss the mechanisms associated with the benefits of using microorganisms as biocontrol agents (BCAs) (Elbanhawy et al. 2019; Guetsky et al. 2002; Junaid et al. 2013; Köhl et al. 2019). However, when it comes to the use of microbial consortium as a group of microorganisms, the benefits depend largely on the synergistic interaction within them.

Microbial consortium use both direct and indirect processes to improve soil fertility. Indirect mechanisms include processes happening outside the plant, whereas direct mechanisms include processes happening inside the plant parts and completely altering plants' metabolisms. In direct mechanism, microbial consortia collectively make nutrients available to the plant to grow and develop while regulating growth hormones such as auxin and cytokinin. In an indirect mechanism, microbial consortia shield indirectly the plant from other devastating pathogens by induction of resistance or immunity in plants via systemic acquired resistance (SAR) or induced systemic resistance (ISR). Several mechanisms were investigated on how a microbial consortium stimulates the defense of host plants and accelerates plant growth during pathogen inoculation (Saeed et al. 2021).

6.6 Direct Methods

6.6.1 Hormone Production in Plants

One of the mechanisms of microbial consortia is the production of bioactive compounds in the rhizosphere such as plant hormones, vitamins, enzymes, and chelators (Kumar et al. 2022; Saeed et al. 2021). Roots produce a series of substances known as root exudates that increase water and mineral uptake, control the microbial population, and modify the structural characteristics of agriculture soils. For example, PGPR and AM fungi can modify the pH and the balance of various biological and chemical interactions of the soil. Precipitation, adsorption, and oxidation of metal cations are among the interactions that regulate plant uptake (Abdul Rahman et al. 2021).

Few microbial consortia produce phytohormones or plant hormones involved in plant growth. These phytohormones are diverse and depend on the species that produce them. Among them are ethylene, cytokinin, gibberellins, auxins, and cytokinins (Abdul Rahman et al. 2021). Understanding the relationship between microbes and phytohormones is crucial when devising microbial consortia as plant growth promotors. Plants react to any type of plant hormone, whether it is injected or produced by microbes. Plant growth is facilitated by phytohormones, which promote cell division, cell elongation, and root hair extension, among other functions (Glick 2014). The most important and thoroughly researched phytohormone is auxin (indole-3-acetic acid, IAA), which promotes the elongation growth of plant root cells (Verma et al. 2013). The synergistic impacts of phytohormone-producing microorganisms on plant growth have not yet been fully understood. The phytohormone-producing by endophytic microoganrisms *Paecilomyces formosus* LHL10 and *Sphingomonas* sp. LK11 showed robust growth and resilience in one in vitro experiment under Al and Zn stresses (Bilal et al. 2018). The in vivo experiments also evidenced considerably higher plant growth attributes than plants solely treated with LHL10 or LK11, as well as control plants that weren't treated, indicating that the synergistic affect assisted plants in surviving metal stress (Bilal et al. 2018). Soybean treated with the microbial consortia showed higher gibberellin levels, but abscisic acid and jasmonic acid levels were much lower compared with single inoculation (Bilal et al. 2018).

The effect of hormones-producing consortia also helps in other abiotic stress. For instance, the development of tropical crops in temperate areas is severely hampered by unfavorable soil temperatures in the spring (Moradtalab et al. 2020). Surprisingly, microbial consortium having *Trichoderma harzianum* OMG16, *Bacillus licheniformis, Bacillus megaterium, Bacillus polymyxa, Bacillus pumilis*, and *Bacillus subtilis* all produced more cytokinin, jasmonic (JA), and salicylic acids (SA) than usual (Moradtalab et al. 2020), thus helping maize crop to overcome climatic challenges.

6.6.2 Nitrogen Fixation

Synthetic nitrogen fertilizers are heavily used in intensive agriculture, yet more than half of the applied amounts are lost as a consequence of ammonia volatilization, runoff, leaching, denitrification, and other factors (Mahmud et al. 2021). This situation poses significant environmental problems, such as nitrate contamination of the ozone layer, groundwater, greenhouse impact degradation, as well as tremendous financial losses to farmers.

Some species of soil microorganisms can absorb nitrogen from the atmosphere and convert it into ammonia, a type of nitrogen that plants can assimilate (Moreau et al. 2019). This process, known as biological nitrogen fixation (BNF), can take place in soil, water, sediments, on or inside some plant roots, stems, leaves, and in the digestive tract of some animals. Estimates of the annual terrestrial BNF vary from 100 to 290 million tons, with 40 to 48 million tons of it biologically fixed in agroecosystems (Pashaei et al. 2022). This demonstrates that BNF makes a significant impact on crop productivity in agricultural systems. Microorganisms that coexist with plants (symbiotic) or that are free to roam in soils carry out BNF in agroecosystems (nonsymbiotic) (Jeffery et al. 2010).

BNF is significantly influenced by symbiotic nitrogen-fixing bacteria that are present in the tissues and roots of legumes, grasses, and cereals (Hoorman and Islam 2010; Jeffery et al. 2010). Rhizobium, or rhizobia or nitrogen-fixing bacteria, is the most well-known example, which lives in legumes such as beans, lentils, soybean, clover, and peanut (Hoorman 2011; Jeffery et al. 2010). Where bacteria infect a growing root hair, visible nodules are produced. The bacteria get simple sugars from the plant and transform nitrogen (N_2) from the air into forms of nitrogen (NO_3^- or NH_4^+) that the plant may utilize. The amount of nitrogen in the soil rises when a plant's leaves or roots degrade. Dinitrogen (N_2) fixing bacteria require anaerobic conditions to fix nitrogen in microsites given by legume nodules, which are characterized by pink or red tissue (aerobic conditions) in nodules (Hoorman 2011). Although these bacteria are found in most soils, their populations may be insufficient or ineffective to establish beneficial interactions with cultivated crops. In these cases, microorganisms should be artificially introduced into the system. Usually, bacteria are introduced to seeds before sowing to achieve this. Legumes are also regularly used in crop rotation to raise the nitrogen content through BNF. In this regard, results indicated that symbiotic bacteria may fix 30–300 kg of nitrogen per hectare a year (Jeffery et al. 2010).

Nonsymbiotic soil bacteria that can restore atmospheric N2 are a sustainable alternative supply of nitrogen. Some types of soil bacteria can fix atmospheric N2 and are in charge of providing agricultural plants with biologically fixed nitrogen, commonly known as ammonium. Some nonsymbiotic nitrogen fixing bacteria—or endophytic bacteria from *Bacillus*, *Paenibacillus*, *Burkholderia*, *Herbaspirillum*, *Clostridium*, *Klebsiella*, *Enterobacter*, *Citrobacter*, and *Pseudomonas*—are also included in this group (Altomare and Tringovska 2011).

It is possible to find nonsymbiotic N2-fixing bacteria in the bulk soil, on the surface and inside of plant roots, and even in their aerial tissues. They utilize root exudates as a source of energy close to the plants, which increases the efficiency of N2-fixation in the rhizosphere compared to bulk soils (Modi and Jha 2022). These bacteria utilize root exudates as carbon sources, which are rich of a variety of chemicals including organic acids, carbohydrates, and amino acids (Aasfar et al. 2021; Engels and Marschner 1995). It is possible for the nitrogen absorbed in dead microbial biomass to be released through mineralization right away or to first become immobilized in the biomass before that. Most of the time, immobilization and mineralization occur simultaneously (Powlson et al. 2001).

Crop plants and diazotrophic bacteria have a successful interaction that has been explored. The combined inoculation of particular microorganisms has demonstrated a more dramatic impact on plant nutrition and growth than the mixture's components. Approximately 29% of the nitrogen in sorghum plants was fixed as ammonium by a consortium of *A. lipoferum*, *A. brasilense*, *Azoarcus* sp., *Pseudomonas* sp., and *Zoogloea* sp., whereas *Azoarcus* sp. alone fixed only 10.7% to shoots and 2.0% to roots (Stein et al. 1997). Nearly 30% of fixed N was produced in the sugarcane plants after being injected with a mixture of *Gluconacetobacter diazotrophicus*, *Herbaspirillum seropedicae*, *Herbaspirillum rubrisubalbicans*, *Azospirillum amazonense*, and *Burkholderia* sp. (Oliveira et al. 2002). To greatly increase ammonium and make it available to plants, mycorrhizal fungi are also crucial. Mycorrhizal fungi's hyphae are smaller than plant roots, allowing them to easily absorb newly mineralized nitrogen and penetrate decaying organic debris (Miransari 2011). These fungi can also indirectly break down soil organic matter by stimulating decomposing bacteria (Miransari 2011).

6.7 Solubilization of Minerals and Nitrogen Mineralization

Many microorganisms inhabiting the rhizosphere exhibit biological activities that mediate the solubility of micronutrients, making them available at the root surface. Because of the rapid depletion caused by intensive farming, solubilization in most soils is generally inadequate (Liu et al. 2022; Mendes et al. 2013). Through acidification and the formation of organic acids, numerous microorganisms of soil and rhizosphere have the ability to solubilize insoluble mineral phosphates. Thus, the majority of soil phosphorus (P) reserves become accessible to plants (Alori et al. 2017; Mendes et al. 2013). More P-solubilizing activity is displayed by fungi than by bacteria, especially fungi belonging to genus *Aspergillus*, *Penicillium*, and *Trichoderma*. Additionally, P-solubilizing fungi, AMF, and bacteria from the *Bacillus* spp. and *Pseudomonas* spp. families produced the best outcomes. When used with mineral fertilizers like rock phosphate, P-solubilizing microorganisms have typically generated positive outcomes (Altomare and Tringovska 2011).

The applications to the rhizosphere with fungal and bacteria consortium enhanced the amount of fresh basil overall and significantly modified the plant metabolome including phenolic acids such as rosmarinic acid content. The use of microbes consortium thus improved both yield and quality (Comite et al. 2021). Nitrogen is a key component of organic matter and is found in a variety of organic forms, including proteins and amino acids (Moreau et al. 2019). Soil microorganisms helped in mineralization process and converted organic compounds to inorganic compounds like ammonium, nitrite, and nitrate. Ammonification is the process of converting proteins and amino acids to ammonium. Most microorganisms engaged in the decomposition of organic matter are capable of performing it. Microorganisms get their energy by the transformation of organic nitrogen into ammonium, which they also utilize as a nutrition. Ammonium is frequently produced by microorganisms over what is needed, and this surplus is then released into the soil where it can be used as a plant nutrient or as a substrate for other microbial processes (Jeffery et al. 2010; Lahlali et al. 2021).

Many plant species, especially those that grow in acidic soils and water, can utilize ammonium as a nutrient. However, most plants in nonacidic soils are unable to use ammonium effectively and must rely on nitrate as a nitrogen source. Nitrification is a process in which nitrogen is converted from one form ammonium to other forms such as nitrate. Bacteria that convert ammonia to nitrite are *Nitrosomonas*, *Nitrosospira*, *Nitrosococcus*, *Nitrosolobus*, *Streptomyces*, and *Nocardia* while those convert nitrite to nitrate are *Nitrobacter*, *Nitrospina*, and *Nitrococcus* (Hoorman and Islam 2010). Ammonium (NH_4^+) is converted by nitrifying bacteria into nitrite (NO_2^-) and eventually nitrate (NO_3^-), which is the preferred form of nitrogen for grasses and the majority of row crops. Well-aerated soils are necessary for nitrifying bacteria. Nitrification inhibitors are sometimes used by farmers to lessen the activity of nitrifying bacteria because nitrate is more readily leached from the soil (Hoorman 2011).

Nitrate is transformed into nitrogen (N_2) or nitrous oxide (N_2O) gas by denitrifying microorganisms. As anaerobic organisms, denitrifiers function in environments without oxygen, such as saturated soils, compacted soils, or inside soil microaggregates. Denitrification may cause 40–60% of the nitrogen in thick clay soils to be lost (Hoorman 2011). Archea may have a substantial effect on the soil nitrogen cycle, although the particular aspects of their involvement with the nitrogen cycle have yet to be completely studied (Gribaldo and Brochier-Armanet 2006; Jeffery et al. 2010; Odelade and Babalola 2019). Exogenous application of consortiums is, therefore, an efficient strategy to improve soil nutrients and lower chemical inputs which in turn help maintain soil structure. Microbial consortiums collectively acting with different mechanisms of action can have a broader effect and benefit on soil health.

6.8 Microbial Mobilization of Phosphorus in Soil and Other Macro and Micronutrients

Because of its special and essential function in plant structure and metabolism, phosphorus (P) is a key nutritional element for plant growth and development (Malhotra et al. 2018). In fact, adenosine triphosphate (ATP) and its derivatives, adenosine diphosphate (ADP) and adenosine monophosphate (AMP), are all engaged in energy transfer activities. Phosphorus is a component of these molecules (Walsh 2021). Phosphorus intermediates in pathways of energy metabolism including the glycolysis, the photorespiratory route, and the Calvin cycle. P also intermediates in amino acid and nucleotide metabolism. Cell membranes, phosphoproteins, and enzymes like phospholipids all include phosphorus. A typical source of P in seeds is phytic acid. Cell vacuoles contain P in inorganic forms. Both the inorganic and organic forms of P act as buffers to maintain the cellular pH (White and Hammond 2008). Importantly, this element is needed in higher amounts at very early stage of a plant's development, root formation, water and nutrients uptake, flower blooming, and grain filling stage (White and Hammond 2008).

Phosphorus exists in soil in two forms, organic and inorganic (Frontela et al. 2008). The most important organic forms of P are inositol phosphate (soil phytate), phosphotriesters, phosphomonoesters, and phosphodiesters (phospholipids and nucleic acids) (Frontela et al. 2008). Because P is immobile in soil, it frequently serves as the nutrient that restricts growth. To utilize poorly accessible soil P, such as P precipitated or immobilized in organic forms, it is crucial to understand root P mobilization processes and associated rhizosphere dynamics.

By secreting organic acids, creating enzymes, and excreting siderophores that can chelate metal ions and form complexes, Phosphate solubilizing microbes (PSMs) can solubilize insoluble phosphates in the soil and make P available for plant absorption. To improve P availability in the rhizosphere, some rhizosphere microorganisms have been examined for their capacity to convert glucose to gluconic acid with the aid of membrane-bound enzymes and turned it into 2-keto-gluconic acid (Verma et al. 2017).

P-mobilization is a process including acidification, chelation, and exchange to solubilize inorganic P (Bilyera et al. 2022). The most common method of mineral phosphate solubilization is soil acidification. P-solubilization, which results in a decrease in pH in the soil matrix, is brought on by the release of organic acids or protons by edaphic biological processes at high pH levels (Barroso and Nahas 2007). P is solubilized by a range of organic acids released by soil microorganisms, including gluconic, lactic, propionic, isovaleric, isobutyric, oxalic, and tartaric acids (Barea and Richardson 2015). In other cases, however, a lack of correlation between the amounts of organic acids produced in the medium and the quantity of mineral P solubilized has been noted (Deubel and Merbach 2005; Rudresh et al. 2005), demonstrating that the acidic dissolution of mineral P may potentially be mediated by mechanisms other than the synthesis of organic acids. In compost, it has been found that P mobilization and microbial biomass are significantly correlated (Marschner 2008; Zhan et al. 2021). For example, in the study of composts, available P reduced the bacterial makeup. When 10% rock phosphate (RP) was injected, the bacterial community increased the P-solubilization process and RP solubilization efficiency was inversely linked with C:P ratio. Soil microorganisms also produce carbonic acid, derived from respiratory CO_2 that solubilizes the P. Phosphates are made more soluble by chelation by organic acids or siderophores, which complexate metal ions (Ca, Fe, and Al) (Hamdali et al. 2008; Prabhu et al. 2019).

Another technique to boost the efficacy of P-solubilizing microorganisms is to mix them as consortia. Numerous studies suggested that soil inoculation with mixed microbe provides certain benefits over single-strain inoculation. For example, one investigation on the P status of maize after the third successive enrichment revealed that plants infected with rhizobacterial suspensions and insoluble P had a P status comparable to plants inoculated with soluble P. Additionally, metabarcoding demonstrated a correlation between the third enrichment's improved P status and a rise in bacteria with properties that promote plant development and dissolve P (De Zutter et al. 2021).

Synergistic effects between fungi and soil bacteria were observed. When combined with AM fungus, P-solubilizing bacteria, also referred to as mycorrhiza-helper bacteria, promote plant growth and P nutrition (Barea et al. 2002). For instance, compared to the AM fungus *Glomus aggregatum*, *Bacillus polymyxa* and *Azospirillum brasilense* solubilize P more readily. Other elements, including soil type, microbial species, and soil environment, have an impact on this synergy. The overall N, P, and potassium (K) uptake did not significantly improve after mixed inoculation on oil palm. It was then discovered that the anticipated synergism between mixed inocula of AM fungus and endophytic bacteria on oil palm development was not present, indicating the possibility of interspecies incompatibility that needs further research (Sundram et al. 2022).

The released P ions are absorbed by mycorrhizal hyphae, which keep the soluble P content low. As a result, bacteria are encouraged to solubilize additional P, causing continued P mobilization (Ratti et al. 2001). After being treated with P-solubilizing bacteria (*Pseudomonas* sp. BR2 and *Aspergillus awamori* Nakazawa C1) and the AM fungus *Glomus intraradices*, wheat seeds produced grain yields that were comparable to wheat that had been inoculated with the pricy fertilizer diammonium P (Babana and Antoun 2006). Soil fungus and bacteria in a consortium have a huge potential to boost the availability of P to plants.

Soil microorganisms have an impact on microbes' capacity to solubilize macronutrients like K, magnesium (Mg), and calcium (Ca), as well as micronutrients like zinc (Zn), iron (Fe), copper (Cu), and sulfur (S) (S). There is proof that AMF can affect the host plant's uptake of Zn, Cu, and Fe as well as other macronutrients such as N, P, and Mg. AMF may facilitate plants' uptake of additional nitrogen from organic sources (Jeffery et al. 2010).

Soil minerals contain inorganic nutrients, in particular Mg, K, Ca, Fe, and P, which are released during weathering. Organisms such as earthworms and fungi both can contribute significantly to the release of nutrients from the basic minerals in the soil. Bacteria and fungus excrete organic acids that serve as weathering factors. By breaking mineral particles with their hyphae (a thread-like structure), fungi can contribute to their physical degradation (Hoorman and Islam 2010).

6.9 Indirect Mechanisms

6.9.1 Mechanism of Abiotic Stress Reduction and Phytopathogen Suppression

Microbial consortia safeguard plants against various stresses, including invading pathogens or abiotic stress (Tiwari et al. 2022). Because of their improved nutritional status, notably in terms of P and N, mycorrhizal tomato plants cultivated in normal and drought-pressured circumstances displayed enhanced drought resistance and fruit quality under stress. Similar findings were found from drought-stressed maize, with mycorrhizal plants displaying noticeably higher shoot biomass and higher N, P, K, Mg, Mn, and Zn content in grains compared to non-mycorrhizal plants (Subramanian et al. 2006).

Likewise, consortia were shown to induce host plant growth and defense responses in a variety of ways. One of these involved boosting defense signaling to increase the transcriptional activity of various metabolic pathways (Sarma et al. 2015). Through modification of the auxin/cytokinin ratios, PGPR has a cumulative influence on the entire plant (Walker et al. 2012).

An example of overcoming abiotic stresses showed that some endophytic and rhizospheric microorganisms prevent the synthesis of ethylene, which is a plant hormone, causing the abscission of leaves, and flowers wilting. The prevention of ethylene synthesis is mediated by the degradation of one substance, ACC. Also known as 1-Aminocyclopropane-1-carboxylic acid, it is an ethylene precursor and blocks the plant's ability to produce ethylene (Glick 2014).

In high-salt soil, some ACC deaminase-producing bacteria promote tomato plant development (Ali et al. 2014) and minimize mini-carnation flower senescence (Ali et al. 2012). Other crops, such *Catharanthus roseus* (Karthikeyan et al. 2012) and French Bean (*Phaseolus vulgaris*), have also shown that ACC deaminase-producing bacteria can reduce the effects of heat stress (Gupta and Pandey 2019, 2020). Microorganisms that produce ACC deaminase are also effective at reducing other abiotic stresses, including osmotic stress in wheat and copper stress in canola (Zhang et al. 2011; Danish et al. 2019).

Low plant diversity (caused by monoculture) and poor agricultural practices in agricultural systems can foster the growth of pests and diseases, potentially resulting in substantial quantitative and qualitative losses to crops (Reddy and Ramu 2013).

Pest populations can be controlled and increased in healthy soil with a diversified soil community. Beneficial soil microorganisms can help reduce soil-borne infections through competition, antibiosis, parasitism, and the induction of plant disease resistance (Lahlali et al. 2022). Under natural, untouched conditions, a vast variety of soil microorganisms can exist in a state of dynamic balance. Infections in plants are an exception. The majority of soil-dwelling fungi and bacteria are thought to be advantageous to higher plants due to their (1) direct association with roots (e.g., mycorrhizae, nodule-forming bacteria); (2) mineral breakdown and release from soil organic matter, increasing the availability of essential elements to plants; and (3) parasitization of disease-causing microorganisms or suppression of their growth through other types of interactions, such as competition for nutrients and production of compounds like ethylene. The following are some examples of organisms that aid in biological pest and disease control: A typical soil fungus known for its antifungal qualities is *Trichoderma harzianum*. Other (pathogenic) fungal hyphae are encircled by their hyphae, which then exude enzymes that weaken the host's cell walls and prevent the host from growing. (1) *T. harzianum* is an active ingredient in some commercial biofungicides; (2) *Drechslerra anchonia* and other nematode-trapping fungi build particular structures on their hyphae with which to capture worms. (3) Bacteria like *Pseudomonas* sp. have been shown to infiltrate the rhizosphere of plants and guard them against a range of diseases by digesting the host from the inside out and taking the nutrients for their development and reproduction.. This is most likely accomplished through competition for resources (especially iron), antibiotic synthesis, and improved plant health, which increases pathogen resistance; and (4) entomopathogenic nematodes are pathogenic nematodes for insects. Because of their pathogenic properties to insect pests, a few genera (such as *Steinernema* sp.) have attracted a lot of interest (Stockwell et al. 2010). It is known that PGPR can both promote plant development and manage fungi-related diseases (Riaz et al. 2022).

Plants' resistance increases when they are challenged by pathogens. SAR is mostly responsible for this growth. SAR is a defense mechanism that first develops locally at the site of infection before spreading throughout the entire plant to protect the healthy tissues and prevent the spread of the infection (Pieterse et al. 2009). The complete mechanism is previously reviewed (Sarma et al. 2015). For instance, when challenged with *S. sclerotiorum*, the microbial consortium of *T. harzianum*, *B. subtilis*, and *P. aeruginosa* boosted SAR in pea over single inoculation (Jain et al. 2012). Small consortia of microbes improve defense signaling cascades, resulting in increased transcriptional activation of various metabolic pathways (Sarma et al. 2015).

Plant defense responses against invading pathogens are also influenced by phytohormonal signals induced by rhizospheric bacteria. When plants are challenged by diseases, they recruit desired microbes in the rhizosphere in addition to native root colonizers. For instance, when the foliar pathogen *Pseudomonas syringae* pv. tomato was introduced to *Arabidopsis* plants, l-malic acid secretion increased and the plants attracted the advantageous rhizobacterium *Bacillus subtilis* FB17 (Rudrappa et al. 2008). Each microbial component's range of biocontrol methods may potentially help to prevent disease. *Botrytis cinerea*, a pathogen that causes gray mold disease, was controlled by *Trichoderma asperellum* GDFS1009 and *Bacillus amyloliquefaciens* ACCC1111060 more successfully than either strain alone (Wu et al. 2018). A consortium made up of *Trichoderma virens* GI006 and *Bacillus velezensis* Bs006 successfully eradicated cape gooseberry Fusarium wilt (Izquierdo-García et al. 2020). *Rhizoctonia solani*, which causes sugar beet rot, was more effectively controlled by a group of *Chitionophaga* sp. and *Flavobacterium* sp. than by the individuals in the community (Carrión et al. 2019). Another example showed that more successfully than a single non-virulent strain, an indigenous bacterial community made up of five non-virulent *Ralstonia* spp. strains was able to stop the spread of the bacterial wilt of tomatoes (Wei et al. 2015). In earlier studies, many bacteria, including *Bacillus*, *Pseudomonas*, *Rhizobium*, *Glomus*, and others, were used to create microbial consortia (Duffy et al. 1997; Jetiyanon 2007; Kannan and Sureendar 2009; Srivastava et al. 2010). In comparison to a single inoculation of *Glomus*, the in vivo treatment of *Trichoderma* fluorescent together with *Pseudomonas* and *Glomus* reduced the incidence of Fusarium wilt in tomatoes by more than half (Srivastava et al. 2010). Application of a combination of *Rhizobium*, PSB, and *Trichoderma* as a consortium improved biocontrol of diseases that are soil-borne in chickpea (Rudresh et al. 2005). *Trichoderma harzianum*, *B. subtilis*, and *P. aeruginosa* were included in microbial consortia that successfully managed *S. sclerotiorum* in pea plants (Jain et al. 2012).

When exposed to *S. sclerotiorum*, the treated pea plants' defense parameters increased by up to 1.4–2.3 and 1.1–1.7 folds, respectively, in comparison to their untreated counterparts (Jain et al. 2012). Following artificial inoculation, the microbial consortium stimulated the phenylpropanoid pathway and antioxidant enzyme activities, raising levels of total phenolics, proline, and pathogenesis-related (PR) proteins. Compared to plants treated with either the single or dual microbial consortium, plants treated with the triple microbial consortium accumulated up to 1.4–4.6 times more phenol.(Jain et al. 2012).

Lignification is a process that strengthens the plant vascular body, making it an ideal antimicrobial and nondegradable attribute to overcome biotic attacks (Rogers and Campbell 2004). In comparison to individual microbiological treatments, exogenous treatment of a wide range of microorganisms consisting of *P. aeruginosa* PHU094, *T. harzianum* THU0816, and *Mesorhizobium* species RL091 resulted in substantial and consistent lignin deposition in chickpea cambial cells during *S. rolfsii* stress (Singh et al. 2013).

Additionally, the microbial consortia boosted the activity of the first enzyme in the phenylpropanoid pathway, phenylalanine ammonia-lyase (PAL), resulting in a larger buildup of phenolic compounds. For lignifications, this is an essential phase. This was also the first report indicating that the microorganisms had synergistic properties that enable the physical protection of chickpea cell walls to *S. rolfsii* (Singh et al. 2013). Treatment of tomato crops with PGPR and *Bacillus sp*. successfully controlled both tomato *Fusarium wilt* and tomato fruit borer (Prabhukarthikeyan et al. 2014), decreasing the utilization of chemical inputs as a result.

6.9.2 Benefits to Improve the Efficiency of Soil Microbes

Most events observed in the apparent aboveground environment are affected, directly or indirectly, by organisms, interactions, or processes in the subsurface soil (Eisenhauer 2012). They are microbial populations involved in nutrient cycling and organic matter decomposition, and as such, they can influence the biodiversity and productivity of aboveground ecosystems. By releasing metabolites with a range of activities, microbes can either stimulate or inhibit plants. All higher life on our planet depends on the microorganisms in the "living soil" (Vogel et al. 2009). The removal of contaminants and the provision of essential environment moderating and supporting activities, such as soil fertility, resilience, and stress resistance, are all predominantly carried out by microbial populations (Saccá et al. 2017), carbon cycle, and nutrient cycling, all of which influence our quality of life (El Maaloum et al. 2020). Even though microorganisms play critical roles in soil, only a small number (less than 0.5%) have been grown in the laboratory, and genome sequences are only accessible for a few (Hemkemeyer et al. 2021).

Consortia utilization in soils has several direct and indirect advantages for the soil, plants, and entire ecosystem, thus maintaining the sustainability of soils and their resident microbes while protecting the environment. The whole ecosystem including soil, plants, and microorganisms should be treated as complex-dependent organisms rather than independent entities. Direct benefits include increased revenues as a result of higher yields, lower fertilization, and chemical pesticide costs (Lahiri et al. 2022). Improved water-holding capacity, which can prevent or delay drought, increased infiltration capacity, which minimizes waterlogging, and improved soil aeration are all benefits of good soil structure for crops (Shaheb et al. 2021). Furthermore, a healthy soil structure offers resistance and resilience to physical deterioration like erosion and compaction and facilitates the movement of soil organisms. Soil structure is determined by the pore size, content, and spatial distribution of soil aggregates and particles. The development of soil structure is influenced by physiochemical processes as well as the actions of living things including bacteria, fungi, meso- and macrofauna, and plant roots. When breaking down organic materials, soil microorganisms excrete chemicals that can serve as binders between soil particles and aid in aggregate formation (Jeffery et al. 2010). It has also been demonstrated that phosphorus-solubilizing microorganisms enhance soil quality (Panwar et al. 2014).

For instance, *T. harzianum*, *Gluconcetobacter diazotrophicus*, and *Pseudomonas fluorescens* treatment increased soil organic carbon, soil microbial population, microbial biomass carbon, microbial biomass nitrogen, and soil respiration (Shukla et al. 2020). For instance, external mycorrhizal hyphae can access places that are normally unreachable to roots or root hairs because they are much finer than plant roots. By serving as elongation of plant roots, external hyphae enhance the amount of soil that may be used by roots and the size of the nutrient-uptake surface (Ness and Vlek 2000). Also, consortiums can protect the plants and the environment from pollution (Abou-Aly et al. 2021) by lowering carbon gas emissions (Chang et al. 2021) and reducing the leaching of NO_3–N to groundwater (Ge et al. 2022). When chemical fertilizer inputs are prohibited in agricultural systems, microbial consortiums may be extremely helpful. In various experiments, consortia outperform single microorganisms. For example, PGPR specifically changes the gene expression in plants, especially the gene for reactive oxygen species (ROS) detoxification (Chi et al. 2010). Some plants overreact when they are stressed, resulting in the release of toxic ROS. As discussed previously, consortiums help plants to cope with various stresses to avoid being toxic to the environment. Plants emit enzymes like superoxide dismutase (SOD) that link to the toxic radical O_2^-, transforming it into the less toxic H_2O_2 (Mittler 2002). After they have been transformed into oxidized and toxic states, antioxidants are transformed back to their inactive and nontoxic state by these plant enzymes. They are overexpressed in the presence of successful consortiums to recycle toxic elements and render them neutral in the plant environment, especially under stressful conditions (Dungan et al. 2021). Subsequently, the interior environment of plant cells is kept in a state of equilibrium and complementarity between the chemical processes of oxidation and reduction (Dungan et al. 2021). However, forming a consortium is difficult, and successful application examples are scarce. The selection of consortium members to enhance performance is a major challenge.

6.9.3 The Biotechnological Applications of Soil Consortia

An innovative biotechnological method for increasing agricultural yields and resilience involves manipulating soil microbes through microbiome engineering (Ke et al. 2021; Lau et al. 2022). Utilizing soil amendmentsor-specific substrates is an indirect method of microbiome engineering. Direct methods include the use of certain probiotic bacteria, introduction of artificial microbial consortia, and microbiome breeding and transplanting (Arif et al. 2020). Systems biology allows for a full understanding of the various physiological processes that cells go through and how they interact, which in turn sheds light on how to establish artificial microbial consortiums that work best (Fig. 6.1). The most recent use of defined microbial consortia in the fields of soil fertility via decreasing pollution in soils was discussed in more detail. Microbial consortia are categorized according to how they are assembled. They may be artificially gathered or isolated from their natural surroundings (referred to as “natural consortia”). The performance of a community’s members can also be enhanced through engineering (referred to as “synthetic consortia”) (Ibrahim et al. 2021).

Microbial consortia that have evolved for generations to survive in the soil or other ecosystem are represented by natural consortia. They are capable of distributing resources among themselves. These naturally occurring consortiums can be exploited and isolated from the soil to be used as bio-fertilizers or biocontrol agents. These consortiums are referred to as “undefined consortiums” because the species they belong to are typically uncharacterized (Eng and Borenstein 2019). However, species identification within consortiums helps to better understand and regulate the dynamics of the metabolic interactions and the community.

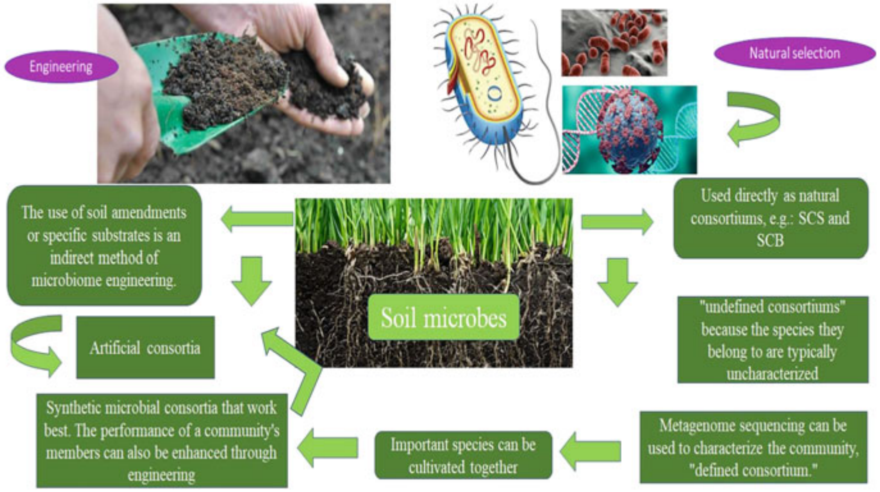

Fig. 6.1 Schematic representation of main types of microbial consortia and how they are exploited

Metagenome sequencing can be used to characterize the community, and the important members can be cultivated together to create a "defined consortium" (Fig. 6.1) (Eng and Borenstein 2019).

The ability of microbes to break down particular substrates or to create particular metabolites can also be used to couple them purely according to our agricultural needs. Although these species cannot coexist in their natural habitat, they are also not metabolically designed. Such groups are referred to as "artificial consortia." Collective performance, under controlled conditions, of multispecies is far better than monocultures. This is through cell signaling and communication pathways that occur between cultures of bacteria strains. The communication is mediated by diffusible signal molecules. Bacteria communicate to monitor their density (quorum sensing (QS)) or their requirement to specific ecological niches. Subsequently, bacteria activate population-wide changes in gene expression and bacterial behavior. For example, in *Escherichia coli*, spatiotemporally controlled cell-cell communication has been successfully engineered using the QS mechanism (Basu et al. 2004).

However, little is currently known about mixed microbial cultures and how they interact with one another (Qian et al. 2020). Microbial consortia may create novel microenvironments for strains that may activate metabolic processes that are inactive under "usual" culture systems, resulting in the finding of new chemicals and giving microbial consortia a promising future, particularly in the development of novel agricultural products (Netzker et al. 2018). An example of natural soil consortia and their role in soil fertility was carried out using two types of consortia, sugarcane bagasse (SCB) consortia and sugarcane soil (SCS) consortia. Both SCB and SCS were naturally occurring in soils of sugarcane (*Saccharum officinarum*) crop fields but with the unknown composition and have good abilities to degrade cellulose. In addition to their ability to thrive on various carbon sources, both consortia were able to release equivalent to 11.90 mol/mL and 15.23 mol/mL of glucose for SCS and SCB, respectively. The latter include various bacteria able to produce amylase and cellulase, among others. Agricultural residues survive in soils and influence the root system and physical properties of soils, making the soil less fertile. These consortiums rapidly decompose bulky residues, making root movement easier (Wongwilaiwalin et al. 2010). Recently, SCS and SCB were characterized and it was found that they contain 80% of Burkholderia as the most prevalent genus. They would offer a fresh alternative for usage in numerous biotechnological applications because of how well they produce enzymes (Constancio et al. 2020). In a consortium, there are several microbial communities, and these are distinguished by various metabolic pathways that can mediate the destruction of biomass in addition to producing biotechnological enzymes (Wetler Tonini et al. 2011). Due to the benefits that microbial enzymes provide in many chemical transformations, as well as their low cost and simple, mass production, the use of microbial enzymes in numerous biotechnological processes has received growing attention. The reactions that microbial enzymes catalyze also enable stability in easily manipulable physical and chemical processes, thus complementing chemical fertilizers and pesticide solutions (Ali et al. 2019). Additionally, these enzymes don't create any harmful by-products or negative effects. These microbial enzymes can be enhanced, modified, and overexpressed in the future to fully use their capabilities (Ali et al. 2019). Also, another way to devise microbial consortial is via the study and characterization of genes responsible for improving soil fertility or other desired trait. For example, in soils with very low nitrogen, the production of hetR protein by heterocysts form of cyanobacteria was higher, suggesting that there is a link between hetR protein and nitrogen deficiency (Zhou et al. 1998). Reduced stress and increased hetR protein expression were seen in Anabaena strains that had copies of the hetR gene beneath the promoter (Chaurasia and Apte 2011). Excellent biotechnological potential for usage as a biofertilizer was shown by the creation of a mutant strain that can manufacture the nitrogen fixation enzyme and release ammonium in the environment (Abinandan et al. 2019; Spiller et al. 1986).

Genetic engineering techniques have been used in the past to produce biomolecules, but they are rarely productive enough for purposes such as commercialization or large-area environmental cleanup (Hoehler and Jørgensen 2013). The metabolic load placed on a single microbial strain serves as a barrier to their properties. Microbial strains don't have enough resources to do multiple jobs at once. Microbes develop in a way that allows them to expand to their maximum potential under particular circumstances. However, certain biotechnological procedures are designed to generate metabolites that are unrelated to proliferation or even those that interfere with it. "Synthesized consortia" are groups of artificial microorganisms (Ibrahim et al. 2021).

The metabolites of cyanobacteria and microalgae have a wide range of biotechnological uses, including proteins, fatty acids, steroids, carotenoids, lectins, amino acids similar to mycosporine, and polyketides (Cardozo et al. 2007). These organisms have been selected, isolated, mutated, and genetically modified to bioremediate organic contaminants efficiently. As a result, the rates of deterioration have increased, and survival and colonization in the contaminated areas have improved (Koksharova and Wolk 2002; Ramakrishnan et al. 2010; Venkateswarlu 1993).

6.10 Opportunities in Engineering and Developing Microbial Consortia

Species diversity often does not guarantee the long-term survival of designed consortia, even though they will function better under shifting environmental conditions. The intercellular signaling in microbial communities is an important mechanism for the stability of consortiums (Venturi et al. 2010). Communication refers to the exchange of metabolites and special molecular signals within each population or between individuals of a consortium (Venturi et al. 2010). The division of labor is the second crucial component that is made possible by this communication (Brenner et al. 2008). Designing microbial consortia should take into account the maintenance of long-term homeostasis, feature of consortia despite genetic exchange, integration of sustainable modifications further into genome sequences of microbial representatives, and fine-tuning of the performance of multiple populations as all these elements determine the success of artificial consortia (Brenner et al. 2008).

Approaches to metabolic engineering provide previously undiscovered mechanisms to comprehend the complex relationships among the microbial components and to determine the most valuable compounds to device new cell factories. Metabolic engineering can be expanded to include many types of microbial consortiums for an effective increase in soil fertility. For a plant to grow and develop sustainably, a microbial consortium must efficiently break down pollutants while simultaneously producing its beneficial metabolites from wastewater (Subashchandrabose et al. 2011). Although it is inhabited by extremophilic bacteria, the desert ecosystem is typically thought of as a dead place with harsh environmental conditions. Cyanobacteria, microalgae, and bacteria could survive in these environments, despite the extreme and rapidly changing climate factors, intense UV exposure, and a scarcity of water that would otherwise result in cell desiccation. They are excellent prospects for industrial uses because they have valuable metabolites that can tolerate harsh environmental conditions. Additionally, the majority of naturally occurring microorganisms in these harsh situations are found in consortia that offer wide metabolic capabilities. Better soil fertility, water-holding capacity, crop production, pollution elimination, and soil durability are some of the capabilities. In dry areas, engineering effective cyanobacterial/microalgal-bacterial consortia would benefit biotechnological applications as well as the development of plants (Perera et al. 2018). In natural habitats, microbes typically coexist with many organisms that carry out sophisticated tasks. Synthetic biologists establish clonal communities to produce complex cellular forms with distinctive characteristics like cell memories, cell differentiation, division, and adaptability. This would be helpful for a variety of biotechnological applications and research into new cellular signaling and communication pathways between various organisms that aid in the construction of consortia for the harsh desert climatic conditions (Brune and Bayer 2012). The key to designing consortia for a certain environment, like deserts, would be a better knowledge of connections like competition and cooperation between bacteria and algae for various types of nutrients present in ecosystems.

6.11 Conclusions and Future Perspectives

Exogenous application of microbial consortia performs better than the treatment with a single microorganism. Due to a variety of methods, such as generated systemic resistance, it can lessen the side impacts of biotic and abiotic diseases and lower the cost of each biological agent when purchased separately. The method is also appropriate for organic farming, which uses the least amount of exogenous biocontrol agents to promote plant development and to control a disease. Indigenous microbial consortium inoculated in the soil is an effective and reliable method that enhances the effectiveness of beneficial soil microbes. To achieve successful outcomes in enhancing the effectiveness of beneficial soil microbes, isolation and commercialization of the biocontrol agent should be region- and topography-specific. Extensive research efforts are needed to identify geographical and topography-specific microbial strains, and to study their compatibility with other biocontrol agents and bio-fertilizers. To improve the efficiency of indigenous soil bacteria, more unique and robust strains must be investigated. Examining microbiological relationships that benefit or hinder the utilization of microbial consortia is crucial to comprehend and predict how well they will operate. Microbial consortia at the biological and genetic levels are needed to be examined. The use of molecular markers would yield a wealth of data that could be used to construct monitoring systems for gathering genetic and environmental data on the secure application of biological control agents in native or foreign soils. Understanding the interplay between foreign applications of microbial consortium and the soil microorganisms already present in the environment will benefit from biological knowledge. How does the introduction of new strains in an ecosystem increase the soil microbiome performance should be very well understood to preserve and enhance the efficiency of the whole ecosystem.

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Novel Biotechnological Interventions in Plant Nematode Management Technologies

7

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Abstract

Plant nematodes are major threat to crop production. They cause significant damage to agricultural crops and suppress their yields. While many methods of control have been proposed for nematode management, only a few have proved effective in the long run. The widespread elimination or restriction on conventional nematicides has compelled the development of new methods of pest and disease control. Biotechnological approaches applied to nematode management show promising and viable options at this juncture. Reduced nematode infection and proliferation in the transgenic host plants have been attributed to the use of nematode resistance genes, protease inhibitors, nematoxic proteins, and chemodisruptive peptides. Furthermore, with the development of RNAi technology, new targets have been discovered that may be exploited for nematode suppression. The present chapter examines the potential of all these biotechnological interventions for their application in commercial nematode management.

Keywords

Heterodera spp. · *Meloidogyne* spp. · Polymerase chain reaction · RNAi technology · Transgenic plants

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7.1 Introduction

Nematodes are pseudocoelomic and the most commonly occurring multicellular animals on the planet, making about 80–90% of all multicellular invertebrates (Khan 2008, 2016). However, only a 5–10% of the world's nematode taxa are known (Haque and Khan 2021). Parasitic and free-living forms of the nematode can be found in any terrestrial or aquatic ecosystem. Free-living species can be classified as bacterivores, mycophagous, algalivores, herbivores, omnivores, or carnivores, and they can be found in both saltwater and freshwater environments, as well as in soil (Mohiddin et al. 2010; Mohiddin and Khan 2013). However, only a small proportion of the known soil nematodes have the ability to parasitize plants, insects, mammals, and humans (Khan 2008, 2023).

Plant-parasitic nematodes have emerged as a serious threat to the world's food supply due to the extensive damage they cause to agricultural and horticultural crops. There are approximately 4100 known species of plant-parasitic nematodes, which inflict crop losses close to US$ 175 billion annually (Haque and Khan 2021). Endoparasitic nematodes (root-knot, root lesion, and cyst-forming nematodes) are major nematode pests of agricultural crops (Mohiddin and Khan 2014; Khan et al. 2022). While many methods of control have been proposed for nematode management, only a few have proved effective in the long run. A concerned grower primarily relies on nematicides to control the disease problem. When infestation level in soil is high, it becomes essential to grow a non-host crop, otherwise soil has to be disinfested with fumigants such as methyl bromide, and metham, etc. Since application of most of the fumigants has been banned or it involves soil covering, etc., granular nematicides such as carbofuran, phorate, thionazin, etc. at a dose of 4–5 kg ai/ha can provide satisfactory decline in the nematode population. These nematicides are relatively safer, hence can also be applied at post-planting stage. In case of transplanting crops, it is always advisable to disinfest the planting materials by root-dip treatment with 100–200 ppm carbofuran solutions. When plants are small and nematode infestation has been detected, a foliar spray with phenamiphos or oxamyl @ 5 l/ha can effectively decrease the level of soil infestation. Khan et al. (2014) reported satisfactory control of root-knot nematode in rice by applying phorate through root dip and soil application. In recent year, some new molecules/chemicals such as fluopyram and fluensulphone have been found effective against soil nematodes (Haque and Khan 2021). The fluensulphone (Nimitz TM) has a novel mode of action by disrupting the nematode feeding and causing paralysis that cumulatively leads to their death. Similarly, Fluopyram selectively blocks cellular energy production in nematodes by inhibiting complex-II system. However, before their wide use, the impact on soil microbial community is needed to be essentially examined. There are other nematicides which also can suppress nematodes. Application of nematicides, however, should be restricted to serious or epidemic situations.

Biotechnological approaches applied to nematode management, however, show promising and viable options. The use of nematode resistance genes, protease inhibitors, nematicidal proteins, chemo-disruptive and elicitor peptides, RNAi technology, and the development of nematode resistance transgenic plants are recent biotechnological approaches which have substantial potential for nematode management. A number of studies and reviews published over the past few decades have attested the success of these methods for nematode control. This chapter provides an overview of the significant breakthroughs on novel biotechnological interventions for managing plant-parasitic nematodes, in agricultural crops.

7.2 Biotechnological Interventions

The advent of biotechnology has opened the door to the exploration of new methods of nematode control. The term "biotechnology" is the result of collaboration between the biological and technological sciences. Technical chemistry and chemical engineering are integral parts of this field, which combines biochemistry and microbiology with an emphasis on practical applications. Managing nematodes is important for food production because nematodes cause substantial losses to agricultural crops. Plant nematodes like other pests and pathogens are controlled with chemicals or natural remedies. Chemicals, besides being costly, create a serious risk of food and environmental contamination. However, the use of non-chemical approaches is a better option in totality. However, traditional farming methods such as cultural practices are slow in action as well as give lower productivity (Rao and Phani 2019). So, to combat the devastating effects of nematodes on agricultural and horticultural crops, the best option is to cultivate resistant varieties. Conventional plant breeding used to create a resistant variety, but this process is much slower. The use of biotechnology presents a viable and efficient option for creating a resistant cultivar. Besides application in host resistance, biotechnology can be applied in various other ways to achieve sustainable nematode management in crops, which are summarized in the following.

7.2.1 Application of Plant Natural Resistance Genes

Host resistance is widely regarded as an eco-friendly and economically viable alternative to chemical treatments. Many R-genes (resistance genes) have been isolated and characterized especially from wild hosts which confer resistance primarily against sedentary endoparasitic nematodes (Table 7.1, Williamson and Kumar 2006; Rao and Phani 2019). Generally, R-genes constitutively occur in plants, albeit at low levels. These genes encode surveillance proteins which detect the effector molecules (pathogen origin) and trigger an efficient defence reaction. The plant R-genes so far identified are part of multigene families with anywhere from a few to over 30 homologs, all of which may contribute to the evolution of resistance specificity (Hulbert et al. 2001).

Natural resistance genes (R-gene) have been isolated from a wide variety of plants and introduced into different economically important crop species, suggesting that resistance or tolerance to nematodes may also be conferred upon other plant

Table 7.1 The list of nematode-resistant genes (Rao and Phani 2019)

Gene	Plant	Nematode
Cre1, Cre3	Wheat	*Heterodera avenae*
Gpa2	Potato	*Globodera pallida* specific populations
Gro1–4	Potato	*Globodera rostochiensis* pathotype Ro1
H1	Potato	*G. Rostochiensis* pathotypes Ro1 and Ro4
Has-1^{Og}	Rice	*Heterodera sacchari*
Hero A	Tomato	*Globodera pallida* pathotypes Pa2 and Pa3 *Globodera rostochiensis* pathotypes Ro1, Ro3, and Ro5;
Hs1^{pro1}	Sugar beet	*Heterodera schachtii*
Ma	Plum	Root-knot nematodes
Me3	Pepper	*Meloidogyne incognita, M. javanica, M. arenaria,* and some *M. hapla* isolates
Mi-1	Tomato	*Meloidogyne incognita, M. javanica, M. arenaria*
Mi-3	Tomato	*Meloidogyne incognita, M. javanica, M. arenaria*
Mi-9	Tomato	*Meloidogyne incognita*
Rhg1, Rhg4	Soybean	*Heterodera glycines* type 0
Rmc1	Potato	*Meloidogyne chitwoodi, M. fallax,* and some *M. hapla* isolates

species through this method. The R-genes occur in monogenic as well as polygenic manner. The nematode single dominant resistance genes encounter with the corresponding avirulence genes (Avr), leading to 'gene-for-gene' interaction. Isolating nematode resistance genes has the practical implication of transferring that resistance to economically significant crop species where it is currently unavailable. Transgenic techniques have proven effective for intraspecific transfer of nematode-resistance genes. However, interspecific transfer has been met with only moderate success. Tomatoes that had been transferred with the *Mi*-1 gene became resistant to the tomato root-knot nematode, but the same gene did not confer resistance to the nematode in tobacco or *Arabidopsis* (Williamson and Kumar 2006). Genotype differences among tomato cultivars were found to impact effectiveness of *Mi*-1 gene even within the cultivated tomato species (Jacquet et al. 2005). It was also determined that map-based cloning and marker-assisted selection methods worked well for nematode resistance breeding. A major quantitative trait locus, *Rhg*4, which imparts resistance against *H. glycines* in soybean was identified and induced through map-based cloning (Liu et al. 2012). The resistant chemical, serine hydroxyl-methyl transferase, was found to be encoded in the *Rhg*4 soybean mutants. For the purpose of marker-assisted selection for root-knot nematode resistance in pepper cultivars, several PCR-based markers closely linked to the *Me*1 gene were developed and demonstrated to be useful (Wang et al. 2018). Moreover, a significant reduction in the adult females population in soybean roots was recorded after over expression of a number of other candidate resistance genes encoding dehydrogenase, ascorbate peroxidase, lipase, ß-1,4-endoglucanase, calmodulin, etc. (Liu et al. 2012). Panella and Lewellen (2007) achieved resistance against *H. schachtii* through transgenic expression of *Hs*1*ro*1, a resistance gene from *Beta* *procumbens*, introduced into sugar beet; however, this was associated with other genes that reduced the yield. Additionally, most R-genes are only effective against a single nematode species or pathotype (Ali et al. 2017). The evolution of novel nematode pathotypes with undetectable effectors (avr genes) due to the R-genes is another major drawback of this approach (Jung et al. 1998). In-depth familiarity with plant and nematode genetics is crucial in this regard. This will pave the way for the creation of new strategies for long-term resistance in crop plants by shedding light on the potential mechanisms by which a resistant phenotype is attained.

7.2.2 Utilizing Genes Encoding Proteinase Inhibitor

Proteinase inhibitors are molecules that are synthesized within plants to counteract the effects of proteases and lysases, two types of enzymes that are commonly secreted by pathogens. Aspartic-, cysteine-, serine-, and metallo-proteinases are the four types of proteinases discovered in nematodes. Hepher and Atkinson (1992) first described the potential of plant-derived proteinase inhibitors to combat nematodes; their study focused on transgenic potatoes expressing a cowpea trypsin inhibitor, which conferred protection against the potato cyst nematode, *G. pallida*. Later, it was discovered that *H. schachtii* and *M. incognita* growth and reproduction could be inhibited by *Arabidopsis* plants that overexpressed cystatin Oc-IΔD86 (Urwin et al. 1997). A transgenic eggplant (*Solanum melongena*) that expresses a modified rice cystatin (Oc-IΔD86) gene under the control of the root-specific promoter, TUB-1, has shown resistance against *M. incognita* and also increased the crop yield (Papolu et al. 2016). Positive results against lesion nematode *Pratylenchus penetrans* infecting lily cv. Nellie White has been observed after treatment with this rice cystatin (Vieira et al. 2015). Bananas that had an overabundance of maize cystatin (CC-II) also showed a significant decrease in *Radopholus similis* and *Helicotylenchus multicinctus* infections (Roderick et al. 2012). *Triticum durum* PDW215, a transgenic wheat line, was able to withstand invasion by the cereal cyst nematode, *Heterodera avenae*, because of the serine proteinase inhibitor (PIN2) gene (Vishnudasan et al. 2005).

Rice, potatoes, tomatoes, alfalfa, bananas, and sweet potatoes are engineered to be resistant to a wide variety of nematodes including *M. incognita*, *M. hapla*, *H. schachtii*, *G. pallida*, *Rotylenchulus reniformis*, *Ditylenchus destructor,* and *Pratylenchus penetrans* and showed significant resistance to multiple species of these pests (Chan et al. 2015; Papolu et al. 2016). Additionally, 60–80% less galling and reproduction of *M. incognita* were observed in tomato transgenic lines expressing the hairpin construct of cathepsin L cysteine proteinase (*Mi*-cpl-1) (Dutta et al. 2015). Tobacco transgenic lines expressing dsRNA for the *Mi*-cpl-1 gene also showed partial resistance to *M. incognita* race 3 (de Souza Júnior et al. 2013). By interfering with the nematode's capacity for sex determination and gall formation, heterologous expression of a taro cystatin conferred significant resistance to tomato against *M. incognita* (Chan et al. 2010). Multiple proteinase inhibitor combinations have been shown to increase resistance to nematodes. Hepher and

Atkinson (1992) and Urwin et al. (1998) reported that the resistance to *G. pallida,* and *H. schachtii* is conferred on transgenic *Arabidopsis* lines expressing a translational fusion protein of CpTI and Oc-IΔD86. Chan et al. (2015) observed that the overexpression of the taro cysteine proteinase inhibitor (CeCPI) and the fungal chitinase (PjCHI-1) regulated by a synthetic promoter, pMSPOA, had a detrimental impact on the egg-laying of *M. incognita* females. Given these results, gene pyramiding becomes a viable strategy for enhancing plant defences against nematodes (Tripathi et al. 2017). Accordingly, proteinase inhibitors are a promising candidate for inducing resistance in crop plants against serious nematode species.

7.2.3 Use of Nematicidal Proteins

The development of nematodes in plants is impeded in part by nematicidal proteins. Some examples of such proteins include lectins, specific antibodies, and Bt Cry proteins and a few reports of their use on a commercial scale are available. Non-immune proteins called lectins have a wide range of biological effects, including anti-inflammatory, antiparasitic, insecticidal, ovicidal, and larvicidal (de Medeiros et al. 2018). The ability of lectin proteins to impede intestinal function in organisms that exhibit or ingest them is a hallmark of their toxicity (Vasconcelos and Oliveira 2004). Concanavalin A, a lectin extracted from the jack bean (*Canavalia ensiformis*), was found to significantly reduce *M. incognita* populations on tomato after being applied (Marban-Mendoza et al. 1987). Soybean agglutinin, wheat germ agglutinin, and Concanavalin A were all used to induce hypersensitivity to *M. incognita* infection in infective juveniles (Davis et al. 1989). The lectin from Snowdrop (*Galanthus nivalis*) expressed in transgenic plants such as potato and rapeseed offered moderate resistance against *G. pallida*, *H. schachtii,* and *P. neglectus* (Burrows et al. 1998; Ripoll et al. 2003). *Moringa oleifera* lectin, recently isolated from *M. oleifera* seeds, has been found to be highly effective against animal nematodes such as gastrointestinal nematodes (de Medeiros et al. 2018). Protein fractionation revealed that lectins were a major determinant of the nematicidal activity of crude protein extracts from *M. oleifera* seeds against *M. incognita* (El-Ansary and Al-Saman 2018).

The Bt toxins from *Bacillus thuringiensis* are suppressive to plant pathogens (Khan and Tarannum 1999; Shahid and Khan, 2019; Khan et al. 2022) and have the potential to impart resistance in plants against nematodes. Marroquin et al. (2000) used Bt toxin as a nematode suppressive protein by exposing *C. elegans* to Cry5B and Cry6A, causing a decrease in nematode reproduction and survival. The reproduction of *M. incognita* was adversely influenced by expressing 54 kDa Cry6A and Cry5B proteins of the hairy roots in tomato (Li et al. 2008). However, the cyst nematode, *H. schachtii,* lacked the digestive capacity to consume this protein due to the limited size of its feeding tube (Urwin et al. 1998). This restriction has prevented the widespread implementation and utilization of this poison. Cheng et al. (2018) reported transformation of the Bt nematicidal cry5Ba3 gene into *Botrytis cinerea* to altered the mycophagous feeding by *Bursaphelenchus xylophilus* and decreased the nematode fitness. Toxin delivery by the fungus to sites where the nematode forages is a promising avenue of research towards the management of pine wood nematodes by using this "sweet poisoning" tactic to interrupt the nematode's life cycle.

7.2.4 Use of Plantibodies

Plantibodies, which are essentially the antibodies expressed in plants, are another candidates for nematode resistance development. The sedentary endoparasites (*Meloidogyne, Heterodera, Globodera*, etc.) use a number of enzymes and effectors secreted from their pharyngeal glands to trick host plants into changing their cells into feeding sites. It may be possible to dampen the nematode's parasitic ability by directing plantibodies in the opposite direction of the active proteins from these secretions (Ali et al. 2017). The movement and invasion of *G. pallida* in potato roots are affected by amphidial and cuticular secretions. Fioretti et al. (2002) reported that this effect can be blocked by using monoclonal antibodies. Polyclonal and monoclonal antibodies that bound to the cuticular surface of *M. javanica* J_2 altered their behaviour and pattern of movements (Sharon et al. 2002). Because of this, it may be useful to characterize surface antigens from various nematodes to aid in the creation of novel nematode control strategies.

7.2.5 Utilization of Peptide Elicitors and Chemodisruptors

Nematodes that parasitize plants use their wide variety of chemoreceptive neurons to detect and enter the host plant. An alternative method to reduce the number of infectious juveniles entering a plant is the application of chemo-disruptive peptides. The chemoreception and locomotion of *H. glycines* and *G. pallida* were found to be disrupted by peptides mimicking the effects of the pesticides aldicarb and levamisole (Winter et al. 2002). Transgenic potato variety was developed in which only a few females of *G. pallida* were able to develop due to the expression of peptide which inhibited the acetylcholinesterase (Liu et al. 2005). Similarly, resistance to *H. schachtii* and *G. pallida* were observed in *Arabidopsis* and potato plants that expressed a chemo-disruptive peptide for acetylcholinesterase controlled by of root tip-specific promoter and CaMV35S (Lilley et al. 2011b). This method was used to create transgenic potatoes resistant to potato cyst nematodes and expressed rice cystatin (Green et al. 2012). Roderick et al. (2012) and Tripathi et al. (2013) developed nematode-resistant transgenic plantain based on protease inhibitor cystatin. Combining cystatins and a chemo-disruptive peptide with a gene pyramiding strategy, transgenic variety of tomato, banana, etc. resistant to *Meloidogyne* spp. has been developed (Chan et al. 2015; Tripathi et al. 2017).

Lee et al. (2018) discovered that treating soybean seeds with exogenous peptides from plant elicitors (specifically GmPep1, GmPep2, and GmPep3) greatly reduced the reproduction of *M. incognita* and *H. glycines*. Additionally, the peptide treatment prevented the root-knot nematodes from damaging the roots and increased the expression of nematode-responsive defence genes. While this method has been shown to be successful in combating insect and fungal pests (Lee et al. 2018), the plant nematodes have received very limited attention.

7.3 Application of RNA Interference

Using biotechnology, scientists have been able to use in vitro silencing of parasitism genes to pinpoint the nematode genes that will be most useful for a host-delivered RNA interference (RNAi) strategy by causing the degradation of messenger RNA (mRNA). The double-stranded RNA (dsRNA) mediates gene silencing in a specific target gene or genes. Resistance to *M. incognita* was first achieved through host-delivered RNAi by Yadav et al. 2006, who used tobacco transgenics to express the dsRNA of integrase and splicing factor genes. Reproduction of *H. glycines* was also significantly reduced in transgenic soybeans expressing PRP17 dsRNA (Li et al. 2010a). The gene Mj-far-1 for fatty acid and retinol-binding protein for *M. javanica* were expressed in tomato hairy roots; it reduced its transcript abundance by about 80% (Iberkleid et al. 2013). Transgenic soybeans expressing dsRNA of the major sperm protein coding gene were shown to reduce *H. glycines* fecundity by roughly 68% (Steeves et al. 2006). The soybean transgenic varieties expressing tyrosine phosphatase gene (RNA hairpin) developed significantly fewer root galls of *M. incognita* (Ibrahim et al. 2011). Similarly, potato cvs. Desiree, Russet, and Burbank for expressing an RNAi construct targeting the effector gene (Mc16D10L) became resistant to *M. chitwoodi* (Dinh et al. 2014).

The expression of cell wall degrading enzyme coding genes was altered after in vitro silencing of five esophageal gland genes expressed either in subventral or dorsal glands of *M. incognita,* resulting in decreased penetration of infective juveniles (Shivakumara et al. 2016). This demonstrates the existence of genetic communication between parasitism-related species. Additionally, transgenic brinjal plants had roughly 70% less *M. incognita* multiplication as a result of host-delivered RNAi silencing of msp-18 and msp-20, the pharyngeal gland-specific genes (Shivakumara et al. 2017). Furthermore, it was found that cell wall modifying enzymes (CWMEs) undergo transcriptional oscillation in both developing and penetrating nematodes, indicating a complex interaction between CWMEs and pioneer genes during parasitism (Shivakumara et al. 2017). *Arabidopsis* has shown significant resistance to a variety of nematodes, prompting the identification and subsequent targeting several candidate genes involved in the resistance (Atkinson et al. 2012; Dutta et al. 2014). For plant-parasitic nematodes, host-delivered RNAi transgenics present a novel and potentially useful management tool; however, RNAi-based management is not without the risk of unintended side effects (Danchin et al. 2013). In addition, the RNAi-engineered plants did not exhibit full resistance against the intended nematodes (Dutta et al. 2014; Rao and Phani 2019). Some important applications of RNAi in nematode management are described below.

7.3.1 Utilization of Neuropeptides as a Therapeutic Target

The neuropeptides responsible for coordinating vital aspects of nematode physiology and behaviour are remarkably conserved across taxonomic groups. The different types of nematode neuropeptides (FLPs, NLPs, ILPs, etc.) have been thoroughly characterized, thanks to the advances in *C. elegans* research (Li and Kim 2008). By interfering with the juvenile stage's host finding ability and invasion into roots, RNAi targeting flp-14 and flp-18 (FMRF amide like peptides) was delivered by the host reduced infection and multiplication of *M. incognita* in tobacco (Papolu et al. 2013). Two FLP genes (flp-14 and flp-18) and a 16D10 (subventral pharyngeal gland-specific gene) were used in a combinatorial in vitro RNAi experiment on *M. incognita*, resulting in a 20–30% reduction in nematode infection and multiplication (Banakar et al. 2015). Silencing neuropeptide genes nlp-3 and nlp-12 in *M. incognita* also resulted in delayed host finding and reduced infection of tomato plants, similar to what was seen with FLPs (Dash et al. 2017). Bioactive neuropeptides from the neuropeptide-like protein (NLP) family have been profiled and targeted in an effort to use them as novel targets for nematode management (Warnock et al. 2017). The feeding activities of *M. incognita* and *G. pallida* (chemosensation, host invasion, stylet thrusting, etc.) were found to be negatively impacted by a large number of separate NLPs. The nematode infection rate in tomatoes was reduced by as much as 90% when transgenic *Chlamydomonas reinhardtii* (terrestrial microalgae) and *Bacillus subtilis* were used to secrete these neuropeptides. This "non-food transgenic delivery" system may be used to deliver neuropeptides, a new type of nematicide that protects plants from pests.

7.3.2 Utilization of Parasitism Genes

The genes responsible for the synthesis and release of certain proteins from the oesophageal glands and introduced into host plants through stylet of the nematode are called parasitism genes. These genes may be crucial for nematodes that invade plants for RNAi. The SKP-1, Ring-H2, ubiquitin-like proteins (proteasome), secreted by some nematodes, control the degradation of protein in host cells. In order to invade and migrate more easily (Sindhu et al. 2009), the β-1-4 endoglucanases secreted by *H. glycines* and *G. rostochiensis* destroy plant tissues (Chen et al. 2005; Bakhetia et al. 2007). In situ hybridization analysis has suggested that the *M. incognita* and *H. glycines* cysteine proteinase genes' products are digestive enzymes, and RNAi of both of them significantly reduced the number of established nematodes on plants.

Four major *Meloidogyne* spp. contain parasitism gene 16D10, which protects secretory peptide of the nematode that promotes root growth (Huang et al. 2006). A macrophage mannose receptor, aggrecan, shares sequence homology with C-type lection (Urwin et al. 2002). The RNAi gene responsible for the synthesis of amphid protein affects the searching and invading ability of *G. rostochiensis* (Chen et al.

2005). It is possible that inhibitors for encoding these genes may be introduced into plants to control the synthesis of the amphid protein.

7.3.3 Utilization of Genes Regulating Development of the Nematode

Certain genes which regulate developmental stages of parasitic nematodes, such as embryogenesis, moulting, reproduction, etc., may be exploited in nematode management. A chitin synthase gene, regulating the production of chitin in the eggshells, was repressed by RNAi, which caused *M. artiellia* egg hatching to be delayed (Fanelli et al. 2005). The gene encoding a key sperm protein that expressed dsRNAs was found responsible for reduced reproductive potential in transgenic soybean plants. The disruption of FLP gene in PCN, *G. pallida,* resulted in motor impairment and exceptional neural sensitivity to RNAi (Kimber et al. 2007). Bioinformatics was used to identify 1508 candidate genes in *H. glycines* (Alkharouf et al. 2007). The contemporary homologous genes in *C. elegans* exhibit lethal phenotypes upon silencing in *C. elegans*. Li et al. (2010a, b) demonstrated using the same method that the RNAi of three genes encoding for a beta subunit of the coatomer (COPI) complex, a pre-mRNA splicing factor, and an unidentified protein resulted in a considerable decrease in the formation of cysts and eggs of *H. glycines*.

7.3.4 Utilizing Genes Regulating the mRNA Metabolism

Inhibiting development or reproduction of nematodes by genes regulating mRNA metabolism may prone to be an effective technique. According to Yadav et al. (2006), tobacco plants were protected from infection by *M. incognita* due to fragments of two dsRNA genes that encoded an integrase and a splicing factor. In another study, *H. glycines* soaked in dsRNA solution of a ribosomal gene Hg-rps-23 exhibited more than 95% mortality to the J_2 population (Alkharouf et al. 2007). Additionally, *H. glycines* cyst counts were reduced by 81 to 88% in soybean roots producing inverted repeat constructions, Hg-rps-3a, Hg-rps-4, and Hgspk-1 genes, which are implicated in the metabolism of mRNA (Klink et al. 2009). Transgenic soybean plants with Prp-17 gene, regulating mRNA splicing, inoculated with *H. glycines* showed 53 and 79% decline in the number of cysts and eggs/g root tissue, respectively. The Prp-17 gene and other similar genes operate the metabolism of mRNA, indicating that RNAi may be sensitive specifically to these genes and that they may be suitable targets for parasitic nematode control.

7.3.5 Genome-Enabled Development of Novel Chemical Nematicides

Using genomic data from *M. incognita*, a bioinformatics pipeline was used to screen candidate gene targets for novel nematicides. With the help of this approach, a shortlist of excellent target genes that might be used as a starting point for the creation of fresh chemical nematicides was produced. Functional studies took the form of in vitro feeding studies where siRNAs targeted at each potential gene were tested for their impact on phenotype or the nematode's capacity to attack and feed on plant roots. Following the identification of the necessary essential nematode target genes, targeted development or chemical testing for compounds that suppress such functions can be carried out to create new pesticides.

7.3.6 Ectopic Delivery of dsRNA: Non-transgenic RNAi

The ectopic application spraying of dsRNA on plants has good potential of introducing genes into a crop for nematode control. The BioDirect Technology, a non-transgenic alternative route of introducing RNAi into a crop for protection against herbicides, insects, and viruses, is quite effective in using this tactic. The challenge in this case is to create stable dsRNA forms and spray delivery methods for foliar part of crop and taken up systemically through the conductive tissue to the roots where they can be ingested by the nematodes. Foliage may also ingest while feeding on the host, and upon ingesting, crucial function and processes of the nematodes are inhibited.

7.4 Nematode Resistance Transgenic Crops

Some of the approaches mentioned above are being applied to cereals, vegetables, and staple crops where nematode control is critically needed. Below is a more detailed discussion of developments of nematode resistance transgenic plants in some most economically important crops.

7.4.1 Banana

Bananas and plantains (*Musa* spp.) suffer considerable production losses due to nematode infestation (Khan and Jairajpuri 2012). *Pratylenchus coffeae, P. goodeyi*, and *Radopholus similis* are commonly encountered in banana plantations, causing 20 to 40% yield losses (Haque and Khan 2021). Similarly, *Meloidogyne incognita* and *M. javanica* are other significant nematodes of banana in areas where *Pratylenchus* and *Radopholus* are less prevalent (De Waele and Davide 1998). Bananas are triploid, which makes them a particularly attractive crop for genetic modification because they of limitation in the cultivar improvement through conventional breeding methods. The plants' sterility is advantageous in this situation because it reduces the possibility of gene flow to related plants. Recent genetic engineering efforts on bananas and other plantains have some success. Various transformation procedures based on particle bombardment, protoplast electroporation (through embryogenic cell suspension), and transformation mediated through *Agrobacterium* are available (Arvanitoyannis et al. 2008), which may prove effective.

Banana and plantain are being used as test crops for the above nematode resistance techniques. The resistance diploid banana hybrid against *R. similis* (Uganda population) is regulated by two dominant genes. Dochez et al. (2009) found that 37 out of 81 hybrids were resistant to the nematode. In a glasshouse test, Cavendish dessert bananas with a 70 ± 10% resistance to *R. similis* expressed the OcIΔD86 transgenic version of rice cystatin (Atkinson et al. 2004). It was found that giant cells in plants which expressed cystatin production exhibited 83.4% resistance to *M. incognita* (Green et al. 2002; Lilley et al. 2004). This technique is being exploited in developing different *Musa* types (Lilley et al. 2011b).

The banana plants in East African Highland expressing the maize cystatin showed suppressed population, while the plantain cv. Gonja has been modified to express cystatin as well as a repellent peptide (Lilley et al. 2011b). Different East African Highland banana types have been introduced with similar additive cystatin plus repellant constructions (NARO, Uganda). Cystatin prevents banana weevils from feeding and growing, it is possible that cystatin-mediated nematode resistance in bananas has additionally benefits in host resistance (Kiggundu et al. 2010). According to Lilley et al. (2011a), *R. similis* quickly absorbs molecules, and uptake of dsRNA results in effective suppression of transcript; however, the degree of silencing can vary depending on the nematode target gene and the environmental conditions (Haegeman et al. 2009). The *R. similis* infestation in *Medicago truncatula* later was reduced by up to 60% when it was soaked in dsRNA identical to xylanase gene (gland cell) (Haegeman et al. 2009).

7.4.2 Potato

Globodera, Meloidigyne, Pratylenchus, and *Ditylenchus* constitute important nematode pests of potato in temperate countries as well as in cooler areas of subtropical and tropical regions (Haque and Khan 2021). The *H*1 resistance gene is found quite effective against the infestation with *G. rostochiensis*, but not effective in preventing reproduction of *G. pallida* on potato. In potato, proteinas inhibitor (PI) based engineered resistance has been thoroughly tested, primarily against *G. pallida*. The serine PI cowpea trypsin inhibitor (CpTI), a plant-based PIs as anti-nematode effectors that has been examined first for effectiveness. Hepler and Atkinson reported that the sexual fate of freshly hatched *G. pallida* was affected by CpTI expressed in transgenic potatoes. This led to development of much greater number of less harmful male individuals in the *G. pallida* population. Successive field tests of transgenic potatoes were conducted for further study on cystatins. Urwin et al.

(2001) reported that best line among healthy susceptible transgenic potato cv Desiree demonstrated 70% field resistance to PCN when it expressed chicken egg white cystatin via the constitutive CaMV35S promoter. Similarly, from potato cv. Sante and cv Maria Huanca, the best lines which exhibited natural partial resistance to PCN were improved to complete resistance when the identical design was applied to them. The field tests later showed that the sunflower cystatin produced in cv. Desiree and modified rice cystatin (OcI-D86) both provided comparable degrees of resistance to chicken egg white cystatin (Urwin et al. 2003). Lilley et al. (2004) observed that potato cultivars with OcI-D86 cystatin expression restricted primarily to the roots, particularly to the syncytia (*G. pallida*), and giant cell (*M. incognita*) exhibiting comparable levels of resistance to both nematodes.

The peptide repellent technique and its potential and application in developing transgenic potato plants have also been evaluated. A containment trial recorded a 52% decrease in the *G. pallida* females in the roots of best line expressing the acetylcholinesterase-inhibiting peptide over control (Liu et al. 2005). Lilley et al. (2011b) further increased the resistance to 95% in best line by employing localized production of the same peptide with a root tip-specific promoter.

7.4.3 Rice

Rice is a major cereal, and is commonly consumed throughout the world, particularly in South-East Asia (Haque and Khan, 2021). About 90% of world paddy is cultivated and consumed in tropical and subtropical regions. Rice is recorded to host around 300 species of nematodes belonging to 35 genera (Khan et al. 2022). About 10 genera are economically significant in rice cultivation, which are accounted for nearly 10% of annual yield decline equivalent to US$ 16 billion in rice world over. Rice is grown in a wide range of ecological climates. Important nematodes infecting rice in irrigated ecosystems include *Meloidogyne graminicola, Aphelenchoides besseyi*, and *Hirschmanniella* spp. (Khan and Ahamad 2020). Deepwater rice is infected with the ufra nematode *Ditylenchus angustus*, while upland rice is attacked by *Pratylenchus* spp. and *M. graminicola* (Khan et al. 2022). Transgenic nematode-resistant varieties offer enormous scope for the production of rice throughout the world. Rice, for having a modest genome size (389 Mb), may serve as a model for monocot. A number of resistance genes against *M. graminicola* have been identified in *O. longistaminata* and *O. glaberrima* which have been introduced into *O. sativa* (Soriano et al. 1999). However, the cystatin-based defence is the only nematode-resistant technology that has yet to be introduced into rice. Vain et al. (1998) observed that modified rice cystatin OcIΔD86 was constitutively expressed in transgenic plants of some important African rice types, and these plants demonstrated 55% resistance to root-knot nematode. Only a minimal amount of cystatin expression was seen, which may be related to a poor CaMV35S promoter in conjunction with the native OcI gene. In order to increase expression levels, a maize ubiquitin gene intron region was additionally incorporated leading 91–97% resistance to *M. incognita* in the best transgenic lines (Green et al. 2002).

7.4.4 Other Crops

Application of biotechnological methods for resistance against nematode has been tried in some other agricultural crops also. To prevent the *Heterodera avenae* invasion in wheat, a potato serine proteinase inhibitor (PIN2) was encoded in transgenic wheat which performed tolerance to the nematode and gave a good yield (Vishnudasan et al. 2005). It has been found that the proteinase inhibitor had a preventive impact against the nematode infection. Chen et al. reported that a tomato cultivar that was sensitive to the root-knot nematode when constitutively expressed a cystatin from the taro root, prevented the nematode attack to a considerable level. Comparing transgenic plants to wild-type plants, *M. incognita* developed 50% fewer galls on the transgenic plants, and these plants also produced lesser egg masses.

7.5 Biosafety Issue of Nematode-Resistant Transgenics

The benefits of transgenic crops for food security can only be realized if every biosecurity concern is scientifically as well as convincingly sorted out, and the crops are grown commercially with access of farmers to seeds at the reasonable cost in third world countries. Numerous individual studies have found that transgenic nematode-resistant crops do not affect non-target organisms (Atkinson et al. 2009). There have also been multiple investigations into whether or not the introduction of transgenic plants modifies the micro-environments of soil, thereby altering the web of life there (Ferris et al. 2001), it has been concluded that transgenic nematodes-resistant crops pose no threat to the natural world. To further alleviate the impact on plants, tissue-specific promoters can be used to lessen the risk to non-target organisms. Depending on the degree of similarity between the target gene sequence of nematode and that of other organisms, it is possible that the host-delivered RNAi technology will have unintended consequences for those organisms. Therefore, in order to reduce the amount of unintended silencing of off-targets, speedy and accurate bioinformatics analysis should be performed to select unique and novel targets (Atkinson et al. 2012). Finally, substantial political support is needed for the widespread adoption of transgenic crops at the field level. In India, Bt cotton has become widely planted as a proof that a transgenic crop can help poor farmers. Similarly, in the U.S.A., over 90% of cultivated maize is transgenic crop and accounted for the annual 33 Mha area (Pellegrino et al. 2018). Moreover, China has established an autonomous capability in the development of transgenics, serving as a model for developing countries (Atkinson et al. 2012).

7.6 Conclusion and Future Perspectives

Plant nematodes are one of the major pests of crops in today's highly mechanized agricultural system. It is not uncommon for nematode populations in the soil to balloon over time due to the pest's microscopic appearance and the farmer's failure to recognize it as a threat, which leads to serious quantitative and qualitative losses to their valuable crops. Research on nematode management has shown that; no single tactic has proven to be effective enough to eradicate the entire problem. Nematode population density and damage decrease considerably with the implementation of different management practices, but to a variable extent. Several new targets and novel technological strategies for nematode control have emerged especially due to advances in biotechnology. All of these measures are aimed to suppress nematode parasitism and to enhance crop yields. However, most targets have been evaluated in the laboratory or a greenhouse on selected model plant species, e.g., *Arabidopsis*. Therefore, in order to use these findings singly or in an integrative manner to achieve maximum nematode management, extensive field-level research is of utmost need to validate technology for commercial application.

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Applications of Omics in the Management of Plant-parasitic Nematodes

8

Leonardo F. Rocha and Vitor V. Schwan

Abstract

Diverse approaches and techniques in omics sciences can serve as tools to address the role of DNA, RNA, proteins, metabolites, and microbial communities on plant–nematode interactions. This assumes that complex systems can be better comprehended when entirely considered. As omics sciences become more popular and datasets are made available for researchers worldwide, these methods can be integrated to study complex plant–nematode interactions. This approach, designated holo-omics, incorporates multi-omic datasets from the plant host and nematode domains to unravel these multifaceted interactions. A multi-omics system can potentially provide a detailed representation of plant–nematode interactions, allowing researchers to forecast responses to these interactions under stress and environmental changes. This chapter discusses novel omics technology applications in the management of plant parasitic nematodes, focusing on the soybean cyst nematode (*Heterodera glycines*) and root–knot nematodes (*Meloidogyne* spp.).

Keywords

Genomics · Metabolomics · Metagenomics · Proteomics · Transcriptomics · Ionomics · Phenomics

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8.1 Introduction to Omics

Omics sciences include genomics (genome), metagenomics (metagenome), transcriptomics (transcriptome), proteomics (proteome), and metabolomics (metabolome). The main objectives are to detect, describe, and measure biological molecules and microbial communities in a cell, tissue, organism, or an environment (meta-omics) in response to time, stimuli or other environmental conditions. The role of DNA, RNA, proteins, metabolites, and microbial communities on plant–nematode interactions can be addressed by different approaches and techniques included in omics sciences, which assumes that complex systems can be better understood when considered as a whole (Horgan and Kenny 2011). Omics sciences include genomics (genome), metagenomics (metagenome), transcriptomics (transcriptome), proteomics (proteome), and metabolomics (metabolome), with overall objectives being to detect, describe, and measure biological molecules (DNA, RNA, proteins, metabolites, etc.) and microbial communities in a cell, tissue, organism, or an environment (meta-omics) in response to stimuli or other environmental conditions (Horgan and Kenny 2011; Omenn et al. 2012; Vailati-Riboni et al. 2017) (Fig. 8.1).

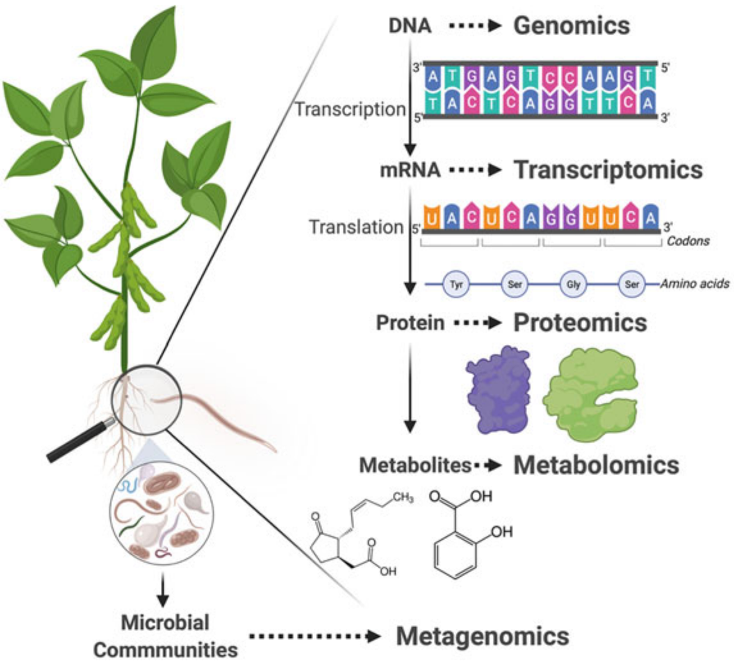

Fig. 8.1 Application of omics sciences in plant nematode management (Created by L. F. Rocha with BioRender® (BioRender.com))

Next-generation sequencing technologies, employed in genomics, metagenomics, and transcriptomics, emerged around 2005 and have been applied to unravel DNA and RNA-based research in plants, microbes, and plant–microbe interactions (Bell et al. 2014). The decreasing cost of next-generation sequencing, the availability of plant and nematode reference genomes, the increase in computational capacities, and curated analysis pipelines made these technologies more accessible to the research community (Montarry et al. 2021). Therefore, omics sciences are implemented to elucidate interactions among nematodes and plants, clearing ways to improve management practices. In the past decades, for example, the introduction and advance of molecular techniques to study microbial ecology brought a new perception of the richness of microbial diversity within plant–microbe interactions, since a soil sample may have one million distinct genomes per gram, and only about 1% of these organisms are culturable (Dubey et al. 2020; Kielak et al. 2016). Omics techniques allow researchers to correlate gene expression and microbial diversity patterns through spatial or temporal grids or by linking them with specific treatments (Brown and Tiedje 2011). By unraveling soil microbial communities, for example, novel structures and enzymes with essential applications for science can be identified and produced (Brown and Tiedje 2011).

8.2 Genomics in Plant Nematode Management

Plant–parasitic nematode management relying on host–plant resistance is often based on a narrow genetic base, which nematodes can overcome through time (Khan 2023; Li et al. 2011). However, advances in genomics, such as sequencing techniques and data analysis, provide substantial datasets of information concerning plant–nematode interactions. Sequenced genomes are available for the most critical nematode species, including the root–knot nematode (*Meloidogyne incognita*) and soybean cyst nematode (*Heterodera glycines*) (Ali et al. 2015). With genomic information, researchers can further understand nematode behavior, plant recognition processes, the establishment of feeding sites, and changes in plant hormone levels in response to infection (Rosso et al. 2009). Based on sequenced genomes, further work can induce, cross, isolate, characterize, and identify specific genes of importance within the plant–nematode interactome (Sommer and Streit 2011).

Genomic approaches may also explore molecular and evolutionary events underlying plant–nematode interactions to better understand the foundations of nematode pathogenicity and plant resistance (Montarry et al. 2021). As of 2022, 202 genomes and their respective annotations and transcriptomes are available through the WormBase ParaSite database (parasite.wormbase.org) (Howe et al. 2017). Still, this number is expected to increase as similar projects aim to sequence 1000 nematode genomes (Kumar et al. 2012). The WormBase ParaSite includes animal parasites and model organisms, such as *Caenorhabditis elegans*, and important plant–parasitic species, including *Heterodera glycines*, *Globodera pallida, Globodera rostochiensis, Meloidogyne arenaria, Meloidogyne enterolobii, Meloidogyne floridensis, Meloidogyne graminicola, Meloidogyne hapla,* *Meloidogyne incognita, and Meloidogyne javanica*. Specific genomic databases are developed to advance genomic resources for a particular nematode species, such as the SCNBase (scnbase.org) for *Heterodera glycines* (Masonbrink et al. 2019b).

The soybean cyst nematode (*H. glycines*) is a significant soybean pathogen in the Midwestern United States (Rocha et al. 2021a). The first build of the *H. glycines* genome (Masonbrink et al. 2019a) comprehends 123 Mb and annotations for 29,769 genes. Lian et al. (2019) later released the annotation of *H. glycines* race X12, with 141.01 Mb and genome assembly at the chromosome level. The availability of both an *H. glycines* and soybean genome has provided tools for identifying novel insights into the mechanisms of resistance and parasitism within this pathosystem, which has the potential to improve management practices. For illustration, Sharma et al. (2020) unraveled the role of exocyst proteins in the defense process of soybean against *H. glycines* infection. Hu et al. (2019b) reported that *Hg16B09,* part of a family of 10 *H. glycines* effectors, has increased activity in the initial stages of parasitism, leading to the suppression of soybean basal defenses.

The root–knot nematode (*Meloidogyne incognita*) is also a destructive obligate parasite in soybean, mainly in warm climate regions, where species such as *M. incognita*, *M. javanica*, *M. arenaria,* and *M. hapla* infect around 95% of the world critical crop production systems (Khan 2008, 2016; Kim et al. 2016). *M. incognita* and *M. hapla* genomes were fully sequenced in 2008, providing critical information regarding the interaction between the nematode and the plant (Sommer and Streit 2011). For example, 486 proteins secreted from *M. incognita* were identified. The hypothesis is that these proteins might regulate the plant cell cycle and growth and reprogram genes in the host plant cells to mediate nematode infection (Ali et al. 2015). Genomics may serve as well to identify sources of host resistance. Li et al. (2011) confirmed the effectiveness of a host-delivered RNAi induced by dsRNAs against *M. incognita*, resulting in resistance in tobacco plants. Another study identified a specific gene, *Mi-1*, conferring resistance to *M. incognita* and other insects in tomatoes (Rosso et al. 2009).

8.3 Metabolomics in Plant Nematode Management

Metabolomics is the systematic documentation and quantitation of metabolic compounds in a cell, tissue, organism, or biological sample (Idle and Gonzalez 2007), supporting advances in health, environmental, and agricultural sciences. These low-molecular-weight metabolites include amino acids, carbohydrates, lipids, nucleic acids, organic acids, peptides, thiols, and vitamins, which play a significant role in biological systems (Zhang et al. 2012). Such compounds are products of the interaction of genome and environment, have remarkably diverse physicochemical properties, and occur as part of the regulatory system at different abundance levels in an integrated manner (Rochfort 2005).

Although the metabolome is complementary to the proteome and transcriptome, the metabolome is often considered closer to the phenotype, as metabolomic changes are often amplified compared to transcriptomic and proteomic levels. Furthermore, metabolic fluctuations are not exclusively controlled by gene expression but also by post-transcriptional and post-translational events (Hollywood et al. 2006; Rochfort 2005). While metabolite profiling was first reported in the literature as early as the 1950s, only after the 2000s did these studies start to be widespread (Rochfort 2005), especially with the progress of detection methodologies. Examples of current technologies used to separate, detect, characterize, and quantify such metabolites, include gas-chromatography (GC), high-performance liquid chromatography (HPLC), ultra-performance liquid chromatography (UPLC), capillary electrophoresis coupled to mass spectrometry (MS), and nuclear magnetic resonance (NMR) (Idle and Gonzalez 2007; Zhang et al. 2012).

Metabolomics has wide applications in nematode management, especially in identifying metabolites produced by both host and pathogen during the infection process. Eloh et al. (2016) performed a GCMS untargeted fingerprint analysis and identified dramatic local changes in the metabolome of tomato plants inoculated with *Meloidogyne incognita*. Willett et al. (2020) used global metabolomic profiling to demonstrate that the upregulation of compounds linked to plant defense, such as pipecolic acid, plays a crucial role in protecting tolerant African bermudagrass (*Cynodon transvaalensis*) cultivars from sting nematode (*Belonolaimus longicaudatus*) infection. *Belonolaimus longicaudatus* was also shown to suppress the production of amino acids in susceptible bermudagrass cultivars. Other studies aim to identify specific compounds responsible for nematode suppression by beneficial organisms or cover crops, such as the use of a GCMS approach to identify several chemical compounds negatively correlated with SCN counts, suggesting a role of these compounds on SCN suppression by wheat in double-cropping soybean production (Rocha et al. 2023a). Additional metabolomics studies have focused on plant and root–knot nematode interactions, focusing on the influence of nematode parasitism on plant metabolism (Ali et al. 2015; Baldacci-Cresp et al. 2020; Behr et al. 2020; Eloh et al. 2016; Willett et al. 2020).

Metabolomics also serves as a tool to identify and characterize novel sources of genetic resistance to nematodes. Mittal (2020) implemented untargeted metabolomics and genetics to investigate the molecular mechanisms underlying resistance to *H. glycines* in wild soybean (*Glycine soja*). Shi et al. (2021) compared an incompatible (resistant) soybean variety (PI437654) and three compatible (susceptible) using LC-MS untargeted metabolomics. Their results revealed potential novel metabolic compounds and associated genes linked to the incompatible interaction in the resistant line, supporting an improved understanding of the interactions among *H. glycines* and soybean. Through a high-resolution LC-MS pipeline, Manohar et al. (2020) reported that *C. elegans* pheromones might function as defense mechanisms analogous to conventional pattern-triggered immunity in *A. thaliana*, highlighting how plants might dynamically operate chemical signaling from soil-borne microorganisms. Medeiros et al. (2015) explored the role of peroxidases on tomato plants against root–knot nematodes. These enzymes contribute to tomato resistance by triggering the production of toxins, which inhibits nematode penetration and development in the root system. Other studies focused on metabolites exudated by biocontrol agents such as *Bacillus* spp., unraveling compounds, and enzymes involved in the interaction of *Bacillus* with root–knot nematodes (Horak et al. 2019).

Although these compounds identified using metabolomics could be assessed individually for their nematicidal activity, research suggests that an array of compounds operating synergistically instead of singular compounds are more likely to suppress nematodes in field conditions (Huang et al. 2003). The biological activity of a group of phytochemicals may produce a more substantial effect equivalent to a much higher concentration of the most active compound.

8.4 Metagenomics in Plant Nematode Management

Soil and plant-associated microbial communities are linked to critical ecological and physiological functions, including plant health and nutrition, contributing to organic matter breakdown and turnover, nutrient cycling and absorption, nitrogen fixation, decomposition of compounds, aggregate formation, protecting against disease pressure, solubilizing mineral phosphates, and several other sympathetic interactions (Gattinger et al. 2008; Chaparro et al. 2012; De-la-Pena and Loyola-Vargas 2014; Sofo et al. 2014; Meena et al. 2017; Taiz et al. 2015). Microbial communities result from spatial/temporal gradients of varying resources, producing diverse populations on small scales (Brown and Tiedje 2011). Plants and associated microorganisms have developed biological communication mechanisms, creating a customized surrounding soil microbiome shaped by the emission of plant exudates.

Metagenomics provides data on the composition, evolution, function, and interaction between diverse microbiomes and their hosts. (Cheng et al. 2013). Metagenomics studies aim to detect changes in microbial communities at the community level and how environmental factors change the soil microbial structure, for example (Feng et al. 2018; Hiraoka et al. 2016; Marchesi and Ravel 2015). Even a family- or genus-level identification would address questions in a community-level study, whereas for research focusing on a single or a limited set of organisms, specific and efficient assays would be required to detect minor changes in a microorganism population (Rocha et al. 2023b). It is possible to get advanced speed and resolution of large specie numbers from different complex communities using metagenomics (Peham et al. 2017; Porazinska et al. 2009). These analyses could also exploit the potential of biological control agents in that microbiome.

Extensive work has been done to characterize the microbial communities associated with cysts of *H. glycines* using metagenomics, highlighting the role of bacteria and fungi in the suppression of *H. glycines* and supporting the development of biological management practices (Hu et al. 2017, 2019a). Haarith et al. (2020a) reviewed studies covering *H. glycines*-associated fungal communities and their potential to be employed as nematode biological control agents. Metagenomics studies also allowed the research community to identify shifts in bacterial community composition due to crop rotation and seasonal variation (Hu et al. 2019a). Haarith et al. (2019) described the *H. glycines* cyst culturable mycobiome in a long-term study with soybean–corn rotation. Promising isolates collected from infected cysts in the previous rotation study were later screened in vitro (Haarith et al. 2020b) and in vivo (Haarith et al. 2021), showing high biocontrol efficacy. Rocha et al. (2022a), employing three distinct DNA markers targeting bacterial (16S, V4-V5 region), fungal (ITS2), and *Fusarium* (*tef1*) communities, proposed an influence of soil-borne beneficial microorganisms suppressing *H. glycines* in soybean fields formerly planted with winter wheat (Rocha et al. 2021b).

Advances in metagenomics lead to a better characterization of microbiome populations in the soil and their association with the root–knot nematodes. Cao et al. (2022) explored the mechanisms of selected potential biological control on root–knot nematodes. Other studies found bacterial strains antagonistic to *Meloidogyne* spp. (Ciancio 2021; Liu et al. 2022), as well as associated soil microbiome communities (Colagiero et al. 2020).

8.5 Proteomics in Plant Nematode Management

Proteomics projects have been developed independently or complemening other omics technologies such as genomics, transcriptomics, and metabolomics, aiming to detect and quantify the proteome from a cell, organelle, tissue, or organism (Aslam et al. 2017). Proteins are studied regarding their pathways, expression, function, and structure (Pierce et al. 2007). Studies in the field of proteomics started in the mid 1990s, with the initial efforts to build a protein complement to the genome, utilizing newly developed high-resolution separation techniques such as two-dimensional gel electrophoresis (Lottspeich 2009). The primary methods used in proteomics are based on mass spectrometry and allow researchers to identify protein structures, interactions between proteins, post-transitional alterations, and variations in expression level and structure (Pierce et al. 2007). Additional methods include two-dimensional polyacrylamide gel electrophoresis and protein arrays.

The proteome of important plant–parasitic nematode species, including *H. glycines*, is relatively well characterized. Chen et al. (2011b) compared three diverse protein extraction methods to identify *H. glycines* proteins via two-dimensional gel electrophoresis (2-DE). Later, that same group implementing two-dimensional gel electrophoresis identified 426 proteins, which were later identified using LC-MS/MS and assigned to nearly 700 gene ontology (GO) molecular function terms belonging to almost 200 distinct functions (Chen et al. 2011a). This study also identified 20 proteins secreted by *H. glycines* during infection through the stylet. Li et al. (2020) elucidated the white to brown color change in adult cysts by identifying two proteins linked to melanin production only in brown cysts.

Protein expression assays are being applied to identify plant–parasitic nematode species (Ahmad and Babalola 2014), distinguish races, and study plant–nematode interactions, further understanding pathogenicity and plant resistance. During infection, plant–parasitic nematodes, especially sedentary species, release a series of effector proteins to manipulate molecular and physiological systems within the plant as they form feeding sites (syncytium and giant cells (Escobar et al. 2011)).

Chen et al. (2014) contrasted the proteome of resistant and susceptible soybean roots parasitized by *H. glycines* at diverse time points. The abundance of over 40 proteins differed among the two soybean genotypes, highlighting the potential of using proteomics to understand plant–nematode interactions further. Wang et al. (2015) and Liu et al. (2011) used 2-DE proteomics-based approaches to identify the mechanisms of *H. glycines* resistance in soybean breeding lines.

Additional studies implemented proteomics to unravel interactions among host plants and *Meloidogyne* species. Patel and Pitambara (2018) assessed protein profiles during the infection phase of *M. incognita* on tomato plants to identify proteomics changes and plant responses to nematode infection. Other studies applied proteomics to characterize plant–nematode interactions in many different areas, from the formation of giant cells, feeding sites, and galls to the use of proteins from soybean seed exudates against *M. incognita* (Rocha et al. 2015; Bhadauria 2016; Ha et al. 2017; Xiang et al. 2020).

8.6 Transcriptomics in Plant Nematode Management

Transcriptomes provide highly dynamic spatiotemporal gene expression patterns composed of multiple regulatory events (Sun et al. 2021). The genome sequence combined with transcriptome analyses allows comparing expression patterns of various organisms, including nematodes, to elucidate biochemical and molecular processes involved in parasitism, nematode development, reproduction, and the interaction with their hosts (Lai et al. 2014; Yan et al. 2012).

Studies in transcriptomes became popular with the advances in genomic and bioinformatic technologies (Cantacessi et al. 2012). An extensive list of publications implements next-generation sequencing technologies to identify novel resistance genes against plant–parasitic nematodes, as genomes and transcriptomes are made available in online databases. An *H. glycines* transcriptome is currently available, serving as a tool for annotating expected genome assemblies. These databases include transcriptome assemblies for critical crops and plant–parasitic nematode species, allowing plant breeders to identify novel resistance mechanisms. Sharma et al. (2020) used gene silencing, RNA sequencing (RNA-seq), and other molecular methods to study 61 genes involved in the exocyst encoding in soybean. The overexpression of exocyst genes was demonstrated to dramatically reduce *H. glycines* infection in a susceptible cultivar (Williams82 - PI 51867). The exocyst suppression through RNAi led to a sharp increase in *H. glycines* infection in a normally resistant cultivar (Peking - PI 548402). Gardner et al. (2018) released a novel de novo transcriptome assembly for *H. glycines* in early developmental stages in both a susceptible and resistant interaction with soybean, opening doors to identifying effector proteins involved in the *H. glycines*–soybean pathosystem. On the plant host side, Rocha et al. (2022b) used an RNA-seq approach to reveal that fluopyram activates systemic resistance in soybean, potentially complementing that compound’s known nematicidal activity.

Transcriptomes studies are also being conducted with *Meloidogyne* spp. Sung et al. (2019) accessed peroxidase genes on sweet potato against nematode infection. The gene expression of the peroxidases *swpal*, *swpa4*, *swpa6*, and *swpb3* was shown to serve as molecular resistance markers of *Meloidogyne* spp. It is documented that *Meloidogyne* spp. infection induces tomato transcription factors WRKY; among them, SlWRKY70 is required for Mi-1-mediated resistance to aphids and nematodes in tomato (Atamian et al. 2012). Sato et al. (2021) examined in detail the transcriptional reprogramming of *Solanum torvum* in response to infection with virulent and avirulent populations of *M. arenaria* at early infection stages by comparative transcriptome analyses. That study highlighted significant expression patterns in response to nematode infection, providing a molecular basis for understanding *S. torvum*–*M. arenaria* interactions. Shukla et al. (2018) found multiple *Meloidogyne* spp. genes involved in different life cycle stages and during the infection process on tomato plants. *Meloidogyne* spp. transcriptomes were evaluated on other crops, such as alfalfa and rice. The host response was found to be more significant on susceptible plants on alfalfa than on the resistant cultivar, where the nematode development is aborted (Postnikova et al. 2015). On rice, the authors found that the resistance in African rice involves a series of different mechanisms, where initial juvenile penetration is restricted, and later, the formation of the giant cells is degenerated (Petitot et al. 2017).

8.7 Ionomics and Phenomics in Plant Nematode Management

Additional omics tools are in their initial application stages in nematode management studies. Ionomics, through the implementation of high-throughput elemental studies, aims to quantify and measure the elemental composition (ionome) of a cell, organelle, tissue, or organism in response to genetic, physiological, developmental, and environmental stimuli (Salt et al. 2008). Ionomics has multiple plant-forward and reverses genetics applications, from screening diversity panels to modeling physiological conditions, serving as a tool to identify genes and regulatory pathways related to these conditions (Ali et al. 2021; Baxter 2010). For example, a plant's physiological status can indicate response to environmental stresses, including cold or drought, perturbed cell walls, and nematode parasitism. This provides insights into processes not easily detected by the other omics sciences (Salt et al. 2008). Thus far, most ionomics studies with plants have been limited to studies on nutrient uptake and movement within tissues, some targeting nutrient and drought tolerance. Still, this approach has the potential to elucidate research questions within the plant–nematode interactome (Deshmukh et al. 2014). The main techniques with the potential to be utilized in ionomics studies are X-ray fluorescence (XRF), neutron activation analysis (NAA), inductively coupled plasma-mass spectrometry (ICP-MS), and inductively coupled plasma-atom/optical emission spectrometry (ICP-AES/OES) (Wu et al. 2013).

Phenomics is the acquisition of high-dimensional phenotypic data (systematic descriptions of phenotypic characteristics) on an organism's genome-wide scale

(Houle et al. 2010; Warringer et al. 2003). It corroborates with genomics and provides knowledge that applies to curation, such as correcting assigning accessions to a taxon, quantifying changes during an ex situ conservation or in situ, and ensuring that the data collected are consistent with the data provided in gene banks and other gene communities (Volk et al. 2021). Studies in the field of phenomics are still limited, as producing phenomics dataset can be expensive and time-consuming (Houle et al. 2010). Technical advances in the area may increase phenomics throughput and lower costs, similar to when sequencing costs decrease, boosting research in transcriptomics, genomics, and metagenomics. Nematode studies are currently mostly limited to model and non-plant parasitic nematode species. Phenomics successfully detected and quantified electrophysiological phenotypes in *C. elegans*, *Ascaris ceylanicum,* and *A. suum* (Weeks et al. 2019). More recently, Cai et al. (2021) implemented a multi-omics (metabolome, phenomics, and transcriptome) study to investigate pine wood nematode pathogenicity associated with culturable microbiota through an artificial assembly approach.

8.8 Conclusions and Future Perspectives

Much of the research conducted to understand plant–nematode interactions is based on genomics and transcriptomics. However, other omics branches, including proteomics, metabolomics and metagenomics, are still limited to selected pathosystems. Imminent methods, such as ionomics and phenomics, are still in early use in nematode pathosystems. As omics sciences become more popular and datasets are made available for researchers worldwide, these methods can be integrated to study complex plant–nematode interactions. This approach, designated holo-omics, would incorporate multi-omics data from the plant host and nematode domains to unravel these multifaceted interactions (Montarry et al. 2021). A multi-omics approach can potentially provide a detailed representation of plant–nematode interactions, allowing researchers to forecast responses of these interactions under stress and environmental changes (Crandall et al. 2020; Kang et al. 2018; Rochfort 2005). Current sample preparation and extraction pipelines allow researchers to obtain DNA, RNA, proteins, and metabolites from a single sample derived from a series of human tissues (Shah et al. 2018). Developing similar sample processing and extraction pipelines for soil and plant nematodes will enhance multi-omics projects targeting plant–nematode interactions.

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Transgenics, Application in Plant Nematode Management

9

Tushar K. Dutta and Victor Phani

Abstract

Plant parasitic nematodes are one of the major biotic stressors to present-day global agricultural and horticultural food production system. Limitations of other management strategies such as chemical (environmentally harmful), cultural (not remunerative), and biological (not feasible for all agro-climatic regions) have necessitated the adoption of novel biotechnological tools to manage nematodes. An advancement made in the genetic and molecular intricacies of plant–nematode interrelationships and continued publications on nematode genomes and transcriptomes have also aided in this cause. A number of strategies, including deployment of *R* genes, transgenic expression of protease inhibitors, chemodisruptive peptides, host-induced gene silencing involving RNAi, etc., have been discussed in greater detail in this chapter highlighting the associated advantages and drawbacks. Additionally, the potential of emerging technologies including genome engineering and biosafety concerns of nematode-resistant transgenic crops is elaborated. As a consensus is yet to be reached regarding the government policy in different countries, a number of genetically modified crops are waiting in the greenhouse for impending field trials till the regulatory guidelines are lifted.

Keywords

RNAi · Protease inhibitors · Chemodisruptive peptides · Host-induced gene silencing · *R* gene

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9.1 Introduction

Plant–parasitic nematodes (PPNs) infect more than 3000 genera of crop plants that immensely affect the productivity which is equivalent to an approximate annual loss of 173 billion US Dollar (Elling 2013). The relationships between PPNs and their hosts are diverse. The PPNs, on the basis of mode of parasitism, can be sedentary or browsing as well as endoparasitic or ectoparasitic. For the migratory ectoparasitic nematodes, host plants remain a transient food source and the interactions between nematode and plant is time-limited. By contrast, for the endoparasitic species, the interaction is far more sophisticated and durable for longer time. The most economically destructive PPNs are obligate biotrophs and elicit permanent alterations in the host root in order to generate hypermetabolic nematode feeding sites (NFS) that serve as nutrient sink for the feeding nematodes (Vieira and Gleason 2019).

Nematodes are quite armored for plant parasitism. All PPNs possess a protrusible, needle-like structure which is useful for puncturing the host tissue. This structure is known as stylet/onchiostyle/odontostylet in different taxonomic groups and is used as a mean to deliver esophageal gland secretions into host cell and to ingest plant metabolites while feeding. As per their feeding style, stylets vary considerably in shape and size in different nematodes. For example, *Trichodorus* spp. contain short onchiostylets as they feed on epidermal cells, while *Xiphinema* spp. and *Longidorus* spp. contain longer odontostylets as they feed on deeper host tissues including the cortical cells (Gheysen and Jones 2006). Notably, plant parasitism has independently evolved at least thrice in the Phylum Nematoda; and in spite of the fact that PPNs are not always correlated phylogenetically, few structural features are common amongst the members belonging to similar clades (Blaxter 2011). Subsequent studies found that plant parasitism arose in “clade 2” lineage (includes ectoparasites like *Longidorus* spp., *Xiphinema* spp., *Trichodorus* spp.) which is quite ancient in evolutionary terms compared to the recent lineages in “clade 10” (includes *Bursaphelenchus* spp.) and “clade 12” (includes endoparasitic nematodes including *Heterodera* spp., *Meloidogyne* spp., *Pratylenchus* spp., and *Radopholus* spp.) (Holterman et al. 2017).

9.2 Role of Nematode Esophageal/Pharyngeal Glands

The secretions of the esophageal gland cells play pivotal role in the plant–PPN interactions. PPNs have two sets of esophageal glands, i.e., subventral and dorsal glands. The exact number and location of each cell type differs in different PPN species. In the sedentary endoparasitic Tylenchids, two subventral and one dorsal gland is present. During the initial stage of plant parasitism, subventral gland cells become large due to abundance of secretory granules in the pre- and post-parasitic second-stage juveniles (J2s). Thereafter, the size of subventral gland cells is reduced in the third- (J3s) and fourth-stage (J4s) juveniles, young and adult female stage. On the contrary, dorsal gland cell becomes larger during the later stage of nematode parasitism specifically in the stages inside plant tissue. Further, these gland cell

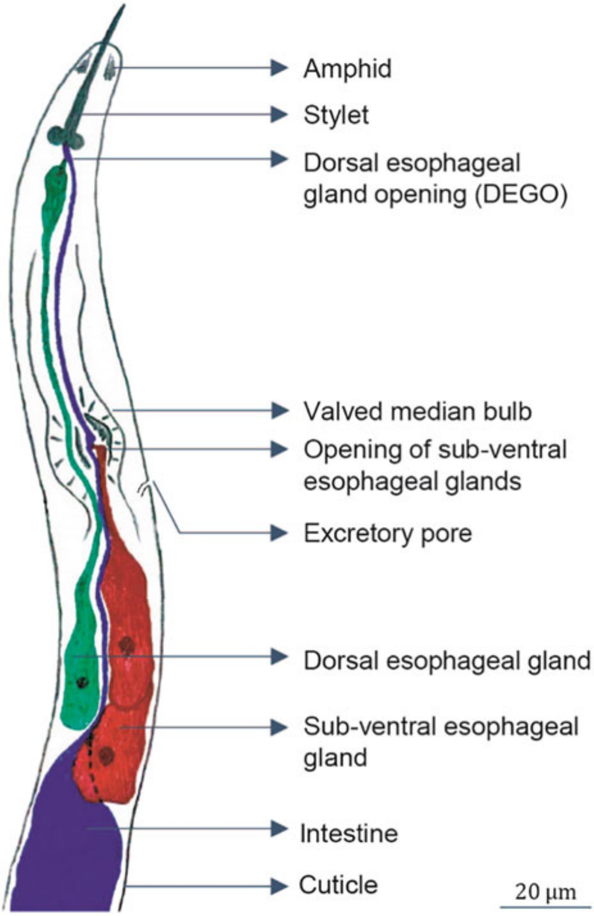

Fig. 9.1 Anterior end of a sedentary endoparasitic PPN at J2 stage is schematically represented. PPN possesses a protractible stylet that introduces effectors and enzymes synthesized in the dorsal and subventral esophageal gland cells into the plant tissue. There are few effectors which are produced via amphidial glands (chemosensory sensillary organs in the cephalic region) and the surface coat or cuticle

products are developmentally regulated; like subventral gland cell products are synthesized during nematode penetration of host tissue and NFS induction, whereas dorsal gland products play important role during NFS development and maintenance (Mejias et al. 2019).

The parasitism genes produced in these gland cells are collectively known as effectors. Although most of the known effectors are synthesized in the pharyngeal glands, a few of them are also synthesized in the nematode olfactory organs including amphidial glands, and nematode body wall or cuticle (Fig. 9.1). Effectors can function as (1) chemotactic factors (e.g., HYP and MAP-1 secreted from amphids) associated in finding the host root and location of potential NFS, (2) cell wall-degrading enzymes (CWDEs), which help in PPN host penetration and browsing in the host tissue, (3) host reprogramming modulators that are involved in the NFS initiation and maintenance, (4) host metabolism regulators that supply nutrients to feeding PPNs, and last but not the least, and (5) immune suppression elements that protect PPNs and feeding sites against plant's innate immune reactions (Shivakumara et al. 2017).

Effector proteins are produced de novo in the nucleus part of esophageal gland cells; N-terminal signal peptides guide them toward secretory pathway to be packaged in spherical, membrane-enclosed secretory granules via mediation of the Golgi bodies. After being delivered into the host cell via stylet orifice, the effectors may have direct interaction with specific cytosolic proteins and alternatively target the host cell nucleus to play its downstream role in compatible plant–PPN interaction. The role of effector molecules in apoplastic region of the plant cell is has also been deciphered (Mitchum et al. 2013). Most recently, the role of effectors as transcription factors has been recognized. The cis-regulatory elements such as "DOG boxes" and "SUG boxes" were identified in the subventral and dorsal gland secretions of cyst (*Globodera rostochiensis*, *Heterodera glycines*) and burrowing (*Radopholus similis*) nematodes, respectively, that may function as master regulators to specifically switch on or off a specific set of effectors (Eves-van den Akker 2021; Vieira and Gleason 2019).

Nematode feeding sites or NFS are astonishingly diverse because these are formed in a variety of root tissues having some dramatically conserved characteristics (Fig. 9.2). NFS have similar structural features of being hypermetabolic tissues with cytoplasm enriched with subcellular organelles. NFS exhibit signs of DNA replication, become enlarged, and possess multiple nuclei. The simplest form of NFS consist of single modified cells; e.g., *Trophotylenchulus* spp. feed on a single cell which becomes hypermetabolic and harbors a single enlarged nucleus. An identical feeding cell is observed with *Cryphodera* spp. infection in cortical cells. PPNs like *Tylenchulus* spp. feed from clusters of uninucleate feeding cells (also known as nurse cells). However, more sophisticated NFS comprises of two categories, i.e., syncytia and giant cells. Syncytia (induced by cyst nematodes such as *Heterodera* and *Globodera* spp.) are large multinucleate cells synthesized due to partial dissolution of plant cell walls and their fusion via adjacent protoplasts. The root-knot nematodes (*Meloidogyne* spp.) induce giant cells which are generated via numerous repetitive cycles of mitotic nuclear division and cell growth sans cell plate separation or cytokinesis (in short acytokinetic mitosis). The detailed view of various NFS is given in the following Figure (Adapted from Gheysen and Jones 2006; Vieira and Gleason 2019).

The existing nematode management strategies with chemical nematicides are considered as a great threat to the environment, non-targets, and human health. The cultural, physical, and biological control tactics though advocated for several PPN species, they cannot be practiced under all types of agro-ecological regimes for all crops. Further, these non-chemical approaches may also become ineffective if not adopted with proper planning. Hence, invention of a novel, environment friendly, cost-effective nematode management tool and its wide-range adoption in the farmers' field will surely prove to be a sustainable approach to combat these hidden enemies. The growing information about PPN–plant interrelationships at genetic/molecular level, supplemented with plethora of information related to the PPN genome and transcriptome, new avenues for engineering PPN resistance in crop

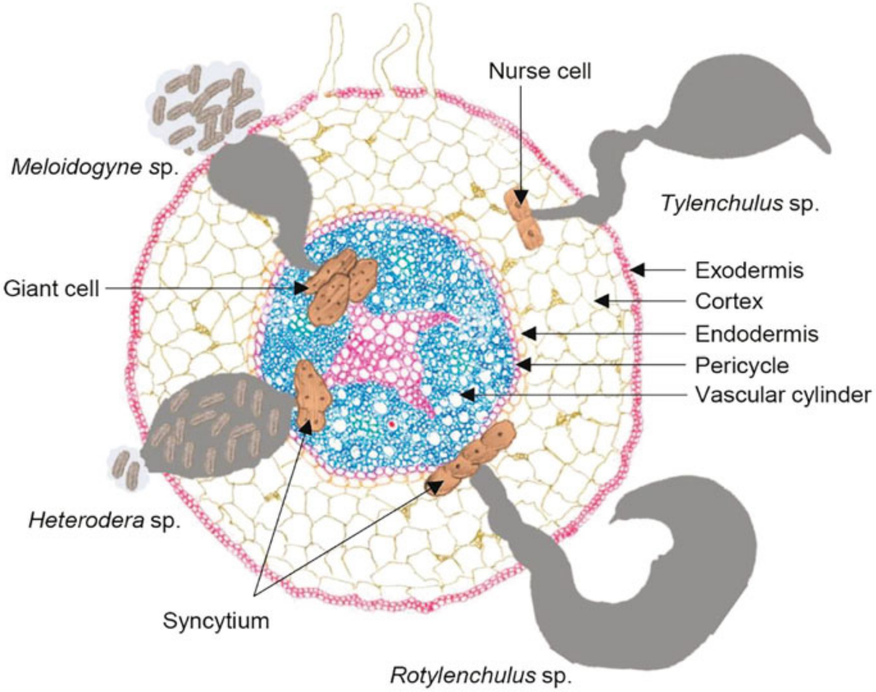

Fig. 9.2 The transverse section of host root is schematically illustrated to represent the highly conserved nature of PPN-induced feeding cells. Although feeding cells are induced in different locations of root tissue (e.g., giant cell and CN-induced syncytia in vascular bundle, nurse cell in cortex, reniform nematode-induced syncytia in endodermis and pericycle) and their biogenesis processes differ greatly (giant cell is formed by endoreduplication and syncytia by joining of adjacent cells due to partial dissolution of cell walls), they all are hypermetabolic and isolated entity that continuously provide nutrition to feeding PPNs

plants are opening day by day. All such avenues and/or strategies are being discussed in greater detail in the following sections.

9.3 Deployment of Plant Resistance (*R*) Genes

Natural resistance genes or *R* genes may function in both polygenic (determined by major and minor quantitative trait loci or QTL combinations; a number of them shows clustered genomic arrangement) and/or single dominant manner. A number of *R* genes were molecularly characterized from a number of plant species that could potentially be transferred to diverse plant species for imparting resistance against pests and pathogens (Table 9.1). The genes there initiate effective defense responses both directly and indirectly. Transgenic expression of *R* genes elicits the pathogenesis-related (PR) protein induction, as a hallmark of nematode resistance. For example, potato plants expressing the *Hero A* gene show greater expression

Table 9.1 Details of the *R* genes characterized from various plants for PPN resistance

R gene	Plant	Target nematode and other pest
Hs1^{Pro1}	Sugar beet	*H. schachtii*
Mi-1	Tomato	*M. incognita*, *M. arenaria, M. javanica*; *Macrosiphum euphorbiae* (potato aphid); *Bemisia tabaci* (white fly)
Gpa2	Potato	*Globodera pallida*
Hero A	Tomato	*G. Rostochiensis* pathotypes Ro1, Ro3, and Ro5; *G. pallida* pathotypes Pa2 and Pa3
Gro1–4	Potato	*G. Rostochiensis* pathotype Ro1
Mi-2 to *Mi-9*	Tomato	Heat stable resistance to RKNs
H1	Potato	*Globodera rostochiensis* pathotypes Ro1 and Ro4
Rhg1, *Rhg4*	Soybean	*H. glycines*
Rmc1	Potato	*M. chitwoodi*, *M. hapla*, *M. fallax*
Cre-1, *Cre-3*	Wheat	*H. avenae*
Ma	Plum	All species of RKN
Has-1^{Og}	Rice	*H. sacchari*
Me3, *Mech1–7*	Pepper	*M. incognita*, *M. hapla, M. arenaria*, *M. javanica*

levels of various salicylic acid (SA)-dependent PR genes during incompatible interaction with *G. rostochiensis*. Similar effects are seen in hexaploid wheat-resistant lines harboring the *Cre2* gene; ascorbate peroxidases were upregulated during incompatible interaction with *Heterodera avenae*. Expression of other candidate *R* genes encoding short-chain dehydrogenases, lipases, ß-1,4-endoglucanases, calmodulins, DREPP membrane proteins, etc., also results in considerable jeopardy in the number of egg-laying nematodes in soybean roots (Liu et al. 2005) that ultimately reduce the multiplication factor and pathogenic potential of the PPN species. The Map-based cloning of a gene at *Rhg4* locus in Soybean, a major QTL encompassing the *Rhg1* gene, exhibits resistance against *H. glycines* by production of serine hydroxymethyltransferase (Ali et al. 2017). Few of the resistance genes provide broad-spectrum protection; for example, the *Mi-1* and *Hero A* exhibit resistance to several *Meloidogyne* spp. and several pathotypes of *Globodera* spp. respectively (Fuller et al. 2008). On the contrary, *Gpa2* and *Gro1–4* (*R* genes identified in potato) show resistance to a narrow spectrum of *G. pallida* and *G. rostochiensis* pathotypes.

The resistant plants, based on the phenotypic characters, are often characterized by inability of the PPN species to develop permanent feeding sites post-invasion inside the root that ultimately reduce the establishment of egg-laying females. The induction of localized hypersensitive response (HR), along with the signaling pathways in resistant hosts, follows several similar trends alike other pathogens (Branch et al. 2004). For instance, the *Mi-1.2* gene governed resistance is recognized

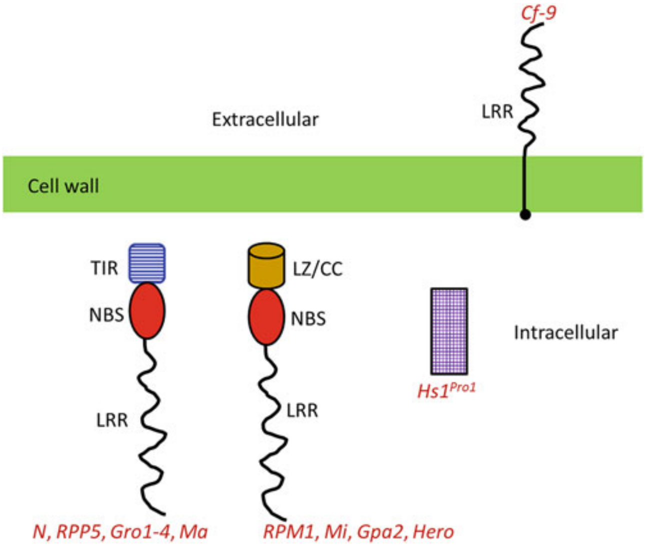

Fig. 9.3 Three classes of *R* genes confer PPN resistance: (*1*) TIR-NBS-LRR family (*Gro1–4*, *Ma,* bacterial *R* gene *RPP5,* viral *R* gene *N*) (*2*) LZ/CC-NBS-LRR family (*Mi*, *Gpa2*, *Hero*, bacterial resistance gene *RPM1*) (*3*) $Hs1^{pro\text{-}1}$, dissimilar from other candidate *R* genes. Fungal *R* gene Cf-*9* is a type of *R* gene that possesses an extracellular LRR domain

as quick localized cell death in tomato around the nematode's head region where it initiates the feeding site development. As a result, the nematode-resistant plants fail to support feeding site (giant cell) formation and inhibit development of the invading root-knot species. However, the mechanism may vary greatly depending on the gene types; like *Hero A*-mediated resistance response starts after feeding site induction by the nematode, but it leads to abnormal development and atrophy of the feeding site (Sobczak et al. 2005). The transfer of resistance gene from the source plant may also pose difficulties under different circumstances. For example, finding the resistance source is itself a tedious task for the breeders that can be viably transferred to the plant of economic interest without loss of any trait(s). Further, intraspecific transfer from wild hosts though successfully achieved through transgenic techniques, interspecific transfer does not include many references. For example, resistance is achieved upon transferring of *Mi-1* gene into susceptible tomato plants, although it is not found effective for the root-knot nematodes while transferred into *Arabidopsis* and tobacco (Williamson and Kumar 2006). Even the efficacy of *Mi-1* is affected by genotype differences of the tomato crop cultivars (Jacquet et al. 2005), which may result in plant–nematode incompatibility. The construction of a number of PCR-based molecular markers tightly linked to *Me1* gene proved to be helpful for marker-assisted selection of root–knot nematode resistance in pepper (*Capsicum annuum*) (Wang et al. 2018).

Majority of *R* genes (Fig. 9.3) encode leucine-rich repeat (LRR) motifs which mediate protein–protein interactions. Some R proteins contain transmembrane and extracellular domains, while few contain completely cytosolic domains. Viral avirulence proteins generally interact with R proteins at the intracellular space, whereas fungal avirulence proteins or effectors interact with R proteins at the intercellular space. A number of *R* genes contain TIR (Toll/interleukin-like receptor) or serine/threonine protein kinase domains, which are associated with intracellular signal transduction. *R* genes perform two major functions, i.e., recognition of pathogen-derived avirulence products or effectors and elicitation of downstream signaling pathways that activate plant defense responses, subsequently leading to localized programmed cell death or PCD (typical of hypersensitive response or HR).

Although the use of host resistance is considered as an economically viable option to be included in the integrated nematode management programs, most of the *R* genes are predominantly successful against a narrow range of PPN species, race, or pathotypes (Ali et al. 2017). Further, most of the researches in this line have been conducting experiments with the endoparasitic and semi-endoparasitic nematodes; not the PPNs with ectoparasitic and foraging habit that continuously change their location of feeding across the root. Sometimes, the introduction of resistant gene in a cultivated host also negatively affects the crop yield. For example, transgenic expression of $Hs1^{pro1}$ gene from *Beta procumbens* into sugar beet imparts resistance against *H. schachtii*, but is linked to other unwanted genes that retard the yield (Panella and Lewellen 2007). Another major drawback about this tactics lies with the origin of race/pathotypes within a PPN species having unrecognizable effectors (avirulence proteins) for the corresponding *R* proteins (Jung et al. 1998). In this context, sufficient information about genetic makeup of both plant and nematode species is required to improve our existing knowledge of the plant–PPN interactions, which would aid in deploying nematode-resistant plants as a more successful candidate for PPN control.

9.4 Exploitation of Protease Inhibitors (PIs)

Proteinase or protease inhibitors (PIs) are plant-derived protein molecules that impede the function of protease or proteinase enzymes produced by the different pathogens (including PPNs). PIs are functional against all the four classes of proteases, i.e., cysteine-, serine-, aspartic- and metallo-proteinases, in PPNs. Anti-nematode efficacy of a PI was initially developed in transgenic potato tubers transcribing a serine PI CpTI (cowpea (*Vigna unguiculata*) trypsin inhibitor) against the golden cyst nematode of potato *Globodera pallida* (Hepher and Atkinson 1992). Compared to other PIs, cysteine PIs or cystatins from various plant species performed better for enhancing PPN tolerance level in different crops. Apart from that, plants expressing sweet potato (*Ipomoea batatas*) serine PI or SpTI-1/sporamin, Oc-IΔD86 or rice (*Oryza sativa*) cystatin, PIN2, and few other cystatins such as taro (*Colocasia esculenta*), maize (*Zea mays*), and sunflower (*Helianthus annuus*) also showed reduction in PPN incidence. The PIs can also be effective against different PPN species having distinct feeding habits. For example, transgenic *Arabidopsis* overexpressing Oc-IΔD86 suppressed the development and reproduction of

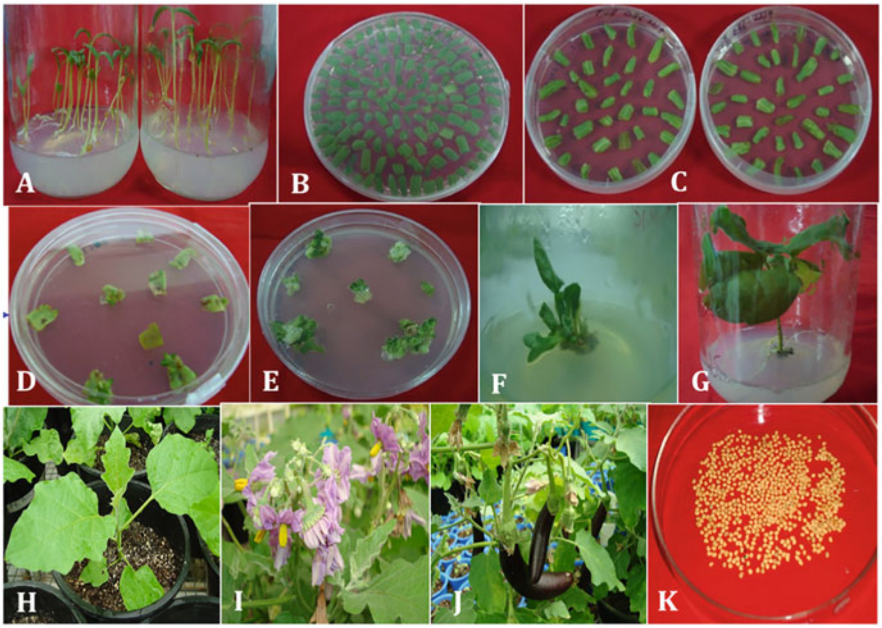

Fig. 9.4 Aubergine variety Pusa Purple Long was transformed with a oryzacystatin construct. (**a**) Raising of seedlings in half-strength Murashige Scoog media, (**b**) leaf disks were pre-cultivated in MS media, (**c**) leaf disks co-cultivated with *Agrobacterium tumifaciens* cells harboring the oryzacystatin construct, (**d**) callus were initiated in the explants, (**e**) callus differentiated in explants, (**f**) shoots were indued via hormonal treatment, (**g**) roots were induced via phytohormone treatment, (**h**) hardening of T_0 plants in pot soil, (**i**) inflorescence formation in T_0 plant, (**j**) fruit formation post-self-pollination, (**k**) T_1 seeds

Heterodera schachtii and *Meloidogyne incognita* together (Lilley et al. 2004; Fuller et al. 2008). Translationally fused two protease inhibitors (i.e., CpTI and Oc-IΔD86) when transgenically expressed in *Arabidopsis thaliana* a resultant additive effect was documented against *Globodera pallida* and *Heterodera schachtii* (Urwin et al. 1997, 1998). In this line, dual expression of taro (*Colocasia esculenta*) cysteine proteinase inhibitor (CeCPI) and fungal (*Paecilomyces javanicus*) chitinase (PjCHI-1) in tomato, driven by a synthetic promoter pMSPOA, reduced the reproductive potential of *M. incognita* to a greater extent (Chan et al. 2015). The research indicated that dual or multiple candidate gene transformation to be better than single gene transformation. The dual transformation of an antifeedant maize cystatin and a chemodisruptive peptide in banana plantain (*Musa* spp. cv. Gonja manjaya) conferred resistance to *Radopholus similis*, *Meloidogyne* spp., and *Helicotylenchus multicinctus* in plantain with enhancement of yield attributes (Tripathi et al. 2015). Oryzacystatin (Oc-IΔD86) transcribed in the of eggplant (*Solanum melongena*) roots driven by a root-specific promoter TUB-1 (tubulin isolated from *Nicotiana benthamiana*) caused detrimental effect on development and pathogenicity of *M. incognita* (Papolu et al. 2016; Fig. 9.4). Oryzacystatin also showed good response in lily (*Lilium longiflorum* cv. Nellie White) against the migratory endoparasitic nematode *Pratylenchus penetrans* (Vieira et al. 2015). Further, transgenic tomato lines expressing the hairpin construct of cathepsin L cysteine proteinase gene (*Mi-cpl-1*) resulted in the considerable decline in growth and fecundity of *M. incognita* race 1 (Dutta et al. 2015a). Identical finding was also reported earlier by de Souza Júnior et al. (2013), where the transgenic tobacco (*N. tabacum*) lines transcribing RNAi construct of *Mi-cpl-1* gene conferred tolerance to *Meloidogyne incognita* race 3. Deployment of the other PIs including transgenic wheat lines of *Triticum durum* PDW215 expressing a serine proteinase inhibitor (*pin2*) gene conferred tolerance to cereal cyst nematode *Heterodera avenae* (Vishnudasan et al. 2005).

Biosafe PIs pose no threat to the humans, other animals, and livestocks and also exist in normal diet. While used as a pest control measure, researchers have found that the PIs are specific to their action and have no adverse effect on non-targets, natural predators, or soil microorganisms (Green et al. 2012).

9.5 Transgenic Expression of Chemodisruptive Peptides

PPNs are extremely reliant on their chemosensory system and olfactory neurons to detect host root exudates for host finding and successful penetration of host tissue. Use of chemodisruptive peptides offers an alternative way to minimize the invasion of infective juveniles into the plants thereby reducing their pathogenic potential. The peptides mimetic of aldicarb and levamisole inhibit the chemotaxis in the cyst nematodes *Heterodera glycines* and *G. pallida*, and these peptides also affect the worm locomotion (Winter et al. 2002). Transgenic potato roots overexpressing a peptide that inhibits PPN acetylcholinesterase (AChE) leads to disorientation of infective juveniles of *Globodera pallida* and results in fewer establishment of adult females (Liu et al. 2005; Wang et al. 2011). This peptide remains effective post-uptake by the chemoreceptor sensilla via retrograde transport across the neurons toward cholinergic synapses. Chemodisruptive peptide for AChE, driven by the constitutive CaMV35S promoter and tissue-specific (root tip) promoter in potato and *Arabidopsis thaliana*, conferred resistance to *Globodera pallida* and *Heterodera schachtii* (Lilley et al. 2011). Similarly, transgenic eggplants expressing the repellent peptide inhibited the *M. incognita* multiplication (Papolu et al. 2020). The strategy led to development of potato transgenic with rice cystatin that maintained greater degree of resistance against potato cyst nematodes *Globodera pallida* and *G. rostochiensis* (Green et al. 2012). Roderick et al. (2012) and Tripathi et al. (2013), in partnership with International Institute of Tropical Agriculture (IITA) and University of Leeds, UK, generated transgenic banana plantains for nematode resistance in Africa by utilizing dual expression of cystatins and chemodisruptive peptides. Gene pyramiding with cystatins and chemodisruptive peptide in tomato, banana, etc., also imparted high degree of resistance to *Meloidogyne* spp. (Chan et al. 2015; Tripathi et al. 2017). Recently, Lee et al. (2018) observed that seed treatment with plant elicitor exogenous peptides (namely

GmPep1, GmPep2, and GmPep3) can substantially reduce the fecundity of *M. incognita* and *H. glycines* in soybean. The peptide treatment also protects the plants from the disruptive effects of *Meloidogyne* spp. on the aboveground plant growth and up-regulates the transcription level of PPN responsive defense genes in plants. Although the technology was found to be effective for fungal and insect pests (see Lee et al. 2018), not much work has been done on nematodes.

9.6 Utilization of Nematicidal Proteins

Nematicidal proteins disrupt the PPN development and reproduction in plants. For example, lectins, few antibodies, and *Bacillus thuringiensis* Cry toxins though used in this aspect, their commercial availability is scant. Lectins are sugar-binding proteins that bind specific monosaccharides or oligosaccharides and are naturally found in plants, animals, and fungi. Lectins can inhibit the digestion process of an organism that has ingested the lectin (Vasconcelos and Oliveira 2004). A jack bean (*Canavalia ensiformis*) lectin concanavalin A caused considerable control of root–knot nematode *M. incognita* in tomato, probably by binding to the chemoreceptive organs (Marban-Mendoza et al. 1987). Similarly, elevated hypersensitivity is recorded in soybean against *Meloidogyne incognita*, when the infective juveniles (J2s) are treated with soybean agglutinin, wheat germ agglutinin, and Concanavalin A (Davis et al. 1989).

Lectins can interact with glycoproteins present in the PPN cuticle, chemoreceptor organs such as amphids or amphid secretions, and thereby interfere with or block the chemoreception processes and other related biological processes of PPNs. Transgenic expression of a snowdrop (*Galanthus nivalis*) lectin or GNA under the control of CaMV35S promoter exhibits nematicidal efficacy in a number of host plants (including potato, *Arabidopsis thaliana*, oilseed rape *Brassica napus*) upon infection of root–knot, cyst, and lesion nematode (Ali et al. 2017). Transgenic potato or rapeseed plants expressing snowdrop lectin (*Galanthus nivalis* agglutinin) showed tolerance to *Globodera pallida*, *Pratylenchus neglectus,* and *Heterodera schachtii* (Burrows et al. 1998; Ripoll et al. 2003). Recently, it was observed that the water-soluble *Moringa oleifera* lectin (extracted from the seeds of *Moringa oleifera*) conferred strong nematicidal activity on gastrointestinal nematodes of goats (de Medeiros et al. 2018). Different crude protein extracts from *M. oleifera* seeds also showed nematicidal properties against *M. incognita*, and fractionation of crude protein extracts identified lectins as one of the major ingredients that determined toxicity (El-Ansary and Al-Saman 2018). However, more research work is needed to exploit this protein in plant nematology-related research.

Plantibodies are nothing but the antibodies produced by plants and are promising candidates for developing PPN resistance/tolerance in the host. Root–knot and cyst nematodes rely on their esophageal gland secretions to hijack host tissue organogenesis in order to develop NFSs including giant cell and syncytium. Expression of plantibodies against the antigens from pharyngeal secretions suppresses the parasitic ability of the nematodes (Ali et al. 2017). Monoclonal antibodies reactive with amphidial and cuticular secretions of *G. pallida* negatively regulate PPN migration and penetration in potato roots (Fioretti et al. 2002). Behavior of *M. javanica* J2s is affected by the polyclonal and monoclonal antibodies that bind to their cuticle and affect the movement pattern (Sharon et al. 2002). In this regard, identification of surface coat antigens from different nematodes can be helpful in devising novel PPN management tactics.

Bt or Cry toxins isolated from the bacterium *Bacillus thuringiensis* maybe transgenically expressed to elicit plant resistance against PPNs. Free-living nematode *Caenorhabditis elegans* when treated with Cry5B and Cry6A toxin, a decline in nematode fecundity and survival, was documented (Marroquin et al. 2000). This is the first report of Bt toxin acting against nematodes. PPN feeding plug near stylet orifice function as a molecular sieve that allows ingestion of particular molecules and excludes the others. *Meloidogyne* spp. can uptake bigger protein molecules than *Heterodera* and *Globodera* spp. Transgenically expressed 54 kDa molecules such as Cry5B and Cry6A toxins in tomato hairy roots negatively altered the fecundity of *Meloidogyne incognita* (Li et al. 2008). The same protein molecules cannot be uptaken by *Heterodera schachtii* because of smaller orifice of the feeding plug having the size inclusion limit up to 23 kDa. These drawbacks restrict the commercial usage of Bt proteins against all types of PPNs (Ali et al. 2017). However, amongst the recent developments, Cheng et al. (2018) found that transformation of Bt nematicidal *cry5Ba3* gene in fungus *Botrytis cinerea* could affect the fungivorous nature of *Bursaphelenchus xylophilus* that ultimately reduced the nematode's infection ability. The fungal transformation with Bt overexpression cassette provided a notable nematicidal efficacy against *B. xylophilus*, that suggested a cornerstone approach for delivering fungal-derived toxins at places where nematodes forage. The strategy of "sweet poisoning" can be utilized to disrupt the life cycle progression of this nematode pest in pine trees and manage the devastating pine wilt disease.

9.7 Barnase (Enzyme)—Barstar (Inhibitor) System

Barnase is a ribonuclease synthesized extracellularly by the bacterium *Bacillus amyloliquefaciens*; barstar inhibits excess production of barnase (which may become toxic to the bacteria itself) and synthesized intracellularly. Barnase degrades RNA, which is utilized as a source of nutrition for the bacterium. It also functions alike of a toxin to ward of predating or competing microflora within the soil environment. Barnase–barstar combinatorial system is primarily used to achieve plant sterility and production of hybrid seeds. Resistance against *H. schachtii* in *A. thaliana* is achieved by using a promoter expression limited to the syncytia for barnase and constitutive expression of barstar. However, expression of barstar must be confined within the nematode feeding cell and leaky expression in other plant parts is to be curbed. Therefore, a promoter, which is switched off during differentiation of nematode-induced feeding cells, will be effective for controlling barstar transcription (Ali et al. 2017).

9.8 RNAi and Host-Induced Gene Silencing (HIGS) Approach

The finding of RNA interference (RNAi) mechanism in a nematode, *C. elegans*, where double-stranded RNA (dsRNA) causes the degradation of target endogenous mRNA and inhibits the production of encoded proteins, has given a novel strategy to investigate gene manipulation and analysis of gene function. RNAi is exploited as a robust reverse genetic tool for developing RNAi-based transgenic plants to minimize PPN pressure in the rhizosphere (Dutta et al. 2015b).

The proposed RNAi pathway in eukaryotes is provided in Fig. 9.5. In short, double-stranded RNAs (dsRNA) upon introduction into the host cell are recognized by RNaseIII class enzyme DICER, that digests the dsRNA molecule in ATP-dependent manner into a stretches of 21–23 bp duplexes known as small RNAs or siRNAs, which possess 2 nucleotide (nt) overhangs at their 3′ end. In parallel, systemic transport of siRNAs across the different cells/tissues occurs via

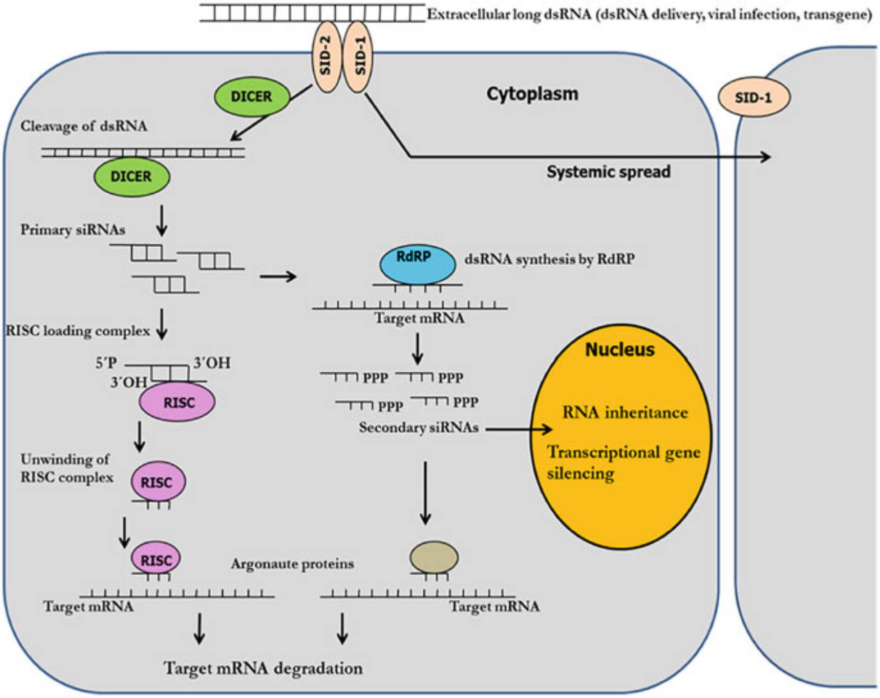

Fig. 9.5 Schematic representation of the basic RNAi pathway in eukaryotes. DsRNAs coming from various sources such as hairpin dsRNA expression, virus infection, or transgenes are processed by DICER enzyme to liberate primary siRNAs. Next, primary siRNAs dock to a multi-component RISC followed by RISC activation, single-stranded siRNA formation, target mRNA recognition, and degradation. Alternatively, argonaute proteins loaded onto primary siRNAs search target mRNAs, generate secondary siRNAs and synthesize dsRNAs via catalytic action of RNA-dependent RNA polymerase (RdRP). SID proteins are involved in dsRNA uptake into all cells

interaction with SID transmembrane proteins. SiRNA duplex complexes with another nuclease protein, i.e., RNA-induced silencing complex (RISC) in ATP-dependent manner. SiRNA duplex unwinds to provide single-stranded siRNAs in order to activate the RISC. Activated RISC finds out homologous mRNA transcripts by simple base pairing rule and digests mRNA at 12 nt apart from the 3′ end of siRNA eventually degrading the mRNA. Release of single-strand (ss) siRNAs may also lead to amplification of RNAi effect; single-stranded siRNA molecules produced from dissociated dsRNAs or liberated from activated RISCs can function as primers on complementary mRNAs resulting in the synthesis of new dsRNA molecules as the DICER substrate (Rosso et al. 2009).

For host-delivered RNAi (alternatively known as host-induced gene silencing or HIGS), plant hosts are genetically modified to transcribe dsRNA sequences which correspond to a nematode gene. RNAi construct is generated by assembling the coding sequences of target gene in sense and antisense orientations; sense and antisense cassettes are joined together via an intronic sequence linker; the entire assembly is driven by either a constitutive promoter or tissue-specific promoter. While transcribed, sense and antisense fragments form duplexes via base pairing and generate an intron-spliced hairpin RNA (ihpRNA) that eventually leads to dsRNA formation (Fig. 9.6). When PPNs suck the transgenic plant tissue for their nutritional requirement, dsRNAs and siRNAs are introduced into the nematode's digestive system (Dutta et al. 2015a).

Target genes for HIGS approach are divisible into three categories based on their biological functions, e.g., effector genes, housekeeping genes, and development or reproduction-related genes. Effectors such as *Ma16D10*, *Mi-Crt*, *Mi8D05* (*M. incognita*, *M. arenaria,* and *Meloidogyne* spp. in *Arabidopsis* and grape hairy root), *Mc16D10L* (*M. chitwoodi* in *Arabidopsis* and potato), *Mimsp40*, *Mimsp18*, *Mimsp20*, *Mimsp1*, *Mimsp2*, *Mimsp3*, *Mimsp5*, *Mimsp24* (*M. incognita* in *Arabidopsis* and eggplant), *Hs3B05*, *Hs4G06*, *Hs8H07*, *Hs10A06* (*H. schachtii* in *Arabidopsis*), *Hg30CO2* (*H. glycines* in *Arabidopsis*), *Gp-hyp* (*G. pallida* in *Arabidopsis*), etc., when targeted for *in planta* RNAi, a substantial disruption in PPN parasitic process is observed. RNAi of FMRF amide-like peptides, major sperm proteins, tyrosine phosphatases, *Cpn-1*, lactate dehydrogenases, *Y25*, *Fib1*, mitochondrial stress-70 protein precursor, *Prp-17,* etc., are involved in development and reproduction and exert deleterious effect on nematode survival. RNAi of housekeeping genes including *Pv010*, integrase, *Mi-Rpn7*, splicing factor, spliceosomal SR protein, ribosomal protein-3a, ribosomal protein-4, coatomers, etc., affect the housekeeping function of parasitizing PPNs (Dutta et al. 2015b).

HIGS reports are mostly confined to the sedentary endoparasitic PPNs including *Meloidogyne* spp., *Heterodera* spp., and *Globodera* spp. although genes of the migratory endoparasitic PPNs including *Radopholus similis*, *Pratylenchus* spp., and *Bursaphelenchus xylophilus* are also successfully targeted. The in vitro dsRNA delivery is achieved by soaking the PPNs in dsRNA solution containing pharyngeal neurostimulants such as serotonin, resorcinol, octopamine, gelatin, lipofectin, spermidine derivatives, or carbamoylcholine chloride, etc. Ingestion of dsRNA molecule through the stylet, esophagus, intestine, excretory pore, or other

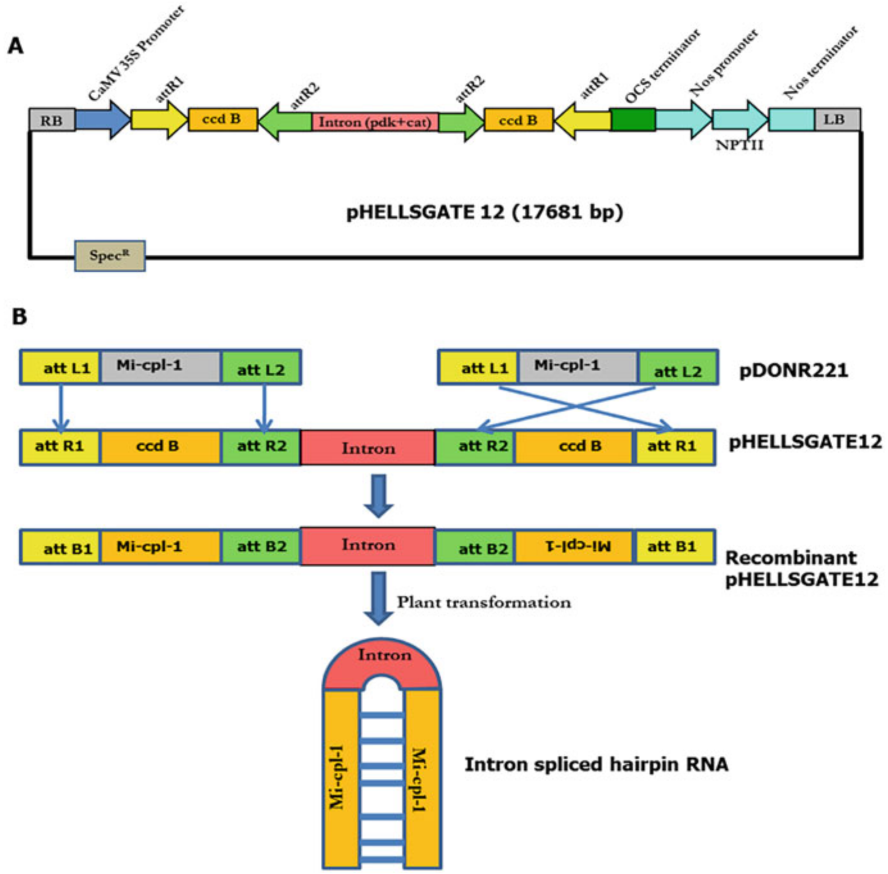

Fig. 9.6 Production of intron-spliced hairpin RNA of target gene in transgenic plant. (**a**) The Gateway-ready RNAi vector pHELLSGATE12 contains the sense and antisense cassette under the control of CaMV35S promoter. (**b**) A nematode protease gene, *Mi-cpl-1* is inserted into the vector using Gateway cloning (recombination-based) method. During plant transformation, dsRNs in hairpin RNA conformation are produced. Feeding nematodes may ingest the hpRNAs directly or may ingest plant Dicer-processed siRNAs. The figure is recreated from Dutta et al. (2015a)

natural openings is tracked using fluorescent dyes such as fluorescein isothiocyanate (FITC), Alexa Fluor, etc., by either mixing dsRNAs with dyes or by labeling dsRNAs with dyes. Using in vitro dsRNA uptake method, attenuated transcription of different candidate genes leads to retardation in PPN infectivity, developmental delay, motility inhibition, and inability to find and penetrate host root (Lilley et al. 2012; Dutta et al. 2020).

The successful in vitro delivery of dsRNAs and induced suppression of the target genes provide the useful lead for *in planta* dsRNA delivery to invading PPNs. For HIGS, host plants are genetically engineered to transcribe dsRNAs by cloning the target gene in sense and antisense orientations (linked by an intronic sequence) in a

RNAi vector driven by a plant promoter (Fig. 9.6). While PPNs suck nutrients from the host during their entire developmental process, dsRNA and siRNA molecules are continually delivered to the PPN. Tobacco transgenic lines constructed with hairpin dsRNA of two target genes encoding for integrase and splicing factor of *Meloidogyne incognita* show RNAi effect due to decreased transcript abundance of the target mRNA (Yadav et al. 2006). This was the first instance of *in planta* RNAi delivery for PPNs. The complementary dsRNA to the parasitism gene *16D10* when transcribed in transgenic *Arabidopsis thaliana* resulted in a considerable decline in root gall formation and fecundity of *Meloidogyne incognita* (Huang et al. 2006). HIGS of a number of *H. schachtii* effectors including 8H07, 10A06, 4G06, and 3B05 in *Arabidopsis thaliana* caused knockdown of the target genes in infecting nematodes *vis a vis* reduction in parasitic ability (Sindhu et al. 2009). Similarly, transgenic soybean expressing PRP17 dsRNA resulted in significant reduction of reproductive fitness in *H. glycines* (Li et al. 2010). Iberkleid et al. (2013) achieved approximately 80% reduction in transcript abundance of *Mj-far-1* (fatty acid and retinol binding protein) in *M. javanica*, when expressed by hairpin construct in tomato hairy roots. Similarly, genetically modified soybeans transcribing dsRNA of MSP (major sperm protein) coding gene provided around 68% reduction in fecundity of *Heterodera glycines* (Steeves et al. 2006). Significantly less number of gall formation is observed for transgenic soybeans carrying RNAi constructs targeting the tyrosine phosphatase gene of *Meloidogyne incognita* (Ibrahim et al. 2011). Dinh et al. (2014) found that three potato cultivars namely Desiree, Russet Burbank, and an advanced breeding line PA99N82–4 harboring the RNAi constructs containing an effector gene, *Mc16D10L*, showed resistance against *M. chitwoodi*. Shivakumara et al. (2016) reported that in vitro silencing of five esophageal gland genes expressed either in subventral or dorsal glands of *M. incognita* affected the expression of cell wall-modifying enzyme coding genes that ultimately resulted in the reduced penetration of infective juveniles. This demonstrates that there is a crosstalk existent between different parasitism genes. Further, host-delivered RNAi of two pharyngeal gland-specific genes, *Mi-msp-18* and *Mi-msp-20*, resulted in approximately 70% decline in *Meloidogyne incognita* multiplication in the transgenic eggplants (Shivakumara et al. 2017). Additionally, transcriptional oscillation of cell wall-modifying enzymes (CWMEs) is also observed in the invading and developing nematodes suggesting the complex crosstalk between CWMEs and *Mi-msp* genes during the parasitism process (Shivakumara et al. 2017). Several other candidate genes were targeted using the model plant *Arabidopsis thaliana*, which showed considerable resistance against different nematodes (Dutta et al. 2015b; Atkinson et al. 2012). Although the host-delivered RNAi can offer a novel and potential management tool for plant–parasitic nematodes, RNAi-based management might have potential off-target effects (Danchin et al. 2013). Further, the engineered plants with RNAi do not show complete resistance against the targeted nematodes, rather partial resistance is achieved (Dutta et al. 2015b). Figure 9.7 depicts the in vitro regeneration of transgenic tomato plants expressing RNAi constructs.

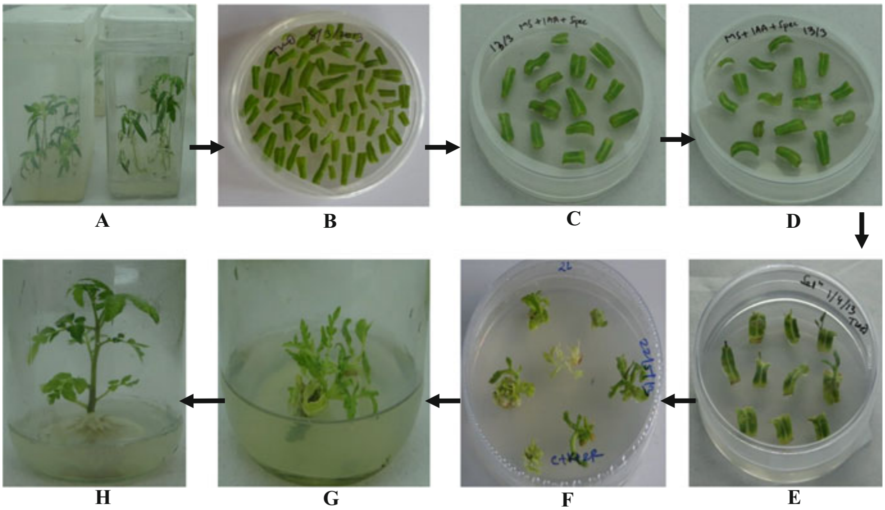

Fig. 9.7 Transformation of tomato plants with RNAi constructs and generation of transgenic lines (T_0). **(a)** 15-day-old tomato seedlings, **(b)** leaf disk pre-cultivated in MS media, **(c)** *Agrobacterium* infection of leaf disk, **(d)** selection plate containing kanamycin, **(e)** callus induction in explant, **(f)** differentiated callus in explant, **(g)** phytohormone-induced shoot initiation in explant, **(h)** hormone-induced root initiation in explant

Neuropeptides are highly conserved amongst different groups of nematodes that coordinate crucial aspects of physiology and behavior. Research progress with *C. elegans* has extensively provided the knowledge of different classes (FLPs, NLPs, ILPs, etc.) of nematode neuropeptides (Li and Kim 2008). HIGS of two FMRF amide like peptides, *flp-14* and *flp-18*, caused significant decline in parasitic success of *Meloidogyne incognita* in tobacco by interfering with juvenile's host finding ability and invasion into roots (Papolu et al. 2013). The combinatorial in vitro RNAi with two FLP genes, *flp-14* and *flp-18*, and a subventral esophageal gland gene, *Mi16D10,* reduced the *M. incognita* infection by 20–30% (Banakar et al. 2015). An array of FLPs when silenced by in vitro and in vivo RNAi in rice root–knot nematode *M. graminicola*, a considerable attenuation in nematode infectivity to rice and wheat was observed (Kumari et al. 2017). In an attempt to use neuropeptides as novel targets for nematode management, bioactive neuropeptides from neuropeptide-like protein (NLP) family have been profiled and targeted (Warnock et al. 2017). A number of discrete NLPs were detected that negatively impacted chemotaxis, root penetration, and stylet movement ability of *Meloidogyne incognita* and *Globodera pallida*. When these neuropeptides transgenically secreted from a rhizobacteria (*Bacillus subtilis*) and terrestrial microalgae (*Chlamydomonas reinhardtii*), they reduced nematode infection up to 90% in tomato. The use of this novel "non-food transgenic delivery" system can be exploited because neuropeptides can function as a novel class of plant protective nematicides. Further, silencing of neuropeptide genes, *nlp-3* and *nlp-12*, in *M. incognita* delayed the host finding and reduced their infectivity in tomato plants (Dash et al. 2017).

RNAi has been established as a promising tool for plant protection during the last decade, but there exist few bottlenecks that need to be duly answered prior extensively utilizing this tool. Notable apprehensions related to the wider deployment of HIGS-based crop protection are the possibility of off-target effect. Although RNAi-based knockdown happens mostly in a sequence-specific manner, a cross-hybridization with the transcript having partial gene homology to the target dsRNA sequence may cause silencing of non-target genes, that lead to unexpected mutant phenotypes. Additionally, the targeted dsRNA must not any sequence identity to the host genes. Majority of the HIGS study are confined to the model plants (e.g., *Arabidopsis thaliana*, *Nicotiana benthamiana*) and performed in confined trials such as greenhouses. In view of this, future HIGS investigations must be directed toward testing the hypotheses at field level in agronomically superior crops. Although most of the HIGS experiments do not confer absolute tolerance or resistance to PPNs, one of the probable ways to overcome it is by deploying fusion RNAi constructs that target multiple genes associated with single or multiple parasitic pathways, driven by the tissue-specific and wound-inducible or PPN-inducible promoter to achieve complete resistance. RNAi is a biosafe approach because no foreign proteins are transcribed *in planta* in HIGS.

A number of RNAi transgenic crops are marketed harboring the novel traits including decaffeinated coffee, nicotine-free tobacco, nutrient-fortified crops, hypo-allergenic crops, banana bract mosaic virus (BBrMV)-resistant crops, etc. RNAi generated healthier cooking oil production by inhibiting the enzyme that catalyzes oleic acid into unsaturated fatty acids (Plenish high oleic acid soybean from DuPont Pioneer). A RNAi corn crop (SmartStax PRO that protects against above- and below-ground insects) developed by Monsanto Bayer is in the pipeline for release during 2022. Genetically modified RNAi plants are currently being assessed and regulated under stricter regulatory guidelines in different countries. As any consensus is yet to be reached, a number of RNAi crops are waiting in the greenhouse for impending field trial till the regulatory guidelines are lifted.

9.9 Other Alternative Strategies

Transgenic plants expressing genes that correspond to resistance or silencing genes that are crucial for feeding site establishment are some newer research areas that maybe explored further. This can be accomplished by precisely delivering the transgenes (either constitutively overexpressing or suppressing the target genes) into the NFS, although this can negatively affect the plant growth and development. In view of this, the use of promoters that are particularly transcribed in the NFS (for example, MIOX5 and Pdf2.1 promoters) may resolve the issue. In addition to NFS-specific promoters, root-specific promoters are also deployed to regulate localized transcription of protease inhibitors (PI) and PPN-repellent peptides in different host plants. Expression of an ethylene-dependent transcription factor

AtRAP2.6 was greatly attenuated in the syncytium of *Arabidopsis thaliana* during compatible interaction with *H. schachtii*. Interestingly, when *AtRAP2.6* was overexpressed under the control of constitutive CaMV35S promoter in *A. thaliana*, transgenic plants displayed reduced susceptibility to *H. schachtii* infection (Ali et al. 2017). Additionally, plants overexpressing the mRNAs of target genes associated with host defense pathways including callose synthase and camalexin have also been tested for nematode resistance. Overexpression of AtPAD4 driven by the promoter FMV-sgt conferred greater tolerance to *H. glycines* and *M. incognita* in soybean. AtWRKY33 and AtPAD3 overexpression in *Arabidopsis* lines conferred resistance against *H. glycines*. Transgenic expression of a soybean (*Glycine max*) salicylic acid methyltransferase (GmSAMT1) gene exhibited resistance to soybean cyst nematode *Heterodera glycines* (Ali et al. 2017). Recently, CRISPR-Cas-based genome editing strategy has gained the momentum to be used for pest management in agriculture. For this, nematode susceptibility genes in plants (should not be any gene essentially involved in plant developmental pathway) need to be precisely identified. The potential deployment of base editing or prime editing, an advanced technology that can install point mutations in the genome to alter a susceptible allele into a resistant one, may also be explored.

9.10 The Biosafety of Transgenic Nematode Resistance

According to the World Bank, the transgenic crops must be cultivated to realize their benefits toward global food security, and it is also important to consider the biosafety of such plants. Many isolated studies have revealed that the transgenic nematode-resistant crops do not harm the non-target organisms (Atkinson et al. 2009). Besides, several studies have also been conducted to analyze whether the transgenic plants divert the structural enrichment of soil food web by changing the soil microenvironments (Ferris et al. 2001). In general, the nematode transgenics were not found to be environmentally hazardous. The effective use of tissue-specific promoters also lowers the burden of bioprotectant synthesis across the whole plant and reduces any adverse effect on non-target organism. It is apprehended that the host-delivered RNAi technology might become hazardous to non-target organism if there exists considerable sequence identity between the targeted gene of the PPN and an orthologue in non-target organism. Hence, rapid and precise bioinformatic analyses should be conducted to select the unique and novel targets so that the unintended silencing of off-targets can be minimized (Atkinson et al. 2012). Lastly, rapid uptake of transgenic crops at field level requires large political support. The popularization of Bt cotton in India indicates that transgenic crops can support the livelihood of poor and marginal farmers. China and other developing countries are continually building independent facilities to develop transgenics (Atkinson et al. 2012). Transgenic crops are now extensively grown in USA, Brazil, Argentina, few European, and African countries (Chaudhary and Singh 2019). It is known that the technological intervention in agriculture is able to promise the “quantity requirement," but the scientists should always look at the "quality requirement" to ensure safe and healthy food as well as environment.

9.11 Conclusion and Future Perspectives

The plant parasitic nematodes cause serious yield loss in modern intensive farming system. The microscopic appearance and also the farmer's negligence to consider nematodes as a potential pest often result in huge population buildup of this soil pest over the years. The heavy toll of nematode population then causes serious yield and quality losses. Historically, several management strategies have been devised for the management of nematodes, but any of those strategies have not been found suitable for complete eradication. Later, the combination of different management practices has also been employed that results in considerable management of nematode population density and damage. With the advent of biotechnological applications, several novel targets and management strategies were developed for nematode control. All these strategies result inconsiderable reduction of the nematode parasitism in plants, and thereby increase the crop yield. Nevertheless, majority of the targets have been tested in model plant *Arabidopsis* and in laboratory or green house conditions. Therefore, extensive field level research is of utmost need to validate the findings in open environment condition in a planned way so that they can be utilized alone or in integrative manner to get maximum nematode management.

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10

Novel Nanomaterials and Nanoformulations for Nematode Management in Agricultural Crops

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Abstract

Economic and sustainable management of pests and diseases is one of the most challenging tasks in the crop production sector. Nematodes with diversified parasitism are considered as an important pest all over the world, causing significant damage to agricultural crops, and reduce their yield valuing over \$175 to 200 billion world over. Farmers use different chemicals, but generally the nematode attack remains significantly uncontrolled. The chemical nematicides create serious issues of human and the environmental toxicity. In view of increasing demand of food and lesser effectiveness of existing methodologies, innovative technologies and materials are needed to be evolved for the management of pest and disease issues including those created by plant nematodes. Nanotechnology is one of the most promising and innovative technologies emerged in recent years, and has great potential for application in agriculture, particularly for pest management. The nanomaterials work as inhibitors against soil populations of nematodes. One significant use of nanotechnology is the synthesis of nematicidal nanocompounds, which penetrate the root system and prevent nematodes from feeding or establishing on the host. Nanomaterials can penetrate the body of nematodes, causing nematodes to die within a short period of time. Similarly, nanofertilizers are available, which are

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absorbed by plants more quickly than conventional fertilizers. An other application of nanotechnology is the development of nanosensors that can precisely detect nematode populations in the soil or plant tissue. The present chapter offers important information on nanomaterials and nanoformulations that can be used to protect plants from nematode invasion or to detect them in the plant or soil.

Keywords

Nanotechnology · Nanopesticides · Plant nematodes · Nanosensors

10.1 Introduction

Recent advancements in nanotechnology have changed the perception of the agricultural industry exposing numerous avenues where nanomaterials can be used with high degree of effectiveness. Pest and disease management is one of such avenues which warrants potential application of nanotechnology. It is expected that suitable nanomaterials will become widely available in the near future, increasing the effectiveness of commercially available products, particularly the bionanopesticides and nanofertilizers (Khan and Rizvi 2014). Nanobiotechnology is not a cure-all for pest-controlling programs but it provides many powerful tools, such as smart delivery systems which shall greatly aid the agricultural sector in the fight against crop pests and pathogens (Khan et al. 2019a, 2020). This tool will be helpful in many areas of integrated pest management programs for better agriculture production.

In this chapter, we attempt to address the questions like what should be the priority areas for nanobiotechnology in applied pest control with special emphasis on plant nematode management, and how this new technique shall benefit the practicing plant pathologists and extension managers. Therefore, our primary objective is to focus on the application of nanotechnology in crop protection and nematode management.

10.2 Plant Parasitic Nematodes

Plant parasitic nematodes constitute only a small portion of the soil nematode species so far identified. Usually, plants in soil are often attacked by a number of nematode species (Khan and Jairajpuri 2012). Nematodes occur in wide geographical areas, ranging from forest to desert soils and from high mountains to the wet banks of lakes and streams (Mohiddin and Khan 2014; Khan 2008). Usually, they aggregate in the root zone of plants and attack roots but sometimes are also found invading stems, leaves, flower, flower buds, and even the plant seeds (Khan 2016). Around 2000 species of plant nematodes are known that have major impact on the human food supply and crop production. Besides causing diseases, phytonematodes synergize soil-borne plant pathogenic fungi and bacteria and also act as vectors to certain plant viruses, such as NEPO and Tobra viruses (Khan et al. 2021a; Khan 2023b). Nematodes exhibit considerable variation in both external and internal structure; this factor enables them adapt almost every kind of environment. In spite of their complexity, all plant parasitic nematodes share a few fundamental structural principles. Although plant parasitic nematodes differ in size and shape of the body, all of them have filiform shape tapering toward end, at least up to second juvenile stage.

10.3 Economic Losses Caused by Nematodes

Major plant nematode genera with regard to economic importance are *Meloidogyne, Pratylenchus, Heterodera, Radopholus, Globodera, Ditylenchus, Tylenchulus, Rotylenchulus, Aphelenchoides, Xiphinema, Helicotylenchus,* etc. (Haroon et al. 2021; Khan 2023a). Plant nematodes are reported to inflict an estimated loss of crop yield equivalent to \$175 billion (Kantor et al. 2022) to \$200 billions (Khan 2023b). In view of this huge economic loss, numerous management strategies are applied against plant nematodes, but none offers satisfactory control in all situations. This necessitates the need of exploring new, innovative, and emerging technologies such as nanotechnology to manage nematode infestations in agricultural crops.

10.4 Nanotechnology in Nematode Management

Nanotechnology, in principle, is a technology that converts a material in to size range of nano, i.e., 1×10^{-9} m. The reduction to the nanosize makes drastic change in the volume as well as property of the material (Cortie 2004). Nanoparticles from 1 cm^3 gold may have a surface area, 2.7 million times larger than that of a single sphere of gold of the same mass (Bond 2005). Similarly, it has been generally found that the nanoform of a material has the properties different form its macroform. For example, silver is non-toxic element but its nanoform becomes antimicrobial (Sampathkumar et al. 2020). Similarly, Cu or Ni nanoparticles become more toxic than their molecular form. Researchers have detected the nematicidal properties of several nanoparticles, such as AgNPs, against root–knot nematode (Shoaib et al. 2022). Cromwell et al. (2014) were among the researcher to explore the nematicidal effect of AgNP against a phytonematode on Bermuda grass. The lab testing demonstrated substantial nematicidal effects, but the field experiments were inconclusive. However, subsequent researches have highlighted positive findings of FeNP (Kalaiselvi et al. 2017) and AgNP (Hassan et al. 2016) against *M. incognita* on tomato, okra, and eggplant. Furthermore, the toxicity of three nanoparticles was detected on tomato plants viz TiO_2NP (20 nm), SiO_2NP (11–14 nm), and AgNP (20 nm) against *M. incognita* was detected on tomato plant (Ardakani 2013).

There has been little or no research on the mechanism on the nematicidal activity of nanoparticles. The majority of the research on this aspect has focused on bacteria or fungus. Further, most of such studies have used Ag, Au, or ZnO nanoparticles and the effects are contradictory and vary with the NP species, its stability, size, concentration, etc., which are discussed under:

10.5 NP Decomposition into Metal Ions

The NPs may return to their precursor state or ionic form, when come in contact with the micrograms during the antimicrobial impact. The antibacterial action of AgNPs was generated by degrading NPs into silver ions (Yang et al. 2020). The silver ions released from AgNPs, easily infiltrate into the bacterial cells, causing cell damage and inhibiting various critical activities. In the same way, the ions may also enter into the nematode body and disturb neuro-muscular transmission. In addition to causing additional defects, the silver ions may block the respiratory enzymes inside the cell. The silver ions also have the potential to interact with the DNA molecule and replace protons in the hydrogen bonds, which may suppress the cell replication as sulfides and phosphates are widely present in the cell. The reactions of AgNPs with DNA may depend on the arrangement of the atoms on the NP, which may cause structural changes in the DNA affecting the transcription and translation (Carriere et al. 2017). These effects may disrupt the development and replication of cell. The hydrogen atoms are Lewis basic elements while Ag is a Lewis acid, which reacts together, on the essential proteins, resulting in their deactivation or cell death (Xu et al. 2012).

10.6 Reactive Oxygen Species (ROS)

Reactive oxygen species (ROS) is a type of unstable molecule containing oxygen and that easily reacts with other molecules in a cell. A buildup of ROS in cells may cause damage to DNA, RNA, and proteins, and may cause cell death. The silver ion may induce the production of ROS in the cell. The silver ions break [Fe-S] clusters in respiratory enzymes, as a result Fe is released that can subsequently induce catalysis in the Fenton processes, giving rise to ROS (Godoy-Gallardo et al. 2021). However, not all studies have reported ROS formation in response to silver treatment (Guo et al. 2013). This shows that ROS formation depends on the microorganism, NP characteristics, etc. Further, all kinds of metal atoms do not catalyze Fenton process and subsequently the Haber–Weiss reactions, because NPs have different characteristics than free ions (Wan et al. 2022). ROS may potentially produce aberrations on the wall of bacterial cells generated by AgNPs (Wu et al. 2020).

10.7 Disruption of the Cell Membrane Integrity

By disrupting the cell wall integrity, AgNPs can enter in to the microbial cells as well as in the cells of nematodes body and sensory organs exposed to NPs. The particles initially cling to the surface, causing irregularities in the cell membrane. The anomalies may impair the membrane permeability to the point of cell death. When aberrations closely occur, they can consolidate and produce micropits on the cell's surface, where NPs can concentrate.

10.8 Signal Transduction Disturbance

Signal transduction is a process by which a cell responds to substances outside the cell through signaling molecules found on the surface and inside the cell. Molecules that lead to signal transduction bind to a specific protein receptor (signaling molecule) on or inside a cell. The signals are then passed from one molecule to another inside the cell, which results in a specific cell response, such as cell division or cell death. Signal transduction is important for cells to grow and work normally. NPs may also disrupt signal transmission in exposed bacterial cells. Protein phosphorylation is an important element in signal transmission. When Gram-negative bacterial cells were exposed to AgNPs, tyrosine residues were dephosphorylated leading to disruption of signal transduction, halting the cell development of bacteria (Flifl and Singh 2021).

10.9 Chemotaxis and Motility

Experiments have shown that NPs can affect chemotaxis and motility in bacteria. Both qualities, however, vary with the length of NP therapy. Within 4 h, a huge swarm colony of *E. coli* grew in the medium supplemented with AuNPs (Khan et al. 2021b). However, *E. coli* motility declined after 8 and 20 h. Although the colonies were larger than the control at the start, the rising concentration of AuNPs progressively reduced their size (Khan et al. 2019b).

10.10 Effect of Nanomaterials on Plant Nematodes

The nanotechnology may be used in the management of crop nematodes in different ways. The application of NPs through drenching in the soil or foliar spray is a most obvious or simple way of NP application as are conventional pesticides applied against the nematodes and other pests or pathogens (Khan et al. 2019a). However, direct application of NPs may also affect the non-target organisms such as mineral fixing/solubilizing microorganisms. Besides as a suppressant, the nanomaterials have potential application in the delivery system, carrying and releasing pesticidal chemicals like inhibitors of polyamine synthesis, pheromones, SAR inducing chemicals, active ingredients, etc. (Khan et al. 2014). Nanotechnological approach may result in multi-site action against nematodes with no phytotoxicity (Khan and Akram 2020). Some nanoproducts or nanomaterials including nanosilver and nanosulfur have been in use against plant nematodes with some significant success (Hardman 2006).

10.11 Metal-Based Nanoparticles and Their Effect on Plant Parasitic Nematodes

In recent years, metal-based NPs have become increasingly popular among nanostructures because of their novel physicochemical characteristics and biological activities, and plant pathology has become infiltrated by NPs of metalloids, metal oxides, and nonmetals, which have been used as fungicides/nematicides or nanofertilizers to control plants diseases (Khan et al. 2021c). Impact of some of the important nanomaterials is discussed under:

10.12 Silver Nanoparticles

Silver as nanoparticle has earned huge interest because of its strong conductivity, chemical stability, catalytic, and antibacterial activity (Khan et al. 2021b). Richards (1981) was the first to study the role of AgNPs in the management of a plant disease. The AgNPs have also proved to be quite effective in suppressing the nematode activity (Roh et al. 2009). The AgNPs induce oxidative stress in the targeted nematode cells (Lim et al. 2012) (Table 10.1).

In addition, AgNPs disrupt multiple cellular mechanisms such as membrane permeability, and ATP synthesis in both eukaryotes and prokaryotes (Zhang et al. 2016) including fungi, bacteria, and nematodes (Choi and Hu 2008). Ardakani (2013) examined the toxicity of AgNPs on *M. incognita* juveniles; it was observed that treatments of AgNP induced considerable mortality to the nematode larvae. In view of high degree of nematicidal action against root–knot nematode, the AgNPs may be used in place of high-risk chemical nematicides in high value-food crops (Cromwell et al. 2014). El-Deen and El-Deeb (2018) reported that application of AgNP treatments on tomato under greenhouse condition substantially reduced the population of *M. incognita* and improved the plant growth parameters. AgNPs toxicity can cause inhibition in the reproduction and plant growth at a dose of 0.05–0.5 mg/mL for 72 h and 5–50 mg/mL for 1–3 days, respectively (Lim et al. 2012). This suggests that the effect of AgNP at low concentrations applied in the field can be gradual and persistent. Hence, AgNPs can be used as a broad-spectrum chemical effective against nematodes, fungi, and bacteria (Fig. 10.1).

10.13 Copper Nanoparticles

Use of copper nanoparticles (CuNPs) is a logical choice for the management of plant diseases because Cu has a long history of controlling plant diseases (Elmer et al. 2018). CuNPs show effectiveness and specificity in suppressing activity of a large number microorganisms (Tamayo et al. 2014). Study on CuNPs as fungicide/bactericide was first made by Giannousi et al. (2013) under greenhouse condition. They observed the disease for 10 days once symptoms developed and revealed that the CuO NP treatment was the most effective in reducing leaf lesions at 150–340 μg/

Table. 10.1 Impact of nanoparticles on plant nematodes

Nanoparticle	NP Size	Plant nematode	Plant	Effect	References
AgNPs	8–19 nm	*Meloidogyne javanica*	Eggplant	Inhibited J_2 population	Abdellatif et al. (2016)
AgNPs	~55 nm	*M. incognita*	–	Inhibited egg hatching	Khan et al. (2021d)
AgNPs	60–112 nm	*M. incognita*	–	Inhibited egg hatching	Nassar (2016)
AgNPs	–	*M. incognita*	Tomato	Mortality to J_2 and inhibited egg hatching	Kalaiselvi et al. (2019)
AgNPs	2 nm	*M. incognita*	Groundnut	Decreased soil population	Fabiyi and Olatunji (2018)
AgNPs	–	*M. graminis*, *M. incognita*	Bermudagrass	Inhibited soil population	Cromwell et al. (2014)
AgNPs	–	*M. incognita*	–	Mortality to nematode	Nazir et al. (2019)
AgNPs	–	*M. incognita*	Tomato	Nematicidal effect	Hassan et al. (2016)
MgO-NPs	100 nm	*M. incognita*	Strawberry	Mortality of nematode	Khan et al. (2022a)
AgNP, SiO_2NP, TiO_2NP	20 nm, 11–14 nm and 20 nm	*M. incognita*	Tomato	Mortality to J_2	Ardakani (2013)
AuNPs	20–45 nm	*M. incognita*	Tomato	Inhibited soil population	Thakur et al. (2018)
ZnONPs	≤50 nm	*Xiphinema vuittenezi*	–	Nematicidal effect	Sávoly et al. (2016)
SiCNPs	50 nm ± 21.5	*M. incognita*	–	Mortality to J_2	Al Banna et al. (2018)
AgBNPs	29.55 nm	*M. incognita*	Tomato	Mortality to J_2	El-Batal et al. (2019)
CuNPs	100 nm	*M. incognita*	–	Mortality to J_2	Mohamed et al. (2019)
CS/γ-PGA	61 ± 4.5 nm and 56 ± 2.6 nm	*Bursaphelenchus xylophilus*	Pine tree	Nematicidal effect	Liang et al. (2018)
CuONPs	5–15 nm	*M. incognita*	Chickpea	Mortality to J_2 and inhibited egg hatching	Khan et al. (2022b)

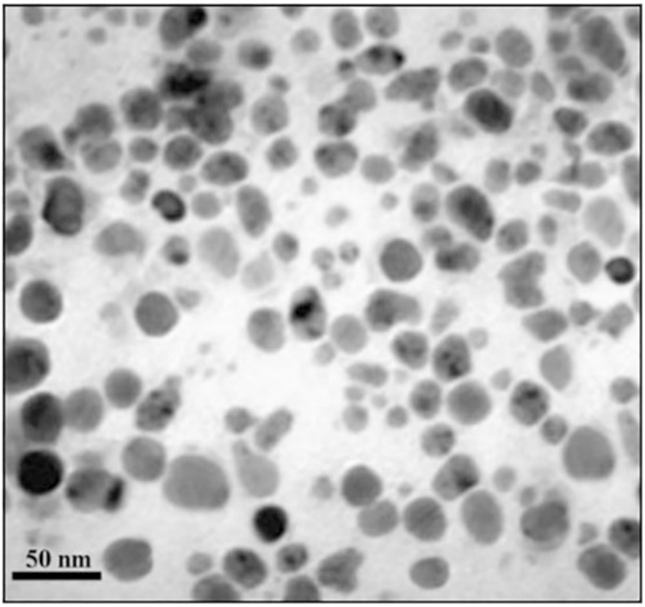

Fig. 10.1 TEM images of AgNPs (5–50 nm; Courtesy: Vahabi et al. 2011)

mL. The study on the effect of CuNPs on nematodes was carried out with *Xiphinema index* infesting grapes (Darago 2014) (Fig. 10.2).

With the given antimicrobial nature, CuNPs are expected to be effective against nematodes. The treatments with of CuNPs against *M. incognita* significantly suppressed the nematode population in laboratory condition (Kausar 2022). In the study, it was further recorded that J_2 mortality was directly proportional to CuNP concentrations and 0.2 g NPs/L concentration caused 100% mortality to the nematode juveniles. Ch et al. (2019) studied the role of CuFe NPs against *Meloidogyne* spp. together with the effect on plant growth of nematode-infested plants. They reported that 0.03 μg CuFe NP most effectively suppressed the nematode and enhanced the fresh weight of shoot and root of tomato plants.

10.14 Zinc Oxide Nanoparticles

Several studies have been made to examine the antimicrobial activity of zinc nanoparticles (ZnO NPs) against plant pathogens (Fig. 10.3; Malandrakis et al. 2019). The ZnO NPs have been recorded to inhibit bacteria (Kaushik and Dutta 2017) and fungi, including *Alternaria alternata, Fusarium oxysporum, Sclerotinia sclerotiorum, Botrytis cinerea, Penicillium expansum, Rhizopus stolonifera, Rhizoctonia solani,* and *Mucor plumbeus* under in vitro condition (Sardella et al. 2017), as well as nematode like root–knot nematode, *M. incognita* (Kaushik and Dutta 2017). The application of ZnO NPs is reported to promote the seed germination, seedling

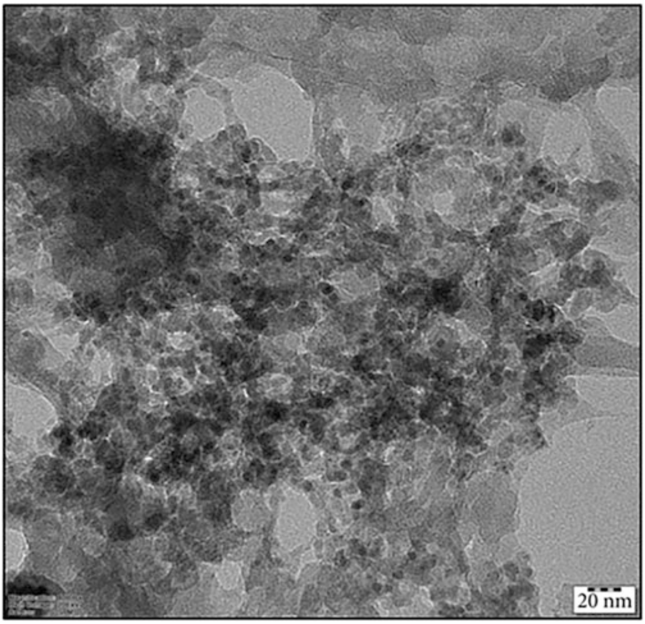

Fig. 10.2 TEM images of CuNPs (Courtesy: Shanmugapriya et al. 2022)

vigor, and plant growth of ground nut (Prasad et al. 2012). The ZnO NPs at 0.10 mg/mL concentration induced highest reduction in the multiplication of *M. javanica* and galling as compared to graphene oxide (Siddiqui et al. 2019). Gupta et al. (2015) also recorded the reduction in nematode multiplication and galling due to spraying of plants with ZnO NPs. The NP sprays may have led to entry of the particles in the nematode cuticle and hypodermis and affected the lipid, glycogen, and mucopolysaccharides that resulted to distorted juveniles of *M. javanica* (Fig. 10.3).

10.15 Other Nanoparticles

Udalova et al. (2018) reported that selenium NPs stimulated the plant growth and development by inducing systemic resistance in tomatoes against *Meloidogyne* spp. and also stimulated the expression of PR-6 gene in the roots and leaves of tomatoes. Consequently, the gene expression to inhibition and enhanced proteinase inhibitor activity. Ardakani (2013) studied the toxicity of silicon oxide nanoparticles (SiONPs) and titanium oxide nanoparticles (TiO_2NPs) against root–knot nematode under lab and greenhouse condition. In the study, it was found that TiO_2NP treatments (0.02%) greatly reduced the nematode population. It was also observed that TiO_2NPs had toxicity to the tomato plants and the nematode lesser than AgNPs. The research carried out by Thakur et al. (2018) has shown that gold nanoparticles (AuNPs) are of great use in the control of root–knot nematode, *M. incognita*

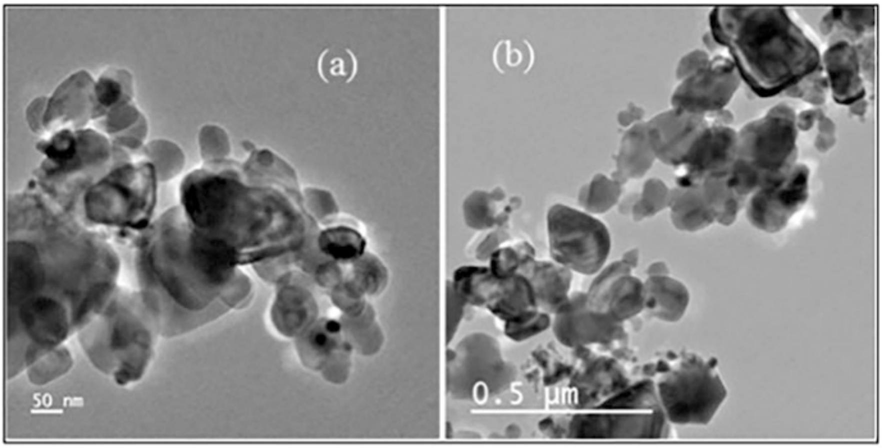

Fig. 10.3 TEM images of ZnONPs (Courtesy: Geetha et al. 2016)

infesting tomato plants. The incubation of *M. incognita* with AuNPs for 3 h, caused 100% mortality to *M. incognita* juveniles in water. The AuNP soil treatment also produced high suppression of the nematode juveniles, evident of low survival rate of the *M. incognita* in the soil. The pot experiment revealed that AuNPs not only proved lethal to *M. incognita* but also stimulated the plant growth of tomato without any phytotoxicity.

10.16 Effect of Green-Synthesized Nanoparticles on Plant Nematodes

Biological approaches of nanoparticle synthesis including green chemistry are non-toxic and environmentally friendly techniques which use plants, algae, fungi, bacteria, etc., for synthesizing nanoparticles (Behravan et al. 2019). This technique was suggested as an alternative to chemical and physical methods. The green synthesis uses microorganisms and plants to mediate the synthesis of nanoparticles (Khan et al. 2019b, 2020). Several bacteria are known to be capable of synthesizing proteins, enzymes, polysaccharides, phenols, etc., which mediate the generation of NPs. Many metal NPs, including gold, silver, selenium, platinum, palladium, silica, quantum dots, etc., are reported to be biosynthesized by using the culture or filtrates of bacteria, actinomycetes, fungi, yeasts, and algae (Khan et al. 2020). The biosynthesis methods have preference over chemical synthesis which leads to generation of toxic residues or bioproducts with heavy expenditures. Further, the NPs appear to clump and become useless and continue to agglomerate (Xia et al. 2006).

Biogenic silver NPs are reported to be around 20 times more antimicrobial in contrast to chemically developed NPs (Sintubin et al. 2011). Nano-Ag has a high degree of suppressive effect on plant nematodes. The impact of nano-Ag on

Meloidogyne species has been studied by a number of researchers (Abdellatif et al. 2016; Nassar 2016). The culture extracts of a plant species, *Urtica urens* induced synthesis of AgNPs, which successfully suppressed *M. incognita*, the suppression was 11 times higher in juveniles than the eggs of the nematodes (Nassar 2016). The Soliman et al. (2017) showed that AgNP formulations were more toxic than crude extracts to the second (J_2) juveniles of *M. incognita*. The inhibiting effects of all extractive materials on egg and larval stages were concentration dependent. The photosynthesized AgNPs formulation contained higher concentrations of secondary metabolites (20- to 30-fold). The AgNPs based on cyanobacteria not only effectively suppressed the nematode populations but also stimulated the plant growth. Apart from its bio-stimulant impact on plant growth, the cyanobacteria-based AgNPs also controlled the *M. javanica* population (Hamed et al. 2019). Abdellatif et al. (2016) examined the impact of AgNPs synthesized using *Ulva lactuca* and *Turbinaria turbinata* (algae) on *M. javanica* infesting eggplants. The treatment with *U. lactuca* NPs (17 mg mL–1) performed better in reducing *M. javanica* second-stage juveniles in the soil up to 69.4%, in boosting the growth of eggplants. No phototoxicity was observed in eggplants during the treatment of GSN. The engineered or biosynthesized silver NPs completely inactivated all the juveniles of *Meloidogyne* spp., typically within 6 h of exposure period. The AgNPs were applied twice a week at 90.4 mg/m^{-2} to a commercial landscape infested with *M. graminis.* The treatment improved the turfgrass consistency and reduced root gall formation (Cromwell et al. 2014). A low concentration of AgNPs suppressed the population of *M. incognita* and *M. javanica* equal to the traditional conventional nematicide treatment (Abdellatif et al. 2016; Ardakani 2013). The nematicide organo phosphorodithioate was supplied with biosynthesized nano-Ag and found that the LC_{50} was lowered from 7.2 to 5.6 for suppression of *M. incognita* (Nassar 2016). Biosynthesized AuNPs for their strong antibacterial and antimicrobial properties could be extremely useful in medicine and agriculture.

10.17 Application of Nanomaterials in Nematode Detection

Correct detection and identification of pathogens is a vital step in selecting and deciding an effective management strategy. Nanoparticles have a great application for use in quick and reliable diagnosis of harmful plant pathogenic bacteria (Zhao et al. 2004), viruses (Tang et al. 2020), and fungi (Khan and Rizvi 2014; Khan et al. 2019c). Nanoparticles have intriguing electrical and optical characteristics and may be used to manufacture various materials for sensing and detection (Yin et al. 2013). The detection limit of a biosensor can be considerably enhanced by employing nanomaterials in biosensing applications. Various nanostructures have been tested in order to create a biosensor capable of recognizing living cells, tissues, bacteria, and so on. DNA, antibodies, enzymes, etc., can be detected using a variety of ways such as biomolecule adsorption, covalent attachment, encapsulation, or a complex mix of these methods (He et al. 2013). The metal NPs, quantum dots, carbon nanomaterials such as carbon nanotubes, graphene, and polymeric nanomaterials have great potential for use in biosensor fabrication (He et al. 2013). The current diagnostic systems use computer-based digital chips. The nanomaterials are being used in preparing the sensor chips (Rocha-Santos 2014). The nanochips are microarrays that incorporate fluorescent oligo capture probes to detect the hybridization. The nanochips are highly sensitivity and can identify single nucleotide change in bacteria and viruses. Generally, plants immediately respond to pathogenic infections through the synthesis of jasmonic acid, methyl jasmonate, salicylic acid, etc. (Khan and Haque 2013). Hence, a sensitive electrochemical sensor using a modified gold electrode with CuNPs to measure the levels of ascorbic acid, salicylic acid, and other compounds in the plant or seeds can identify the pathogen responsible for chemical response of the plant. Minute change in the level of salicylic acid can be detected successfully and precisely using gold electrodes and a CuNP sensor (Wang et al. 2011). The detection devices based on the sensitivity of the nanosensors sensitive to detect infections in plants/seeds have become available for some plant pathogens (Choudhary et al. 2018) and are expected to be available soon for nematodes and other pathogens at reasonable prices. The NPs can be integrated with biological components, antibodies etc.; for example, SiNP biomarker was created by employing silica nanoparticles and antibodies, to develop a SiNP-probe to detect *Xanthomonas axonopodis* pv. *vesicatoria,* the causal pathogen of bacterial leaf spot (Yao et al. 2009). Similarly, an optical immunosensor based on gold nanoparticles was developed to detect Karnal bunt in wheat (Sharma et al. 2022). Singh et al. (2010) developed an immunosensor based on AuNPs for detecting *Tilletia indica,* causing wheat Karnal bunt. The application of NP-based sensors in seed certification and plant quarantine might be extremely successful and precise in identifying microbial infestations. The nanosensors for plant pathogens are in the developing stage, whereas for plant nematodes, hardly a few attempts have been made, but it is very much expected that in near future NP-based diagnostic kits especially against PCNs and other seed-borne nematodes shall become a practical reality that will greatly increase the detection efficiency in quarantine departments and ease their working with greater preciseness.

10.18 Conclusion and Future Perspectives

Nanotechnology has proved to be extremely helpful and profitable in agriculture particularly in crop production and protection, although minimum efforts have been done to exploit its potential. The bionanofertilizers may provide efficient and target delivery of nutrients to all plant cells due to the nanoproperties as well as their stability in the environment. Besides, treatment of seeds with nanomaterials may stimulate their germination faster, and shall enhance their resilience to environmental stress. The nanopesticides may effectively suppress the pests and pathogens at extremely low doses. The nanobiosensors shall certainly improve efficiency, accuracy, detection time, and sensitivity compared to conventional biosensors. To enhance agricultural production, it is vital to exploit different applications of nanotechnology with optimism, but with adequate biosafety verification and care.

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Nematode Disease Diagnosis: Application of Nano-Sensors

11

Al-kazafy Hassan Sabry

Abstract

Nanotechnology plays a vital role in agriculture development. This role exists in many agricultural aspects such as fertilizer production, pesticide production, and pest management. Using nanotechnology, it could reduce the cost of food production and environmental pollution and at the same time increase the yield of crops. Nano-sensors are considered one of the most important components in nanotechnology branches. The nano-sensors used in diagnosis or detection of diseases may be chemical, biological, or mechanical components. Sometimes biological sensors are used so are called nanobiosensor. Recently, nano-sensors were used for monitoring of plant diseases. It was known that the sooner the disease is detected, the sooner the treatment is possible. Some nano-sensors have been synthesized for detection of plant parasitic nematode infection and also gave a fast solution to the management of this pest. So, many scientists considered that the nano-sensors are the main tolls in disease mentoring.

Keywords

Nano-sensors · Root–knot nematode · Diseases · Monitoring · Detection · Control

11.1 Introduction

Nano-sensors play an important role in disease detection and also application. These nano-sensors may be chemical or mechanical agents. These nano-sensors can be used in detection of pollution in atmosphere or detect and diagnosis of diseases in

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human blood. The nanosizes of these nano-sensors enable it to movement easily. By nano-sensors, it can be monitored plant metabolism and signaling.

In the past, there were conventional methods to detect the plant diseases. These methods divided into two divisions. The first method is a direct detection such as detection by polymerase chain reaction (PCR), LFA, ELISA, DNA sequence, and cell culture; the second is indirect detection which carried out by volatile profiling hyperspectral imaging and thermographical imaging.

Nanotechnology is considered the magic solution for the most of problems including plant disease management (Khan and Rizvi 2014; Khan et al. 2019a, b, c) and disease detection (Khan and Akram 2020; Khan and Rizvi 2016; Khan et al. 2020). Nano-sensors are the most important product of nanotechnology, and have great potential for use in plant disease diagnosis (Khan 2023). Sellappan et al. (2022) developed nanobiosensor to early detection and prevention of agricultural crops from harmful microorganisms. These nano-sensors may be chemical nano-sensors or biological nano-sensors (Kaushal and Wani 2017).

Using specific nanoparticles as nano-sensors to detect the plant pathogen early can reduce the plant disease damage and overcome the pathogen (Khan and Rizvi 2018). Singh et al. (2010) prepared gold nanoscales for early diagnostic and characteristic of Karnal bunt, *Tilletia indica* (fungal disease) which is causes wheat disease. Nano-sensors also were used for viral disease detection. Lin et al. (2014) used gold nanorods for viral detection in ornamental crops. The nano-sensors not only used to quickly detect the fungal and viral diseases but also the bacterial infection. Silica nanoparticles were used as nano-sensors for detect of the bacterial infection. Yao et al. (2009) used the silica nanoparticle for detection of bacterial infection in solanaceous crops such as eggplant and green pepper. Choi et al. (2019) used *Agrobacterium* as a nanobiosensor to detect the difference between the galls formed by *Agrobacterium* or by root–knot nematode. This nanobiosensor is consisting of *Agrobacterium* mannitol (ABM) agar media and X-gal. This work depends on the exogenous nopaline created by the galls in plant root.

Plant parasitic nematodes (PPNs) are the most destructive pest for plants (Khan 2007, 2008, 2016). It causes a $157 billion loss each year in crop yields worldwide (Chariou and Steinmetz 2017). The plant parasitic nematodes infested vegetables, crops, and fruit trees in all the world regions (Haque and Khan 2021; Khan et al. 2021). This pest has many genus and species. More than 4100 species of plant parasitic nematodes were described until now (Poveda et al. 2020). The most destructive genuses of pathogenic plant nematodes are called as the root–knot nematode*, Meloidogyne incognita a*nd potato cyst nematodes (*Heterodera and Globodera* spp. (Jones et al. 2013). This pest infested plants by forming wounds in plant roots causing dark spots on these infected roots and tumors or molding in the infected fruits over the upper surface of the infested leaves (Tuncsoy 2021). Nematode not only feed on roots but also feed on stems, leaves, and seeds. It uses special spear called the stylet to penetrate the plant tissues (Fig. 11.1). By this stylet, it can withdraw the fluid of plant cells and kill it. Due to this mechanism, the browning spots were formed on the plant parts. Many conventional methods used to control this pest: chemical control, mechanical control, and biological control.

Fig. 11.1 The stylet of plant—parasitic nematode

Unfortunately, all these methods were failed to suppress this pest. So, it is a dire need to find a new method for control this pest.

In this chapter, the role of nano-sensors in nematode disease detection and application will discuss and clear.

11.2 What Are Nano-Sensors?

According to Chauhan and Saxena (2021), the nano-sensors are nanotechnology-sensors relay on the measure physical or mechanical quantities and converted it to signals to detect and analyze those quantities. Nano-sensors also can define as sensing device that have a nanosize shape smaller than 100 nm. The nano-sensors not only chemical or physical compound but also there is a biological nano-sensor such as bacteria, protein, or DNA (Smith et al. 2015). DNA as a nanobiosensor was synthesized for detection and characterization and/or multigenic characterization of the diseases caused by nematodes infection. Furthermore, nano-sensor can be defined as an indicator that is able to carry the obtained data and information about the action and behavior of the detected nanoparticles from the nanosizes parameter to the macrosizes parameter (Scoville 2013).

Nano-sensors also defined as a measurement system created for detection and estimation of any materials by using interaction among all estimated materials and evaluating these changes into a readable form (as a report) by the helping of a transduction and electromechanical interpretation (Malik et al. 2013).

Nano-sensors also can be defined as a measurement tool converted into measurable signals (Butnariu and Butu 2019). The size of these nano-sensors ranged between 10 and 100 nm.

11.3 How the Nano-Sensors Work?

The nano-sensor's detection type can be improved by using of selective and specific chemical agent or by the using of bionanospecific sensor estimation agents such as DNA molecule, aptamers, antibodies, or specific enzymes. The characterization and diagnosis sensors can be enhanced by using of surface-improved optical options (e.g., surface plasmon resonance (SPR)) or electron-conductive nanoscope agents such as carbon nanoparticles (carbon nanotubes or graphene nanoscales) which used as transducers device (Mukherjee et al. 2016). The principal mechanism of nano-sensors is converting of the response of bioanalyte interactions converted to an electrical interaction through the using of transduction mechanisms (Malik et al. 2013). So, there are three main components in nano-sensor detectors (Fig. 11.2). The first one is the receptor of the nano-sensors which is disease or material or protein or any agent. The second one is the transducer which is converting the interaction to an electrical report or electrical signal. The third device is called detector.

On the other hand, the nano-sensors work by detecting the physical signals and convert these signals into standardized signals (Butnariu and Butu 2019). Nano-sensors interact with proteins (Ta et al. 2018), nucleic acids (Peng et al. 2005), and also with ions or respond directly to physiological changes (Narayanaswamy et al. 2016).

11.3.1 The Advantage of Nano-Sensors in Diagnosis and Application of Diseases

There are many advantages of nano-sensor in diseases detection such as:

(a) High stability.
(b) High specificity.
(c) Fast dynamic.
(d) High accuracy.

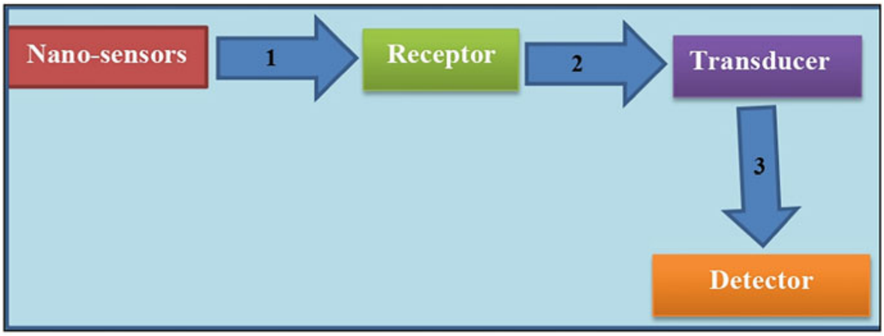

Fig. 11.2 The components of nano-sensors measurement

(e) High sensitive.
(f) Low-cost tests and portability.
(g) Remote control.

11.4 Types of Nano-Sensors

There are many types of nano-sensors used in diagnosis and detection of diseases. In this review, the nano-sensors related to plant diseases were selected.

11.4.1 Chemical Nano-Sensors

The chemical nano-sensors depend on two components. The first one is the chemical element (Zn, Ag, . . . etc.) and the condenser. In this section, metal nanoparticles were used in detect of plant diseases. The infection which caused by *Xanthomonas axonopodis* pv. *Vesicatoria* (a distractive plant disease which causes a dangerous bacterial infection in tomatoes and peppers fruits) has been diagnosis and characterized by the using of fluorescent silica nanoparticles interacted with antibody molecules (Yao et al. 2009). Silica nanoparticles were selected due to its high photostability. When these particles were exposed to the pathogenic organisms such as bacteria, it showed a high significantly fluorescence intensity as compared with the control (untreated sample). So, gold nanoparticles can be used as a nano-sensor to many pathogenic organisms.

Carbon nanotubes also used as nano-sensors to many phytopathogenic organisms. *Caenorhabditis elegans* are belonging to plant pathogenic nematodes that used as a test model pathogenic for many estimated treatments with the type of single-walled carbon nanotubes (SWCNs) (Tissenbaum 2015). The single-walled carbon nanotubes are considered fluorescent nanoscales which forming the near-infrared (NIR) spectral region. The wavelengths of these wives are between 900 and 1400 nm (O’Connell et al. 2002). So, these carbon nanotube particles were created and synthesized for imaging within the gastrointestinal track of *C. elegans*. There is high affinity between the using of SWCNs and *C. elegans* nematodes. Also, *C. elegans* have been selected as an experimental organism for scientific studies on Huntington infection, which is considered a neurodegenerative disease existed due to poly glutamine (polyQ) repeat increasing in the Huntington protein (MacDonald et al. 1993). The huge autofluorescence in *C. elegans* nematode can be characterized and diagnosis throughout the optical spectral range. This optical is caused by two main major reasons that located in the uterus and the intestine.

11.4.2 Physical Nano-Sensors

These types of nano-sensors depend on the electronic movement and transport from the sender and receiver. This type is related to the industry only.

11.4.3 Mechanical Nano-Sensors

These types are types of nano-sensors that can measure the quantities and volumes, such as mass, pressure, force, or displacement (Eswaraiah et al. 2011).

11.4.4 Biological Nano-Sensors

This type depends on a biological system as a receptor such as protein or DNA or enzyme, and the transducer mechanism such as an electrochemical detector. Similarly, zinc oxide nanoparticles as nano-sensors can be used for detecting the fungal pathogen *Trichoderma harzianum* (Siddiquee and Suryani 2014).

11.5 Role of Nano-Sensors in Nematode Disease Diagnosis

Recently, there are many methods to detect and diagnosis the plant parasitic nematode diseases not by morphology, feasibility, and accuracy but by using small size sensors called nano-sensors. The nematode called unseen organisms or enemies because it very tiny in size and not visible by naked eyes.

11.5.1 DNA as a Nanobiosensor for Nematode Disease Diagnosis

DNA molecule was used as a nano-sensor for detection and diagnosis of the nematode diseases (Carneiro et al. 2017).

11.5.1.1 Advantages of DNA Using

- Cheap.
- Fast.
- Accurate.
- High-throughput manner.

The using of DNA contributes in detection and diagnosis many types of plant parasitic nematodes such as *Heterodera, Meloidogyne, Pratylenchus,* and *Globodera* (Subbotin et al. 2000). Using of DNA not only for nematode detection but also can be used in nematode eggs detection, infested or infected tissue, egg cluster, soil samples after infection, and any abnormal changes in the soil fauna (Nega 2014). The sample soils were collected and tested for nematode detection by PCR. The mixtures of amplificated and digested (PCR and RFLP) of a single line of DNA strand have been estimated for useful DNA comparing of among all nematode individuals (Powers et al. 1997).

On the other hand, DNA as a biosensor was used in detection of nematodes in potato tuber tissue (Susiˇc et al. 2020). This technique also was used for fast detecting of the pine wood nematode (PWN), *Bursaphelenchus xylophilus* (Zhou et al. 2022). Fast detection can decrease the nematode damage. Omer et al. (2022) discovered a new technique to diagnosis the root–knot nematode, *Meloidogyne hapla*. This mechanism is called loop-mediated isothermal amplification (LAMP). This strategy depends on extraction of DNA from different soils. LAMP carried out by expansion and special diagnosis of nucleic acids by using 4 to 6 marker and a polymerase with auto-displacement activity under isothermal conditions (Notomi et al. 2000). This method was very fast, accurate, and cheap determination for diagnosis and determination of volume to *M. hapla* DNA in infected soil.

11.5.2 pH-Sensitive Ratiometric Nano-Sensors

Developing sensors at the nanoscale has several benefits as particles in this size range exhibit special characteristics. pH-sensitive ratiometric nano-sensors were used to detect the parasitic nematode, *Caenorhabditis elegans*. These nano-sensors were able to carefully showing the all image of physiological pH levels. This image has been estimated and characterized to determine the pH range of the pharyngeal and intestinal lumen of *C. elegans* recently (Chauhan et al. 2013). The size of nano-sensors used was 40 nm. In this case, the nano-sensors were developed by integrate Oregon Green (OG) and pH-sensitive fluorophores, carboxyfluorescein (FAM) in a percent 1:1, and a slandered fluorophore, 5-(and-6)-carboxytetramethylrhodamine (TAMRA) to an inert polyacrylamide matrix.

11.5.3 Soil PCR

The main advantage of using PCR in nematode diagnosis pathogen is more specific, susceptible, fast, easy, and costless as compared with the other diagnostic protocols (Ahuja and Somvanshi 2021). In this method, the nematodes can be diagnosed by analysis of soil sample only. The soil sample was used to detect and diagnose the infected nematodes species (Castagnone-Sereno 2011). The soil sample was collected, and commercial kits were utilized to diagnose the presence of nematode species in the soil.

11.6 Nano-Sensors and Pathogenic Fungi Detection and Diagnosis

Pathogenic fungi are considered one of the most destructive pests for agriculture (Savary et al. 2012). About 70 to 80% of plant diseases are caused by the pathogenic fungi (Li et al. 2017). These fungi caused approximately 10–16% of global annual productivity loss. So, early detection of the pathogenic fungi is playing an important role in overcoming of this pest. Using nano-sensors can be played an effective role in fungi detection (Wang et al. 2020). These nano-sensors are used in this diagnosis as graphene, carbon nanotubes (Schroeder et al. 2019), and nanofibers (Mercante et al. 2017). So, there are many nano-sensors used in pathogenic fungi detection such as:

11.6.1 Gold Nanoparticles as Nano-Sensors in Pathogenic Fungi Detection

Nanogold has many advantages in pathogenic fungi detection because it has very high electro-activity and very conductive (Cao et al. 2011). Wang et al. (2010) used mixture of modified gold electrode (Au) with copper (Cu) nanoscales to determine the salicylic acid which produced by oil seeds infected by the *Sclerotinia sclerotiorum* fungus. Nanogold also was used in detection of *Aspergillus niger* fungus by interaction between the spores of this fungus and gold nanoparticles (Lee et al. 2021). This interaction produced a special color indicated to fungal infection (Fig. 11.3).

11.6.2 Carbon Nanotubes as Nano-Sensors in Pathogenic Fungi Detection

It was known that the carbon nanotubes divided into two principle types. Single-walled carbon nanotubes (SWCNs) and the second is multiwalled carbon nanotubes (MWCNs). The second type (multi walled carbon nanotubes) was used in detection of pathogenic fungi in agriculture. These nanotubes were used in detection of *Fusarium graminearum* fungus. The carbon nanotubes have carboxyl groups which play important role in fungal detection (Migliorini et al. 2020).

11.6.3 Quantum Dots Nanoparticles

Quantum dots (QDs) are synthetic nanoscale particles that can move and inducing the electrons. The quantum dots have many optical properties. So, it used as very

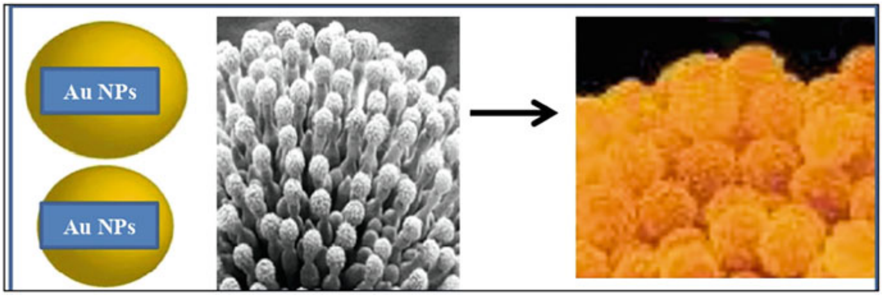

Fig. 11.3 Using of gold nanoparticles in fungal infection detection

sensitive and specific nano-sensors in detection and diagnosis of pathogenic fungi. Rad et al. (2012) used the quantum dots in detecting and diagnosing the broom disease that causes by *Phytoplasma aurantifolia* fungus.

11.6.4 Nanopore and Pathogenic Fungi Detection and Diagnosis

Nanopore sequencing platforms have been used in pathogenic fungi diagnosis such as *Penicillium digitatum* in lemon (Li et al. 2020).

11.6.5 Nanosilicon Oxide

Nanosilicon oxide was used as a nano-sensor in *Aspergillus ochraceus* detection (Kaushik et al. 2009).

11.6.6 Copper Oxide Nanoparticles

Copper nanoparticles were used in detection of some pathogenic fungi such as *Aspergillus niger* (Etefagh et al. 2013).

On the other side, Mahlein (2016) used the red, blue, and green lights as optical sensors for detecting of *Cercospora* leaf spot disease in sugar beet fields. This strategy was also used in detecting of wheat head blight disease which caused by *Fusarium graminearum* (Moshou et al. 2004). Wang et al. (2017) prepared nanobody for detecting of *Aspergillus flavus.*

11.7 Nano-Sensors and Bacterial Diseases Detection and Diagnosis

11.7.1 Silica Nanoparticles

Silica nanoparticles were used as nano-sensor for detecting of the bacterial plant pathogenic. Yao et al. (2009) developed silica nanoscales in detecting and diagnosis of *Xanthomonas axonopodis* in tomato plants. These bacteria were very dangerous on tomato production.

11.7.2 Gold Nanoparticles

Used gold nanoparticles in detection of *Pantoea stewartii* subsp. *Stewartii* as a bacterial disease (Zhao et al. 2014).

11.8 Nano-Sensors and Viral Detection and Diagnosis

11.8.1 Nanopore and Viral Detection

Nanopore was used in detecting of both *Liberibacter asiaticus* and *plum pox* virus, in the peach (Badial et al. 2018). This method was very effective in virus detection compared with the conventional methods.

11.8.2 Nanowire and Viral Detection

Nanowire used as a nano-sensor detector for virus diseases. Ariffin et al. (2014) developed a nanowire as an effective and promising detector for cucumber mosaic virus disease (CMV) and papaya ring spot virus (PRSV).

11.9 Conclusion and Future Perspectives

Nanotechnology has a potential role in agriculture development and crop production. With many crops, saturation levels have reached, where no further economic yield enhancements are expected with the existing crop production technologies. However, nanotechnology has potential to break these barriers, and may greatly enhance the crop productivity, reduce the cost of food production and improve environment quality, if integrated with conventional technologies. Nanopfertilizers, nanopesticides and nanosensors are the major avenues where the agriculturists have to hit to harnes the benefit of this technology, and these areas are the areas which may prove to be a gane changer in the near future to deal with the most serios issues of food shortage and hunger.

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Part II

Nematode Problems and Their Management

Root–Knot Nematodes in Vegetables and Ornamentals and Their Management by Novel Biological and Biotechnological Tools

12

Mujeebur Rahman Khan, Faheem Ahamad, Tanveer Fatima Rizvi, and Mohammad Akram

Abstract

Root–knot nematode is a significant pest of crops all over the world. It seriously harms vegetables and ornamentals. The vegetables with the worst infestations include tomatoes, eggplants, potatoes, pepper, okra, lettuce, cucumber, bottle gourd, etc., and may exhibit up to 60% crop losses. The nematode is a sedentary endoparasite, and forms galls on the root system. As a result, there is a significant disruption in the absorption of water and nutrients by the roots and subsequently to the entire plant body. The nematode may be managed through a variety of strategies, including cultivation of resistant crops/cultivars, use of nematicides, cultural practices, physical methods, etc. However, none of these methods are completely successful against *Meloidogyne* spp. Keeping in view the commercial value of the crops, and economic importance of this nematode, the present chapter elaborates updated information on application of novel biological and biotechnological tools for managing root–knot in ornamental and vegetable crops.

Keywords

Root-knot nematodes · Management · Biocontrol · Biotechnological tools · Ornamental and vegetable crops

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12.1 Introduction

Nematodes from the genus *Meloidogyne* constitute a most significant global pest of vegetable and ornamental crops (Khan et al. 2012, 2018a, b; Mohiddin and Khan 2014). First species of *Meloidogyne* was reported on cucumbers in a greenhouse by Berkeley (1885) in England. This pest was well known in numerous parts of Western Europe by the end of the eighteenth century (Siddiqui 2005). Root–knot nematodes affect several economically important species and have over 3000 recognized hosts (Buenna et al. 2007) and present significant challenge to all kinds of cultivated crops. Several researchers have conducted surveys to analyze the disease infestation and association of various root–knot nematode species. But the most significant efforts in this direction at the global basis were made through USDA International *Meloidogyne* Project (IMP) in which over 50 countries collaborated during 1979–1986 under the leadership of Prof. Sasser (Sasser et al. 1983). Four species, viz., *M. incognita, M. javanica, M. arenaria,* and *M. hapla* on the basis of global distribution and economic significance, were recognized as major *Meloidogyne* species responsible for 5–43% yield loss in vegetables (Sasser and Carter 1983; Sasser 1989). For having unique ability of interacting with plant pathogenic fungi and bacteria, root–knot nematodes assume additional significance in aggravating disease severity and subsequently greater damage to agricultural crops (Khan 1993; Khan and Sharma 2020). Crop losses due to *Meloidogyne* spp. have been estimated by a number of researchers. Duncan and Noling (1998) introduced the approach for calculating crop losses and damage functions. In the tropics, Sasser (1979) assessed yearly crop losses of 29%. Lamberti (1979) estimated 50–60% losses caused by root–knot nematode in eggplant and tomato. Khan et al. (2021) reported 7–35% yield loss to different kinds of crops due to root–knot disease. Further, average agricultural losses in areas/fields where root–knot is not managed, expected to be more than 20%, with losses in particular fields may reach to 60% even more depending on the crop species (Khan 2008), and sometimes farmers plow the field without taking any harvest (Khan et al. 2023).

Rapid advancements in biological and biotechnological methods have had an impact on the study and treatment of plant diseases during the last two decades. The genome-sequencing programs for *Caenorhabditis elegans* have offered new methodologies and solutions for nematode problems. However, biotechnology alone is not a cure-all for nematological issues, but it does give numerous strong tools with potential applications in applied nematology and management. The implements will make it easier to research on challenges, in precise identifying species and pathotypes and developing resistant cultivars that were previously unavailable due to technological constraints. However, for people who are inexperienced with or are not in a direct association with new methods and their comprehensive terminology, the advantages of biotechnology advancements may be challenging to recognize. Future agriculture will require ecology-based management, and integrated nematode management will rely on a variety of management strategies to minimize nematode populations especially though biological and biotechnological approaches. Various important aspect of root knot disease along with the management covering biological and biotechnological approaches are discussed under.

12.2 Symptoms

The *Meloidogyne* spp. are sedentary endoparasites and are polyphagous in nature. The nematodes infect all higher plants including vegetables and ornamentals. The second-stage juveniles penetrate roots and migrate intercellularly. The migration and feeding in the root tissue lead to the formation of peculiar galls on roots (Bird 1885). The galls are cancerous outgrowth especially on lateral roots (Mohiddin and Khan 2014; Fig. 12.1). The size and shape of the galls vary with the nematode and host species (Khan 1997). The galls are large and fleshy on tomato, eggplant, okra, etc. (Fig. 12.1a, b) whereas small and diffused on chili, potato tuber, etc. (Fig. 12.1c). The galls become complex and form bead-like structure in bottle gourd, sponge gourd, etc. (Fig. 12.1d, e; Khan et al. 2023). Similarly, in ornamental plants, the gall become complex or fused or small but numerous (Fig. 12.1f, g; Crow and Mitkowski 2010; Khan et al. 2023). Due to the formation of galls, intake of water and nutrients is hampered and the plants show symptoms of water and nutrient deficiency such as stunted growth, sparse, and yellowish foliage (Khan et al. 2007), which appear in patches of plants. The nematode infection ultimately decreases the economic value, and quality of vegetable (Desaeger et al. 2023) and ornamental produce (Crow and

Fig. 12.1 Galling incited by *Meloidogyne* spp. on tomato (**a**), brinjal (**b**), and okra (**c**) (source: Z. Haque, Aligarh Muslim University), bottle gourd (**d**), (source: M. W. Khan, Aligarh Muslim University), sponge gourd (**e**) (source: M. R. Khan, Aligarh Muslim University), balsam (**f**), kochea (**g**) (source: M.R. Khan, NASI, India)

Mitkowski 2010). Wilting also sets due to occlusion of xylem vessel, but plant retains normalcy during night when transpiration rate decreases. The nematode infection predisposes the plants and exposes the root tissue to greater infection by plant pathogenic bacteria and fungi, resulting in formation of disease complex (Khan 1993; Khan et al. 2023) and subsequently greater yield losses (Khan and Sharma 2020).

12.3 Epidemiology

Although root–knot nematode was first observed in UK on greenhouse grown cucumbers (Berkeley 1885), the nematode is highly prevalent and damaging in warmer and moderately warmer areas all over the world, where summer lasts much longer than winter. For this reason, root–knot is a major nematode problem in vegetables (Khan et al. 2021; Desaeger et al. 2023) and ornamental plants (Khan et al. 2012) in tropical and subtropical regions (Shakeel et al. 2020). According to Abad et al. (2003), *Meloidogyne* spp. have a host range of over 3000 species of plants. The crop damage caused by root–knot nematodes extends up to 80%; nevertheless, global losses of up to $500 million have been estimated due to this nematode. Khan (1997) determined the distribution and yield loss to vegetable crops in North India and found 21% occurrence of root–knot nematodes in vegetables with dominance of *M. incognita*, followed by *M. javanica, M. hapla*, and *M. arenaria*. Root–knot nematodes inhabit the soil and are able to migrate maximum 1 m throughout the lifespan. Their spread from one field to the other or even in the same field is enabled by any agent that disperses parts of plant and/or soil debris, such as agricultural tools, shoe dirt having infested soil, floodwater, and irrigation (Lehman 1994). The nematodes may survive in harsh conditions and spread even in the lack of water moisture due to wind, plant debris, or birds.

12.4 Biology

Females of root–knot nematode deposit eggs in the gelatinous matrix (egg mass) secreted by rectal cells. Temperature can greatly influence the egg hatching. Hatching is occasionally promoted by the root diffusates. Six stages in the life cycle of *Meloidogyne* spp. occur, starting from egg, four juvenile stages, and one adult stage. After the post-embryonic development, the first-stage juvenile (J_1) undergoes first molt to become J_2 which emerges from the egg. Freshly emerged larvae move in the soil to search young lateral roots of a susceptible plant and penetrate through the root tips. The juvenile (J_2) moves to reach it head in the phloem region and induces the formation of giant cells preferably in primary phloem on which the J_2 feeds (Bird 1885). Concurrent with the giant cell formation, the surrounding root tissue undergoes hyperplasia and hypertrophy. After continuous feeding, the J_2 undergoes through morphological changes and molts thrice before developing to a sedentary pear-shaped adult female. However, male nematode at fourth molt become vermiform and migrate out of the root. *Meloidogyne* spp. complete life cycle in 3–8 weeks, depending on temperature, host, and nematode species. A female can deposit normally 200–500 eggs (up to 2000 eggs/egg mass; Akitt 1978).

12.5 Host Range

Meloidogyne is a polyphagous genus and has the widest host range, making it difficult for farmers to control the problem through crop rotation (Oyetunde et al. 2022). Over 100 *Meloidogyne* species have been described globally (Kim et al. 2022). *M. incognita, M. javanica,* and *M. arenaria* are three highly polyphagous apomictic species (Karajeh 2015) and can infect multiple hosts in the same fields. Besides, there is also a high chance of specificity of distinct pathogenicity variants on the specific crop. Over 3000 plant species from all kinds and groups of crops are categorized as susceptible host plant species to root–knot nematode (Ralmi et al. 2016). Vegetables and ornamentals are among the highly susceptible hosts of root–knot nematodes (Khan et al. 2023).

12.6 Yield Loss

Plant nematodes are reported to inflict an estimated loss of crop yield equivalent to 175 billion dollars to 200 billion dollars (Kantor et al. 2022; Khan 2023). Majority of these nematodes attack underground parts, causing 12–24% or even greater yield loss to horticultural crops (Rao 2007). According to an estimate, annually on an average 6% loss in field crops, 12% in fruit and nut crops, 11% in vegetables, and 10% in ornamental crops occur due to nematode infections. The crop losses, however, vary greatly with the nematode and crop species. Root–knot nematodes, *Meloidogyne* spp., are most devastating group of nematode pests of vegetable crops and reduce their yield by 5–43% in tropical and subtropical regions (Sasser 1989). Bhati and Baheti (2021) recorded yield loss up to 67% by *M. incognita* in polyhouse.

12.7 Biological and Biotechnological Tools of Management

There are different methods and techniques of root–knot management in vegetables and ornamentals. Generally, cultural practices, host resistance, and chemicals are used to control the plant nematodes (Khan et al. 2023), but none is fully effective, reliable and fails to offer satisfactory control. Chemical pesticides are relatively more effective but have serious environmental, toxicological, and food safety issues (Shahid and Khan 2019). In this situation, biological and biotechnological approaches may play major role in dealing effectively and successfully with nematode problems in vegetables and ornamentals, which are discussed under:

12.8 Biological Control

The damage caused by nematodes remained unrecognized and is generally unnoticed by the growers. As a result, the growers fail to implement the proper management practices. However, in recent years, farmers have begun to recognize the significance of nematode management to achieve good yield and employ pesticides. Most of these chemicals are effective in controlling nematode populations, although they do not always deliver a financial return to farmers. In addition, the consequences of chemicals on food, human health, and the environment are becoming increasingly evident (Khan 2009; Shahid and Khan 2019). Biological control has the potential to be a useful and environmentally friendly strategy to deal with nematode infestation in food crops, especially at a time when people are becoming more conscious of the risks associated with pesticides. Several microorganisms are known to be suppressive toward plant nematodes (Stirling 1991). Soil-borne fungi have been found to possess potential for significant antagonism against plant nematodes (Khan et al. 2022). However, soil bacteria have also been found quite effective in successfully controlling phytonematodes. The biocontrol agents may also be applied successfully along with pesticides (Mohiddin and Khan 2013). In addition to direct parasites, phosphate-solubilizing bacteria can also contribute to inhibit nematodes (Khan et al. 2009, 2017). The scope of biocontrol fungi and bacteria is elaborated under:

12.9 Biocontrol Fungi

Fungi constitute the most ubiquitous group of parasites of plant nematodes. On the basis of parasitism, biocontrol fungi have been categorized into the following subgroups:

12.10 Predacious Fungi

Around 150 fungal species, reported to show nematode predation are categorized as predacious fungi, nematode-trapping fungi, or nematophagous fungi (Khan 2016). Majority of the nematophagous fungi belongs to the family Moniliaceae (Hyphomycetes). Predacious fungi are typically found infesting free-living nematodes (Hastuti et al. 2022). Although they are capable of devouring phytonematodes, their role in eradicate plant parasitic nematodes or significantly suppressing their population has not proven successful. Introduction *of Arthrobotrys dactyloides* and *Dactylaria thaumasia* along with *Meloidogyne incognita* in the soil did not represent discernible decrease in the nematode infection on tomato or okra (Mankau 1961; Khan et al. 2002). Application of *Dactylaria eudermata* was also found ineffective in suppressing development of *Globodera rostochiensis* infection in potato. *Arthrobotrys oligospora*, a nematode-trapping fungus, is a well-studied biocontrol agent, which is largely attributable to the existence of a range of trapping structures (Khan and Answer 2011; Singh et al. 2013). The thick, collagen-containing cuticle of phytonematodes and the proteinaceous structure of their eggshells serve as mechanical barriers that prevent infection by soil bacteria. Nematophagous fungus can breach such barriers and establish themselves successfully. There are a number of lytic enzymes, such as extracellular proteases, that hydrolyze the proteins present in the cuticle and eggshell of phytonematodes, and these have been identified as key contributors to the penetration (Jansson 1982). The predacious fungus, *A. irregularis,* has been found effective against plant nematodes; hence, its commercial formulation was first prepared in France and the trade name Royal 350 in France (Caudal and Morin 1983).

12.11 Nematode Parasitic Fungi

The most effective biocontrol agents of phytonematodes fall under this category (Stirling 1991). The fungal spores or mycelium enter the nematode body through natural openings or at any point on the cuticle. Endozoic or endoparasitic fungi are also included under the nematode parasitic fungi. Some endozoic fungi are known to be present in soil and contribute in the natural control to keep the nematode populations in balance; for this reason, they are often referred to natural antagonists (Bilgrami and Khan 2022). It is known that fifty endozoic fungi classified under Phycomycetes and a few under Deuteromycetes are nematode parasites (Askary 2015). All of these fungi undergo a brief saprophytic phase, and many of them are obligate parasites. Typically, they complete their life cycles inside the body of the host nematode and do not form mycelium in the soil. Depending on the type of nematode and the stage of its development these fungi attack vermiform stages, eggs and adult females of phytonematodes which are discussed under:

12.12 Parasites of Vermiform Nematodes

This group of fungi shows diversity in the parasitism for having some degree of variation in the initiation of infections. The motile zoospores produced by some fungi move through exudates of nematode to approach the nematode. The germ tube enters in the nematode through natural opening or at any point on the cuticle (Gray 1988). *Catenaria anguillulae* and *Lagenidium caudatum* have been found to parasitize *Xiphinema* spp. Conidia of *Verticillium balanoides* and *Drechmeria coniospora* were found to synthesize some sort of sticky substance that aided in the adhesion to the nematode cuticle (Saikawa 1982; Dowsett et al. 1982). Nematotoxic substances released by the developing spores of *Nematoctonus* spp. promote the mobilization of nematodes (Guima et al. 1973).

12.13 Parasites of Eggs and Adult Females

Fungi belongs to this group preferentially colonize and damage nematode reproductive structures (Jiang et al. 2017). These fungi are also known as opportunists since they easily penetrate eggs whenever they have the chance, otherwise live saprophytically in soil (Li et al. 2022). *Exophilia, Phoma, Dactylella, Catenaria, Purpureocillium, Pochonia, Nematophthora, Fusarium, Cylindrocarpon, Cephalosporium, Trichoderma*, and other species have been documented to infect phytonematode eggs and adult females (Khan et al. 2012). Root–knot nematodes in sedentary life stages are especially susceptible to the attack by these fungi when exposed on the root surface (Khan 2007, 2008). The opportunistic fungi preferentially parasitize egg masses or eggs. The spore of these fungi disperses into soils where they develop and wait for a susceptible host nematode (Rodriguez-Kabana et al. 1987). Numerous *Trichoderma* species were discovered in close proximity to the egg masses of the root–knot nematodes that were infecting black pepper. The best colonists among them were *T. harzianum, T. hamatum, T. viride, T. koningii, T. virens*, and *T. longibrachiatum* (Khan and Akram 2000; Khan and Mohiddin 2018; Mohiddin et al. 2010). *Trichoderma* spp. reduced the cardamom nursery galling by 58–69%. (Eapen and Venugopal 1995). In the past years, more and more cases of *Trichoderma* species parasitizing plant parasitic nematodes have emerged (Khan 2016). A few opportunistic fungi, including *Purpureocillium lilacinum, Pochonia chlamydosporia*, and *Cylindrocarpon destructans*, have also been thoroughly assessed for nematode biocontrol (Khan and Tarannum 1997; Khan and Akram 2000; Khan et al. 2011). In view of their potential of parasitizing plant parasitic nematodes, the commercial formulations of most of these fungi are available which are quite effective against root–knot nematodes (Khan 2016).

12.13.1 *Cylindrocarpon* Species

Some *Cylindrocarpon* species were identified by Tribe (1977) as significant pathogens of nematode eggs and adult stages. Some studies have shown the biocontrol capability of these fungi, particularly against *Heterodera* and *Meloidogyne* spp. (Rodriguez-Kabana and Morgan-Jones 1988; Meyer 1990; Khan et al. 2002). Two species, *C. destructans* and *C. radicicola,* attack eggs and cysts (Tribe 1979; Crump 1987). However, both these species are uncommon, hence are thought to be the insignificant contributors in the control of nematode populations in the soil. Khan and Kounser (Khan and Kounsar 2000) and Khan et al. (2002) found that adding of *C. destructans* to the soil or seeds of mungbean decreased the root galls incited by *M. incognita* in mungbean and tomato. Decrease in the root gall in citrus and tomato was also recorded due to treatment with above biocontrol agent (Freitas et al. 1996).

12.13.2 *Pochonia chlamydosporia*

Pochonia chlamydosporia is an excellent biocontrol agent which parasitizes nematode eggs and unhatched larvae (Manzanilla-Lopez et al. 2013; Khan 2007). The fungus produces diffusible toxic compounds that antagonize the nematodes by enzymatic breakdown of the egg shell and larval cuticle, as well as physiological disruptions (Meyer 1990; Stirling 1991). Prior to infection, *P. chlamydosporia* creates a branching mycelial network in the close proximity to the nematode eggs, which pierces the egg shell with the help of lateral branch/ hyphae (Atkins et al. 2003). The hyphae spread easily inside eggs and in unhatched juveniles. *P. chlamydosporia* infected eggs and larvae fail to develop and enter into the plant roots (Stirling 1991). The fungus needs a distinct biological niche comprising of particular mycoflora and favorable conditions, which makes *P. chlamydosporia* less effective under tropical and subtropical climates. However, in Europe, *P. chlamydosporia* appears to help maintain the population of nematodes in a healthy equilibrium (Stirling 1991). In crop pathosystem, soil application of *P. chlamydosporia* colonized oat kernels significantly suppressed the soil population of *Meloidogyne* and *Heterodera* spp. (Dallemole-Giaretta et al. 2012; Kerry 1997). Substrates like Leaf litter, saw dust-soil-molasses and bagasse-soil-molasses have been evaluated to mass culture the fungus which revealed 10^{6-7} CFUs/g material (Khan et al. 2001). The liquid suspension of the fungus encapsulated with sodium alginate pellets was found successful in carrying the fungus to the field (Davies et al. 1988). In another study, Kerry (1997) produced granules of the fungus and their application to field revealed, 9×10^4 and 4×10^4 CFUs/g soil, 1 and 12 weeks after application. Khan et al. (2012) developed powder formulation of *P. chlamydosporia* on a material containing fly ash, saw dust, and molasses, and its application as seed or soil treatment significantly suppressed the galling in chickpea and pigeon pea.

12.13.3 *Purpureocillium lilacinum* (= *Paecilomyces lilacinus*)

Paecilomyces lilacinus is an important parasite of eggs and adult females of nematodes (Jatala 1985), which has been synonymized by *Purpureocillium lilacinum* (Spatafora et al. 2015). A number of nematodes, particularly from the genus *Meloidogyne* and *Pratylenchus* infesting vegetable crops, have been successfully controlled by the *P. lilacinum*. The fungus prevalently occurs in warmer climates (Jatala et al. 1979; Stirling 1991). Lysek (1976) was the first to identify *P. lilacinum* as (*Paecilomyces lilacinus*) infecting eggs of root–knot nematodes. Since then, the fungus has been discovered at different geographic locations parasitizing eggs of different plant nematodes (Mankau 1980; Manzanilla-Lopez et al. 2013; Jatala 1986; Stirling 1991). The infection process of *P. lilacinum* starts with colonization on the gelatinous matrix of eggs or egg mass. Over the smooth egg surface, the hyphae become prostrate or spiral. The hyphae with appressorium readily penetrate nematode eggs, mostly through a minute pore in the vitelline layer (Morgan-Jones et al. 1984; Jatala 1986; Dunn et al. 1982). The eggs swell due to a change in shell permeability caused by *P. lilacinum* infection, and several ultrastructural alterations take place (Zaki 1994). Due to infection, the growth and development of the juvenile are stopped (Stirling 1991). Fungus also frequently attacks sedentary saccate females of *Meloidogyne, Heterodera, Globodera*, and other species by entering through the natural opening (Jatala 1986). The effectiveness of *P. lilacinum* against root–knot nematodes has been examined on a range of crops (Verma and Khan 2004; Khan et al. 2005; Khan 2016). The soil application of *P. lilacinum* effectively reduced the galling incited by *M. javanica* in tomato (Khan and Esfahani 1992). The percentage of infected eggs was significantly higher than the females. The fungus also colonized on unhatched larvae inside the eggs.

Khan and Akram (2000) observed that soil application of *P. lilacinum* successfully minimized the galls/ root system incited by *M. incognita* and increased production of tomato plants. In other studies also, the soil treatment with *P. lilacinum* was recorded to control root–knot in tomato (Khan and Saxena 1997), okra, betel vine (Nakat et al. 1995), cardamom, and black pepper (Sosamma and Koshy 1997). Khan and Ejaz (1997) carried out various field trials and examined the effectiveness of *P. lilacinum*, dried neem leaves, and aldicarb treatment in soil on root–knot in okra caused by *M. incognita*. The fungus treatments reduced gall formation, egg mass production, and fecundity less than the aldicarb treatment, but increased the yield of okra more than the nematicide treatment. They also found the eggs, egg masses, and adult female *M. incognita* infected with *P. lilacinum*. The fungus can be raised on a variety of waste materials, food grains, and oil cakes for field application. The studies on this aspect have shown that *P. lilacinum* can grow quickly on mung bean husks, sesame oil cake, wet rice, or wheat grains (Sharma and Trivedi 1987). The fungus colonized wheat or rice seeds with significantly decreased tomato and eggplant infection (Sharma and Trivedi 1989). Application of 2 g fungus-infested bran (4.14×10^8) decreased 89% root galling caused by 1000 juveniles of *M. incognita* on chili.

12.14 Mycorrhizal Fungi

Mycorrhizal fungi are a highly efficient group of plant root symbionts, invariably infect the roots of those that grow in their native environments (Xie et al. 2022). The symbiotic relationship that develops between these fungi and roots boosts capacity of plants to absorb phosphorus, other minor nutrients, and water without causing any disease (Harley and Smith 1983). In addition to producing plant growth hormones, mycorrhizae defend host roots against pathogenic infections (Benjamin et al. 2022), including those caused by nematodes (Francl and Wheeler 1993). On the basis of hyphal development and association on the root, mycorrhizal fungi and grouped as ecto or endo-mycorrhizal fungi. Ecto-mycorrhizal fungi create a "fungus mantle," or outwardly on the root surface, a densely interwoven association network. This type of association is developed by several Agaricales of Basidiomycetes, including *Amanita, Leccinum, Russula, Suillus*, as well as a few genera of Ascomycetes and Zygomycetes. The "lower fungi" that create endo-mycorrhizae are typically members of the Zygomycete family Endogonacea. These fungi typically cohabit with angiosperms and develop internally in the cortical cells of feeder roots, either by developing huge, swelling food-storing hyphae termed "vesicles" or by developing specialized feeding hyphae (haustoria) known as "arbuscules." The vesicles may or may not be formed; hence, it is more appropriate to call endomycorrhizal fungi as arbuscular mycorrhizal (AM) fungi. Five genera, *Acaulospora, Endogone, Gigaspora, Glomus*, and *Sclerocystis,* are the important AM fungi (de Moura et al. 2022).

Studies have shown that mycorrhizal fungi have an antagonistic impact on nematode parasitism (Jalali and Jalali 1991; Sharma and Trivedi 1994; Siddiqui and Mahmood 1995; Ambo et al. 2010; Khan et al. 2017). The spores and mycelium of *Glomus* spp. have frequently been seen in the eggs and adult sedentary female nematodes (Tribe 1979). Kellam and Schenck (1980) recorded that inoculation of AM fungus, *G. macrocarpus* on the soybean, resulted to formation of fewer galls of *M. incognita*. Sharma and Trivedi (1994) and Bagyaraj et al. (1979) observed reduced *M. incognita* or *M. hapla* infection on tomato roots colonized by *G. fasciculatum*. In the field tests, it was discovered that *Glomus intraradices* and *Gigaspora margarita* successfully compensated cotton yield loss caused by *M. incognita*. The reduction in the gall formation was greater due to pre-inoculation of cowpea plants with *G. fasciculatum* (Jain and Sethi 1988). It has also been observed that mycorrhizal fungi cause degeneration of nematode-feeding sites (Sikora 1979). In tomato roots, it was noticed that the multi-nucleated giant cells induced by *M. incognita* developed relatively slower which affected the nematode development. Further, it has been observed that mycorrhizal fungi caused adverse allelopathic effect on the movement and host-finding capacity (probing) of *M. incognita* which resulted to decrease in the nematode penetration in egg plants (Khan et al. 2017; Curtis et al. 2009).

12.15 Root Nodule-Forming Bacteria

The nodule-forming bacteria, *Bradyrhizobium* and *Rhizobium* species, which form important symbiotic associations with plant roots from Fabaceae, cause adverse impact on the pathogenesis of nematodes (Taha 1993; Khan et al. 2016; Khan et al. 2017). However, a lower portion, 7–10% of the known rhizobia strains have proven to be able to combat plant nematodes (Sikora and Carter 1987). The nodulation partially prevents the galling and egg mass production of *Meloidogyne* spp. (Huang 1985). In contrast, *Heterodera, Meloidogyne* spp. have been frequently observed invading the nodules. Such nodules disintegrate earlier than the healthy ones (Taha and Raski 1969), as a result the sedentary female dies prematurely before producing the egg masses (Khan et al. 2018a, b). The soybean, chickpea, and lentil plants treated with rhizobium developed lesser galling incited by *M. incognita/ M. javanica* (Taha 1993). Negative effects were also observed on the nematode reproduction. It shows that the root nodulation offers some self-defense to plants against the invasion of *Meloidogyne* species in pulse crops with 9–23% lesser galling in pulse crops (Khan et al. 2017).

12.16 Biocontrol Bacteria

Relative to fungi, lesser number of biocontrol bacteria have been found antagonistic to phytonematodes, although bacteria constitute a major component in the microbial community in soil. The biocontrol bacteria can suppress plant nematodes in different ways; accordingly, three categories of biocontrol bacteria can be formed, i.e., nematoxic metabolite-producing bacteria, nematode-parasitizing bacteria, and plant growth-promoting bacteria.

12.17 Nematoxic Metabolite-Producing Bacteria

Usually, metabolites produced by microorganisms are toxic to other living organisms. Nevertheless, nematode management can make use of metabolic byproducts, enzymes, toxins, and other substances that are detrimental to nematodes (Khan et al. 2012; Kumar et al. 2016). *Tylenchorhynchus martin* population declined in saturated rice field because of volatile fatty acids, particularly butyric acid produced by *Clostridium butyricum* (Johnston 1957). Other toxic substances such as hydrogen sulfide and ammonia are synthesized by *Desulfovibrio desulfuricans*, *Nitrosomonas,* and *Nitrobacter* species which may adversely affect the nematode activity in the soil (Rodriguez-Kabana et al. 1965; Zavaleta-Mejia 1985; Roblin et al. 2023). Avermectins, an antibiotic produced by some bacteria, particularly those that belong to the actinomycetes, have been discovered to be extremely toxic to nematodes (Burg et al. 1979; Omura 1986). More than 800 actinomycetes were subjected to a screening program, and it was discovered that about 10 to 15 of them exhibited nematicidal activity. It was discovered that the tested nematode species were suppressed by the antibiotics, valinomycin produced by *Streptomyces annulatus* (Mishra et al. 1987). Juvenile mortality was caused by the metabolites produced by some rhizobacteria (Becker et al. 1988).

Bacillus thuringiensis produces delta endo toxin, which is very harmful to insects and other invertebrates. The same toxin can also cause death to root–knot nematode juveniles (Khan and Tarannum 1999). The *B. thuringiensis* sub sp. *kurstak*, strain Dipe and SAN 415, suppressed *M. javanica* infection (Osman and Viglierchio 1988). The root–knot of tomato in greenhouses and fields was successfully controlled by another isolate of *B. thuringiensis* CR-371 (Zuckerman et al. 1993). A bioproduct of *B. thuringiensis*, Thuricide at 0, 0.25, 0.5, and 0.75% concentration significantly inhibited *M. incognita* juvenile emergence from eggs (Chahal and Chahal 1993). *Bacillus subtilis* produces bulbiformin (Brannen 1995) and other toxins (Keuken and Sikora 1995), which have been reported to cause mortality to *Meloidogyne* juveniles (Merriman et al. 1974; Azlay et al. 2022). The chickpea plants treated with *B. subtilis* showed increased growth and decreased root galls induced by *M. incognita* (Siddiqui and Mahmood 1995). The *B. subtilis* treatments applied to the field as soil or root-dip treatment greatly increased the growth and yield of tomato plants and significantly lowered the galling and multiplication of *M. incognita* (Khan and Tarannum 1999).

12.18 Nematode Parasitizing Bacteria

Pasteuria species are true parasites of plant parasitic nematodes. The *P. penetrans* (Sayre and Starr 1985), *P. thornei* (Starr and Sayre 1988), and *P. nishzawae* (Sayre et al. 1991) are the three nematode parasitic species, and the former has shown the highest promise for being an effective parasite of phytonematodes. The *P. penetrans* is considered as group which contains obligate nematode parasites that develop mycelium and endospores (Davies et al. 1988; Stirling 1991). The bacterial life cycle begins with the attachment of spores with the cuticle of nematode larvae (Sayre and Wergin 1977). The spores incumbered nematode juveniles show relatively slow movement but, by and large, they penetrate and feed normally (Davies et al. 1988). The spores germinate and the germ tube enters the nematode cuticle at least a week after root invasion (Davies et al. 1988). The nematode larvae infected with *P. penetrans*, molts, and grow normally, but their ability to reproduce is impaired, and the females are unable to lay eggs (Sayre and Wergin 1977). Hence, root galls develop without egg masses. The bacterial spores are released into the soil when dead roots and infected females disintegrate. There may be up to million spores in a single female (Stirling 1991). Air-dried powder of *P. penetrans* parasitized root–knot nematode-infested roots is used as bacterium inoculum for lab or field application. The bacterium not only prevents nematode reproduction but also reduces the capability of the juveniles to spread infection (Davies et al. 1988). Without losing their infectiousness or viability, spore in air-dried root powder may resist a wide range of environmental conditions (Stirling et al. 1986; Bird et al. 1990). Although spores of the *P. penetrans* group adhere to the cuticle of up to 200 nematode species, each population of the nematode has a limited host range, with some being very specialized (Sayre and Starr 1985; Sturhan 1988); for example, *P. penetrans* parasitizes *Meloidogyne* spp. (Sayre and Starr 1985), *P. thornei* parasitizes *Pratylenchus* spp. (Sayre and Starr 1985), and *P. nishizawae* parasitizes *Heterodera glycines* (Sayre et al. 1991).

12.19 Plant Growth-Promoting Bacteria

The rhizobacteria being important biotic component of soil are involved in maintaining soil health. These microorganisms play active part in improving soil nutrition and plant growth, hence commonly called as plant growth-promoting rhizobacteria (Rizvi et al. 2022). The PGPR can also adversely affect the plant nematodes (Khan and Kounsar 2000; Khan et al. 2003). Recent researches have shown that plant growth-promoting bacteria, nitrogen-fixing bacteria (*Azotobacter,* *Azospirillum, Beijerinckia* species), phosphate-solubilizing microorganisms (*Aspergillus awamori, Bacillus megaterium, Penicillium digitatum, Pseudomonas striata*), iron-chelating pseudomonads, blue green algae, etc., may contribute in the biocontrol of nematodes (Khan et al. 2009, 2017). Santhi and Sivakumar (1995) reported that root-dip treatment with *P. fluorescens* PF- I significantly increased tomato growth and decreased galling by *M. incognita*. The field application of *P. fluorescens* PRS-9 or root-dip treatment had similar effects on *M. incognita* and yield of tomato plants. Khan and Akram (2000) observed that application of *Azotobacter chroococcum* and *Bacillus polymyxa* suppressed *M. incognita* and accelerated the plant growth of tomato. Under field conditions, the effects of seed treatment with *A. chroococcum*, *Azospirillum lipoferum, Bacillus subtilis*, and *Beijerinckia indica* were evaluated. The soil application with *B. subtilis* significantly decreased the galls and increased mungbean yield by 20–26% (Khan and Kounsar 2000; Khan et al. 2003). The treatments with blue green algae, *Nostoc calcicola,* resulted in significant enhancement in the plant growth and reduction in egg mass production. However, a combination of *Anabaena oryzae*, *N. calcicola*, and *Spirulina* spp. were found significantly better in controlling the nematode infection and stimulating cowpea plant growth (Youssef and Ali 1998). Khan et al. (2017) examined relative antagonism of different *Pseudomonas* spp. against *M. incognita* infecting mungbean and observed that seed treatment with *P. fluorescens* caused 20–30% decline in the galling and reproduction of the nematode, and 31% increase in the grain yield.

12.20 Biotechnological Approaches

Biotechnological approaches with regard to nematode management are mainly aimed to exploit some of the fancy and novel approaches at gene and sub-gene level, like resistance for disruption of feeding cites, expression of specific peptides or proteins, transfer of toxic compounds to the infesting nematode or gene silencing, etc. (Abd-Elgawad 2022). The existing biotechnological approaches largely rely on large-scale germplasm screening, along with using molecular markers to identify genes of desired characters, and their ultimate transfer into a plant species/cultivar. Transgenic methods for root–knot control, on the other hand, use knowledge of nematode-host interactions to target the nematode, such as disorienting infective stages to prevent nematodes from locating host root, affecting the ability to sense the host stimuli for mobility toward the susceptible host tissues, limiting invasion in host cells, or decreasing nematode ability to move and feed, (Lu et al. 2022). One main goal of discovering pathogen resistance genes (R genes) is to incorporate them into the other economically important vulnerable crops/ cultivars in order to increase agricultural output and quality while reducing expenses and dependency on chemical nematicides. Some pathogen resistance genes have been successfully deployed, e.g., Mi gene-bearing tomato plants. However, effective transfer of defined R genes into a new cultivar is limited to a few instances (Williamson and Kumar 2006). It seems that the efficiency of the genes in heterologous system depends on the successful signaling in the pathways that produce a hypersensitive response may require numerous components, some of which may not be found in a species. Transferring the Mi gene to eggplant, for example, provides resistance against *M. javanica* but not against the aphids. Even in Mi gene-bearing tomato plants, resistance against *M. incognita* varies due to genomic background of the cultivar (Jacquet et al. 2005). A deeper knowledge and more research on nematode resistance mechanisms are needed that could provide effective insertion of R genes into the other profitable crops. Some of the important biotechnological approaches are discussed under:

12.21 Transgenic Approaches to Control Root–Knot Nematodes

Disruption in the establishment or operation of feeding sites: Since it was discovered that the reproduction of sedentary nematodes, *Meloidogyne*, *Globodera, Heterodera,* etc., depends on the successful development and maintenance of nurse cells (Jones 1981), concerted efforts have been in progress to induce disruption in feeding cell development using RNAi-based techniques that target nematode capacity to induce feeding sites (Menezes et al. 2022). The success of this technique is highly dependent on discovering plant promoters that are responsible for nurse cell development and may be associated to production of a gene toxic to feeding cell development causing death or damage to the cells. Opperman et al. (1994) were the first to show that the shortened (D0.3 kb) water channel protein promoter TobRB7 was produced particularly in the giant cells produced by the root–knot nematodes and resulted in cell death when connected to the cytotoxic ribonuclease barnase. Although some genes have been identified which may dysregulate nurse cells, like the thermal shock promoter Hahsp17.7G4 (Escobar et al. 2003). Wang et al. (2008) proposed an alternate technique using two nematode-sensitive promoters, which may circumvent the problem of specific cell specificity of expression.

Nematode resistance based on RNAi: The finding of RNAi in nematodes and thus silence gene expression crucial for their expansion or invasion processes is a viable and eco-friendly approach to supplement current nematode control methods (Tan et al. 2013). Urwin et al. (2002) observed that soaking of nematode juveniles (J_2s) in the neurostimulant solution shall lead to the absorption of enough dsRNA in the J_2 body to cause RNAi. The dsRNA soaking/feeding technique has been employed to study the consequences of downregulating with more than 30 essential and parasitism genes in *M. incognita, M. hapla, M. javanica, M. artiellia,* and *M. arenaria* (Tan et al. 2013). However, it has recently been established that the use of neurostimulant and other substance is not required to induce RNAi using dsRNA (Kimber et al. 2007). This way of dsRNA distribution seems to be an appropriate and cost-effective method of controlling obligate sedentary nematodes. Yadav et al. (2006) demonstrated that delivery of dsRNA to two specific genes to decrease *M. incognita* replication on genetically engineered plants. Huang et al. (2006) successfully transferred dsRNA to an *M. incognita* activator enzyme in transgenic crops. As a result, reproduction of nematode was inhibited.

12.21.1 RNAi and Its Application in Root–Knot Nematode Management

RNA interference (RNAi) involves the use of sequence-specific, homologous RNA molecules to decrease gene expression. Guo and Kemphues (1995) were the first to report this phenomenon in *C. elegans*. Fire et al. (1998) demonstrated that the improvement of dsRNA, in the interfering action. Silencing of gene occurs in a variety of eukaryotic species, including plants and nematodes (Hammond et al. 2001). Klink and Wolniak (2001) revealed that dsRNA generated in vitro could knockdown centering mRNA and that dsRNA was at least 10 times more efficient than sense RNA or antisense RNA for knockout effects.

Dicer generates siRNAs in plants by dsRNAs derived from nematode genes. Plant-derived dsRNAs or siRNAs are picked up via stylets as they feed on plants, and RISC binds siRNAs to promote the destruction of particular nematode genes. Chapman and Carrington (2007) reported that siRNAs may subsequently get intensified in the nematode using RNA-dependent RNA polymerase. The gene silencing induced by siRNA is very sequence-specific. For example, it was revealed that a single nucleotide mismatch between a siRNA and its mRNA target inhibited gene silence (Elbashir et al. 2001). The RNAi travels throughout the plant body (Kehr and Buhtz 2008; Yoo et al. 2004) through the phloem and spreads via plasmodesmata (cell to cell) (Mlotshwa et al. 2002). Hence, the RNAi impact may migrate both locally and long distances (Himber et al. 2003). Limpens et al. (2004) discovered that silencing signals were carried systemically from roots to shoots of Arabidopsis. However, releasing these GM nematodes into the environment would face severe regulatory barriers.

Kimber et al. (2007) observed that dsRNA molecules of 42 to 1300 bp sizes have been found to be efficient in inducing RNAi in *Meloidogyne* spp. For example, the transgenic *Arabidopsis* plants that expressed dsRNAs from the full-length (271-bp) and truncated (42-bp) 16D10 genes showed a significant decrease in root–knot galls (Huang et al. 2006). Sopping nematodes in dsRNA can result in RNAi because or it may be ingested during soaking. Ingestion of dsRNAs is problematic since sedentary nematodes normally feed only after establishing giant cells within the root tissue. Several groups have effectively repressed mRNAs of specific genes of nematode and decrease the quantity by soaking root–knot nematodes in dsRNAs (Shingles et al. 2007). Other group of researchers have successfully reduced nematode growth with or without the use of various substances to stimulate absorption (Park et al. 2008).

12.21.2 Host-Delivered RNAi to Silence Nematode Genes

Evidences support the viability and efficacy of host-delivered RNAi for management of nematodes (Lilley et al. 2012). The RNAi was prompted in *M. incognita* by utilizing dsRNAs of two encoding genes, a splicing factor and an integrase, resulting in protection against nematode infection (Yadav et al. 2006). Resistance to four main root–knot species was achieved by expressing the gene 16D10 dsRNA in transgenic

Arabidopsis plants (Huang et al. 2006). Real-time RT-PCR research revealed direct molecular evidence of target nematode genes are downregulated by host-derived RNAi in nematodes living on transgenic roots (Li et al. 2010a). Chimeric hairy root systems or a composite have been constructed in numerous systems, that includes soybean, sugar beet, and tomato, to swiftly analyze target genes in plants (Remeeus et al. 1998). Li et al. (2010b) used a composite hairy root technique to cut the screening procedure by 8 months when compared to the typical soybean transgenic methodology. Li et al. (2010a, b) and Klink et al. (2009) developed the gateway cloning approach for RNAi building, which significantly shortened the time required to convert genes into RNAi constructs.

12.21.3 Characterization of Target-Specific Genes

Many groups of researchers have attempted to use an RNAi technique to inhibit the nematode life cycle, prevent infection, or to block the translation or transcription procedure of nematode genes in order to control root–knot. A variety of genes from the nematode have been targeted for silencing of gene in recent years. As a consequence of gene silencing studies, the specific genes have been identified and successfully targeted to suppress the nematode population. The target genes are classified into three types: genes parasitism, developmental genes, and metabolism-related mRNA genes, which are discussed under:

Parasitism genes: Several enzymes encoded by nematode parasitism genes are released into plant tissues via the nematode stylets during the feeding on the host tissue. The investigations show that the RNAi parasitism genes have critical roles to play in plant invasion by nematodes. Bakhetia et al. (2005) studied the significance of twin oxidases (peroxidase and NADPH oxidase) linked with nematode extracellular matrix in *M. incognita* using in vitro RNAi experiments. Some phytonematodes secrete proteasome members, including SKP-1 and Ring-H2, along with ubiquitin-like enzymes, which may influence host cell enzyme breakdown for parasitic purposes (Sindhu et al. 2009).

Developmental genes and RNAi genes: Genes governing in the development of nematodes, particularly those involved in the embryogenesis, molting and reproduction, are of specific interest as they have the potential to disturb the parasitic phase of nematode life cycle. Egg hatching of *M. artiellia* was delayed as RNAi repressed a chitin synthase gene, which is vital for formation of chitin in egg membrane (Fanelli et al. 2005). RNA interference (RNAi) of nematode genes appears to be particularly efficient to reduce the development of nematodes.

12.22 Other Functional Genomics Approaches for Helping Target Gene Selection for Gene Silencing

12.22.1 Microarrays with Laser Capture Microdissection

Expression of some genes, such as microarray and laser capture microdissection, were used to explore plant nematode interaction in order to increase host resistance against nematodes. A comparative microarray study was conducted utilizing detection call methods to discover genes that may be cell-type particular and engaged in crucial elements of nematode plant interactions (Klink et al. 2010). The sequencing of key genes from root–knot nematodes the unrestricted sequencing of approximately 250,000 ESTs from 30 species of nematodes, with >100,000 plant parasitic ESTs (McCarter et al. 2003). The genome of *M. hapla* (Opperman et al. 2008) and *M. incognita* (Abad et al. 2008; McCarter 2008) have been mapped and sequenced. All these genomic and EST sequences have given incredibly useful information that will allow investigators to locate viable target genes for future RNAi trials.

12.22.2 Artificial microRNAs (amiRNAs)

MicroRNAs (miRNA) are another approach for gene silencing. Brodersen and Voinnet (2006) reported that dicer synthesizes miRNAs from small MiRNA precursors (pre-miRNA) with hairpin shapes were formed from longer main miRNA transcript (pri-miRNA). Mature miRNAs are single-stranded small RNA molecules that exist in the body that are 20–24 nucleotides long and are integrated into RNA-induced silencing complex (RISC) to direct mRNA deprivation. Changing a few nucleotides inside a sequence of 21-nt miRNA may not affect its biogenesis (Vaucheret et al. 2004). Following this research, artificial miRNAs (amiRNAs) have been demonstrated to selectively control expression of gene through RNA silencing in a variety of plant species including rice (Wang et al. 2010), *Nicotiana benthamiana* (Tang et al. 2010), and *Arabidopsis* (Li et al. 2010a, b). The key benefit of amiRNA carrier over standard RNAi vectors having inverted repetitions of specific genes is that amiRNA carrier can result in more precise silencing of gene since they include a single 21-nt complementary sequence to the specific gene and can be a significant factor in RNAi research (Sukno et al. 2007).

12.22.3 Small RNA Sequencing

Small RNAs, particularly microRNAs (miRNAs), are involved in a variety of morphological and physiological processes through computational and cloning techniques. The deep sequencing in mammals, plants, fungus, and protozoa has aided in understanding the small RNA regulation processes (Wu et al. 2010). Small RNA sequencing data may provide information on, for example, how does endogenous gene suppression work in a crop (Houmard et al. 2007; De et al. 2009).

Sequencing of small RNAs from transgenic plants may give insight into the populations of siRNA species created and may aid in the identification of particular target sites for the building of artificial miRNA vectors. No data on short RNA sequencing from transgenic plants producing dsRNAs from nematode genes or from nematodes parasitizing such plants have been reported. Nonetheless, short RNA sequencing is an emerging discipline that would yield further knowledge on small RNA regulation systems in crop plants and other organisms in the future.

12.23 Conclusions and Future Perspectives

There are several promising research prospects in fundamental and applied nematology that make use of novel techniques and tools of molecular biology for root–knot management in vegetable and ornamental plants. These tools may make possible study on previously intractable topics in nematode biology that were previously unavailable due to technological constraints. Nematicides, cultural methods, resistant cultivars, and crop rotations are conventional methods of nematode management and do not always provide effective control. Future integrated nematode management will rely on a variety of control measures to reduce nematode populations in agricultural fields. Novel biological approaches such as biocontrol, and the application of biotechnology will have a impact on nematode identification, development of resistant cultivars, improving effectiveness of biocontrol agents, etc., which would ultimately improve efficiency of nematode management programs, especially aimed against root–knot nematodes in vegetables and ornamentals.

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Root–Knot Nematodes in Cereal and Pulse Crops, and Their Management by Novel Biological and Biotechnological Approaches

13

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Abstract

The root–knot nematode, *Meloidogyne* spp., is a most significant pest of food and commercial crops world over. The infestation with *Meloidogyne* spp. in pulse and cereal crops is an important constraint in the production of these two very important groups of crops. All pulse crops especially chickpea, pigeon pea, moong bean, urd bean, pea, and lentil are severely invaded by *M. incognita, M. javanica,* and *M. arenaria,* whereas the cereals are attacked by *M. graminicola, M. oryzicola*, *M. naasi, M. incognita,* etc. The root–knot nematodes cause substantial yield loss to cereals and pulses, particularly under tropical and subtropical climates. Various strategies, including cultivating resistant crops, using nematicides, and cultural practices practised world over for nematode management, are conventional methods, and do not always prove effective. The present chapter offers up-to-date information on biological and biotechnological methods for management of *Meloidogyne* infestation in cereal and pulse crops along with distribution, symptoms, biology, and life cycle of the nematode.

Keywords

Meloidogyne spp. · Biocontrol · Biotechnology · Cereals · Pulses

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13.1 Introduction

Cereals belong to Poaceae (grass family) and are grown for their endosperm, which provides food and energy to humans and animals more than any other crop (Arvanitoyannis and Tserkezou 2008). Productivity of cereals is higher than other crop groups, and are directly consumed by humans and constitute a significant portion of livestock feed, contributing significantly to dairy and meat production. The seven most important cereals grown worldwide are rice (*Oryza sativa*), wheat (*Triticum aestivum*), maize (*Zea mays*), barley (*Hordeum vulgare*), oats (*Avena sativa*), *Secale cereale* (rye), and sorghum (*Sorghum bicolor*). Similarly, pulses are also very important crops as they add important proteins and minerals to the human diet as well as from animal feed. Plant diseases incited by fungi, bacteria, viruses, and nematodes cause significant yield loss to all kind of agricultural crops including pulses and cereals. Plant–parasitic nematodes are potential pathogens of cereal and pulse cultivation.

Rice is the most important staple food crop in Asia, where over 90% of the global rice is produced. The processed rice (milled) contains carbohydrate (10%), protein (5%), and small amounts of fats as well as thiamine, niacin, riboflavin, iron, and calcium. The cereal next to rice is wheat which is widely cultivated for its grains and forms a staple food world over (Thurston 2020). Wheat is a primary nutrition source to millions of people globally. Nutritionally, it contains proteins, vitamins, and carbohydrates which provide a balanced food diet. The world leading countries in wheat production are Russia, USA, China, and India. However, wheat is cultivated throughout the world.

Maize (*Zea mays*) also called as “Queen of Cereals” is a highly adaptable crop, as it thrives in a wide range of agro-climates. Besides, being world’s third most important cereal food for humans and high-quality animal feed. It also provides raw materials for numerous industrial products. Barley (*Hordeum vulgare*) is an important rabi cereal crop grown world over after wheat, rice, and maize. It is primarily cultivated in temperate and semi-arid regions. It is believed that barely is originated from the Middle East. Barley was once widely grown for human consumption, but today it is also produced for animal feed and malt products. Asia is the largest producer of barley, after Europe. Barley is rich in starch, minerals, vitamins, and protein. Oat (*Avena sativa*) is an important cereal and fodder crop. Oats can thrive in high-altitude tropical climates and are grown in temperate and subtropical regions. Oatmeal is a typical food, and oats are well known for their health advantages. It contains a lot of fiber and proteins. Additionally, they support blood pressure management, weight loss, and immune system development. Major producers of oats are European countries, Russia, Canada, Australia, United Kingdom, and Brazil (STATISTA 2022).

Pulse crops, also known as grain legumes, belong to the family Fabaceace. These crops are grown throughout the world with India being the largest producer of pulses. These crops require less moisture to survive and can grow even in dry conditions. Pulses crops also help restore soil fertility by fixing nitrogen from air. Important pulse crops are chickpeas (*Cicer arietinum*), pigeon peas (*Cajanus cajan*), beans (*Phaseolus vulgaris*), mung beans (*Vigna radiata*), urd beans (*Vigna mungo*), lentils (*Lens culinaris*), pea (*Pisum sativum*), etc. Pulses supply extremely good quality protein to the human diet, as well as animal feed.

Chickpea is primarily grown in India, Pakistan, Ethiopia, Burma, Turkey, Mexico, Iran, and other regions of the Indian subcontinent. India is the topmost producer of chickpea, followed by Pakistan. In India, chickpea accounts for near 50% of all pulse production and about 35–40% of the land used for pulse farming. It is fed to animals and used for human consumption. Fresh green leaves and grains are used as vegetable, while chickpea straw forms an excellent fodder for cattle. Pea (*Pisum sativum*) is grown for its green pods, from which green grains are used as vegetable or in soup, canned, frozen, or dehydrate grains. Dried split grains are commonly used for dal. Pea is third most important winter season crop next to chickpea and French bean. Pigeon pea is commonly known as red gram or Arhar, in tropical and semitropical regions and is particularly popular in South Asia, Southeast Asia, Africa, Latin America, and the Caribbean. It is mainly consumed as a split pulse known as "dal." Pigeonpea seeds contain iron, iodine, and essential amino acids such as lysine, threonine, cystine, and arginine.

Green gram or mung bean or moong has an origin in Indian subcontinent. It is grown in Asia, Africa, South America, etc. The largest producer is India, followed by China. It can be cultivated as a Kharif crop as well as a summer crop. It is a proven fact that mung beans are fat controllers and blood pressure regulators. Black gram also known as Urd bean is an important drought-tolerant pulse crops, grown in diverse climatic conditions in America, Africa, and Asia. It is rich in protein and carbohydrates. Lentil, also known as masoor, is originated from the Middle East. India is the largest producer of lentils. Lentil is a good source of protein and rich in dietary fibers, which helps in weight control and healthy digestion. Its straw is fed to the animals.

13.2 Nematode Infestation in Cereals

The plant parasitic nematodes associated with cereals have diversified parasitism, habitats, feeding behaviours, and pathogenicity (Owen et al. 2023). Some of these nematodes feed on roots as migratory endoparasite such as *Pratylenchus* and *Hirschmanniella,* whereas *Ditylenchus angustus* and *Anguina tritici* feed migratory ecto and endoparasitically on foliage. *Tylenchorhynchus*, *Helicotylenchus, Criconemella* are ectoparasites on roots, while *Aphelenchoidees basseyi* feeds ectoparasitacially on leaves and floral parts. In addition, sedentary endoparasites of roots, *Meloidogyne* spp. and *Heterodera* spp., are commonly encountered on cereals and pulses attacking their roots.

Plant nematodes have been reported to be highly harmful to temperate cereals and economically affect their productivity especially wheat, barley rice etc. (Dababat and Fourie 2018; Dababat et al. 2020). The *Heterodera* and *Pratylenchus* are two important genera in cereals because of their wide distribution and host range (Rivoal and Cook 1993; Jones et al. 2013). *Pratylenchus thornei* and *P. neglectus* are frequently encountered in cereal fields (Castillo and Vovlas 2007; Riga et al. 2008). The attack of *Heterodera avenae*, *H. oryzae*, *H. filipjevi*, *H. hordie,* etc., is quite prevalent in cereals (Subbotin et al. 2010). *Meloidogyne artellia*, *M. naasi, M. incognita, M. graminicola,* etc., are important root–knot nematodes infesting cereals (Owen et al. 2023).

Pulses are highly susceptible to the invasion of nematodes, and over 100 species of plant nematodes are recorded from the root zone of pulse crops (Ali et al. 2010). The nematodes from *Heterodera*, *Meloidogyne*, *Paratylenchus*, *Rotylenchulus*, *Helicotylenchus, Tylenchorhynchus,* etc., are commonly found invading pulse crops world over (Khan et al. 2023). *Meloidogyne incognita, M. javanica, M. arenaria* and *Heterodera ciceri, H. cajani,* and *H. glycines* are the major species of sedentary endoparasites prevalent in pulse cultivation (Brair et al. 2023). In addition, infestation with *Rotylenchulus reniformis* (Sitaramaiah 1984), *Ditylenchus dipsaci* (Greco and Di Vito 1987), *Pratylenchus thornei, P. penetrans,* and *P. mediterraneus* have been quite prevalent in pulse fields (Di Vito et al. 1994). Detailed information on major nematodes attacking cereals and pulse crops has been presented under:

13.3 Infestation of *Meloidogyne* spp., in Cereal and Pulse Crops

13.3.1 Distribution

The cereals crop, including rice, wheat, maize, barley, ray, and oats, are vulnerable to the attack of *Meloidogyne* spp. especially *M. graminicola*, *M. graminis, M. kikuyuensis*, *M. spartinae, M. triticoryzae, M. arenaria, M. incognita, M. oryzae,* and *M. javanica* (Khan et al. 2014; Sikora et al. 2018a, b). In recent decades, *M. graminicola* (Golden and Birchfield 1968) has become serious pest of rice (Brair et al. 2023). In wheat and barley, *M. naasi* and *M. artiellia* inflict significant damage to winter-growing varieties (Nicol 2002; Owen et al. 2023). Similarly, *M. incognita*, *M. javanica,* and *M. arenaria* and *M. artiellia* are most economically important in pulse crops in tropical and subtropical areas (Khan et al. 2016) (Table 13.1).

13.4 *Meloidogyne* spp. Infesting Cereals

The important species of *Meloidogyne* infesting the cereal crops are elaborated under

13.4.1 *Meloidogyne graminicola*

M. graminicola is a significant pest of rice which is distributed widely in major rice-growing areas throughout the world in upland, deep water, and irrigated rice (Golden and Birchfield 1968; Bridge et al. 2005; Haque and Khan 2022a; Khan et al. 2023).

Table 13.1 *Meloidogyne* spp. infesting cereals and pulses

Meloidogyne spp.	Crop species	Yield loss	References
M. graminicola	Rice	16–80%	Khan et al. (2014), Peng et al. (2018)
	Wheat	2–20%	Dababat and Fourie (2018)
	Barley	Up to 15%	Dababat and Fourie (2018)
	Oat	2–8%	Dababat and Fourie (2018)
M. triticoryzae	Rice	17–30%	Peng et al. (2018), Bridge et al. (2005)
	Wheat	8–16%	Peng et al. (2018), Sikora et al. (2018a, b)
	Barley	Up to 10%	Peng et al. (2018), Sikora et al. (2018a, b)
M. oryzae	Wheat	7–10%	Peng et al. (2018), Dababat and Fourie (2018)
	Rice	6–19%	Peng et al. (2018), Sikora et al. (2018a, b)
	Barley	5–9%	Peng et al. (2018), Bridge et al. (2005)
M. chitwoodi	Wheat	10–15%	Santo and O'Bannon (1981), Dababat and Fourie (2018)
	Oat	Up to 37%	Santo and O'Bannon (1981), Dababat and Fourie (2018)
	Barley	Up to 25%	Santo and O'Bannon (1981), Dababat and Fourie (2018)
	Maize	0–5%	Santo and O'Bannon (1981), Dababat and Fourie (2018)
M. naasi	Wheat	15–20%	Suresh et al. (2017), Allen et al. (1970)
	Barley	Up to 10%	Suresh et al. (2017), Allen et al. (1970)
	Sorghum	Up to 25%	Suresh et al. (2017), Bélair et al. (2006)
M. artiellia	Wheat	3–7%	Imren et al. (2014)
	Barley	Up to 3%	Dababat and Fourie (2018), Nicol (2002)
M. incognita	Urd bean	19–22%	Ali and Askary (2005), Sikora et al. (2018a, b), Ali (1995)
	Chickpea	Up to 60%	Sikora et al. (2018a, b), Ali (1995), Freire et al. (1972)
	Lentil	17–20%	Sikora et al. (2018a, b), Ali (1995)
	Mung bean	17–57%	Ali and Askary (2005), Sikora et al. (2018a, b), Ali (1995)
	Pigeonpea	13%	Ali and Askary (2005), Sikora et al. (2018a, b), Ali (1995)
M. javanica	Urd bean	17–23%	Sikora et al. (2018a, b), Freire et al. (1972)
	Chickpea	19–22%	Sikora et al. (2018a, b), Freire et al. (1972)
	Lentil	8–12%	Sikora et al. (2018a, b), Siddiqui (2007)
	Mung bean	14–29%	Nadakal (1964)
	Pigeonpea	Up to 12%	Nadakal (1964)
M. arenaria	Urd bean	15–25%	Edwards (1956)
	Chickpea	16–22%	Sikora et al. (2018a, b)
	Lentil	Up to 13%	Edwards (1956), Ali (1993)
	Mung bean	42–90%	Nath et al. (1979)
	Pigeonpea	Up to 16%	Sikora et al. (2018a, b)

(continued)

Table 13.1 (continued)

Meloidogyne spp.	Crop species	Yield loss	References
M. artiellia	Urd bean	8–14%	Greco et al. (1984), Oteifa (1987)
	Chickpea	40–50%	Ali (1995)
	Lentil	18–29%	Greco et al. (1984), Oteifa (1987)
	Mung bean	18–75%	Greco et al. (1984), Oteifa (1987)
	Pigeonpea	Up to 12%	Greco et al. (1984), Oteifa (1987)

The wide occurrence of *M. graminicola* in rice fields has been recorded throughout Asia, especially in India (Prasad et al. 2010; Khan and Ahamad 2020), China (Zhao et al. 2001), Pakistan (Munir and Bridge 2003), Bangladesh (Page et al. 1979), Nepal (Pokharel 2009), Sri Lanka (Nugaliyadde et al. 2001), Malaysia (Zainal-Abidin et al. 1994), Indonesia (Netscher and Erlan 1993), Philippines (Plowright and Bridge 1990), Thailand (Buangsuwon et al. 1971), Vietnam (Khuong 1983), Singapore, Italy, and Ecuador (Torrini et al. 2020), U.K. (Yik and Birchfield 1979). However, root–knot in rice occurs in a severe form in Asia, particularly in the rice-wheat cropping system (Rao et al. 1986; Padgham et al. 2004; Bridge et al. 2005; Khan and Ahamad 2020). It has been reported that *M. graminicola* populations in the Indo-Gangetic plains in Bangladesh, India, and Pakistan cause damage to wheat also (Soomro and Hague 1992).

13.4.2 *Meloidogyne naasi*

Root–knot nematode, *Meloidogyne naasi,* is an important nematode found infecting barley, wheat, and sorghum in temperate climates (Kort 1972; Suresh et al. 2017). The nematode is reported to in cereals Belgium (Gooris 1968), England and Wales (Franklin 1965), France (Caubel et al. 1971), Serbia, Yugoslavia (Grujiĉiĉ 1967), Malta (Inserra et al. 1975), Holland (Franklin 1971). In USA, the nematode has been recorded attacking wheat and barley in California (Allen et al. 1970), Illinois (Golden and Taylor 1967), Kansas (Aytan and Dickerson 1969), and Michigan and Oregon (Jensen et al. 1968). Further, *M. naasi* is found infesting barley in Maltese islands in the Mediterranean areas (Inserra et al. 1978). Oat is a poor host to *M. nassi* compared other cereals in Europe, whereas in the USA, oat is recorded to be highly susceptible to the nematode (Kort 1972). Similarly, barley crop is susceptible to *M. naasi*. and its yield is severely affected in northern Europe, USA, Canada, and former USSR (Bélair et al. 2006). However, barley has been found to be moderately affected by the nematode in Chile, Iran, the Maltese islands, New Zealand, and Turkey (Kort 1972; Inserra et al. 1975; Jepson 1987).

13.4.3 *Meloidogyne artiellia*

The nematodes attack cereals, crucifers (Di Vito et al. 1985), and legumes (Sikora 1988). The nematode has been reported to attach wheat in Greece (Kyrou 1969), wheat and barley in Syria (Mamluk et al. 1983), and wheat in the Mediterranean region (Philis 1978). This nematode has been found infesting wheat and barley in France, Greece, U.K., Italy, Spain, Russia, Israel, Syria, Morocco, Algeria, Tunisia, and Turkey (Imren et al. 2014; Dababat and Fourie 2018).

13.4.4 *Meloidogyne incognita* and *M. javanica*

Meloidogyne incognita and *M. javanica* are reported to infect rice especially the upland rice in many countries (Khan et al. 2023). The former species has been found infesting the upland rice in Costa Rica, Cuba, Egypt, Ivory Coast, Nigeria, South Africa, and Japan (Bridge et al. 1990) whereas *M. javanica* infecting paddy is reported in Brazil, Egypt, Comoro Islands, Nigeria, and Ivory Coast, *M. arenaria* in Nigeria, Egypt, and South Africa. In Costa Rica and Panama, *M. salasi* (Bridge et al. 1990). The irrigated rice is attacked by *M. oryzae*, in Surinam.

13.4.5 *Meloidogyne chitwoodi*

Meloidogyne chitwoodi is also considered an important pest of cereals in the USA (Northwest), Australia, Argentina, Turkey Mexico, and South Africa (Fourie et al. 2002; Elling 2013). This species attacks wheat, oat, barley, maize, and several dicots (Santo and O'Bannon 1981; Dababat and Fourie 2018).

13.5 *Meloidogyne* spp. Infesting Pulse Crops

Root–knot nematode, *M. incognita, M. javanica, M. Arenaria,* and *M. artiellia* are four major species attacking the pulse crops (Brair et al. 2023). These species inflict significant yield loss in chickpea, pea, pigeon pea, mung bean, urd bean, lentil, etc. (Ali et al., 2010; Sikora et al. 2018a, b; Brair et al. 2023).

13.5.1 *Meloidogyne incognita*

Meloidogyne incognita is the major pest of pulse crops in the pulse-growing areas world over (Niu et al. 2010). The nematode is a significant pest of chickpea in India, Pakistan, Brazil, Ethiopia, Bangladesh, and Nepal (Khan et al. 2018a).

13.5.2 *Meloidogyne javanica*

Meloidogyne javanica also creates a major problem in pulse crops world over, especially in Ethopia, India, Pakistan, Syria, Nepal, Brazil, Spain, Ghana, Italy, Malawi, USA, Zambia, and Zimbabwe (Nath et al. 1979; Ali 1995). The nematode has been reported to infest almost all pulse crops, pigeon pea and chickpea (Khan et al. 2011; Sikora et al. 2018a, b), black gram (Freire et al. 1972), lentil (Siddiqui et al. 2007), green gram (Khan and Khan 2000; Khan et al. 2002), etc.

13.5.3 *Meloidogyne arenaria*

Meloidogyne arenaria is considered as an important nematode pest of pulse crop in India, Pakistan, Nepal, Syria, Brazil, Ghana, Italy, Malavi, USA, Zambia, and Zimbabwe (Ali 1995). It is prevalent in all pulse-growing area worldwide. Its host range includes ground nut, chickpea, pigeon pea, mungbean, urdbean, and lentils (Sikora et al. 2018a, b).

13.5.4 *Meloidogyne artiella*

Meloidogyne artiella is reported to be the pest of economic significance in chickpeas in Spain, Brazil, Italy, USA, India, Nepal, Pakistan, Syria, Malawi, Ethopia, Ghana, Zambia, and Zimbabwe (Nath et al. 1979; Ali 1995).

13.6 Symptoms of *Meloidogyne* Infestation

The infected plants show stunting, and wilting and yellowing of leaves, which can be confused with the signs of nutritional deficiency (Fig. 13.1). *Meloidogyne graminicola* causes severe damage in nursery beds of paddy. The seedlings become stunted, sparce, and distinctly chlorotic (Fig. 13.1a).

The typical symptom incited by *Meloidogyne* spp. on underground parts is the formation of root galls (Figs. 13.2 and 13.3; Khan et al. 2021). The plant shows aboveground symptoms in patches, which are irregularly distributed in the field. In cereal crops, *Meloidogyne* spp. cause terminal galls at the tip of the monocot roots. For example, *M. graminicola* causes terminal galls on the roots of rice, which are curved, horseshoe, or spiral-shaped (Fig. 13.1; Khan et al. 2011; Haque and Khan 2022b). In heavily infected plants, the emerging leaves appear fuzzy, distorted, and wrinkled along the margins, and mature early (Dutta et al. 2012; Haque and Khan 2022b). On the wheat roots also, horseshoe to complete spiral/curly galls are formed (Fig. 13.1b). The nematode at high population density induces wilting to rice seedlings, and at later stage, severe reduction in the growth parameters may occur. Barley roots show galls that are horseshoe or spiral in shape and vary in size (Fig. 13.1c).

Fig. 13.1 Aboveground symptoms caused by *Meloidogyne graminicola* to rice nursery (**a**), and paddy field (**b**) and *M. incognita* to chickpea field (**c**). (Source: **a**–**c**: M. R. Khan, Aligarh Muslim University, India)

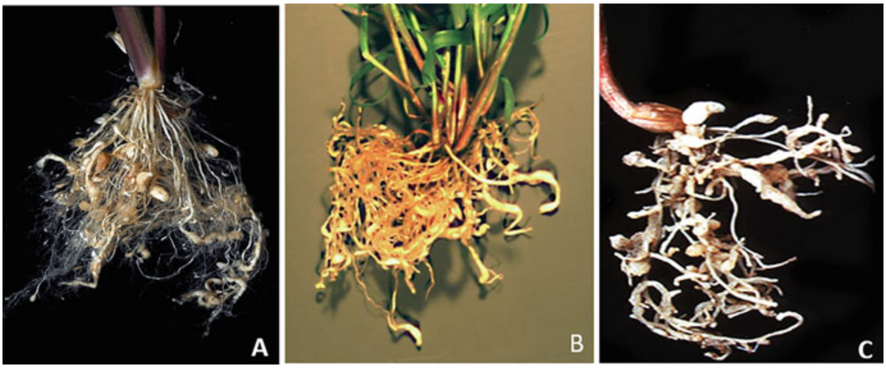

Fig. 13.2 The root galls caused by root–knot nematode on rice (**a**), wheat (**b**), and barley (**c**), (Source: **a**: M. R. Khan, Aligarh Muslim University, India; **b**: Jonathan D. Eisenback, Virginia Polytechnic Institute and State University Bugwood.org; **c**: G. Caubel, Institute National de la Recherche Agronomique, Bugwood.org)

Contrary to cereals, the root galls in pulse crops are oval, semi-oval, oblong, small or large, and fleshy (Fig. 13.3b). The galls in pulses generally have brownish gelatinous egg mass attached to the gall, whereas in cereals eggmass may not be visible on the surface of the gall. The galls on the roots of pulse crops are oval, large, fleshy, and rarely terminal in position but never hook-like (Fig. 13.3a–c; Khan et al. 2005, 2007; Brair et al. 2023). The nematode infection also affects the root

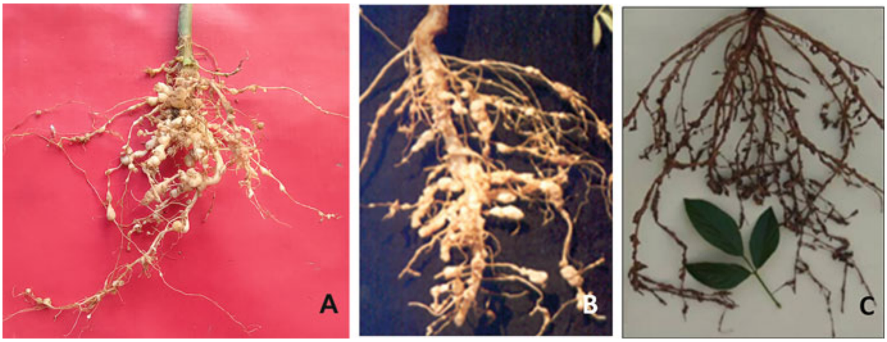

Fig. 13.3 The root galls caused by root–knot nematode on mungbean (**a**), chickpea (**b**), and pigeonpea (**c**) (source: **a**–**c**: M. R. Khan, Aligarh Muslim University, India)

nodulation in pulse crops. The rhizobial nodules became smaller and fewer and sometimes invaded by the nematode (Khan et al. 2018a; 2019a, b).

13.7 Biology and Life Cycle

The infection is incited by the second stage juvenile (J_2), which emerges from the egg after having first moulting inside (Khan 2007). The J_2 invades the young lateral root and moves intercellularly to reach the cortex. The larva moves further towards the stelar tissue till the head reaches in the phloem region, where it induces specialized nurse cells called "giant cells" (Bird 1972). The juvenile becomes immobile after the formation of the nurse cells and gradually assumes obesity through three successive moults. The males after the third moult re-assume vermiform shape and free-living phase, while the females remain saccate. The giant cells are multinucleated bigger in size and rich in cytoplasm, carbohydrates, protein, and other cell organelles (Huang 1985) and support the food requirement of the female.

Concurrent with the giant cell formation, the adjoining cortical cells undergo hyperplasia and hypertrophy which lead to development of root galls. The conversion of normal root parts in to galls leads to disruption of absorption of water and nutrients by the root system (Hussey 1985). Gall formation occurs within 48 h of infection. Under severe infection, adjacent galls coalesce, and multiple nematodes can be found in the same gall especially in pulse crops. Galled tissue shows considerable variation in carbohydrate and protein contents. Insoluble polysaccharides and starch contents are decreased in primary galls. With the increase in gall size, the lipid, proteins, and carbohydrate contents increase (Sarna 1984). Generally, there is a greater allocation of photosynthates to roots with increased supply to galls and giant cells, in particular. The mature female reproduces parthenogenetically, and lay eggs in a gelatinous matrix secreted by rectal cells (Siddiqui 2005). The egg mass is attached to the posterior end of the saccate female and appears on the root surface. An egg mass generally contains 200–500 eggs (up to

2000). The *M. incognita, M. javanica,* and *M. graminicola* complete the life cycles in 20–30 days, at 25–30 °C, hence taking two or more life cycles in one cropping season (Khan et al. 2023). However, *M. artiella,* completes one generation in a season/year because it requires cool temperature for complete development of eggs (Greco 1987; Tobar Jiménez 1973).

13.8 Novel Approaches for Biocontrol of Root–Knot Nematodes

Biological control has emerged an effective method for managing the nematode populations (Khan 2016). This method involves introduction of certain microorganisms or manipulation of the environmental conditions to support multiplication of naturally occurring biocontrol agents (BCA) or antagonists. There are several biocontrol fungi such as *Purpureocillium lilacinum* (Jatala 1985; Luangsa-Ard et al. 2011), *Pochonia chlamydosporia* (Kerry 1993; Khan et al. 2005), *Trichoderma* spp. (Khan and Mohiddin 2018; Khan et al. 2018b), *Aspergillus niger* (Khan and Anwer 2007), and biocontrol bacteria, *Pasteuria penetrans* (Chen and Dickson 1998), *Pseudomonas putida* (Haque and Khan 2021), *P. fluorescens* (Rhodes 1959), and *Bacillus subtilis* (Basyony and Abo-Zaid 2018), which are efficient antagonists of nematodes and may suppress *Meloidogyne*, *Heterodera,* etc., infesting cereal and pulse crops (Khan 2007; Dababat and Fourie 2018; Sikora et al. 2018a, b). Some important biocontrol agents effective in suppressing *Meloidogyne* infestation in cereal and pulse crops are discussed under separately.

13.9 Bacterial Antagonists

Biological management using various endophytic bacteria which complete their life cycle within the host is quite successful in controlling endoparasitic nematodes. The biocontrol agents have potential for suppressing upto around 90% soil population of *Meloidogyne* species (Khan 2007; Askary 2015). Hence, several bacterial biocontrol agents, including *Pseudomonas putida, Pseudomonas fluorescens,* and *Bacillus subtilis,* are being used in commercial crop production. Bacteria like *P. putida* and *P. fluorescens* have been found to effectively control the root–knot in cereal and pulse crop (Haque et al. 2018). The chitinolytic microorganisms, such as the plant growth-promoting rhizobacterium *P. fluorescens*, *B. subtilis,* etc., can also be considered for controlling root–knot nematodes in cereals and pulses (Srinivasan et al. 2011; Khan et al. 2019a, b, 2023; Singh et al. 2019; Brair et al. 2023). Biocontrol bacteria have also been found successful against disease complexes involving *Meloidogyne* and wilt or root-rot causing fungi in pulses. Siddiqui and Shakeel (2006) tested 20 fluorescent *Pseudomonas* isolates against wilt disease complex caused by *M. incognita* and *Fusarium udum* in pigeonpea and recorded varied effectiveness. In another study, effect of six isolates of *Bacillus* and *Pseudomonas* were evaluated against the above disease complex of pigeonpea in pot and field condition (Siddiqui et al. 2008). In both the experiments, the isolates Pa324 and B18 caused significant decrease in *M. incognita* multiplication and a reduction in plant wilting. The application of *P. fluorescens* (5 g/kg seed) reduced galls and egg masses/root system (23% and 18%) and enhanced the chickpea yield by 31% and 34%, respectively, due to infection with *M. incognita* (Khan et al. 2011). Application of *Pseudomonas fluorescens, P. variotii*, *Purpureocillium lilacinus,* and *A. niger* significantly decreased the galling and soil population of *M. incognita* (Wani and Bhat 2012a; Khan et al. 2016). The treatment with *P. fluorescens* and *B. thuringiensis* decreased the galls incited by *M. incognita* and *M. javanica* in pulses (Khan and Tarannum 1999; Ali Siddiqui and Ehteshamul-Haque 2001).

The root–knot nematodes in cereals have been demonstrated to be substantially suppressed by the biocontrol agents (Lamovšek et al. 2013; Stirling 2017). Bacteria like *Bacillus* and *Pseudomonas* spp. have been identified as efficient biocontrol agents against plant pathogens among the biocontrol agents (Khan et al. 2009; Shafi et al. 2017). Both of these microbes are rhizobacteria that promote plant growth and are commonly found in soil. Numerous researchers have shown that *B. subtilis* has the ability to reduce the infection of root–knot nematodes on various crops (Khan et al. 2009). It was observed that seed inoculation with *Bacillus* spp. and *Pseudomonas* spp. was very effective in reducing *M. graminicola* galling and nematode growth (Pankaj et al. 2010). In a similar way, *B. megaterium* treatment reduced nematode penetration and gall formation by more than 40% when compared to untreated rice (Padgham and Sikora 2007). Siddiqui et al. (2007) recorded that combined treatment with rhizobium and *P. putida* significantly reduced the galls caused by *M. javanica* in lentils plants.

13.10 Fungal Antagonists

A number of opportunistic biocontrol fungi including *T. harzianum A. niger, Purpureocillium lilacinum,* and *Pochonia chlamydosporia*, have shown tremendous potential for suppressing the endoparasitic nematodes and decreasing their reproduction and soil population (Quintanilla and Fazlabadi 2023). In addition to above biocontrol agents, AM fungi especially *Glomus fasciculatum* can effectively reduce root–knot in cereals and pulses. Application of *Trichoderma* spp. (Khan and Haque 2011; Haque et al. 2018), *Aspergillus niger*, and *Pochonia chlamydosporia* (Podestá et al. 2016; Hidalgo-Díaz et al. 2017) can effectively manage the root-knot nematode infection. Against plant-parasitic nematodes, these BCAs react in a number of ways. *T. harzianum* hyphae digest the chitin layer through an enzymatic activity to penetrate the cuticle of eggs and juveniles (Santos et al. 1992). The fungus parasitizes the egg *M. graminicola* in cereals (Manzanilla-Lopez et al. 2013). However, *Aspergillus niger* colonizes and sporulates on nematodes eggs as well as on juveniles, and it has been observed to inhibit egg development (Khan and Anwer 2008). The biological control agents including *T. virens*, *T. harzianum*, and *Catenaria anguillulae* (Singh et al. 2013a, b) have demonstrated positive potential against *M. graminicola* (Deng et al. 2018). Priya (2015) used biocontrol fungi against *M. graminicola* in aerobic rice and found that *T. viride* was superior to

P. lilacinum, *P. fluorescens*, and *B. subtilis* over control. Bhat et al. (2012) reported that treatment with *P. lilacinum* along with *Bradyrhizobium* minimized the damage to black gram plants from *Meloidogyne* spp. Treatments with *T. harzianum, A. niger*, and *P. lilacinum* singly or jointly reduced the nematode attack and root zone population of *M. incognita* in chickpea (Pant et al. 2004). Using *Pochonia chlamydosporia* and *Purpureocillium lilacinum* along with PGPR decreased the galls and *M. javanica* population in chickpea (Siddiqui and Akhtar 2009). The seed priming with *P. chlamydosporia* (5 g/kg seed) significantly controlled root–knot of chickpea and pigeonpea also decreased the population of *M. incognita* in soil by 30–40% (Khan et al. 2011, 2019a, b; Sikora et al. 2018a, b; Reddy 2021). *T. harzianum* and *T. virens* were found to suppress infestation of *M. graminicola* and other nematodes in rice (Pathak and Kumar 2003; Pathak et al. 2005). In addition, the biocontrol agents can effectively be used under IPM modules (Haque and Khan 2022a, b, c)

13.11 Biotechnological Approaches for the Management of *Meloidogyne* spp.

Biotechnological approaches may greatly enhance efficiency as well as reliability of searching the gene for resistance against plant nematodes, and introducing into a suitable crop variety. Biotechnological approaches applied for root–knot nematode management show a promising and viable option. There are various techniques like RNAi technology, RNAi-based nematode resistance genes, protease inhibitors, nematicidal proteins, chemodisruptive, elicitors peptides, and the development of nematode-resistant transgenic plants which have tremendous scope of application in the management of root–knot nematodes. Some of the important ones are presented under:

13.12 RNAi-Based Nematode Resistance Genes/RNAi-Based Technology

Traditional breeding efforts to induce nematode resistance in plants are slow and non-stable. RNA-based techniques may greatly help the researchers in identifying the natural genes used to develop nematode-resistant plants. The RNA interference (RNAi) technology is considered reliable for mitigating nematodes (Tamilarasan and Rajam 2013). The relationship between host plants and nematode resistance primarily involves: passive and active resistance. During passive resistance, nematode infestation is impacted by anatomical, physiological, and chemical barriers. For example, a nematode may starve to die due to necrosis of host cells around the nematode triggered by the genes responsible for such reaction (Giebel 1982). Similarly, the gene (HS1pro1) can protect tomatoes from root–knot nematode (Mi-1.2) and sugar beet cyst nematode (Mi-1.2). The GPa2 gene exhibits resistance to *Globodera* (Briar et al. 2016; Ralmi et al. 2016). The genes responsible for synthesis of salicylate, 2-isopropyl-3-methoxypyrazine tridecane, limonene, etc., which affect nematode ability to find the susceptible host roots may be used in the breeding programmes to develop resistant rice and pulse cultivars (Sikder and Vestergård 2019).

The suppression of gene expression by sequence-specific, homologous RNA molecules is known as RNA interference (RNAi), which was observed first in *Caenorhabditis elegans* (Guo and Kemphues 1996). Nearly all genes of the *C. elegans* have been temporarily silenced using RNA interference (RNAi), and thousands of the genes of *C. elegans* have shown phenotypic effects, including lethality (Maeda et al. 2001; Kamath et al. 2003). *C. elegans* can be made to undergo RNAi by ingesting bacteria that express dsRNA (Timmons et al. 2003; Tabara et al. 1998), or its microinjection (Mello and Conte 2004). The two approaches that have received the most attention are soaking nematodes in dsRNA solutions and host-delivered RNAi. Since plant nematodes are obligate parasites, feeding via RNAi on bacteria expressing dsRNAs is nonpractical.

13.13 Proteases Inhibitors Coding Genes

Proteinase/proteases secreted by nematodes can function improperly if proteinase inhibitors (PIs) are used. These PIs become active against all nematode proteinases, including aspartic, cysteine, metalloproteinase, and serine, as nematode invades plant. PIs have substantial potential for their exploitation against plant nematodes. Ali et al. (2017) examined several PI applications against plant nematodes. They emphasized that using multiple PIs simultaneously could have an additive effect. To use a synthetic promoter to pyramid taro cystatin and fungal chitinase genes, tomatoes may become more resistant to *Meloidogyne* spp. (Chan et al. 2015).

13.14 Nematicidal Proteins

Development of nematode inside host plants can be prevented by antinematode proteins like antibodies, lectins, Bt Cry proteins, etc. These proteins vary in the synthesis as well as mechanism of nematode inhibition. Toxic lectins can block nematode infestation function (Vasconcelos and Oliveira 2004). Since many lectins bind with glycans, it is essential to understand the mechanism used by the lectins. To provide antinematode efficacy against *Heterodera*, *Globodera*, *Meloidogyne*, and *Pratylenchus* spp. in plants like potato, oilseed rape, and Arabidopsis, overexpression of a lectin related to *Galanthus nivalis* agglutinin (GNA) driven by cauliflower mosaic virus promotor is used (Ali et al. 2017). *Bacillus thuringiensis* toxin could directly suppress *M. javanica* root population in tomato plants by applying the bacteria or its crystal mixture (Khan and Tarannum 1999; Ravari and Moghaddam 2015; Antil et al. 2022).

13.15 Chemodisruptive

Plant parasitic nematodes typically use chemoreceptor neurons to move towards or away from a plant species. These neurons can identify specific chemical stimuli that are released by a plant species (Khan 2008). The nematode nervous system and transmission of various signals (stimuli) are controlled by the acetylcholinesterase and nicotinic acetylcholine receptors (Wolstenholme 2011). Selected peptides can bind with these receptors at very low concentrations, impairing the ability of chemoreceptors to respond to chemical signals and thereby disrupting their ability to locate the susceptible plants (Winter et al. 2002). The use of chemodisruptive peptides alone or in combination with cystatins in the signals of different plant species has provided high levels of resistance against *Meloidogyne* spp., which led to significant increase in the crop yields (Dutta et al. 2015; Papolu et al. 2020).

13.16 Conclusion and Future Perspectives

Cereals and pulses are the major food crops grown world over. Improvement in the global productivity of these food crops has become essential to meet the increasing demand for carbohydrates, proteins, and other nutrients as well as to fight against hunger. However, abiotic and biotic factors have become a major constraint in obtaining yield to the genetic potential of a cultivar, and nematodes are one of these factors, significantly affecting the productivity of food crops. The plant nematodes assume greater significance because of their hidden nature of occurrence and crop damage. The root–knot nematode, *Meloidogyne* species, is a major nematode pest, which attacks a wide range of crops and significantly decreases their yields. Hot spots of this nematode in cereal and pulse-growing areas should be identified through well-planned and coordinated survey and surveillance efforts using soil sampling, remote sensing and drone technology. Adequate breeding programmes should be launched based on biotechnological tools to develop resistant crop varieties. Indigenous isolates of biocontrol agents, especially those with additional phosphates solubilizing capability, should be exploited on performance and priority basis. The existing management technology is needed to be evaluated for effectiveness under prevailing and future climatic situations, and their sustainable combinations be devised and tested for implementation under IPM strategies. However, foremost important is to create awareness among farmers towards the economic significance and seriousness of plant nematodes in crop production especially in cereals and pulses.

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Management of Cyst-Forming Nematodes in Agricultural Crops Through Novel Biological and Genetic Engineering Technologies

14

Leonardo F. Rocha and Priyanka Duggal

Abstract

Cyst nematodes are obligate sedentary endoparasites characterized by developing pear or lemon-shaped protective structures for their eggs. Cyst-forming nematodes embrace nearly 100 known species across six genera, but *Heterodera* and *Globodera* represent the main genera of cyst-forming plant parasitic nematodes world over. Novel biological and genetic-based management practices are discussed for *Heterodera glycines* (soybean cyst nematode), *Heterodera avenae* (cereal cyst nematode), *Heterodera schachtii* (sugar beet cyst nematode), *Heterodera zeae* (corn cyst nematode), and *Globodera pallida* and *Globodera rostochiensis* (potato cyst nematodes).

Keywords

Plant–parasitic nematodes · *Heterodera* spp. · *Globodera* spp. · Soybean cyst nematode · Cereal cyst nematode

14.1 Introduction

Soil organisms are classified into three major groups: microfauna, mesofauna, and macrofauna (Moncada and Sheaffer 2010). Nematodes, part of the microfauna, are worm-like organisms comprising the most abundant group in Metazoan (Ferris et al.

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2001). Free-living nematodes are found in the rhizosphere, rhizoplane, and endosphere of plants, influencing soil structure and nutrient cycling and playing an essential role in the mineralization and breakdown of complex organic compounds and nutrients (Bohlen and Edwards 1994; Nair and Ngouajio 2012). Since soil-borne nematodes are relatively easy to sample and well categorized into feeding groups based on their vermiform shape and standardized morphometric characteristics, they are widely used as effective soil health bioindicators (Neher 2000; DuPont et al. 2009; Ferris 2010). The main groups of soil nematodes are bacterial feeders, fungal feeders, predatory nematodes, omnivores, and plant–parasitic nematodes (PPN) (Ferris and Ferris 1974; Hoorman 2011).

Plant–parasitic nematode species cause losses to major crops worldwide and substantially constrain worldwide food security. Annual yield losses caused by PPN are estimated to be 12.3% (or $157 billion) worldwide and are intensified in areas with low use of technology in agriculture and aggravating problems such as food uncertainty and undernourishment (Singh et al. 2015; Bernard et al. 2017). The major PPN groups worldwide are cyst-forming (*Globodera* and *Heterodera* spp.), root-knot (*Meloidogyne* spp.), and root lesion (*Pratylenchus* spp.) nematodes.

The population densities and richness of these plant–parasitic nematode species are shaped in response to crop history (Sun et al. 2018). Kimenju et al. (2009) reported the dominance of PPN in soils under intensive agricultural production, while saprophytic nematodes were more frequently isolated in forest sites. Figure 14.1 summarizes biotic and abiotic factors influencing soil nematode populations from the perspective of the disease triangle. A diversified toolbox of practices is recommended to minimize PPN-related crop losses, including implementation of resistant/tolerant cultivars, crop rotation, adoption of cover crops, nematicides, biological control, weed management, and other principles of integrated nematode management (Gavassoni et al. 2007; Mueller et al. 2016; Niblack and Tylka 2008; Niblack 2009; Rodriguez-Kabana and Canullo 1992; Werle et al. 2015). Novel biological- and genetic-based management practices are further discussed for major cyst-forming nematodes.

14.2 Major Cyst Nematode Species Worldwide

Cyst nematodes are obligate sedentary endoparasites characterized by developing pear or lemon-shaped protective structures for their eggs (Cook and Noel 2002). Females, upon maturity, may enclose hundreds eggs inside their bodies, which become a protective structure until hatching. Upon plant infection, these organisms establish specialized and complex relationships with their hosts, forming a feeding site denominated syncytium, characterized by cells with numerous due to wall dissolution-mediated cell fusion, resulting in the loss of large vacuoles, higher numbers of organelles, and boosted metabolic activity (Hewezi et al. 2009; Ohtsu et al. 2017).

Nematode genera in the Heteroderidae family are generally divided into cyst-forming nematodes (the female body becomes a hard-walled protective structure)

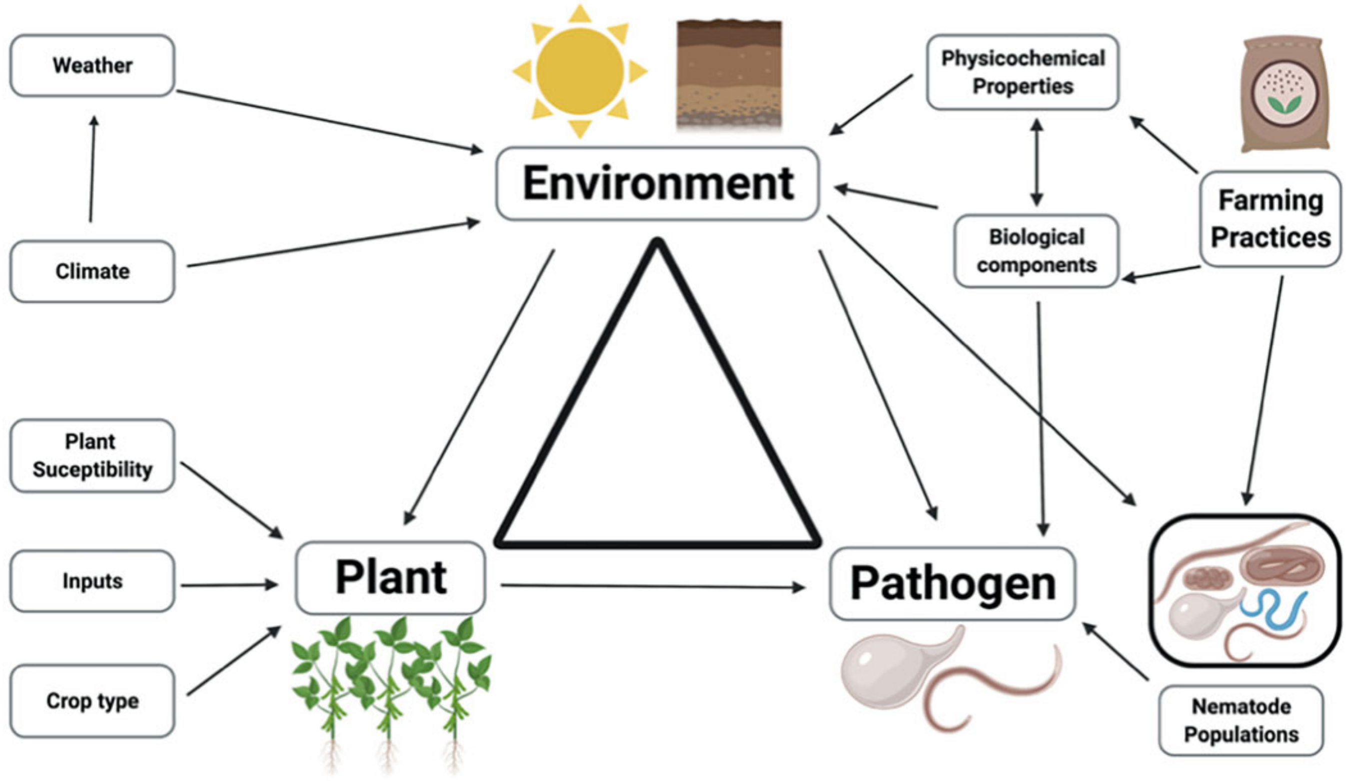

Fig. 14.1 A schematic of major biotic and abiotic factors influencing populations of plant–parasitic nematodes. (Created with biorender.com)

and cystoid nematodes (female body without hardened wall) (Subbotin et al. 2017; NEMAPLEX 2022a). Cyst-forming nematodes embrace nearly 100 known species across six genera, including *Afenestrata*, *Betulodera*, *Cactodera*, *Globodera*, *Heterodera*, *Punctodera*, *Thecavermiculatus,* and *Vittatidera* (Mulvey and Golden 1983; Turner and Rowe 2006; NEMAPLEX 2022a). Across the globe, the major genera of cyst-forming nematodes are *Heterodera* and *Globodera*. The nomenclature of these genera is derived from terms indicating the characteristics, shape, and marking of the adult female (cyst). *Hetero* for "changing," *Globo* for "spherical," and *Deros* for "skin" (NEMAPLEX 2020).

Speciation of cyst nematodes is usually based on the morphology of the adult female (size, color, and cuticular patterning) and the host information (Cook and Noel 2002; NEMAPLEX 2022b). The critical differences between *Heterodera* and *Globodera* spp. rely on the morphology of the adult female. While lemon-shaped cysts characterize *Heterodera*, *Globodera* females develop into a characteristic round form (Cook and Noel 2002). Furthermore, *Globodera* does not have either a vulval cone or egg sac, while *Heterodera* develops egg sacs and a posterior protuberance in the vulval cone (Luc et al. 1988; NEMAPLEX 2020). Molecular markers, such as the ITS2, are also implemented to speciate cyst nematodes based on minor sequence variation (Clapp et al. 2000).

14.2.1 Major *Heterodera* spp. Worldwide

A broad list of *Heterodera* species has significant economic importance worldwide, including in soybean, cereals rice, sugar beet, and potato production, from temperate regions to the tropics. The most important species are *Heterodera avenae* (cereal cyst nematode), *H. filipjevi* (cereal cyst nematodes), *H. cajani* (pigeon pea cyst nematode), *H. cruciferae* (cabbage cyst nematode), *H. glycines* (soybean cyst nematode), *H. goettingiana* (pea cyst nematode), *H. latipons* (Mediterranean cereal cyst nematode), *H. oryzicola* (rice cyst nematode), *H. sacchari* (sugarcane cyst nematode), *H. schachtii* (sugar beet cyst nematode), *H. sorghi* (sorghum cyst nematode), *H. trifolii* (clover cyst nematode), and *H. zeae* (corn cyst nematode) (Turner and Rowe 2006). Novel biological and genetic engineering-based management practices are discussed in this chapter for *H. glycines*, *H. avenae,* and *H. schachtii,* as these species are widely spread and cause the most yield losses worldwide.

14.2.2 Major *Globodera* spp. Worldwide

Potato cyst nematodes (PCNs), included in the genus *Globodera*, are critical pests of potatoes worldwide in temperate areas and are believed to have originated from the Andes Mountains in South America, the center of genetic diversity for potatoes (Stone 1979). The genus *Globodera* encompasses 13 species worldwide, viz. *G. rostochiensis* Wollenweber, 1923; *G. leptospira* Cobb & Taylor, 1953, Skarbilovich, 1959; *G. tabacum* Lownsbery & Lownsbery, 1954, Skarbilovich, 1959; *G. millefolium* Kirjanova & Krall, 1965, Behrens, 1975; *G. artemisiae* Eroshenko & Kazachenko, 1972, Behrens, 1975; *G. pallida* Stone, 1973, Skarbilovich, 1959; *G. mali* Kirjanova & Borisenko, 1975, Behrens, 1975; *G. zelandica* Wouts, 1984; *G. mexicana* Subbotin, Mundo-Ocampo & Baldwin 2010; *G. ellingtonae* Handoo, Carta, Skantar & Chitwood 2012; *G. capensis* Knoetze, Swart & Tiedt 2013; *G. agulhasensis* Knoetze, Swart, Wentzel & Tiedt 2017; and *G. sandveldensis* Knoetze, Swart, Wentzel & Tiedt 2017. Among these, *G. rostochiensis* and *G. pallida* are major quarantine pests on potatoes (Hockland et al. 2012). The former possesses five pathotypes (Ro1 to Ro5), and the latter has only three (Pa1 to Pa3) (Prasad 2008). The other two species of parasitizing potato, G. *ellingtonae,* were initially reported from Oregon (Handoo et al. 2012) and later from S. America (Lax et al. 2014), while *G. leptospira* is believed to be native to South America. *G. tabacum* (tobacco cyst nematode) is important species of tobacco in the Eastern United States.

Despite strict quarantine regulations, PCN species have been identified in many areas producing potatoes worldwide. *Globodera rostochiensis* and *G. pallida* were reported in 70 and 47 countries until 2019, but within 1 year, *G. rostochiensis* reached 83 countries (CABI/EPPO 2020a) and *G. pallida* in 64 countries (CABI/EPPO 2020b). The PCN host range includes nearly 90 *Solanum* species and their hybrids, including *Solanum tuberosum*, *Solanum lycopersicum*, and *Solanum melongena* (Mai and Lownsbery 1948).

14.3 Soybean Cyst Nematode (*Heterodera glycines*)

The soybean cyst nematode (SCN—*Heterodera glycines* Ichinohe) was first described in Japan in 1915, with the first identification in the United States in North Carolina, in 1954 (Noel 1986). SCN is the most critical PPN in soybean production, with widespread dissemination across all significant growing areas across the United States (Niblack and Tylka 2008). SCN is also present in all important soybean-producing regions in South America, including Brazil and Argentina (CABI 2022).

Overall, SCN is the main biotic factor causing yield losses in US soybean production. Despite its distribution within all major US soybean-growing areas, higher latitude areas create favorable conditions for SCN. From 2010 to 2019, SCN was always the number one disease in Northern US regions and first or second in the Southern United States, alternating with charcoal rot (*Macrophomina phaseolina*) (Allen et al. 2017). As temperature increases, SCN may reach even further north areas. However, conditions in the lower Midwest may also become favorable for other foliar diseases and nematode species common in the South, such as *Hoplolaimus columbus*, *Rotylenchulus reniformis, Belonolaimus longicaudatus, Meloidogyne* spp.*, and Paratrichodorus* spp., which could reduce local SCN pressure.

Losses caused by SCN may reach over 60% when susceptible varieties are planted (Hershman 2014), with frequent losses of up to 30% occurring without presenting visible aboveground evidence (Mueller et al. 2016; Tylka and Marett 2017). Therefore, proper sampling is fundamental when establishing an SCN management program. Management of fields with elevated levels of SCN pressure is complex and requires many crop rotation seasons, and suppression of populations might fade after one soybean season, demonstrating the importance of implementing a group of management practices to maintain populations under control (McSorley 2011). Another critical aspect of SCN management is the capacity of this organism to parasitize on other crops and weed hosts. Rocha et al. (2021a) reported 116 weed species from 23 families as potential hosts of SCN, with the majority included in the Fabaceae family. Further work is also being conducted to explore the ability of SCN to reproduce on emerging crops, such as pennycress (*Thlaspi arvense* L.) (Hoerning et al. 2022).

14.3.1 Novel SCN Biological Management Practices

Multiple factors are pointed to explain reduced disease caused by beneficial soil microbes, involving antagonism for resources, mycoparasitism, the release of antimicrobial compounds, and stimulation of plant defense mechanisms that are effective against pathogens (Selosse et al. 2014; Pozo and Azcón-Aguilar 2007; Pimentel et al. 2020; Jung et al. 2012; Topalovic et al. 2020). Variations in levels of nematode suppression between fields are commonly observed since it depends on indigenous antagonist microorganisms (Timper 2014). Plant-associated fungi and bacterial species might be a source of biological control agents (BCAs) targeting plant parasites and other biotic and abiotic stresses (Zhou et al. 2020). Therefore, identifying specific antagonists will ultimately predict where suppressive effects will be naturally observed and if antagonistic isolates may need to be applied to increase efficacy and duration of suppression (Timper 2014). For illustration, Zhou et al. (2020) examined fungi isolates from the cotton phytobiome with the potential to serve as BCAs of *Meloidogyne* spp.

The two-body openings in cyst nematodes (mouth and vulva) allow hatched juveniles to leave and open ports of entry for soil microorganisms (Nour et al. 2003). An estimated bacterial density of one billion bacterial cells is present in 1 ml of cyst volume (Nour et al. 2003). Cysts of SCN are often found to support a rich community of microorganisms, which can be screened for their ability to reduce reproduction rates and their prospective to serve as biological control agents (Haarith et al. 2020a). Research demonstrates that SCN developmental stage affects parasitism by fungi, with higher infection rates in early SCN developmental stages, indicating that the time of BCA release may play a role in their effectiveness against SCN (Chen and Chen 2003).

Bacterial and fungial communities are associated with SCN cysts collected from fields, and research indicates their role in SCN suppression in disease-suppressive soil (Nour et al. 2003; Song et al. 2016). Microbial strains colonizing SCN cysts in field conditions are a starting point to identify BCAs and possible biological-based nematicides to manage SCN (Haarith et al. 2020a). Nour et al. (2003) retrieved a rich collection of 290 bacterial isolates from SCN cysts, including species from genera *Lysobacter, Variovorax, Microbacterium, Ultramicrobacterium, Rhizobium, and Streptomyces*. Rocha et al. (2022a), employing three distinct DNA markers targeting bacterial (16S), fungal (ITS2), and *Fusarium* (*tef1*) communities, proposed a likely involvement of soil microorganisms in the reduction in SCN counts in soybean production plots formerly planted with winter wheat, in a double-cropping system (Rocha et al. 2021b). A GCMS-based metabolomics study later revealed several chemical compounds negatively correlated with SCN counts, suggesting a role of these compounds on SCN suppression (Rocha et al. 2023).

Haarith et al. (2020a) reviewed fungal communities associated with SCN and their prospective to be implemented as BCAs. *Fusarium, Cylindrocarpon, Neocosmospora, Ilyonectria, Neonectria, Clonostachys, Pochonia, Lecanicillium, Hirsutella, Purpureocillium, Exophiala, Leptosphaeria, Phoma, Setophoma,* and *Mortierella* are listed as the most common fungal species isolated from SCN cysts in the United States (Haarith et al. 2020a). Haarith et al. (2019) described the SCN cyst culturable mycobiome in a long-term soybean–corn production system, pointing to *Fusarium* as the major genera in a total of 14 frequently isolated. In that study, the abundances of *Alternaria, Clonostachys, Fusarium*, *Leptosphaeria, Neonectria,* and *Pochonia* varied significantly due to crop succession. Promising isolates collected from infected cysts in the previous rotation study were later screened in vitro (Haarith et al. 2020b) and in vivo (Haarith et al. 2021), showing high biocontrol efficacy. *Pochonia chlamydosporia,* frequently isolated from SCN cysts, was reported to parasitize up to 97% of *Meloidogyne* eggs in greenhouse conditions (Gine et al. 2016).

14.3.2 Novel SCN Genetic Engineering Technologies

Over the last decades, extensive work has been developed to identify genes governing the multifaceted molecular exchanges among soybean and SCN (Lilley et al. 2005). SCN was proposed as a model nematode to investigate nematode behavior and plant–nematode interactions since *Caenorhabditis elegans*, the nematode model organism, is a free-living species (Opperman and Bird 1998; Niblack et al. 2006).

A complete SCN genome is currently available for the research community, with 123 Mb and annotations for close to 30,000 genes (Masonbrink et al. 2019a). The SCN genome includes a high number of repeats (34%), tandem duplicates (18.7 Mb), and horizontal gene transfer events (151 genes) (Masonbrink et al. 2019a). SCN was also the first nematode to have a greater spliced leader trans-splicing rate using a species-specific SL over well-conserved spliced leader-like sequences in *C. elegans* (Barnes et al. 2019). The SCN-Base (*scnbase.org*) serves as a hub for the SCN research community, summarizing and allowing researchers to access findings and genomic resources with minimal need for further curation

(Masonbrink et al. 2019b). The availability of an SCN and soybean genome has provided tools for identifying novel insights into the mechanisms of resistance and parasitism within this pathosystem. For illustration, Sharma et al. (2020) unraveled the involvement of exocyst proteins in the soybean defense response to SCN infection. Hu et al. (2019) reported that *Hg16B09,* part of a family of 10 SCN effectors, has increased activity in the initial stages of parasitism, leading to suppressed soybean basal defenses.

Further work has been done within their hosts to understand better the factor influencing syncytium formation. Ohtsu et al. (2017) reported that syncytia cells could be classified into two forms due to arrangements of root cell wall configurations that seemed to be governed by the syncytium inside soybean roots. Sharma et al. (2020) used next-generation sequencing technologies coupled with overexpression and RNA to demonstrate that the soybean exocyst, encoded by 61 genes, plays a significant role in syncytium formation and defense in the soybean-SCN pathosystem (Sharma et al. 2020).

Genetically modified plants have long been used to manage insect populations, but more recently, studies are targeting these tools, such as *Bt* proteins, to manage plant–parasitic nematodes (Kahn et al. 2021). Soybean transgenic plants producing a *Bacillus thuringiensis* Cry protein (Cry14Ab) drastically reduced SCN counts in greenhouse and field studies, demonstrating this protein as an excellent tool to manage SCN in soybean (Kahn et al. 2021).

14.4 Cereal Cyst Nematode (*Heterodera avenae*)

Around ten genera of PPN parasitize wheat to the level of causing yield losses in wheat (Donald et al. 2017). Still, among these, the crop’s most destructive soil-borne pathogen is the cereal cyst nematode (CCN). The genus *Heterodera* was described by Schmidt in 1871. Kuhn noted cyst nematodes parasitizing cereals in Germany in 1874, later known as cereal cyst nematodes. Earlier, this group encompassed only 12 species infecting cereals and grasses (Andres et al. 2001), but the number increased to 67 over time. Out of these, *Heterodera filipjevi*, *Heterodera latipons*, *Heterodera australis*, *Heterodera avenae*, *Heterodera sturhani,* and *Heterodera hordecalis* are considered primary pests of cereals (Owen et al. 2023). In contrast, the others are known to parasitize grasses only. *Heterodera filipjevi*, *Heterodera avenae,* and *Heterodera latipons* are the primary species that attack cereals (Nicol et al. 2002) and are economically important pests on cereals (Nicol and Rivoal 2008). Cereal cyst nematodes cause 20–90% yield losses of wheat in various parts of the world (Wu et al. 2014). The initial incident of CCN in India was related to the Sikar District of Rajasthan (Vasudeva 1958), inciting *Molya* disease. Subsequently, the nematode was reported from Delhi, Haryana, Himachal Pradesh, J&K, MP, UP, and Punjab.

14.4.1 Novel CCN Biological Management Practices

In the past, management strategies were mainly focused on using chemicals to control disease spread due to their effectiveness and fast response. However, environmental pollution due to the indiscriminate use of agrochemicals is driving a change toward more sustainable management practices, including biological control. Since cysts survive in the soil for a more extended period, different fungi have been found in their association (Kerry and Crump 1980), which may act as major factors limiting cyst population in various environmental conditions through the production of nematicidal or nematistatic compounds.

Fungal strains have activity against PPN through many means, i.e., trapping, endoparasitic, and opportunistic approaches, which may capture the nematodes, parasitize secondary-stage juveniles, or colonize the females and cysts, respectively. *Paecilomyces* sp., *Fusarium* spp., *Pythium* sp., and *Verticillium* sp. isolated from *Heterodera avenae* cysts and eggs suppressed the cysts up to 98% (Stein and Grabert 1992). *Paecilomyces lilacinus* (the egg–parasitic fungus) and *Monacrosporium lysipagum* (predatory fungus) significantly reduced cyst density by up to 65% in barley (Khan et al. 2006). *Chaetomium* sp., *Fusarium* spp., *Penicillium oxalicum,* and *Stemphylium solani* are very effective against *H. avenae* (Yuan et al. 2011).

Vesicular arbuscular mycorrhiza (VAM) and plant growth-promoting rhizobacteria (PGPR) are very well known for their effectiveness against plant–parasitic nematodes through plant growth promotion and production of secondary metabolites to inhibit nematodes by various mechanisms (Ingham 1988; Mhatre et al. 2019a, b). Endophytic bacteria, PGPR, and obligate parasites (*Bacillus*, *Pseudomonas,* and *Pasteuria*) have been studied by various researchers for the biological management of CCNs (Kloepper et al. 1992; Davies 1998). Bacterial suspensions of *Achromobacter xylosoxidans* (09X01) and *Bacillus cereus* (09B18) effectively minimized the CCN female population in wheat roots (Zhang et al. 2016). The *Bacillus cereus* strains controlled 30% of the nematode population during in vivo trial (Ahmed et al. 2019). More than 290 *Bacillus* strains showed complete mortality of CCN J2 under in vitro screening (Li et al. 2011). *Pasteuria nishizawae* proved promising against cyst nematodes (Sayre et al. 1991). *Azotobacter chroococcum*, *Azospirillum lipoferum*, and *Pseudomonas* sp. suppressed cyst formation by up to 60% in wheat infected with *H. avenae* (Bansal et al. 1999). Actinomycetes, belonging to the genus Streptomyces, have been reported to inhibit the motility of J2s by 60%, indicating their potential to serve as BCA targeting CCN (Yavuzaslanoglu et al. 2011).

14.4.2 Novel CCN Genetic Engineering Technologies

The most economical and cost-effective practice for controlling cereal cyst nematodes is host–plant resistance, the capacity of a plant or its genotype to prevent or minimize nematode reproduction (Trudgill 1991). In commercial cultivars, resistances reported against CCNs are primarily based on introgressions of single-

Table 14.1 Resistance genes against *Heterodera avenae* from various sources

Resistance gene	Source of gene	References
Cre2-Cre7, Rkn2	*Aegilops variabilis*	Jahier et al. (2001)
Rha2, Rha4	*Hordeum vulgare*	Barr et al. (1998)
CreR	*Secale cereale*	Asiedu et al. (1990)
Cre1, Cre8	*Triticum aestivum*	Slootmaker et al. (1974)
CreX, CreY	*Wild relative*	Delibes et al. (1993)

dominant genes (Smiley et al. 2017). Resistant genes against *H. avenae* have been identified in wheat (Lewis et al. 2009) and barley (Andersson 1982). Fourteen CCN resistance genes have been reported from wild grasses and bread wheat relatives (Table 14.1).

The broad specificity of *Cre1* as highly effective against *H. avenae* makes it most widely used in commercial cultivars (Mokabli et al. 2002). By in vitro soaking the larvae in homologous dsRNA, it was possible to inhibit the expression of HaEXPB2 in *H. avenae*, which in turn decreased its parasitism of *Nicotiana benthamiana* (Liu et al. 2016). Cowpea trypsin inhibitor and serine protease inhibitor, PIN2, from *Solanum tuberosum* into *Triticum durum* induced resistance against *Heterodera avenae* (Vishnudasan et al. 2005). CRISPR/Cas9 technique imparts immense opportunities to improve resistance to CCN in cereals (Baltes et al. 2015). To detect *H. avenae* resistance in wheat, one RFLP marker csE20-2 and one PCR marker Cre3spf/r have been developed (Ogbonnaya et al. 2001). Forty *H. avenae* target genes were tested for their susceptibility to RNAi (in vitro soaking); seven genes showed upregulation, 18 genes were downregulated, and 15 genes gave unaltered expression. In total, 37 genes altered nematode behavior negatively and reduced the juvenile penetration, development, and reproduction in wheat (Dutta et al. 2020). Seven additive quantitative trait loci (QTL) with a maximum of 9.42% morphological differences on chromosomes 1A–3A, 2B, 6B, 2D, and 6D and five QTLs on 2A, 2B, 6B, 2D, and 6D chromosomes have been identified (Dababat et al. 2021), having resistance genes against nematode. Many researchers have reported resistance against *H. avenae* on the 2D chromosome (Eastwood et al. 1994; Moustafa et al. 2015). QTL, *QCcn.ha-2D,* was mapped on a 2D chromosome having 23% of the variation and co-localized with *Cre3* locus out of 19 QTLs, in which 13 were novel, and six were found to be localized with the earlier reported *Cre* gene (Pundir et al. 2022). Epn-1, emb-4, pab-1, and nhr-91 genes were cloned and sequenced for testing against different stages of *H. avenae* in wheat. The expression of epn-1 and nhr-91 was recorded maximum in feeding females on plants, while pab-1 expressed highest in the J2s stage while emb-4 in the egg stage. siRNA soaking of epn-1, emb-4, and pab-1 resulted into reduced development by 71%, 60%, and 26.5% (Gantasala and Rao 2021).

14.5 Potato Cyst Nematodes (*Globodera pallida* and *Globodera rostochiensis*)

The potato cyst nematode (PCN) is among various nematodes associated with the potato rhizosphere. In India, Jones reported potato cyst nematode in 1961 for the first time. Since then, both species (*Globodera rostochiensis* and *G. pallida*) have been identified exclusively in potato-producing areas of India, specifically in the Nilgiris and Kodaikanal hills of the state (Seshadri and Sivakumar 1962). The prevalence of the Ro1 pathotype of *G. rostochiensis* and Pa2 pathotypes of *G. pallida* has been reported in India (Prasad 1996). Since the seed tubers are the primary mode of spread for PCNs, domestic quarantine has been regulated in Himachal Pradesh, Uttarakhand, and J&K (Mhatre et al. 2019a, b), so transportation of seed is legally restricted from these areas to other parts of the country. Depending on the initial inoculums, yield losses may vary from 5 to 80% in India (Prasad 1996).

14.5.1 Novel PCN Biological Management Practices

Earlier nematicides were used to control potato cyst nematodes in many temperate countries (Been and Schomaker 1999). Still, many countries have revised and prohibited the use of various chemicals on different crops. Therefore, more awareness is toward chemicals. In this direction, the biological management strategy is in prime position due to its specificity toward specific pathogens, low cost, and environmentally safe practices.

For the management of PCNs, various biological control agents like bacteria, fungi, nematodes, viruses, and mites have been used. Among these, fungal and bacterial biocontrol agents like *Purpureocillium lilacinum*, *Pochonia chlamydosporia*, *Trichoderma* spp., *Bacillus* spp., *Pseudomonas fluorescens,* and *Pasteuria* spp., are recognized as potential candidates to suppress the PPN population. The potential of antagonistic fungus *Trichoderma viride,* the nematode egg parasitic fungus, *Purpureocillium lilacinum* (syn *Paecilomyces lilacinus*), and the PGPR *Pseudomonas fluorescens* in managing PCNs are well described (Cronin et al. 1997; Seenivasan et al. 2007). *Pochonia chlamydosporia* produces some extracellular enzymes helping penetration followed by digestion of eggshell and cuticle (Huang et al. 2004). *Catenary auxiliaries*, *Nematophora gynophile*, *Hirsutella minnesotensis,* and *H. rhodesiensis*, being endoparasitic in nature (Chen and Liu 2005), complete their life cycle inside the nematode body after producing adhesive spores and get attached to the nematode cuticle. Trichoderma isolates from potato rhizosphere have antibiotics and egg-hatching inhibitors, successfully parasitizing *G. rostochiensis* and *G. pallida* eggs (Devarjan et al. 2011). The application of *Metarhizium carneum* (nematophagous fungus) and faba bean crop rotation reduced nematode counts by 81% and showed a significant increase in yield (Lima López et al. 2020). The treatments comprising biocontrol fungi/bacteria alone (Stirling 1991; Khan 2007; Khan and Anwer 2011) or along with oil, neem cakes (Sikora and Roberts 2018; Khan et al. 2021) or pesticides (Mohiddin and Khan 2013) are getting popularity in achieving sustainable nematode management in agricultural crops (Khan 2023; Khan et al. 2023). The microbial antagonists, *Aspergilus niger, Pochonia chlamydosporia, Purpureocellium lilacinum, Pasturia penetrans* etc. (Jatala 1986; Stirling 1991; Kerry 2000; Khan 2016), and phosphate solubilizing microorganisms such as *Aspergillus, Bacillus, Penicillium, Pseudomonas* etc. (Khan et al. 2009, 2016a, b; Sikora and Roberts 2018) may significantly contribute in the sustainable management of plant nematodes. The well known mycoparasitic fungus, Trichoderma has also been found effective in suppressing plant nematodes (Mohiddin et al. 2010; Khan and Mohiddin 2018), and formulations of *T. harzianum* and *T. hamatum* of are available (Khan et al. 2011), and provide satisfactory control of nematodes (Mohammed and Khan 2021; Sikora and Roberts 2018; Shahid and Khan 2019). Some commercial formulations, i.e., Rizotec® (Brazil), KlamiC® (Cuba), PocharTM (Italy), and Nematofree+ (India), are based on *P. chlamydosporia,* and BioAct® (Spain), PL Plus® (South Africa), MeloCon® (USA), and Paecilo® (India), based on *P. lilacinum* are available in markets for the management of PPNs (Kumar and Arthurs 2021). A liquid formulation of *Purpureocillium lilacinum*, *Pseudomonas fluorescens,* and *Trichoderma viride* reduced the reproduction and population density of PCNs in potatoes (Nagachandrabose 2020).

The culture filtrate of *Pseudomonas pseudoalcaligenes*, *B. cereus,* and *B. carotarum* resulted in more than 80% mortality of *G. rostochiensis* under laboratory conditions and more than 70% reduction in nematodes population in the field of (Istifadah et al. 2018). 2–4 DAPG secreted by *Pseudomonas* has been proven inhibitory to *G. rostochiensis* (Cronin et al. 1997). *P. fluorescens,* along with neem cake and mustard as intercrop, reduced potato cyst nematode population (Devarjan et al. 2004); the mode of action may be linked to the antagonistic activity of bacteria (Cronin et al. 1997), the nematicidal activity of organic cake (Alam et al. 1979), and the toxic compound produced by roots (Haque and Gaur 1988). *P. fluorescens* combined with neem cake + *T. viride* has proved to be a promising biocontrol agent for the PCN in the Nilgiris region (Umamaheswari et al. 2012).

Vesicular arbuscular mycorrhiza (VAM) fungi induce tolerance against PCNs in potatoes and improve root biomass compared to non-mycorrhized plants (Ryan et al. 2003). Avermectins (AVM, extracted from *Streptomyces avermitilis*) has insecticidal, acaricidal, and nematicidal properties (Putter et al. 1981). Vertimec®EC (commercial product based on abamectin) was demonstrated to have great nematicidal activity against *G. pallida* Pa3 (Sasanelli et al. 2019), with moderate persistence and low toxicity to non-target beneficial organisms (Lumaret et al. 2012). The degradability of this compound by soil-borne microbes combined with low leaching risk makes it a potential bio-nematicide. *Solanum sisymbriifolium* reduced egg counts of *G. tabacum*, *G. pallida,* and *G. ellingtonae* in one growing season when used as a trap crop (Dandurand et al. 2019).

14.5.2 Novel PCN Genetic Engineering Technologies

Cultivars with pyramiding the R genes/QTLs with multiple resistances against both the species of *Globodera* are practically the economically viable and environmentally safe practice to manage PCNs (Gartner et al. 2021). QTLs and R genes provide multiple resistances to different PCN species, and their pathotypes like Grp1_QTL have resistance against Pa2 and Pa3 pathotypes of *Globodera pallida* and Ro5 of *Globodera rostochiensis* (Voort et al. 1998). The Hero gene of tomato has >90% resistance against *G. rostochiensis* and all pathotypes of the same (Ganal et al. 1995) and >80% resistance against *G. pallida*. Gro1-4-1, a more specific marker, has resistance against all pathotypes of *G. rostochiensis* and is very useful in PCN resistance (Asano et al. 2012). The HC marker has been used for screening the resistance against the Pa2 and Pa3 pathotypes of *G. pallid* (Sudha et al. 2019). CAPS marker TG432 is used to detect the Grp1 locus, responsible for broad-spectrum resistance to pathotypes Ro5, Pa2, and Pa3 (Finkers-Tomczak et al. 2009). Out of three DNA markers, viz. HC-I2 and C237-I for *G. pallida* and N195 for *G. rostochiensis, the C237-I* marker helps identify the resistance allele of $GpaIVs_{adg}$ to develop *G. pallida* resistance (Asano et al. 2021).

RNAi may prove a promising approach for developing resistance against nematodes in plants. RNAi was applied by targeting the cysteine proteinase of *Heterodera glycines,* and *G. pallida* changed the sex ratio (female to male) of the nematode from 3:1 to 1:1 (Urwin et al. 2002). A significant correlation between cyst counts after extraction and the average count of white females on roots indicates the significance of the tissue culture technique in screening for resistance to *G. pallida* (Mwangi et al. 2019).

14.6 Sugar Beet Cyst Nematodes (*Heterodera schachtii*)

The sugar beet cyst nematode (SBCN) (*Heterodera schachtii*) is a critical cyst-forming species limiting yield in sugar beet, brassicas, and further similar plants (Fosu-Nyarko et al. 2016). Like other cyst nematode species, SBCN eggs are protected by the cyst, allowing them to stay viable for several cropping seasons without a host, waiting for hatching when optimal environmental conditions are available, and a susceptible crop is planted (Huang et al. 2021). These cysts are often suppressed and parasitized by a large taxonomically diverse group of antagonistic microorganisms (Haj Nuaima et al. 2021). The literature lists much larger publications covering SCN biological and genetic engineering management than SBCN. However, as life cycles and developmental stages are like SCN, much of these research finds may apply to these two pathosystems.

14.6.1 Novel SBCN Biological Management Practices

Cover crops have been extensively studied to suppress plant–parasitic nematode populations, including SBCN. Research has demonstrated that cover crops often suppress SBCN populations by altering the surrounding microbial communities (Haj Nuaima et al. 2019). As previously discussed for SCN, microorganisms isolated from infected cysts and suppressive soil might be employed as BCAs. A method commonly used to pinpoint potential microorganisms linked to nematode suppression is correlating nematode counts with the abundance of microbial species (Yin et al. 2003; Rocha et al. 2022a). Haj Nuaima et al. (2021) evaluated fungi isolates (*Exophiala* sp., *Pochonia chlamydosporia,* and *Pyrenochaeta* sp.) isolated from cysts collected from the cover crop study previously mentioned (Haj Nuaima et al. 2019). Data from in vivo and *planta* confirmed that these fungal strains infected SBCN cysts and highlighted these as potential biocontrol agents of SBCN in field conditions.

Sugar beet cyst nematode populations were shown to significantly increase in conducive compared to suppressive soils (Westphal and Becker 2001). Fungal isolates frequently infect SBCN cysts in that study, including *Fusarium* spp., *Dactylella oviparasitica,* and *Paecilomyces lilacinus*. Interestingly, the suppressiveness effect in that soil was eliminated after soil samples were autoclaved, confirming that beneficial microorganisms were linked to reduced SBCN counts. Yin et al. (2003) also successfully transferred the suppressive effect by fixing varying ratios of the suppressive soil with an autoclaved sample, with later studies confirming the association of *Rhizobium* with the suppression of SBCN. Research has been done to characterize further the mechanisms of action involved in the suppression of SBCN by microorganisms. Huang et al. (2021) demonstrated that *Bacillus firmus* I-1582 not only disturbed SBCN reproduction ratios but also the development and pathogenicity of the next generation of nematodes previously exposed to *B. firmus. Arabidopsis thaliana* recruits *B. firmus* through root exudates, which act as a plant growth-promoting bacterium (PGPR).

14.6.2 Novel SBCN Genetic Engineering Technologies

As early as 2002, molecular biology methods were deployed to identify SBCN, using the internal transcribed spacer (ITS) PCR-RFLPs and species-specific PCR primers (Amiri et al. 2002). The capacity of SBCN to parasitize *Arabidopsis thaliana*, the model organism for plant research, allowed researchers to take advantage of the first plant genome to be sequenced and annotated in the early 2000s. Near that timeframe, efforts were also put into obtaining details on SBCN genetic variability (Plantard and Porte 2003).

Two recently sequenced and annotated genome versions are available for SBCN through NCBI (National Center for Biotechnology Information), with 179.2 (PRJNA722882) and 190.2 MB (PRJNA767548). SBCN has the largest sequenced cyst nematode genome to date, compared to 106, 158, 124, and 92 MB for

G. ellingtonae, H. glycines, G. pallida, and *G. rostochiensis*, respectively (Siddique et al. 2021). The SBCN transcriptome profile was closer to SCN than PCN and *Meloidogyne* spp. (Fosu-Nyarko et al. 2016).

Numerous studies aimed to provide further information on the interactions among SBCN and *A. thaliana*. Szakasits et al. (2009) indicated that the transcriptome of SBCN syncytia differs from other cells in the root system. Elashry et al. (2020) used next-generation sequencing to detect close to 200 SBCN putative effectors. After some of these effectors were knocked down, *A. thaliana* plants displayed reduced nematode parasitism and smaller syncytia size. In a different study, the 32E03 effector protein was demonstrated to inhibit the activity of histone deacetylases in *A. thaliana*, comprising the HDT1 enzyme. The effector protein adjusts plant gene expression through these alterations, facilitating SBCN parasitism (Vijayapalani et al. 2018). An SBCN secretory protein (10A06) is an effector during the initial stages of syncytia development, affecting *A. thaliana* morphology and increasing plant susceptibility by disrupting the salicylic acid pathway signaling (Hewezi et al. 2009).

Infective-stage juveniles (J2s) are also primed for root infection. Fosu-Nyarko et al. (2016), after sequencing and annotating the transcriptome of SBCN J2s, found a series of critical genes linked to the modulation of host defenses and syncytia formation even before host recognition. Theoretically, early stimulation of the resistance pathways state may enable plants to become more resistant to pathogen infection, as they would be induced to a sensitized state, responding more efficiently to subsequent pathogenic attacks (Rocha et al. 2022b; Shoresh et al. 2005). Several examples are described in the literature, mainly focusing on priming resistance by beneficial microorganisms. Plant-associated bacteria, including *Pseudomonas* sp., were shown to induce the priming of resistance-related phenolic compounds, resulting in reduced infections of *Hymenoscyphus fraxineus* in European ash trees (*Fraxinus excelsior*) (Striganaviciute et al. 2021).

14.7 Corn Cyst Nematodes (*Heterodera zeae*)

Corn cyst nematode is another important pest in India and other countries (Koshy and Swarup 1971). The occurrence of corn cyst nematode, *Heterodera zeae* Koshy et al. 1971, was initially reported from corn crops in Rajasthan (India) (Koshy et al. 1970). Around 17–29% yield losses have been reported in maize by *Heterodera zeae* in India, particularly in Rajasthan state, due to congenial soil factors, monoculture of host crops, and lack of efficient management approaches (Srivastava and Chawla 2005).

14.7.1 Novel CCN Biological Management Practices

The biological management of plant–parasitic nematode species with various antagonistic organisms has increased in the present era (Jatala et al. 1986). Fungal parasites, i.e., *Purpureocillium lilacinum*, *Pochonia chlamydosporia,* and *Trichoderma viride,* reduce the nematode population and promote plant growth. *P. lilacinum* (4%) reduced cyst population by up to 35.71% followed by *Pochonia chlamydosporia* (4%) up to 25% and *Paecilomyces lilacinus* (2%) up to 22.62% (Baheti et al. 2015). Aak, Neem, and water hyacinth leaf powder (each four g/plant as soil amendment) significantly increased the growth of maize plants infected with *H. zeae,* which may be because of the production of phenols, amino acids, and HCN compounds (Mehta et al. 2015). *Calotropis Procera* at 4% was found to be most effective in managing *H. zeae*, followed by the same at 2% (Kumhar et al. 2018a, b). The same treatments enhanced plant growth and reduced the nematode population, possibly linked to phenols, enzymes, and substances with nematicidal activity such as calactin, calotropin, and mandarin. Integrating biological control agents and botanicals has been reported to promote plant development and protect against plant–parasitic nematodes. *Purpureocillium lilacinum* and neem leaf powder reduced the population of the corn cyst nematode (Baheti et al. 2017). Five phytoceramides from the yellow flower extract of *Tagetes patula* have been identified. One ceramide (HFA) showed total mortality against *H. zeae*, while *β*-sitosterol and a commercial product, stigmasterol at 1%, were 40–50% active after 1 day of exposure (Samina et al. 2019). Organic pesticides originating from plant products may have excellent nematicidal properties. Petroleum ether extract from *Ceriops topga*llant (CTPM-IN-2) resulted in 91% mortality at 0.5% and 93% mortality at 1% concentration against *H. zeae* (J2) when exposed for 72 h (Ahmad et al. 2022).

14.7.2 Novel CCN Genetic Engineering Technologies

A limited number of studies focus on genetic engineering technologies to manage the corn cyst nematode compared to other cyst-forming species. A series of molecular markers and the detection of this species, including the rDNA internal transcribed spacer regions (ITS1 and ITS2), the 28S large ribosomal subunit (LSU) D2–D3 expansion segment, and partial 18S small ribosomal subunit (SSU) (Skantar et al. 2012).

Genetic resistance and crop management practices are well understood for the corn cyst nematode. Corn varieties with varying resistance levels are available to farmers (Hashmi et al. 1993; Shahina et al. 1989). Ismail (2009) assessed the influence of barley, broad bean, clover, and wheat on corn cyst nematode populations in rotation with corn. Winter wheat and barley increased initial inoculum pressure at corn planting more than broad bean and clover. Root exudates from other plants also can potentially suppress this nematode's field populations (Haroon et al. 2009).

A sequenced and annotated genome is not currently available for the corn cyst nematode, which limits the advances in understanding plant–nematode interactions and resistance genes. Field populations of this nematode have genetic and geographical variations, suggesting strong isolation among different populations (Grace et al.

2009). These genetic variations can be detected through RAPD (random amplified polymorphic DNA) markers and microsatellites (Meher and Venkatesan 2004). Further work classified Indian populations of corn cyst nematode into cluster-based on egg and second-stage juveniles' morphometric readings, highlighting differences in these parameters among geographically diverging populations.

14.8 Conclusion and Future Perspectives

Novel biological and genetic-based management practices were discussed for the management of soybean cyst nematode (*Heterodera glycines*), cereal cyst nematode (*H. avenae*), sugar beet cyst nematode (*H. schachtii*), potato cyst nematode (*Globodera pallida and G. rostochiensis*), and corn cyst nematode (*H. zeae*). Within these species, many publications target the soybean cyst nematode, as this pathogen has global distribution and is pathogenic to soybean, the main oilseed worldwide. Biological management practices often investigate bacterial and fungal isolates as potential biological control agents. Some of these isolates were identified through culture-based or metagenomic methods after characterizing the cyst microbiome. Genetic engineering-based methods frequently aim to unravel genes and nematode effectors involved in parasitism and syncytium formation. Although the different cyst nematode species discussed above are pathogenic to various plants as hosts, since their life cycles and developmental stages are similar, research finds may apply to additional cyst nematode pathosystems.

Further research is necessary to better characterize the microbiome of cyst species other than *H. glycines*, *H. avenae*, and *H. schachtii* and test potential biological control agents in field conditions and under long-term studies. On the genetic aspect, assembled and annotated genomes are still not available for significant cyst nematodes, as examples of *H. zeae*. While some species have annotated genomes, more work is required to characterize additional pathogenicity genes.

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Stem and Bulb Nematodes in Agricultural Crops and Their Management by Biological and Biotechnological Methods

15

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and Raman Kumar Walia

Abstract

The stem and bulb nematode, *Ditylenchus dipsaci* is a voracious pathogen of crops and displays a broad host range. It has been designated a quarantine pest in several countries, including India. For the management of this nematode, crop-specific methods are recommended. However, the information on natural resistance, biotechnological approaches, and specific biocontrol agents for this nematode is scant. The 227.2 Mb genome of *D. dipsaci* was sequenced in 2019, and further identification of nematode genes and processes essential for parasitism are expected to push the use of biotechnological approaches for managing the stem and bulb nematode.

Keywords

Ditylenchus dipsaci · Management · Pathogen · Plant–parasitic nematode · Stem and bulb nematode

15.1 Introduction

With the ever-increasing world population and looming climate change, agriculture is under high pressure from biotic and abiotic stresses. Global warming is expected to affect many areas of crop production through changes in climatic extremes. The pests and pathogens characterized previously as minor constraints can emerge as major ones, as the increase in average temperature will lead to more generations of those pests per year. Globalization has also increased the risk of invasive species dispersal into new habitats due to worldwide trade, which climatic changes will

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promote by providing suitable environments for exotic pests. In the absence of natural enemies, these introduced pests would spread rapidly. Therefore, crop protection would be critical to secure the nutritional security of the masses.

Among the variety of pathogens and pests attacking crops, plant parasitic nematodes (PPNs) have established themselves as a significant constraint to global food production systems, with an estimated $183 billion in global economic losses (Elling 2013). To date, ~4100 PPN species are described (Decraemer and Hunt 2006), affecting all types of crops and their shoot and root system (Khan 2008, 2023). When the nematode-associated scientific community was asked about the top 10 PPNs in the world, root-knot nematodes (*Meloidogyne* spp.), cyst nematodes (*Globodera* and *Heterodera* spp.), root lesion nematodes (*Pratylenchus* spp.), *Radopholus similis*, *Ditylenchus dipsaci*, *Bursaphelenchus xylophilus*, *Rotylenchulus reniformis*, *Xiphinema index*, *Nacobbus aberrans*, and *Aphelenchoides besseyi* were adjudged as the top 10 PPN problems (Jones et al. 2013). Among these, while the majority of PPNs from the list are soil dwellers that feed on root systems, only three PPNs affecting the aboveground parts of their respective host plant made it into the list. *Ditylenchus dipsaci*, commonly known as the stem and bulb nematode was adjudged as one of the most destructive non-telluric PPNs among the lot.

The family Anguinidae contains mycophagous nematodes and PPNs that attack aerial plant parts, bulbs, and tubers. Among these, the genus *Ditylenchus* comprises more than 60 species, a few of which are parasitic on higher plants (Duncan and Moens 2013). *Ditylenchus angustus* (rice-stem nematode), is ectoparasite of rice leaves and stems and causes Ufra disease in Southeast Asia including India. In addition to being fungivorous, *D. destructor* (potato rot nematode), and *D. africanus* (peanut pod nematode), parasitize potato tubers in Europe and peanuts in South Africa, respectively. *D. myceliophagus*, the most prominently known fungivorous nematode of the genus, is a key nematode problem of mushroom production in the world. *D. gigas* (Vovlas et al. 2011), which was earlier considered as *D. dipsaci* giant race, later turned out to be a new species parasitizing *Vicia faba* (broad bean) in Europe, Asia, and Africa. *D. arachis* was identified as a new species in China, isolated from groundnut seeds and hull. However, the most popular species of the genus is *Ditylenchus dipsaci* due to its wide host range, distribution, and ability to survive severe desiccation.

15.2 The Stem and Bulb Nematode, *Ditylenchus dipsaci*

15.2.1 Distribution and Host Range

D. dipsaci is widespread in an extensive range of climatic conditions and has been recorded in 82 countries in Africa, America, Asia, Europe, and Oceania (CABI 2009; Khan et al. 2020). In addition, it is widespread in Austria, Chile, Czechia, Germany, Israel, Mexico, New Zealand, Slovakia, Sweden, Switzerland, the United

Table 15.1 Major hosts of stem and bulb nematode *D. dipsaci* (EPPO 2017)

Family	Host plants
Gramineae	Oat (*Avena sativa*), rye (*Secale cereale*), maize (*Zea mays*), wheat (*Triticum aestivum*)
Liliaceae	Onion (*Allium cepa*), garlic (*Allium sativum*), tulip (*Tulipa* spp.)
Fabaceae	Bean (*Vicia faba*), lucerne (*Medicago sativa*), pea (*Pisum sativum*), clover (*Trifolium* spp.)
Solanaceae	Potato (*Solanum tuberosum*), tobacco (*Nicotiana tabacum*)
Cruciferae	Cabbage (*Brassica oleracea var. capitata*), turnip (*Brassica rapa subsp. rapa*), mustard (*Brassica* spp.)
Moraceae	Hemp (*Cannabis sativa*)
Amarilidaceae	Narcissus (*Narcissus* spp.)
Others	Carrots (*Daucus carota*), sugarbeet (*Beta vulgaris*), strawberry, (*Fragaria ananassa*), leeks (*Allium porrum*), *Phlox drummondii*, *P. paniculat*a, *Hyacinthus orientali*s, carnations (*Dianthus caryophyllus*), celery (*Apium graveolens*), *Hydrangea*, lentils (*Lens culinaris*), rape (*Brassica napus*), parsley (*Petroselinum crispum*), sunflowers (*Helianthus annuus*), apples (*Malus* spp.), and peaches (*Prunus persica*) in nurseries

Kingdom, the United States, and Uruguay. Over 500 plant species from around 40 angiosperm families including vegetables, field crops, and weeds are known as hosts for *D. dipsaci* (Subbotin et al. 2005). Initially, Ritzema Bos (1888) observed numerous host plant species for *D. dipsaci* for the first time. *D. dipsaci* has the highest economically impact on cash crops like lucerne, onion, garlic, clover, narcissus, and faba bean (Beyerinck 1883; Godfrey and Scott 1935; Ormerod 1886; Ritzema Bos 1888). Several weed species are also good hosts of *D. dipsaci* for, e.g.., chickweed (*Stellaria media* L.), purple dead-nettle (*Lamium purpureum* L.), cleavers (*Galium aparine* L.), and *Anagallis arvensis* L., the scarlet pimpernel (Goodey 1947; Johnson 1938; Staniland 1945) (Table 15.1).

15.2.2 Morphological and Molecular Diagnosis of *D. dipsaci*

Morphological diagnosis of *D. dipsaci* depends on the following characteristics (Sturhan and Brzeski 1991; Wendt et al. 1995). When relaxed, the nematode body is straight. The lateral field contains four incisures. The nematode head is continuous, and the labial area contains the amphidial apertures. Hemizonid is approximately six annules wide and is located next to the excretory pore. The labial region is square shaped, and lips are medial and large and divided by a small bulge into submedial lobes. The head contains labial disk and four annules. The stylet length in females is 10–13 μm and in males 10–12 μm. Stylet knobs are rounded, and the cone is approximately half of the length of the stylet. The median bulb is muscular, with 4–5 μm thick lumen wall. The basal bulb either marginally overlaps the intestine or is offset with two inconspicuous and three prominent gland nuclei. Excretory pore is located near the posterior part of the glandular bulbar isthmus. The ovary of *D. dipsaci* is outstretched, with one or two flextures, mostly reaching up to the basal bulb but occasionally may reach up to the median bulb. The outstretched testis and the spermatocytes are mostly arranged in a single file. Three-quarters of the male tail is enveloped by the bursa. Spicules are 23–28 μm in length. Tails are conical in males and females and have a pointed tip. Diagnostic keys for various species of *Ditylenchus* genus have been provided by Sturhan and Brzeski (1991) and Viscardi and Brzeski (1993).

This species can also be characterized using PCR-based techniques using the DNA sequence of several marker genes including D2–D3 region and ITS-rRNA region (Mollov et al. 2012; Testen et al. 2014). Subbotin et al. (2005) developed species-specific primers for amplifying ITS-rRNA region in a study using 30 *D. dipsaci* populations. Species-specific assays and SCAR PCR assay have also been developed and used for either differentiating normal and giant race (Esquibet et al. 2003) or identification of various species (Zouhar et al. 2007).

15.2.3 Different Races of *D. dipsaci*

D. dipsaci has extensive intraspecific variations with at least 30 distinguishable host races (Sturhan and Brzeski 1991). Many physiological and biological races, such as 15 (Kirjanova and Krall 1971) and 30 races (Ladygina 1982), were identified and named according to their main hosts. This led researchers to regard *D. dipsaci* as “a species complex” with two groups. The diploid populations as characterized by having a “normal” size were considered as the first group called “*D. dipsaci sensu stricto*.” The second set of polyploid populations was subdivided into *Ditylenchus* spp. B or giant race (now described as *D. gigas*); *Ditylenchus* sp. C (now known as *D. weischeri* (Chizhov et al. 2010)); and *Ditylenchus* sp. D, E, and F (have hosts from Fabaceae, Asteraceae, or Plantaginaceae) (Jeszke et al. 2014).

15.2.4 Biology and Lifecycle

D. dipsaci is a migratory endoparasite and completes its life cycle within leaves, stem, and bulbs (but rarely in roots). Reproduction in *D. dipsaci* is by amphimixis after four molts of developmental stages (Hooper 1972), and a single male can fertilize multiple females (Sturhan and Brzeski 1991). At 15 °C, *D. dipsaci* completes its life cycle in 19–23 days (Yuksel 1960). It takes 2 days for the J2s to hatch and develop into females in 4–5 days (Duncan and Moens 2013). Each mature female is known to produce 200–500 eggs and can survive for 45–73 days (Yuksel 1960). The host plant growth rate, ambient temperature, and moisture can affect the reproduction of *D. dipsaci*. *D. dipsaci* develops optimally at 15 °C, but the temperature can vary according to hosts; for, e.g., on onion the optimal temperature for its development is 21 °C (Sayre and Mountain 1962); on alfalfa 20 °C (Griffin 1968);

and on oats 15 °C (Blake 1962). *D. dipsaci* is highly resistant to low temperatures, and some juveniles could survive exposure to −150 °C for 18 months (Sayre and Hwang 1975). *D. dipsaci* produces multiple generations in each growing season, and therefore, the nematode populations may cross economically damaging threshold levels.

All developmental stages except the egg can infect their host. However, the major infective stage is J4 (the fourth-stage juvenile) as it can survive desiccation by undergoing anhydrobiosis as "nematode wool" (EPPO 2017). Nematode wool is a lump of juvenile nematodes (generally J4), which have undergone anhydrobiosis, and are found on the infested plant or tissue surface (Hooper 1972). Dry nematodes are metabolically reactivated by moisture when the infested planting material is used. J4s desiccated in 50% relative humidity can regain activity after exposure to water (Wallace 1962). This allows the nematode to survive in unsuitable environmental conditions including low temperature and moisture. In soil, some races (e.g., garlic and bean) can survive up to 2 and 9 years, respectively (Tenente 1996).

15.2.5 Symptoms

D. dipsaci causes twisting, distortion, and discoloration of stems and foliage of ornamentals and distorted and cracked bulbs. Stunted growth, failure to bloom, and swelling of hypocotyl and epicotyl and premature death are major symptoms of nematode infestation (Caubel et al. 1994). This nematode attacks young buds of field crops, including oats, alfalfa, and clover, preventing normal stem elongation. Infected stems are shorter than healthy stems due to decreased internodal length. Usually, nematodes penetrate the base of stem, leaf axils, the bulb scales, or through stomatas and feed on cellular contents, leading to necroses and distortion of the plant tissues.

D. dipsaci typically invades parenchyma and causes enzymic degradation of pectic/ middle lamella between cells leading to rounding and separation. Enzymatic secretions by nematode also lead to hormone imbalance, cellular hypertrophy, and intercellular cavities typical of migratory endoparasites (Duncan and Moens 2013). This can also cause infested tissue to appear as glistening and mealy in texture (like an over-ripe apple's flesh) (Southey 1993). Nematode infestation can economically impact storage, and they can continue to decompose infested onion bulbs (Macias and Brzeski 1967). Heavy infection may reduce number of stems and make plants susceptible to winterkill and other maladies such as bacterial wilt and *Fusarium* wilt. Symptoms caused by *D. dipsaci* on its different hosts are briefly described in Table 15.2.

Table 15.2 Symptoms caused by *D. dipsaci* on different hosts (EPPO 2017)

Sr. no.	Host	Symptom
1.	*Allium* spp. (onion, garlic, and shallots)	• Basal swelling and distorted leaves in young plants
		• Young plants killed at high infestations
		• Older bulbs are swollen (bloat), scales with cracks at the root disk
		• Infested onion plants have frosted appearance due to cellular dissolution by nematode feeding
		• Leaf yellowing and death of infested garlic plants bulbs soften as season advances and show browning of scales in concentric circles when cut
2.	Lucerne/alfalfa	• "White flagging" due to loss of chlorophyll in moisture stress condition • Predisposes infected plants to *Phytophthora megasperma*
		• Swelling of the basal region, and twisting and stunting of stalks and leaves
		• Shorter internodes with numerous axillary buds
		• Abnormal tillering causing bushy appearance of plant
		• Failure to produce flower spikes
		• Irregular areas of sparse growth in infested fields
3.	Narcissus	• Presence of blister-like pale-yellow swellings on leaves (known as spikels)
		• Concentric rings of brown color in bulbs upon transverse sectioning
		• Transverse cut in bulb reveals necrosis start at the neck and spreads downward
4.	Carrot	• Skin with transverse cracks along with white patches in sub-cortex tissue
		• Decay and rot due to secondary infections by fungi and bacteria
		• Severe crown rot in autumn
5.	Sugar beet	• Damage to both seedlings and mature plants
		• Development of multiple crowns in seedlings owing to death of the growing points
		• Twisting, swelling, and distortion of cotyledons and leaves
		• In somewhat older plants, galling of leaves or petioles
		• "Crown rot, crown canker or collar rot" in mature plants due to nematode feeding on crowns
6.	Rye and oats	• Short, stunted, and bushy plants due to leaf distortion, thickening of stems, and abnormal tillering
		• Heavy infestation leads to death of seedlings causing patchy appearance in the field
		• "Rye race" is common in Europe and "oat race" is economically important in Britain

(continued)

Table 15.2 (continued)

Sr. no.	Host	Symptom
7.	Red and white clover	• Symptoms similar to alfalfa
		• Stunting and hypertrophy of infected leaves and petioles, shortening of internodes with the proliferation of stolons
		• Patches of stunted growth are a good indication of damage
8.	Hyacinth and other bulbs	• Bulb symptoms in hyacinth are similar to narcissus (however, spikels are usually not visible on leaves)
		• Distorted leaves with pale-yellow streaks and some swelling
		• Nematode infestation in Amaryllidaceae is similar to narcissus
9.	Tobacco	• Small, yellow swellings (galls) in young infected seedlings that might spread up to 40 cm or above the soil level
		• Low leaves might drop off and yellowing of upper leaves
		• Nematode invasion of lower stem leads to plant stunting and deformation, afterward causes "stem break" triggering plants to fall over
10.	Faba beans, vetch, pea	• Infected pea and bean plants are short, shrubby, and several of these plants die
		• Deformation and swelling of tissue of stem, or reddish-brown to black lesions
		• New formed pods are dark-brown
		• Leaf and petiole necrosis
		• Seeds infected by nematodes are smaller in size, distorted, dark, and may contain speckle-like spots
		• "Nematode wool" visible on seeds in cases of heavy infestation
		• Heavy infestation kills main shoots causing secondary tiller formations
		• Total necrosis of stem on vetch
11.	Potato	• "Funnel-shaped rot" which is deeper than the surface rot
		• Invasion of stem/leaves in wet weather leads to typical stunting with severe distortion of stems and petioles
12.	Tulips	• Infestation begins in new bulbs at the base
		• Outer scales show gray or brown areas
		• Brown rings like narcissus and hyacinth are not present
13.	Strawberry	• Small and distorted leaves with petioles that are thick, short, twisted
		• Foliage dries and falls off
14.	Maize	• Maize is a poor host
		• Young plant's stem is invaded resulting in necrosis causing plants to die or fall over

(continued)

Table 15.2 (continued)

Sr. no.	Host	Symptom
15.	Phlox and ornamentals	• Stems typically thick and brittle, internodes short and prone to splitting
		• Crinkling of leaves and reduced upper leaf laminae may become just diminished filaments
		• Angular interveinal necrosis patches in some hosts (similar to *Aphelenchoides* spp. infestation)

15.3 Traditional Approaches for Management of Stem and Bulb Nematode

The presence of intraspecific diversity of *D. dipsaci* and large host range is critical in choosing an effective management method. It has been suggested that no method can prevent the nematode spread or manage it completely (Hughes et al. 2013). Traditionally, the management of *D. dipsaci* infestation is done through good cultural practices such as using clean seeds and planting material, treatment with heat, rotation of crops, soil solarization, and cleaning equipments used for agricultural operations. Chemical measures like fumigants and nematicides are ever more problematic because of environmental and uneconomical concerns in most cases. New nematicide fluopyram demonstrated poor results when tested against *D. dipsaci* (Storelli et al. 2020).

15.3.1 Nematode-Free Planting Material

Certified nematode-free planting material and seeds are most essential to prevent *D. dipsaci* infestation. Fields can be quickly infested due to its fast life cycle, so avoiding contamination of agricultural fields and nurseries with contaminated seeds, water, soil or plant debris from previously infested crops is critical.

15.3.2 Physical Measures

Using hot-water treatments for various combinations of temperature and time, based on state and type of seed/planting material, is an effective and affordable method to avoid initial inoculum for the disease (Gratwick and Southey 1972). Hot-water treatment either alone or in combination with chemicals has been investigated in many crops to manage this nematode (Green 1964; Hanks and Linfield 1999; Qiu et al. 1993; Roberts and Matthews 1995). Narcissus bulb hot-water treatment is achieved either by storage at 25–30 °C for 1–2 weeks, soaking for 24 h in water followed by treatment in hot water for 4 h at 45 °C or 47 °C. Roberts and Matthews (1995) demonstrated hot-water treatment (49 °C) along with 20 ppm abamectin for 20-min managed stem and bulb nematode in garlic. In narcissus, peroxyacetic acid at 0.5–1.5% added to hot water at 44.4 °C post-exposure of 2 h, 1 h, and 30 min caused 100% nematode mortality (Hanks and Linfield 1999). As this nematode has short persistence in warm moist soil, control can be achieved by soil solarization and crop rotation (Duncan and Moens 2013). Soil solarization is a simple but effective measure for nematode management in warmer climatic conditions as the soil temperature can increase up to 50 °C (Katan 1987). Soil solarization as a control measure providing reduction in stem nematode population was utilized in Israel (Siti et al. 1982) and Italy (Greco et al. 1985; Greco and Brandonisio 1990). However, this cannot be utilized in temperate countries where stem and bulb nematode is a major menace.

15.3.3 Crop Rotation

In case of *D. dipsaci*, crop rotation's success would depend on the race and its host range, non-host crop availability, and control of weeds. Some *D. dipsaci* races are polyphagous and can persist in clays. However, in New York State, crop rotation with lettuce completely controlled this nematode (Lorbeer 1997). Similarly, it is expected that rotating alfalfa with small grains, corn, or beans can manage this nematode.

15.3.4 Biofumigation

Biofumigation is also effective in reduction in nematode population in field, where mechanically cut brassica plants are incorporated into soil (Dutta et al. 2019). Secondary metabolites like dhurrin, poly-thienyls, and glucosinolates are antagonistic to the nematodes. The biofumigant effect is primarily due to toxic and volatile isothiocyanates produced because of glucosinolate hydrolysis in tissues of Brassica. *Brassica juncea* L. Coss, also known as cutlass mustard, has shown nematicidal activity against this nematode. A preliminary study pertaining to using brassica and *Tagetes* sp. as green manure for control of *D. dipsaci* also showed promising by arresting mobility in the juveniles (Yavuzaslanoğlu et al. 2021).

15.3.5 Host Resistance

The most cost-effective and competent approach for managing PPNs is host resistance. Sources with genetic resistance against many of the *D. dipsaci* races are available, with commercial resistant cultivars or genotypes reported in alfalfa, clover, faba bean, garlic oat, and potatoes (Charchar et al. 2003, McDaniel and

Barr 1994; Mwaura et al. 2015; Peng and Moens 2003; Starr et al. 2013; Stanton et al. 1984). Complete resistance against *D. dipsaci* has not been reported in onion or sugarbeet (Storelli et al. 2021; Yavuzaslanoglu 2019). A positive correlation on red clover between symptoms expressed at 3 weeks and reproduction of *D. dipsaci* at 10 weeks was detected (Caubel et al. 1994). Resistant cultivars are reported for various crop plants; however, the resistance is *D. dipsaci* race or population-specific.

15.4 Biological Control of Plant–Parasitic Nematodes

Biological control refers to the utilization of microbial antagonists or use of natural products to suppress diseases (Pal and Gardener 2006). Biocontrol agents manage nematode infection by hyperparasitism and antibiosis. Biological control of PPNs has been in practice since long (Ahmad et al. 2021; Hay and Bateson 1997; Khan 2016; Mendoza et al. 2008; Moosavi and Zare 2020; Zouhar et al. 2009). The biocontrol fungi/bacteria alone (Stirling 1991; Khan 2007; Khan and Anwer 2011) or along with oil, neem cakes (Sikora and Roberts 2018; Khan et al. 2021) or pesticides (Mohiddin and Khan 2013) are getting popularity in achieving sustainable nematode management in agricultural crops (Khan 2023; Khan et al. 2023). The microbial antagonists, *Aspergilus niger, Pochonia chlamydosporia, Purpureocellium lilacinum, Pasturia penetrans* etc. (Jatala 1986; Stirling 1991; Kerry 2000; Khan 2016), and phosphate solubilizing microorganisms such as *Aspergillus, Bacillus, Penicillium, Pseudomonas* etc. (Khan et al. 2009, 2016a, b; Sikora and Roberts 2018) may significantly contribute in the sustainable management of plant nematodes. The well known mycoparasitic fungus, Trichoderma has also been found effective in suppressing plant nematodes (Mohiddin et al. 2010; Khan and Mohiddin 2018), and numerous formulations of *T. harzianum*, *T. hamatum* etc. are available in market (Khan et al. 2011), and provide consistently satisfactory control of soil-born pathogens (Mohammed and Khan 2021; Sikora and Roberts 2018; Shahid and Khan 2019). Major types of biocontrol agents (BCAs) used against plant–parasitic nematodes are given in Table 15.3.

15.4.1 Biocontrol of Stem and Bulb Nematode

Very few studies pertaining to the effect of biocontrol agents against *D. dipsaci* are available in the literature. It is possible that nematophagous fungi, for example, *Verticillium balanoides,* are useful for biological control of *D. dipsaci* as found in white clover (Hay and Bateson 1997). In vitro activity of *Bacillus firmus* against *D. dipsaci* showed that the bacteria produced secondary metabolites toxic to the nematode (Mendoza et al. 2008). These metabolites showed paralytic to lethal effect on nematode juveniles. Application of *Beauveria bassiana*, an entomopathogenic

Table 15.3 Some major fungal and bacterial biocontrol agents of plant–parasitic nematodes

BCA	Mode of action	Example
Fungi	Predatory fungi	*Arthrobotrys oligospora, A. superb, A. dactyloides, Dactylaria brochopaga, Monacrosporium cionopagum, Dactylaria candida*
	Egg parasites	*Pochonia chlamydosporia* and *Paecilomyces lilacinus*
	Adhesive spore-forming nematophagous fungi	*Nematodontous teliospores, Hirsutella* sp., *Syncytium lenticular, Catenaria anguillulae, M. anomalum*
	Arbuscular mycorrhizal fungi	*Rhizophagus irregularis, Scutellospora heterogama, Funneliformis mosseae*
	Paralyzing toxin-secreting fungi	*Cyathus striatus, Fomitopsis pinicola, Gymnopilus junonius*
Bacteria	Parasitic bacteria	*Pasteuria* spp.
	Opportunistic parasitic bacteria	*Brevibacillus laterosporus, Bacillus* sp. *B16* and *RH219*
	Plant growth-promoting rhizobacteria	*Pseudomonas* sp., *Bacillus* sp., *Bacillus firmus T11, Bacillus cereus N10w, Bacillus aryabhattai A08, Bacillus subtilis*
	Parasporal cry protein-forming bacteria	*Bacillus thuringiensis var. thuringiensis*
	Trojan horse mechanism	*Bacillus nematocida (B16)*

fungus, reduced damage of potato tubers by *D. dipsaci* and *D. destructor* (Mwaura et al. 2017). Several natural enemies such as *Drechmeria coniospora* and *Hirsutella sinensis* (Cayrol and Frankowski 1986) are natural pathogens of *D. dipsaci*. Moreover, *Dactylella lysipaga* and *Rhizoglyphus echinopus* (bulb mite) are natural predators of this nematode. These predators and parasites can be tested further for their efficacy against *D. dipsaci* for which future studies are warranted.

In addition to biocontrol agents, several essential oils and crude plant extracts have also been tested against stem nematode. *Origanum vulgare*, *O. compactum*, and *Thymus vulgaris* essential oils caused higher mortality of *D. dipsaci* in 3 h of exposure at 1500 ppm (Zouhar et al. 2009). A product containing *Thymus* spp. extracts marketed as ProMax® is also effective against several plant–parasitic nematodes including *D. dipsaci*. Another in vitro screening of plant extracts of *Inula viscosa* and against *D. dipsaci* led to >70% mortality in juveniles (Hassan et al. 2015). The effect of *Morina persica* extracts in an in vitro screen also showed promising results against *D. dipsaci* and several pathogenic fungi in Turkey (Onaran and Sağlam 2017). These results show new ways that may be explored to search alternatives of synthetic fumigants for PPN management.

15.5 Biotechnological Approaches for Stem and Bulb Nematode Management

One prerequisite for the use of any biotechnological approach for the management of *D. dipsaci* is the knowledge of nematode effectors and genes important for plant parasitism and nematode biology. The recent advances in sequencing of plant–parasitic nematode genomes and transcriptomes have been instrumental in ushering use of biotechnology in the management of plant–parasitic nematodes. The genome and transcriptome of *D. dipsaci* were sequenced in 2019 (Mimee et al. 2019). The genome size of *D. dipsaci* was 227.2 Mb, with the GC content of 37.5%, and 26,428 putative genes were annotated (Mimee et al. 2019). However, a more detailed analysis of the genome is yet to be done to identify the effectors and other important target genes. Biotechnology is useful in several ways for the management of plant–parasitic nematodes. Primarily, it has been utilized through the transgenic approach by creating host-delivered RNA interference targeting the nematode feeding site, various nematode effectors, and chemosensory genes (Dutta et al. 2015; Fosu-Nyarko and Jones 2015; Sivasubramaniam et al. 2020). It may also be used to transfer the natural nematode resistance genes to different crop species or popular cultivars. A thorough literature search has revealed that so far none of the prevailing biotechnological approaches have been used for the management of stem and bulb nematodes. In addition to the existing approaches, topical application of RNAi and genome-editing-based approaches are promising and are expected to emerge as major approaches to fight PPNs in future.

15.6 Conclusion and Future Perspectives

D. dipsaci has one of the largest host ranges after *Meloidogyne* spp. It is an important quarantine pest in several countries and is not yet present in India. Therefore, exclusion is the best approach for managing this nematode. An important biological characteristic of this nematode species is the presence of several races and high genetic variation in its populations, making managing this nematode extremely difficult. The cultural methods are the most economical in the absence of nematode-resistant cultivars. The information on the biological control agents is inadequate for this species, and more research is required. The genome of *D. dipsaci* was sequenced in 2019, but the studies on the effectors and molecular host–plant interactions are needed to utilize biotechnological approaches for its management. Table 15.4 provides a summary of strategies that may be used for the management of this nematode.

Table 15.4 Possible approaches to be utilized for the management of *D. dipsaci*

S. No.	Objective	Strategy	Tool/effect	Effectiveness against *D. dipsaci*
1.	Exclusion avoidance	Quarantine	Avoiding nematode spread to new localities	Large host range might exclude crops to be tested for stem nematode during phytosanitary inspection
2.	Reduce initial population density	Cultural control	Certified seeds and planting material	Nematode-free seeds and planting material can avoid initial inoculum for pest
			Farm sanitation	Avoids nematode infestation
			Soil amendments	Effective to some degree
			Weeding	Avoids buildup of initial inoculum
			Crop rotation	Not very effective due to several biological races and ability of nematode to undergo anhydrobiosis
			Biofumigation	Effective against *D. dipsaci* and other soil-borne pathogens
		Physical control	Hot-water treatment	Economical and effective but depends on amenability of planting material to heat exposure
			Soil solarization	Not effective in temperate regions
		Biological control	Use of nematode antagonistic fungi, bacteria, PGPR, VAM	No commercial formulation targeted for *D. dipsaci* exists
		Resistance and tolerance	Cultivation of genetically resistant and nematode-tolerant varieties	Very few resistant varieties exist; resistance can vary to different populations/races of *D. dipsaci*
		Transgenic plants	Host-induced gene silencing of a selected nematode target gene	Effectiveness of target gene has to be the same for biological races
3.	Suppress nematode reproduction	Transgenic/genome-edited plants	Targeting nematode or host gene	
		Tolerant cultivars	Reduce economic damage to crops	Population can build up rapidly which can affect standing crop and provide large initial inoculum for next season
4.	Restrict current crop damage	Nematicide application	Eradicate current nematode population	No nematicides registered for *D. dipsaci*

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16

Leaf and Bud Nematodes in Agricultural Crops and Their Management by Biotechnological Approaches

B. B. Westerdahl and Oluwasesan M. Bello

Abstract

Leaf and bud nematodes in the genus *Aphelenchoides* constitute an important limiting factor in the production of a number of important agricultural crops. The *Aphelenchoides* species which attack aboveground parts of plants cause major economic loss to rice, strawberry, mushrooms, and ornamentals. Losses on rice due to nematode infestation are estimated at 10%. On strawberry, the losses can be as high as 65% in the infested fields. Depending on the level of infestation, losses on mushrooms can be as high as 42%. Important species include *A. besseyi* on rice and strawberry, *A. fragariae* and *A. ritzemabosi* on strawberry, and *A. composticola* on mushrooms. Symptoms of damage are often not recognized because of their similarity to those caused by other pests, diseases, and cultural problems. Molecular methods of identification have been developed to assist with the identification of species. Leaf and bud nematodes can be managed by preventive, physical, cultural, biological, and chemical means. Utilizing certified nematode-free planting materials can prevent infestation. High-temperature treatments can be used to eradicate nematodes from infested planting materials. The use of resistant or tolerant varieties can minimize damage. To help manage *Aphelenchoides*, it is important to have extension personnel to assist growers with recognizing the problem and developing management programs. Because the crops of interest are raised, exported, and imported

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worldwide, it is important to have regulatory programs to minimize the possibility of nematode-infested planting material being exported or imported.

Keywords

Aphelenchoides · Rice · Strawberry · Mushroom · Phytonematodes

16.1 Introduction

Leaf and bud nematodes in the genus *Aphelenchoides* are important limiting factor in the production of agricultural crops. These nematodes attack the aboveground parts of plants causing major economic loss to rice, strawberry, mushrooms, and ornamentals. Rice, *Oryza sativa*, is an important source of food for many people, particularly in Asia, Sub-Saharan Africa, and South America. It is an annual grass that originated in Asia and has been cultivated for thousands of years. It is planted either from seeds or transplants. It grows best with high daytime temperatures and cool nights. It utilizes large amounts of water and is typically planted in a soil type that limits percolation in order to maintain a flooded condition. The ten largest producing countries in order from most to least are China, India, Indonesia, Bangladesh, Vietnam, Thailand, Burma, Philippines, Brazil, and Japan with a total production of 421.3 million metric tons each year. These same countries are also the top ten rice-consuming countries. India, Thailand, Vietnam, Pakistan, the United States, China, Burma, and Cambodia are the largest exporters of rice. Sub-Saharan Africa is the largest importer, followed by the Middle East and Southeast Asia, East Asia, and South Asia (Childs 2022).

The commercial strawberry, *Fragaria* × *ananassa,* was bred in France in the 1700s by crossing plants from eastern North America and from Chile. Commercial strawberries for fruit production are planted from stolons also called “runners” that are horizontal stems arising from a “mother” plant. The runners are produced by the strawberry nursery industry and sold to the fruit production industry. For fruit production, strawberries are grown either as annuals on raised beds that are covered with plastic or as perennials. Annual cropping requires greater initial investment but returns a higher yield (Darrow 1966). The largest producing countries from highest to lowest are China, United States, Egypt, Mexico, Turkey, and Spain with a total production of 8.9 million metric tons. Fruit is produced both for the fresh market and for processing (Tridge 2022). Global exporting of bare root plants is a major market for the nursery industry, and this is a significant concern for the regulatory industry.

Mushrooms are the fruiting body of a fungus known to have been grown in China since 600 AD. The white button mushroom, *Agaricus bisporus*, is the most widely grown variety and was first grown in France beginning in the 1600s (Miles and Chang 1997). Globally, mushroom cultivation has a value in excess of 16.7 billion US dollars. In 2014, China raised more than 30 million metric tons of mushrooms, for an 87% share of the market. Other areas of Asia raised 1.3 million metric tons, while other countries raised 3.1 million metric tons (Taylor 2018). For all of these crops, diseases and pests cause significant loss to the industries. To help manage *Aphelenchoides*, it is important to have extension personnel to assist growers with recognizing the problem and developing management programs. Because the crops of interest are raised, exported, and imported worldwide, it is important to have regulatory programs to minimize the possibility of nematode-infested seeds and plants being exported or imported.

16.2 Plant–Parasitic Nematodes

Plant–parasitic nematodes are microscopic, non-segmented, vermiform aquatic organisms. They utilize a hollow spear or stylet to feed on the cells of plants (Khan 2023). As parasites of plants, they function as either ectoparasites or endoparasites (Khan 2008). As ectoparasites in soil, they live within the film of water that lines the soil pores and feed on roots. As ectoparasites of aboveground parts of plants, they live within protected surfaces of flower and leaf buds or move within a film of water on the surface of stems, leaves, and flowers. As endoparasites, they live either belowground within roots, or aboveground within stems, leaves, and flowers. The life cycle of bud and leaf nematodes is similar to that of other nematodes, consisting of an egg stage, four juvenile or larval stages, and adults (Fig. 16.1). Juveniles and adults have been observed to be active swimmers able to swim up the stems of plants when a film of water is present. Reproduction is thought to be bisexual. The life cycle of bud and leaf nematodes is relatively short lasting approximately 2 weeks. Some species have the ability to become anhydrobiotic during dry conditions and have the ability to survive for several years in this condition. In addition to parasitizing plants, some species are also able to live on fungi (Hesling 1977a, b; Jenkins and Taylor 1967; Siddiqi 1974, 1975).

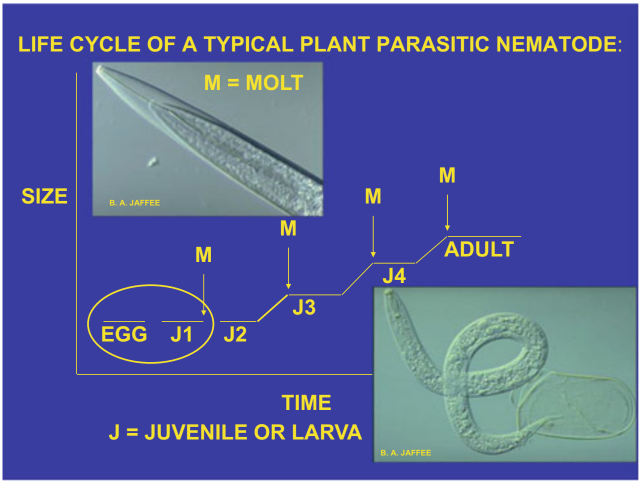

Fig. 16.1 Life cycle of a typical plant parasitic nematode. (Redrawn from Lee 1964)

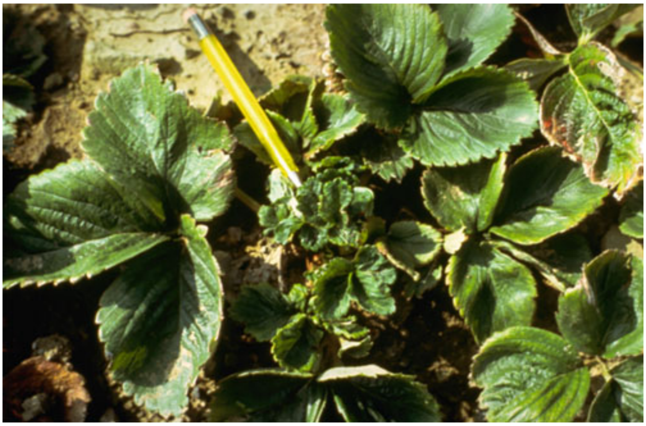

Fig. 16.2 Symptoms of *A. fragariae* on strawberry plant. Department of Entomology and Nematology University of California Davis slide collection

The bud and leaf nematodes are in the Genus *Aphelenchoides*. As suggested by their common name, they generally feed on the aboveground parts of plants. Four nematode clades (groups of organisms that evolved from a common ancestor) have evolved to parasitize plants. *Aphelenchoides* sp. are in Clade 10, the Aphelenchoididae grouped with other species that share similar traits biologically, morphologically, and molecularly. The other clades are the Trichodoridae (Clade 1), Longidoridae (Clade 2), and Tylenchida (Clade 12) (Holterman et al. 2017). Historically, *Aphelenchoides* were placed in a group of nematodes that have the outlet of the dorsal esophageal gland duct orifice located in the metacorpus rather than behind the stylet base as is found in Tylenchida. This grouping has been validated by molecular means. Members of Clade 10 include parasites of plants, parasites of insects, predators of other nematodes, and species that feed on fungi, algae, lichens, and mosses. *Bursaphelenchus xylophilus*, the "pine wood nematode," is another important parasite in Clade 10 that can feed on both plants and fungi (Chap. 25). Also, in Clade 10 is the "red ring nematode" currently known as *Bursaphelenchus cocophilus* (but discussed in older literature as *Bursaphelenchus cocophilus*) that causes "red ring disease of palm."

The important plant parasites of *Aphelenchoides* are typically found aboveground where they are either ectoparasites or endoparasites depending on the species of nematode and of the plant host. *A. fragariae,* for example, is an ectoparasite on strawberries where it feeds on flower and leaf buds causing a disease known as "spring dwarf" (Fig. 16.2), but an endoparasite of ferns (Fig. 16.3) and other ornamental plants where it enters into and feeds within the leaves. *A. ritzemabosi,* a pest of chrysanthemum, enters the leaves through stomata and feeds endoparasitically (Fig. 16.3). For both species, when within the leaves, their movement is initially restricted by the veins of the leaf on which characteristic necrotic lesions form. The necrosis can later spread throughout the leaf. Although not a focus

Fig. 16.3 Symptoms of *Aphelenchoides* on ferns (left) and chrysanthemum (right) illustrating interveinal necrosis. Department of Entomology and Nematology University of California Davis slide collection

Fig. 16.4 Symptoms of *A fragariae* on Easter lily (left) and African violet (**b**) illustrating stunting of infested (left) vs non-infested plants (right). Department of Entomology and Nematology

of this chapter, for management purposes, it is important to consider that leaf and bud nematodes have ornamental plants, aquatic plants, and fungi that can be alternate hosts for the species being covered in detail (Hague 1972; Siddiqi 1975) (Fig. 16.4). *A. besseyi* feeds ectoparasitically both on the growing tips of rice plants where it causes a disease known as "white tip of rice" (Fig. 16.5) and on strawberries where it feeds on young leaves within buds and causes a disease known as "crimp" or "summer dwarf." *A. composticola*, a fungal feeder, is ectoparasitic on mushrooms. *Aphelenchoides* sp. have been able to interact with the bacterium *Corynebacterium fascians* to produce cauliflower disease on strawberry (Hesling 1977a, b; Jenkins and Taylor 1967; Siddiqi 1974, 1975).

Fig. 16.5 Symptoms of *A besseyi* on rice (Khan et al. 2021, p 8). University of California Davis slide collection

Recently, Subbotin et al. (2021) studied several populations that had been previously identified as *A. besseyi*. They determined that morphologically and molecularly *A. besseyi* was a complex of species. They identified a population feeding on strawberries in Florida as matching the characteristics of *A. besseyi*, but a population on rice from Louisiana was identified as being *A. oryzae*, a species that had been previously synonymized with *A. besseyi*. Three populations feeding on ornamental plants in Florida were determined to be different from the two previously described species and were identified as a new species *A. pseudobesseyi*. Several populations from other countries that were previously identified as *A. besseyi* were determined to be *A. pseudobesseyi*. These three species are difficult to distinguish without molecular analysis. These recent findings complicate the situation for regulatory agencies and for the development of non-chemical management programs such as crop rotation and development of nematode-resistant varieties.

16.3 Infestation of Food Crops and Its Management

Leaf and bud nematodes cause serious economic damage to several important crops including rice, strawberry, and mushroom. Reductions are found in both growth and yield. Typical damage symptoms include stunting, wilting, and yellowing of the crop, but these are not diagnostic as such symptoms could be caused by lack of irrigation or fertilization, for example. To diagnose a nematode infestation, samples of infested plant parts and soil need to be taken and sent to a diagnostic laboratory. Nematodes are often irregularly distributed in a field resulting in patches of poor growth.

16.3.1 Rice

Nematodes cause significant economic damage to rice, which is a major source of food in many countries, particularly in Asia. Worldwide, losses from nematodes have been estimated to be 10% (Owen et al. 2023; Sasser and Freckman 1987). This is highly variable from country to country. In the United States, losses due to *A. besseyi* are 1% or less (Koenning et al. 1999). In other locations, losses can be as high as 30–50% (Ichinohe 1972). Variability is affected by variety, the initial level of the nematode population, and cultural practices.

16.3.1.1 White Tip Caused by *Aphelenchoides besseyi*

Aphelenchoides is one of 14 different genera of plant–parasitic nematodes that have been reported to be associated with rice (Ferris 2022a). Depending on where it is found, *A. besseyi* has several common names including rice white tip nematode, crimp nematode, strawberry summer dwarf nematode, strawberry bud nematode**,** fire-fly blast, heart blight, black grain, and ear blight (Ichinohe 1972; Khan 2010).

A. besseyi is found in at least 71 countries including nine in Europe, 25 in Asia, 27 in Africa, two in North America, five in Central America and Caribbean, three in South America, and five in Oceania (CABI 2021a).

Infection with *Helminthosporium sigmoideum*, a causal agent of stem-rot disease, was less if plants were infected with *A. besseyi*. It was hypothesized that nematode infestation increased the rate of respiration in the plant (CABI 2021a). *A. besseyi* also interacts with *Acrocylindrium oryzae* (previously known as *Sarocladium oryzae*), *Curvularia* spp., and *Fusarium* spp. (Khan 2010).

Leaves and seeds of rice are infected by *A. besseyi* (Jenkins and Taylor 1967). Sixteen days after seeds germinate, white tip disease symptoms become apparent on leaves. The leaf tips first turn pale yellow to white. Later, they may appear shortened and twisted, turn brown, and become necrotic and frayed. Reductions in height, vigor, weight, and number of grains have been reported (CABI 2021a; Khan 2010). Wang et al. (2020) utilized transcriptome sequencing of *A. besseyi* and rice to elucidate their interactions. Their study suggested that *A. besseyi* suppressed the photosynthetic system of rice. In laboratory trials, Liu et al. (2018) determined that hyperspectral reflectance of rice leaves could be utilized to differentiate *A. besseyi* infestation from that of insect and fungal infestations.

Worldwide, it is estimated that three million acres are infested with *A. besseyi* with yield losses of from 2 to 71% (Hollis and Koeboonrueng 1984; Khan et al. 2021).

Management relies on an understanding of the biology of the pest species and begins with an accurate species identification. Molecular techniques are being developed for accurate identification of *A. besseyi,* which is important for breeding programs, certification of seed, and quarantine programs for seed. One method that has been developed is KASP (kompetitive allele-specific PCR) technique using SNP (single-nucleotide polymorphisms) (Devran and Göknur 2020). To detect and quantify *A besseyi* in rice fields in Turkey, Çelik et al. (2019) developed a real-time PCR assay. Rice and fern pathotypes of *A besseyi* can be distinguished by a LAMP (loop-mediated isothermal amplification assay) utilizing the mitochondrial COI (cytochrome oxidase subunit I) gene when as few as five nematodes are present (Yang and Yu 2019).

A besseyi survives in and is disseminated in rice seed where it survives in a quiescent, dehydrated anhydrobiotic stage of reduced metabolism (Hoshino and Togashi 2021). Nematodes become reactivated upon exposure to water. At 25–30 °C, this occurs within 3–4 days (Ichinohe 1972). Nematodes are attracted to rice seedlings and feed ectoparasitically on meristematic tissues (CABI 2021a). As plants grow, nematodes are carried upwards (Christie 1959). They feed ectoparasitically on reproductive tissues and their numbers rapidly increase as plants mature (Christie 1959). At harvest, as rice seeds slowly desiccate, *A. besseyi* aggregate, coil up, and become anhydrobiotic. Up to 64 nematodes, mostly adult females, per seed, have been reported. The economic damage threshold has been reported to be three live nematodes per seed (CABI 2021a). In stored seed, nematode survival was lower at 20–25 °C (3.6 years) than at −5 to 10 °C (18 years) (Hoshino and Togashi 2020).

Reproduction is typically amphimictic but can also be parthenogenetic, and males are common (Huang et al. 1979). The optimum temperature for the development of *A besseyi* is 21–23 °C. A life cycle is reported to take 10 days at 21 °C, 8 days at 23 °C, and 8–12 days at 30 °C. Development does not occur below 13 °C (CABI 2021a).

In the absence of a host, *A besseyi* is thought to not be able to survive in soil between crops of rice. However, it has been shown that it can be cultured on several species of fungi indicating that it could survive and possibly reproduce in the soil in the absence of rice (Jenkins and Taylor 1967).

Seeding directly into water or irrigating seed beds reactivates nematodes and can reduce subsequent infestation by causing nematodes to use up energy stores prior to germination that typically occurs in 3–5 days (Christie 1959). Low seeding rates and planting in cooler temperatures may reduce levels of infestation (CABI 2021a).

Planting nematode-infected seed is the main route of infestation by *A. besseyi.* Surveys of rice production areas demonstrated that between 2 and 80% of tested seed lots were infested. A number of different hot-water treatments have been developed to eliminate *A. besseyi* from rice seed (Ichinohe 1972). The IRRI (International Rice Research Institute) recommends soaking in cold water for 3 h and then treating in hot water for 15 min at 55 °C (CABI 2021a). Ultrasound and gamma irradiation have also been tested for treatment of seed (CABI 2021a). Exposing seed to a mixture of 97.5% nitrogen and 2.5% oxygen at 25 °C for 10 days has also been utilized to disinfest seed (Khan et al. 2021).

In addition to rice, *A. besseyi* is known to be associated with at least 32 genera of plants. Important crops that are infested include cotton, tobacco, sugarcane, strawberry, soybeans, millet, sorghum, onion, oat, corn, yam, and beans (Ferris 2022a). Ornamental hosts include ferns, gladiolus, chrysanthemum, and tuberose (Ferris 2022a).

Varieties of rice may possess a range of susceptibility. Susceptible varieties may or may not display typical white tip symptoms, while other varieties may be immune to nematode invasion (Ichinohe 1972). It has been observed that nematodes are more attracted to susceptible varieties and that the rate of reproduction is slower in resistant varieties (Jenkins and Taylor 1967). Resistant varieties have been found in studies conducted in Japan, the United States, Italy (Fortuner and Williams 1975), and India (Khan et al. 2021).

Regulations and registrations of pesticides vary with location. Local authorities are consulted to determine those that can be legally used, and product labels are followed. Different methods tested for treating seeds prior to planting include fumigation, or soaking or dusting with various organophosphate and carbamate materials (Jenkins and Taylor 1967). Foliar applications have been less successful than preplant seed treatments.

The supernatant of the bacterial symbiont *Xenorhabdus bovienii*, infective juveniles of the entomopathogenic nematode *Steinernema feltiae*, and the entomopathogenic fungus *Purpureocillium lilacinum* were shown to suppress *A. besseyi* based on observed white tip symptoms and yield (Tülek et al. 2018). Rice utilizes the hormones ethylene, salicylic acid, and jasmonate in defense and immune responses to *A. besseyi* (Xie et al. 2022).

16.3.2 Strawberry

Damage reported to strawberries is serious, but highly variable. In Europe, *Aphelenchoides* was reported to be the seventh most damaging nematode after *Heterodera*, *Globodera*, *Meloidogyne*, *Ditylenchus*, and *Pratylenchus* (Sasser and Freckman, 1987). In Germany, *A. fragariae* reduced yields by 45% and *A. ritzemabosi by 65%* (Blank 1985). *A. fragariae* has also caused significant losses in both France and Italy (Clerfeau et al. 1983; Tacconi 1985). In tests in Poland, on seven strawberry varieties tested, A. *fragariae* and A. *ritzemabosi reduced yields by 32–61%* (McElroy 1972; Szczygiel 1967). In Australia, yields were reduced by 50% by *A. besseyi* (McCulloch 1978). In the United States, losses are infrequent but serious when they occur. For example, in Massachusetts losses as high as 60–70% from *A. fragariae* were reported following the planting of infested planting stock. For *A. besseyi*, infested plants typically yielded 7–10% less than non-infested ones with losses in Louisiana reported at 1–2% and in Florida losses in individual fields were as high as 75% (Brown et al. 1993; Plakidas 1964).

16.3.2.1 Foliar Nematode Disease of Strawberry Caused by *Aphelenchoides* sp.

Three species of *Aphelenchoides* (*A fragariae, A ritzemabosi, and A besseyi*) are aboveground parasites on strawberry (McElroy 1972). Common names for *A fragariae* are spring dwarf (because it is prevalent during cool weather), spring crimp, and red plant (CABI 2022). *A fragariae* is found in eight countries in Asia, 22 in Europe, four in North America, three in Oceania, and one in South America (CABI 2022).

Common names for *A ritzemabosi* are chrysanthemum foliar eelworm, leaf wilt nematode of chrysanthemum, leaf and bud nematode, chrysanthemum foliar nematode, and leaf and bud nematode (CABI 2021b). *A. ritzemabosi* is found in two countries in Africa, seven in Asia, 19 in Europe, three in North America, two in Oceania, and three in South America (CABI 2021b).

Common names for *A. besseyi* are rice white tip nematode, crimp nematode, strawberry summer dwarf nematode (because it is prevalent in warm weather), strawberry bud nematode**,** fire-fly blast, black grain disease, ear blight, and heart blight (CABI 2021a; Khan 2010). *A. besseyi* is found in at least 71 countries including nine in Europe, 25 in Asia, 27 in Africa, two in North America, five in Central America and Caribbean, three in South America, and five in Oceania (CABI 2021a).

Cauliflower disease of strawberry results from the interaction of either *A. fragariae* or *A. ritzemabosi* with the bacterium *Rhodococcus fascians.* In earlier literature, this bacterium is referred to as *Corynebacterium fascians*. In this disease, the flowers of stunted plants look like miniature cauliflowers (Crosse and Pitcher 1952; Pitcher and Crosse 1958).

A. fragariae is most active in cool spring weather when plant growth is beginning. Ectoparasitic feeding activity begins in the crown of the plant from which distorted buds and puckered leaves with short stems emerge. Brown patches may be visible near the mid-rib of leaves. Fruit either fails to develop from the distorted buds or is small and deformed. If runners are allowed to develop, they will likely be infested. During warmer weather, nematodes may become quiescent and symptoms may disappear (McElroy 1972).

A. ritzemabosi and *A. fragariae* may occur separately or together on the same plant (CABI, 2021b). Plants infested with *A. ritzemabosi* are stunted with deformed and crinkled leaves. During warmer weather, symptoms of summer dwarf caused by *A. besseyi* may appear. The symptoms are similar to *A. fragariae* with stunted plants and deformed leaves with short stems. The edges of younger leaves may curl upward, and those of older leaves may curl downward (McElroy 1972).

Yield losses from infestation with *A. fragariae*, *A. ritzemabosi,* and *A. besseyi* can be serious but highly variable. Losses from *A. fragariae* typically occur early in the growing season during cool weather, while losses from *A. besseyi* are seen in warmer weather. Infestations have been shown to reduce the weight of crowns by more than 50%, of fruit yield by more than 80%, and to reduce the number of runners produced by 30% (McElroy 1972).

Management relies on an understanding of the biology of the pest species and begins with an accurate species identification. Sánchez-Monge et al. (2017) utilized the cytochrome oxidase I gene (COI) to distinguish *A. besseyi*, *A. fragariae*, and *A. ritzemabosi*. Wang et al. (2019) utilized a LAMP (loop-mediated isothermal amplification) technique for the identification of individual life stages either alone or in a mixed population of nematode species or in samples of plant tissue.

On strawberry, *Aphelenchoides* species live ectoparasitically on buds and leaves. They move across plant surfaces when a film of water from high humidity, dew, or rain is present (McElroy 1972). Anhydrobiosis permits survival in the absence of moisture (Zhen et al. 2020). *A. fragariae*, *A. ritzemabosi,* and *A. besseyi* are bisexual and reproduce sexually (CABI 2021b). The life cycle of *A fragariae* takes

10–11 days at 18 °C in Lorraine begonia. A female nematode lays about 32 eggs that hatch in 4 days. Juveniles mature into adults in 6–7 days (Strümpel 1967). The life cycle of *A. ritzemabosi* takes 10–13 days and has been studied in chrysanthemum leaves. Female nematodes lay 25–30 eggs that hatch after 3–4 days. It takes 9–10 days for juveniles to mature (Wallace 1960). The life cycle in *Senecio vulgaris* (groundsel) takes 14–15 days (Siddiqi 1974). The life cycle of *A. besseyi* on strawberry takes 2–3 weeks (McElroy 1972).

A. ritzemabosi and *A. fragariae* did not increase in numbers when infested crowns were stored at 14–15 °C, but did increase when stored at 20 °C. They survived at 4 °C for at least 3 years (French and Barraclough 1962). *A. fragariae, A. besseyi,* and *A. ritzemabosi* can all reproduce on fungi making it possible for them to survive in soil in the absence of a host plant (Hooper and Cowland 1988). De Oliveira et al. (2022) demonstrated that *A. besseyi* can reproduce on pathogenic and non-pathogenic fungi as alternate hosts in the soil**.**

Studies have demonstrated that hot-water treatments of runners prior to planting can effectively control *Aphelenchoides* sp. Treatment of 15 min at 47 °C or 10 min at 46 °C followed by cooling in cold water has been recommended. Qiu et al. (1993) found that exposures of 44.4 °C for 20–30 min, 46.1 °C at 10–15 min, and 47.7 °C at 8–10 min were effective against *A. fragariae*. *A. ritzemabosi was* controlled on strawberry by a 10 min treatment at 46 °C. Strawberry cultivars may have different thermal tolerances, and this should be tested prior to large-scale treatments. Pre-heating runners in warm or room temperature water before treatment and immersing in cold water after treatment are recommended (CABI 2022).

Strawberry plants are propagated vegetatively by means of runners produced from mother plants, and this can lead to the distribution of *Aphelenchoides* species in the planting stock. Worldwide, there are highly effective regulatory programs in place to minimize the spread of *A. fragariae, A besseyi,* and *A. ritzemabosi* on plants (O'Bannon and Esser 1987). Examples of programs to certify that planting stock is nematode-free can be found in EPPO (European and Mediterranean Plant Protection Organization) and CDFA (California Department of Food and Agriculture) documents that provide details for producing nematode-free planting stock and methods to inspect plants and planting sites for strawberry runner plant inspection for both exporting and importing (OEPP/EPPO 2017; CDFA 2009). Studies in Italy conducted over an 8-year time frame have demonstrated the effectiveness of these types of programs (O'Bannon and Esser 1987; Tacconi and Lamberti 1994).

Removing (roguing) and disposal of symptomatic plants as soon as they appear can help to minimize the spread of nematodes to other plants (McElroy 1972). In addition to rogueing of infested plants and propagating from clean mother plants, minimizing surface moisture on plants and contact between plants, planting on ridges, avoiding splashing water from sprinkler irrigation, and rooting runners in containers placed in the row can help to minimize nematode spread in the field (McElroy 1972; Siddiqi 1975).

Over 250 plants in 78 genera in 47 families have been reported to be hosts of *A. fragariae* (CABI 2022; Ferris 2022b). In addition to strawberry, hosts include plants in the families Liliaceae, Primulaceae, and Ranunculaceae and ferns. Wheat has been shown to be a good rotation crop in Japan (CABI 2022).

A fragariae and *A. ritzemabosi* have been reported to occur together in at least 28 hosts. These include strawberry, aster, and begonia. At least 124 genera are alternate hosts of *A. ritzemabosi,* and these are mainly in the Compositae (Ferris 2022c; CABI 2021b). Several weeds including goosegrass, chickweed, buttercup, sowthistle, and speedwell are hosts of *A. ritzemabosi* highlighting that for crop rotation to succeed it is important to control weeds in the alternate crops (CABI 2021b).

In addition to strawberry, *A. besseyi* is known to be associated with at least 32 genera of plants. Important crops that are infested include rice, cotton, tobacco, sugarcane, soybeans, millet, sorghum, onion, oat, corn, yam, and beans (Ferris 2022a). Ornamental hosts include ferns, gladiolus, chrysanthemum, and tuberose (Ferris 2022a).

More than 100 varieties of strawberries grown in various areas of the world have been tested and found to have a range of resistance/tolerance to foliar nematodes, but none have been found that are immune. This indicates the potential for future breeding of varieties with more effective resistance (CABI 2022).

Regulations and registrations of pesticides vary with location. Local authorities are consulted to determine those that can be legally used and follow product labels. Preplant soil fumigation is widely utilized in strawberry fruit production for control of soil-dwelling nematodes, weeds, and fungi and is currently essential for the production of nematode-free planting stock. Because strawberry is a high-value crop, chemical treatments can be cost-effective for improving yields (McElroy 1972).

Various organophosphate and carbamate products have been tested for post-plant treatment of *Aphelenchoides* species on strawberry. Additional chemical tests on ornamentals that have demonstrated efficacy against *A. besseyi* include oxamyl, chlorfenapyr, and spirotetramat (Wheeler et al. 2022), and Rotifa and Evans (2021) tested spirotetramat, abamectin, and azadirachtin alone and in combination with ASM (acibenzolar-*S*-methyl an elicitor of plant defenses) for the management of *A. fragariae* on ornamental plants. All treatments reduced nematode populations compared to an untreated control. In trials on the ornamental plant hosta, Pylon (24% chlorfenapyr) and Nemakill (32% cinnamon oil, 8% clove oil, 15% thyme oil mixture) demonstrated efficacy against *A. fragariae* (Ruisheng et al. 2017).

Thirteen different nematophagous fungi including *Hirsutella rhossiliensis* have been shown to feed on *A. fragariae. H. rhossiliensis* reduced populations of *A. fragariae* by 45–65% (CABI 2022).

16.3.3 Mushroom

Several species of Aphelenchoides are pests of mushroom with A. composticola being the most serious. For example, it is a major factor-limiting mushroom production in India (Richardson and Grewal 1993; Sharma and Seth 1986). Crop loss in Europe, China, Australia, New Zealand, and the United States has also been reported. Infesting mushroom compost with 1, 10, or 50 *A. composticola* per 100 g compost resulted in yield losses of 26, 40, and 42%, respectively (Arrold and Blake 1968; Richardson and Grewal 1993).

16.3.3.1 Mushroom Disease Caused by *Aphelenchoides composticola*

Worldwide, several species of *Aphelenchoides* have been identified as parasites of commercial mushrooms (*Agaricus bisporus*). Of these, *A. composticola* has been found to cause the most damage. Common names in Germany are Aelchen, Champignon-Blatt (CABI 2019).

An infestation is often not noticeable until patches of the surface of the mushroom bed sink, become foul smelling, and possibly become covered with nematophagous (nematode-eating) fungi (Hesling 1977a, b). Nematodes feed by piercing mycelia with their stylets and sucking out the contents.

In addition to *A. bisporus*, *A. composticola* has been shown to feed on more than ten genera of fungi including several that are plant pathogens (Grewal 1990; Richardson and Grewal 1993), but has not been reported to parasitize plants. Reproduction is bisexual and occurs most rapidly from 23 to 25 °C. The time required to complete a generation is temperature-dependent, requiring 8, 10, and 22 days at 23, 18, and 13 °C, respectively (Arrold and Blake 1967; Cayrol 1967; Okada and Ferris 2001). It can survive without a host for at least 6 weeks and is not killed by freezing or by slow desiccation. From 10,000 to 100,000 nematodes can be found in 1 g of mushroom compost. At high levels, the nematodes can swarm out of the mushroom bed and collect on equipment where they can be easily spread by workers and insects (Hesling 1977a, b).

Good sanitation throughout the growing process is needed to minimize infestation. Pasteurizing wet compost and casing at 60 °C for 2 h is needed to kill nematodes. Dry compost requires temperatures as high as 71 °C to kill nematodes (Sharma and Seth 1986). A number of chemical treatments have been tested to manage *A. composticola* including formulations of neem, dazomet, thiabendazole, and benomyl (Gahukar 2014; Gitanjali 2001; McLeod 1978), but these run the risk of killing the crop or possibly leaving toxic residues. Regulations and registrations of pesticides vary with location. Local authorities are consulted to determine those that can be legally used, and product labels are followed.

16.4 Conclusions and Future Perspectives

Important agricultural species of leaf and bud nematodes include *A. besseyi* on rice and strawberry, *A. fragariae* and *A. ritzemabosi* on strawberry, and *A. composticola* on mushrooms. *Aphelenchoides* species affecting rice, strawberry, and mushrooms feed ectoparasitically on aboveground parts of plants or on fungal mycelia. The major means of nematode dispersal are infested seed for rice, infested runners for strawberry, and infested compost for mushrooms.

Biotechnological approaches are important components of management programs for leaf and bud nematodes. For example, recent advances in molecular identification have determined that *A. besseyi* is a complex of species (Subbotin et al. 2021). This reinforces the need for the accurate identification of species.

When economical and available, crop rotation and use of resistant cultivars are optimum methods for nematode management. Screening of cultivars of rice and strawberry has shown that a range of resistance and tolerance is present in local cultivars. This shows promise for use in breeding programs using biotechnological approaches to develop additional resistant and immune cultivars.

Heat treatment is an effective means for controlling *Aphelenchoides* on rice seed, strawberry runners, and mushroom compost. A cooperative effort involving growers, university research and extension, and government agencies utilizing biotechnological approaches could expand the use of heat treatments by growers for disinfesting planting materials in local cropping systems. Times and temperatures required to kill *A. besseyi*, *A. fragariae, A ritzemabosi,* and *A. composticola* have been experimentally determined. Potential differences in the susceptibility of local rice and strawberry cultivars could be evaluated through university research. Qiu et al. (1993) provided an example of this in which the survival and flowering of local strawberry cultivars not infested with nematodes were evaluated to determine thermal tolerances at predetermined times and temperatures that kill *A. fragariae*. Cooperative programs involving growers, extension, and government agencies could develop equipments from local sources and provide quality control for the treatment of planting materials. These and other cultural and physical methods can reduce the need for using chemical management practices.

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17 Dagger and Stubby Nematodes in Agricultural Crops and Their Bio-Management

Linnley Mulusa

Abstract

Plant diseases due to plant parasitic nematodes (PPNs) are among the leading causes of significant agricultural losses, which eventually affect the global economy and millions of households depending on agriculture as an income and food source. Therefore, effective control of PPNs is necessary. The increasing environmental concerns and development or resistance to chemical agents by PPNs inform the advocacy of bio-management techniques by researchers and other stakeholders. Among economically and agriculturally significant PPNs are stubby-root and dagger nematodes. The effects of these nematodes are double-edged because they are pathogens, directly affecting the host plants' physiological processes adversely, and vectors, transporting pathogenic microorganisms to susceptible plants eventually causing plant diseases and yield reduction or loss. These ectoparasites exhibit different characteristics, and understanding them is a crucial factor for their effective bio-management. For example, while soil solarization can be effective against dagger nematodes, it is not suitable for stubby-root nematodes, which occur at greater depths than dagger nematodes. Other bio-management techniques for dagger nematodes are the application of green manure, trap crops, crop rotation, bio-fumigation, and biocontrol agents. For stubby-root nematodes, effective bio-management techniques are improving soil conditions, crop rotation, proper transplanting procedures, and use of resistant cultivars as green manure. The present chapter offers a detailed account on the crop damage and management of dagger and stubby nematodes.

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Keywords

Stubby-root nematodes · Plant–parasitic nematodes · Dagger nematodes · Bio-management · Agriculture

17.1 Introduction

Agriculture is a vital economic activity worldwide. According to the World Bank, approximately 65% of salaried adults living in developing countries make a living through agriculture (Mulusa 2021). Additionally, the global gross domestic product benefits significantly from the practice since, even in the modern era of scientific and technological advancements, many countries continually depend on agriculture to create numerous employment opportunities and reinforce the national income (Mulusa 2021). Farmers engage in diverse forms of agriculture, including subsistence, cash crop, horticultural, intensive, and mixed farming. Farmers can then use their agricultural products differently. For example, they can feed their families, sell the produce for profit, trade them locally, or package them for export. Over the years, the global population has increased significantly, demanding an equal increase in agricultural products to sustain the population and prevent food scarcity, undernutrition, high food prices, and limited food reserves. Consequently, it is crucial for farmers, governments, researchers, and other stakeholders to identify, develop, implement, and sustain farming technologies with increased efficiency and effectiveness to counter the challenges of agriculture that contribute to reduced yields and, ultimately, food shortage.

Natural disasters, climate change, pests, and unfavorable growth conditions contribute to significant agricultural losses. Still, diseases caused by biotic and abiotic factors are a leading cause of reduced yields, affecting plants' psychological activities (Mulusa 2021). Psychological activities like metabolism, water, nutrient uptake, and photosynthesis are critical plant functions that facilitate healthy plant growth and development. Therefore, interfering with these fundamental activities adversely affects plants by hindering normal growth processes, ultimately resulting in poor or undesirable outcomes. Abiotic agents of plant diseases include mesobiotic factors like viroids and viruses that exhibit an intermediate state between living and non-living organisms, inadequate nutrients, and extreme temperatures.

Conversely, the biotic agents of disease, which can be prokaryotic or eukaryotic microorganisms, are living and pathogenic. Categories of these microbial pathogens are algae, fungi, bacteria, protozoa, and nematodes. These cause plant diseases that lead to reduced yields and disrupt the natural ecosystem, disturbing the environment's balance of living things (Mulusa 2021). Even though fungi cause the highest number of plant diseases, nematodes are significant plant pathogens because of the difficulty farmers encounter in managing them due to misdiagnosis of symptoms and delayed application of prevention and control measures. Furthermore, ineffective and deleterious farming practices like crop rotation using different host crops, reusing contaminated farm implements, and applying nematicides contribute to the difficulty of managing plant parasitic nematodes. As a result, scientists recommend applying bio-management techniques to prevent and control nematode-borne plant diseases (Khan 2016).

The advocacy for biocontrol techniques arises from the distinct and desirable advantages offered by the strategies, including increased efficiency, effectiveness, and safety. These advantages benefit farmers, crops, and the environment, indicating the wholesomeness of nematode bio-management in agriculture. Foremost, biocontrol agents attack specific disease-causing organisms, meaning that they do not harm beneficial organisms within the ecosystem. Additionally, bio-management of plant–parasitic nematodes offers farmers a long-term solution to stubborn pathogens, reducing farming costs and improving plant health and yields. Bio-management practices also reduce environmental pollution and adverse health effects on consumers due to their non-chemical nature.

Furthermore, even though stubby-root and dagger nematodes can develop eventual resistance to chemical nematicides and pesticides, they cannot develop resistance to bio-management strategies that function according to the pests' physiological processes. Consequently, it is essential to understand the biology of these microbes for the effective application of bio-management techniques against stubby-root and dagger nematodes. Extensive research exists on the bio-management of stubby-root and dagger nematodes due to the significance of bio-management in controlling environmental pollution, improving plant health and yields, and offering alternative and effective pest control techniques. Therefore, this chapter explores existing research to provide a comprehensive and detailed overview of the biology of plant–parasitic nematodes and their effects on agricultural crops, particularly stubby-root and dagger nematodes, the existing bio-management techniques effective against them, and prospects for their biocontrol.

17.2 Plant–Parasitic Nematodes

Plant parasitic nematodes are soil-borne microorganisms that damage agricultural crops by feeding on every plant part, including the yield, flowers, roots, and stems; however, most pathogenic species attack and damage plant roots (Khan 2008; Poveda et al. 2020). The nematodes use protrusible stylets when feeding and penetrating plant cells. Plant parasitic nematodes are one of the most recognized and devastating pathogens of agricultural crops as they affect different crops of great economic and consumption value (Khan 2023). Sato et al. (2019) indicate that the annual losses caused by plant–parasitic nematodes amounted to approximately 80 billion US dollars in 2013. However, in more recent research, Poveda et al. (2020) indicate that these losses skyrocketed to $157 billion by 2015, a significant increase. Still, Poveda et al. (2020) indicate that it is impossible to estimate the global economic losses due to nematode damage as many times, and farmers are not aware of the pathogens since the general symptoms they present on affected crops make it challenging to attribute the losses to nematode infestation. Furthermore, economies realize these large losses because plant–parasitic nematodes have a wide host range, increased virulence, complex virulence strategies, and good survival strategies like food reserves and dauer stages. Scientists have identified and classified 4100 plant–parasitic nematode species (Poveda et al. 2020). The most common classification system identifies these nematodes into three groups according to their feeding characteristics: ectoparasites, endoparasites, and semi-endoparasites.

Ectoparasites usually live outside the host plant, invade its cells, and feed on cellular contents using a stylet. Therefore, these pathogens cause plant diseases by depriving plants of essential nutrients (Khan et al. 2021). Conversely, endoparasites, further categorized as sedentary or migratory endoparasites, invade and feed on the host's internal tissues. The difference between the two endoparasitic nematodes is their activity following the invasion of a suitable host. While sedentary endoparasites like *Meloidogyne* spp. and *Globodera* spp. move into the host's vascular tissue and trigger the formation of multinucleated giant feeding cells where the nematode completes its lifecycle, migratory endoparasites like *Pratylenchus* spp. and *Radopholus* spp. move through plant tissues while feeding, damaging the tissues extensively (Khan and Jairajpuri 2010; Mulusa 2021; Sato et al. 2019). Therefore, endoparasites damage crops by interfering with critical physiological processes like water, mineral, and nutrient uptake and causing a nutrient deficiency. As the name suggests, semi-endoparasitic nematodes spend part of their lifecycle within the host plant and part within the soil. When feeding, these nematodes penetrate plant tissues partially, leaving their posterior end in the soil. Following host penetration, semi-endoparasites like *Tylenchulus semipenetrans* and *Rotylenchus reniformis* become immobile because they trigger the formation of a permanent feeding cell from where they acquire their nutrients and complete their lifecycle.

From their lifecycles, plant–parasitic nematodes depend on their host plants' presence and relative abundance. However, as Jagdale et al. (2021) indicate, other factors like soil characteristics favor the occurrence of these pathogens. For example, research highlighted by Jagdale et al. (2021) indicates that *Meloidogyne* spp. is abundant in sandy soils, *Xiphinema americanum* in silty clay-loam soils, and *Pratylenchus* and *Criconemella* in fine silt and sandy soils. Therefore, the soil properties in different agricultural areas significantly impact the species of plant–parasitic nematodes observed and the related plant diseases. Furthermore, plant–parasitic nematodes (PPNs) are also vectors of other pathogenic microorganisms, mostly viruses that increase the incidence of plant disease and lower yields for farmers. Stubby-root and dagger nematodes are important in agriculture because they damage crops directly and act as vectors of several viruses that affect diverse agricultural crops, hence the need to understand their biology and effective bio-management strategies.

17.2.1 Dagger Nematodes

The scientific classification of dagger nematodes identifies them as *Xiphinema* spp. Generally, these ectoparasitic nematodes are 5 mm long and feed on the root tips and sides of roots by penetrating root cells with their stylets in fields with clay soils

(Evans 2007; Lehman 1981). It is possible to attribute the high population density of dagger nematodes in clay soils to their sensitivity to soil moisture and temperature changes and their preference for deep soils to topsoil, which often has desiccating conditions (Heve et al. 2017). The nematodes exhibit a low reproduction rate; however, their ability to survive for close to 5 years compensates for this shortcoming (Evans 2007; Lehman 1981). Crops susceptible to attack by *Xiphinema* spp. include grasses, raspberries, celery, roses, and strawberries. The Arabis mosaic, grape fanleaf strawberry latent ringspot, cowpea mosaic, and bromegrass mosaic viruses vectored by the nematodes affect more crops, including blackcurrants, cherries, cucumbers, peaches, plums, cereals, and grasses, increasing the concern for the effective management of dagger nematodes (Evans 2007; Lehman 1981; Heve et al. 2017). To increase bio-management strategies' effectiveness, it is essential to understand the salient features of dagger nematodes that this chapter presents in the next section.

17.2.1.1 Distribution of Dagger Nematodes

Different species of *Xiphinema* occur in tropical and temperate regions. Researchers have identified the pathogens in New Zealand, North and South America, Africa, Australia, Europe, and Asia. Lehman (1981) documents the specific distribution of three *Xiphinema* species:

- *X. basiri*—Indian River County, Florida, Zimbabwe, Nigeria, Sudan, Mexico, Ceylon, India, and Puerto Rico.
- *X. brevicolle*—California, Spain, France, Guadalupe, Chile, South Africa, Brazil, Czech Republic, Hungary, Poland, Bulgaria, and Israel.
- *X. index*—North and South Africa, USSR, Greece, California, Kansas, West Germany, Hungary, Chile, Argentina, Algeria, Poland, France, Australia, Iraq, Portugal, Israel, Spain, Iran, Turkey, and Italy.

17.2.1.2 Biology and Life Cycle

The life cycle of dagger nematodes typically includes six stages. The life cycle of dagger nematodes resembles that of other ectoparasitic nematodes, involving an egg stage, four juvenile stages, and an adult stage. Most species exhibit parthenogenesis, a type of reproduction that does not involve males (Heve et al. 2017). The females lay the eggs in the soil in a gelatinous matrix or membrane to protect them from desiccation, predators, and other environmental factors. The juveniles that emerge from the eggs molt four times. Each molt results in a larger juvenile until it becomes an adult. When juveniles can transfer viruses by feeding on virus-infected plants during their development, they obtain plant–pathogenic viruses, also known as nematode polyhedral viruses or nepoviruses, forming a commensal relationship (Heve et al. 2017). Heve et al. (2017) state that the viruses line the nematodes' pharynx stylet tube, and the nematode injects them into root tissues during subsequent feeds.

Since dagger nematodes are ectoparasites, each life form in the lifecycle, except the eggs, can attack and feed on the host plants' roots (Heve et al. 2017). The nematodes feed by inserting a long stylet deep into the host's root tissue, while the rest of the body stays in the soil. As the stylet penetrates the root tissues, it perforates the cell walls due to enzymes secreted by the nematode, causing extensive damage to the host's root system that results in root malformation. The enzymes that the nematodes produce to aid in the stylet's penetration include cellulases, chitinases, hemicellulases, and pectinases (Heve et al. 2017). The nematodes' continued feeding and extensive damage cause the root cells to collapse, hence diseased plants with poor yields.

17.2.1.3 Symptoms of *Xiphinema* spp. Infestation

When dagger nematodes feed on the host's meristematic root tips, they damage root cells and cause reduced root volume. In woody plants, the nematode-feeding activity causes terminal galling of the roots (Heve et al. 2017). Like other plant–parasitic nematodes, the aboveground symptoms associated with dagger nematode infestation are patchy fields and stunted growth. Apart from these direct effects, the viruses vectored by the nematodes produce symptoms that researchers can associate with the nematode's presence. Still, as Heve et al. (2017) report, it is highly possible to observe the symptoms, including mosaic and wilting shoots in woody plants compared to grasses.

17.2.1.4 Identification of Dagger Nematodes

The length of an adult dagger nematode is approximately 2–6 mm (Heve et al. 2017). Furthermore, the lip region in adults is smooth and flat with a non-offset head (Heve et al. 2017). Nematodes in the genus have an odontostylet, which is a long stylet with flanges instead of stylet knobs that reinforce the odontophore's basal region (Heve et al. 2017). The odontophore is the stylet's rearmost region. The long stylet stays in position due to the presence of a guiding ring at its center (Heve et al. 2017).

While it is challenging to use the nematodes' tail region for identification, it is possible to use the region to distinguish between the sexes: An adult male's tail region has paired spicules and a cloaca, while that of an adult female bears an anus and vulva (Heve et al. 2017). The position of the vulva varies among species; however, its general location is the female's mid-body (Heve et al. 2017). It is impossible to tell the juvenile sexes apart because, at this stage, the nematodes' sex organs have not yet developed.

17.2.1.5 Detection and Density Approximation

Nematode infestation results in patchy fields. Therefore, soil samples can be obtained from the patchy regions in a zigzag manner, as Heve et al. (2017) suggest. Since dagger nematodes avoid desiccating conditions, researchers or farmers should use wider spades to obtain soil samples from deeper in the ground, preferably 60 cm, during the dry season (Heve et al. 2017). However, during the wet season, when the nematodes migrate upward due to the top soil's high moisture content, it is possible to obtain nematode samples from soil obtained from a 40 cm depth (Heve et al. 2017). The soil samples are then packaged and labeled correctly before transportation to the laboratory for analysis.

The sugar floatation method and Bearmann funnel methods are some methods that researchers can use to extract dagger nematodes from the soil samples. Following extraction, researchers with specialized training study the nematodes' minute morphological features under a 400–1000× magnification. Besides morphology, morphometrics and molecular identification are used to study the nematodes for accurate classification. According to their morphometrics, some features used to classify nematodes are the body, pharyngeal, tail length, the distance of the vulva from the tail, and maximum body width. The three steps for molecular identification are DNA extraction, polymerase chain reaction, and gel electrophoresis. Nanotechnology can also be effectively used in the detection of nematode infestation in plant tissue and soil by using the nanosensors (Khan and Akram 2020; Khan and Rizvi 2016; Khan et al. 2020). Nano-sensors are the most important product of nanotechnology, and have great potential for use in plant disease diagnosis (Khan 2023; Khan and Rizvi 2014). The nano-sensors may be chemical nano-sensors or biological nano-sensors (Kaushal and Wani 2017; Khan and Rizvi 2018). Sellappan et al. (2022) developed nanobiosensor for early detection and prevention of agricultural crops from harmful pathogens.

For density approximation, one obtains a specific soil sub-sample, for example, 100 ml, from the sample collected from the field. Nematode extraction using one of the identified methods follows. After extracting the maximum number of nematodes, the researcher picks a specific volume, for example, 20 μl, from the nematode mixture using a micropipette and places it on a microscope slide, and then counts the number of nematodes in the sample under the dissecting microscope thrice for improved accuracy. After determining the number in the sample, the researcher uses deductive techniques to calculate the density of nematodes in the soil sub-sample, main sample, and field, hence determining the severity of nematode infestation.

17.2.1.6 Economic Importance of Dagger Nematodes

In landscape areas, the nematode population density ranges between 0 and 500 in 100 cm^3 of soil; still, dagger nematode activity results in moderate damage to susceptible host plants in such fields (Heve et al. 2017). Nevertheless, Heve et al. (2017) report that the findings of a 2013 study show that in agriculture, dagger nematodes rank eighth in terms of their economic significance. The root damage associated with dagger nematode infestation can cause plant roots to lose their density by 65%, eventually causing significant yield reduction (Heve et al. 2017). The viruses vectored by dagger nematodes also cause severe damage and increased crop losses since the viruses hinder plant development. Since cultivars resistant to dagger nematodes are non-existent, Heve et al. (2017) state that many countries include the virus-vectoring *Xiphinema* spp. on their quarantine lists.

17.2.2 Stubby-Root Nematodes

Stubby-root nematodes belong to the family Trichiuridae (Crow 2019). Their common name stubby-root nematodes arises from their feeding activity that gives roots a stubby or stunted appearance. *Trichodorus obtusus*, also known as *Trichodorus proximus*, is the most studied species because of the extensive destruction it causes to crops, especially turf grasses. Studies on *Nanidorus minor* also exist because it damages plant roots directly and transmits some plant viruses, causing significant agricultural and economic losses.

17.2.2.1 Distribution of Stubby-Root Nematodes

Crow (2019) states that *T. proximus* occurs in the United States only. The States plagued by the Nematodes are New York, Florida, Michigan, Kansas, South Dakota, Virginia, and Iowa (Crow 2019). On the other hand, *N. minor* has a more widespread distribution, occurring globally in various tropical and subtropical countries. These include the United States, Netherlands, Canary Islands, Philippines, Argentina, Cuba, Afghanistan, Upper Volta, Mauritania, West Germany, Taiwan, Java, Japan, Egypt, Greece, India, Fiji, Russia, Nicaragua, Ivory Coast, Brazil, Belgium, Puerto Rico, Senegal, Venezuela, Italy, Israel, and Sweden (Crow 2014).

17.2.2.2 Biology and Life Cycle of Stubby-Root Nematodes

Stubby-root nematodes are microscopic ectoparasites with a six-layered cuticle. Mostly, they feed on plant roots' meristematic cells. A unique characteristic of these PPNs is the possession of an onchiostylet, which is a firm and curved stylet that they use to feed on cellular components (Crow 2019). The onchiostylet also functions in perforating plant cells for access. Following perforation, the pathogen secretes a salivary substance into the damaged cell. The salivary substance solidifies, forming a feeding tube that the nematode uses to draw and ingest the host's cellular contents. After draining one cell, the nematode migrates to another cell, leaving the used feeding tube in the damaged cell and forming a new one during each subsequent feed.

N. minor reproduces by parthenogenesis, a form of asexual reproduction. Therefore, the nematodes mostly occur as females since there is no need for males during reproduction. On the other hand, *T. proximus* exhibits amphimixis, a form of sexual reproduction, meaning that populations comprise male and female nematodes (Crow 2014). The females lay eggs that hatch into second-stage juveniles (J2s) in the soil following fertilization. Since stubby-root nematodes are obligate PPNs, they locate susceptible host plants soon after hatching and begin feeding on the meristematic cells (Crow 2014). Feeding causes the J2s to molt thrice to become female adults. At higher temperatures, the life cycle of *N. minor* lasts for approximately 16 days (Crow 2014). Lower temperatures can prolong this duration.

17.2.2.3 Stubby-Root Nematode Hosts

T. proximus attacks bermudagrass, tomato, zoysia grass, St. Augustine grass, potato, saw palmetto, seashore paspalum, sweetbay magnolia, eucalyptus, big bluestem, rhododendron, sideoats grams, Kentucky bluegrass, sorghum sudangrass, and little leaf linden (Crow 2019).

N. minor damages more than 100 crops, including corn, sorghum, sugarcane, cabbage, bermudagrass, peanut, mustard, St. Augustine grass, soybean, tomato, and eggplant (Crow 2014).

17.2.2.4 Symptoms of Stubby-Root Nematode Infestation

The patches caused by stubby-root nematode damage in the field occur irregularly (Crow 2019). The severity of the symptoms is higher in sandy soils than in more compact soil types (Crow 2019). The aboveground symptoms of *N. minor* attack are lodging, stunting, poor stand, nutrient deficiency, and wilting (Crow 2014). In turfs, *T. proximus* causes wilting and death when the plant experience additional stressors like drought. Belowground, the nematodes cause a stubby appearance to the roots.

17.2.2.5 Detection and Identification of Stubby-Root Nematodes

Khan et al. (2021) indicate that it is vital to consider the sampling time when determining the population density of stubby-root nematodes because the population varies significantly according to a host's presence or absence. Furthermore, the researchers indicate that the harvest season is the best for sampling because the nematode population is at its highest then (Khan et al. 2021). The best sampling method for stubby-root nematodes involves the following steps as Khan et al. (2021) suggest:

1. Using a zigzag pattern.
2. Sectioning the sample area into smaller divisions according to soil texture, drainage patterns, moisture, and crop growth differences.
3. Taking away the top 2 in. of soil before digging out soil cores at a depth of 12–20 in. since the nematodes avoid the topsoil because of its desiccating or freezing temperatures.
4. Collecting soil core samples from the rhizosphere. Researchers should collect soil samples from affected and unaffected regions for comparison.
5. Storing and labeling the soil core samples correctly until transported to the laboratory for analysis, identification using molecular, morphological, and morphometrical procedures, and density determination.

17.2.2.6 Importance of Stubby-Root Nematodes

T. obtusus damages turf grasses' root systems significantly, increasing the hosts' vulnerability to environmental stresses. The extensive damage also increases water and fertilizer input as farmers try to boost the grasses' growth or herbicide use as the affected plants exhibit reduced competitiveness against weeds (Crow 2019).

N. minor was the first ectoparasite associated with plant damage (Crow 2014). When the nematode feeds on the root tip cells of the host plant, it causes the cessation of the roots' elongation and development, hence stubby roots (Crow 2014). The damage reduces the roots' adequacy in water and nutrient uptake and supply, leading to the symptoms identified in this chapter. As viral vectors, *N. minor* transmits the tobacco rattle virus that causes corky ringspot disease of potatoes in

Florida (Crow 2014). Potato farmers encounter significant losses since it is impossible to market the diseased tubers.

17.3 Bio-Management of Plant–Parasitic Nematodes

In the past, farmers used chemicals including pesticides, nematicides, and herbicides to control plant–parasitic nematodes and enhance plant growth. However, these chemicals are largely ineffective because of the difficulty associated with delivering them to the phytonematodes' immediate environment for optimal results (Abd-Elgawad 2016; Khan et al. 2014). Additionally, the chemicals present health and environmental hazards, making environmentalists, governments, and other concerned parties advocate against their application (Khan 2016). The reduced appeal and viability of chemical control methods necessitates increased research and use of biocontrol agents (BCAs), which have several advantages including target specificity, increasing plant growth, and maintaining the ecosystem's balance by not destroying beneficial organisms (Abd-Elgawad 2016; Khan and Mohiddin 2018; Mulusa 2021). Farmers should apply BCAs to their fields before sowing or when transplanting seedlings to optimize BCAs' effects, especially in cases where the nematode density surpasses the threshold (Khan 2005). The BCAs use various antagonistic strategies including mycoparasitism, plant growth promotion, antibiosis, cell wall degradation, inductive resistance, competition, and rhizosphere colonization ability (Abd-Elgawad 2016). Overall, an effective BCA should use multiple mechanisms to antagonize the pathogen. The known classes of BCAs are fungi, bacteria, mites, predatory nematodes, arbuscular mycorrhizal fungi, and plant growth-promoting bacteria. This chapter discusses these BCA classes next to highlight important aspects including their mode of action, competitiveness, ideal surroundings, and biocontrol effectiveness since understanding them is necessary for their correct and effective application.

17.3.1 Fungi

Scientists have conducted extensive studies on nematophagous, endophytic, and arbuscular mycorrhizal fungi as antagonists of plant –parasitic nematodes, hence the successful exploitation and commercial production of some fungal species as BCAs. Examples of fungal species included in commercial products as active ingredients are *Trichoderma hamatum, Trichoderma asperellum, Aspergillus niger, Paecilomyces lilacinus,* and *Pochonia chlamydosporia*. Further, classes of nematophagous fungi depending on their mode of action are egg and female-parasitic fungi, nematode-trapping fungi, toxin-producing fungi, and endoparasitic fungi (Abd-Elgawad 2020). Laboratory and greenhouse experiments show promising results for fungal biocontrol. Additionally, these experiments show how fungi antagonize PPNs. For example, filamentous fungi produce lytic enzymes and secondary metabolites, hence antibiosis, or compete with the PPNs for space on the plant roots (Poveda et al. 2020). On the other hand, arbuscular mycorrhizal fungi increase the host plant's water and nutrient uptake ability, alter the roots' morphology, alter the rhizosphere interactions, or compete for colonization or photosynthesis sites (Poveda et al. 2020). Further, endophytic fungi lessen PPN activity through parasitism, antibiosis, inducing nematode paralysis, space competition, or production of lytic enzymes (Poveda et al. 2020). Still, these fungi exhibit limited biocontrol activity in agricultural fields where scientists cannot control the ecosystem to favor the BCAs' activity, compromising their antagonistic capacity.

The biocontrol fungi alone (Stirling 1991; Khan 2007; Khan and Anwer 2011) or along with oil, neem cakes (Sikora and Roberts 2018; Khan et al. 2021) or pesticides (Mohiddin and Khan 2013) may provide sustainable nematode management in agricultural crops (Khan 2023; Khan et al. 2023). The microbial antagonists, *Aspergilus niger, Pochonia chlamydosporia, Purpureocellium lilacinum*, etc. (Jatala 1986; Stirling 1991; Khan 2016) may significantly contribute in the nematode management. In recent years, *Trichoderma* has also been found effective in suppressing plant nematodes (Mohiddin et al. 2010; Khan and Mohiddin 2018), and its formulations are available in market (Khan et al. 2011), which are quite effective against soil nematodes and other pathogens (Mohammed and Khan 2021; Sikora and Roberts 2018; Shahid and Khan 2019). However, the virulence and viability of some BCA fungal isolates vary significantly when introduced into the soil, indicating a potential inconsistency in their field applications. Furthermore, antagonists of these fungi exist in the soil, weakening their biocontrol capacity when applied in the field (Abd-Elgawad 2016; Poveda et al. 2020). It is also difficult for nematophagous fungi to control PPNs when the PPN density in the soil is too high. Still, it is possible to reduce the PPN population to an insignificant number, leading to increased plant yields. Abd-Elgawad (2016) and Poveda et al. (2020) indicate that maximizing the effectiveness of fungal BCAs requires the simultaneous application of BCAs and other pest management strategies, hence integrated pest management (IPM). Abd-Elgawad (2016) further indicates that previous research posits that treating seeds with a fungal BCA, botanicals, and pesticides concurrently yields more BCA effectiveness, a safer, and more economical and viable solution for PPN control using fungi.

17.3.2 Bacteria

Scientists have also conducted extensive research on bacteria such as *Pasteuria* spp., *Bacillus subtilis*, *Pseudomonas* spp., and *Paenibacillus* spp. as biocontrol agents of PPNs. These studies have yielded information on the mechanisms that the antagonistic bacteria for PPN biocontrol. Abd-Elgawad (2016, 2020) identify various classes of these bacteria according to their mode of action: opportunistic parasitic bacteria, endophytic bacteria, opportunistic bacteria, parasporal crystal-forming bacteria, rhizobacteria, and symbionts of entomopathogenic nematodes. The bacteria also produce metabolites that suppress PPN activity and induce positive host plant responses. The advantages of bacterial BCAs include the production of undetectable or biodegradable harmful residues hence environmental safety, cost-effectiveness, and target specificity. Concerning target specificity, Abd-Elgawad (2016, 2020) posits that the high specificity exhibited by some bacterial agents such as *Pasteuria penetrans* is advantageous because it promotes the identification and classification of the target PPN species. Additionally, high specificity reduces the BCA probability of attacking other species, which might be beneficial to the ecosystem.

The treatments with some biocontrol bacteria (Kerry 2000; Khan 2007; Stirling 1991), and phosphate solubilizing microorganisms such as *Bacillus, Pseudomonas* etc. (Khan et al. 2009; Sikora and Roberts 2018) may significantly contribute in the sustainable management of plant nematodes. It has been well established that application of rhizobium to legume plants (Khan et al. 2016a), and *Pseudomonas, Bacillus* etc. to non-leguminous plants (Khan et al. 2016b) may subside nematode attack and improve plant yield (Sikora and Roberts 2018). However, like with other biocontrol agents, bacterial BCAs act and establish significant population densities slowly, reducing their appeal to farmers and stakeholders who might prefer a faster solution for their PPN challenges. Additionally, bacterial BCAs exhibit variability in their effectiveness and reproduction due to the effects of biotic and abiotic factors within an unregulated environment. Still, the bacteria, like other living organisms, try to adapt to their new environment. Therefore, if the identified factors do not eliminate or render the bacterial BCAs incapable of reproduction, they can develop adaptive mechanisms that increase their tolerance to environmental factors producing an evolutionary strain or race (Abd-Elgawad 2016). Due to the evolutionary processes and time involved, it is difficult to predict bacterial multiplication, which varies significantly, making it difficult to apply the results for large-scale exploitation. Thus, additional fundamental and applied research on the interactions among bacterial strains, PPN targets, soil characteristics, soil microbes, host plants, and the environment will facilitate the productive exploitation of bacterial BCAs for the effective application in the management of PPNs in different agricultural systems.

17.3.3 Nematophagous Mites

Abd-Elgawad (2016) indicates that a 1977 study by Sturhan & Hampel identified *Rhizoglyphus echinopus*, an acarid mite, as the sole predator of *Aphelenchoides* spp., which attacks the buds and leaves of various plants. Nevertheless, the author also indicates that a 2015 study by Gerson indicates that nematophagous mites and other predators do not offer long-term and cost-effective PPN management, necessitating the application of several techniques as integrated pest management. Examples of these strategies are chemicals such as soft pesticides, soil aeration, green manure, crop rotation, use of soil amendments, solarization, use of resistant or tolerant crop cultivars, and natural enemies (Abd-Elgawad 2016). Furthermore, studies emphasize the need for research on PPN distribution patterns and their agricultural chemical compatibility because effective exploitation of these BCAs requires a substantial understanding of the PPN species, their natural enemies, ecology, biology, and soil conditions (Abd-Elgawad 2016). Abd-Elgawad (2016) also indicates that previous research highlights the importance of increased precision in reporting the mites' predation rates in soil as such knowledge enables stakeholders to make sound predictions about a predator's effects on PPN population density.

17.3.4 Predatory Nematodes

Most studies on predatory nematodes focus on their biology, prey-searching capacity, and predation ability instead of their viability as BCAs in terms of durability, stability in storage, mass production, and easy application (Abd-Elgawad 2016). The actual number of prey eliminated from an ecosystem due to predatory nematode (PN) activity indicates the PN's control efficacy (Abd-Elgawad 2016). The measures used to determine this outcome are the PN's predation and prey-searching abilities. While monorchid predators belonging to the Monochidae, Anatonchidae, and Iotonchidae families encounter prey nematodes accidentally, other PNs like aphelenchids, dorylaimids, and diplogasterids encounter their prey intentionally through chemosensory receptors triggered by the kairomones produced by the prey (Abd-Elgawad 2016). Monorchid predatory activity reduces their prey capture probability, decreasing their preference as BCAs. The phases of prey predation are encountering the prey, attack reaction, and feeding, which comprises attack, extra-corporeal digestion, and ingestion (Abd-Elgawad 2016). Apart from a better prey-capturing probability, aphelenchid PNs inject toxins into their prey to immobilize them, enhancing their BCA effectiveness. Moreover, as Abd-Elgawad (2016) highlights, the high colonization capacities, short reproduction time, and high reproduction rate increase aphelenchid and diplogasterid BCA appeal and effectiveness. Nonetheless, under favorable conditions and using practical techniques, it is possible to use all PN as biocontrol agents. The main setback of PNs as BCAs involves the challenges encountered in their mass production and storage. Thus, to reduce these difficulties, Abd-Elgawad (2016) suggests that researchers and other stakeholders should use practical and viable techniques, for example, those used with entomopathogenic nematodes.

Apart from biocontrol agents, other bio-management strategies for nematodes are agronomic and physical methods. Using these strategies together with BCAs, a process called integrated pest management increases the outcomes of the applied bio-management strategies.

17.3.5 Agronomic Methods

Crop rotation is one agronomic technique that farmers have used for many years to control PPNs, improve soil quality, and prevent nutrient depletion (Sasanelli et al. 2021). Application of the practice involves growing a series of different crop types within the same area in PPN-infested soil (Sasanelli et al. 2021). The farmers alternate host crops and non-susceptible crops to reduce the PPN density to insignificant levels. Despite this basic principle, crop rotation is difficult to use on polyphagous nematodes since they attack diverse crops and even weeds (Sasanelli et al. 2021). However, researchers have shown the technique's effectiveness on PPNs including cyst nematodes that have specific host plants (Sasanelli et al. 2021). Consequently, when considering crop rotation, farmers should be knowledgeable about a PPNs' host specificity.

Bio-fumigation, which involves incorporating green manure material into the soil to reduce PPN density while improving soil fertility, is also another agronomic technique (Sasanelli et al. 2021). The plants used as green manure contain wide-spectrum biocidal compounds that exhibit biological activity against several plant pests including PPNs. The plants also inhibit weed seeds from germinating (Sasanelli et al. 2021). This implies that the method might be effective against polyphagous nematodes because it prevents the growth of weeds that act as intermediary hosts. Sasanelli et al. (2021) indicate that using bio-fumigation together with soil solarization or soil plastic film coverings optimized the outcomes.

Cover crops inhibit the growth of PPN populations through three techniques. Firstly, they are non-host or low-susceptible crops that prevent PPNs from completing their lifecycles (Sasanelli et al. 2021). They also produce secondary metabolites that antagonize PPN density and create favorable factors allowing the growth of microflora and microfauna that antagonize PPNs.

Furthermore, researchers also recommend trap crops, a method that involves planting a host plant of sedentary nematodes, for example, rice, cauliflower, tomato, and mustard (Sasanelli et al.). Females that develop from the juvenile stage within the plant root cannot leave the roots. The subsequent step involves the trap crop's destruction before egg-laying begins, preventing the PPNs' spread and multiplication. Finally, as Sasanelli et al. (2021) indicate, soil amendments like biochar, farmyard manure, peat, and composted mixture of household waste are effective against several PPN groups including dagger nematodes, root lesion nematodes, and stem nematodes.

17.3.6 Physical Methods

Farmers usually use physical disinfestation by hot water and steam in areas with continuous monocultures such as sheltered cropping systems and intensive field crops (Sasanelli et al. 2021). Despite this frequent application, these methods, in addition to microwave soil radiation, are costly, hence the high preference for soil solarization, which is cheaper and more environmental-friendly. Soil solarization is increasing the soil's temperature using solar energy and plastic films (Sasanelli et al. 2021). The resulting high temperatures destroy PPNs due to the microbes' sensitivity to high temperatures. Sasanelli et al. (2021) state that research shows the technique's effectiveness against root-knot nematodes, cyst nematodes, stem bulb nematodes, reniform nematodes, and root lesion nematodes.

In addition to the methods listed above, farmers also use biopesticides, which are secondary metabolites derived from microorganisms including bacteria and fungi and plant extracts from cloves and garlic in the bio-management of PPNs (Sasanelli et al. 2021). These exhibit different modes of action against PPNs, including toxicity, reducing hatching rates, and inhibition of nematode life cycle completion.

17.3.7 Bio-Management of Dagger Nematodes

Heve et al. (2017) indicate that soil solarization could be an effective bio-management technique for dagger nematodes because the heat generated from solar energy and the plastic bags kills the phytonematodes, which exhibit sensitivity to high temperatures. Further, agronomic techniques including use of trap crops such as *Sesbania* and *Crotalaria*, decomposing organic mulch, and green manure facilitate the growth of microbes such as *Pochonia*, a fungus, that destroy PPNs. Moreover, research recommends bio-fumigation using *Euphorbia* spp. extracts, mustard bran, and mustard seed meal (Heve et al. 2017). Additionally, studies indicate that using *Tagetes* spp. (marigold) as cover crops or alternative crops in an infested field reduces dagger nematode density significantly as the marigolds produce inhibitory root exudates (Heve et al. 2017). Natural enemies of *Xiphinema americanum* that can be used as BCAs also exist. These are *Aphanomyces*, *Catenaria anguillulae*, *Lagenidium caudatum*, *Mononchoides fortidens*, *Mononchoides longicaudatus*, and *Pseudomonas denitrificans* (Center for Agriculture and Bioscience International 2022). These microbes are either parasites or pathogens of different life stages of dagger nematodes. Consequently, their effective and efficient application requires adequate research.

17.3.8 Bio-Management of Stubby-Root Nematodes

Physical methods that improve soil quality can be used to reduce *T. obtusus* populations. Consistently, Crow (2019) indicates that improving stress-related conditions like poor light and irrigation, inadequate mowing, and poor coverage where turf grasses are grown can reduce damage caused by *T. obtusus*. Further, Crow (2019) suggests replacing the nematode-infested crops with alternative non-host plants. Since the stubby-root nematodes cannot invade the non-host plants, they lack nutrition, leading to their eventual death and immature termination of lifecycles, which gradually reduces the nematode population in the field. Similarly, Hajihassani et al. (2020) suggest that when transplanting plants that can regenerate roots such as onions, farmers should uproot the plants by hand, ensuring they remove the transplanted plant's entire root mass, before transplanting it into the field. If transplanted into nematode-free fields, the method helps in preventing the spread of stubby-root nematodes because they survive in the soil rather than plant roots and systems. Other agronomic methods that can be used for the bio-management of *P. minor* are using resistant cultivars of pearl millet, sun hemp, cowpea, and oat as cover crops or green manure (Hajihassani et al. 2020). Still, it is important to study the relationship between resistant cultivars and stubby-nematode population growth as some resistant cultivars, for example, sorghum sudangrass, contribute to the PPN's population growth.

17.4 Conclusion and Future Perspectives

Agriculture is an important global industry as it contributes significantly to the GDP and household incomes. However, abiotic and biotic factors undermine agricultural output. Still, plant parasitic nematodes (PPNs) are one of the leading causes of significant losses since farmers can misdiagnose the plant symptoms, hence the apply improper management strategies. Proper diagnosis, however, facilitates proper management of PPNs. Today, environmental concerns and the development of chemical resistance by PPNs warrant extensive use of bio-management techniques including biocontrol agents, agronomic methods, physical methods, plant extracts, and biopesticides to reduce or eliminate PPN populations. Research shows that using these techniques in combination yields more bio-management effectiveness against PPNs rather than using a single technique at a time. Similarly, this chapter finds that it is possible to use various bio-management techniques like soil solarization, bio-fumigation, crop rotation, green manure, and trap crops to suppress stubby-root and dagger nematode populations in infested fields. These nematodes destroy important agricultural crops as pathogens and vectors, necessitating their effective bio-management. Still, despite this significance and the extensive research conducted on the bio-management of PPNs, limited literature on the biocontrol of stubby-root and dagger nematodes exists. Therefore, greater and oriented research efforts should be made to evalute the effectiveness of different bio-management strategies against stubby-root and dagger nematodes, and be desseminated to the farming communities to protect their agricultural crops from these notorious nematode pests.

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18

Burrowing Nematode in Spice and Fruit Crops and Their Management by Novel Biocontrol Strategies

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Abstract

The burrowing nematode, *Radopholus similis* is one of the tropics' ten most important plant parasitic nematodes, limiting the productivity of banana and black pepper plantations. Nematode-related losses are estimated to range between 20 to 80%. Chemical nematode control is unfavorable and detrimental to both human health and environmental health. Biological nematode control is one of the most effective alternatives to chemical nematicide. The fundamental tenet of *R. similis* biocontrol is the utilization of nematode-antagonistic fungi and bacteria as active bionematicides for its management, including mycorrhizal fungi from the *Glomus* and *Trichoderma* genera, *Fusarium oxysporum* endophytes, *Purpureocilium lilacinum*, and bacteria from the fluorescent pseudomonads, *Bacillus* spp., *Corynebacterium paurometabolum*, and *Serratia marcescens.* This group has been expanded to include *Blattisocius dolichus* (Acari: Blattisociidae) as a potential biocontrol agent for the nematode due to its predation ability on *R. similis*. Some biocontrol agents are available as commercial formulations such as wettable powder (WP), water dispersible granular (WG), talc-based products, vermiculite flakes, and dry root powder. This chapter highlights the parasitism of the burrowing nematode, the beneficial microorganisms' antagonistic bioactivity, as well as its benefits and drawbacks in the biocontrol of the burrowing nematode. The strategic affective applications of the biocontrol agents against the nematode were also discussed.

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Keywords

Radopholus similis · Biocontrol agent · Mode of action · Strategic application · Effectivity

18.1 Introduction

Burrowing nematode, *Radopholus similis* (Cobb, 1893; Thorne, 1949), is one of the tropics' ten most important plant–parasitic nematodes (PPNs) (Haegeman et al. 2010; Khan 2008). The nematodes are major constraints to black pepper and banana plantation productivity. Losses due to the nematode are estimated to be between 20 and 90%. Bananas, black pepper, citrus species, and coffee are some of its best-known hosts (Moens and Perry 2009; Campos and Villain 2005; Khan 2023).

With 138 million tons produced globally in 2010, bananas and plantains (Musa spp.) are among the most important food and economic crops in the world. These crops provide a significant source of food and an important part of a healthy diet for more than 400 million people in the tropics (FAOSTAT 2011). Black pepper is also one of the most significant agricultural products in Indonesia and other countries that produce it, particularly in terms of foreign exchange. Because smallholder farmers manage 95% of the pepper acreage, the plant plays an important role in local economies (Deciyanto et al. 1998). *R. similis* is found in all of the world's major banana-producing regions and severely reduces banana yields by up to more than 75% (Sarah and Vilardebo 1979 *in* Sarah 1989). Extensive root lesions caused by R. similis might cause banana trees to topple over (Leach 1958). The yellow disease of *Piper nigrum* (black pepper), which caused losses in Bangka ranging from 32% to 80%, was similarly brought on by the nematode in Indonesia (Yolanda 2013; Sitepu and Mustika 2000). Furthermore, some migratory endoparasitic species, such as *R. similis*, are classified as quarantine pests, which are defined as a "pest of potential economic importance to the area endangered thereby and not yet present there, or present but not widely distributed and being officially controlled" (Holdeman 1986).

Nematode control in commercial banana plantations in Latin America is primarily accomplished by using granular nematicides such as carbamate and organophosphate (Marín 2005). Nematicide-based chemical control of nematodes is undesirable. In addition to polluting the environment, they leave toxic residues in the product that are harmful to human health. In light of changes to EU legislation controlling the use of pesticides on agricultural crops, low-impact management techniques are required (Migunova and Sasanelli 2021). For alternatives to chemical nematicides and overall improvement of IPM (integrated pest management) programs, biological control using natural enemies of nematodes with low environmental impact has been proposed. As a result, it is needed to develop biological control agents as a substitute for nematode control measures (Kerry 1990; Sikora 1992).

Research findings on beneficial microorganisms for *R. similis* suppression and plant growth have been compiled (Declerck et al. 1995a, b; Jaizme-Vega and Azcon

1995). Rhizobacteria, endophytic microbes, mycorrhizal fungi, obligate parasites, and predator mites are some of the organisms used to combat plant-parasitic nematodes (PPNs). Beneficial microbes that are competitors with *R. similis* have been shown to act through a number of different mechanisms, including hyperparasitism, penetration restriction, population growth inhibition due to nutrition competition, and the antibiosis impact linked to bioactive metabolites. According to several experiments, there may be possibilities for the suppression of phytoparasitic nematodes by advantageous microorganisms. For instance, natural PPN enemies may contribute to the pesticidal effects of bio-nematicide compounds. A few examples of microbial substances with the potential to be used as biopesticides against pests, plant diseases, and PPN include antibiotics, inhibitors, toxins, and lytic enzymes (Bhattacharyya et al. 2016). Several investigations of microbial substances over the past two decades on sedentary endoparasitic nematodes like cyst and root-knot nematodes, as well as migratory endoparasitic nematodes like burrowing and root lesion nematodes, have led to the development of efficient biological control agents (Kerry 2000; Siddiqui and Mahmood 1999). More commercial biopesticide products have recently been marketed globally.

This chapter highlighted the parasitism of the burrowing nematode, the beneficial microorganisms' antagonistic bioactivity, as well as its benefits and drawbacks in the biocontrol of the burrowing nematode. The strategic affective applications of the biocontrol agents against the nematode were also discussed.

18.2 The Burrowing Nematode

18.2.1 Biology and Parasitism

Understanding the biology of *R. similis* is helpful for constructing nematode pest control program that more effectively reduces burrowing nematodes and, as a result, supports the sustainable production of bananas, black pepper, and other important host crops.

The burrowing nematode is an amphimictic species with observable sexual dimorphism. Males and females have distinct morphologies, with males having poorly formed stylets and a knobby head from an elevated, narrowed lip area. Long, tapering tails with rounded or indented tips are present on both sexes. The spicule, which is the male reproductive organ, is located in a bursa, or sac, in the male (Brooks 2008). Because females retrieved from male-dominated populations typically have sperm in their spermatheca, fertilization is typically considered to be bisexual; however, parthenogenesis does occur. Table 18.1 lists the morphometrical characteristics of *R. similis* associated with the yellow disease of black pepper in Bangka, Indonesia (Mustika 1990).

R. similis at various stages was found in soil and plant root samples. All stages of the nematode were vermiform (wormlike), transparent, and less than 1 mm in length. The burrowing nematode is a migratory endoparasitic nematode that spends its entire life cycle within the root cortex, though the nematodes may emerge from the roots in

Table 18.1 Morphological characteristics of *Radopholus similis* associated with yellows disease of black pepper (Mustika 1990)

Type	Body length (μm)	Stylet length (μm)	Tail length (μm)	Body width (μm)	Spicule length (μm)	Vulval site (%)
Female	614	19	64	24	–	59%
Male	614	13	70	17	18	–

adverse conditions. Nematodes can migrate the entire length of the root, but the majority of their penetration occurs close to the root tip. The nematode burrows between cortical cells of the roots (and rhizomes) (Fig. 18.1), punctures cell walls with its stylet, and feeds on the cytoplasm to create enormous cavities within the roots where it lays eggs (Gowen 1995; Blake 1961, 1966; Loos and Loos 1960). Cavities coalesce to form red-brown lesions as the nematode migrates and destroys the cells (Gebremichael 2015; Mateille 1994; Blake 1961, 1966). The nematode's life cycle takes around 21 days to complete at 25 °C, and each female lays four to five eggs every day for 2 weeks.

Djiwanti and Wahyudi (2015) reviewed the parasitism of the burrowing nematode on black pepper. *R similis* penetrate pepper roots 24 h after nematode inoculation or after the nematode adhered to roots. Usually, *R. similis* penetrate tissue near root tip, but sometimes penetration evidenced at 1.0–1.5 cm away from the root tip. While penetrating into roots, *R. similis* release enzymes, i.e., hydrolase (cellulase), invertase (saccharose, sucrase, or fructofuranosidase), and pectolytic (Duebert and Rohde 1971 *in* Mustika 2005). Tissue cells around nematode penetration cites turned dark brown in color, and 72 h after penetration, lesions were formed. In 5 days after penetration, the female lays eggs in cavities along damaged root cortex tissues caused by nematode activity. The root stele is not invaded, but was deposited with gum-like substances (Mustika 1990). The optimum temperature for population development is 27 °C. At 20–30 °C, *R. similis* completes its life cycle in 35 days (Mustika 1990). In soil fungus pathogens such as *Fusarium* spp., invasion of the lesions results in necrosis that penetrates the stele and damages the roots. *R. similis* has a significant economic impact, and interactions with other diseases increase crop damage and yield loss (Gebremichael 2015).

In contradiction to *R. similis* sensu stricto (formerly the banana race of *R. similis*, which infects bananas but not citrus), *R. citrophilus* (formerly the citrus race of *R. similis*, which infects both bananas and citrus) invades the stele and lodges in the phloem and cambium ((Esser et al. 1984, Huettel et al. 1984, Gebremichael 2015). Not all nematologists agree that the two species are distinct from one another (Esser et al. 1988). *R. citrophilus*, on the other hand, is not covered in this chapter.

R. similis is a species with significant quarantine value with a broad host range (Karssen and den Nijs 2001). More than 350 plant hosts are found in tropical and subtropical areas (Brooks 2008; Haegeman et al. 2010). Its primary hosts are bananas, black pepper, citrus, Araceae plants (anthurium, philodendron, and taro), Marantaceae plant (calathea), and Zingiberaceae plants (ginger and turmeric). The

Fig. 18.1 Damage caused by *Radopholus similis* on banana and black pepper plant. Above left: banana plants falling over or being uprooted (CABI 2018, https://www.plantwise.org/KnowledgeBank/pmdg/20187800464); above right: banana roots have developed lesions as a result of R. similis feeding (CABI 2018); below left: severe yellow diseased-black pepper vines; below center: dying pepper vine shown defoliation as further progressed symptom; and below right: a burrowing nematode *R. similis* (Ferris 2019, In Nemaplex, http://nemaplex.ucdavis.edu/Taxadata/G111s2.aspx)

other hosts include tropical palms (betel nut palms, coconuts, parlor palms), sugarcane, tea, and coffee plant.

The nematode is dominant at lower altitudes (<1000 m dpl) (Fogain et al. 1998). However, most banana-growing locations in the world are affected by the burrowing nematode, which is also observed in temperate glasshouses (EPPO 2022). *R. similis* cases have been reported in Africa (Egypt, most all of the sub-Saharan, and Indian Ocean islands), Asia (India, Indonesia, Japan, Malaysia, Pakistan, the Philippines, Sri Lanka, and Thailand), Central America and the Caribbean, North America (Canada and USA), South America, and Oceania (Australia, Fiji, France, Papua New Guinea, and Polynesia).

18.2.2 Symptom, Damage, and Loss

The nematode parasitized the root system; as a result, the plant loses its capacity to absorb nutrients and water, which is likely to have an adverse effect on growth and yield (Manzanilla-López et al. 2002; Fogain 2000; Fogain et al. 1996). The nematode-infected banana plants exhibit poor growth, fewer and smaller leaves, early defoliation, and smaller fruits as symptoms. Deep cracks on the root surface appear after browning and cavities in the cortex are first visible in main banana roots. In the banana-growing regions of Australia, Central and South America, Africa, and the Pacific and Caribbean Islands, the burrowing nematode plays a significant economic role by developing root rot, blackheads, toppling disease, decline, and predisposing trees to fungal infection. The burrowing nematode can cause significant damage to the primary root system of the banana plant, which can result in total yield loss of the crop of up to more than 75% due to toppling over (Sarah and Vilardebo 1979 *in* Sarah 1989; Leach 1958). *R. similis* also is a significant pest of the East African cooking banana species *Musa* spp. in Uganda. Yield losses can be as high as 20–75% as demonstrated by the use of nematicides (Gowen 1994; Sarah 1989; McSorley and Parrado 1986; Broadley 1979). In Cameroon, yield loss due to this nematode on plantain can be more than 50% in the case of severe infestation (Fogain and Njifenjou 2003).

The nematode is also a significant constraint on sustainable black pepper production in Bangka Island—Indonesia, Thailand, India, and Vietnam (Phan Quoc Sung 2000 *in* Ton and Buu 2013; Sitepu and Mustika 2000; Sharma and Loof 1974; Sher et al. 1969). The aerial symptoms of yellow disease caused by *R. similis* show a slow wilt disease that causes slow decline (van der Vecht 1950). These include foliar yellowing, defoliation, and dieback (Fig. 18.1). The first indication of yellowing disease is the appearance of occasional yellowed leaves, which increase in numbers until, within a year, a large portion or even all of the foliage may become yellow. The larger branches of roots gradually develop extensive necrosis because the main roots lose smaller feeder roots. The root damage will inhibit the absorption and translocation of water and nutrients. Plants that have been severely affected have decreased vigor and rarely yield berries. The plants eventually experience severe dieback and die. The root damage caused by *R. similis* infection will inhibit water and nutrition translocation in plants. When nutrients were analyzed from the leaves of healthy and damaged (yellowing) vines, significant variations were found in a number of nutrients. Large variations with potential deficiencies were detected in the K, Fe, Mn, and Zn contents, and toxicity was seen in the component Al content (Wahid 1976) (Table 18.2). Slow decline or slow wilting in pepper has also been observed in India, Vietnam, and Thailand as being related problems (Phan Quoc Sung 2000 *in* Ton and Buu 2013; Sharma and Loof 1974; Sher et al. 1969). In advance, the yellow disease is considered as nematodes (*R. similis* and *Meloidogyne* spp.) and fungal pathogen *Fusarium* spp. complex coupled with soil moisture stress and malnutrition, with the key causal agent being burrowing nematode, *R. similis* (Mustika 2005;

Table 18.2 Chemical analysis of pepper leaves of healthy and affected (yellows) vine in Bangka Island[a]

Leave samples	N (%)	P (%)	**K (%)**	Ca (%)	Mg (%)	Na (%)	**Fe (ppm)**	**Al (ppm)**	**Mn (ppm)**	Cu (ppm)	**Zn (ppm)**
Healthy plant	2.33	0.10	**2.55**	0.72	0.44	0.04	**385**	**115**	**309**	8	**19**
Affected plant	2.35	0.11	**1.42**	0.97	0.44	0.03	**121**	**187**	**129**	8	**13**

[a] Analyzed by Soil Research Institute, Bogor—Indonesia (Source: Wahid 1976); *N* nitrogen, *P* phosphorus, *K* kalium/potassium, *Ca* calcium, *Mg* magnesium, *Na* natrium/sodium, *Fe* ferrum, *Al* aluminum, *Mn* manganese, *Cu* copper, *Zn* zinc

Momota et al. 1991). Estimated losses caused by yellow disease infection in Bangka were about 32–80% a year (Sitepu and Mustika 2000; Yolanda 2013).

Infestation restricts the root systems of Calathea, which lowers productivity. Reduced leaf size and color changes result in lower-quality plants that can be marketed (Hamlen and Conover 1977). It is a significant pest on ginger (in Fiji), cardamom, and turmeric. In contrast to aubergine, coffee, tomatoes, and potatoes, which produced moderate damage, inoculation studies on soybeans, sorghum, maize, and sugarcane resulted in severe damage.

18.3 Biological Control of the Burrowing Nematode

18.3.1 Definition of Biological Control

There are various methods to control burrowing nematodes, such as applying chemical and botanicals, using natural enemies, and enhancing cultural practices, including crop rotation, fallowing, the use of organic fertilizers, and irrigation (Navasca et al. 2020; zum Felde et al. 2006). Due to their hazardous properties, the most effective chemical control treatments (such methyl bromide) against PPN infestation have recently been banned (Lamovšek et al. 2013). A potential nonchemical alternative of plant disease control is the biological control of plant–parasitic nematodes by natural enemies or antagonistic microbes (EPA 2022; Khan 2016; Stirling 1991). Natural enemies of the nematode are including predators, parasitoids, and diseases pathogen of the nematode. A wide range of bacterial and fungal agents have been investigated to reduce the burrowing nematode (Table 18.3). In recent years, the back-to-nature, healthy-eating, and environmental-friendly agricultural production movements have influenced the research community to focus on biological control agents as alternative disease management tactics.

Biocontrol agents of plant diseases are most often referred to as antagonists. Antagonists of pests and pathogens, as well as biocontrol agents, may contribute to the pesticidal effects of biopesticides. Furthermore promising as biopesticides against plant pathogenic and pests are microbial products such as enzymes, antibiotics, inhibitors, and toxins (Bhattacharyya et al. 2016). Microbial pesticides may contain live organisms (such as beneficial bacteria, fungus, nematodes, viruses, and protozoan) and/or the products of their fermentation (EPA 2022; Cao et al. 2019; Bhattacharyya et al. 2016). When considering the sensory mechanism of nematode pathogen detection, using microbial secondary metabolites such as enzymes, antibiotics, inhibitors, and toxins rather than whole organisms for nematode control can be advantageous (Bhattacharyya et al. 2016; Rahul et al. 2014; Zabaketa-Mejia 1985).

Table 18.3 Biocontrol agent of the burrowing nematode and their mechanisms of bioactivity

Biocontrol agents	Mechanisms of action	Effectivity	References
A. Bacteria group			
Fluorescent pseudomonads (*Pseudomonas fluorescens*)	Antibiosis [secondary metabolites of *P. fluorescens* (phenazine, pyrrolnitrin, tropolone, phycocyanin), 2,4-diacetylphloroglucinol (DAPG) antibiotics, and hydrogen cyanide, adversely affected nematode], induced systemic resistance (ISR) of plant against nematode, plant growth-promoting rhizobacteria (PGPR), repellent effect in in vitro, and the pseudomonads alter the polysaccharides and amino acids present in various root exudates, which alters nematode behavior, and bind the root surface with carbohydrate–lectin to prevent PPNs from recognizing the host	Reduced population in roots, increased yield, and prevented early invasion of tomato, maize, and banana roots by *R. similis* and *Meloidogyne* spp.	Raymaekers et al. (2020), Köhl et al. (2019), Khabbaz et al. (2019), Chowdhury et al. (2015), Maheshwari et al. (2013), Akhtar and Siddiqui (2010), Siddiqi and Shaukat (2002, 2004), Aalten et al. (2003), Racke and Sikora (1992), Oostendorp and Sikora (1990), Becker et al. (1988)
Bacillus thuringiensis var. kurstaki	Antibiosis (toxins produced by the sporulated bacteria), PGPR, lytic enzyme (lipases), ISR	Reduced the average population of *R. similis* in plantations by 87%	Fernández et al. (2005), Rodríguez et al. (1991)
Bacillus firmus	antibiosis, PGPR, ISR	In an in vitro test, all developmental stages of *R. similis* juveniles were paralyzed and killed in bacterial-free water extracts (bacterial filtrate) from formulations of the strain *B. firmus*. The highest mortality was 96–99%. *R. similis'* survival in bioassays in sand was similarly decreased by 41% when pure bacterial cell suspensions were added to the sand	Khabbaz et al. (2019), Radhakrishnan et al. (2017), Mendoza et al. (2008)

(continued)

Table 18.3 (continued)

Biocontrol agents	Mechanisms of action	Effectivity	References
Serratia marcescens	Antibiosis (volatile metabolites prodigiosin, and serratamolide-like lipopeptides), and PGPR	Inhibition on nematode egg-hatching and effectiveness against *R. similis* and *M. javanica* juvenile stages at low concentrations (LC50 values of 83 and 79 g/mL, respectively)	Marques-Pereira et al. (2022), Raymaekers et al. (2020), Rahul et al. (2014), Maheshwari et al. (2013). Zabaketa-Mejia (1985)
Corynebacterium paurometabolum	Toxins and antibiosis [its mode of action is primarily caused by the interaction of the gases (sulfides) released by the bacteria and the chitinases found in the culture media where they were grown	Testing of *R. similis* antibiosis under controlled settings revealed a population decline of over 85%, and a yield increase of 106%	Fernández et al. (2005)
Pasteuria penetrans	Hyperparasitism (the endospore attaches to the nematode's cuticle and penetrates the body wall and then parasitizes the body)	The bacteria could reduce the population of *R. similis* by about 68.89–93.33% and the incidence of yellow disease by as much as 26.67–50.0% on black pepper plant	Abd-Elgawad and Askary (2018), Ciancio (2018), Tian et al. (2007), Mustika et al. (1997)
B. Fungi group			
Glomus spp. (AMF)	The AMF colonization increased plants' tolerance to nematode, competition for host photosynthates, and colonization sites. The tolerance of mycorrhizal plants to soil pathogens could be explained by microbial changes in the mycorrhizosphere and the activation of plant defense mechanisms in the root system. However, increased P nutrition, though controversial, has also been postulated as one of the mechanisms of tolerance of plants to nematodes	The AMF suppressed the *R. similis* population by almost 50% in AMF-colonized plant roots	Fogain and Njifenjou (2003), Azcon-Aguilar and Barea (1996), Hussey and Roncadori (1982)

Glomus mosseae	ISR	*G. Mossae* significantly suppressed the population of Ugandan population of *R. similis* in Grand Naine and Pisang Jari Buaya cultivars	Elsen et al. (2003a)
Glomus intraradices	ISR	Research conducted under dixenic culture conditions revealed that the AMF colonization significantly reduced the number of *R. similis* population in roots by about 50%	Elsen et al. (2008), Elsen et al. (2001)
Trichoderma asperellum	Antibiosis (by the antibiotics gliotoxin, gliovirin, and peptaibols), lytic enzymes (mainly chitinases, glucanases, and proteases), and PGPR	*R. similis* in greenhouse conditions was significantly reduced, and the yield of 1 year of production was increased	Anonymous (2020), Lorito et al. (1996)
Trichoderma spp.	PGPR, antibiosis (antibiotics gliotoxin, gliovirin, and peptaibols), and lytic enzymes (chitinases, glucanases, and proteases) make the nematode easier to penetrate the host roots and use its nutrition, altering root exudates or by producing phytoalexins in the root such as glyceollin as nematicidal effect	It has been demonstrated that some Trichoderma isolates promote plant development while minimizing burrowing nematode damage	Vu (2005), Meyer et al. (2004), Sikora et al. (2003), Lorito et al. (1996), Hallmann and Sikora (1996), Morandi (1996), Reißinger (1995), Stirling (1991), Windham et al. (1989), Kerrry (1987), Graham and Menge (1982)
Fusarium oxysporum NP (nonpathogenic endophytes)	ISR, antibiosis (toxin gliotoxin fermentation medium from their secondary metabolism causing paralysis of motile stages, and inhibitory of hatching egg), competition for space and nutrients, and improved plant growth	The damage and nematode population in nematode-infected root segments of treated plants decreased by 51% and 99%, respectively	Lisnawita et al. (2013), Viljoen et al. (2007), Paparu et al. (2007), Dubois et al. (2007), Mandeel (2007), Carñizares Monteros (2003), Meneses Hérnandez (2003), Niere et al. (1999)

(continued)

Table 18.3 (continued)

Biocontrol agents	Mechanisms of action	Effectivity	References
Paecilomyces lilacinus (Thom.) Samson	Antibiosis (toxins paecilotoxins, leucinostatins, hydrolytic enzymes polysaccharides, proteases and chitinases, and leucinostatins) The parasitic fungus immobilized the nematode larva within 48 h, caused digestive system and vacuoles abnormalities Neurotropic mechanism action on the nervous receptors of nematodes was also proposed (*P. lilacinus* isolate 23N5-2 caused greatest paralysis of male and juvenile, which was not influenced by filtrate concentration)	Ten and 18 weeks after nematode inoculation, respectively, *P. lilacinus* reduced *R. similis* by 21 and 86%, and increased yields by 25% The populations of *R. similis* and *M. incognita* did not significantly rise over the course of a year compared to the control	Mendoza et al. (2007), Kilama et al. (2007), Park et al. (2004), Gupta et al. (1993), Cayrol et al. (1989)
Rhizophagus irregularis	ISR (increased tolerance or the activation of plant defense mechanisms)	Reductions in the nematode population and surface root necrosis of 60 and 56%, respectively, were seen in the plantlets exposed to the AMR	Koffi et al. (2012)
C. Insect group			
Blattisocius dolichus (Acari: Blattisociidae)	The mite feeds on nematodes in all of its active developmental stages	Ten days after the application of 500 mites per pot in both the experimental field and on potted plants, the nematode density had decreased by 66%	Chen et al. (2013)

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[a] Antibiosis: an antagonistic association between two organisms (especially microorganisms), in which one is adversely affected

18.3.2 Biological Control Agents, Their Antagonistic Bioactivity, and Effectivity

Biological control using natural enemies of *R. similis* has been investigated by some researchers. It has been consistently demonstrated that antagonistic bacteria are promising antagonistic microorganisms for use in the control of sedentary and migratory endoparasitic nematodes both in vitro and in vivo (Khan 2007; Sikora et al. 2007). Depending on the bacteria and microorganisms engaged, the modes of action can include obligate parasitism, reduced penetration, growth inhibition due to resource competition, and antibiosis associated with bioactive metabolites (Table 18.3). Arbuscular mycorrhizal fungi (AMF) are now widely recognized for their role in the control of migratory endoparasitic nematodes. AMF have been reported to reduce damage of nematodes including migratory endoparasitic nematodes (Schouteden et al. 2015; Koffi et al. 2012; Siddiqui and Mahmood 1995; Lindermann 1994). Through in vitro testing, greenhouse tests, pot experiments, and field tests, biocontrol agents' antagonistic bioactivity and efficacy are demonstrated at various stages of nematodes' lifespans.

Migunova and Sasanelli (2021) described how the antagonist and the nematode interact in agroecosystem. In the habitat comprised of soil and plants, the natural enemies and the nematode coexist as part of agroecosystems. PPN population levels are influenced by the immune-genetic properties of the host plant, as well as by the composition and characteristics of the biological regulators of nematodes (Berg et al. 2017). Then, crop rotation practices and decreased biodiversity of PPN natural regulators are adverse effects of agriculture's intensification (Migunova and Shesteperov 2007).

18.3.2.1 Bacteria as Biological Control Agent

From the group of bacterial antagonist to *R. similis*, there were several types of bacteria, i.e., endophytic bacteria, rhizosphere bacteria (rhizobacteria), and soil bacteria. Among these treatments, fluorescent pseudomonads (a key bacterial group that promotes plant growth) and *Bacillus* spp. were found to be successful in reducing the burrowing nematode in several crops (Cannayane and Rajendran 2001; Shanthi and Sivakumar 1995; Oostendorp and Sikora 1990). It is widely known that there are bioactive chemicals produced by bacteria isolated from the soil or rhizosphere that can suppress plant–parasitic nematodes (Kerry 2000) (Table 18.3). Several studies have shown that bacterial endophytes also aid in the development and well-being of a range of plants. (Brown 1974), and it is called as plant growth-promoting rhizobacteria (PGPR).

The treatments with some biocontrol bacteria (Kerry 2000; Khan 2007; Stirling 1991), and phosphate solubilizing microorganisms such as *Bacillus, Pseudomonas* etc. (Khan et al. 2009; Sikora and Roberts 2018) may significantly contribute in the sustainable management of plant nematodes. Endophytic bacteria are increasingly seen as promising candidates for biological control of a number of pests and diseases since they stay within living plant tissues without causing significant harm or acquiring anything other than a habitat to call residence (Aravind et al. 2009).

Because they share the same niche as plant diseases, they may be better suited than rhizosphere bacteria to either outcompete or actively antagonize pathogens (Ryan et al. 2008). A wide variety of bacterial species, including *Pseudomonas fluorescens* Migula, *Bacillus* spp., *Enterobacter* spp., *Herbaspirillum* spp., *Serratia marcescens* Bizio, *Streptomyces* spp., etc., are found in endophytic bacterial communities (McInroy and Kloepper 1995), that are capable of controlling nematodes (Mekete et al. 2009; Hallmann et al. 1995, 1998, 1999). Through the exchange of information at the cellular and molecular levels, the co-evolution of plants and endophytic bacteria results in a close interaction (Bacon and Hinton 2006; Hallmann 2001).

Bacillus, Pseudomonas, and *Serratia* are the most effective as PGPR (plant growth-promoting rhizobacteria) in their biological control during the past 20 years when it comes to the impact of bacteria on PPN population density (Raymaekers et al. 2020; Radhakrishnan et al. 2017; Maheshwari et al. 2013), and on plant yield (Brown 1974; Lifshits et al. 1987). PPNs are suppressed, although plant growth and productivity are also promoted by PGPR (Migunova and Sasanelli 2021). PPN-affected plants are stressed because of a shortage of nutrients and water. PGPB can constitute for this shortage by producing more nutrients available (nitrogen, phosphorus, and others). However, study by Brown (1974) and Lifshits et al. (1987) revealed that the production of plant growth regulators including gibberellins, cytokinins, and indole acetic acid may be responsible for an increase in yield caused by the introduction of *P. fluorescens*.

Bacilli and pseudomonad bacteria trigger induced systemic resistance (ISR) pathways, preventing plants from PPNs and microbial diseases (Khabbaz et al. 2019; Anita and Samiyappan 2012). Numerous interactions between bacteria and nematodes also involve plants and other microorganisms. As a result, in a complex disease, PGPB can reduce both PPNs and plant pathogenic bacteria or fungus.

R. similis with antagonist bacteria is brought on by a variety of mechanisms, such as direct activity with *Pasteuria penetrans*, obligate parasitism, synthesis of secondary metabolites with antibacterial or phytotoxic effects, like with *Pseudomonas* spp., and *Serratia marcescens* (phenazines, pyrrolnitrin, tropolone, pyocyanin, and 2,4-diacetylphloroglucinol antibiotic, hydrogen cyanide, lytic enzyme lipases, and prodigiosin), and combined effects of the bacteria's chitinases-producing culture media and sulfides-producing gases, as in the case of *Corynebacterium paurometabolum* and indirect mechanisms, such as alterations in root exudate patterns caused by *P. fluorescens* that hinder nematode host identification processes and systemic resistance in nematode hosts produced by *P. fluorescens*-mediated indirect activity against nematode (Table 18.3).

Pseudomonas fluorescens

The *Pseudomonas fluorescens* strains and the type strain *Pseudomonas putida* CFBP2066 prevented the burrowing nematode *Radopholus similis* and the root-knot nematode *Meloidogyne* spp. from penetrating the roots of banana, maize, and tomato plants. It was determined that *P. putida* CFBP2066 either induced systemic resistance in plants or other chemical bacterial metabolites impaired nematode infectivity because it was negative for the enzymatic activities and HCN productivity tests (Aalten et al. 2003). The fluorescent pseudomonads alter certain root exudates such polysaccharides and amino acids, which modify nematode behavior, to develop plant systemic resistance to nematode pests and prevent phytonematode early root penetration. The bacteria can also enclose or bind the surface of the root with carbohydrate–lectin, which prevents plant–parasitic nematodes from regularly recognizing the host (Racke and Sikora 1992; Oostendorp and Sikora 1990).

Bacillus firmus

The effectivity of *Bacillus firmus* on *R. similis* was observed by Mendoza et al. (2008). Cell-free filtrate and bacterial cultures may have quite different effects on PPNs. In laboratory tests, two *B. firmus* formulations (Bf-125 and Bf-106) with cell-free water extracts (bacterial culture filtrate) killed 99 and 96% of the nematode, respectively, indicating that *B. firmus* metabolite secunder substances act as a bioantagonist against nematodes. In field tests, *R. similis* was killed by various concentrations of *B. firmus* cell solution concentrations when applied to non-sterile soil, but only 41% of it. These varying mortality percentages could have a causal aspect related to competition with other microorganisms in the soil environment.

Bacillus thuringiensis var. kurstaki

The bacterium *B. thuringiensis* var. kurstaki (LBT 3 strain) has insecticidal properties as well as nematicidal properties, primarily against *R. similis*. Its observation in the field, in pots, and in the laboratory experiments decreased considerably the nematode's root penetration. An average reduction of 87% was observed in plantations with various populations, based on an evaluation of its effectiveness in more than 185 ha of banana-producing fields (Shanthi and Rajendran 2006; Fernández et al. 2005). The early inoculation of healthy in vitro plants (tissue culture plants) with *B. thuringiensis* during the hardening period produced the best effects. It is considered that the effects of Bt on nematodes are caused by the activities of the toxins produced by the sporulated Bt bacteria, lytic enzymes (lipases), and ISR (Migunova and Sasanelli 2021; Anita and Samiyappan 2012; Khabbaz et al. 2019; Rodríguez et al. 1991).

Serratia marcescens

Targeted nematodes of *Serratia marcescens* are *Meloidogyne incognita*, *Meloidogyne javanica,* and *R. similis*. The mode of action involved in nematode suppression is volatile bioactive metabolites, red pigment prodigiosin (Zabaketa-Mejia 1985; Rahul et al. 2014). When compared to the chemical copper sulfate, which has a bioactivity of 380 and 280 g/mL, respectively, the prodigiosin isolated from *S. marcescens* was found to be effective against juveniles of *R. similis* and *M. javanica* at low concentrations (LC_{50} values of 83 and 79 g/mL, respectively). Furthermore, it prevented nematode eggs from hatching. Prodigiosin was the only substance that was discovered to directly affect infective juveniles of the sugarbeet cyst worm *Heterodera schachtii* (Habash et al. 2020). Prodiginines are potential metabolites that could be employed to generate novel antifungal and antinematodal products. Other new compounds introduced as by-products of the prodiginine production process may strengthen bioactivities or aid in better microbial resistance management (Rahul et al. 2014). Concentrated supernatants of Serratia strains with serratamolide-like lipopeptides were able to kill pine wilt nematode *Bursaphelenchus xylophilus by* more than 77% after 72 h. Supernatants from various Serratia strains and eight particular amino lipids had high nematicidal action against *B. xylophilus*, and they may be beneficial and studied in the future against *R. similis* (Marques-Pereira et al. 2022).

Corynebacterium paurometabolum

Fernández et al. (2005) investigated the biocontrol agent *C. paurometabolum* (C-294 strain) against *R, similis* under controlled conditions indicated that an almost 85% reduction in the population. In field observation, *R. similis* decreased following treatments with the bacteria or fenamiphos. The yields of treated plants were significantly higher than those of control plants, with increases of 106% for the bacterium and 66% for fenamiphos (Fernández et al. 2005). Its mode of action is primarily caused by the interaction of the gases produced by the bacteria (sulfides) and the culture media where they grow (chitinases).

Pasteuria penetrans

P. penetrans is an obligatory parasitic bacterium. *P. penetrans* can infect several types of plant–parasitic nematodes and has great potential as a biological control agent for the *R. similis, Meloidogyne* spp. (*M. incognita, M. arenaria, M. javanica*), cyst-forming nematodes, *Pratylenchus scribneri*, *P. brachyurus, Helicotylenchus* sp., and *Xiphinema diversicaudatum* (Sayre and Starr 1985; Stirling 1984; Sayre 1980; Mankau 1975). The bacteria are widespread in various regions and are very persistent, and their spores are drought-resistant and resistant to agricultural products such as pesticides and fertilizers.

The mechanism of action of *P. penetrans* against *R. similis* was hyperparasitism (Tian et al. 2007; Abd-Elgawad and Askary 2018; Ciancio 2018). *Pasteuria* species are endospore-forming bacteria with septate mycelium, gram-positive, and dichotomously branched (Mankau and Imbriani 1975). Endospores are nonmotile and lie in the soil matrix. The endospore attaches to the cuticle of a compatible nematode host when it entered its territory. Per nematode, one to several 100 endospores can adhere. But just one endospore is required to infect the nematode host. A germ tube is formed during the infection phase, and it pierces the nematode's body wall. After the germination tube enters the nematode pseudocoelom, primary colonies are generated from it. These colonies resemble clusters of elongated grapes or cauliflower florets. The optimum growth temperature for the mesophyllic bacteria *P. penetrans* is between 28 °C and 35 °C (Serracin et al. 1997; Hatz and Dickson 1992). The bacteria carry out this suppression by preventing root penetration and sterilizing the nematode host. Furthermore, there was a significant correlation between the community composition of *Pasteuria* spp. and soil carbon, moisture, bulk density, and pH. These findings suggest that metabarcoding can be used to sensitively, specifically, and semi-quantitatively profile *Pasteuria* species from eDNA.

In black pepper, the bacteria could reduce the population of *R. similis* by about 68.89–93.33% and the incidence of yellow disease by as much as 26.67–50.0% (Mustika et al. 1997). *P. penetrans* increased the fresh weight of ginger rhizomes by up to 16.11–54.06% while suppressing the number of *M. incognita* and *R. similis* in ginger (Mustika 1998).

18.3.2.2 Fungi as Biological Control Agent

The biocontrol fungi alone (Stirling 1991; Khan 2007; Khan and Anwer 2011) or along with oil, neem cakes (Sikora and Roberts 2018; Khan et al. 2021) or pesticides (Mohiddin and Khan 2013) may provide sustainable nematode management in agricultural crops (Khan 2023; Khan et al. 2023). The microbial antagonists, *Aspergilus niger, Pochonia chlamydosporia, Purpureocellium lilacinum*, etc. (Jatala 1986; Stirling 1991; Khan 2016) may significantly contribute in the nematode management. In recent years, *Trichoderma* has also been found effective in suppressing plant nematodes (Mohiddin et al. 2010; Khan and Mohiddin 2018), and its formulations are available in market (Khan et al. 2011), which are quite effective against soil nematodes and other pathogens (Mohammed and Khan 2021; Sikora and Roberts 2018; Shahid and Khan 2019). In addition to above biocontrol agents, mycorrhizal fungi especially arbuscular mycorrhizal fungi such as, *Glomus intraradices*, *G. mosseae*, *Rhizophagus irregularis*) and nonpathogenic *Fusarium* species/isolates may prove quite effective in reducing *R. similis* populations in banana (Table 18.3).

The interaction between the mycorrhizal fungi AMF/VMF and migratory endo-parasitic nematodes in plants has been reported on *Pratylenchus coffeae* and *R. similis*. The mycorrhizal fungi that interacted with *R. similis* are from the genus *Glomus* spp., *Trichoderma* spp., and *Rhizophagus irregularis* (Table 18.3).

One of the primary mechanisms for suppressing plant–parasitic nematodes is the direct pathogenicity of fungal biocontrol agents (Stirling 1991; Kerrry 1987). Culture filtrate of different biocontrol agents, effective against plant–parasitic nematodes, was previously studied by many researchers (Vu 2005; Meyer et al. 2004; Hallmann and Sikora 1996; Reißinger 1995). This finding was related to fungi's secondary metabolites that contain substances that are harmful to plant–parasitic nematodes (Sikora et al. 2003; Hallmann and Sikora 1996).

The Mycorrhizal Fungus

Most plant species develop a symbiotic association with arbuscular mycorrhizal fungus (AMF) (Wang and Qiu 2006). The fungi promote host growth and resistance against several soil-borne disease pathogens, including PPNs. One of the advantages of the symbiosis between AMF/ VMF and plants has long been recognized as being the protection of plants from pests and disease pathogens (Krishna and Bagyaraj 1983; Graham and Menge 1982; Hussey and Roncadori 1982). Umesch et al. (1989) reported that *R. similis* populations were significantly lower in banana plants with mycorrhiza than those without it. The nematode population was significantly decreased in plants that had experienced mycorrhizal inoculation prior to infection with *R. similis*. *R. similis* had no effect on the 6-week colonization of tissue-cultured banana plants (cv. Grande Naine) by AMF from the genus Glomus sp. (Fogain and Njifenjou 2003). Root mycorrhization of tissue-cultured plantain plantlets during the weaning phase leads to plant growth enhancement. Several mechanisms for the decrease in harmful nematodes in the AMF-colonized plants have been proposed by Elsen et al. (2001, 2003a). In an interaction known as mycorrhizal association, plants release organic substances to fungi in exchange for the fungi absorbing soil nutrients for plant use (Koide and Mosse 2004). Vesicular arbuscular mycorrhiza (VAM) have vesicles which serve as storage organs of the fungus and arbuscules which act as exchange points between the host plant and the fungi. This partnership results in improved host growth and plant vigor and was attributed to the enhanced capability of the roots to absorb soil minerals, particularly phosphorus, zinc, sulfur, and copper (Koide and Mosse 2004; Borowicz 2001; Azcon-Aguilar and Barea 1996; Mosse 1981). Additionally, according to Graham and Menge (1982), AMF impacts nematodes through modifying root exudates or by synthesizing phytoalexins in the root, such as glyceollin, which may have nematicidal effects (Morandi 1996).

Glomus spp.

Elsen et al. (2008) determined that AMF can induce systemic resistance and reduce *R. similis* and *P. coffeae* by more than 50% in a root system even when the AMF and the plant–parasitic nematodes are spatially separated in a split-root compartmental setup. The presence of the nematode in the roots had no effect on *Glomus intraradices* colonization on plant roots under conditions of dixenic culture (Elsen et al. (2001). The findings showed that both the nematode's and AMF *G. intraradices* life cycles were completed in the presence of one another and a modified carrot root was allowed to serve as the host.

In a more related study involving mycorrhiza and migratory endoparasitic nematodes, *G. mosseae* significantly suppressed the population of a Ugandan population of *R. similis* in Grand Naine and Pisang Raja Buaya cultivars (Elsen et al. 2003a). The nematode count per root system reached 10,425 per gram for non-mycorrhized bananas but only 1080 for mycorrhized bananas. *R. similis* per gram of root for VAM mycorrhized bananas. In mycorrhized Obina l'Ewai cultivars inoculated with both Ugandan and Indonesian nematode isolates, without VAM, 1336 and 218 nematodes were recorded, but with mycorrhization, only 314 and 74 nematodes were counted for the Ugandan and Indonesian populations, respectively (Elsen et al. 2003b). Other Glomus species reported to effectively reduce *R. similis* is *Glomus fasciculatum* (Shanthi and Rajendran 2006; Umesch et al. 1989). However, the most consistent results have been shown for the species *G. intraradices, G. manihotis*, and *G. mosseae* in *R. similis* population suppression.

The tolerance of mycorrhizal plants to soil pathogens and nematode could be explained by a number of mechanisms, including competition for host photosynthates and colonization sites, root system morphological changes, mycorrhizosphere microbiological changes, and the activation of plant defense systems. Increased P nutrition, though controversial, has also been postulated as one of the mechanisms of plants' nematode tolerance (Azcon-Aguilar and Barea

1996). Hussey and Roncadori (1982) reported that increased tolerance is the single most common effect of AMF on nematode-susceptible plants. The effect of the low intensity of VAM mycorrhizal colonization VAM on nematode population and reproduction factor was still exhibited because the mode of action of mycorrhiza, particularly against migratory endoparasitic nematodes, was reported to be systemic (Elsen et al. 2008). Additionally, the low intensity of mycorrhizal colonization may be attributed to the early stage of mycorrhizal colonization (Elsen et al. 2003a, b).

Trichoderma spp.

Some Trichoderma isolates have been demonstrated to reduce root-knot and burrowing nematode damage while simultaneously enhancing plant growth (Meyer et al. 2001; Sharon et al. 2001; Windham et al. 1989). To eradicate the disease-causing pathogen, *Trichoderma* spp. generates and releases lytic enzymes, primarily chitinases, glucanases, proteases, as well as toxins (such as the antibiotics gliotoxin, gliovirin, and peptaibol). These enzymes make it easier for Trichoderma species to enter the host and use the nutrients inside (Lorito et al. 1996).

Trichoderma asperellum ENDO 4 has the potential to be developed into a commercial product to control the burrowing nematode in Costa Rican banana crops (Anonymous 2020). *T. asperellum* strain ENDO 4 has been tested in several trials on banana in greenhouse conditions and in the field as a biocontrol agent of the burrowing nematode. Results showed that Grande Naine and Williams cultivars' annual production yields rose and that ENDO 4 considerably decreased the population of *R. similis* in greenhouse settings. They indicated that the bunch weight in cv Grande Naine was 2 kg higher in plants protected with ENDO 4 and 1.14 kg in the case of cv Williams, in comparison with the control. *R. similis's* nematode populations were also greatly reduced by the application of species *T. viride*; however, this reduction was only moderate (Shanthi and Rajendran 2006).

Rhizophagus irregularis MUCL 41833 AMF

In an early in vitro experiment, *Rhizophagus irregularis* MUCL 41833 was able to reduce the reproduction capacity of *R. similis* in excised-root carrots culture (Elsen et al. 2001). In vitro culture system developed by Koffi et al. (2009), which combined autotrophic micropropagated banana plantlets with *R. irregularis* MUCL 41833, they found nematodes and AMF were both able to complete their life cycles in an in vitro banana plantlet (cultivar cv. Grande Naine, particularly sensitive to *R. similis*). Surprisingly, the nematodes' presence considerably enhanced the proportion of arbuscules. Furthermore, they observed reductions of 60% and 56%, respectively, in the nematode population and surface root necrosis in the plantlets exposed to the AMF (Koffi et al. 2012).

R. similis reproductive ratio is reduced by *R. irregularis* MUCL 41833 in the partially resistant banana cultivar Yangambi km5 (Anene et al. 2013). The nematode population in the roots and medium decreased by 52.6% when the AMF was present; however, the nematode had no impact on the proportion of total hyphae and spores/vesicles in the roots. The fact that the percentage of arbuscules discovered in the banana plantlets exposed to the nematode larva significantly increased suggests that the plant may have altered its defense response to enhance its resistance to the nematodes. Early colonization of the banana cv. Yangambi km5 by AMF of *R. irregularis* MUCL 41833 may enable it to outcompete *R. similis* (prior to nematode infestation).

Nonpathogenic *Fusarium oxysporum* (*F. oxysporum* NP)

Nonpathogenic *Fusarium* is one of the endophytic fungi known as biocontrol agent for *R. similis*. The addition of the fungi did not cause disease symptoms to appear on the plants (Dababat and Sikora 2007). Endophytic microbes live out the most of their lives in the benign confines of host plant tissues (Wilson 1995). Many endophytes interact mutualistically with plants that serve as their hosts, from which they receive nutrients and in exchange provide the plants with defense against biotic and abiotic stressors (Schulz and Boyle 2005).

Fusarium NP has been shown to be effective in suppressing *R. similis* on bananas. (Lisnawita et al. 2013; Viljoen et al. 2007; Niere et al. 1999). The *F. oxysporum* NP fungus-inoculated banana plants all had fewer nematodes than non-inoculated plants. The initial nematode inoculum in the root segments of those plants was significantly decreased, by 51–99% (Niere et al. 1999). When several *F. oxysporum* NP isolates were injected into tissue culture plants, plant growth was enhanced while nematode populations and damage were decreased (Viljoen et al. 2007; Athman 2006; Dubois et al. 2004). Viljoen et al. (2007) obtained the same result on the greatest suppression of *R. similis* reproduction by endophytes F. oxysporum NP Strain V5w2. The findings of this study indicate that the antagonistic effects of endophytic *F. oxysporum* NP against *R. similis* in banana plants are controlled by post-infectional and mediated nematode reproduction disruption (Athman 2006; Dubois et al. 2004; Gold and Dubois 2005; Sikora et al. 2003). When given the option of endophyte-treated or untreated plants or roots, *R. similis* did not exhibit any preference, with a similar proportion migrating in the direction of either plant type (Athman et al. 2006).

In order to lessen nematode root invasion, endophyte-treated plants may release chemicals that interfere with the nematode's receptor activity during host search (Perry 1996). According to Cañizares Monteros (2003) and Meneses Hérnandez (2003), chemicals of toxic metabolite products from their secondary metabolism displayed nematicidal and nematistatic effects on *R. similis*, paralyzing its motile phases and inhibiting its ability to hatch eggs. Mortality in all *R. similis* bioassays was caused by fungal filtrates from all strains and ranged from 84 to 100%, while it was only 14–36%, in pH-adjusted control media.

These mortality rates are a strong indicator that the tested strain produces nematicidal metabolites because fungal filtrates do not include spores or mycelium (Dubois et al. 2004). Previous studies carried out by Amin (1994) and Hallmann and Sikora (1996) showed that metabolites of endophytic *F. oxysporum* strains decreased the mobility of migratory endoparasitic nematodes in vitro. The metabolites of *F. oxysporum* isolate B20 from a gliotoxin fermentation medium at a concentration of 50% strongly affected the mobility of *R. similis,* and the metabolites of *F. oxysporum* strain 162 from a gliotoxin fermentation medium at a concentration of 90% inactivated approximately 65% of J2 and adults of *Pratylenchus zeae* and *R. similis*. Second-stage juveniles of *R. similis* were more sensitive to fungal metabolites than adults (Amin 1994).

Another inhibition mechanism of plant pathogens by *Fusarium* NP is generally in the form of competition for space and nutrients (Mandeel 2007) and induced systemic resistance (Vu et al. 2006). However, some researchers concluded that induced systemic resistance was demonstrably the principal aspect of the overall mode of action of *F. oxysporum* NP on banana plants, affecting nematode behavior, and that this led to a decrease in the *R. similis* population invading the plants (Vu et al. 2006). The inoculation of some *Fusarium* NP isolates on one side of the split-root system considerably reduced *R. similis* invasion on the other side of the system (Vu et al. 2006). The penetration of *R. similis* in the Fusarium untreated responder root portion was significantly reduced by 30–38.5% 5 days after nematode inoculation. Fifteen (13) days later, the effect of induced resistance was still noticeable in comparison to the roots in the responder root section of the non-inoculated controls. Timing is crucial since induced systemic resistance often only offers temporary protection (Fuchs et al. 1997). On tolerant and susceptible cultivars, Paparu et al. (2007) described the induction of systemic resistance by *Fusarium* NP. The tolerant cultivar's roots were colonized by endophytes, which temporarily increased POX (peroxidase) expression while suppressing that of *pathogenesis-related protein* PR-3, lectin, PAE (pectin acetylesterase), PAL (phenylalanine ammonia-lyase), and *plant inducible regulatory* protein PIR7A. The catalase and PR-1 activities of the tolerant cultivar increased 33 days following endophyte colonization of the roots; however, their expressions were also increased after nematode challenge. Except for POX and lectin, none of the other genes investigated in the susceptible cv. Nabusa responded to endophyte colonization or *R. similis* challenge (Paparu et al. 2007).

Paecilomyces lilacinus

The fungus parasitizing the eggs, juveniles, and adult females of several sedentary (*Meloidogyne* spp., *Heterodera* spp., *Globodera* spp.) and migratory endoparasitic nematodes (*R. similis* and *Pratylenchus* spp.) (Kilama et al. 2007; Viaene et al. 2006; EPA 2005; Domsch et al. 1993). Moreno-Gavíra et al. (2020) reviewed the bio-control mechanisms of the Genus Paecilomyces. Following interaction between the Paecilomyces and the nematode, enzyme complexes are secreted, enabling the Paecilomyces to expand quickly at the expense of its host. Enzymes such cellulase, glucanase, laccase, leucinoxin, lipase, pectinase, protease, chitinase, or xylanase are generated throughout the infection process and may result in mechanical or enzymatic penetration. *P. lilacinus* spores were observed parasitizing the tail region of *R. similis* (Generalao and Davide 1992). *P. lilacinus* strain 251 was isolated from a *Meloidogyne* egg mass in Los Banos, Philippines (EFSA 2007).

In an in vitro investigation, motile stages of the *P. lilacinus* isolate 23N5-2-submerged *R. similis* consistently exhibited paralysis (Kilama et al. 2007). In newly established plantations with low initial nematode populations, the *P. lilacinus* treatments resulted in considerable decreases in *R. similis* and *M. incognita of* about 75% and 85%, respectively, over a period of more than a year, as well as a yield increase of about 25%. Consequently, this fungus was used commercially on more than 5500 acres of land (Fernández et al. 2005). This fungus demonstrated effective nematode control in a variety of crops. However, female *R. similis* often have a lower mortality rate following *P. lilacinus*-induced paralysis than juveniles and males have. This might be the result of the structural variations between juveniles or females, such as stronger physical characteristics in females (Van Weerdt 1960). For instance, females may be more resistant to fungal filtrates than males because females are shorter and have a sclerotized cephalic framework. *P. lilacinus* was both more and similarly as effective as the nematicide Nemacur 10G at 200 ppm in nematode number reduction in the soil and roots of Giant Cavendish bananas, according to observations for mortality and infectivity. When used directly on the nematode, the fungi propagated in ipil-ipil leaves, rice hulls, banana leaves, and leaf sheaths were more effective than when used as spores and mycelial suspension in a soil drench (Generalao and Davide 1992). *R. similis* on banana dose–response relationship and method of application studies with a commercial *P. lilacinus* (strain 251) product demonstrated that nematode infestation diminished in the presence of *P. lilacinus*. The best control was generated by the method in which plantlets and soil were preinoculated with *P. lilacinus* and reinoculated after transplantation (Mendoza et al. 2004).

Toxins present in the fungus' filtrate would be the source of *P. lilacinus* antagonistic relationship with *R. similis*. Paecilotoxins, hydrolytic enzymes such polysaccharides, proteases, chitinases, and leucinostatins (peptide mycotoxins) are a few of the toxin products that *P. lilacinus* has been documented to produce against 17 important nematode species (Gupta et al. 1993; Park et al. 2004; Mikami et al. 1984). The bioassays showed that *P. lilacinus* 23N5 filtrates rendered *R. similis* paralyzed (Kilama et al. 2007). There was a potential neurotropic mechanism of action of the *P. lilacinus* bioactivity that was proposed since the nematodes were only temporally paralyzed by the toxic compounds in the filtrates used in the current study by Cayrol et al. (1989).

Another Paecilomyces species that is antagonistic to *R. similis* is *P. marquandii.* Esnard et al. (1998) also demonstrated that wheat substrate culture of *P. marquandii* application suppressed *R. similis* population in a Costa Rica banana field. It has been determined that the paecilotoxins produced by *P. lilacinus* and *P. marquandii* are identical (Khan et al. 2003).

Attention to *P. lilacinus* was being paid for it toxicology test. In Cuba, *P. lilacinus* is being used after the completion of toxicology tests (Fernández et al. 2005). According to a toxicity study on a rabbit infection model, leucinostatins (peptide mycotoxins) of *P. lilacinus* strains obtained from soil and a case of human oculomycosis may be involved in the inflammatory response of invading tissues (Mikami et al. 1984). However, from some investigations by some researcher indicated that not all strains of *P. lilacinus* produce mycotoxin that can affect human. *P. lilacinus* strain 251 does not generate mycotoxins that can affect other organisms, according to chromatographic studies and a lack of toxic effects to mammals and other test organisms (EPA 2005). *P. lilacinus* strain 251 grows optimally in a laboratory setting between 21 and 27 °C; the fungus cannot survive or reproduce at human body temperature. As a result, this strain is thought to be safe to be developed into a commercial biopesticide product. The product is a 6.0% active component by weight water dispersible granule.

18.3.2.3 Insect as Biocontrol Agent

Blattisocius dolichus **(Acari: Blattisociidae)**
Blattisocius dolichus is beneficial insect mite from Acaricide for controlling the nematode as observed by Chen et al. (2013). Both in the field test and on potted test, ten (10) days after the introduction of 500 mites per plant, the nematode density was reduced by 66%. All active development stages of the mite prey on nematodes. During its pre-adult development phases (larval and nymphal instars), each *B. dolichus* killed or consumed about 76 nematodes. Within a day (24 h), female and male adults could consume 28 and 22 nematodes, respectively. However, adult female mites had stronger predatory ability than males (Chen et al. 2013). The optimal temperature for the growth, reproduction, and invasion of *R. similis* was between 24 and 27 °C. Both male and female adults of *B. dolichus* reached their peak predation at this temperature (Duncan 2005). On *R. similis*, *B. dolichus* was capable of complete its life cycle (Chen et al. 2013). Three steps are involved in predation: probing, holding and eating, and cleaning of the mouth. A nematode may be consumed in around 60 s. With ongoing growth, the mite's size rose and its capability for predation strengthened. Adult males and females had the highest consumption rates at 25 °C after 96 and 72 h of starvation, respectively.

The predatory mite *B. dolichus* has the potential to operate as a biological control agent of R. similis based on some similarities between the bioecologies of *B. dolichus* and *R. similis* (Chen et al. 2013). *B. dolichus* vigorously searched the area surrounding the testing site for nematodes. Live *R. similis* was favored by *B. dolichus* to live *C. elegans*, dead *R. similis*, and *T. putrescentiae* eggs. The rhizosphere of plants is inhabited to *B. dolichus* and *R. similis*. As a result, it's critical that *B. dolichus* keep the nematodes under control in the plant's rhizosphere. However, due to its sensitivity to nematicide, this mite cannot be used in combination with nematicide to control nematodes (Chen et al. 2013).

18.4 Formulation and Commercial Product of the Biopesticide for the Nematode

18.4.1 Formulation

Nematicides are frequently necessary to develop the banana crop economically (Gowen et al. 2005). Commercial products of bionematicides for *R. similis* are important for the farmer for economically and sustainably commercial banana production and other commercial crops, especially if they are required to produce environmental-friendly agricultural products. Soil rhizobia (Khan et al. 2016a) and phosphate solubilizing bacteria (Khan et al. 2016b) have been found quite effective in suppressing a range of nematodes and improving plant yield (Sikora and Roberts 2018). Some biological control agents that have been found to be effective in reducing the *R. similis* population were bacterial *P. fluorescens*, *B. firmus*, *Bacillus* sp., *B. thuringiensis* var. kurstaki, *C. paurometabolum, S. marcescens,* and *P. penetrans,* and fungal VAM *Trichoderma* spp., AMF *Glomus* spp., *P. lilacinus* (Thom.), *F. oxysporum* NP., *R. irregularis* MUCL 41833 AMF, and predatory mites *B. dolichus* (Acari: Blattisociidae). Some formulations of those beneficial microorganisms were prepared on wettable powder (WP), water dispersible granular (WG), talc-based products, vermiculite flakes, sorghum grains, and wheat substrate. Live microorganisms (such as beneficial bacteria, fungi, nematodes, and viruses) and/or their fermentation products are employed as the active ingredient in biopesticides against plant pathogens and pests in microbial products like cell-free filtrate, bacterial culture, spores, lytic enzymes, antibiotics, inhibitors, and toxins (Cao et al. 2019; Bhattacharyya et al. 2016). Many biopesticides on the market include nematode control mechanisms that rely on bioactive substances generated by the bacteria during fermentation rather than direct parasitism.

P. lilacinus was recently in vitro cultivated on sorghum grains, and the WP formulation of *B. firmus* contains around 5% lyophilized bacterium spores and 95% additives. Formerly, talc-based products were used for the commercial manufacture of *P. fluorescens* (Pf 1), *T. viride*, and *B. subtilis*, and vermiculite flakes were used for the commercial manufacture of *G. fasciculatum* (Keren-Zur et al. 2000). *P. lilacinus* (strain 251) was also prepared on a commercial water dispersible granule-formulated product (Mendoza et al. 2004).

P. penetrans was prepared as dry root powder of tomato root infected by *P. penetrans*-infected *Meloidogyne* spp. (Harni and Mustika 2003). *P. penetrans* cannot be multiplied in vitro (in artificial media) (Sayre and Starr 1985). *P. penetrans* can only be multiplied conventionally by inoculating tomato plants in the greenhouse or the field with nematodes that are infected by *P. penetrans*. At the age of 1 month, tomato plants are inoculated with these bacterial spores. As an inoculum, root powder which already contains spores is used and packaged in capsule or pellet form. After the fruit is harvested, the tomato roots are cut into pieces, dried in the sun for about 3 days, or dried in an oven at 50 °C for several hours. After drying, the roots are made into powder with a blender or grinder. Root powder is then formulated in the form of capsules, pellets, or compost. From 0.25 ha of tomato plants, 16,000 capsules containing root powder containing 25×10^6 P. *penetrans* spores/capsule can be produced (Mustika 1998; Sharma and Stirling 1991).

The weakness of biopesticide formulations prepared from organisms and/or biomaterials are usually susceptible to desiccation, ultraviolet light inactivation, or even heat. Microbial-based and other biopesticide formulations need stabilization and directed delivery mechanism toward identified targets. Nanoformulations using biomaterials may provide new ways to enhance the stability and to develop smart delivery system of these biological agents (Chandra et al. 2013). Chitosan nanoparticle-coated fungal metabolite was found to be more effective than uncoated fungal metabolite and fungal spores of entomopathogenic fungi *Nomureae rileyi* against pest *Spodoptera litura* (Chandra et al. 2013). Chitosan nanoparticle-coated fungal/bacterial metabolite technology innovation could be developed for engineering the biopesticide formulation in the burrowing nematode control.

18.4.2 Commercial Product of the Biopesticide for the Nematode

It was determined from the review by Wolfgang et al. (2019) that a single strain represents the majority of the current commercial products. These products primarily target cyst and root-knot nematodes. Bionematicide *P. lilacinus* strain 251 against the banana burrowing nematode *R. similis* was described by Mendoza et al. in 2007. The product has been produced and registered for sale in various countries under the trade name Bioact® WG for nematode control. Under the trade name MeloCon® WG, this product has also received EPA registration approval for use as a biological nematicide in the USA (Kiewnick and Sikora 2006). This demonstrated that the MeloCon® WG product was suitable for the biological control of *R. similis* in organic farming systems and was both safe and efficient.

For the suppression of the root-knot nematodes, further formulations with two or more bacterial components, such as BioYield, Biostart, Micronema, Equity, and Ag-Blends, have been proposed (Migunova and Sasanelli 2021). A formulation of two *B. firmus* strains (Bf-125 and Bf-106) mixed with powdered organic substrate from fermentation tests against *R. similis* has been commercialized and registered as BioNem. Two (2) g of BioNem formula containing living cells of *B. firmus* suspended in 1 L of water was effective for the *R. similis* population reduction (Keren-Zur et al. 2000).

Existing commercial formulations, both as single strains and as a consortium of complex strains/beneficial microorganisms, including beneficial fungi that have been known antagonists to *R. similis*, were mostly targeted at root-knot nematode and cyst nematode (Migunova and Sasanelli 2021). There is little information available about the effectiveness of marketed bacterial formulations for the burrowing nematode. The bio-formulations based on *Pasteuria penetrans* are of particular significance. From a rough calculation, commercial bionematicide products on the market that were reviewed by some researchers were mostly targeted at root-knot nematodes *Meloidogyne* spp. (about 54.54%), followed by at *Heterodera* spp., *Rotylenchulus reniformis* and *R. similis* (about 9.0%), *Belonolaimus longicaudatus* and plant–parasitic nematodes (about 6.0%), and at *Tylenchulus semipenetrans* and *Helicotylenchus* spp. (about 3.0%). One of the most promising strategies for RKN control involves using a group of bacterial, fungal, and other nematode antagonists (Wolfgang et al. 2019). Products based on group of endophytic bacterial *Bacillus*, *Pseudomonas*, and *Serratia* are more promising because they not only suppress PPNs but also encourage plant growth and control plant pathogenic microorganisms. More investigation of these products against *R. similis* should be carried out.

However, some products have been tested and verified to effectively reduce the burrowing nematode population (Table 18.4). More investigation will be required to evaluate the efficacy of commercial formulations, both simple and complex, used to

Table 18.4 Commercially formulated beneficial microorganisms against the burrowing nematode *Radopholus similis*

Biocontrol agents	Commercial formulation/ product name	Bioactivity to *Radopholus similis*	References
Bacillus firmus (Bf-125 and Bf-106)	BioNem-WP	Effective	Keren-Zur et al. (2000)
Paecilomyces lilacinus (PL251)	Bioact® WG and MeloCon® WG	Effective	Mendoza et al. (2007), Kiewnick and Sikora (2006)
Pasteuria penetrans	Econem—Syngenta, Econem—Pasteuria Bioscience—USA, Econem—Nematech, Japan	It was targeted at *P. penetrans* product, but the effectiveness of the products against *R. similis* has not been investigated yet	Migunova and Sasanelli (2021)

Note: *WP* wettable powder, *WG* water dispersible granulate

combat *R. similis*, including their ability to inhibit associated pathogenic bacteria and promote plant growth.

18.5 The Strategic Application of the Biocontrol Agent for Their Effective Bioprotection Activity

The management of the burrowing nematode by biocontrol agents has shown promising results in several laboratory, greenhouse, and field trials, suggesting potential applications. There are some biocontrol products available for nematode management in commercial banana plantations, such as Blue Circle™ (contains the bacteria *Burkholderia cepacia*), Paecil™ (contains the fungus *P. lilacinus*), or DiTera™ (contains the toxic fermentation products of the fungus *Myrothecium verrucaria*), but banana producers rarely use them due to a lack of effective control measures (APS Biological Control Committee 2005). Application of biocontrol agent in the field affected by some ecological (biotic and abiotic) factors. Interactions with nontarget organisms, damage from nontarget pathogens and pests, the degree of rhizosphere and/or soil colonization by a biocontrol agent, the population size of the target pests, and the susceptibility of the host plant species and cultivar are examples of biotic factors that have been recognized, while the rhizosphere's physical and chemical composition, as well as the environment, are examples of abiotic factors (Meyer and Roberts 2002; Sikora and Hoffmann-Hergaten 1993). The duration of this activity may be limited by competition with other microbes in the soil ecosystem, which depends on the amount of organic material present in the soil (Mendoza et al. 2008). According to Mendoza et al. (2008), a low control level was attained by applying *B. firmus* BCA cells to the sand at high population densities, nutrient synthesis in the substrate, and the potential release of toxins after cell death. These facts might point to bacterial multiplication and survival on organic material, which would improve levels of control after application. Agbenin (2011) suggested that biocontrol through the use of natural enemies be explored as an integral part of environmentally acceptable PPN management, in addition to cultural practices such as crop rotation and organic amendment, to include the use of beneficial microorganisms.

These facts allow for the construction of new integrated pest management techniques that rely on the employment of biocontrol agents with additional mechanisms of action to provide longer-lasting defense. Numerous researchers have attempted to improve the bioactivity stability and duration, as well as the efficacy and reliability of the biocontrol method, by implementing effective application techniques of each type of beneficial microorganism and/or their integration system with other control method components in an IPM (integrated pest management) system.

18.5.1 Organic Matter Incorporation Along with Biocontrol Agent Application

Based on some research, *B. firmus* BCA can survive and reproduce on organic debris, which could increase levels of control after treatment (Mendoza et al. 2008). The use of organic amendments is one of several methods that have been developed to protect bananas against *R. similis* (zum Felde et al. 2006). The direct application of *P. lilacinus* BCA, which was cultured in organic materials such as ipil-ipil leaves, rice hulls, and banana leaves and leaf sheaths, was more efficacious against the nematode than spores and mycelial suspension (Generalao and Davide 1992). A different approach to managing the plant–parasitic nematode-induced yellowing disease on black pepper (caused by a combination of *R. similis, Meloidogyne* spp., and *Fusarium* spp.) is the incorporation of endophytic bacteria along with commercial organic fertilizer (Munif and Harni 2020). The application of *P. penetrans* BCA with cow dung manure may decrease the black pepper yellow disease incidence caused by *R. similis* by 26.67–50.0% (Mustika et al. 1997). In many cases, the addition of organic material can enhance the activity of beneficial microorganisms to inhibit plant–parasitic nematodes and soil-borne plant pathogens, promote plant growth, and improve biomass accumulation (Katan 2017; Xia et al. 2015; Widmer and Abawi 2000).

18.5.2 Healthy In Vitro Plants Early or Preventive Inoculation During the Hardening Phase

Massive production of in vitro tissue culture plants produces healthy seedlings that can be utilized to grow bananas. Field investigations in plantations with low initial infestations showed that nematode populations expanded more slowly than in control plots and that plants with early AMF inoculation grew better than plants without inoculation. Damage from *R. similis* and *M. incognita* was reduced by early inoculation of in vitro plants with several species of the Glomus genus during the hardening stage. Studies in controlled conditions showed that populations of both species had reduced to various degrees, with *G. intraradices, G. manihotis*, and *G. mosseae* being the most promising species (Fernández et al. 2005). Plants that had mycorrhizal colonization before becoming infected with *R. similis* had significantly fewer nematode populations (Fogain and Njifenjou 2003). When *P. fluorescens* and *G. mosseae* are applied early in the crop, whether as a soil application, a sucker treatment, or a bio-priming of plantlets, they colonize and establish well on banana roots (Rodriguez-Romero et al. 2008; Jaizme-Vega et al. 1997). Similar results were achieved when *P. lilacinus* BCA, a potent biocontrol fungus of *R. similis* and *M. incognita*, was used to preventively inoculate healthy in vitro plants throughout the hardening phase (Mendoza et al. 2004).

Better tissue culture planting material can be produced in large quantities by dipping tissue culture plants at the nursing stage in a spore suspension, *F. oxysporum* NP. There is no need for additional applications, and only a little amount of inoculum is required (Niere et al. 1999). The inoculation of the fungal isolates barely changes the weaning process, and producing fungal spores from *F. oxysporum* NP is rather simple and affordable. The advantage of seed treatment, such as root immersion (tissue culture plants), seed soaking, or the introduction of bacteria into the soil before planting, is that it is a protective measure at the start of growth (Hallmann et al. 1997).

18.5.3 Sequential Inoculation and Re-Introduction of Beneficial Microorganisms

When compared to using just one biocontrol agent, Zum Felde et al. (2006) found that using compatible biocontrol agents in combination may improve protection against *R. similis*. To avoid any potential detrimental interactions among fungal conidia prior to inoculation, Zum Felde et al. (2006) used consecutive inoculations and set a 5 min inoculation time for each fungus.

Reintroducing beneficial microbes to sterile tissue grown plants may significantly increase the success of beneficial microbe infestation in plant roots and, as a result, may help to support sustainable banana production (Niere et al. 1999). The technique in which plantlets and soil were preinoculated with *P. lilacinus* and reinoculated during transplantation resulted in the best control of *R. similis* by *P. lilacinus* (Mendoza et al. 2004). *P. lilacinus* (6×10^6 cfu/g dry soil) was added to the soil at inoculation, 6 days prior to planting, at planting, and as a plantlet drench in order to achieve the greatest level of *R. similis* suppression (Mendoza et al. 2007).

18.5.4 Optimal Concentration/Dose of the Biological Agent Formula

Based on the study by zum Felde et al. (2006), in comparison to the duration of the dip inoculation, the concentration of the conidia suspension was more crucial for the efficient root colonization by the beneficial fungi, *F. oxysporum* NP. An effective inoculation technique involves immersing tissue culture plants' root systems for 5 min in a conidia suspension containing at least 1×10^5 cfu/ml (Pocasangre et al. 2004; Carñizares Monteros 2003; Meneses Hérnandez 2003; zum Felde 2002). When compared to treating soil, treating seedlings with these endophytes is a very effective and economically viable method of biocontrol (Pocasangre 2006). Root colonization of tissue culture banana plants required a 5-min dip in a suspension concentration of 1×10^6 cfu/ml (Pocasangre 2000). For significantly suppressing *R. similis*, the optimum dose of *P. lilacinus* was determined to be 6×10^6 cfu/g dry soil (Mendoza et al. 2007). This dose was sprayed three times: at 6 days prior to planting, during planting, and as a plant drench.

18.5.5 Consortia Application of Compatible Beneficial Microorganisms with Different Mechanisms of Action that Target Various Phases of the Infection Process

As shown by Guetsky et al. (2002), the employment of multiple biocontrol agents increases the efficacy and consistency of biocontrol. Additionally, Guetsky et al. (2001) suggested that combinations of agents with different ecological needs will probably improve dependability and minimize variability in biocontrol as long as biocontrol agents have a variety of ecological requirements. Mendoza and Sikora (2009) reported that the combination application of *B. firmus* and *F. oxysporum* strain 162 was the most Both the presence of FO162 in the roots, which prevents nematode penetration and exposes *R. similis* to metabolites produced by *B. firmus* in the soil, and the presence of these bioactive metabolites, which have the potential to kill nematodes in soil, may help to improve the level of control of *R. similis* in the presence of both agents (Giannakou et al. 2004, 2007). When *F. oxysporum* (FO162) and *P. lilacinus* 251 (PL251) were introduced together, greater *R. similis* biocontrol was achieved (68.5% decreased nematode density) than when they were applied alone (27.8% and 54.8% decreased nematode density over the controls, respectively) (Mendoza and Sikora 2009). According to Dube and Smart (1987), the combination of antagonists using several modes of action was what facilitated the higher levels of control. However, the application of the two biocontrol agents together did not result in either an additive or a synergistic improvement in nematode control (Mendoza and Sikora 2009). The use of *P. lilacinus* and *Monacrosporium lysipagum* biocontrol agents together significantly reduced *M. incognita* infection in banana plantlets, but not in an additive or synergistic manner that would have improved control (Khan et al. 2006).

However, the synergistic combination of beneficial microorganisms against *R. similis* should be done through try and error evaluation before attempting to obtain a synergistic antagonist effect from the consortia of beneficial microorganisms formulation. Some researches show that combinations are not always advantageous since antagonistic interactions between biocontrol organisms might result in control levels that are lower or unchanged from individual biocontrol agent applications (Chen et al. 2000; Esnard et al. 1998; Zaki and Maqbool 1991). According to a number of studies, beneficial microorganism compatibility is crucial for enhancing biological control when using multiple agents simultaneously (Meyer and Roberts 2002; Baker 1990). Meyer and Roberts (2002) suggest that the adverse impacts of biocontrol agent combinations are caused by the fact that the control mechanisms employed by these combinations target both the companion biocontrol agent and the plant pathogen.

18.5.6 As Component of Integrated Pest Management

Nematode management techniques that are dependable, efficient, economically feasible, and able to be included in overall banana production systems are necessary for commercial banana production. It has been demonstrated that biological control utilizing nematode natural enemies is a successful alternative that may be used in integration with other supplementary measures in order to optimize the effectiveness of biocontrol agents application against *R. similis* in the field (Anene and Declerck 2016; zum Felde et al. 2006; Harish and Nanje 2001; Loos' 1961).

Harish and Nanje (2001) investigated the integrated pest management (IPM) of *R. similis*, which infests bananas, by integrating ecofriendly components including botanical pesticide oil cakes (neem and pongamia), biocontrol agents, and a nematicide (carbofuran). Under field conditions, the treatments were assessed singly and in combination against *R. similis*. Neem cake with carbofuran plus *T. viride* outperformed all other treatments in terms of nematode population reduction, plant development, and fruit yield with a favorable cost-benefit ratio.

The use of biocontrol organisms like endophytes is important in circumstances where nematicides are prohibited, such as in organic farming and areas where low nematode populations have been regularly recorded over time (zum Felde et al. 2006). According to Loos' (1961) study, *R. similis* was completely eradicated after a 5-month rotation to sugarcane that came right after the removal of contaminated bananas. After a sugarcane rotation, Lakatan banana plants grown from nematode-free rhizomes were free of *R. similis* 9 months later. After the whole banana crop was removed, the nematode-infested ground was either left to grow weeds and grasses or was planted with sugarcane, which completely eradicated *R. similis* in 10 weeks. It may enhance the effectiveness of the biopesticide formula in decreasing *R. similis* penetration in roots and population in soil when applied in combination with a 5-month rotation and contaminated banana removal.

Anene and Declerck (2016) observed a combined effect of AMF *Rhizophagus irregularis* MUCL 41833 and cover crop push-pull plants (*Crotalaria spectabilis*) in suppressing the *R. similis* population in the management of the burrowing nematode *R. similis* in banana. Banana plants pre- or post-colonized with *R. irregularis* MUCL

41833 were cultivated in 3 L-pots with or without *Crotalaria spectabilis*. A fixed talpa net was used to isolate the two plants' shoots and leaves from their above-ground components. Similar to this, the pots were split into two sections below ground by wrapping the banana roots in a nylon mesh pocket to prevent the roots of both plants from competing with one another (30 m). The *C. spectabilis* plant was grown 3 weeks after the banana plants. Nematode inoculation was carried out concurrently with *C. spectabilis* planting. Based on the study, AMF and C. spectabilis are effective in reducing the pressure of *R. similis* in banana roots, and their application integration even more effectively decreases the surface area of necrotic cortical tissues caused by the nematodes.

18.6 Conclusion and Future Perspectives

A number of biocontrol agents included PGPR, viz., *P. fluorescens*, *Bacillus* sp., *B. firmus*, *B. thuringiensis* var. *kurstaki*, hyperparasite *P. penetrans*, *C. paurometabolum*, and *S. marcescens* from the bacterial group; mycorrhizal fungi VAM *Trichoderma* spp. and *T. asperellum*; AMF *Glomus* spp. (*Glomus* sp., *G. mosseae*, *G. intraradices*); and *R. irregularis* MUCL 41833 AMF; egg parasite fungus *P. lilacinum*, endophyte *F. oxysporum* NP from the fungal group; and predatory mite *B. dolichus* (Acari: Blattisociidae) from the insect group have been found suppressive to *R. similis*. Other species of potential biocontrol agents that have significantly reduced *R. similis* populations are: *Pseudomonas putida, B. subtilis, Paecilomyces marquandii, T. viride, G. fasciculatum,* and *G. manihotis*. However, among these reported effective beneficial microorganisms against the burrowing nematode, the PGPR P. fluorescens and *Bacillus* spp., the mycorrhizal fungus (*Glomus* spp. and *Trichoderma* spp.), the fungus endophyte *F. oxysporum*, and the parasite *P. lilacinum*, were admitted for commercial products, and are potential alternatives for chemical (nematicide) to control *R. similis*.

Some formulations of biocontrol agents were prepared using wettable powder (WP), water dispersible granular (WG), talc-based products, vermiculite flakes, dry root powder, and bioproduct suspension. For mass production, the biocontrol agents were multiplied in sorghum grains, wheat substrate, and tomato plants inoculated by *P. penetran*-infected root-knot nematode. Live organisms (cultures of beneficial bacteria, fungus, and insects) are utilized as the active ingredient in microbial pesticides as well as their secondary metabolites or fermentation by-products. The active components in biopesticide products include microbial substances such cell-free filtrate, bacterial culture, spores/conidia, lytic enzymes, antibiotics, inhibitors, and toxins. Some existing commercial biopesticide products contained known broad-spectrum beneficial microorganisms for the plant–parasitic nematode bioprotection, but the target for these products for the burrowing nematode was scarce in compared to root-knot nematodes and root lesion nematodes. More exploration into these bioproducts' efficacy to control *R. similis* should be conducted to determine how effectively they can interact with pathogenic microorganisms, control the nematode, and promote plant growth.

Future challenges for the sustainable biocontrol of the burrowing nematode include: 1) investigating the use of high- and broad-spectrum bioactive ingredients and developing the forrmulation of compatible biocontrol agent consortiums that target various life stages of the burrowing nematode and or various nematode species, and its associated pathogens as well; 2) developing bio-agents' mass production technologies which can enhance the bio-agents' shelf life; 3) developing the formulation of compatible biocontrol agent consortia, and developing the nanoformulation of bioactive compound/agent using biomaterials; and 4) developing its practical synergistic integration methods with other IPM components for enhancing and extending the bioprotection level of biocontrol agents after application in the field.

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Reniform Nematode in Agricultural Crops and Their Management by Novel Biocontrol Technologies

19

Priyanka Duggal and R. Sharmila

Abstract

The use of chemical pesticides for the management of insect-pests, diseases, and nematodes is not new but environmental awareness and human health safety have assumed special significance in disease management programs. Nematicides are expensive, hazards to living being, a notable cause of environmental pollution, and their toxic residues in agricultural production system led to increased attention and to explore possibilities of developing other methods of nematode control. In this direction, we can manipulate agricultural practices in such a way which not only control plant pathogens but also reduce the unnecessary expenditure on harmful chemicals. Biological control is a safe and feasible alternative to control plant pathogens where one organism may be pathogenic, parasites, directly consume the harmful organism or may have other mechanisms for their pathogenic or parasitizing abilities. There are numerous fungi, bacteria, predatory nematodes, mites, etc. which play very significant roles in pest management strategies. The genetic engineering methods aim to harness resistance present, naturally in gene pools of crop species or their wild relatives to be introduced in already well doing cultivar in breeding programs. Various other alternatives can be feeding cells destruction, presence of specific toxic compounds/proteins to the nematodes or post-transcriptional gene silencing or RNAi technologies, etc.

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Keywords

Rotylenchulus reniformis · Biocontrol · Rhizobacteria · Genetic engineering · RNAi

19.1 Introduction

The global cultivation of crops depends on the geographical location and dietary habits of a particular area; however, the majority of the countries produce cereals as their main food source while other countries prefer potato, corn, millets, etc. (Khan 2023). In addition to food crops cultivated for eating, there are certain crops grown for industrial purposes and allied agricultural sectors. Numerous diseases, insect-pest, and plant-parasitic nematodes attack all these crops. The nematodes having a broad host range such as root-knot nematode, root lesion nematode, and reniform nematodes are important nematodes pest of most of the agricultural crops (Khan 2008). The common name "reniform nematode" for genus *Rotylenchulus* indicates the kidney shape of adult females which is attached to the roots and becomes sedentary. Reniform nematode is a damaging pest of about 300 plant species, including cotton, maize, soybean, castor, tea, coffee, carrot, cucumber, okra, betel vine, brassicas, lettuce, olive, potato, sweet potato, banana, mango, papaya, pine apple, passion fruit, pea, beans, chickpea, cowpea, mung bean, and a wide range of cultivated and uncultivated plants. Reniform nematode is prevalent to Alabama, Africa, Asia, Australia, China, Florida, Ghana, Jamaica, Georgia, Guam, India, Japan, Louisiana, Netherlands, Pakistan, Peru, Texas, and other tropical, subtropical, and some warmer areas in temperate countries.

Preadult stage (immature female) is the infective stage in case reniform nematode which enters inside the root and only the front half portion of the body is fixed in the root. Reniform nematode is a sedentary semi-endoparasites, infective stage starts feeding and creates a permanent feeding site known as syncytium (Rebois et al. 1975) which provides water and food to the growing nematode (Rebois 1980). The female of reniform nematode can lay on an average 50–75 eggs, which are in a cluster of a sticky material (egg mass) to hold all the eggs together (Robinson 2007). The total life cycle (Fig. 19.1) duration may vary from 17 to 23 days, depending on temperature and soil conditions. This nematode can be found in fine as well as coarse-textured soils (Rebois 1973). This chapter's goal is to provide an insight of the many management alternatives for reniform nematodes, including biological methods, genetic engineering approaches, and the use of trap crops or nonhost crops to lower their incidence in various agricultural crops.

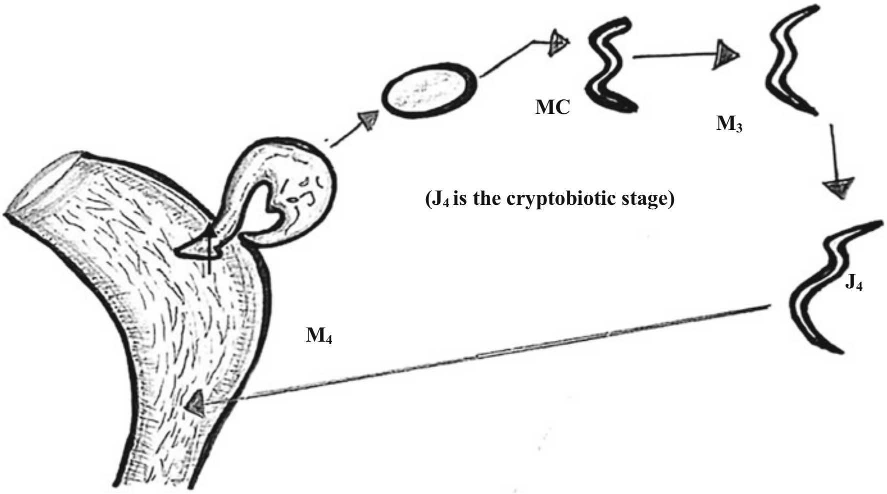

Fig. 19.1 Life cycle of reniform nematode. (Source: TNAU)

19.2 Major Reniform Nematodes Species Worldwide

Around ten species have been reported globally but *Rotylenchulus reniforms* (Linford and Oliveira 1940) is the economically important species. Dasgupta et al. (1968) collected nematode specimens across the world and described six new species, *i.e.*, *Rotylenchulus anamictus* (Dasgupta et al. 1968), in association of acacia; *Rotylenchulus clavicaudatus* (Dasgupta et al. 1968, Berg and Spull 1981), associated with strelizia and sugarcane; *Rotylenchulus leptus* (Dasgupta et al. 1968), associated with bamboo; *Rotylenchulus macrodoratus* (Dasgupta et al. 1968), associated with grape; *Rotylenchulus macrosoma* (Dasgupta et al. 1968, Cohn and Mordechai 1988), associated with olive; and *Rotylenchulus variabilis*, later synonymized with *Rotylenchulus borealis* (Loof and Oosternbrink 1962), associated with maize. *Rotylenchulus parvus* (Williams 1960, Dasgupta et al. 1968), transferred from genus Helicotylenchus, is a pest of sugarcane. Two more species, *R. sacchari* (Berg and Spull, 1981) (pest of sugarcane) and *R. brevitubulus* (Berg, 1990) were reported from South Africa.

In India, first occurrence of reniform nematode was reported by Siddiqi and Basir (1959) and later two host "races" based on their reproduction on castor, cowpea, and cotton were also reported, where out of ten, nine morphologically similar populations (Race A) reproduced on all host plants and remaining one multiplied only on cowpea (Race B) (Dasgupta and Seshadri 1971). Four physiological races of reniform nematode were reported by Rao and Ganguly (1996), which can reproduce on five host plants, i.e., cotton (H-777), bajra (Pusa-23), castor (CH-1), mustard (Pusa Bold), and cowpea (Pusa Komal). Singh (2009) reported Race-2, Race-3, and Race-4 in Aligarh (UP), using host differential.

19.3 Novel Biological Management Practices for the Management of Reniform Nematode

Biocontrol is an efficient mechanism against various pathogens including plant-parasitic nematodes due to several advantages over application of conventional pesticides. Various microorganisms can be found associated with host plants in rhizosphere which may be harmful or beneficial for the other organism including pathogens also. Among all of them, fungi and bacteria are more prominent which can be found in same rhizosphere and control other pests and disease-causing pathogens. On cotton roots, *Arthrobotrys dactyloides*, *Aspergillus fumigatus*, *A. glaucus*, *Cladosporium cladosporioides*, *C. herbarum*, *Dactylaria brochophaga*, *Fusarium oxysporum*, *Purpureocillium lilacinum*, *Penicillium waksmanii*, *Phoma exigua*, *Torula herbarum* has been reported from vermiform stages and eggs of *Rotylenchulus reniformis* (Castillo et al. 2010) in which, *P. lilacinum* (egg-parasite fungus) has efficient biocontrol activity (Reddy and Khan 1988).

19.3.1 Cotton

Cotton is an important cash and fiber crop of India which is grown in semi-arid areas and shares a major role in agricultural and industrial economy of the country, with largest area under cotton cultivation (36% of the world's area under cotton cultivation). Cotton production is hampered by many insect-pests, diseases, and nematodes including reniform nematode. Two tropical species of genus *Rotylenchulus* viz., *R. parvus* and *R. reniformis* are pathogenic to cotton. *R. reniformis* is prevalent across the tropical and subtropical regions globally while *R. parvus* was found in Africa only. Reniform nematode is more prominent in fine textured soils having more clay or silt content. Infected cotton plants produce less number of bolls with smaller size (Lawrence and McLean 2001) which reduce the percentage of lint (Cook and Namken 1994) and causing yield losses from 40 to 60%. The tolerance level of reniform nematode is 100 nematode/100 g soil.

Fungus and bacteria having the biocontrol activities seem a good option to manage reniform nematode. Arkansas fungi (ARF) (nematophagous fungi) reduced the numbers of *R. reniformis* with parasitism range from 48% to 79% (Wang et al. 2004). *Catenaria auxiliaries* (Castillo and Lawrence 2011) and *Pochonia chlamydosporia* parasitized eggs and reduced the number of reniform nematodes on cotton plant and in field (Wang et al. 2005). Seed treatment with combined application of *Bacillus firmus* and *Paecilomyces lilacinus* 251, considerably lowered the *R. reniformis* population in cotton (Castillo et al. 2013). On cotton, *Pasteuria* spp. can parasitize reniform nematode (Hewlett et al. 2009) and complete their life cycle in all stages of the nematode (Schmidt et al. 2010). A rotation with corn crop for at least 2 years not only reduced reniform nematode but also increased cotton yield (Stetina et al. 2007). *Bacillus subtilis* @10 g/kg seed as seed treatment and @ 2.5 kg/ha as soil treatment, is also useful against nematodes.

19.3.2 Vegetable Crops

Rotylenchulus sp. is the second destructive nematode to affect vegetable cultivation after root-knot nematode, is frequently underestimated in areas where it coexists with RKN. This nematode has reported in vegetable crops in many countries, i.e., Hawaii, the Southern United States, Mexico, S. America, India, Africa, and South East Asia. Stunting, leaf curling, root necrosis, cortical necrosis, and yield losses are among the damage symptoms. Numerous vegetable and melon crops have been found to be affected by this nematode. After 100 J2/plant of inoculation, tomato production decreased and inoculation with 1000 nematodes, snake gourd plants exhibited stunting, reduced leaves, and brown roots with cortical necrosis. Okra yellow vein mosaic virus (OYVMV) and Brinjal Mosaic Virus (BMV) were reported higher on plants infested by reniform nematode. According to the reports, this nematode can survive in soil for 7 months in moisturized soil, for 6 months in dry soil; the duration can be longer up to 29 months in the absence of hosts.

Fungi including VAM are effective in minimizing the penetration, reproduction of reniform nematode attacking cucumber and tomato (Stirling 1991; Khan 2007, 2016; Khan and Anwer 2011; Sikora and Roberts 2018; Khan et al. 2020a). The nematode antagonists such as *Pochonia chlamydosporia, Purpureocillium lilacinum, Aspergillus niger,* and *Pasteuria penetrans* are effective antagonists of plant nematodes (Jatala 1986; Stirling 1991; Kerry 2000; Khan 2016). *Purpureocillium lilacinum* minimized *R. reniformis* population in tomato, under greenhouse as well as microplot trial (Walters and Barker 1994). *P. lilacinum* is an egg-parasite fungus and with the help of an appressorium, it penetrates the nematode egg shell and secretes enzymes, acids, and toxins (Park et al. 2004) and PL protease (Lopez-Llorca et al. 2008). Commercial product based on *P. lilacinum* strain 251, MeloCon® WG, and NemOut WP, has been developed to control *R. reniformis* in tomato (Schenk 2004).

The phosphate solubilizing microorganisms such as *Pseudomonas, Bacillus*, *Penicillium,* and *Aspergillus* species/strains may also contribute to suppressing soil populations of plant nematodes (Stirling 1991; Khan et al. 2016a, b). Plant growth-promoting rhizospheric bacteria promote plant growth and reduce reniform nematode in soil by inducing systemic resistance (Khan et al. 2009; Sikora and Roberts, 2018). Two isolates, Pfbv22 (*Pseudomonas fluorescens*) and Bbv57 (*Bacillus subtilis*) significantly reduced *R. reniformis* infestation, when applied as soil and seed treatment (Jonathan et al. 2009). Rotation which includes soybean, maize, sugarcane, and Pangola grass reduces nematode population. A number of other crops like Finger millet, peanut, chillies, sugarcane, grasses known to be resistant to the nematode may also be included in crop rotation. Short period of flooding, hot water treatment with 50 °C hot water for 5 min are also effective for nematode management. *Crotoleria juncea* followed by *Brassica napus*, and *Tagetes erecta* as soil amendments enhanced trapping fungi, egg-parasitic fungi, and parasites against reniform nematode (Wang et al. 2001). Crotalaria, tagetes, and brassica produce allelochemicals having nematicidal properties, i.e., monocrotaline, alpha-terthienyl (Gommers and Bakker 1988), and glucosinolates (Brown and Morra 1997). In recent decades, *Trichoderma* spp. have emerged as an effective biocontrol agent of plant nematodes (Mohiddin et al. 2010; Khan and Mohiddin 2018). The available commercial formulations of *T. virens, T. harzianum,* and *T. hamatum* are found to offer satisfactory control of nematode infestations in vegetables in particular (Khan et al. 2011; Sikora and Roberts 2018; Mohammed and Khan 2021). The biocontrol agents may become more effective if applied in integration with compatible pesticides (Mohiddin and Khan 2013). Nanotechnology also has potential application in plant protection, and offers satisfactory solutions for plant disease management (Khan and Rizvi 2014; Khan et al. 2019a, b, c) and disease detection (Khan and Akram 2020; Khan and Rizvi 2016; Khan et al. 2020b). Nano-sensors are the most important product of nanotechnology, and have great potential for use in plant disease diagnosis (Khan 2023). Sellappan et al. (2022) developed nanobiosensor to early detection and prevention of agricultural crops from harmful microorganisms. Using specific nanoparticles as nano-sensors to detect the plant pathogen early can reduce the plant disease damage and help in proper management of the disease (Khan and Rizvi 2018).

19.3.3 Fruit Crops

19.3.3.1 Grapes

Reniform nematode, *Rotylenchulus reniformis* mostly damages the secondary and the feeder roots. Affected roots show brownish discoloration and rotting due to secondary infection, interrupting nutrient uptake, making the vines appear sick.

19.3.3.2 Banana

The reniform nematode feeds on the secondary or tertiary roots of banana and formed necrotic lesions where females are attached to the root portion. High population of the nematodes causes severe necrosis and destruction of feeder roots. Management tactics include cultural practices, physical methods, chemical application, biocontrol, host resistance, and integrated pest management. Cultural methods include summer ploughing, organic manuring, rotation of crop, and destroying infected crop residues. The promising biological agents include the fungus (*Paecilomyces lilacinum*), VAM, and bacterium (*Pasteuria* spp.).

19.3.3.3 Papaya

Rotylenchulus reniformis (Fig. 19.2) causes severe damage, toppling, and death of fruit plants. Affected plants become stunted with reduced chlorotic foliage. Nematodes penetrate the secondary and tertiary root and cause necrotic lesions. Nematode facilitates easy entry of soil-borne pathogens like *Phytophthora* and *Fusarium* causing root rot disease.

19.3.3.4 Pineapple

Two varieties of pineapple, i.e., Victoria (Queen) and MD2, were selected and two separate treatments, i.e., Methyl jasmonate (10^{-4} M) and a bacteria isolated from

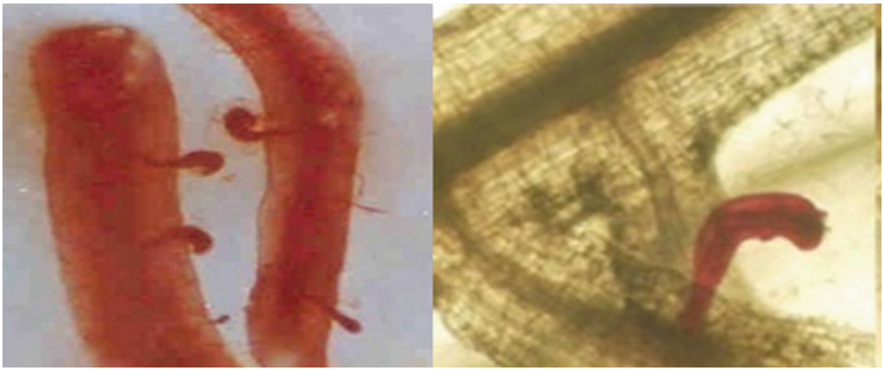

Fig. 19.2 Reniform nematodes on papaya roots. (Source: TNAU)

roots of pineapple plant, *Bacillus* sp. GVS2 (10^8 CFU per mL), both @ 10 mL per plant, were applied once in a month in the field condition. The nematode densities on MD2 and Victoria (Queen) were recorded after 8 months, and 58% and 50% reduction was observed in case of methyl jasmonate and 60% and 54% reduction in case of bacteria, as compared to check, may be due to induced systemic resistance or antibiosis (Soler et al. 2021).

19.3.4 Oilseed Crops

19.3.4.1 Soybean

Soybean crop reported to be infested with several nematode pest species, i.e., cyst nematode, *H. glycines, Meloidogyne* spp., and *Rotylenchulus reniformis*. In a trial against *R. reniformis*, two bacterial isolates viz. Bmo3 (*Bacillus mojavensis*) and Bve2 (*B. velezensis*) effectively minimized total numbers of eggs (Xiang et al. 2018). Sugarcane, wheat, maize, oats, rice, peanuts, and sorghum act as nonhosts to reniform nematode and can be used in rotation with soybean (Hartman et al. 2015).

19.3.4.2 Castor

In India, castor crop is reported to be infected by *M. incognita, R. reniformis* causing chlorosis, stunting of the plants, necrosis on the roots, and predisposes of the entry of other microorganism.

19.3.5 Pulses Crops

19.3.5.1 Cowpea

This crop is cultivated around the world primarily for seed, vegetable, fodder production, and also as a cover crop. Reniform and root-knot nematodes are the major restrictive biotic factors of cowpea crop in India as well as across the globe

(Sikora and Fernandez 2005). *R. reniformis* is known to reduce the growth of cowpea (Yassin and Ismail 1994). *Trichoderma harzianum* @ 2 g/kg soil as soil treatment, combined with seed soaked in 30% lantana leaves extract, reduced nematode population and improved plant growth (Patil et al. 2013). Application of *Paecilomyces lilacinus* @ 2.0 g mycelium/pot reduced RKN, reniform nematode, and fungus-causing root-rot (*Rhizoctonia solani*) infestation in cowpea (Khan and Husain 1990). Aqueous neem (*Azadirachta indica*) leaves extract as foliar spray and soil application @ 10% and 15% inhibited reniform nematode population in cowpea (Umamaheswari et al. 2005). Seed soaking with leaves extracts of lantana, calotropis, and withania @ 30% w/w was reported as most effective in reducing *R. reniformis* population (Patil et al. 2016).

19.4 Genetic Engineering Technologies for the Management of Plant-Parasitic Nematodes

Reniform nematode is an important nematode pest which reduces agricultural crop yields (Robinson 2007). To maintain a successful host parasite, syncytium, a permanent feeding site for the survival of nematode, is initiated and maintained by nematodes secretions, injected by their mouth part to be called as stylet or more specifically stomatostylet. Plant-parasitic nematodes pose a continuous danger to food security and annual crop losses about $100 billion USD (Nicol et al. 2011). The most problematic PPNs, i.e., primarily root-knot nematodes and cyst nematodes are obligate parasites in nature and become sedentary inside the roots after penetration, are the most studied group (Jones et al. 2013).

The development of functional genetic tools is hindered by the limited use of forward genetics in most advanced form of PPNs, i.e., sedentary endoparasitic nematodes, *Meloidogyne hapla* in RKN group, and relies on natural variants as the source of mappable polymorphisms (Thomas and Williamson 2013), while reverse genetics is solely dependent on RNAi (Bakhetia et al. 2005). The development of useful genetic tools would hasten the study of the biology of PPNs and, indirectly, the process of new control strategies. Viable genetic tools in nematodes are hampered by knowledge gaps and nematodes biology.

19.4.1 Molecular Approach

Synthetic forms of resistance like disrupting feeding cells, expression of a particular peptide or proteins, or delivery of toxic substances to the nematode during initial period can be used to supplement natural resistance, to be found in gene pools of crop species and their wild relatives. Large-scale screening of germplasm is done in association with genetic markers to find R (resistance) genes or metabolites conferring resistance to some nematodes. The desired germplasm is then introgressed with the identified sources of resistance. Contrastingly, transgenic approaches to nematode control take full advantage of understanding of nematode-host interactions to target the nematode like disorientation of the nematodes movement to stop chemotaxis (host-finding mechanism), reducing migration of nematodes through host tissues, establishment of feeding sites, feeding and egg laying capacity of nematodes on a susceptible or tolerant host. Reniform nematode has been reported to have cellulase genes (Wubben et al. 2010; Nyaku et al. 2013, 2014).

19.4.2 Resistance Mechanism

Against nematodes, mechanism of active resistance is not known in all crops but its use is economically viable, environmentally safe to reduce the crop losses resulting from nematodes attack. Marker-assisted breeding generally involved the screening, for evaluation of resistance from wild progenitors of a plant. The molecular sequence technologies used are, Restriction Amplified Length Polymorphisms (RFLPs), Amplified Fragment Length Polymorphisms (AFLPs), Random Amplified Polymorphic DNA (RAPDs), Sequenced Characterized Amplified Regions (SCAR), and Sequence-Tagged Site (STS)-based approaches.

19.4.3 Reniform Nematode Resistance in Cotton

Reniform nematode has become more prevalent in USA and around 50% of the cotton fields are infected with nematode (Blasingame 1993). The effective resistance mechanism has not been found within G*ossypium hirsutum,* but resistance source was identified in its other species. However, efforts to introgress these resistances into *G. hirsutum* have been difficult by incompatibility barriers. No useful resistance to *R. reniformis* was identified among lines of *G. hirsutum* that carried a single monosomic addition from the highly resistant, but genetically incompatible *G. longicalyx* (French 1995). The moderately resistant *G. barbadense* line TXllO, crossed with the *M. incognita*-resistant *G. hirsutum* genotype M315 resulted in fertile F1 progeny with high levels of resistance to *M. incognita* and moderate resistance to *R. reniformis*. CRISPR-Cas9 technology is used for genome editing and commonly studied in the *Caenorhabditis elegans* (Frøkjær-Jensen 2013). The resistance sources were identified through 104 SSRs makers-based resistance mechanism.

19.4.4 RNA Interference (RNAi)

The RNAi technique is not new to manage the attacks caused by PPNs in various crops. In this technique, plants produce dsRNAs to silence particular genes which are essential for nematodes growth and development or may contribute in parasitism. During feeding on these these modified plants, the nematode ingests siRNAs, along with cytoplasm, once it is inside the nematode, it would inactivate the gene targeted by the dsRNA through the RNAi process and inhibit subsequent growth and development and in some case nematodes parasitism (Ganguly and Rao 2009). RNAi was used by targeting the Cystein-proteinase region of the nematode; it is resulted in altering the sex ratio from 3:1 to 1:1 of the nematode from female to male in *Heterodera* and *Globodera* (Urwin et al. 2002). In transgenic tobacco plant, infected with female of *Meloidogyne javanica,* a reduction was observed in eggs development and hatching (Fairbairn et al. 2007).

19.5 Conclusions and Future Prospects

After studying the management of plant-parasitic nematodes, more specifically reniform nematode, it is essential to know about the pros and cons of the various methods. Biocontrol is an efficient mechanism due to host specificity and environmentally sustainability but rearing of biocontrol agent and its survival in absence of its host pathogen may cause some problems in their uses. One should have the broad knowledge about its ecology, life cycle, and taxonomy too which cannot be done without the help of expert. Biocontrol agent itself must be free from its natural enemies, in that particular environment. Use of trap crops and nonhost crops depends on crop requirement, ecology, and food habits of that particular area. Genetic engineering technique including host plant resistance provides a good opportunity to control reniform nematodes but there are certain drawbacks in applying these techniques to their management. We should look for the source of resistance in grown or wild plant species, and new techniques should be developed for better management options which not only control the nematode pests but also are economically viable, environmentally safe, and in the favor of farmers.

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Citrus Nematode in Fruit Crops and Their Management by Biological and Biotechnological Interventions

20

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Abstract

One of the most common fruit crops cultivated worldwide is citrus, which is also a significantly traded horticulture product. In places of the world where citrus is grown, *Tylenchulus semipenetrans*, one of the main nematode pest that parasitizes plants, significantly reduces yields in the world where citrus is grown. The management of plant parasitic nematodes in citrus can be done alternatively by using biological control because of its lower toxicity to the environment, specificity of the target, and safety for nontarget organisms. Even though various bacteria, mites, and fungi have been employed to reduce *T. semipenetrans* population in citrus, a dedication to the creation of high-quality products, extension programs, and industrial partnerships will help to promote the widespread use of biological control agents.

Keywords

Nematode · Biological control · Citrus · Management

20.1 Introduction

One of the most popular fruit crops and a significant horticulture traded commodity in the globe is citrus (Matheyambath et al. 2016). Oranges account for 55% of all citrus production worldwide, followed by 25% mandarins, 13% lemons, and 7%

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grapefruits (Global Citrus Outlook 2019). One of the main reasons restricting citrus production globally is plant-parasitic nematode infection. Since citrus crops become perennial, they nourish and encourage nematode population growth throughout the year around (Reddy 2018). There are many plant nematodes associated to the citrus rhizosphere; however, only a small number of species affect the trees (Khan 2023). *Tylenchulus semipenetrans, Pratylenchus coffeae, Radopholus citrophilus*, and *Meloidogyne indica* are among the groups of plant-parasitic nematodes that significantly reduce citrus crop yields worldwide (Kumar and Das 2019; Duncan 2009; Verdejo-Lucas and McKenry 2004).

The main pathogenic species almost every region where citrus is grown in the world is the citrus nematode, *T. semipenetrans*. The young adult females enter the cortical cortex more deeply, settle down, and create nurse cells, which serve as a permanent feeding site and food sink for the nematode (Khan 2008). Depending on the level of infestation, yield losses brought on by this nematode are predicted to range from 10% to 30% globally (Verdejo-Lucas and McKenry 2004). However, researchers estimate those orchard infestations in many regions of the world range from 50% to 90% due to insufficient regulatory exclusion measures (Sorribas et al. 2008, 2000; Maafi and Damadzadeh 2008; Iqbal et al. 2006; de Campos et al. 2002). Citrus is slowly declining as a result of it, and it is also responsible for other complexities like citrus dieback. The age and health of the tree, the nematode population density, and the rootstock's vulnerability all have a role in how much damage a nematode infection causes (Ravichandra 2014). Some of the symptoms that are noticeable include chlorosis, leaf defoliation, smaller fruit, fruit loss before maturity, and twig dieback from above branches. In contrast to healthy roots, the branch rootlets on the infected feeder roots are shorter, darker, and covered in soil (Abd-Elgawad 2020; Duncan 2009).

The most effective preplant nematicides employed in citrus nurseries and orchards against *T. semipenetrans* were fumigants such as 1,3-dichloropropene, metham sodium, and methyl bromide (Shokoohi and Duncan 2018). Due to their toxicity to the environment and negative effects on human health, many pesticides have been taken off the market. In some cases, using resistant rootstocks to control *T. semipenetrans* has been effective (Verdejo-Lucas and McKenry 2004; Verdejo-Lucas et al. 2000; Gottlieb et al. 1987; Kaplan and O'Bannon 1981), but these hybrids perform poorly in alkaline soils, and over time, resistance-breaking biotypes were developed (Abd-Elgawad 2020). Citrus plant-parasitic nematodes can be managed through biological control since these are less hazardous to the environment, more particular in their target species, and safe for nontarget creatures. The world's citrus-growing regions have been subject to biological control methods based on fungi, bacteria, and mites or their bioactive components.

The current chapter examines the potential for biological control agents in citrus to evolve in the future and provides an overview of the biological control agents now being used in citrus to combat *T. semipenetrans*.

20.2 Biological Control of *T. semipenetrans* in Citrus

Biocontrol fungi/bacteria alone (Stirling 1991; Khan 2007; Khan and Anwer 2011) or along with oil, neem cakes (Sikora and Roberts 2018; Khan et al. 2021) or pesticides (Mohiddin and Khan 2013) are getting popularity in achieving sustainable nematode management in agricultural crops (Khan 2023; Khan et al. 2023). The microbial antagonists, *Aspergilus niger, Pochonia chlamydosporia, Purpureocellium lilacinum, Pasturia penetrans* etc. (Jatala 1986; Stirling 1991; Kerry 2000; Khan 2016), and phosphate solubilizing microorganisms such as *Aspergillus, Bacillus, Penicillium, Pseudomonas* etc. (Khan et al. 2009, 2016a, b; Sikora and Roberts 2018) may significantly contribute in the sustainable management of plant nematodes.

20.2.1 Fungi

20.2.1.1 *Trichoderma* spp.

As a biocontrol agent, *Trichoderma* spp. has been utilized extensively against plant pathogens like bacteria, plant and soil nematodes, and fungus. The chitinases, glucanases, and proteases generated by fungi are crucial in the fight against diseases (Sharon et al. 2001). In recent years, *Trichoderma* has also been found effective in suppressing plant nematodes (Mohiddin et al. 2010; Khan and Mohiddin 2018). Various species of *Trichoderma* were employed to combat citrus nematodes. The effectiveness of *Trichoderma* spp. has been established in numerous experiments carried out under various circumstances. According to Narendra et al. (2008), when *T. harzianum* (4 kg/soil) was applied to *C. jambhiri* under pot conditions, the juvenile and female populations of *T. semipenetrans* were reduced by 30.58% and 64.85%, respectively, in comparison to the untreated control. The commercial formulations of *Trichoderma* spp. are available in market (Khan et al. 2011), which are quite effective against soil nematodes and other pathogens (Mohammed and Khan 2021; Sikora and Roberts 2018; Shahid and Khan 2019).

T. hamatum, however, significantly reduced the amount of delicious orange under greenhouse conditions (86.68% and 61% at 3×10^8 spore/mL, respectively) (Hanawi 2016). While applying *T. harzianum* (3×10^8 spore/mL) to citrus *cv.* volkameriana resulted in the highest control (91.1%) in J_2 population compared to other treatments, according to Montasser et al. (2012). The same findings were reported by Shawky and Al-Ghonaimy (2015) who found an 86.3% decrease in *T. semipenetrans* J_2 on volkameriana seedlings when *T. harzianum* was administered at the highest rate (5×10^8 cfu/pot). According to recent studies, combining *T. harzianum* with Nemastop (natural oils) boosted the mortality rate of *T. semipenetrans* juveniles from 46% to 80% in vitro experiments (Ibrahim et al. 2019).

Based on field trials on sweet orange, *T. viride* (3×10^8 spores/mL) decreased J_2 and female *T. semipenetrans* populations by 64.9% and 44.8%, respectively (Hanawi 2016). While a month after, *T. harzianum* (5×10^8 cfu) was applied to volkameriana. According to Shawky and Al-Ghonaimy (2015), citrus nematode in the soil as well as roots had decreased by 55%. However, 4 months after application in the field, a striking suppression (72%) in the nematode population was seen in comparison to untreated control plots. Ibrahim et al. (2019) investigated the effectiveness of *T. harzianum* alone or combined with nemastop (natural oils), other biocontrol agents, and chemical pesticide to maintaining citrus nematode under control on Washington navel orange trees in Menia EL-kamh, Sharkia governorate, Egypt (Nemaphos). After 12 months of treatments in the field, *T. harzianum* mixed with Nemastop and when applied as a soil drench (500 mL/tree), compared to 33.1% for Nemaphos and 35.85% for *T. harzianum* alone, it caused a 51.7% reduction in the number of nematodes.

More successful nematode control was achieved by combining hostile bacteria with agricultural waste, such as compost, than by employing only one microbial strain or compost. It was reported that *T. semipenetrans* population density was less in the soil and roots by the application of *T. harzianum* mixed with neem, karanj, and castor oil cakes, and acid lime seedling growth was found to be boosted (Reddy et al. 1996). El-Mohamedy et al. (2016) reported that the population of citrus nematode that developed on sour oranges under greenhouse conditions decreased from 0.73 to 0.80 after the application of compost containing either *T. harzianum* or *T. viride* (1×10^6 cfu/mL) to 0.38 and 0.41, respectively. Similar results were achieved by combining compost with *T. viride* or *T. harzianum* at the same rate, which led to a lower nematode population (0.40, 0.42) as a comparison to the fungal cultrate alone (1.1, 0.76) in volkameriana (Hammam et al. 2016).

20.2.1.2 *Purpureocillium lilacinum* (=*Paecilomyces lilacinus*)

This fungus can parasitize citrus nematode eggs, egg masses, and females (Kumar 2020). Seven different *P. lilacinus*-based treatments are utilized globally to control citrus nematodes at different phases of their life cycles. According to Maznoor et al. (2002), the application of *P. lilacinus* (8 g/kg soil) made with rice bran-reduced nematode populations on khasi mandarin in India by 64.4% compared to nematode populations reduced by formulations with mustard oil cake (63.9%). However, in terms of nematode population decrease, the bioefficacy of the fungi developed in both environments was comparable. While Narendra et al. (2008) reported that when *P. lilacinus* (4 kg/soil) was applied to *C. jambhiri* plant, J_2 and the female population of *T. semipenetrans* were significantly reduced (64.7% and 75.7%, respectively), compared to the control under pot conditions. When *P. lilacinus, T. harzianum*, and *G. fasciculatum* were all applied together, the population of *T. semipenetrans* was decreased by 73.04% and 89.08%, respectively. This helped *C. jambhiri* plants grow more quickly. Similar to this, applying 10 g of *P. lilacinus*, 10 g of *Pseudomonas fluorescens*, and 250 g of neem seed cake per tree once every 6 months for 2 years decreased the plant nematodes and increased the yield (30.24 kg/tree) in comparison to control (17.20 kg/tree) (Rao 2008). In future, market growth for *P. lilacinus*-based commercialization products manages the domestic citrus nematode strains. Verdejo discovered 20 fungal strains from citrus rhizosphere in Spain, among them

P. lilacinus and *Talaromyces cyanescens* showed promising against citrus nematode infesting Carrizo citrange and Cleopatra mandarin in greenhouse conditions.

20.2.1.3 *Pochonia chlamydosporia*

P. chlamydosporia-based talc formulation was applied to the soil, Kumar and Prabhu (2009) claim that this resulted in a considerable reduce citrus nematode after 30 days over control (52.5, 9.5, respectively), under nursery conditions. As *P. chlamydosporia* (20 g/tree) was applied in orchard, Deepa et al. (2011) reported that the population of citrus nematode decreased by 42.76% when compared to untreated controls. Successful reduction of the *M. javanica* infects root gall in nursery by adding *P. chlamydosporia* and *P. lilacinus* to the soil @ 5 and 10 g/kg, respectively (Rao 2005).

20.2.1.4 *Mycorrhizae* (*Glomus* spp.)

These are the obligate root symbionts, which increase nutrient intake to promote plant growth and reduce plant stress brought on by nematodes that parasitize plants (Schouteden et al. 2015; Vos et al. 2012). Nematode and mycorrhizal fungal interactions depend on the association of plant cultivars, fungi, and nematode species and appear to be highly particular (Ingham 1988). In a preliminary greenhouse investigation, rough lemon seedlings grew more quickly than nonmycorrhizal seedlings after being transplanted into soil contaminated with *Glomus mosseae* and infected with *T. semipenet*rans (O'Bannon et al. 1979). *Radopholus citrophilus*, a citrus-burrowing nematode, was later found to have lower population densities in mycorrhizal-infested or nonmycorrhizal, high-phosphorus plants than in nonmycorrhizal, low-P plants of rough lemon. However, there was no discernible difference in the seedlings' growth. According to Reddy et al. (1995), citrus nematode was successfully controlled after *G. fasciculatum* was treated with neem cake in nursery. The soil treatment of *G. fasciculatum* (@ 500 spores/kg soil) reduced the citrus nematode infesting *C. jambhiri* by 66.77–82.22% (Narendra et al. 2008). While Ravichandra (2014) reported that, *T. semipenetrans*-infesting citrus might be controlled by applying *G. fasciculatum* or *G. mossae* @ 50–100 g/plant. Despite having a biocontrol effect on PPN, arbuscular mycorrhizal fungi's usage in citrus is fairly limited because of variable results.

20.2.1.5 Nematophagous Fungi

Nematophagous fungus and citrus nematodes coexist in the rhizosphere of the soil. They are successful in controlling these nematode species. According to Martinelli et al. (2012), the abundance of the *Pratylenchus jaehni* in pera orange under natural conditions in Spain was successfully reduced by applying formulations of *Arthrobotrys robusta, A. musiformis, A. oligospora, Monacrosporium eudermatum,* and *Dactylella leptospora*, enriched with sugarcane bagasse and rice bran mixture separately at doses of 1 and 2 L/plant. However, Noweer (2018) reported that the use of a combination of egg-parasitizing fungus *Verticillium chlamydosporium* and nematode-trapping fungus *Dactylaria brochopaga* (0.5 kg/tree) for 2 seasons caused a significant decline in the population of *T. semipenetrans* (97% and 70%, respectively) compared to control in mandarin trees.

20.2.2 Bacteria

Among the most effective and extensively used bacteria against several plant nematodes infecting citrus over the world include *Bacillus* spp., *Pseudomonas* spp., and *Streptomyces* spp. *Pasteuria* species have also been utilized as biocontrol agents in addition to these *Serratia marcescens.*

20.2.2.1 *Bacillus* spp.

This genus has successfully controlled plant nematodes at an amazing level on a variety of horticulture crops in multiple instances. *B. thuringiensis*, *B. firmus, B. subtilis*, and *B. megaterium* species have all been investigated in citrus under various circumstances. According to Montasser et al. (2012), of the seven isolates of fungi and bacteria used as biocontrol agents that were tested in vitro*, B. subtilis* had the highest level of success against *T. semipenetrans* (J_2) (100% mortality at 3×10^8 cfu/mL), followed by *S. marcescens* (99.9%) after 72 h of exposure. When compared to untreated plots, field tests in Egypt using the commercial formulation of *Bacillus thuringiensis*—Agerin® (3 kg/4200 m^2) grafted onto 15-year-old baladi mandarin (*Citrus reticulata*) trees on sour orange (*Citrus aurantium*) trees boosted yields by 52.9–69.2% over two seasons (El-Nagdi et al. 2010). On 16-year-old Valencia sweet orange trees, Abd-Elgawad et al. (2010) showed a sharp decrease in *T. semipenetrans* juveniles and the maximum fruit output (85.6–90.2 kg/tree) following the application of *B. subtilis* (107 cells/mL).

Hammam et al. (2016) reported that, the effectiveness of *T. semipenetrans* population was higher when *B. subtilis* combined with compost (10^{16} cfu/mL) was administered to the soil of volkameriana seedlings in Egypt after 3 months of treatment under greenhouse conditions. *B. subtilis* had similar results in sour oranges (1×10^6 cfu/mL) mixed with compost, which caused 73.7% more *T. semipenetrans* death across all life stages than *B. subtilis* alone (66.8%) (El-Mohamedy et al. 2016). El-Tanany et al. (2018) found that soil treatment of a combination of commercial formulations including *B. megaterium* and *T. album* (Bio Arc + Bio Zeid) over two seasons boosted fruit yield and significantly decreased (66.20–78.79%) *T. semipenetrans* populations in Washington navel orange trees over two seasons under field conditions in Egypt.

B. megaterium, a similar species, has become a promising citrus biocontrol agent. According to Elzawahry et al. (2015), the use of the commercial formulation Bioarc TM (30 g/L) resulted in 90.5% *T. semipenetrans* J2 mortality following a 72-h exposure period in the laboratory. While a greenhouse study revealed a considerable reduction (89.0%, 89.5%, and 76.6%, 82.9%) in juvenile in the soil and females in the root of baladi orange and lime, respectively.

20.2.2.2 *Pseudomonas fluorescens*

It is possible to control *Meloidogyne* spp. and *T. semipenetrans* in citrus successfully by using *P. fluorescens* as a biocontrol agent (Rajendran et al. 2001). For instance, after 72 h of exposure under in vitro conditions, Montasser et al. (2012) reported the maximum death (99.9%) of *T. semipenetrans* juveniles. Hanawi (2016), however, found that after 48 h of exposure, 94% of juveniles died at a dosage of 3×10^8 cfu/mL. In comparison to untreated control plots under natural conditions in India, the application of commercial talc-based *P. fluorescens* formulation (20 g/tree) to the soil decreased *T. semipenetrans* infesting *C. limon* and increased the yield (Deepa et al. 2011). Despite the fact that the use of experimental culture filtrate led to a sharp decline in *T. semipenetrans* juveniles on sweet orange trees in Egypt when compared to control plots (48.2% at 25 mL/tree—3×10^8 cfu/mL) (Hanawi 2016). Applying neem cake (25 g/plant) and *P. fluorescens* (2×10^9 spores) together considerably decreased the population of *T. semipenetrans* in the soil and on the roots of acid lime seedlings as compared to the control (Reddy et al. 2000).

20.2.2.3 *Streptomyces avermitilis*

The naturally occurring fermentation byproduct of *S. avermitilis*, Abamectin, has enormous promise as a biocontrol agent for a variety of plant nematodes (Saad et al. 2017). El-Nagdi et al. (2010) reported that the application of commercial formulations of *S. avermitilis*—abamectin to mandarin trees grafted on sour orange (*Citrus aurantium*) enhanced yield by 84.6–115.4% over two seasons compared with control plots under field conditions. El-Tanany et al. (2018) evaluated abamectin (Tervigo®), oxamyl (Vydate®), and botanical insecticide to manage *T. semipenetrans* infesting Washington navel orange trees in Egypt. In comparison to oxamyl and azadirachtin (Achook®), the substance used in the soil (2.5 L per feddan) caused a reduction of 78.12–87.06% throughout two growing seasons. However, compared to abamectin (41.45 kg/tree), the average fruit output was much higher with oxamyl treatment (51.87 kg/tree). A similar reduction in *T. semipenetrans* population was found by El-Saedy et al. (2019) following the administration of Tervigo® (15 mL/tree), which also led to an increase in fruit yield (71.1 kg/tree) throughout two seasons among orange trees in Valencia

20.2.2.4 *Pasteuria* spp.

The *Pasteuria* sp. has been associated to *T. semipenetrans* in reports from various citrus-growing regions across the globe (Ciancio et al. 2016; Sorribas et al. 2000, 2008; Gené et al. 2005; Kaplan 1994; Ciancio and Roccuzzo 1992). It could function as an efficient biocontrol agent for *T. semipenetrans* and other plant nematodes due to the density of its endospores and their long-term persistence in soil under challenging conditions (Ciancio 2018). The population of *T. semipenetrans* was effectively reduced by the combined application of *P. penetrans* (2109 spores/plant) and *P. lilacinum* (50 g/plant with 4107 spores/g) (Reddy and Nagesh 2000). The limited host range and obligatory character of this genus have limited the experimental investigations that have been done utilizing it to combat *T. semipenetrans*. The application of these bacteria in the biocontrol of citrus nematode will be further improved by further knowledge of their biology and field ecology as well as artificial culturing of the bacteria employing fermented technology.

20.2.3 Mites

It has been determined that mites may be used as plant nematode biocontrol agents. Investigations on various species of mites such as *Macrocheles muscaedomestica, Cosmolaelaps simplex, Macrocheles matrius*, and *Gaeolaelaps acul*e against *T. semipenetrans* have been undertaken on citrus, with the majority of the studies taking place in greenhouses. Al Rehiayani and Fouly (2005) found that the simultaneous application of *C. simplex* (200 individuals/pot) and *T. semipenetrans* juvenile inoculation to citrus seedlings significantly reduced the nematode's reproduction capacity, although mite individuals were less effective than aldicarb (614 juveniles/ 100 cm^3 soil). Salehi et al. (2014) found that key lime plants that were not treated (398.25 J_2/100 cm^3 soil) produced considerably more juvenile *T. semipenetrans* plants than those that were treated (20 individuals/pot), ranging from 126 to 161. Similar research was conducted by Abo-Korah (2017) on the efficiency of *M. matrius* against citrus nematode and found that it reduced *T. semipenetrans* juvenile population by the highest percentage (77.5%) when compared to carbofuran (76.9%), and that seedling growth was also increased. However, compared to *T. semipenetrans*, *P. penetrans* had a reduced predation efficiency. Despite the possibility of managing *T. semipenetrans*, problems with mass production, soil delivery, and nonspecificity prevent predatory mites from being widely used in the biocontrol of plant nematodes (Cumagun and Moosavi 2015; Viaene et al. 2006). However, the advancement of mass production, delivery, and soil ecological knowledge may boost the use of these agents shortly.

20.3 Biotechnological Interventions in Citrus Nematode Management

There is a dearth of information on biotechnological methods for controlling *T. semipenetrans*. To handle *T. semipenetrans*, methods including gene silencing (RNAi) and the introduction of harmful substances to the invading nematode should be taken into consideration. Natural variation for resistance, extensive germ plasm screening, and genetic markers should all be investigated to find the genes that confer resistance to *T. semipenetrans*. To reduce effective establishment in host cells on a sensitive or tolerant host, transgenic techniques that take advantage of an understanding of nematode-host interactions and direct the infective stage to prevent locating host roots are used (Fosu-Nyarko and Jones 2015). To counteract *T. semipenetrans*, citrus breeding programs are using genomic editing techniques like CRISPR/Cas (Abd-Elgawad 2022). Nanotechnology is a most recent branch of science and offers satisfactory solutions for plant disease management (Khan and Rizvi 2014; Khan et al. 2019a, b, c) and disease detection (Khan and Akram 2020;

Khan and Rizvi 2016; Khan et al. 2020). Nano-sensors are the most important product of nanotechnology, and have great potential for use in plant disease diagnosis (Khan 2023). Sellappan et al. (2022) developed nanobiosensor to early detection and prevention of agricultural crops from harmful microorganisms. Using specific nanoparticles as nano-sensors to detect the plant pathogen early can reduce the plant disease damage and help in proper management of the disease (Khan and Rizvi 2018).

20.4 Conclusion and Future Perspectives

In addition to acting as a safer alternative to toxic chemical pesticides in citrus, biological control is crucial in the management of nematode infections. Although several fungi, bacteria, and mites have been used in citrus, a dedication to the development of high-quality products, extension programs, and collaboration between researchers, farmers, and industry will more strongly advocate the use of biological control agents against plant nematodes in citrus. To create cultivars with long-lasting resistance, major resistance genes or quantitative trait locus (QTLs) must be introgressed alongside low-impact QTLs. The next goal is to precisely identify these low-effect QTLs. This suggests that to acquire a high heritability trait, all resistance testing must be taken into account. Due to the availability of complete genome sequences for the major crops, nematode resistance genes can be found, localized, diagnosed, and cloned, a goal that is likely to be accomplished shortly. This will give breeders a flexible tool for precise resistance breeding.

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Spiral and Other Minor Ectoparasitic Nematodes in Agricultural Crops and Their Biomanagement

21

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Abstract

Ectoparasitic nematode damage to agricultural crops has received little attention from researchers due to difficulty in estimating individual nematode impacts. This is because the degree of damage depends on the nematode pathotype, crop species, nematode population densities, nematode management practices, soil attributes, and climatic conditions. Furthermore, damage symptoms and effects are sometimes mistaken for those caused by drought, nutrient deficiencies, and other factors, and are not necessarily immediately associated with the nematodes. Limited investigations conducted so far have revealed damage to important crops such as maize, soybean, cowpea, sugarcane, rice, sugarcane, grapevine, sugar beet, potato, yam, strawberry, bermudagrass, woody vines, and vegetables, with *Xiphinema, Trichodorus, Paratrichodorus, Helicotylenchus, Tylenchorhynchus,* and *Belonolaimus* being the most economically important ectoparasitic nematode genera. Generally, impacts on crops may also be challenging to predict or grossly understated as plant-parasitic nematodes (PPNs) may damage plants in several ways. These include direct feeding action characterized by root galls, root stunting, or direct injury. In specific, ectoparasitic nematodes primarily injure plants by interacting with other soil pests namely fungi, bacteria, and other PPNs developing disease complexes through synergistic relations. Notably, *Longidorus* spp., *Xiphinema* spp., *Paratrichodorus* spp., *Paralongidorus* spp., and

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Trichodorus spp. can transmit some significant plant viruses. This chapter thus aims to highlight the threat posed by ectoparasitic nematodes to the agricultural crops including legumes/pulses, cereals and tubers as well as their potential management strategies.

Keywords

Ectoparasitic nematodes · Nematode biomanagement · Spiral nematodes

21.1 Introduction

There is a wide variety of interactions between plant parasitic nematodes (PPNs) and their hosts. Each of the parasitic forms has hollow, protruding stylets or spears with protruding points that are used to penetrate cells for feeding (Khan 2008). It is important to note that some nematodes are migratory ectoparasites, meaning they do not enter the host but feed on roots when they encounter them as they migrate through the soil. The PPNs are categorized into three major groups namely ectoparasitic, endoparasitic, and semi-endoparasitic nematodes depending on their feeding habits (Decraemer and Hunt 2013; Khan and Jairajpuri 2010; Smant et al. 2018). The ectoparasitic, endoparasitic, and semi-endoparasitic nematodes together represent 14% of the global crop yield losses, which is projected at the US $173 billion per year (Gamalero and Glick 2020; Mesa-Valle et al. 2020). It has been hypothesized that the PPNs mode of feeding began from an ectoparasitic to an endoparasitic and from a migratory to sedentary life cycle (Luc 1987). This has been confirmed using evidence based on small subunit rDNA sequence data analysis (Holterman et al. 2009).

An ectoparasitic nematode develops its life cycle completely outside the plant, and in order to feed, it perforates different host plant tissues, such as epidermis, root cortex, root hairs, and vascular tissues with the stylet thereby feeding on the cytoplasm (Khan 2023). The feeding depth is generally determined by the length of the stylet. As a result, the damage is often denoted by necrosis of cells penetrated by the stylet. Ectoparasitic nematodes that feed on meristematic cells in the root tip cause the most damage. After feeding, they then shift to another plant or another feeding location of the root. They primarily occur in the soil but they can also be found in stems, leaves, seeds or flowers. Compared to endoparasitic nematodes, individual ectoparasitic nematodes induce relatively lesser damage to the host plant tissues due to their feeding lifestyle (Holbein et al. 2016). Jones et al. (2013), for instance, highlighted that ectoparasitic nematode, *Xiphinema* spp., parasitized different important crops such as figs and grape vine.

There are limited or no studies that have revealed the direct effects of ectoparasitic nematodes on important crop plants; however, there are recent studies involving stunt, stubby root, and dagger nematodes addressing this issue (Abdulsalam et al. 2021; Sikora et al. 2018a; Sikora and Fernandez 2005). Economically important ectoparasitic nematode genera namely *Trichodorus, Xiphinema*, and

Paratrichodorus serve as vectors of some crucial crop viruses (Khan and Sharma 2020). It is well-known that some Longidoridae and Trichodoridae species transmit nepo- or tobraviruses, respectively. Sikora and Fernandez (2005), for instance, recorded yield losses in vegetables grown in sandy soils due to these nematodes. They also parasitize many plant species such as potatoes and weeds by attacking root tips causing galls on root hairs and other root tissues. Most nematode families consisting of the ectoparasites including Longidoridae and Trichodoridae are categorized by low reproduction rate, long fecundity, and low number of offspring.

21.2 Ectoparasitic Nematodes

Ectoparasitic nematodes attack a wide array of plant species, including monocots as well as dicots, which is the main reason for restricting the production of most important crops, causing moderate to significant crop yield loss in the world each year. They constitute a major limiting factor for the production of cereals, vegetables, and tuber crops by directly attacking the plant roots, preventing the uptake of water and nutrients, which lowers agronomic performance, overall quality, and yields (Sikora et al. 2018a).

Although the list of 17 ectoparasitic nematode genera in this book chapter are representative of the major genera, it might not include all ectoparasitic nematode genera of economic significance in agricultural crops (Table 21.1). Some genera, like *Trichodorus, Tylenchorhynchus*, and *Helicotylenchus*, are well-represented on the list with more than three species each. These genera are viewed as having economic significance, which is evident in the fact that some of these genera have had numerous publications on their species (Abdulsalam et al. 2021; Sikora et al. 2018a, b). It is also challenging to anticipate the possible impacts of many less well-known species owing to the limited information on their economic value and pathogenicity. While there are over 100 confirmed species in genera *Xiphinema*, *Hemicycliophora,* and *Tylenchorhynchus* (Siddiqi 2000; Singh et al. 2013), only a small number of these species have had their economic value studied.

21.3 Damages and Threats They Pose

A common warm-season turfgrass, bermudagrass, in Florida is seriously damaged by the sting nematode (*Belonolaimus longicaudatus*). As an example, Fig. 21.1 shows injury on the artichoke root system due to the sting nematode. Previously, chemical methods have been used to control such nematode pests, for instance, the use of 1,3-Dichloropropene at the rate of 46.8 L/ha reduced the abundance of *Belonolaimus longicaudatus* (Crow et al. 2003).

Bermudagrass, *Cynodon dactylon* L. and *Cynodon hybrids*, in the southeastern U.S. are susceptible to the sting nematode *Belonolaimus longicaudatus* (Perry and Rhoades 1982). The grass can also suffer considerable root reduction from

Table 21.1 List of economically important ectoparasitic nematodes in agriculture

S/ no	Common names	Scientific names	Important crops	Reference
1	Stubby root nematodes	*Trichodorus* spp., *Trichodorus cedarus, Trichodorus cylindricus, Trichodorus primitivus, Trichodorus similis, Trichodorus viruliferus*	Potato, sugar beet, wheat, rye, maize, barley, strawberry, spinach, tobacco, and apple	Sikora et al. (2018a), Sikora and Fernandez (2005), Singh et al. (2013)
2	Stubby root nematodes	*Paratrichodorus* spp., *Paratrichodorus allius, Paratrichodorus anemones, Paratrichodorus minor, Paratrichodorus porosus*	Barley, wheat, rice, sorghum, cowpea, vegetables, apple, potato, cowpea, sugarcane, eggplant, cotton, and cranberry	Sikora and Fernandez (2005), Singh et al. (2013)
3	Dagger nematode	*Xiphinema* spp., *Xiphinema americanum, Xiphinema basiri, Xiphinema brasiliense, Xiphinema brevicolle*	Tomato, soybean, cowpea, vegetables, citrus, woody vines, and many annuals, e.g., grapevine and fig	Jones et al. (2013), Singh et al. (2013)
4	Stunt nematodes	*Tylenchorhynchus agri, Tylenchorhynchus* spp., *Tylenchorhynchus annulatus, Tylenchorhynchus clarus, Tylenchorhynchus claytoni*, *Merlinius* spp., *Bitylenchus* spp., *Amplimerlinius* spp., *Quinisulcius* spp.	Maize, rice, sugarcane, wheat, cotton, tobacco, and sorghum	Abdulsalam et al. (2021), Singh et al. (2013)
5	Spiral nematodes	*Helicotylenchus* spp., *Helicotylenchus dihystera, Helicotylenchus multicinctus, Helicotylenchus microcephalus, Helicotylenchus vulgaris*	Yam, maize, sorghum, millet, rice, wheat, tomato, eggplant, banana, and sugar beet	Abdulsalam et al. (2021), Sikora et al. (2018a), Singh et al. (2013)
6	Sting nematodes	*Belonolaimus* spp., *Belonolaimus gracilis, Belonolaimus longicaudatus Ibipora* spp.	Maize, soybean, potato, turfgrass, citrus, cucurbit, strawberry, peanuts, and root vegetables	Mandal et al. (2021), Stirling et al. (2013)
7	"Awl" nematodes	*Dolichodorus* spp., *Dolichodorus heterocephalus*	Maize, lettuce, cotton, beans, cabbage, potato, celery, tomato, spearmint, carrots, pepper, and turfgrass	Crow and Brammer (2018), Geraert (2011)

(continued)

Table 21.1 (continued)

S/no	Common names	Scientific names	Important crops	Reference
8	Ring nematodes	*Criconemella* (formerly; *Criconema* and *Criconemoides*)	Peach tree	Nyczepir et al. (1985)
9	Sheathoid nematodes	*Hemicycliophora* spp., *Hemicycliophora arenaria* Raski, *Hemicycliophora poranga*, *Hemicycliophora similis, Hemicriconemoides cocophillus, Hemicriconemoides litchi, Hemicriconemoides mangiferae*	Maize, millet, rice, sorghum, tomato, carrots, citrus, mango, and litchi	Babatola (1984), Singh et al. (2013)
10	Seed gall nematodes/ ear cockle nematodes	*Anguina* spp.	Wheat, rye, and barley	Mandal et al. (2021)
11	Rice stem nematode	*Ditylenchus angustus*	Rice	Peng et al. (2018)
12	Lance nematodes	*Hoplolaimus* spp.	Corn, sugarcane, cotton, and alfalfa	Koenning et al. (1999)
13	Needle nematodes	*Longidorus* spp., *Paralongidorus* spp.	Sugar beet, and grapevines	Malik et al. (2022), Mitiku (2018)
14	Pin nematodes	*Paratylenchus* spp.	Parsley, celery, peas, lentils, and pine tree	Kantor et al. (2021), Singh et al. (2013)
15	Spiral nematodes	*Rotylenchus* spp.	Pine, olives, peas, carrots, and lettuce	Singh et al. (2013), Vovlas et al. (2008)
16	Citrus nematodes	*Tylenchus* spp.	Maize, millet, rice, and sorghum	Abdulsalam et al. (2021), Jibrin et al. (2014)
17	Foliar nematodes	*Aphelenchoides* spp.	Chrysanthemum, strawberry, begonia, coconut, and rice	Davis and Nendick (2014)

B. longicaudatus (Giblin-Davis et al. 1992). This leads in nutrient and water stress, which in turn causes significant damage and loss of grass.

A study by Aryal et al. (2016) revealed rotating *Bacillus firmus* strain I-1582, abamectin, furfural and *Brassica juncea* markedly regulated nematode population levels when compared with the 1,3-dichloropropene (standard nematicide) and the integrated pest management (IPM) program.

Fig. 21.1 Sting nematode symptoms on the artichoke root system. Note stunted, matted, and necrotic (brown, dying tissue) root system as well as lateral root pruning and proliferation. The shoot is also severely stunted. (Credits: Photograph courtesy of Zane Grabau, UF/IFAS)

Dolichodorus spp., also known as awl nematode, was first described in 1914 from samples obtained from Florida, Douglas Lake, Silver Springs, and Michigan in the USA. There are many *Dolichodorus* species globally, but the two most prevalent in Florida are *D. heterocephalus* and *D. miradvulvus*. Awl nematodes typically occur in damp to wet places in the field such as near freshwater sources and next to irrigation canals. In this regard, these nematodes are less well-studied and less commonly found in the cultivated crop fields as compared with other PPNs. Similar to the sting nematode (*Belonolaimus longicaudatus*), awl nematodes can cause damage to multiple crops such as cotton, beans, potato, and corn by causing root stunting, hence poor yields (Crow and Brammer 2018). Further, their damage results in the root system being completely depleted, which severely stunts the entire plant. The roots frequently have stubby, coarse tips, and the few remaining secondary roots are also stubby.

Awl nematodes frequently signal an abundance of soil moisture. In some instances, lowering irrigation or improving drainage may help to lessen or even resolve problems caused by this nematode. When top-dressing agricultural fields or creating planting beds, dirt dug up from ditches, ponds, or other water sources may contain awl nematodes.

21.4 General Biocontrol Strategies for Nematode Parasites of Crops

Nematicides are an effective way to manage nematodes, but over the past two decades, most known nematicides have been considered hazardous to both humans and the environment, leading to a search for an environmentally friendly, economically feasible alternative. The new common nematode control strategies include the use of biocontrol agents, and plant-based products among others (Khan 2016). The term biocontrol refers to those natural living enemies that are deliberately used in pest management to reduce target nematode pest populations. Such include predaceous and parasitic fungi, plant growth-promoting rhizobacteria (PGPR), endoparasitic bacteria, predaceous mites, predatory nematodes, and arbuscular mycorrhizal fungi (AMF). There a number of biocontrol fungi/bacteria which can suppress soil nematodes, and their application either alone (Stirling 1991; Khan 2007; Khan and Anwer 2011), or along with oil, neem cakes (Sikora and Roberts 2018; Khan et al. 2021) or pesticides (Mohiddin and Khan 2013) is getting popularized in achieving sustainable nematode management in agricultural crops (Khan 2023; Khan et al. 2023). The microbial antagonists, *Aspergilus niger, Pochonia chlamydosporia, Purpureocellium lilacinum, Pasturia penetrans* etc. (Jatala 1986; Stirling 1991; Kerry 2000; Khan 2016), and phosphate solubilizing microorganisms such as *Aspergillus, Bacillus, Penicillium, Pseudomonas* etc. (Khan et al. 2009, 2016a, b; Sikora and Roberts 2018) may significantly contribute in the sustainable management of plant nematodes. The well known mycoparasitic fungus, *Trichoderma* has also been found effective in suppressing plant nematodes (Mohiddin et al. 2010; Khan and Mohiddin 2018), and numerous formulations of *T. harzianum*, *T. hamatum* etc. of are available in market (Khan et al. 2011), and provide consistently satisfactory control of soil-born pathogens (Mohammed and Khan 2021; Sikora and Roberts 2018; Shahid and Khan 2019).

21.5 Management of Nematode Parasites of Legumes/Pulses

The major food legumes, including soybean, chickpea, cowpea, pigeon pea, and common bean, among others are parasitized by various PPN species. The most harmful PPN genera of legumes include endoparasites; *Meloidogyne* spp., *Heterodera* spp., *Ditylenchus* dipsaci, *Pratylenchus* spp., the semi-endoparasites; *Rotylenchulus* spp., and the ectoparasites; *Helicotylenchus* spp. and *Tylenchorhynchus* spp. (Askary 2017; Sikora et al. 2018b). During parasitism, PPNs inhibit rhizobium root nodulation and nitrogen-fixing activity in some legumes, resulting in a reduction in grain quality and yield. The PPNs may also associate with other soil-borne pathogens such as fungi and bacteria in attacking pulse crops further exacerbating pathogen disease severity. Worldwide, PPNs cause crop losses valued at the US $100–173 billion a year (Gamalero and Glick 2020). Different integrated approaches to manage PPNs such as cultural, chemicals, botanical, host plant resistance, sanitation, crop rotation, and biopesticides are commonly used due to their low-cost and high effectiveness (Roopa and Gadag 2020). In this section, the biomanagement of PPNs, particularly ectoparasitic nematodes in major food legumes in the field, is discussed in detail.

21.5.1 Soybean (*Glycine max* L.)

Soybean is a significant oil seed crop globally, with Brazil being the leading soybean producer country with a production volume of about 138 million metric tons in the year 2020/2021. Per region, the Americas, Asia, Europe, and Africa accounted for 85.7, 11.5, 2.1, and 0.7% of the total world production of soybeans, respectively (FAOSTAT 2020). The crop is a crucial source of food, oil, animal feed, protein, and other purposes, including the manufacture of lubricants, plastics, soaps, biodiesel, and candles (Pagano and Miransari 2016). Among the soybean problems, PPN represents a major constraint for the production of soybean in the world. About 30–100% yield losses of soybean have been reported in previous studies (Barker 1998), relying on factors such as cropping systems, region, soil attributes, pathogenic levels, cultivar susceptibility, and temperatures. The most economically important spiral and ectoparasitic nematodes of soybean include *Helicotylenchus* spp., *Hoplolaimus* spp., *Helicotylenchus dihystera, Belonolaimus* spp., *Aphelenchoides* spp., and *Tubixaba tuxaua* (Lima et al. 2017).

In Brazil's soybean fields, *Helicotylenchus dihystera, Aphelenchoides* spp*.,* and *Tubixaba tuxaua* have been recorded in unusually high abundance, in which pronounced characteristics of nematode infection have been observed on infected plants (Favoreto et al. 2015; Furlanetto et al. 2010). The species *Tubixaba tuxaua* was first described as a possible pest of soybean roots by Monteiro and Lordello (1980). With time, this nematode has been found to parasitize soybeans in the state of Tocantins and in four municipalities in Maranhao state (Lima et al. 2009; Machado 2014). Furlanetto et al. (2010) reported soybean damage due to *Tubixaba tuxaua* under field conditions in Parana state. Currently, the real association between *Tubixaba tuxaua* and soybean remains elusive although its damage is evident as depicted in Fig. 21.2. *Helicotylenchus* spp., a polyphagous spiral nematode, have been recorded in high occurrence and population levels on soybean fields in Brazil and South Africa (Fourie et al. 2015; Machado 2014). *Helicotylenchus dihystera* occurred at a frequency of 85% of the samples collected from different soybean cultivars in the Acre state (Sharma et al. 2001). It has been found to cause stunted growth in soybean, although direct damage requires quantification. Other important ectoparasitic nematodes reported in association with soybean include *Xiphinema* spp., *Trichodorus* spp., *Tylenchorynchus brevilineatus*, *Rotylenchus* spp., *Hemicycliophora* spp., *Hoplolaimus* spp., and *Belonolaimus* spp. (Durand et al. 2012; Fourie et al. 2015; Machado 2014).

21.5.1.1 Control

Literature especially on soybean about host resistance, biopesticides, and management of nematodes is urgently needed in order to identify whether these ectoparasitic nematodes possess a real threat. Due to a lack of information regarding the management of soybean ectoparasitic nematodes, previous work has noted with concern the need to establish the real abundance, pathogenicity, and diversity of nematodes in soybeans and the ways to manage them (Lima et al. 2017).

Fig. 21.2 Damaged soybean field by *Tubixaba tuxaua*. (Credits: Photograph courtesy of Neucimara Rodrigues Ribeiro)

21.5.2 Chickpea (*Cicer arietinum*)

Chickpea is an ancient pulse crop, originally cultivated around 7000 BC in Turkey. Presently, it is now commonly grown in India and the Mediterranean region. After bean *(Phaseolus vulgaris)* and pea *(Pisum sativum),* chickpea is ranked third accounting for 11.67 million tons annually. It provides a cheap source of protein, carbohydrates, minerals, and vitamin B.

The crop is attacked by several PPNs (around 100 species) causing 13.7% yield losses (Abd-Elgawad and Askary 2015). The main ectoparasitic nematodes related to chickpea are *Hoplolaimus* spp., *Tylenchorhynchus* spp., *Helicotylenchus* spp., *Xiphinema* spp., and *Longidorus* spp. The obligatory root ectoparasitic nematode, *Tylenchorhynchus* spp., was reported in India, Syria, Morocco, Tunisia, Netherlands, and Spain in relation with chickpea (Askary 2017). Compared to chickpea endoparasitic nematodes, *Tylenchorhynchus* spp. are considered of less economic importance because their pathogenicity impact on chickpea has not been studied. In Mediterranean countries, *Hoplolaimus* spp. and *Helicotylenchus* spp. have been recorded in fields with chickpeas (Sikora et al. 2018b). *Longidorus* spp. and *Xiphinema* spp. act as a vector in transmitting viruses in chickpea.

21.5.2.1 Control

The use of nonplant hosts such as wheat and barley in all cropping sequences in Pakistan reduced the population of *Tylenchorhynchus annulatus*, resulting in a 10–15% increase in chickpea yield. Sikora et al. (2018b), however, opined that stringent control measures particularly for these ectoparasitic nematodes need to be developed.

21.5.3 Pigeon Pea (*Cajanus cajan*)

Pigeon pea is an important food grain legume grown mostly in developing countries in Asia and Africa. The biggest producer of pigeon pea, India, contributes around 67% of the pigeon pea produced globally. Among the other producers, Kenya, Tanzania, and Malawi contribute about 4.6, 5.3, and 6.3%, respectively (Rawal and Navarro 2019). Many PPN species have been identified to parasitize pigeon pea with their impact ranging from affecting the physiological functioning of the plant to the extent of declining yield. Abd-Elgawad and Askary (2015) estimated yield losses in pigeon pea to the extent of 13.2% annually in the world. The major PPNs reported together with pigeon pea include *Heterodera* spp., *Meloidogyne* spp., and *Rotylenchulus* spp. (Sikora et al. 2018b). Ectoparasitic nematodes such as *Tylenchorhynchus* spp., *Helicotylenchus* spp., *Tylenchus* spp., and *Hoplolaimus* spp. have been recorded in pigeon pea fields in India, Kenya, and Jamaica as significant nematode pathogens (Maina et al. 2022; Sharma and McDonald 1990; Singh 2015).

21.5.3.1 Control

In general, ectoparasitic nematodes are highly susceptible to summer plowing which expose and break their reproduction cycle. Their soil population levels can also be reduced by cultural practices such as the application of organic amendments, crop rotation, and clean cultivation (Khan 2015).

21.5.4 Common Bean/Haricot Bean (*Phaseolus vulgaris* L.)

Common bean remains a commonly grown pulse crop in the word. It is mainly grown in Europe, the Americas, Asia, and Africa, of which, Asia is the biggest producer of common beans accounting for almost half of the world's total production. Since *Phaseolus* spp. are highly sensitive to low temperature, they are primarily cultivated during the warm season and thus often become a suitable host of nematodes that prefer higher temperature ranges. Most ectoparasitic nematodes of common beans are *Hemicycliophora* spp., *Helicotylenchus* spp., *Tylenchus* spp., *Aphelenchus* spp., *Tylenchorhynchus* spp., and *Trichodorus* spp. (Askary 2017).

Helicotylenchus spp., *Tylenchorhynchus* spp., *Tylenchus* spp., *Hemicycliophora* spp., and *Criconemella* spp. have been reported in association with common beans in Kenya and in North and South Carolina (Karanja et al. 2002; Ye 2018). The pathogenicity of these ectoparasitic nematodes on common bean plant growth remains unknown although their impacts on other crops have been demonstrated (Khan 2015).

21.5.4.1 Control

Crotalaria juncea intercrop with banana reduced the population densities of *Helicotylenchus multicinctus* and *Hoplolaimus indicus* compared to the carbofuran treatment, causing an increase in banana yield (Wang et al. 2002). In another study, organic material application, including chicken manure and tagetes regulated *Meloidogyne* spp. and improved bean growth (Kimenju et al. 2004). Some bacterial complex treatments such as *Bacillus amyloliquefaciens* FR203A and *Pseudomonas fluorescens* FP805PU suppressed reproduction and disease development of *Xiphinema* spp. than the untreated control (Migunova and Sasanelli 2021).

21.6 Management of Nematode Parasites of Cereals

21.6.1 Effects of Nematode Parasites on Cereals

In general, farmers cultivate important cereal crops like rice, wheat, maize, oats, barley, sorghum, millet, etc. in most parts of the world, yet more or less than $80 billion has been predicted to be lost each year owing to PPNs globally. The impacts of nematodes are huge on the food chain in the tropical and subtropical ecology (Bernard et al. 2017; Sikora et al. 2018a). Here are a few effects of nematodes on rice, wheat, and maize, and the biomanagement of ectoparasitic nematodes affecting cereals is discussed below.

21.6.1.1 Rice (*Oryza* spp.)

Rice is the most commonly consumed food globally by almost half the population, with Asia growing and consuming >90% of the total rice worldwide. Currently, rice is grown on around 159 million hectares (Mha), yielding 700 million tons of paddy or 470 million tons of milled rice in the world.

Plant-parasitic nematodes, more than 100 different nematode species, affect rice production. Among the PPNs, *Meloidogyne* spp., *Hirschmanniella* spp., and *Heterodera* spp. are important endoparasitic nematodes of rice in temperate and tropical regions (Sikora et al. 2018a; Khan et al. 2023). Stunting, fewer tillers, hook-shaped galls, and poor growth and reproduction are all symptoms of nematode-infected rice (Pokharel et al. 2007; Sikora et al. 2018a). These PPNs are known to cause serious yield loss and are mostly found in both upland and irrigated rice production systems (Mandal et al. 2021; Sikora et al. 2018a). There are numerous PPNs of rice in addition to those already mentioned, although few ectoparasitic nematodes (*Ditylenchus angustus, Aphelenchoides besseyi, Criconemoides onoensis, Paralongidorus australis*, and *Xiphinema ifacolum*) have been associated to damage and are of economic significance (Peng et al. 2018).

21.6.1.2 Wheat (*Triticum* spp.)

There are several PPNs associated with wheat and barley (*Hordeum vulgare*), although only a small number are thought to be economically significant: (a) *Heterodera* spp.; (b) *Pratylenchus* spp.; (c) seed gall (*Anguina tritic*i); (d) root-knot (*Meloidogyne* spp.); and (e) *Ditylenchus dipsaci* nematodes (Owen et al. 2023). The *Heterodera avenae* group of *Heterodera* spp. reduces wheat yields as well as that of barley and oats. Losses in wheat productivity are also caused by *Anguina tritici, Pratylenchus neglectus*, and *Pratylenchus thornei*.

Other ectoparasitic nematode pests, including *Merlinius brevidens, Tylenchorhynchus* spp., *Litylenchus* spp., *Longidorus elongatus,* and *Paratrichodorus* spp., may also reduce wheat yield in many parts of the world like Nigeria, although details regarding their global distribution and destructive capabilities are yet unknown (Abdulsalam et al. 2021; Dababat and Fourie 2018). In some areas of India and the USA, poor growth is attributed to *Tylenchorhynchus nudus, Tylenchorhynchus vulgaris,* and *M. brevidens*. Wheat crops in the USA are similarly vulnerable to *Paratrichodorus anemone* and *Paratrichodorus minor* (presently known as *Nanidorus minor*). In particular, it has been outlined that wheat cultivated on sandy soils early in the autumn is highly vulnerable to *Paratrichodorus minor*.

21.6.1.3 Maize (*Zea mays* L.)

Maize is a crucial cereal food crop, which is used as both human food and as feed for animals. According to data from the FAO (2016), the average annual production of maize has surpassed 900 Mt., outpacing that of paddy rice and wheat during the 2010–2014 period. The USA is the largest producer, followed by Asia (30%), South America (12%), and Africa (8%).

Plant nematodes significantly reduce crop development and increase crop loss during the dry periods and other stressful situations (Coyne et al. 2009; Lopez-Nicora et al. 2023). Prior to this, maize was considered as nonpoor host for several PPNs species, perhaps as a result of yield losses going undetected because of broad root systems, insufficient management efforts (Koenning et al. 1999), or a lack of characteristic symptom (McDonald and Nicol 2005). Although, due to the widespread use of maize in rotation systems and the potential impact of nematode parasitism, it is crucial to understand the crop's status as a host to significant nematode pests. Many nematode species are associated with maize around the world, but there is generally little knowledge about their biology and pathogenicity (Sikora et al. 2018a). *Meloidogyne, Pratylenchus*, and *Heterodera* species are the three most significant genera of PPNs in terms of global economic importance. According to Koenning et al. (1999), the most common genera found on maize in the USA are *Pratylenchus, Meloidogyne,* and *Hoplolaimus*, whereas in South Africa, Nigeria, and Kenya, *Meloidogyne* and *Pratylenchus* are most prevalent (Abdulsalam et al. 2021; Maina et al. 2019; McDonald et al. 2017). Other significant ectoparasitic nematode pests of maize include *Aphelenchoides* spp., *Paratrichodorus* spp., and *Longidorus breviannulatus* (Mary et al. 2013; Nicol et al. 2011).

21.6.2 General Management Practice of PPNs in Cereal Fields

21.6.2.1 Cultural Practice

The use of resistant crop cultivars, crop rotation, and application of organic soil amendments are some of the commonly applied nematode management techniques. In addition, cover crops can control PPNs density as a component of IPM. Bernard et al. (2017) demonstrated cover crops namely *Crotalaria spectabilis* and *Mucuna pruriens* being resistant to various species of *Meloidogyne*. Additionally, *Xiphinema* and *Trichodorus* abundance in maize-cover crop intercrop were regulated by use of cover crops and tillage systems (Jibrin et al. 2014). Crop rotation involving maize, asparagus, garlic, and onion helps to control root-knot nematode (RKN) infestation.

Continuous cultivation of suitable hosts to *P. minor* such as maize and sorghum can rapidly increase their number to damaging levels. Conversely, growing poor hosts, for instance, cowpea and velvet may help to reduce *P. minor* population densities, hence minimizing reliance on nematicides (Crow 2017). Resistant crops to RKN include velvet beans, rye, and *Crotalaria*. Moreover, allelochemicals like dhurrin can be transformed to hydrogen cyanide and employed as potent nematicides (Mandal et al. 2021). A single antagonistic crop, such as marigold, can reduce up to 14 PPN genera such as *Meloidogyne* spp. (Mandal et al. 2021).

21.6.2.2 Biological Control

Biocontrol is delineated as the involvement of beneficial organism genes or their various products, which aid in alleviating adverse effects on plants and enhancing favorable effects. On the other hand, biopesticides are described as “the products intended to protect the plants made from living organisms or natural substances from species co-evolution, not produced by chemistry and use of which is recommended for control of pests or bio-aggressor for a better response of the biocenosis and environment” (Villaverde et al. 2014). In specific, *Pasteuria penetrans, Paecilomyces lilacinus*, *Bacillus subtilis,* and organic amendments such as neem seed powder and manure are regarded as primary biocontrol agents for various PPNs (Mandal et al. 2021). Additionally, the decomposition of complex nitrogenous and organic materials present in animal and plant manure by soil microbes can reduce PPNs influence. The input of these compounds also improves levels of soil fertility and microflora (Agbenin 2011).

21.6.2.3 Host Plant Resistance Method

Previous work has established a distinction between the several natural gene types that are used to produce nematode resistance in plants. Nematode resistance plant conventional breeding programs have shown limited success. However, RNA interference (RNAi) technology has been regarded as one of the most effective methods in PPNs regulation (Tamilarasan and Rajam 2013). The two main categories of nematode resistance, active and passive resistances, involve the association between nematode resistance and host plant. The nematode infestation is impacted by passive resistance in terms of anatomical, physiological, and chemical barriers. The nematode dies as a result of the necroses that grow around it as a result of active resistance's histological alteration (Mandal et al. 2021). The HS1pro1 gene confers resistance against the sugar beet cyst nematode, while in tomatoes, the Mi-1.2 gene provides resistance against various RKN species. Additionally, the GPa2 gene exhibits resistance to *Globodera pallida* (Briar et al. 2016). The nematode's mobility to establish a favorable host is affected by different metabolites such as methyl salicylate and limonene, among others (Sikder and Vestergård 2020).

21.7 Management of Nematode Parasites of Tubers

One of the most important food commodities is root and tuber crops. After maize, rice, and wheat, potato (*Solanum* spp. L.) is ordered fourth as the most imperative food crop in the world. It is grown across many continents, but is mostly grown in Europe, America, and Asia. The primary consumers are Asia, Europe, and the US, with Europe and Asia being the two largest producers, accounting for about 80% of global potato production. Nearly 400 million tons of potatoes are produced each year on an area of cultivation of 20 million hectares, and these are either consumed raw or processed (Lima et al. 2018).

Food security and availability are in more demand. Intensive planting, monocultures, and the extension of crops into recently opened areas are unsustainable crop production practices that have led to an increase in pest and disease issues (Abdulsalam et al. 2021).

In potato production, PPNs are a major biotic stress, causing reduced yield and deformities in potato tubers. Potato yield losses of up to 12% can be attributed solely to nematodes (Lima et al. 2018). Furthermore, potato yield losses at specific cropping systems due to PPNs rely on several variables, such as cultivar, climatic conditions, crop growth duration, and soil properties (Niere and Karuri 2018).

Multiple PPNs species are known to attack potatoes, of which some induce huge yield losses whereas others may only induce minimal damage and are significant locally. *Globodera rostochiensis* and *G. pallid*a are the two globally significant PPNs for potato cultivated in temperate zones. These are the principal nematode species associated with potatoes. Additionally, *Meloidogyne* spp., *Pratylenchus* spp., *Nacobbus aberrans*, and *Ditylenchus destructor* can greatly reduce potato yields (Lima et al. 2018; Niere and Karuri 2018). Other minor ectoparasitic nematode species, for example, *Xiphinema* spp. and *Hoplolaimus galeatus* (Cobb), among others, can pose a threat to potato fields depending on the nematode growth-favoring conditions. Research on ectoparasitic nematodes in areas where crops will be planted is crucial because pathogens like PPNs represent significant losses in various agricultural crops under different cropping systems, particularly when the crops are managed unsustainably. This chapter goes into details regarding the biomanagement of PPNs, in particular the ectoparasitic nematodes (*Trichodorus* and *Paratrichodorus* spp.) that are prevalent in the field's main dietary potato tubers.

The family Trichodoridae (Thorne) contain genera *Paratrichodorus* and *Trichodorus* spp. (Trichodorids). This family's nematodes consist of several important PPNs that compose of 5 genera and almost 100 known species. These are ectoparasites that typically gather around the terminals of the roots. During feeding, they use onchiostyle to puncture plant cells such as root meristem cells. Their direct feeding may stimulate enlarged roots and atrophy, early senescence, and stunting, which is referred to as "stubby root."

Trichodorids are widespread worldwide, while certain species are localized to a specific area. Globally, sand-based soils are where *Trichodorus* spp. are most frequently found. They are regarded as a major potato nematode in the tropics and subtropics, and they also feed on monocots and dicots. However, their distribution, extent of the damage, and monetary losses under field conditions have not been determined.

Despite the role of these nematodes as plant parasites, they are also economically important pests to agricultural crops including potatoes due to their ability to transmit and spread certain plant viruses. *Trichodorus* spp., for example, could spread viruses such as Tobacco Rattle Virus (TRV) to different cultivars of potato, which causes the corky-ring spot disease in potatoes. After feeding on the infected plants, *Trichodorus* spp. can carry the virus with them for around 4 months after which it is shed from the nematode. Potato plants that have been infected with TRV are characterized by symptoms such as chlorosis, necrosis, stunting, brittle tissues, and eventually low-quality yields (Lima et al. 2018).

Other economically significant potato nematodes in specific places, such as some regions in the USA include *Tylenchorhynchus claytoni*, *Xiphinema* spp., *Helicotylenchus pseudorobustus*, and *Hoplolaimus galeatus*, among others (Table 21.1). *Belonolaimus longicaudatus* exhibits the most important impact among these ectoparasitic nematode species (Lima et al. 2018). However, these other nematode species may also have standalone significance.

21.7.1 General Methods for Controlling PPNs in Potato Fields

Management of potato PPNs, especially ectoparasitic nematodes is challenging due to the complex biology of these nematodes, which includes their soil-dwelling habitat, short generation time, rapid population growth, and rapid multiplication. Additionally, only a small number of crop cultivars are antagonistic to them. The synthetic nematicides are either avoided because of their unfavorable impacts on humans and the environment or have a limited impact due to their interactions with soil components. The success of nematode management measures for potatoes, depends on meticulous planning. To maximize control effectiveness, it is advisable to use multiple control strategies (integrated management). The following information is necessary for effective nematode management: (a) accurate nematode species identification; (b) nematode yield losses quantification; (c) PPN biology; and (d) host range; among others (Lima et al. 2018).

In general, the following methods are employed to control potato PPNs: (a) prevention of nematode dispersal; such as cleaning of equipment and machinery;

(b) planting potatoes during a dry and cold season, which is less favorable to nematode reproduction; (c) quarantine policies for *G. pallida* and *G. rostochiensis*; and (d) elimination of diseased infested plants, and others (Niere and Karuri 2018; Sikora et al. 2018a). In addition, the nanotechnology can also offer satisfactory solutions for plant disease management (Khan and Rizvi 2014; Khan et al. 2019a, b, c) and disease detection/diagnosis (Khan and Akram 2020; Khan and Rizvi 2016; Khan et al. 2020). Nano-sensors are the most important product of nanotechnology, and have great potential for use in plant disease diagnosis (Khan 2023). Sellappan et al. (2022) developed nanobiosensor to early detection and prevention of agricultural crops from harmful microorganisms. Using specific nanoparticles as nanosensors to detect the plant pathogen early can reduce the plant disease damage and help in proper management of the disease (Khan and Rizvi 2018).

21.8 Conclusion and Future Perspectives

The limited amount of work done with ectoparasitic nematodes suggests that the activity of most species has rarely caught the interest of researchers in nematode problems. In spite of the fact that multiple ectoparasitic nematodes are emerging and pose a great risk to various crops, especially when in concert with other soil pathogens, hence rendering crops susceptible to weak pests. For example, in Brazil's soybean fields, *Helicotylenchus dihystera, Aphelenchoides* spp., and *Tubixaba tuxaua* have been recorded in unusually high abundance, in which pronounced characteristics of nematode infection have been observed on infected plants. Compared to endoparasites, these nematodes occur in the soil in great abundance. Several ectoparasitic nematode genera such as *Trichodorus*, *Xiphinema*, *Paratrichodorus*, *Paralongidorus*, and *Longidorus*, which are predominant in the rhizospheric soils of multiple agricultural crops, can transmit some significant viruses to crops causing huge yield losses. A major explanation why ectoparasitic nematodes have received little attention is because of the difficulty in estimating individual nematode impacts, whereby the degree of damage relies on the nematode pathotype and population densities, crop species, and climatic conditions such as soil attributes. In terms of nematode management strategies, some achievements in managing plant-parasitic nematodes (PPNs) have been documented with organic materials (chicken manure and neem seed powder), biocontrol agents (*Pasteuria penetrans, Paecilomyces lilacinus*, and *Bacillus subtilis*), and antagonists hosts (velvet, barley, and cowpea). Nevertheless, development of host plant resistance to ectoparasitic nematodes is still difficult because their feeding requirements are minimal as compared to the endoparasitic nematodes that usually spend more than half of their life cycle within the host. Future research should therefore explore and focus on the development of sound and effective holistic nematode biomanagement techniques integrating ectoparasitic nematodes along with other soil-borne pests namely fungi, bacteria, and other PPNs, which are currently lacking.

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Pine Wood Nematode in Coniferous Forests and Their Management by Novel Biological and Biotechnological Interventions

22

Yanan Zheng and Mujeebur Rahman Khan

Abstract

Coniferous trees constitute a major flora in the temperate forests world over. Although a number of nematodes have been recorded infesting coniferous trees, but pine wood nematode (PWN) is a most severe and serious nematode pest in temperate forests. The nematode, *Burshaphelenchus* spp., attacks Pinaceae family (*Pinus*, *Abies*, *Picea*, *Larix,* etc.). The PWN, *Bursaphelenchus xylophilus* may cause wilting and drying of a branch or the entire tree within a few months and it may die within a year or die in the next spring. The nematode is transmitted by the beetle, *Monochamus* spp. The nematode attacks *Pinus* spp. in a number of countries in all continents of the world, and inflicts wood loss of over USD 100 million annually. The present chapter describes detailed information on the pine wood nematode and novel methods of its management.

Keywords

Coniferous trees · *Burshaphelenchus* · *Monochamus* · Vector · *Pinus* · *Abies* · *Picea* · *Larix*

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22.1 Introduction

Forests are spread over around 38% of the world terrestrial having 95% naturally growing flora and 5% man-planted flora (Uprety et al. 2012). Major portion of the forests are tropical (47%), subtropical (9%), temperate (11%), and boreal (33%) (Ameray et al. 2021). On the basis of type of vegetation, the forests can be recognized as evergreen forests, deciduous forests, coniferous forests, boreal forests, etc. The coniferous forests occur in cooler regions of the globe (temperate climate) as well as hilly areas of tropics and subtropics where summers are warm and winters are cool, coupled with adequate rainfall (Fig. 22.1). The coniferous forests chiefly comprise cone-bearing or gymnospermic trees, which are the toughest and longest-living trees such as, pines, fir, spruces, cedars, etc. The leaves of such trees are small, scale-like or needle-like, and generally evergreen. The coniferous trees contain softwoods, and possess ability to survive well in acidic soils and cold temperatures. The average temperature in coniferous forest areas ranges from 40 to 20 °C during winter and from 7 to 21 °C during summer, and experiences long, snowy winters, and hot wet summers. The prevalent tree flora in coniferous forests are *Pinus* spp., *Picea* spp., and the shrub (*Juniperus communis*) as well as the deciduous trees such as *Alnus* spp., *Betula* spp., *Populus* spp., *Salix* spp., *Sorbus* spp., etc. (Engelmark et al. 1999).

The nematode infestation in forest flora has been the neglected and unexplored area of research, apparently due to distant and isolated location of forests coupled with cumbersome and risky accessibility. Further, it is a bit more confusing to

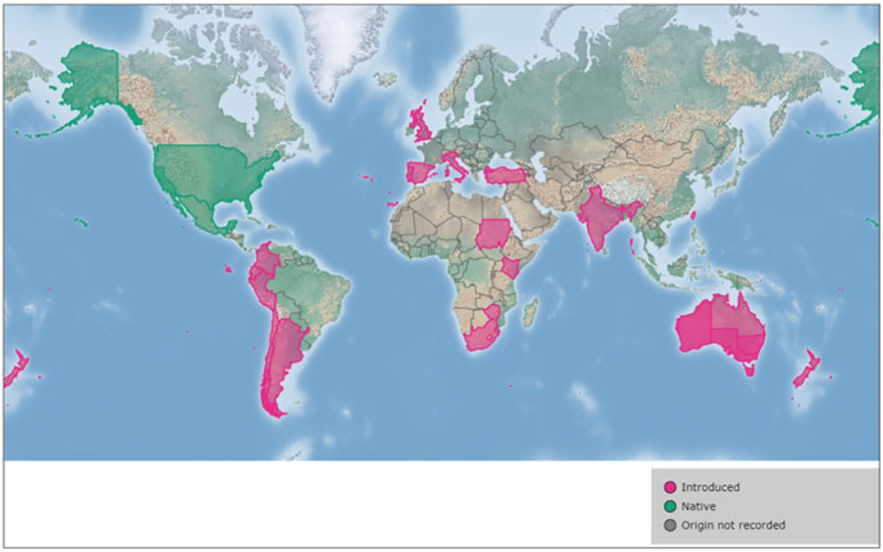

Fig. 22.1 Distribution of coniferous forest trees. (Source: CABI 2023. Pinus radiata. In: CABI Compendium. Wallingford, UK: CAB International)

recognize the nematode-infected forest trees as the symptoms are not so indicative as in the crop field. However, the symptoms of pine wilt are highly discernable, and can be easily recognized on forest trees even from a distant place (Khan 2020).

22.2 Distribution of Pine Wood Nematode

The pine wood nematode (PWN) also known as pine wilt nematode or timber nematode invades coniferous trees especially pines, and the nematode is widely distributed in the conifer forest in all continents of the world (Pimentel et al. 2023). The PWN belonging to the genus *Bursaphelenchus,* is a serious pest of gymnospermic forest trees (Netscher 1970), and its infestation in conifers forest is a serious issue of concern world over (Khan 2010). The nematode has been classified as a highly invasive nematode with a score of 14 out of 15 (Haque and Khan 2021). The genus *Bursaphelenchus* contains more than 50 species, among these around 75% invade the conifers (Braasch 2001). However, pathogenicity of only *B. xylophilus* and *B. mucronatus* is established, former being the most important species in this regard. All species of *Bursaphelenchus* have ancestral habit of being microbivorous (Khan 2008). The species which attack conifers need an insect vector for reaching the tree trunk.

The PWN is originated from the North America, from where it has spread to all continents of the world (Fig. 22.2). The nematode dose not invade native pine species in North America, rather, the invasion and the resulting damage occur to

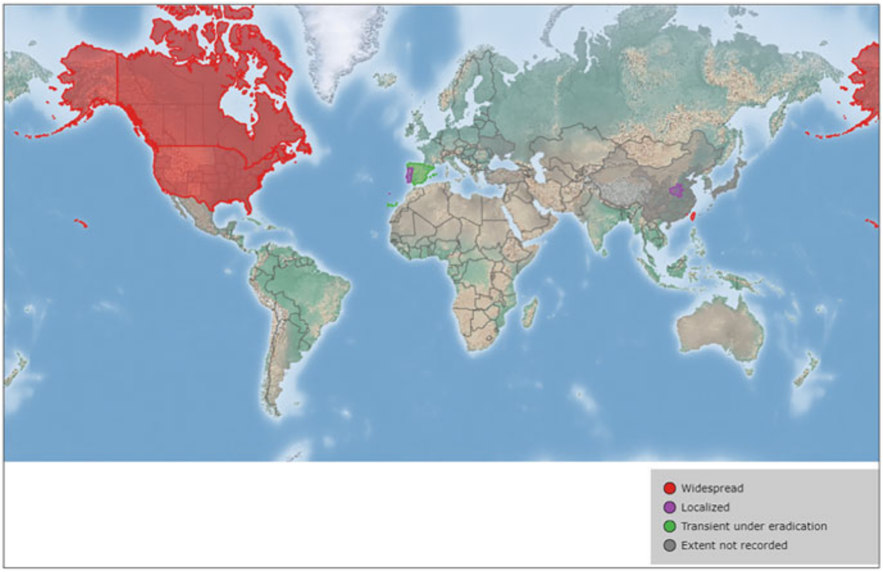

Fig. 22.2 Distribution of pine wood nematode. (Source: CABI 2023. Bursaphelenchus xylophilus. In: CABI Compendium. Wallingford, UK: CAB International)

Table 22.1 List of pine species susceptible to the infection by *Bursaphelenchus xylophillus* (Yang et al. 2003)

Susceptible pine species	Country
Pinus luchuensis, P. densiflora, P. thunbergii	Japan
P. densiflora, P. luchuensis, P. taiwanensis, P. thunbergii, P. massonia, *P. kesiya*, *P. yunnanensis, P. tabulaeformis*, *P. armandii*, *P. koraiensis*, *P. sylvestris* var. *Mongolica*	China
P. banksiana, P. sylvestris, P. contorta, P. resinosa, P. ponderosa, P. strobus	Canada
P. nigra, P. sylvestris, P. taeda, P. densiflora, P. thunbergii, Pinus elliottii	USA
P. densiflora, P. thunbergii, P. koraiensis	South Korea
P. pinaster, P. radiata, P. caribaea, P. elliottii	Australia
P. contorta, P. Radiata, P. ponderosa, P. sylvestris, P. muricata, P. pinaster, P. nigra, P. mugo	New Zealand
P. sylvestris, P. mugo, P. nigra, P. pinaster, P. pinea, P. wallichiana, P. cembra	Netherland
P. sylvestris, P. sibirica, P. cembra, P. pumila	Russia
P. nigra, P. sylvestris, P. pinaster, P. pinea	Spain
P. ayacahuite, P. cembroides, P. durangensis, P. engelmannii, P.greggii, P. hartwegii, P. leiophylla, P. lumholtzii, P. devoniana, P. montezumae, P. nelsoni, P. patula, P. pseudostrobus, P. teocote	Mexico
P. pinaster, P. sylvestris, P. halepensis, P. thunbergii	Portugal
Pinus spp.	Finland

Table 22.2 Nonpine coniferous hosts of *Bursaphelenchus xylophilus* (Evans et al. 1996; Yu et al. 2019)

Abies ambilis	*Larix olgensis*	*Picea stichensis*
Abies balsamea	*Larix gmelinii* var. *principis-rupprechtii*	*Picea pungens*
Abies firma	*Picea abies*	*Pseudotsuga menziesii*
Abies grandis	*Picea englemannii*	*Chamaecyparis nootkatensis*
Abies sachalinensis	*Picea canadensis*	*Cedrus atlantica*
Larix deciduas	*Picea glauca*	*Cedrus deodara*
Larix kaempferi	*Picea jezoensis*	
Larix laricina	*Picea mariana*	
Larix occidentalis	*Picea rubens*	

only non-native pines, such as *P. nigra* (Austrian pine), *P. sylvestris* (Scotch pine), *P. densiflora, P. thunbergii,* etc. throughout world (Table 22.1). In addition to non-native pine species, other conifers tree species such as *Abies, Larix*, *Picea,* etc. are susceptible to the nematodes (Table 22.1), and exhibit substantial damage, and sometimes mortality may also occur to *Picea* and *Pseudotsuga* due to severe infection by *Bursaphelenchus* spp., as reported in USA (Malek and Appleby 1984; Table 22.2). But, by and large, pines are the principal susceptible hosts to *B. xylophilus* and exhibit severe damage. The major *Pinus* spp. highly susceptible to *Bursaphelenchus* spp., are enlisted in Table 22.1.

The wilt disease of pine is a major threat to forests in North America and Europe because of highly damaging effect of PWN on trees as well as difficulty in controlling and preventing its spread (Økland et al. 2010). The nematode was detected in pine logs in France, Sweden, Norway, and Finland in 2001 imported from North America (Braasch 2001). The PWN was first time recorded in 1999 in Portugal (Mota et al. 1999), and in 2008 in Spain (Abelleira et al. 2011), and despite of regular control measures, the nematode has spread hundreds of kilometers infesting a major portion of pine flora in the country (Mota et al. 2009).

In Asia, Japan was the first country where *B. xylophilus* was reported in 1905. The nematode is believed to be introduced there through pine logs arriving from the North America. Within Japan, the PWN spread to different islands and now has become a serious threat to pines flora in Japan (Shi et al. 2008). It is further believed that *B. xylophillus* got introduced into South Korea and China, from Japan through infested pine logs. China was the country next to Japan where the *B. xylophilus* was recorded infesting *P. thunbergii* in 1982 (Sun 1982). In South Korea, the nematode was first recorded infesting *P. densiflora* and *P. thunbergii* in 1989 (Yi et al. 1989). The pine wilt has assumed to be a most serious disease in Japanese forests, and around 800,000 m^3 wood is damaged annually by the PWN. In China, around three million pines trees die annually due to PWN infection (Yang 1995). The pine species, *P. taiwanensis* and *P. thunbergii* constitute major pine flora in Asia and are prone to PWN attack. In addition to the natural habitats, a very high incidence (at least 50%) of PWN infestation has been reported in the nursery of above two pine species in Asian countries (Chang and Lu 1996). The molecular study on *B. xylophilus* isolates collected from some Asian, European, and North American countries conducted by Zhang et al. (2008) has suggested that PWN presented in Asia has originated from the North America and it spread to China from Japan. The RAPD analysis further suggested that within China the nematode dispersed from Nanjing, where the PWN was recorded first (Sun 1982).

22.3 Vector Role

Since PWN invades the trunk of susceptible trees, it cannot reach there at its own. Hence, it needs a vector for its transmission from the diseased tree to the healthy tree. The beetle *Monochamus* spp. serve as vector to disseminate *B. xylophilus*. The nematode is introduced into a healthy pine when the nematode-infested beetle makes wounds into the bark or leaf axil for feeding or egg laying. A number of species of *Monochamus* are reported to transmit PWN. The distribution of these species varies with the location and host species (Table 22.3), for example the nematode is transmitted by *M. alternatus* largely in Asia and by *M. carolinensis* in USA. The nematode transmission can be described under primary transmission and secondary transmission.

Among *Monochamus* spp., *Monochamus alternatus* (Fig. 22.3a), *M. saltuarius* (Fig. 22.3b), *M. grandis* (Fig. 22.3c), and *M. sutor* (Fig. 22.3d) distribute in Asia, *M. spruce* and *M. galloprobincialis* are in Europe, and the other eight species live in

Table 22.3 The geographical locations in relation to *Monochamus* spp. and susceptible conifers (EPPO pest data sheet, Skarmoutsos and Michalopoulos 2000)

Geographical location	Vector (*Monochamus* species)	Conifers species
Japan, China, Siberia, Russia, Finland, Poland	*M. urussovii*	*Abies*, *Larix*, *Picea*, *Pinus*
USA, Canada	*M. marmorator*	*Abies*, *Picea*
USA, Canada, Mexico	*M. carolinensis*	*Pinus*
USA, Canada	*M. clamator*	*Pinus contorta*
USA, Canada	*M. mutator*	*Pinus*
USA, Canada	*M. notatus*	*P. strobus*
USA, Canada	*M. obtusus*	*Pinus*, *Abies*, *Pseudotsuga*
USA, Mexico, Guatemala, Honduras	*M. rubigeneus*	*Pinus*
Eastern North America	*M. scutellatus* subsp. *scutellatus*	*Pinus*, *Picea*, *Abies*, *Larix*
USA, Canada	*M. scutellatus* subsp. *oregonensis*	*Picea*
USA, Canada	*M. titillator*	*Pinus*, *Abies*, *Picea*
Japan, South Korea, China	*M. alternatus*	*Pinus*, *Cedrus*, *Abies*, *Picea*, *Larix*
Japan	*M. nitens*	*Pinus*
Japan, China, Siberia, Lithuania, Europe, Italy	*M. saltuarius*	*Picea*
China, Japan	*M. tesserula*	*Pinus*
Portugal, North Africa, Italy, France, Greece, Germany, Poland, Sweden, Finland, Russia, Siberia	*M. galloprovincialis*	*Pinus*
Central Europe	*M. sartor*	*Picea, Pinus*
China, Siberia, Russia, Georgia, the Nordic countries, Europe	*M. sutor*	*Pinus, Picea, Larix*

North America (Zhang et al. 2007). The vector insects are usually the dominant species in *B. xylophilus* distribution area. *M. alternatus*, *M. carolinensis*, *M. galloprobincialis* are main vectors of *B. xylophilus* in Asia, North America, and Europe (Portugal) respectively (Yang et al. 2003). *M. saltuarius* is the vector of *B. xylophilus* in the middle temperate zone of Asia (Yu and Wu 2018; Fan et al. 2021).

22.4 Primary Transmission

The adult beetle, *Monochamous* spp. emerging from the diseased pine in spring carries fourth-stage dispersal juveniles (J_4) of *B. xylophilus* (Mamiya 2008). *B. xylophilus* larvae spread from the beetle to the wound while the adult beetle

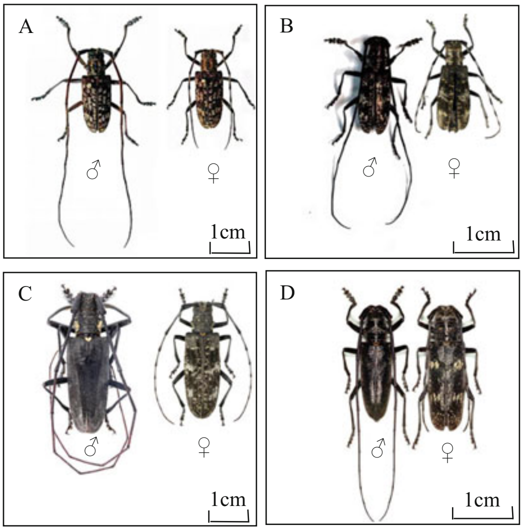

Fig. 22.3 The vector insects of *Bursaphelenchus xylophilus* in Asia (**a**) *Monochamus alternatus*, (**b**) *Monochamus saltuarius*, (**c**) *Monochamus grandis.* (Source: Makapoba K.B.), (**d**) *Monochamus sutor.* (Source: Kacatknh A.R)

feeds on young emerging twigs of the tree. Once inside the trunk, the larva molts quickly and their population increases exponentially which usually causes wilting and drying of the host within 2–3 months.

22.5 Secondary Transmission

The nematodes grow further and reproduce during the feeding on Saprophytic fungi like *Botrytis*, *Ceratocystis*, etc. As the conditions in the pine trunk deteriorate, the nematode begins to produce a third-stage dispersal juveniles (L_{III}), this stage is also referred to as resting stage of the nematode to survive during winters in the infected and dead trunk of pine tree. At the beginning of spring, the fourth instars of beetle pupate in small chambers in the trunk wood. The L_{III} aggregates around the pupal chamber, and molts to become L_{IV} just before termination of pupation, then moves into the tracheae of the adult beetle (Haque and Khan 2021). On the advent of the spring, the adult vector beetle containing thousands of PWN larva makes a bore in the trunk and flies out.

22.6 Symptoms and Damage

22.6.1 Symptoms

After pine trees are infested with *B. xylophilus*, they usually appear abnormal morphological characteristics called the symptoms of pine wilt disease, including external and internal symptoms.

22.6.2 External Symptoms

The change of needles is the only external symptom that can be observed before the death of infected pine trees. At beginning, chlorosis of needles appears on single and a few branches (Fig. 22.4a) or on the whole tree (Fig. 22.4b). The needles then wither and turn reddish-brown without abscission, hanging neatly on branches. The severe infection of *B. xylophilus* can destroy the entire pine forest (Fig. 22.4c). In general, the phenomenon of blue stain can be observed at late stages of pine mortality (Fig. 22.4d). The appearance of the symptoms and death of the tree may show some variation as detailed under.

22.6.2.1 Appearance of the Symptoms

- In the early stages of the disease, no obvious change appears, but secretion of resin begins to decrease.
- Needles appear chlorosis, and secretion of resin stops.
- Most of the needles turn yellow and the infected tree begins to wilt.
- The needles throughout the crown turn brown and the whole tree dies.

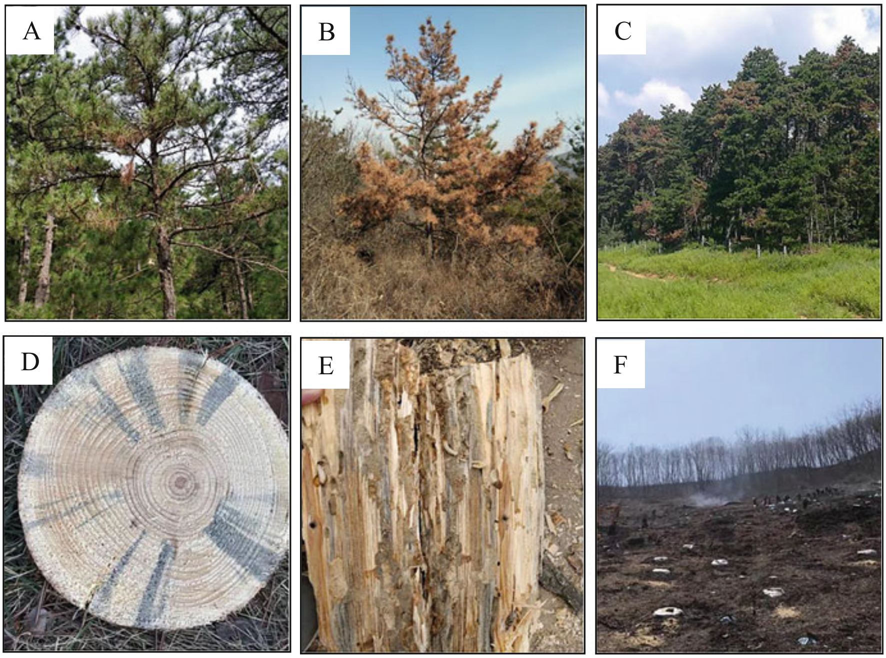

Fig. 22.4 Pine trees infected with *Bursaphelenchus xylophilus* (**a**) branches withered; (**b**) dead pine tree; (**c**) pine forest infected; (**d**) blue stain; (**e**) epidemic wood; (**f**) felling infected pine trees

22.6.2.2 Withering and Death of the Tree

- Dying in current year: Most infected pine trees die in the autumn of current year.
- Dying in next year: In warm areas, a few infected trees (about 10%) do not die in current year, but will die in the next spring or early summer. In the middle temperate zone of China, the rate of dead pine trees infected with *B. xylophilus* in next year is 30–40%, which is significantly higher than that in southern China.
- Branches withering: This symptom differs from the two previously described. The whole infected plant does not die completely in 1–2 years. Generally, a few branches on the crown wither, and the number of dead branches gradually increase over time until the whole tree withers.

22.7 Internal Pathological Reactions

Abnormal internal pathological reactions of infected pine tree appear before the external symptoms appear, for example, water transmission barrier and abnormal respiration. The development of internal reactions leads to anatomical and morphological changes and ultimately to the death of the infected pine trees. Internal pathological responses include:

- Resin production decreases and stops.
- Water physiological indexes decrease.
- Photosynthesis weakens.
- Transpiration weakens.
- Respiration weakens.
- Ethylene production increases.
- Antioxidant enzymes production increases.

22.8 Damage

B. xylophilus does not cause significant damage to the native pine species in America, but it has caused huge economic losses, which include the loss of wood production and high financial cost of management in Asia. In total, about four million cubic meters of pine forests in Japan had been damaged by *B. xylophilus* since 1978 (Hirao et al. 2019). In the 20 years from 1977 to 1997, the Japanese government spent 87.04 billion yen on *B. xylophilus* control, accounting for 93.62% of the total fund spending on forest pest control (Mota and Vieira 2008). In South Korea, 7811 hm^2 of pine forest have been damaged by 2006, and the annual cost of *B. xylophilus* control exceeds \$10 million and is increasing every year (Kulinich et al. 2020). In China, over the past 40 years from 1982 to 2020, billions of pine trees died by *B. xylophilus*, and more than 1.81×10^6 hm^2 forests have been infected. As the most serious and costly invasive pest in the past 20 years, *B. xylophilus* causes direct and indirect economic losses of hundreds of billions of yuan (NFGA

2021a, b). *B. xylophilus* has caused serious plant epidemics in three countries in Asia, and it does not only cause huge economic losses but also threaten the ecological security. As pine is a pioneer tree species, forests are difficult to regenerate naturally after pine trees died caused by *B. xylophilus*. And it is difficult to recover from environmental damage, which may lead to a series of ecological disasters such as soil erosion, flash floods, and mudslides (Zhou and Cao 2022).

22.9 Life Cycle

22.9.1 Developmental Stages

Life history of the *B. xylophilus* passes through three developmental stages: egg, larva, and adult, including propagative cycle and dispersal cycle. The reproduction period has three stages: egg, larva of first to fourth instar, and adult (Chai 2003). Male adult and female adult mate to produce fertilized egg, which develops into first instar larva after about 14 h (Sun et al. 2022), the lip region, stylet, and esophageal glands of the first instar larva have not formed. After 1 h, the first instar larva starts to exuviate for the first time and entered into the second instar. The second instar larva is constantly wriggling inside the eggshell, and its lip region, stylet, esophageal glands, and other organs are clearly visible. Four hours later, the eggshell breaks and the larva hatches (Ye and Feng 1993).

The newly hatched second instar larva of the *B. xylophilus* is light in color. After feeding, particles are visible in the intestine, and then its color gradually deepens, the labial region becomes constrict, the esophageal bulb becomes larger, and the body length is about 220 μm, and develops to third instar larva 1 day later. The length of third instar larva is about 450 μm and its germ cells are clear, 1 day after feeding the larva molts to fourth instar. The constriction of the labial region of the fourth instar larva is obvious, the gonad is rapidly enlarged, the reproductive organs of male and female are formed, the body length reaches to 600 μm, and the fourth instar lasts one and a half days (Ye and Feng 1993). After molting to adult, *B. xylophilus*'s body length continues to increase, female adult has a vulva flap, and male adult has a spicule. The mating period lasts about 23 min (Zhu et al. 2016; Liu et al. 2014), and then female adult starts to oviposit immediately after mating, the average oviposition period lasts over 1 week, the average total egg production per female is about 79 (Mamiya and Furukawa 1977). The peak oviposition period is generally the first 4 days at the beginning of oviposition (Mamiya 1975), female adult dies soon after laying eggs, in which average longevity is 15 days and maximum longevity is 32 days (Zhao et al. 2005).

22.10 Effect of Temperature on the Distribution and Development

The spread of pine wilt disease is closely related to temperature. It is found that worldwide areas with an average annual temperature above 15 °C are suitable for *B. xylophilus*, and the areas have a high risk of pine wilt disease when the average mean monthly temperatures of warmest 3 months reach to 19 °C by analyzing meteorological data and current distribution of *B. xylophilus* using the MaxEnt model and the thermal model (Ikegami and Jenkins 2018). In China, areas with an average annual temperature above 10 °C are suitable for *B. xylophilus* (Mamiya 1983), and areas with an average annual temperature above 14 °C are high-risk (Yang et al. 2003). With the rise of global temperature, the global fitness range of *B. xylophilus* has gradually expanded (Ikegami and Jenkins 2018, Fig. 22.5). Meanwhile, *B. xylophilus* has well temperature adaptability, and its cold tolerance improves significantly with the extension of domestication time (Huang 2015). In

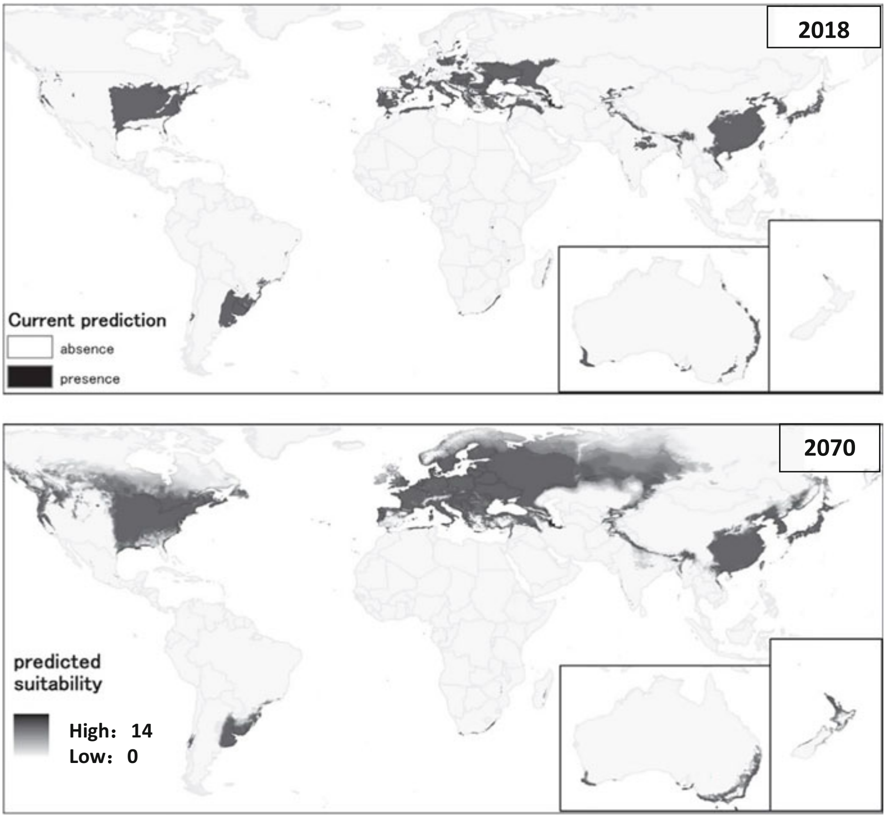

Fig. 22.5 Prediction of potential distribution of *Bursaphelenchus xylophilus*. (Modified from Ikegami and Jenkins 2018)

2018, pine wilt disease was found for the first time in areas with an average annual temperature below 10 °C in China, which further confirmed that the cold tolerance of *B. xylophilus* has been significantly improved after long-term low-temperature domestication during its continuous northward spread in China (Yu and Wu 2018; Zheng et al. 2021b).

Temperature also affects the developing rate of *B. xylophilus*, so its life history period depends on temperature. Laboratory test showed that it took 12 days, 4 days, and 3 days to complete one generation under 15, 25, and 30 °C culture conditions, respectively (Wang et al. 2005). The initial temperature for the growth of *B. xylophilus* is 9.5 °C, the development inhibits over 33 °C, and it developed the fastest at 30 °C (Rutherford et al. 1992; Tomminen 1993).

22.11 Infection Cycle

The PWN, *B. xylophilus* is not able to spread by itself in nature and need to be carried by vector insects to transmit from infested trees to healthy ones (Mamiya and Enda 1972). *Monochamus* spp. is a main vector of *B. xylophilus*, and the two have formed a symbiotic complex during the long-term evolution. The spread of *B. xylophilus* to new host pine tree must depend on the carrying of the vector beetle, and the pine tree infested by *B. xylophilus* provides a suitable ovipositing place for the vector beetle (Feng et al. 2022).

The reported number of *B. xylophilus* carried by vector beetle were different in different studies. For example, the average number of *B. xylophilus* carried by each *M. alternatus* was a minimum of 171 and a maximum of 19,590 in Japan (Mamiya and Enda 1972; Kobayashi 1984). The average number of *B. xylophilus* carried by each *M. alternatus* were a minimum of 1165 and a maximum of 18,445 in southern China (Zhang 2007; Song et al. 1992). Compared with *M. alternatus*, *M. saltuarius* carries less *B. xylophilus*, the average number of *B. xylophilus* carried by each *M. saltuarius* in Japan, South Korea, and China were 9284 (Sato and Guan 1991), 3297 (Kim et al. 2009), and 337, respectively (Zheng et al., unpublished). There are many reasons for the difference in the number of *B. xylophilus* carried by vector beetle, including the species, geographical location, body size, sex of the vector beetle (Chai 2003). The specific relationship between the vector long-horned beetle's ability to carry *B. xylophilus* and the occurrence of pine wilt disease needs further research.

B. xylophilus completes its life history on infected pine tree, and the larva can differentiate into propagative mode and dispersal mode. When food and water content are abundant, *B. xylophilus* develops to dispersal mode and molting from larva into adult to reproduce rapidly (Mamiya 1984). Under unfavorable conditions such as drought, low food, and temperature, *B. xylophilus* enters the dispersal mode of its life cycle, by molting from second-stage propagative juveniles (L_{II}) into third-stage dispersal juveniles (L_{IV}) (Jung et al. 2010), and spreads with the help of vector beetles (Linit 1990). The growth of vector larva stagnates in winter, and L_{III} is attracted to pupal chamber by volatiles of vector larva, and feeds on fungi around the

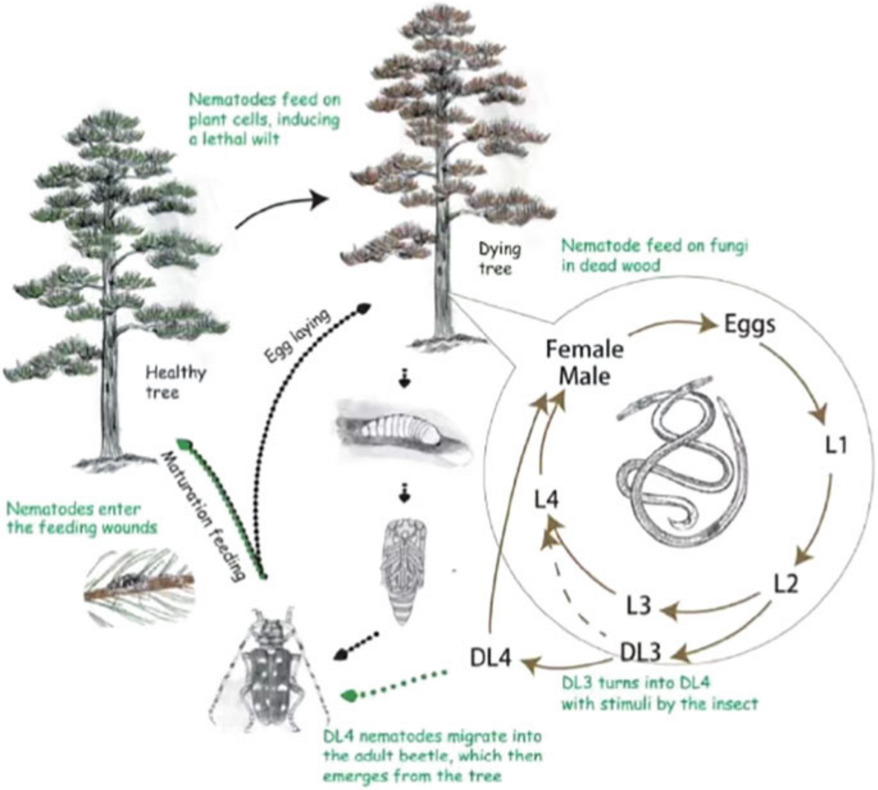

Fig. 22.6 The life cycle and transmission route of *Bursaphelenchus xylophilus* (Kikuchi et al. 2011). Brown arrows show the life cycle of *Bursaphelenchus xylophilus*, black arrows show the development and ovipositing process of vector beetle, and green arrows show the route of *Bursaphelenchus xylophilus* transmission

pupal chamber (Zhao et al. 2014). From May to July of the following year, vector beetle larva pupates in the pupal chamber and emerges into adult 2–3 weeks later. During this period, L_{III} larva is induced to develop into fourth-stage dispersal juveniles (L_{IV}), and L_{IV} enters the body of the beetle through the trachea of the beetle (Futai 2013; Zhao et al. 2013b). Vector beetles transmit *B. xylophilus* mainly through wounds of healthy pine trees caused by feeding (Linit and Edwards 1992; Yang et al. 2003). About 3–4 weeks after *B. xylophilus* infects the pine tree, the pine resin decreases, and the release of volatiles attracts the vector beetle to locate and oviposit on the pine tree (Futai 2013). *B. xylophilus* L_{IV} larva in the dead tree entered the trachea of its vector again and completed the infection cycle (Fig. 22.6).

Chemical signal plays a key role in the symbiotic complex of *B. xylophilus* and vector beetle. The L_{IV} formation in *B. xylophilus* is induced by fatty acid ethyl esters (FAEEs), which are produced by the vector beetle specifically during the late development pupal and emerging adult stages (Futai 1980; Miyazaki et al. 1977;

Zhao et al. 2013a). The ascarosides can help to synchronize the beetle's development with that of *B. xylophilus* (Choe et al. 2012; Zhao et al. 2016). High concentration of CO_2 produced by vector beetle respiration can trigger a CO_2 avoidance mechanism that serves as a signal to the nematodes to disembark from their vector and infect healthy pine trees (Miyazaki et al. 1978; Wu et al. 2019).

Associated microorganism also plays an important role in the symbiotic complex of *B. xylophilus* and its vector. *B. xylophilus*-associated bacteria can reduce the defense response of host pine tree (Kawazu et al. 1996; Neves et al. 2010), and can provide nutrients for the growth and reproduction of the nematode, so then form a favorable living environment for it, improving the compatibility and pathogenicity of *B. xylophilus* (Tian et al. 2011; Zhao et al. 2005). Associated bacteria can also promote the nematode to oviposit (Chi 2003), and increase the *B. xylophilus* reproductive rate (Zhao and Lin 2005). The dominant fungal species in infected wood can influence the number of *B. xylophilus* carried by its vector beetle during the dispersal period of the nematode. *Pestalotiopsis microspora*, *Sphaeropsis sapinea*, *Ceratocystis* spp., *Trichoderma* spp., *Aspergillus* spp., *Verticillium* spp., etc. can significantly increase the number of *B. xylophilus* carried by its vector beetle (Maehara and Futai 2000; Niu et al. 2012). For example, when the dominant fungus is blue-stain fungi (such as *Ceratocystis* spp.), the growth of *B. xylophilus* is the fastest and the population is the largest. It can also increase the invasive of *B. xylophilus* by increasing the population of *B. xylophilus* in vector beetle's pupal chambers (Niu et al. 2012).

22.12 Management

22.12.1 Quarantine and Monitoring

The *B. xylophilus* is a destructive invasive species worldwide, and has been listed as a quarantine object by 52 countries (Mota and Vieira 2008). The widespread spread of *B. xylophilus* disease mainly relies on anthropogenic activities. In another word, the infected pine wood is brought from infested areas to noninfested areas, inside which the vector insects emerge to adult and carry the nematode out of the infected wood, then transmit them to healthy pine trees, and cause new infestations. Thus, the most important task for noninfested area is preventing the entering of infected wood from epidemic areas. The first key step of pine wilt disease prevention and control is quarantine, which is currently being strengthened worldwide. Since the mid-1980s, European countries have banned the logs imported from countries where pine wilt disease is a serious threat (Kulinich et al. 2020).

In China both internal and external quarantine policies have been implemented for *B. xylophilus*. The NFGA issued the latest revised version of the "Pine Wilt Disease Epidemic Areas and Infected Trees Management Rules" in 2019, which further clarified the management of infected areas, management of felling infected wood, management of safe use of infected wood, and related responsibilities for pine wilt nematode (NFGA 2019). The General Administration of Customs P.R. China

(GACC) issued the latest "Announcement on Quarantine Requirements for Imported Pine Wood from Pine Wilt disease Epidemic Countries" in 2021, which clarified the requirements of *B. xylophilus* quarantine (GACC, No. 110, 2021).

Monitoring that detects the invasive pest escaping from epidemic area in time is an important part of integrated pest management (IPM). Since the first detection of *B. xylophilus* invasion in Portugal, European countries have carried out monitoring of pine wilt disease to prevent and control the further spread of the disease (Mota et al. 2009). In 2009, automatic molecular detection and identification techniques were developed to identify and monitor *B. xylophilus* in quarantine work, and molecular detection techniques of pine wilt disease were the popularized in China. It takes less than 1 h to detect *B. xylophilus* by the rapid amplification of nuclear acid under room temperature. The technology has been successfully applied to the investigation of pine wilt disease, and greatly improves the speed and detectability of the disease (Anhui Forestry Bureau 2021). During ground-based monitoring, trapping can reduce the population density of vector insects and monitor *B. xylophilus* effectively to protect forest healthy. A variety of plant volatile and pheromone have been developed as attractant and have been widely used to monitoring and controlling. In addition, aerial monitoring, especially unmanned aerial vehicles (UAV) start to be used in monitoring of the concurrence of pine wilt disease in recent years, and high-resolution satellite image has also achieved clear interpretation of the health status of pine trees on the ground, and has been applied to large-scale, long-distance monitoring of *B. xylophilus* (Huang et al. 2018).

22.13 Removal of Infected Wood

In epidemic area, the removal and treatment of pine wood nematode-infected wood are an important management measure of *B. xylophilus*. In Japan and Portugal, felling infected wood in time, prohibiting transport of infected wood, and fumigating or burning are main measures to clear pine wilt disease-infected wood (Mota et al. 2009). Current national standards of *B. xylophilus* quarantine and monitoring in China include the General Administration of Quality Supervision, Inspection and Quarantine of the People's Republic of China (AQSIQ) issued the "Quarantine technical rules of Pine Wilt Disease," the "Technical Specification for the Treatment of Pine Wilt Disease Epidemic Wood" and the "Technical regulations of the general investigation and monitoring of Pine Wilt Disease" in 2009. In the "Technical Scheme of Pine Wilt Disease Control" revised by the NFGA in 2021, the treatment of epidemic wood is further standardized, and it is suggested that the dead and dying infected tress should be cut in time. And the felling trees should be crushed or burnt in situ as soon as possible. The principles of "centralized felling" and "timely felling" should be followed when felling infected pine trees. During the nonemergence period of vector insects in winter and spring (usually from November of the current year to April of the following year), the pine trees that died of pine wilt disease should be cut and be cleaned up. The sporadic-infected pine trees found after centralized felling should be also cut as soon as possible (NFGA 2021a, b).

22.14 Vector Insect Control

The control of vector insect plays an important role in blocking transmission of *B. xylophilus*. The control strategies of vector insects include chemical control, trapping by sentinel traps and bait-tree, and biological control.

22.15 Chemical Control

Chemicals are widely used to control *M. alternatus* vectoring *B. xylophilus* (Lai et al. 2000; Liu et al. 2006). Spraying to the canopy on ground and spraying by airplane during the periods of complementary nutrition, mating and egg-laying, are effective to kill vector adults. The control efficacy of the following insecticides has been tested in forest: 1% thiacloprid microencapsulated granules (Zhang et al. 2010; Liao et al. 2012), thiacloprid touch-break preparation (Peng and Yan 2011), 8% cypermethrin (Shi et al. 2005), 30% chloramine phosphorus emulsifiable concentrate, 2% avermectin emulsifiable concentrate, 40% omethoate emulsifiable concentrate (Liu et al. 2006).

22.16 Trapping

Sentinel trap can monitor emergence and occurrence of vector adult and develop the best control period and also can reduce the population of vectors. Vector trapping protocol is applied to control *B. xylophilus* in epidemic areas in China. A variety of attractants of volatiles from host trees and vector long-horned beetle pheromones have been developed, including (1) botanical attractant: M-99, A-3, PA, PE, Mat, FJ-Ma, HYP-SH, ZM-60, and BF-I (Tian et al. 2008; Sun 2013), (2) pheromones: APF-I, F-2 for *M. alternatus,* and ZL-I traps for *M. saltuarius* (Chen et al. 2020; Zheng et al. 2021a, b). The aggregation-pheromone components of *M. saltuarius* have been successfully identified (Lee et al. 2017). However, at present most applied attractions target to *M. alternatus,* specific attractions for *M. saltuarius* are needed to develop, and further work is needed to optimize trap design and trapping protocols for adult vectors (Fig. 22.7a).

The vector insects prefer to oviposit on the weakened pine trees. Bait-trees set in forest can attract vector insects to lay eggs on it, and then are disinfested together. Bait-trees can also be used in combination with parasitoid of vector insects and provide hosts and breeding places for natural enemy insects (Wen et al. 2017). In forest, weak or smaller pine trees are selected to be bait-tree in the early stage of vector insect emergence. Trunk of bait-tree needs to be cut 2–3 grooves at an angle of 30° from different sides at a height of 30–50 cm from the ground, and the diluted attractants are injected into the wound to make the tree weak (Yang et al. 2012) (Fig. 22.7b).

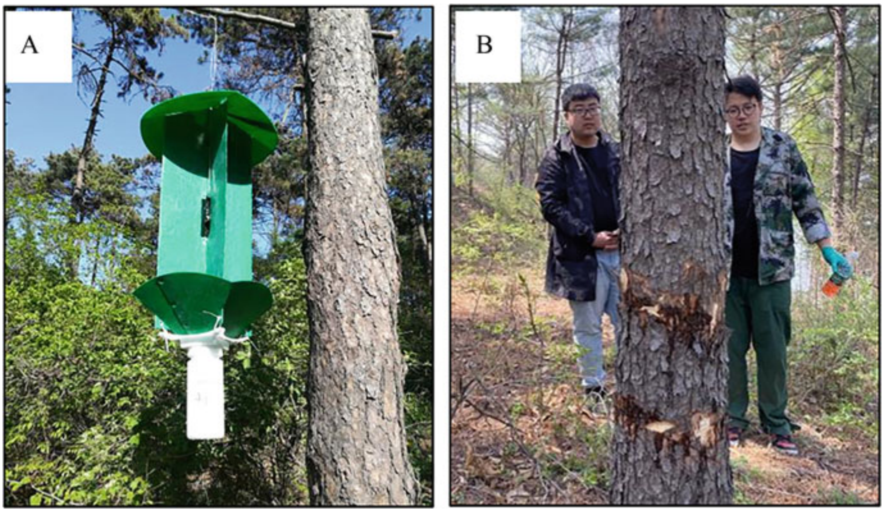

Fig. 22.7 Trapping. (**a**) Sentinel trap composed of ZL-I attractants and ZM-80 trap hung on *Pinus koraiensis* in Dahuofang Forest in Fushun. (**b**) Bait-tree in made of *Pinus koraiensis* in Dengta Forest in Liaoyang

22.17 Biological Control

As a sustainable control strategy, biological control is an important part of integrated pest management (IPM) of pest. Entomopathogen and natural enemy insects are widely used in vector insect control. Among them entomopathogenic fungi, that can control vector insect include *Beauveria bassiana*, *B. brongniatii*, *Metarhizium anisopliae*, *Aspergillus flavus*, *Acremonium* sp., and *Verticillium* spp. In addition, both the entomopathogenic bacteria *Serratia marcescens*, and the entomopathogenic nematode *Steinernema feltiae* can parasitize on *M. alternatus* (Zhang 2006). Among them *B. bassiana* is the most widely used in forest. During the adult period of *M. alternatus*, spreading the bacterial powder of *B. bassiana* can be used to control *M. alternatus* (Liu et al. 2007).

Many natural enemy insects of *M. alternatus* have also been found, such as *Alaus putridus*, *Denticollis miniatus*, *Pectocera fortunei*, *Spheniscosomus cete*, *Stenagostus umbratilis* (Zhang 2006; Yang 2012), *Cosmophorus klugii*, *Megarhyssa* sp., *Spathius radzayanus* (Zhao and Yang 2019), *Scleroderma* spp. (Zhang et al. 2022; Dang et al. 2022), and *Dastarcus helophoroides* (Yang et al. 2012; Zhang and Sun 2010). *D. helophoroides* (Fig. 22.8a–e) and *Scleroderma* spp. (Fig. 22.8f–i) are the most widely used to control *M. alternatus*, effectively in forests, and pupal stage is the best period to control. The control efficiency of *S. alternatus* and

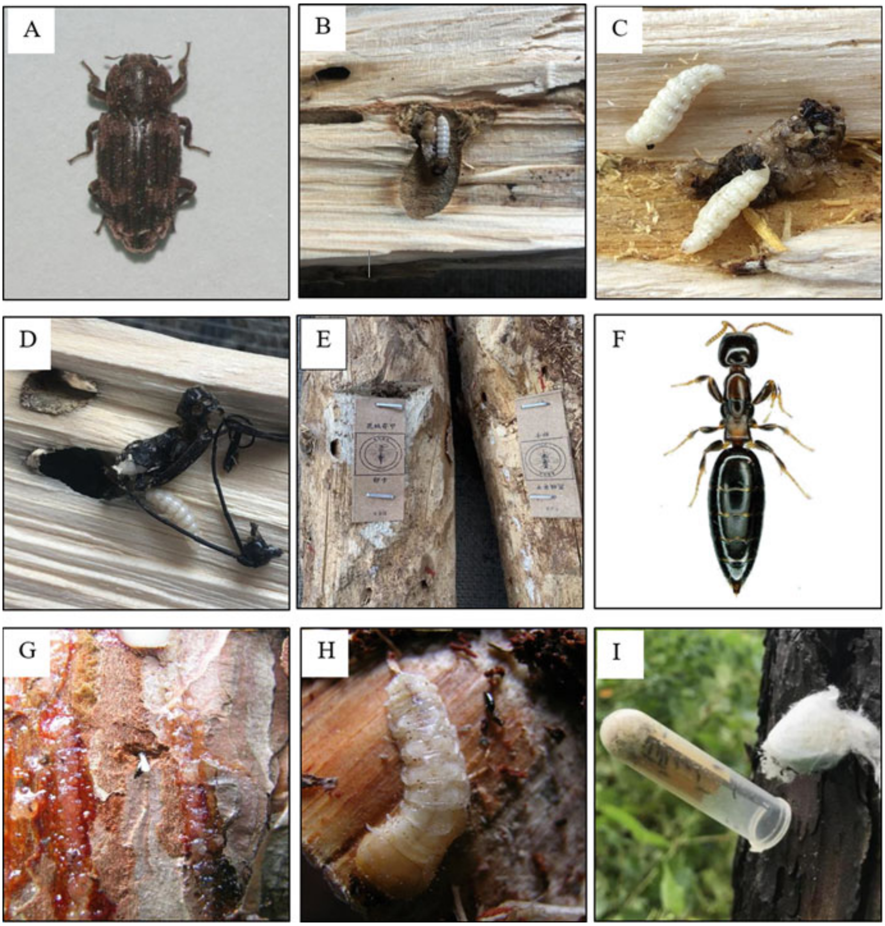

Fig. 22.8 Biological control of *Monochamus saltuarius* and *Monochamus alternatus*. (**a**) *Dastarcus helophoroides* adult, (**b**) *Monochamus saltuarius* larva parasitized by *Dastarcus helophoroides*, (**c**) *Monochamus saltuarius* pupa parasitized by Dastarcus helophoroides, (**d**) Monochamus saltuarius adults parasitized by Dastarcus helophoroides, (**e**) Dastarcus helophoroides eggs released, (**f**) Scleroderma alternatusi adult (Source: Cao L.M.), (**g**) Scleroderma alternatusi searches for oviposition scar of Monochamus alternatus (Source: Zhang Y.L.), (**h**) Monochamus alternatus parasitized by Scleroderma guani larva (Source: Zhang Y.L.), (**i**) Scleroderma alternatusi adults released

D. helophoroides against *M. alternatus* were 57.88% and 82.37% respectively (Yang et al. 2013; Wen et al. 2017). The control efficiency of *S. guani* (Zheng et al. 2022) and *D. helophoroides* (Zheng et al., unpublished) against *M. saltuarius* were 71.43% and 74.15% respectively.

22.18 Control Protocol Targeting the Pine Wilt Nematode

Except for the above control strategies targeting vector insects, chemical and biological control methods are directed against *B. xylophilus*. Tree trunk injection of nematicide, like emamectin benzoate or avermectin, is applied to protect ancient and famous trees and trees in important ecological areas (Yang 2018). Spraying biological control agents on trunks of trees can also prove effective against nematode. Smal-007 that is a strain of *Stenotrophomonas maltophilia* isolated and selected from the body surface of the *B. xylophilus* in the United States (Han 2014), and *Esteya vermicola* have been patented and been proven effective to control pine wilt disease (Liou et al. 1999; Fang et al. 2012; Wang et al. 2014). Further, nanotechnology may also work well in providing satisfactory solution for PWN management (Khan and Rizvi 2014; Khan et al. 2019a, b, c) and its detection (Khan and Akram 2020; Khan and Rizvi 2016; Khan et al. 2020). Nano-sensors are the most important product of nanotechnology, and have great potential for use in plant disease diagnosis (Khan 2023). Sellappan et al. (2022) developed nanobiosensor to early detection and prevention of agricultural crops from harmful microorganisms. Using specific nanoparticles as nano-sensors for early detection of the nematode and its vector can help in reducing the damage to trees and help in proper management of the disease (Khan and Rizvi 2018).

22.19 Silvicultural Measure

Replanting evergreen broad-leaved tree species after pine tree died of pine wilt disease in forests can increase the diversity of tree species, adjusting the stand structure, and forming a relatively stable multilayer mixed forest community containing evergreen species, which is helpful to improving stability of forest system (Liu and Ji 2006). In addition, the selection of resistant tree species is also very important. The resistance resources of *B. xylophilus* and breeding technology of *P. massoniana* have been initially accumulated in China (Ye 2019). Advances have been achieved on introduction of resistant resources, resistance evaluation, and utilization of *P. thunbergii* and *P. densiflora* (Wang et al. 2015; Sun et al. 2015; Chen et al. 2019).

22.20 Conclusion and Future Perspectives

Since *B. xylophilus* was first reported in 1934 (Steiner and Buhrer 1934), nematologists have carried out extensive research on it, mainly focusing on the biological characteristics (Sun et al. 2022), detection technology (Li et al. 2022a, b), distribution and host (Yang et al. 2003), pathogenic mechanism (Li et al. 2022a, b), vector insects (Zhang et al. 2019), and control technology (Khan 2020; Ye and Wu 2022). In view of rapid and extensive damage to a number of forest tree species, afforestation with resistant or nonhost species becomes essentially necessary which may serve as a very important strategy to deal with the PWN. However, the nematode is still spreading globally, especially in East Asia at an alarmingly rapid pace. Special consideration should be given on monitoring of PWN in the noninfested areas to prevent its further spread. Strict measures should be taken to prevent movement of raw and untreated logs from the countries and regions with PWN history to the areas not having the nematode occurrence. In addition, PWN, vector insect, and host tree are closely related to the occurrence, spread, and damage to the pine flora. The further study on the interaction mechanism among *B. xylophilus*, vector insects, and host trees shall help in blocking the spread route of *B. xylophilus*.

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Major Nematode Problems in Direct Seeded Rice and Their Management

23

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Abstract

Nematode infestation in direct seeded rice (DSR) is a significant constraint in the popularization of this method which significantly saves water, time, and labor in rice cultivation. Although all nematode species which attack irrigated rice are important in DSR, root-knot nematode, *Meloidogyne graminicola*, and stem nematode, *Ditylenchus angustus*, are highly prevalent and damaging in DSR, especially under upland and deepwater conditions, respectively. Infestation level and severity of both the nematodes are generally found relatively greater in DSR over transplanted rice. The management of these nematodes in DSR is possible if integrated management approaches are executed timely. Farmers generally overlook the nematode infestation in rice cultivation which leads to severe damage to the crop. Hence, it is extremely important to sensitize farmers about the economic consequences of nematode infestation in rice and the cost-benefit of management strategies. The present chapter offers detailed information on economic importance, distribution, symptoms, life cycle, and management of the rice root-knot nematode and rice stem nematode especially in DSR.

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Keywords

Rice · *Meloidogyne graminicola* · Direct seeded rice · DSR · *Ditylenchus angustus* · Management etc.

23.1 Introduction

Rice, *Oryza sativa* L., is a staple food for over 70% of human population globally. Rice is cultivated in about 140 million hectares in Asia, Africa, Australia, Europe, North and South America. However, the leading producers of rice are China, India, Bangladesh, Burma, Pakistan, Japan, the Philippines, and Thailand. In India, rice occupies around 23% of the national cropped area with a total production of 195 million tons during 2020–2021 (FAOSTAT 2022). Rice is largely cultivated through traditional method under saturated and semi-saturated conditions. In view of water and labor demanding nature of traditional transplanting method, the alternative methods of rice cultivation to increase water use efficiency and productivity have been on a large demand. The direct seeded rice (DSR) is one of the best options to enhance the water utilization efficiency and to reduce the unproductive water flows as well as to reduce the labor requirement and duration.

The DSR involves establishing rice plants from the seeds directly sown in the field in place of traditional method of raising the seedlings in nursery followed by their transplanting in the field. The DSR is, in fact, an oldest method of paddy cultivation, but in recent times it is being promoted for its low water demand. In this method, the pre-germinated seeds of rice are sown into a wet seeding (puddled soil), water seeding (standing water), or dry seeding (prepared seedbed). The major benefit of DSR is that it requires significantly less labor, water, and drudgery and gives early maturity of the crop (Kaur and Singh 2017). As a result, the overall production cost is considerably low. This method is also ecologically safe, because DSR involves less physical disturbance to the soil and less methane emission.

Several pest and disease constraints are associated with DSR, such as high weed infestation (Singh et al. 2006a, b; Rao et al. 2007), increase in the incidence of pathogen infestation (Kreye et al. 2009), nutritional disorders (Singh and Singh 1988; Kirk et al. 1998; Gao et al. 2006), poor crop establishment, and more prone to lodging compared to puddle transplanted rice (Setter et al. 1997). The important pests and diseases observed in DSR are blast, brown leaf spot, sheath blight, nematode infestation, dirty panicle, plant hopper, etc. (Bonman 1992; Pongprasert 1995; Prabhu et al. 2002; Savary et al. 2005). In this chapter, important nematode problems are presented and their nonchemical methods of management are discussed.

Among plant nematodes which attack irrigated rice, root-knot nematode and rice stem nematode pose serious disease problem in DSR. Root-knot nematodes, *Meloidogyne* spp., are highly damaging pests in direct seeded aerobic rice (Kreye et al. 2009), although the nematode can survive for considerable period under flooded water, but generally unable to penetrate rice roots under such conditions

(Bridge and Page 1982). Root-knot nematode *M. graminicola* extensively invades the rice roots under aerobic conditions. Rice stem nematode *Ditylenchus angustus* is another important nematode which is widely spread in the conditions where soil is least disturbed such as DSR in wheat-rice cropping sequence (Pankaj et al. 2006). Deep-water rice or *Bao* rice is generally sown directly in low land areas and this rice is very much prone to the infection of rice stem nematode (Prasad et al. 2010). The literature shows that root-knot nematode problem in DSR under upland condition and rice stem nematode problem in DSR in deep water are common in occurrence, and hence, are discussed in the present chapter alongwith their management strategies.

23.2 Root-Knot Nematode

Around 13 species from the genus *Meloidogyne* have been reported to infect rice world over. *M. graminicola* is the most important and highly damaging species in rice cultivation in several countries (Khan 2023; Khan et al. 2023a), and has assumed the status of major pest of rice (Arayarungsarit 1987a, b; Bridge et al. 1990; Padgham et al. 2004b; Pokharel et al. 2007; Khan et al. 2020b, 2021a; Haque and Khan 2021a). The rice root-knot nematode (RRKN) is a very destructive pest of nursery bed and upland rice in well-drained soils (Rao et al. 1986a, b; Khan et al. 2023b). Severe frequent attacks of RRKN have been recorded in the deepwater rice (Prasad et al. 1985; MacGowan and Langdonm 1989; Bridge et al. 1990; Jairajpuri and Baqri 1991; Khan et al. 2023a) and irrigated rice (Khan and Ahamad 2020). The nematode is also a potential constraint in the cultivation of DSR under upland condition, and inflicts heavy yield losses to rice. The root-knot nematode infestation becomes severe and widespread where DSR is grown in infested puddled fields (Padgham et al. 2004a).

23.3 Distribution and Host Range

The RRKN, *M. graminicola* widely occurs in major rice growing countries in South East Asia (Golden and Birchfield 1968; Sontirat 1981; Page and Bridge 1978; Mohidin and Khan 2014; Khan and Ahamad 2020). In India, *M. graminicola* is reported to occur in the states of Assam, West Bengal, Bihar, Gujarat, Delhi, Haryana, Madhya Pradesh, Orissa, Kerala, Karnataka, Jammu, Uttarakhand, Andhra Pradesh, and Tripura (Prasad et al. 1987, 2006; Sharma and Prasad 1995; Sheela et al. 2005; Khan and Anwer 2011a, b; Sehgal et al. 2012; Kumar et al. 2014; Haque et al. 2019).

Golden and Birchfield (1965) were the first to describe the *M. graminicola* infection (galling) on the roots of barnyard grass (*Echinochloa colonum*) in the USA. Later it was found that *M. graminicola* can infect several other host plants such as cereals and grasses. Among cereals, rice *Oryza sativa* is highly susceptible to *M. graminicola* and exhibits severe crop damage (Rao et al. 1986a, b; Bridge et al.

1990; Padgham et al. 2004b; Prasad et al. 2010; Haque et al. 2018; Khan et al. 2019a). Other hosts of RRKN are *Andropogon* sp., *Blumea* sp., *Cyperus compressus*, *C. deformis, Echinochloa crusgalli*, *E. colconum*, *E. colona*, *Eclipta alba*, *Eleusine indica*, *Fimbristylis miliacea*, *Grangea madraspatensis*, *Jussieua repens, Paspalum sanguinola*, *Phyllanthus urinaria*, *Panicum miliaceum*, *Ranunculus pusillus*, *Vandellia* sp., wheat, banana, chilies, onion, tomato, etc. (Yik and Birchfield 1979; Khan and Khan 2000; Reversat and Soriano 2002; Gergon et al. 2002; Prasad et al. 2010; Haque and Khan 2022).

23.4 Symptoms of Infestation

The RRKN symptoms on aboveground parts of rice are generally not specific and include stunting of plants, yellowing of foliage, curling of leaves, reduction in numbers of tillers with few numbers of effective tillers, and delayed emergence of panicles by 10–15 days (Prasad et al. 2010; Haque and Khan 2022). The young leaves distort and crinkle along the margins. In heavy infestation, ear head production is drastically reduced (Roy 1973; Khan and Anwer 2011b). The panicle becomes undersized bearing numerous poorly filled grains, leading to significant yield reduction (Rao and Biswas 1973a, b). Overall, the rice field shows uneven growth, and plants in patches become stunted with yellowish leaves (Prasad et al. 2006; Haque and Khan 2021b; 2022). It is generally found that plants in the infested patches dry early during moisture stress (Biswas and Rao 1971a, b; Khan et al. 2014). Further, it may be noticed that ear heads have poorly filled or no grain in heavily infested fields. On the rice roots, the nematode inflicts characteristic hook shaped or spiral terminal galls in nursery as well as in the field (Fig. 23.1; Khan and Ahamad 2020). Due to nematode infestation, linear growth of roots is retarded leading to the emergence and development of side roots.

23.5 Life Cycle

The infective stage of *M. graminicola* is the second-stage juvenile which penetrates close to root tip (Mohidin and Khan 2014). The penetration and feeding by J_2 leads to disruption of root physiology and also hypertrophy in the cortical cells. The secretions from esophageal glands incite endomitosis without cytokinesis leading to the formation of giant cells in steeler tissue around the nematode head (Nguyễn et al. 2014). The juvenile invades the young roots emerging from the germinating rice seeds. Heavy infection at this time might prevent the seed from germinating and growing into heavy seedlings later cause them to quickley die (perish). The female J_2 eventually develops to a saccate individual, whereas male J_2 assumes vermiform shape and migrates out of the root (Fig. 23.1). The saccate female lays eggs inside a gelatinous sac secreted by specialized rectal cells. The egg masses do not appear on the root surface unlike other *Meloidogyne* species (Khan et al. 2014). Hence, the progeny moves internally and infects new sites (Mantelin et al. 2017).

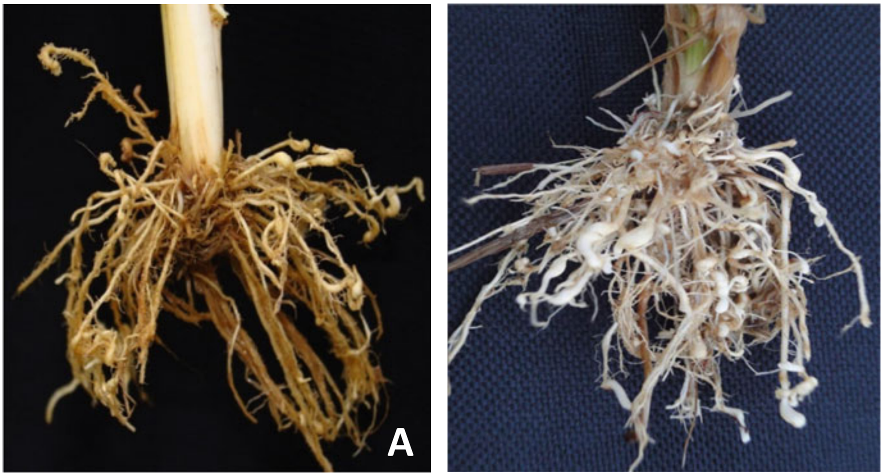

Fig. 23.1 Root-knots caused by *Meloidogyne graminicola* in irrigated (**a**) and direct seeded rice (**b**) (**a** and **b**). (Source: M. R. Khan, Aligarh Muslin University, Aligarh; B: B. Bhagawati, Assam Agriculture University, Jorhat)

M. graminicola completes the life cycle in 19–27 days on rice (Bridge and Page 1982; Yik and Birchfield 1979) and may take 2–3 generations on irrigated rice and 3–4 generations on direct seeded rice, in a season.

23.6 Yield Losses

The rice root-knot nematode, *M. graminicola* causes significant yield losses in rice cultivation, both in transplanted and DSR (Khan et al. 2023b). Prasad et al. (1987) observed up to 21% yield loss due to infestation by *M. graminicola* in rainfed and well-drained soils in India (Prasad et al. 1987), whereas 16–32% lower grain yield was recorded in upland rice due to root-knot nematode infection (Biswas and Rao 1971a, b; Rao and Biswas 1973a, b). Other researchers have reported 17–32% yield decline in paddy (MacGowan and Langdonm 1989; Jain et al. 2007; Khan et al. 2014, 2016a, b, 2017). Much higher yield reduction in rice due to root-knot nematode such as 75% in Ivory Coast (Diomonde 1981) and 65% in Vietnam has been recorded (Kinh et al. 1982).

In Thailand, 12–33% increase in the yield due to application of nematicides in *M. graminicola*-infested upland rice field was recorded (Arayarungsarit 1987a, b). Similarly, Netscher and Erlan (1993) reported 28–87% increase in the rice yield in Indonesia due to application of various management treatments. Overall, the root-knot nematode inflicted 20–80% yield decline in rice (Plowright and Bridge 1990). In Bangladesh 16–20% yield enhancement after application of nematicide in *M. graminicola*-infested lowland rainfed rice field was recorded (Padgham et al. 2004b). Similarly, Haque et al. (2019) recorded 19–42% yield enhancement after application of *Pseudomonas* spp. in RRKN-infected rice fields and Khan et al. (2014) recorded 32–36% yield enhancement due to due to application of phorate. Relatively greater crop damage and yield suppressions due to root-knot nematode have been reported in DSR (Khan et al. 2023b).

23.7 Management Strategies

Management of RRKN *M. graminicola* in rice is a challenging task because of high water requirement of this crop which causes dilution of the applied material. Therefore, the measures should target to reduce the pre-plant population of the nematode below the economic threshold level. The root-knot nematode management in DSR becomes more important because young roots of the germinating seeds become available to the nematode juveniles for invasion, whereas, in the transplanted rice, well-developed seedlings of around 1 month age are exposed to the nematode attacks. Hence, RRKN invesion in the DSR may lead to poor seed germination as well as mortality to the seedlings. Considering the farmer's suitability, following management strategies could be suggested for management of *M. graminicola* infecting direct seeded rice.

23.7.1 Cultural Method of Management

Flooding Flooding of rice field is one of the effective methods of management of infestation of *M. graminicola* in DSR. Soriano et al. (2000) observed that RRKN-induced crop damage can be subsided if the field is kept flooded from the early stage till the late stage. However, this practice can be applied in the area where surplus natural water is available for flooding.

Crop Rotation Crop rotation involves cultivation of tolerant or non/poor host crop after a susceptible crop for a specific period. This practice has proved extremely effective in managing RRKN in rice (Khan et al. 2023b). In continuous rice cropping system, taking a non-host crops in between the rice crops drastically reduced the nemaotde infection in the subsequent host crop (e.g., rice-mungbean-rice, rice-tobacco-rice, rice-watermelon-rice, rice-cotton-rice etc.,) (Davide and Zorilla 1983). Similarly, cultivation of sweet potato, cowpea, sesamum, castor, sunflower, soybean, turnip, and cauliflower is also reported to suppress *M. graminicola* soil population without any damage to the rotation crop (Rao et al. 1984; Rao 1985). Ramakrishnan (1995) reported 94–98% decline in the soil population of *Hirshmanniella oryzae* and *Meloidogyne* spp. with rotation of rice by brinjal. Other sequences such as rice-mustard-rice followed by rice-maize-rice and rice-fallow-rice significantly suppressed the RRKN soil population (Kalita and Phukan 1996). The rotation of rice with jute, mustard, and chickpea may also reduce the infestation of *M. graminicola* (Pankaj et al. 2010).

Soil Amendments Organic materials like cakes, composts, etc. may prove suppressive to plant nematodes in soil. Additionally, decaffeinated tea leaves or compost made from water hyacinth significantly decreased nematode galling and enhanced seedling growth (Roy 1976). Similarly, soil amendment with fresh tissues of *Eclipta alba* caused complete mortality of *M. graminicola* juveniles (Prasad and Rao 1979). In situ decomposition of marigold resulted in remarkable decline in root galling and 46% increase in the rice grain yield (Polthanee and Yamazaki 1996).

The organic amendments in rice field resulted in decline in galling and juvenile population (Poudyal et al. 2001). Poultry manure can also be suppressive to *M. graminicola* (Amarasinghe et al. 2007; Amarasinghe 2011). Soil application of different oil cakes suppressed RRKN infestation in rice (Huang et al. 2015). The soil amendments with the oil cakes of mustard, neem, and castor effectively improved plant growth and suppressed RRKN galling and reproduction, with greater impact of castor cake (Devi et al. 2019).

Soil Solarization Soil solarization before sowing of seeds has been reported to be suppressive to the RRKN juvenile in the soil (Gaur 1994; Ganguly et al. 1996; Khan et al. 2020b, 2021a). The deep plowing before the solarization for 2 weeks caused significantly greater decline in RRKN soil population in comparison to without plowing. The polythene covering or mulching may further increase the effectiveness of the treatments (Khan et al. 2012).

23.7.2 Host Resistance

Growing the resistant cultivars/varieties of crops is a most economic and effective method of reducing crop losses from plant pathogens. Screening of rice varieties or lines to identify resistance against RRKN and their recommendation for cultivation is a continuous process. A number of varieties of rice such as TKM 6, Patnai 6 and N 136 (Israel and Rao 1971), Garem and Dumai (Cox 1980) were identified as resistant, as root galls were not formed. Prasad et al. (1986) reported some TNAU (ADT) lines of rice to be resistant to rice root-knot nematode. Similarly, rice cv. Loknath 505 and M-36 were found resistant to RRKN (Hassan et al. 2004) whereas Poudyal et al. (2001), Devi and Thakur (2007), Devi (2014), and Amarasinghe (2011) reported most of the rice cultivars included in the screening program to be susceptible to RRKN. Srivastava et al. (2011) reported only rice cvs. Achhoo and Naggardhan out of 87 cultivars screened to be resistant to RRKN (Narasimhamurthy 2014). Similarly, out of 20, one genotype KMP-179 expressed resistance against *M. graminicola*. A study was undertaken to screen 33 genotypes of Asian rice against *M. graminicola* under DSR condition (Devaraja et al. 2017). On the basis of root-knot index and multiplication factor, the cv. NDR-97 expressed strong resistance to RRKN with less than 2 galls per plant.

23.7.3 Biological Method of Management

Biocontrol is an important and effective method to suppress nematode infection including root-knot nematodes (Khan 2008). This method is aimed to promote multiplication of naturally occurring nematode antagonists or to introduce the inocula of microbial antagonists to achieve parasitization of plant nematodes leading to decline in the population to a level that crop damage and nematode population are significantly reduced (Khan 2007). The antagonists (biocontrol agents) can be applied alone or along with organic material even with chemical pesticides and offer satisfactory nematode control in agricultural crops (Stirling 1991; Khan and Anwer 2011a, b; Khan et al. 2019a). The important biocontrol agents (BCA) which can effectively and efficiently parasitize and antagonize plant nematodes are *Pochonia chlamydosporia, Purpureocillium lilacinum, Trichoderma harzianum, Pasturia penetrans,* etc. (Jatala 1986; Stirling 1991; Kerry 2000; Khan 2016). In recent years use of plant growth-promoting microorganisms (PGPR) has gained popularization in nematode management, such as phosphate solubilizing microorganisms like fluorescent Pseudomonads, Bacilli, *Penicillium, Aspergillus,* etc. (Khan et al. 2009, 2016a, b, 2017). The antagonists or PGPR may significantly contribute in protecting DSR in the early stage if applied as seed dressing. Further, *Trichoderma* spp. have been found effective against nematodes also (Mohiddin et al. 2010; Khan and Mohiddin 2018). Formulations of *T. harzianum, Pseudomonas fluorescence,* and *Bacillus subtilis* are available in the market (Khan et al. 2011, 2016a, b). The continued increase in market demand of these biopesticides indicates the adaptability of the formulation and their effectiveness against plant diseases (Sikora and Roberts 2018; Khan and Anwer 2011a; Shahid and Khan 2016, 2019; Mohammed and Khan 2021).

Several microbial isolates summarized above have been found to be promising for successful management of RRKN (Haque et al. 2018; Khan et al. 2021b). Pathak and Kumar (1995) found around 96% mortality to RRKN juveniles due to treatments with 50% and 100% culture filtrates of *T. harzianum and Purpureocillium lilacinum* were found to be effective against second-stage juveniles of *M. graminicola* at 100%, 75%, 50%, and 25% concentration. Singh et al. (2019) reported highest mortality to RRKN with *Trichoderma* isolates followed by *P. lilacinum.* Kumar et al. (2020) also observed greater effectiveness of *Trichoderma* isolates S13 and S7 and *P. lilacinum* against *M. graminicola* in vitro condition. The culture filtrates of several other fungi have also been reported to cause nematicidal effect on phytoparasitic nematodes, by producing toxic metabolites, antibiotics, enzymes, etc. (Chet et al. 1997; Sayre 1971; Limon et al. 1998; Blaxster and Robertson 1998; Sharon et al. 2001; Khan et al. 2018; Mohammed and Khan 2021).

The biocontrol agents may be applied by different methods to control RRKN depending on the method of cultivation (Khan et al. 2021b). In DSR, seed treatment and soil application in the main field may prove highly effective. The biocontrol fungi viz. *T. harzianum*, *Pochonia chlamydosporia,* and *Pseudomonas syringe* have been found to be highly suppressive to *M. graminicola* (Pathak and Kumar 2003; Singh et al. 2007a, b; Haque and Khan 2021a). *Pseudomonas stutzeri*, *Pochonia* *chlamydosporia* and *Aspergillus niger* applied as seed dressing and soil application significantly prevented galling on rice roots produced by RKN under pot condtions according to Khan et al. (2021b). The treatments with *P. chlamydosporia* provided highest degree of RRKN control and reduced the galling by 60–64% under field condition. Seed treatment with three strains of *P. fluorescens* (PF1, TDK1, and PY15) caused significant decline in the galling on rice roots. Similarly, the treatment of *T. virens* decreased the root galls and RRKN J_2 population in soil (Bhagawati and Choudhury 2018). Soil application of bacterial biocontrol agents viz., *Bacillus subtilis, B. pumilus,* and *P. fluorescens* when applied in soil significantly improved the plant growth of rice in RRKN-infested soil (Subudhi et al. 2019). The mechanism of nematode supression has been drescribed as a results of antibiosis, synthesis of nematotoxic metabolites/ enzymes, parasitiztion on nemaotdes, induced systemic resistance etc. (Spiegel and Chet 1998; Viterbo et al. 2002; Verma et al. 2007; Khan 2016).

23.8 Integrated Method of Management

The concept of integrated nematode management (INM) has gained importance against plant nematodes and appears particularly applicable in DSR because of handy application of INM components in this cultivation method. The INM approach may also minimize limitation of individual methods, and increases the success rates of the management strategy. Several workers have tried to manage *M. graminicola* infecting rice through integrated approach (Narasimhamurthy et al. 2016; Kumar et al. 2017; Haque and Khan 2022).

Combined application of 2.5 kg *P. fluorescens*, 1 ton neem cake, and 1 kg a.i. carbofuran per ha greatly reduced the RRKN soil population and improved the rice yield (Anitha and Rajendran 2005). The combined application of cinnamon leaf oil and poultry manure resulted in a higher drop in *M. graminicola* population decline than either treatment alone (Amarasinghe 2011). The 15 days continuous solarization preceded by 1 kg *a.i.* Furadan 3G per ha or 50 g *P. fluorescens* 1% WP enhanced the seedling growth and decreased RRKN galling and fecundity (Pankaj et al. 2015). The combined application of 20 g *P. fluorescens* and 0.3 g *a.i* carbofuran per m^2 significantly enhanced the growth and yield of rice and up to 79% decline in the RRKN soil population (Narasimhamurthy et al. 2016).

Integration of organic amendments, *Trichoderma* and carbofuran was found quite effective in checking the galling on rice roots (Kumar et al. 2017). Combined treatment of neem cake + mustard cake + FYM produced highest decline in RRKN infestation compared to individual effects (Anupam et al. 2023). Haque and Khan (2022) observed 68–73% control in the galling caused by *M. graminicola* in rice due to integrated treatment with fluopyrum and *P. putida*/ *T. harzianum*. In addition, nanotechnology (Khan et al. 2019b, 2019c), biotechnology (Khan et al. 2023c; d'Errico and Silvia 2023), and omics technology (Rocha and Schwan 2023) can also be exploited to develop novel methods which may be integrated with other approaches to develop effective INM modules in DSR (Khan et al. 2019b, 2019c; Khan and Rizvi 2014, 2017, 2018)

23.9 Rice Stem Nematode

The rice stem nematode (RSN) *Ditylenchus angustus* also called as ufra nematode shows migratory ecto-parasitism on rice (Khan 2008). The nematode is considered to be the most destructive pest of DSR under deepwater condition (Bora and Rahman 2010). Rice stem neamotde is a seed born in nature, and the rice crop residue in the field acts as a primary source of infestation (Rahman et al. 1994). The rice stem nematode invades the young rice plants at collar region, and under humid condition, the nematode migrates upward on the surface of rice plants to feed on tender growing tissues (Rahman and Evans 1987). The nematodes survive in the fallow field on wild rice ratoons, weed grasses, and leftover stubbles of the crop and wait for the next crop (Rao et al. 1986a, b).

23.10 Distribution and Host Range

The rice stem nematode was first recorded in deepwater rice in Naokhali district of then East Bengal (now in Bangladesh) in 1913 (Butler 1913a). Thereafter, occurrence of this nematode has been reported from many other countries viz., Burma, Malaysia, the Philippines, Vietnam, Thailand, Egypt, and Madagascar (Bora and Rahman 2010). The RSN causes the disease which is known with different local names such as "*Ufra*" (India), "*Dak pora*" (Bangladesh), "*Okhet Pet*" (Myanmar), "*Yad Ngo*" (Thailand), and "*Tim Dot San*" (Vietnam). Due to intensification of rice cropping and introduction of irrigation facilities, infestation of rice stem nematode has also spread to transplanted boro and Sali rice (Bakr 1978; Cuc and Kinh 1981; Rao et al. 1986b). Cultivated rice is the main host of rice stem nematode. Apart from cultivated rice, the wild *Oryza* spp. and several weed species such as *Leersia hexandra, Hygroryza aristata, Echinochloa colona,* and *Sassiolepsis interrupta* are hosts of this nematode (Hashioka 1963; Miah and Bakr 1977; Cuc 1982).

23.11 Symptoms of Infestation

Rice stem nematode feeds on the growing tissues of leaf, leaf sheath, peduncle, and spikelets. The first characteristic visible symptoms of *D. angustus* infestation is appearance of splash-patterned chlorosis on the leaf and leaf sheath, especially near leaf base (Hashioka 1963). The young leaves may develop pale longitudinal streaks and plants may become somewhat stunted. The yellowish areas later turn brown to dark brown. Further, the leaves may be slightly thinner and more flaccid than the normal. The leaves often wilt. The young leaves may become twisted and crinkled with corrugated leaf margins under severe infection. Sometimes lateral

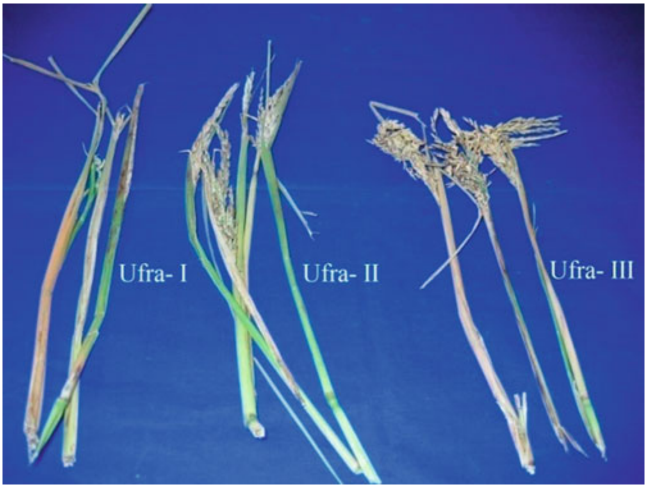

Fig. 23.2 *Ufra* symptoms at reproductive stage of rice plants infected with *Ditylenchus angustus*. (Source: B. Bhagawati, Assam Agriculture University, Jorhat)

branching occurs from the infected nodes giving a bushy appearance to the plants (Bora and Rahman 2010). The peduncle coils and pedicels bear distorted sterile spikelets. The stem bears characteristic lesions just above one or more of the upper nodes. The infested inflorescence usually becomes crinkled, with empty shriveled glumes, especially at the base. The panicle becomes spirally distorted (Ou 1972; McGeachie and Rahman 1983). Two types of ufra symptoms are categorized at the reproductive stage: “thor ufra” or “swollen ufra,” in which the panicle fails to emerge out of flag leaf sheath, whereas, in “pucca ufra” or “ripe ufra,” the panicle emerges partially or completely but most of the grains are unfilled chaffy (Butler 1913b). However, Cox and Rahman (1980) classified three ufra symptoms viz., *ufra I, ufra II,* and *ufra III* depending on the extent of emergence of the panicle. In case of *Ufra I*, panicle does not emerge; in *Ufra II,* panicle partially emerges, and in *Ufra III*, panicle emerges completely but most grains are chaffy (Fig. 23.2).

23.12 Life Cycle

The fourth stage larvae of *D. angustus* under quiescent state survives on crop residue left in the field whereas on wild rice, ratoons, and weeds, the nematode remains active. The dormant J_4 coil together inside peduncles under upper leaf sheaths and within the glumes of the lower grains of the panicle. The number of nematodes varies from 1 to 30,000, in single infested stubble and 5.3–2400 under seed husk per seed (Haque and Khan 2022). The nematode may occur in soil but the RSN is not soilborne (Pathak 1992). In a rice seed, the J_4 are found at the overlapping region of the rice husk. The J_4 larvae become active under moist condition. Under wet and humid condition, the larvae (J_4) move on the seedling surface and feed

Fig. 23.3 SEM of eelwool sowing individually coiled quiescent fourth stage juveniles of *Ditylenchus*. (Source: M. W. Khan, Aligarh Muslin University, Aligarh)

ectoparasitically on the tender growing tissues. The J_4 readily attack coleoptiles of the germinating seed. Later the J_4 larvae reach up to the inner portion of the growing tissue by moving between the folded leaves and leaf sheaths, but remain ectoparasitic (Bora and Rahman 2010).

The J_4 larvae undergo fourth molt under the compact leaves and become adults, and the males and females mate. The reproduction is amphimixtic, and a female lays 50–100 eggs and three generations may be completed in a cropping season (Cox and Rahman 1979). *D. angustus* is reported to take 21 days from J_2 to egg. The life cycle may be completed in 24 days (Cuc 1982) whereas Plowright and Gill reported 10–27 days duration for life cycle at 30 °C. When the crop is near the maturation, predominantly J_4 larvae individually coil, become inactive, and undergo quiescence, forming the typical eelwool state (Fig. 23.3).

23.13 Yield Losses

The yield loss inflicted by rice stem nematode in deepwater rice varies from year to year depending on the time of infection, severity of the disease, and the environmental conditions prevailing at the time of disease development (Haque and Khan 2021a). However, on average *D. angustus* is reported to cause 10–15% (India), 20–90% (Thailand), 50–100% (Vietnam), and 40–60% or occasionally 100% yield losses in Bangladesh (Hashioka 1963; Miah and Bakr 1977; Cuc and Kinh 1981; Rao et al. 1986b). Yield loss also varies with the *ufra* symptoms. Of the three ufra categories, *ufra* II is considered as disease index. When there is more than 40% *ufra* II symptom in a field, yield loss approaches to 100% (Cox and Rahman 1980). In all these studies, it was observed that loss of panicle density in a unit area is the major component for the yield loss. For instance, *D. angustus* causes 40–90% yield loss or sporadically 90% to deepwater rice in Bangladesh (Latif et al. 2011).

23.14 Management Strategies

23.14.1 Cultural Method of Management

Burning of Stubble and Deep Plowing Since *D. angustus* is primary stubble-borne, the burning of stubble is a very effective method for reducing the soil population of the nematode and eventually the inoculum level of the nematode for next rice crop. The effectiveness of the method may be further increased by deep plowing the field before stubble burning (McGeachie and Rahman 1983; Hashioka 1963; Ou 1972). The uprooting and sun exposure drying of stubble are very important and shall greatly increase the effectiveness of burning strategy. The plowing must be done soon after harvest of deepwater rice, which shall also help in early decomposition of stubble materials. During harvesting, adequate amount of straw be left in the field to facilitate proper burning of infested stubble. Proper and complete burning of entire stubble should be ensured. This practice should be adopted in all the fields over a large area because the rice stem nematode shall soon spread from unburnt areas when fields get submerged (Catling et al. 1979). Sometimes plowing and burning become difficult in deep-water rice because the field may remain submerged after harvest. In such situation, practice may be delayed. However, in view of serious environmental issues the stubble burning at a mass scale is not recommended, hence, alternative cultural options should be adopted.

Crop Rotation To reduce the soil infestation of rice stem nematode, cultivation of resistant or non-host crop such as jute which is good option to replace deepwater rice should be adopted for 2–3 years (McGeachie and Rahman 1983). In *rabi* season growing of mustard, wheat, and millet (November to March), followed by sowing of deepwater rice may considerably reduce the soil population of rice stem nematode. Under such cropping sequences, plowing in combination with sun heat drying may reduce the initial population of *D. angustus* in the field leading to much lower incidence of *ufra* in the next deepwater rice.

Escape Cropping and Delayed Sowing The population of active nematodes starts decreasing from the beginning of January reaching to less than 1% of the initial population by the middle of April (Cox and Rahman 1979). This shows that *D. angustus* can hardly survive for a period of about 4 months after the harvest of deepwater rice. But the field must be kept free of alternate/weed hosts on which the RSN survives during these 4 months. Delay in the sowing time from the normal leads to proportionate decline in the nematode number per stem and the number of infested stem (McGeachie and Rahman 1983). Short duration deep-water rice cultivars such as Padmapani and Digha mature early and thus escape post-infection damage by *D. angustus*. Such rice cultivars may be cultivated in *ufra* endemic areas (Mondal and Miah 1987; Rathaiah and Das 1987). Das and Bhagawati (1994) also reported lower incidence of *ufra* in late sown deepwater rice.

Destruction of Alternate Host and Ratoon Crops Destruction of alternate host in deep-water rice field such as *Hygrorysa aristata, Sacciolapis interrupta,* and *Echinocola colanata* may greatly help in checking the survival of stem nematode in the absence of rice (Miah and Bakr 1977; Sein and Zan 1977; Cuc 1982). Generally, *D. angustus* in the absence of rice is able to survive during January to April on alternate hosts. Similarly, rice stem nematode can live an active state in ratoons and voluntary crops grown after the harvesting of main crop. Butler (1919) observed heavy infection of rice stem nematode in main crops from marshy patches with diseased ratoon rice present in the field during off-season. Therefore, before sowing of main crop destruction of such ratoon crops must be ensured to prevent the passing of nematode infestation to the next season crop.

23.14.2 Host Resistance

Growing of resistant varieties or lines is a highly economical and effective method of *D. angustus* management in deep-water rice. Rahman (1987) tested more than 3000 deepwater entries for resistance against *D. angustus*. Of these, few lines of Rayada, Bazail, Gowal, and Karkati showed less than 20% infestation and were considered to be resistant. Later on these entries were again tested in different locations of South East Asian countries and confirmed the resistance of the varieties/lines, especially Bazail-65, Rayada 16-011, Rayada 16-013, Rayada 16-05, Rayada 16-06, Rayada 16-07, Rayada 16-08 and Ba Tuc (Anonymous 1986).

Miah and Bakr (1977) screened several wild rice and some Rayada lines and found that *Oryzae subulata* and Rayada R 16-06 were resistant to *D. angustus*. Rahman and McGeachie (1982) evaluated a number of rice varieties and lines for resistance against rice stem nematode and found some lines of Lakhi, Bazail, Karkati, and BR 308-3-3-2 to be resistant. Pathak (1992) reported 3 out of 62 varieties/lines screened against rice stem nematode, namely AR-9©, IR 13437-20-4E-Pl, and IR-17643-4 to be resistant. The Rayada B3 has been recommended as a high-yielding, and ufra-resistant deepwater rice variety for cultivation in Assam, India. Latif et al. (2011) observed that 4 out of 53 entries, namely Fukuhonami, Hyakikari, Akiyu Taka, and Matsuhonami, expressed resistance to *D. angustus.* Similarly, Khanam et al. (2016) screened 85 rice genotypes against rice stem nematode and reported rice cv. Manikpukha to be highly resistant and six varieties to be resistant against the nematode.

23.14.3 Biological and Integrated Method of Management

Literature pertaining to management of rice stem nematode through the application of biocontrol agents and biopesticides is scanty. Only a few biocontrol fungi and bacteria are reported to effectively suppress the rice stem nematode. Some studies have shown that treatments with *Bacillus subtilis, Pseudomonas fluorescens, Pochonia chlamydosporia,* etc. when incorporated in soil or on foliage suppressed the population of rice stem nematode in the soil and reduced the disease incidence.

Different neem-based pesticides in combination can effectively manage the *ufra* disease (Chakraborti 2000a). Chakraborti (2000b) reported that application of neem-based pesticides in combination with cultural practices significantly reduced *ufra* incidence. An integrated approach involving burning of diseased stubbles followed by soil application of carbofuran before seed sowing and two foliar sprays of neem product have been found highly effective in controlling ufra in direct seeded deepwater rice (Das and Saikia 2005). Another integrated module in DSR comprising a resistant variety Rayada 16-06 or an early maturing variety Padmapani and seed soaking in 0.2% monocrotophos and hostathion for 6 h and spraying with 0.2% at 45 and 80 days after sowing proved quite effective against *D. angustus* (Bhagawati and Bora 1993). Nanotechnology may also be applied in plant disease management (Khan and Rizvi 2014; Khan et al. 2019b, c, d) and disease detection (Khan and Akram 2020; Khan and Rizvi 2016; Khan et al. 2020a). Nano-sensors are the most important product of nanotechnology, and have great potential for use in plant disease diagnosis (Khan 2023). Sellappan et al. (2022) developed nanobiosensor to early detection and prevention of agricultural crops from harmful microorganisms. Using specific nanoparticles as nano-sensors for advance detection of plant nematodes so as to achieve their timely control (Khan and Rizvi 2018).

23.15 Conclusion and Future Perspectives

The rice root-knot nematode, *M. graminicola* poses a serious threat to DSR under upland conditions. Likewise, rice stem nematode *D. angustus* is also a destructive pest in DSR under deep-water conditions. Infestation level and severity of both the nematodes are generally found relatively greater in DSR over transplanted rice. This is apparently due to the fact that in the DSR, the plant is exposed to the nematode invasion just from the seed germination whereas, in transplanted rice, the well-developed seedlings are exposed to the nematode. For this reason, the germination percentage of seeds is also affected in DSR. Management of both major nematodes in DSR is possible by taking integrated measure starting before the crop is sown. However, the damage to rice cultivation is usually overlooked by farmers because of lack of awareness. Further, farmers assume that the chemicals they apply for other purposes in rice crop shall also control the nematode problem. Hence, in addition to developing novel biological and biotechnological techniques for management of nematode infestation in DSR, there is great need to sensitize the farmers about the need and significance of independent measures to manage nematodes in rice as well as in other crops.

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