Chapter 17
Fungal Bioinoculants for Plant Disease Management

Mujeebur Rahman Khan and M. Arshad Anwer

Abstract Plant diseases are among the major constraints in the production of food crops and inflict significant losses to global agriculture. Pesticides are widely used to control plant diseases but their application is costly and, in some cases, may bring more disadvantages than benefits. Use of bioinoculants to control plant diseases is an economically viable and ecologically sustainable method of disease management. A large number of bioinoculants is available; among them, bioinoculant fungi constitute the majority and are widely used in different cropping systems. Important bioinoculants that directly parasitize plant pathogens include *Trichoderma* spp., *Paecelomyces lilacinus,* and *Pochonia chlamydosporia*. Plant growth-promoting fungi such as *Aspergillus* spp. and *Penicillium* spp. may also suppress plant pathogens. In general, bioinoculants are effective against seed- and soil-borne fungi and nematodes. However, an important limitation in their commercial use in crop protection is nonavailability of efficient immobilizing systems for delivery and survival of bioinoculants. This chapter describes important bioinoculants, their effects, and their mechanisms of action against plant diseases caused by fungi, bacteria, and nematodes. State-of-the-art technology available for the production of commercial formulation of bioinoculants, along with important lacuna, is also discussed.

17.1 Introduction

Plant diseases are a common component of natural systems and are among many ecological factors that keep plant and animal populations in balance. When a plant suffers from an infection, its normal development and functioning are affected and

M.R. Khan (✉)
Department of Plant Protection, Faculty of Agricultural Sciences,
Aligarh Muslim University, Aligarh 202002, India
e-mail: mrkhan777in@yahoo.co.in

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it becomes diseased. Kuhn, in 1858 (Wilhelm and Tietz 1978), was probably the first to scientifically define plant disease as "abnormal changes in physiological processes which disturb the normal activity of the organs." Ward (1896) defined disease as a condition in which the function of the organism is improperly discharged or, in other words, it is a state which is physiologically abnormal and threatens the life of the being or organ. The British Mycological Society defined disease as a harmful deviation from the normal functioning process (Wallace et al. 1950). Plant disease can also be defined as "a physiological disorder or structural abnormality that is harmful to the plant or to any of its parts or products that reduces the economic value" (Stalkman and Harrar 1957). According to Horsfall and Cowling (1977), disease is a malfunctioning process that is caused by continuous irritation which may result in some suffering, and this produces symptoms. More scientifically, "disease is any malfunctioning of host cells and tissues that results from continuous irritation by a pathogenic agent or environmental factor and leads to development of symptoms" (Agrios 2005).

Crop plants are known to be affected by over one hundred diseases (Agrios 2005). However, only a few, usually a single pathogen, at a given time can multiply to an extent to cause the disease. Diseases of crop plants are among the most important constraints in the production of adequate quantities of food. Approximately half of the world`s total agricultural production is lost due to various pests and diseases at planting and postplanting stages (Khan 2008). The incidence of crop losses due to disease is much lower in developed countries because of awareness among farmers for disease management. In developing countries, greater yield losses occur due to plant diseases because of unplanned agricultural practices such as use of marginal lands, low agricultural inputs, and lesser concerns by farmers toward plant disease management. On average, losses inflicted by weeds, plant diseases, and insect pests upon agricultural crops have been estimated as 33, 26, and 22%, respectively (Khan 2008). According to another estimate, plant diseases, weeds, and insects contribute to a 14.1, 10.2, and 12.2%, respectively, decline in crop production (FAOSTAT 2003; Agrios 2005; Table 17.1). Among different kinds of pathogens, the greatest losses are inflicted by fungi (42%) followed by bacteria (27%), viruses (18%), and nematodes (13%) (Khan and Jairajpuri 2010; Fig. 17.1).

Table 17.1 Estimated annual crop losses caused by pests and diseases worldwide[a]

Practice	Losses (US $)
Attainable cop production (2002 prices)	$1.5 trillion
Actual crop production (−36.5%)	$950 billion
Production without crop protection	$455 billion
Losses prevented by crop protection	$415 billion
Actual annual losses to world crop production	$550 billion
Losses caused by disease only (14.1%)	$220 billion

[a]FAOSTAT (2003); Agrios (2005)

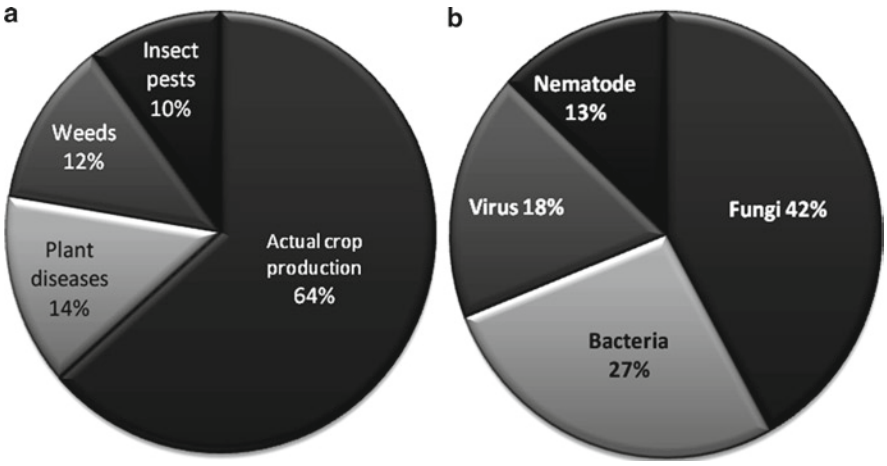

Fig. 17.1 Actual crop production and annual crop losses due to plant diseases, insect pests, and weeds (**a**) and breakdown of crop losses caused by fungi, bacteria, viruses, and nematodes (**b**)

17.1.1 Management of Plant Diseases

Continual increases in global human population have put twofold pressure on agriculture. Precious agricultural lands are being diverted from crop production to urbanization and industrialization. As a result, the net area under crop production is shrinking, whereas demand for food products continues to increase at an alarming pace. According to one estimate, the present global land area under crop production would produce much greater quantities of food than present requirements if pest- and disease-free crops were grown (Khan and Jairajpuri 2010). Hence, the primary requirement to meet food requirements of both present and future populations is to integrate plant protection techniques into crop production systems. Numerous methods of pest and disease management are available including chemical, cultural, physical, and biological, which are used according to the crop, pathogen, availability of material, and demand of the situation.

Pest control methods involving chemical pesticides is one of the most effective and reliable means of disease management; however, in an environmentally conscious world, the use of pesticides is under criticism because of several real and perceived ill effects. Age-old cultural practices like crop rotation, mixed cropping, green manuring, etc. to combat plant diseases are slow in action and are of no benefit during epidemic situations. The pace of development and durability of resistant/tolerant crop cultivars has been slow and unreliable in spite of tremendous advancements in plant genetic engineering. Considering these limitations, there has been a growing emphasis on the development of novel management practices that alone or in integration with other practices result in a good degree of reduction in pathogen inocula and disease severity coupled with sustainability in the production system, cost-effectiveness, and eco-friendliness. Biological control is an important approach

in this direction. The most obvious and environment-friendly alternative to pesticides is to use naturally occurring beneficial bioinoculants to manage pests and diseases.

17.1.1.1 Biological Control

Consensus is developing that chemical-based farming is nonsustainable; as a result, ecological approaches are being researched more intensively. The most obvious environment-friendly alternative to pesticide application for managing agriculturally important diseases is the use of biological approaches. Biological control is based on the phenomenon that every living entity has an adversary in nature to keep its population in check (Khan 2005). In 1965, Garrett defined biological control as "any condition under which, or practice, whereby, survival and activity of a pathogen is reduced through the agency of any other living organism (except man himself) with the result that there is a reduction in the incidence of disease caused by the pathogen." Baker and Cook (1974) defined biological control as the "reduction of inoculum density or disease producing activities of a pathogen or parasite in its active or dormant state, by one or more organisms, accomplished naturally or through manipulation of the environment, host, or antagonists, or by mass introduction of one or more antagonists." In 1983, they revised the definition to "the reduction of the amount of inoculum or disease producing activity of a pathogen accomplished by one or more organisms other than man."

Biological control can be achieved either by introducing bioinoculants (biocontrol agents) directly into a natural ecosystem or by adopting cultural practices that stimulate survival, establishment, and multiplication of the bioincoulants. Hence, more scientifically, biological control of pests and diseases can be defined as reduction in disease severity, crop damage, population or virulence of the pest or pathogen in its active or dormant state by the activity of microorganisms that occur naturally through altering cultural practices which favors survival and multiplication of the microorganisms or by introducing bioinoculants.

In 1874, Roberts demonstrated the first evidence of antagonistic action of microorganisms in liquid cultures between *Penicillium glaucum* and a bacterium and introduced the term "antagonism" (Baker 1987). Since then, a great deal of data has been generated to demonstrate that biological control is a realistic proposition for disease management. The first attempt to control a plant disease with microorganism introduced to soil was by Hartley in 1921 where introduction of isolates of saprophytic fungi and one bacterium resulted in significant reduction in severity of damping-off of pine seedlings caused by *Pythium debaryanum* (Baker 1987).

Bioinoculant Fungi and Mechanisms of Action

Bioinoculants or biocontrol agents are the microorganisms that induce stimulatory effects on plant growth and/or suppressive effects on pests or pathogens through

a variety of mechanisms when applied in an ecosystem. A large number of bioinoculants have been investigated to harness their beneficial effects on crop productivity. Bioinoculants are primarily fungal and bacterial in origin. Bioinoculant fungi basically work through parasitism (Papavizas 1985; Stirling 1993) against plant pathogenic fungi and nematodes (Khan 2005). The important genera of biocontrol fungi that have been tested against plant pathogenic fungi and nematodes include *Trichoderma*, *Aspergillus, Chaetomium, Penicillium, Neurospora, Fusarium* (saprophytic), *Rhizoctonia, Dactylella, Arthrobotrys, Catenaria, Paecilomyces, Pochonia,* and *Glomus*. Other kinds of biocontrol agents such as plant growth-promoting organisms have also been evaluated for disease management (Papavizas 1985; Nair and Burke 1988). A number of fungi such as *Aspergillus* spp., *Penicillium* spp., and *Trichoderma* spp. are active phosphate-solubilizing microorganisms (PSM), which also suppress plant pathogens. Application of PSM can control soil-borne pathogens such as *Fusarium oxysporum, Macrophomina phaseolina, Pythium aphanidermatum, Rhizoctonia solani, Sclerotinia sclerotiorum*, and *Meloidogyne incognita* (Sen 2000; Khan and Anwer 2007, 2008; Khan et al. 2009).

Bioinoculants suppress plant pathogens by direct parasitism, lysis, competition for food, direct antibiosis or indirect antibiosis through production of volatile substances, viz., ethylene, hydrogen cyanide, alcohols, monoterpenes, and aldehydes (Juan et al. 2005). Activity of bioinoculants mainly depends on the physicochemical environmental conditions to which they are subjected. These mechanisms are complex, and what has been defined as biocontrol is the final result of varied mechanisms acting antagonistically to achieve disease control. Some important mechanisms in disease suppression by bioinoculants are discussed below.

Fungistatic

An effective antagonist is usually able to survive in the presence of metabolites produced by other microorganisms and plants, and multiply under extreme competitive conditions. *Aspergillus* spp., *Penicillium* spp., and *Trichoderma* spp. were found to be most resistant to herbicides, fungicides, pesticides, and many toxic heavy metals at minimum inhibitory concentrations (MIC) of 125–850 μg/ml (Baytak et al. 2005; Yuh-Shan 2005; Ahmad et al. 2006; Braud et al. 2006). Dose–response relationships of fungicide resistance in agar growth tests were examined with *Aspergillus niger, A. nidulans*, and *Penicillium expansum* to pentachloronitrobenzene (PCNB), 3-phenylindole, benomyl, or thiabendazole, and resistance was measured at high concentrations of these chemicals (van Tuyl 1977). When *A. niger* was included with Foltaf SOW (Captafol 80%) for the treatment of pigeonpea wilt, the disease was more effectively controlled than when the fungicides were used alone (Bhatnagar 1995).

Trichoderma strains grow rapidly when inoculated in soil because they are naturally resistant to many toxic compounds such as DDT and phenolic compounds (Chet et al. 1997). *Trichoderma* strains are efficient in controlling several phytopathogens such as *R. solani*, *P. ultimum* and *S. rolfsii* when alternated with methyl

bromide, benomyl, captan, or other chemicals due to the presence of the ABC transport system (Vyas and Vyas 1995; Harman et al. 2004). When *Trichoderma harzianum* was included with Blue Copper-50 for the treatment of pigeon-pea wilt, the disease was more effectively controlled than when the fungicides were used alone (Bhatnagar 1995).

Competition for Nutrients

Starvation or shortage of nutrients is one of the most common causes of death of microorganisms (Chet et al. 1997). Competition resulting in limiting the nutrient supply to fungal phytopathogens results in their biological control (Chet et al. 1997). For instance, in most filamentous fungi, iron (Fe) uptake is essential for viability (Eisendle et al. 2004), and under Fe-deficient condition, most fungi excrete low-molecular-weight ferric iron-specific chelators termed siderophores to mobilize environmental Fe (Eisendle et al. 2004). Siderophores play a considerable role in biocontrol of soil-borne plant pathogens (Leeman et al. 1996) and as a supplier of Fe nutrition to crop plants (Jadhav et al. 1994). Since plant pathogens may not have the cognate ferri–siderophore receptor for uptake of the Fe–siderophore complex, they are prevented from proliferating in the immediate vicinity because of Fe deficiency (O'Sullivan and O'Gara 1992). Hence, siderophore-producing bioinoculants can confer a competitive advantage to interactions in the rhizosphere (Raijmakers et al. 1995). One of the most sensitive stages for nutrient competition in the life cycle of *Fusarium* is chlamydospore germination (Scher and Baker 1982). In soil, the chlamydospores of *F. oxysporum* require adequate nutrition to maintain a germination rate of 20–30%. Germination may decrease due to sharing of nutrients with other microorganisms. Root exudates are a major source of nutrients in soil. Thus, colonization in the rhizosphere by an antagonist might reduce infection by *Fusarium*-like pathotypes (Cook and Baker 1983). *Aspergillus niger* AN27, a potential biocontrol agent, produced both hydroxamate and catecholate groups of siderophores (Sen 1997; Mondal and Sen 1999).

Trichoderma has a superior capacity to mobilize and take up soil nutrients compared to other microorganisms. The efficient use of available nutrients is based on the ability of *Trichoderma* to obtain ATP from the metabolism of different sugars, such as those derived from polymers widespread in fungal environments, for example cellulose, glucan, and chitin among others, all rendering glucose (Chet et al. 1997). High-affinity glucose transporter, Gtt 1, has been isolated from *T. harzianum* CECT 2413. Role of this transport system is yet to be discovered properly, but its efficiency is considered to be crucial in microbial competitions (Delgado-Jarana et al. 2003). The strain CECT 2413 was present in nutrient-poor environments and relied on extracellular hydrolases for survival. The Gtt 1 is only expressed at very low glucose concentrations, that is, when sugar transport is expected to be limiting in nutrient competition (Delgado-Jarana et al. 2003).

By the same mechanism, soil composition influences the biocontrol effectiveness of *Pythium* by *Trichoderma* (i.e., according to Fe availability). Some *Trichoderma* strains produce highly efficient siderophores that chelate Fe and stop

the growth of other fungi (Chet and Inbar 1994). In addition, *T. harzianum* T35 controls *F. oxysporum* by competing for both rhizosphere colonization sites and nutrients, with biocontrol becoming more effective as the nutrient concentration decreases (Tjamos et al. 1992). Competition for carbon has also been involved in the occurrence of antagonism expressed by different strains of *Trichoderma* spp. against plant pathogens, particularly *F. oxysporum* (Sivan and Chet 1989). The advantage of using *Trichoderma* to control *Botrytis cinerea* is the coordination of several mechanisms, the most important being nutrient competition, since *Botrytis cinerea* is particularly sensitive to low nutrient levels (Latorre et al. 2001).

Antibiosis

Antibiosis is the phenomenon of suppression of one organism by another due to release of toxic substances/metabolites into the environment. Antibiosis is important in determining the competitive saprophytic and necrotrophic ability of antagonists. The bioinoculant fungi may suppress plant parasitic nematodes through antibiosis and by stimulating host defense. Low-molecular-weight compounds and antibiotics (both volatile and nonvolatile) produced by *Trichoderma* species and *Aspergillus* spp. impede colonization of harmful microorganisms including nematodes in the root zone (Eapen and Venugopal 1995). Harzianic acid, alamethicins, tricholin, peptaibols, 6-pentyl-α-pyrone, massoilactone, viridin, gliovirin, glisoprenins, heptelidic acid, oxalic acid, and enzymes are some of the chemicals possessing antibiotic properties produced by *Trichoderma* and *Aspergillus* species (Mankau 1969a, b; Benitez et al. 2004; El-Hasan et al. 2007).

Aspergillus spp. and *Trichoderma* spp. are well known for producing antifungal and antibacterial agents (Buchi et al. 1983; Fujimoto et al. 1993). An antifungal butenolide, harzianolide has been isolated from *Trichoderma harzianum* (Claydon et al. 1991). Most *Trichoderma* strains produce volatile and nonvolatile toxic metabolites that impede colonization by antagonized microorganisms; among these metabolites, the production of harzianic acid, alamethicins, tricholin, peptaibols, 6-pentyl-α-pyrone, massoilactone, viridin, gliovirin, glisoprenins, heptelidic acid, and others have been described (Vey et al. 2001). In some cases, antibiotic production correlates with biocontrol ability, and purified antibiotics mimic the effect of the entire agent. Volatile substances from *Trichoderma* spp. inhibited mycelial growth of *Macrophomina phaseolina* by 22–51% (Angappan 1992). The volatile antibiotics of *T. harzianum* and *T. atroviride* significantly decreased growth of canker fungal pathogens of poplar, *Cytospora chrysosperma* and *Dothiorella gregaria*, but nonvolatile metabolites in the culture filtrate of *Trichoderma* spp. inhibited the linear growth of pathogens (Deshmukh and Pant 1992; Pandey 1988). There are also examples of antibiotic-overproducing strains such as gliovirin-overproducing mutants of *T. virens*, which provide controls similar to that of the wild type and of gliovirin-deficient mutants, which failed to protect cotton seedlings from *Phythium ultimum*, whereas the parental strain did (Chet et al. 1997). *Trichoderma* spp. are reported to produce carbon monoxide, ammonia (Dennis and Webster 1971b), carbonyl compounds, and acetaldehyde (Robinson and Park 1966), which may enhance antagonistic activity in soil.

Aspergillus niger, *Trichoderma* spp., and *Penicillium* spp. that parasitize eggs prefer eggs which are deposited in cyst or a gelatinous matrix. The oviposition nature of *Heterodera* spp. and *Meloidogyne* spp. makes them more vulnerable to attack by these fungi. As soon as the fungi identify a cyst or an egg mass, they rapidly grow and colonize those eggs where larval formation is not complete. However, when larva is formed, the egg becomes less vulnerable. It has been suggested that this differential vulnerability of egg and larval stage is due to chitinolytic activity of these fungi. Chitin is a major constituent of the egg shell, which is lacking in the larval cuticle.

The fungus *P. chlamydosporia* (i.e., *Verticillium chlamydosporium*) produces nematicidal metabolites. The culture filtrate of *P. chlamydosporia* in yeast extract medium showed pronounced nematicidal and nematostatic effects. A dilution of 1:1 culture filtrate caused 100% mortality of *G. rostochiensis, G. pallida*, and *Panagrellus redivivus* (Saifullah 1996c). The actively growing mycelium of *P. chlamydosporia* infects eggs and females of nematodes (Morgan-Jones et al. 1983). Egg hatching in the presence of the fungus was inhibited probably due to the effect of toxins secreted by the fungus (Meyer et al. 1990) or disintegration of the eggshell's vitelline layer and also partial dissolution of the chitin and lipid layers due to activity of exoenzymes (Lopez-Llorea and Duncan 1988; Saifullah and Thomas 1997; Stirling 1991). Serine proteases have been identified in *P. chlamydosporia* (Segers et al. 1994). These extracellular enzymes are synthesized in the presence of nematode eggs and repressed by glucose (Segers et al. 1999). In a chemical investigation of one fungal strain of *P. chlamydosporia*, YMF 1.00613, isolated from root knots of tobacco infected by *M. incognita,* four aurovertin-type metabolites were isolated and identified, including a new compound, aurovertin I (A1), and three known metabolites, aurovertins E, F, and D (A2–A4). The results suggest that the aurovertin-type metabolites produced by *P. chlamydosporia* might be one of the pathogenic factors involved in the suppression of nematode *M. incognita* (Niu et al. 2010).

Paecilomyces lilacinus is an effective parasite of nematode eggs and adults (Jatala et al. 1979) and its mode of action involves recognition phenomena (e.g., chemotaxis and adhesion), signaling and differentiation, and penetration of the nematode cuticle/eggshell using mechanical as well as enzymatic (protease and chitinase) means (Lopez-llorca et al. 2008).

Mycoparasitism

Mycoparasitism involves direct parasitism of one fungus by another and involves recognition, attack, and subsequent penetration and killing of the host fungus (Harman et al. 2004). In a necrotrophic association, there is direct contact between two fungi, and a nutrient exchange channel is established between them. Typical examples are the association of *Arthrobotrys oligospora* with *R. solani* (Persson et al. 1985), *Trichoderma hamatum* with species of *Phythium,* and *Rhizoctonia* with *Sclerotium* (Bruckner and Pryzybylski 1984).

Observations using scanning electron microscopy revealed that *A. niger* coiled around the pathogen hyphae and penetrated within. Presence of *A. niger* hyphae

inside pathogen hyphae has been confirmed using fluorescent microscopy repeatedly in *F. oxysporum* f.sp. *melonis* and *ciceris*, and other pathogens (Sen et al. 1997; Sharma and Sen 1991a, b). Further studies have revealed that *A. niger* could kill *Macrophomina phaseolina,* several species of *Pythium, Rhizoctonia solani, Sclerotinia sclerotiorum* (Sen et al. 1995), and *Sclerotium rolfsii* (Palakshappa et al. 1989). The dead hyphae of the pathogens were eventually invaded. These observations confirm that *A. niger* is a contact and invasive necrotroph (Mondal and Sen 1999).

Trichoderma spp. may detect a host fungus before contact and grow toward it. Such remote sensing is partly due to the sequential expression of cell wall-degrading enzymes, mostly chitinases, glucanases, and proteases (Harman et al. 2004). *Trichoderma* attaches to the pathogen with cell wall carbohydrates that bind to pathogen lectins. Once *Trichoderma* is attached, it coils around the pathogen and forms the appressoria. Production of cell wall-degrading enzymes and peptaibols (Howell 2003) follows, which facilitates both the entry of *Trichoderma* hypha into the lumen of the parasitized fungus and the assimilation of the cell-wall content. *Trichoderma* spp. reacts vigorously with hyphae of the *Fusarium* species. The hyphae of *Trichoderma* spp. when near a pathogen induce morphological deformities in the host hyphae. Many times bursting of hyphae and vacuolation have frequently been observed (Komatsu 1968; Gao et al. 2001). In addition, granulation, coagulation, disintegration, and finally lysis of the pathogen occurs (Lim and Teh 1990; Elad et al. 1983; Nigam et al. 1997; Gao et al. 2001). In vitro studies have revealed greatly suppressed synthesis of endochitinase, chitobiosidase, *n*-acetyl-b-glucosidase, and glucan 1, 3-β-glucosidase, and combinations thereof, during spore germination and germ tube elongation in *Trichoderma* spp. (Lorito et al. 1993; Di Pietro et al. 1993; Lorito et al. 1994a, b).

Stimulation of Host Defense Response

Association of *Trichoderma* spp., *Aspergillus* spp., *Penicillium* spp., and other phosphate-solubilizing fungal antagonists also stimulates plant defensive mechanisms (Howell et al. 2000; Hanson and Howell 2004). An elicitor of plant disease resistance, pectinase, was produced by *A. niger*, which elicited disease resistance in cucumber and tomato seedlings (Bai et al. 2004). Cervone et al. (1987) showed that the active endo-polygalacturonase (EPG) of *A. niger* formed oligosaccharides from pectin, which were capable of eliciting resistance response in *Vigna unguiculata*.

Species or strains of *Trichoderma* amended to the rhizosphere may also protect plants against aerial infections including those of viral, bacterial, fungal, and nematode pathogens, due to induction of resistance mechanisms similar to the hypersensitive response (HR), systemic acquired resistance (SAR), and induced systemic resistance (ISR) in plants (Harman et al. 2004). At the molecular level, resistance results in an increase in concentration of metabolites and enzymes related to defensive mechanisms, such as production of the enzymes phenylalanine ammonia lyase (PAL) and chalcone synthase (CHS), which are involved in the biosynthesis of phytoalexins (HR response), chitinases, and glucanases. These enzymes comprise pathogenesis-related proteins (SAR response) and enzymes involved in response to

oxidative stress. *Trichoderma* metabolites may act as elicitors of plant resistance or induce the expression in transgenic plants of genes whose products act as elicitors. The metabolites may also be instrumental in the synthesis of phytoalexins, PR proteins, and other compounds that may impart greater resistance against several plant pathogens, including fungi, bacteria, and nematodes (Elad et al. 2000; Howell et al. 2000; Dana et al. 2001; Hanson and Howell 2004), as well as resistance to stressful abiotic conditions (Harman et al. 2004). An ethylene-inducing xylanase (EIX) produced by *T. viride* (Dean and Anderson 1991) elicited the production of the phytoalexin resveratrol in grapevine cells (Calderon et al. 1993). Hanson and Howell (2004) reported that culture filtrates from effective biocontrol strains of *T. virens* stimulated significantly greater terpenoid levels in cotton, and the elicitors were most likely proteins or glycoproteins. *T. harzianum* also induced resistance in bean and cucumber (Koike et al. 2001).

Fungal Diseases and Their Management by Bioinoculants

Fungi are eukaryotes and constitute a group of plant pathogens that incite the most economically significant diseases of agricultural crops. Fungi infect all types of crops including cereals, vegetables, legumes, and ornamentals and cause specific symptoms (Fig. 17.2). Important diseases caused by fungi are rusts (*Puccinia* spp., *Hemileia* spp.), smuts (*Ustilago* spp., *Tilletia* spp.), seed-rot (*Pythium* spp.),

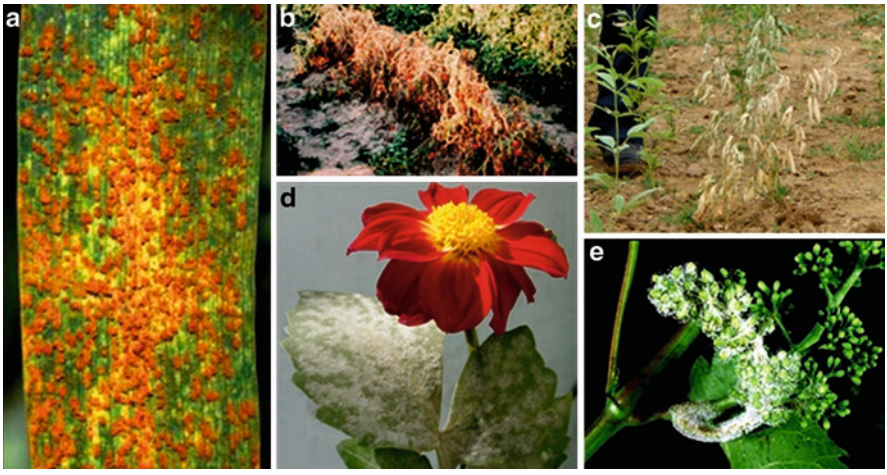

Fig. 17.2 Symptoms of some common plant diseases caused by fungi. (**a**) Leaf rust of wheat caused by *Puccinia recondite* f. sp. *tritici*, (**b**) Fusarial wilt of tomato caused by *Fusarium oxysporum* f.sp. *lycopersici*, (**c**) Fusarial wilt of pigeon pea caused by *Fusarium udum*, (**d**) Powdery mildew of dahlia caused by *Erysiphe cichoracearum*, (**e**) Downy mildew of grapes caused by *Plasmopara viticola*. (Courtesy photo: (**a**) http://www.ars.usda.gov/.../leaf%20rust%20poster.jpg; (**b**) http://www.mobot.org/.../images/Pests/Pest182.jpg; (**e**) http://www.plantmanagementnetwork.org/.../image/1sm.jpg)

damping-off (*Pythium* spp.), root rot (*Rhizoctonia* spp.), wilt (*Fusarium* spp.), blight (*Phytophthora* spp.), powdery mildew (*Erysiphe* spp., *Shaerotheca* spp.), and downy mildew (*Plasmopara* spp., *Peronospora* spp.), which attack crops under a varied range of agroclimatic conditions (Agrios 2005). Generally, moderately cooler climates with higher relative humidity are favorable for pathogenesis of fungi. Numerous studies have been conducted to test the effect of bioinoculants, and on several occasions, their application has proved quite effective in controlling fungal-induced plant diseases. The effects of the bioinoculants have been tested under in vitro, pot, and field conditions.

In Vitro

The antagonistic potential of *Trichoderma* spp. against plant pathogenic fungi has been widely explored. Bell et al. (1982) demonstrated in vitro antagonism of *Trichoderma* species against fungal pathogens. Cell-free culture filtrate of *T. virens* proved inhibitory to *Pythium ultimum* (Howell and Stipanovic 1983). *T. harzianum* strain C184 was tested in vitro for its antagonism against *Cylindrocladium pteridis*, which causes root necrosis in banana and plantain, and *Fusarium solani, F. oxysporum*, and *Aspergillus* sp., which are secondary colonizers of the root system of these crops (Ngueko 2002). *T. viride* and *T. harzianum* were screened for their antagonistic ability against the rice sheath blight pathogen, *Rhizoctonia solani,* and their culture filtrate inhibited the growth of *R. solani* (Krishnamurthy et al. 1999; Xu and Qin 2000).

Among five species of *Trichoderma*, *T. harzianum* and *T. viride* greatly suppressed the growth of *Macrophomina phaseolina* in a dual culture test (Khan and Gupta 1998). In a similar study, *T. virens* strongly antagonized *P. aphanidermatum,* the pathogen responsible for tomato damping-off disease. In fungal growth tests, the isolates *T. harzianum* 1, *T. harzianum* 2, *T. viride* 1, *T. viride* 2 and *T. viride* 3 inhibited growth of the *Helminthosporium* (*Bipolaris*) spp. by 79, 69, 84, 83 and 74%, respectively (Jegathambigai et al. 2009). *T. harzianum* was found antagonistic to *Rhizoctonia solani* and *Verticillium dahliae* at 15 and 25°C, respectively, and in vitro inhibited the development of *R. solani* and *V. dahliae* at both temperatures (Santamarina and Rosello 2006). Chaudhary and Prajapati (2004) reported antagonism of *T. harzianum* and *T. virens* against *F. udum.* The antagonists reduced colony growth of *F. udum* through saprophytic competition. *T. harzianum* showed maximal growth in a dual culture test and effectively inhibited the growth of *Macrophomina phaseolina* (65%) (Malathi and Doraisamy 2004). Similar effects of *T. harzianum* have also been reported on *S. rolfsii* (Prasad et al. 2003) and *F. udum* (Singh et al. 2002). In a dual culture test, *T. harzianum* caused severe vacoulation, shrinkage, and coagulation of the cytoplasm of pathogen hyphae.

In an in vitro study, *T. viride* inhibited the radial growth of *Aspergillus flavus* (51%), *A. fumigatus* (52%), *Fusarium* sp. (64%), and *Penicillium* sp. (54%) in dual culture (Rajendiran et al. 2010). *T. hamatum*, *T. pseudokoningii*, and *T. virens* inhibited *Phytophthora cinnamomi*, the causal organism of root rot of

chestnut, by mycoparasitism with evidence of parallel growth and coiling, and overgrowth, preventing further pathogen growth (Chambers and Scott 1995). Kucuk and Kivanc (2008) reported in vitro mycoparasitism of *Gibberella zeae* and *Aspergillus ustus* by *T. harzianum* strains. In another study, *Trichoderma* isolates were evaluated by the dual culture method, where competition by substrate, mycoparasitism, and antibiosis were observed. The *Trichoderma* spp. isolates inhibited the radial growth of *R. solani* between 60 and 98% (Martinez 2008).

Pot Culture

Species of *Trichoderma* provided protection to seeds during germination against seed rot fungi in pot culture (Elad and Chet 1987). In an in vitro experiment, Elad and Chet (1987) demonstrated that application of spores of *Penicillium oxalicum* on seeds, seedling roots, corms, bulbs, and tubers provided protection against *Pythium ultimum* (Elad and Chet 1987). Significantly lengthy protection from *Penicillium expansum* infection (up to 2 months) was obtained when intact apples were dipped for 30 s in formulated *T. harzianum* conidia before being inoculated by *P. expansum, as* compared to untreated fruits (Benitez et al. 2004).

Muskmelon seeds were soaked overnight in *Aspergillus niger* AN 27 (Kalisena SD) spore suspension and grown in sand for 6 days. The roots of seedlings (with fully opened cotyledonary leaves) were washed thoroughly in water to remove *A. niger* spores. The seeds were suspended in *F. oxysporum meloni* (aqueous) spore suspension. These Muskmelon seedlings raised from the *A. niger*-treated seeds showed 56% resistance to *F. oxysporum melonis* without physical presence of *A. niger* in the root zone. These seedlings were 58, 26, and 2% higher in peroxidase, polyphenol oxidase, and phenylalanine ammonia lyase activity, respectively, over controls (Radhakrishna and Sen 1986; Angappan et al. 1996). The lignin content was also higher in the tissues of treated plants and resulted in the induced resistance (Kumar and Sen 1998).

Application of *Trichoderma* spp. has been found effective in pot conditions against a large number of fungi such as *Fusarium* spp. (Khan 2005), *Rhizoctonia* spp. (Olson and Benson 2007), *Macrophomina phaseolina* (Khan and Gupta 1998), *Pythium* spp. (Pill et al. 2009), *Phytophthora* spp. (Hanada et al. 2009), *Botrytis* spp., and other pathogenic fungi (Olson and Benson 2007). Greenhouse experiments showed that plant growth media based on grape marc compost (compost peat 1:1, v/v) amended with *T. asperellum* T34 suppressed Fusarium wilt of carnation (Sant et al. 2010). In another study, *T. koningii* (TNAU) was used to control chickpea blight caused by *Colletotrichum dematium* with seed treatment (10^8 cfu/ml) (Rao and Narayana 2010). In a greenhouse experiment, *Trichoderma* spp. isolates significantly controlled sheath blight of rice caused by *R. solani* (Martinez 2008) and Fusarium rot of bean caused by *Fusarium solani* (using a combination of *T. harzianum* and *T. asperellum*) (Ibrahimov et al. 2009). *T. asperellum* strain T34 also suppressed *Fusarium* wilt of carnation better than standard chemicals (Sant et al. 2010).

Field Conditions

In a field study, seed treatment with *T. harzianum* decreased incidence and severity of Fusarium wilt in chickpea by 30 and 60%, respectively (Khan et al. 2004). In another trial, the same antagonist provided the highest control of *F. oxysporum* f. sp. *ciceris*, which causes wilt in chickpea under field conditions (Singh et al. 2003). *T. harzianum* had superior antagonistic efficiency against ten isolates of *F. oxysporum* f. sp. *ciceri* compared to *T. viride* (Gurha 2001). Prasad et al. (2002) evaluated *T. harzianum* PDBCTH 10 and *T. viride* PDBCTV against natural incidence of chickpea wilt. The wilt incidence was highest (12 and 16%) in control plots, and in plots treated with *T. harzianum*, only 4 and 5.1% wilt incidence was observed at 60 and 90 days, respectively. Upadhyay and Mukhopadhyay (1986) demonstrated the suppression of *Sclerotium* root rot of sugar beet by application of *T. harzianum* in field soil. Singh and Singh (2004) reported that *T. harzianum* controlled *S. rolfsii*, the incidence of collar rot in mint by 67–100%. Khan and Akram (2000) observed a significant decrease in wilt of tomato caused by *F. oxysporum* f. sp. *lycopersici* by soil application of *T. virens.* In another trial, soil application of *T. koningi, T. hamatum*, and *T. virens* controlled tomato wilt caused by *F. oxysporum* f. sp. *lycopersici* (Cipriano et al. 1989). Khan and Gupta (1998) reported superior control of root rot of eggplant caused by *Macrophomina phaseolina* following soil application of *T. harzianum* and *T. viride* in comparison to *T. koningi.* Satisfactory control of tomato damping-off has been reported by seed treatment with *T. virens* (De and Mukhopadhyay 1994).

Seed treatment with *T. harzianum* or *P. lilacinus* controlled wilt of tomato (Shahida and Gaffar 1991). Seed treatment with *T. harzianum* also checked root rot of chickpea caused by *R. solani*, and subsequently, the yield of chickpea varieties increased by 40–65% (Khan and Rehman 1997). Soil application of a *T. virens* pellet formulation controlled damping-off caused by *R. solani* (Papavizas and Lewis 1989). Coating seeds with *T. harzianum*, *T. viride*, and *T. virens* significantly controlled *F. oxysporum* f. sp. *ciceri* wilt by 30–46%, and integration of biocontrol agent and carboxin increased seed yield by 25–43% (Dhedhi et al. 1990).

Helminthosporium (Bipolaris) causes leaf spot disease in cane palm, *Chrysalidocarpus lutescens,* and losses could reach 90% during rainy weather conditions. Field experiments were carried out to test the efficacy of seed treatment of cane palm against *Helminthosporium* infection. Isolates of *T. harzianum* and *T. viride* obtained from soil and having antagonistic activity against *Helminthosporium* were used in field trials. Seed treatment with spore suspension completely eliminated the disease and also significantly increased seed germination, seedling growth, and seedling vigor (Jegathambigai et al. 2009). Commercial formulations of *T. harzianum* (Plant Guard and Biocide) successfully controlled *F. solani*, *F. oxysporum*, and *Macrophomina phaseolina*, the main pathogens of root rot disease in grapevines. A complete elimination of these pathogens was recorded with Plant Guard, and a 51 and 48% increase in yield/vine was recorded with Plant Guard and Biocide, respectively (Riad et al. 2010). In another study, black rot caused by *Thielaviopsis paradoxa* in pineapple was controlled by *T. harzianum* (Wijesinghe et al. 2009).

A good deal of work has been conducted in field trials of *Aspergillus niger* against soil-borne fungal pathogens. In a field where muskmelon and watermelon crops were suffering from *Fusarium* wilt (sometimes *R. solani* and *Pythium* spp. were associated with the disease), treatment of seeds with *A. niger* (Kalisena SD) at 8 g/kg and soil with *A. niger* (Kalisena SL) at 30 g/pit resulted in 81% control of the disease. The vines were more vigorous, and even with 15% incidence of disease, yield was approximately 5% greater as compared to that in disease-free areas (Chattopadhyay and Sen 1996). Seed treatment with Kalisena SD also provided 30% less sheath blight disease over control plants (Kumar and Sen 1998). Problems of pre-and postemergence damping-off incited by *P. aphanidermatum* and *R. solani* in fruit and vegetable farms were successfully overcome by a combined treatment of seed and soil application of Kalisena SD and Kalisena SL (Majumdar and Sen 1998). Similarly, 93% control of charcoal rot of potato in a *Macrophomina phaseolina*-infested field was obtained with *A. niger* (Kalisena SD and Kalisena SL) (Mondal 1998). Winter sorghum can be strongly damaged by *Macrophomina* infection; however, *A. niger* (Kalisena SD) seed treatment brought down incidence of the disease from 30 to 7% (Das 1998).

Many filamentous fungi and yeasts have been shown to be effective antagonists of fungi infecting the aerial parts of plants (Blakeman and Fokema 1982; Blakeman 1985). Hyseik et al. (2002) reported that a *T. harzianum*-based commercial product (Supresivit) applied at 0.5 g/kg of mineral fertilizers could suppress foliage diseases in wheat, barley, maize, oil rape, and potato, and therefore increase yields. Several foliar diseases have also been reduced significantly (by more than 50%) when leaves were sprayed with spores of common phylloplane fungi, e.g., *Alternaria, Cochliobolus, Septoria, Colletotrichum*, and *Phoma* or with spores of hyperparasites (Omar and Heather 1979). Examples include the cucumber powdery mildew fungus *Sphaerotheca fuliginea* treated with spores of *Ampelomyces quisqualis* or *Tilletiopsis* (Hijwegen 1986), the wheat leaf rust fungus *Puccinia triticina* with spores of *Darluca filum* (Devay 1956), and the carnation rust fungus with *Verticillum lecanii* (Fleming 1980). Similarly, spraying a spore suspension of common bark saprophytes such as *Cladosporium* sp. and *Epicoccum* sp. (Fokkema 1971), and *Trichoderma* spp. on pruning cuts of fruit trees has prevented infection by canker-causing pathogens such as *Nectria galligena* and *Leucostoma* (*Cytospora* sp.). A spray with *Trichoderma* in the field reduced *Botrytis* rot of strawberries and grapes at harvest and in storage (Dubos and Bulit 1981) and dry eye rot of apple fruits (Tronsmo 1986). Andrews et al. (1983) showed that *Chaetomium globosum* was able to control scab (*Venturia inaequalis*) development when applied to apple leaves under experimental conditions.

Postharvest rot of several fruits could be reduced considerably by spraying the fruit with spores of antagonistic fungi and saprophytic yeasts at different stages of fruit development, or by dipping the harvested fruits in a spore suspension. Control of postharvest diseases caused by *B. cinerea* and *A. alternata* of apple and tomato has been successful by using culture filtrates of *T. harzianum* T22 (Ambrosino et al. 2005). Yeast such as *Metschnikowia pulcherrima* (Irina et al. 2006) reduced postharvest rotting of peach and apricot. Also, significant reduction of citrus green

mold (*Penicillium digitatum*) was obtained by treating fruits with antagonistic yeasts or the fungal antagonist *T. virens* (Zamani et al. 2006), whereas post-harvest *Botrytis* rot of strawberries or grapes was reduced by several sprays of *Trichoderma* spores on blossoms and young fruits (Sesan et al. 1999). Postharvest black rot caused by *Thielaviopsis paradoxa* of pineapple fruit has been controlled by *T. harzianum* (Reyes et al. 2004; Wijesinghe et al. 2009). *Penicillium* rot of pineapple was reduced considerably by spraying fruits with nonpathogenic strains of the pathogen (Singh et al. 2009). Similarly, several antagonistic yeasts protected grapes and tomatoes from *Botrytis*, *Penicillium*, and *Rhizoctonia* rots (Janisiewicz and Jeffers 1997). The film-forming *Saccharomyces cerevisiae* strain M25 showed a significant ability to reduce postharvest decay in apples caused by the phytopathogenic fungus and patulin-producer *Penicillium expansum* (Ortu et al. 2005). One such yeast, *Candida saitoana*, controlled postharvest decay of apples by inducing systemic resistance while at the same time increasing chitinase and β-1,3-glucanase activities in the fruit (El Ghaouth et al. 2003).

Bioinoculants in IPM

Some bioincoulants, especially *Trichoderma* spp., have been found to be quite compatible with common fungicides and nematicides such as Thiram, Vitavax, Carbendazim, Nemacur, and Furadon; hence, they can be used in integrated disease management programs. Chickpea and lentil seeds treated with *T. virens* (10^7 conidia/ml) and subsequently with 0.1% carboxin effectively reduced soil-borne populations of *F. oxysporum*, *R. solani*, and *Sclerotium rolfsii* (Mukhopadhyay et al. 1992). In the field, integrated use of *T. harzianum* with fungicidal seed treatments significantly reduced incidence of chickpea wilt complex and increased crop yields. Bean seeds sown in soil heavily infested with *B. cinerea*, *R. solani*, and *P. ultimum* and treated with conidia of the transgenic *Trichoderma* strain germinated, but the seeds treated with wild-type spores did not germinate (Brunner et al. 2005). Transgenic strain SJ3-4 of *T. atroviride* not only exhibited threefold greater inhibition of spore germination of *Botrytis cinerea* but also overgrew and caused lysis of *R. solani* and *P. ultimum* (Brunner et al. 2005). Seed treatment with Vitavax and Ziram resulted in 30% disease control. Disease control increased to 63% when *T. harzianum* was applied with the fungicides (Kaur and Mukhopadhyay 1992).

Bacterial Diseases and Their Management

The first evidence of bacteria being responsible for plant diseases was reported in 1982 when the association of a bacterium (now known as *Erwinia amylovora*) was established with fire blight disease of pear. Since then, numerous plant pathogenic bacteria have been identified. The bacteria that cause diseases in plants are facultative saprophytes and can be grown artificially on nutrient media; however, fastidious vascular bacteria are difficult to grow in artificial media and some do not grow in culture (Agrios 2005). Plant pathogenic bacteria are rod-shaped, the only exception

being *Streptomyces*, which is filamentous. *Streptomyces* produce spores, called conidia, at the end of the filament. Other bacteria, however, do not produce spores. Bacterial pathogenicity depends primarily on spore/conidia production in the shortest possible time. Bacterial diseases of plants occur at any location that is reasonably moist and warm. Under favorable environmental conditions, they may be destructive in any geographical region.

Plant pathogenic bacteria induce different kinds of symptoms in plants depending on causal agent and host, such as leaf spots and blights; soft rots of fruits, roots, and storage organs; wilts; overgrowths; scabs; and cankers (Fig. 17.3), and cause severe yield losses. There are eight major bacterial genera that are plant pathogenic and cause significant economic losses to plants: *Pseudomonas*, *Xanthomonas*, *Erwinia*, *Agrobacterium*, *Clavibacter*, *Curtobacteium*, *Rhodococcus*, and *Streptomyces* (Singh 2008). Bacterial canker of tomato is distributed throughout the world and may cause up to 60% yield loss (Chang et al. 1992). Yield reduction due to other important bacterial diseases may reach 5–25% (bacterial blight of cotton, Verma 1995), 6–60% (bacterial leaf blight of rice, Srivastava and Rao 1966), 10–15% (bacterial blight of mango, Kishun 1987), 8–16% (bacterial spots of chilli and tomato, Singh 2008), 10–70% (bacterial brown rot and wilt of potato, Verma and Shekhawat 1991), and 11–91% (bacterial wilt of tomato and eggplant, Kishun 1987).

Data on control of plant pathogenic bacteria with the application of bioinoculants is limited; however, a few studies conducted thus far have shown that bacterial

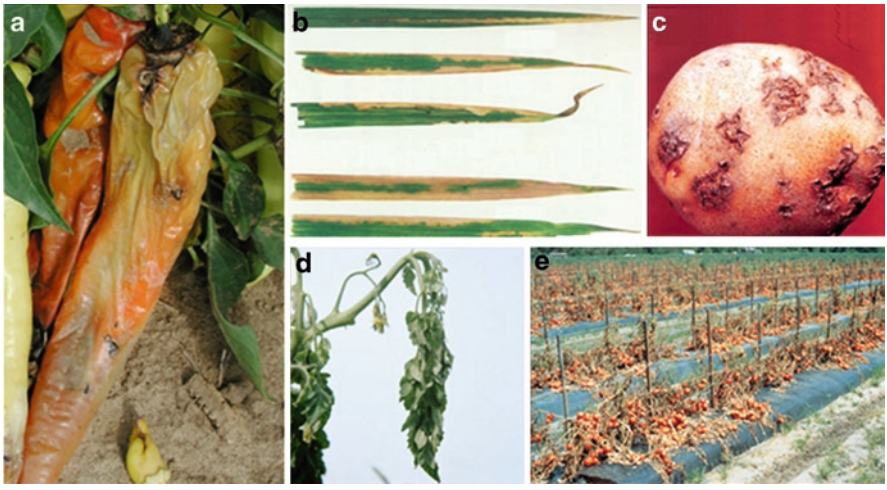

Fig. 17.3 Symptoms of some common plant diseases caused by bacteria. (**a**) Soft rot of chilli caused by *Erwinia carotovora* subsp. *Carotovora.* (**b**) Bacterial leaf blight of rice caused by *Xanthomonas oryzae* pv. *oryzae*, (**c**) Potato scab caused by *Streptomyces scabies*, (**d**) Bacterial wilt of tomato caused by *Ralstonia solanacearum* (symptoms on youngest leaves), (**e**) Bacterial wilt of tomato in the field, (Courtesy photo: (**a**) http://www.omafra.gov.on.ca/.../bacterial-soft-rot.html; (**b**) http://www.jxny.com/bctk/2009-4-15/sdbykb.htm; (**c**) http://www.hort.uconn.edu/ipm/veg/htms/scabpot.htm; (**d**) University, USDA Cooperative Extension Slide Series, Bugwood.org; (**e**) Courtesy J. P. Jones (http://www.apsnet.org/.../bacteria/text/fig02.htm))

diseases of plants can be successfully managed with bacterial antagonists. For example, bacterial crown gall has been controlled by treating seeds or nursery stock with bacteriocin-producing strain *Agrobacterium radiobacter* K-1026 (Jindal 1990). Some information is also available on management of bacterial plant pathogens with fungal bioinoculants. Treatment of tubers and seeds with fungal antagonists has proved effective against plant pathogenic bacteria, but not under field conditions (Agrios 2005). Kalita et al. (1996) reported 47.5% reduction in citrus canker incidence (*Xanthomonas campestris* pv. *citri*) after application of a strain of *Aspergillus terreus*. Bacterial wilt of tomato (*Ralstonia solanacearum*) and soil populations of the pathogen were reduced by soil application of *Glomus mosseae* together with *P. fluorescens* (Kumar and Sood 2002).

Nematode Diseases and Their Management

Parasitic nematodes are considered important pathogens of agricultural crops. Nematodes damage plants by injuring and feeding on root hairs, epidermal cells, cortical, and/or stelar cells (Khan 2008). A significant number of nematodes like *Rotylenchus*, *Hoplolaimus, Helicotylenchus*, *Tylenchorhynchus*, *Belonolaimus*, *Trichodorus*, and *Longidorus* are ectoparasites, which feed on the root surface. However, a considerable number of nematodes fully enter the host root and are termed endoparasites. Examples include root-knot nematodes (*Meloidogyne* spp.), cyst-forming nematodes (*Heterodera* spp.), and root-lesion nematode (*Pratylenchus* spp.). Some nematodes such as citrus nematode (*Tylenchulus semipenetrans*) and reniform nematode (*Rotylenchulus reniformis*) are considered semi-endoparasites as they only partly enter the host tissue.

Nematodes are documented to cause up to 7–12% yield loss to various crops. Yield losses vary greatly, depending on inoculum level and host species. Severe infection may result in as much as 80–90% yield decline in an individual field, and sometimes, plants fail to produce any yields of economic value. Nematode damage usually remains hidden and is not recognized by growers or scientists. This is not always the case, however. When fields are heavily infested, characteristic symptoms appear on roots or shoots. Specific symptoms include root lesions, root rot, root pruning, root galls, and cessation of root growth (Fig. 17.4).

Some nematodes also cause characteristic symptoms on aboveground parts. *Aphelenchoides* spp. cause necrosis and whitening of leaves of chrysanthemum, strawberry, and rice. *Ditylenchus dipsaci* attacks bulbs as well as buds of tulip and lily (Fig. 17.5). In addition to direct damage, nematodes often aid or aggravate diseases caused by fungi, bacteria and viruses or may break the resistance of cultivars to pathogens. Hairy root of rose, caused by *Agrobacterium rhizogenes*, is of minor importance, but in the presence of *Pratylenchus vulnus*, the disease becomes severe (Sitaramaiah and Pathak 1993). Fusarium wilt-resistant cultivars of cotton become susceptible in the presence of root-knot nematodes (Atkinson 1892). The degree of crop damage, however, depends largely on plant species or cultivar, nematode species, level of soil infestation, and prevailing environmental conditions.

Fig. 17.4 Symptoms of some common nematode diseases on roots. (**a**, **b**) Root lesion of tobacco caused by *Pratylenchus penetrans*, (**c**) Root-knot disease of tomato caused by *Meloidogyne incognita*, (**d**) Cysts of *Globodera rostochiensis*, the golden nematode on potato roots, (**e**) Blistered and cracked onion bulbs caused by *Ditylenchus dipsaci.* [Courtesy photo: (**a**) R. J. Reynolds Tobacco Company Slide Set, R. J. Reynolds Tobacco Company, Bugwood.org (http://www.forestryimages.org/.../3072x2048/1402035.jpg); (**b**) *C. C. Russell* (http://www.nematode.unl.edu/extpubs/kanfig3e.jpg); (**c**) *R. S. Hussey,* (http://www.apsnet.org/.../images/fig08.jpg); (**d**) courses.cit.cornell.edu/.../Golden_nematode.html; (**e**) http://www.inra.fr/.../HYPPZ/RAVAGEUR/6ditdip.htm]

Plant nematodes may also act as vectors for bacteria, fungi, and viruses. For instance, *Anguina tritici* carries *Clavibacter tritici* and *Dilophospora alopecuri* to the shoot meristem of wheat (Khan and Dasgupta 1993).

Biological control of nematodes may be achieved with two kinds of microorganisms, i.e., classical parasites or predators, and plant growth-promoting (PGPR) microorganisms. Classical parasites or predators such as *Paecilimyces lilacinus*, *Dactylaria candida*, and *Pasteuria penetrans* have been used in nematode control during the last few decades and reduce nematode population by direct action (De Bach 1964). PGPR may suppress rhizoshpheric nematode populations by promoting host growth, inducing systemic resistance, and/or producing nematoxic metabolites such as bulbiformin (Brannen 1995), phenazin (Toohey et al. 1965), and pyoleutorin (Howell and Stipanovic 1980).

In recent years, considerable research has been carried out on the use of bioinoculants to control nematode populations in soil. Effects of microorganisms have

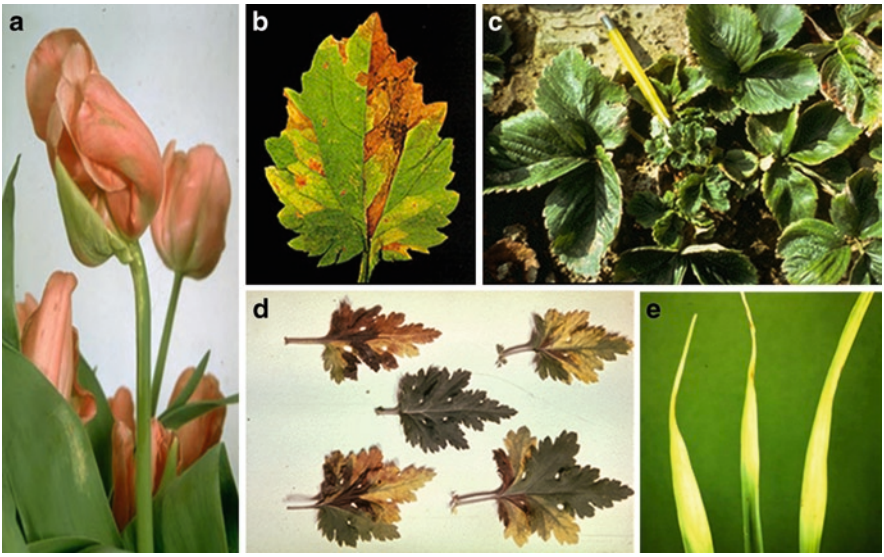

Fig. 17.5 Some common foliar symptoms of nematode diseases. (**a**) Damage to tulip flower due to *Ditylenchus dipsaci*, (**b**) *Aphelenchoides ritzemabosi* on chrysanthemums, (**c**) Cauliflower disease of strawberry caused by *Aphelenchoides fragariae*, (**d**) *Aphelenchoides fragariae* on carnation leaf, (**e**) White tip of rice caused by *Aphelenchoides besseyi*. [Courtesy photo: (**a**) Central Science Laboratory, Harpenden Archive, British Crown, Bugwood.org (http://www.forestryimages.org/browse/detail.cfm?imgn); (**b**) http://www.floranazahrade.cz/poradna/poradna2003_12.htm; (**c**)ucdnema.ucdavis.edu/.../204NEM/2FOLIAR.htm;(**d**)ucdnema.ucdavis.edu/.../204NEM/2FOLIAR.htm; (**e**) Donald Groth, Louisiana State University AgCenter, Bugwood.org (http//www.forestryimages.org/browse/detail.cfm?imgn)]

been evaluated against different nematodes under in vitro, pot, and field conditions. Some important fungal bioinoculants are listed in Table 17.2.

In Vitro Studies

Culture filtrate of *Pochonia chlamydosporia* in yeast extract medium has demonstrated pronounced nematicidal and nematostatic effects. A dilution of 1:1 culture filtrate resulted in 100% mortality of *Globodera rostochiensis*, *G. pallida*, and *Panagrellus redivivus* (Saifullah 1996c). Strains of *T. virens* and *Burkholderia cepacia* (bacteria) were found to produce extracellular factors that decreased *M. incognita* egg hatch and juvenile mobility (Siddiqui and Shaukat 2004). Eapen and Venugopal (1995) have shown that isolates of *Trichoderma* spp. have broad-spectrum biocontrol activity against a number of pathogenic fungi and nematodes. A serine protease of 28 kDa with trypsin activity was isolated from *Trichoderma* strain 2413. The enzyme reduced the number of hatched eggs of root knot nematodes and showed synergistic effects with other proteins produced during antagonistic activity of the strain (Benitez et al. 2004). The number of hatched eggs of the root-knot nematode, *M. incognita,* was significantly reduced after incubation with

Table 17.2 Effect of different bioinoculant fungi on plant nematodes infesting agricultural crops

Antagonistic fungi	Nematode managed	Host plant	References
Aspergillus niger	*Meloidogyne* spp.	Tomato	Singh et al. (1991)
A. niger	*M. incognita*	Okra	Sharma et al. (2005)
A. niger	*M. incognita*	Eggplant	Khan and Anwer (2008)
A. niger	*M. incognita*	Tomato	Khan et al. (2007)
Paecilomyces lilacinus	*Meloidogyne* spp.	Tomato	Khan and Tarannum (1999); Pal and Gardener (2006); Schenek (2004)
P. lilacinu and *T. virens*	*M. incognita*	Tomato	Khan and Akram (2000)
P. lilacinus and *P. chlamydosporia*	*M. incognita*	Mung bean	Khan and Kounsar (2000)
P. lilacinus	*M. javanica*	Tobacco	Hewlett et al. (1988)
P. lilacinus	*R. reniformis*	Tomato	Lysek (1966)
P. lilacinus	*Meloidogyne* spp.	Various	Jatala (1986)
P. lilacinus	*Meloidogyne* spp.	Okra	Khan and Ejaz (1997)
Penicillium anatoticum	*Globodera* sp.	Potato	Jatala (1986)
T. harzianum, P. lilacinus	*M. incognita*	Chickpea	Pant and Pandey (2002)
T. harzianum	*M. javanica*	Tomato	Siddiqui and Shaukat (2004)
T. harzianum	*M. arenaria,*	Corn	Windham et al. (1989)
T. harzianum	*Meloidogyne* spp.	Cardamom	IISR 1995
Trichoderma asperellum -203 and *Trichoderma atroviride*	*M. javanica*	In vitro	Sharon et al. (2009)
T. pseudokoningii, T. viride, P. lilacinus, A.niger, G. mosseae	*M. incognita*	Soybean	Oyekanmi et al. (2008)
T. harzianum, P. lilacinus	*Meloidogyne javanica*	Okra	Zareen et al. (2001)
T. atroviride	*R. similis*	Banana	Zum Felde et al. (2006); Pocasangre Enamorado et al. (2007)
T. harzianum (T014)	*M. incognita*	Gladiolus	Khan and Mustafa (2005)
T .harzianum and *P. chlamydosporia*	*Globodera rostochiensis* and *G. pallid*	Potato	Saifullah (1996a, b)
T. harzianum and *P. chlamydosporia*	*M. incognita*	Chickpea	Khan et al. (2005a)
T. harzianum and *P. chlamydosporia*	*H. cajani*	Pigeonpea	Siddiqui and Mahmood (1996)

pure PRA1 (trypsin-like protease) preparations of *T. harzianum* CECT 2413 (Suarez et al. 2004). In another study, *T. asperellum*-203 *and T. atroviride* suppressed *M. javanica* populations by direct effect on various developmental stages of nematodes, viz., eggs, larvae, and adults (Sharon et al. 2009). Culture filtrates of *Aspergillus niger* soil isolates AnC2 and AnR3 efficiently suppressed hatching of eggs and mortality of juveniles of *M. incognita* (Khan and Anwer 2008).

Pot Conditions

The majority of studies exploring the potential of bioinoculants against plant nematodes have been carried out under pot conditions (Khan 2007). In a pot experiment, chilli (*Capsicum annum*) seedlings were inoculated with *Meloidogyne javanica*, *Aspergillus niger*, and *Rhizoctonia solani* alone or in various combinations. All growth parameters were significantly greater with *A. niger* and lower with *M. javanica* or *R. solani* (Shah et al. 1994). Singh et al. (1991) showed that application of *A. niger* decreased the damage caused by *M. incognita* and *R. solani* singly or together on the tomato cultivar, Perfection. Similarly, inoculation with *A. niger*, *Epicoccum purphurascum*, *Penicillium vermiculatum*, and *Rhizopus utgricans* effectively diluted the adverse effect of *R. solani* and *M. incognita* resulting in an increase in germination of the tomato cultivar, Pusa Ruby (Rekha and Saxena 1999). In a pot experiment, application of *A. niger* isolates (AnC_2 and AnR_3) significantly suppressed galling, egg mass production, and soil populations of *M. incognita*. The isolates AnC_2 and AnR_3 produced the greatest quantities of siderophores, HCN and NH_3, and solubilized the greatest quantity of soil phosphorus (Khan and Anwer 2008). Windham et al. (1989) reported a suppressive effect of *T. harzianum* on *M. arenaria* resulting in an increase in root fresh weight and decrease in number of eggs per gram of root. Significant reduction in *H. avenae* populations and increase in wheat growth were recorded with *P. chlamydosporia* (Bhardwaj and Trivedi 1996). In another study, application of the same fungus decreased the number of eggs, juveniles, and galls of *M. hapla* in tomato plants (De leij et al. 1993).

Application of *T. virens* and *Burkholderia cepacia* (bacteria) as a seed coat followed by root drenches suppressed root-knot nematode infestation in bell pepper compared with untreated plants (Meyer et al. 2000). Pant and Pandey (2001) reported maximum reduction in populations of *M. incognita* with *T. harzianum*, *P. lilacinum*, and *A. niger* applied in sterilized soil in pots at 5,000 spores/pot. In a greenhouse test, *P. chlamysosporia* provided 75% control of the first cropping of *Heterodera schachtii.* Ashraf and Khan (2008) evaluated the efficacy of wastes of apple (*Malus pumila*), banana (*Musa paradisiaca*), papaya (*Carica papaya)*, pomegranate (*Punica granatum*), and sweet orange (*Citrus sinesis*) at 20 g/plant and *Paecilomyces lilacinus* at 2 g (mycelium + spores)/plant against the reniform nematode, *Rotylenchulus reniformis,* on chickpea. The best protection of chickpea against *R. reniformis* was recorded using integration of *P. lilacinus* with papaya wastes, followed by apple and pomegranate wastes. Control of *M. javanica* was accomplished by inoculating soil with *P. chlamydosporia*-colonized rice medium at a rate of 30 g/kg soil (De leij et al. 1993). Introduction of the fungus 2 weeks before nematode inoculation provided significantly greater control of *M. javanica* (De leij et al. 1993). Application of culture filtrate of *T. harazianum, T. viride, T. koningii, T. reesei* and *T. hamatum* resulted in effective control of the reniform nematode (*Rotylenchulus reniformis*) and root-knot nematode (*M. javanica*) on the eggplant cultivar, Black Beauty (Bokhari 2009).

Field Conditions

Relatively few field trials have been conducted to evaluate the effectiveness of bioinoculants against nematode infestations. These studies, however, have demonstrated that nematode control to a level can be exploited commercially (Khan 2005). Soil treatment by *A. niger* in castor beans abated the population of *Rotylenchulus reniformis* up to 71% (Das 1998). Suppression of root-knot nematodes resulting in improved growth of cardamom seedlings in nurseries due to application of *T. harzianum* has been reported (IISR 1995). *Pochonia chlamydosporia* var. *catenulate* integrated with other strategies reduced soil populations of plant parasitic nematodes (51–78%) in vegetable crops (Garcia et al. 2004). Under natural soil conditions, nematode eggs appear to be an important source of nutrients for *P. chlamydosporia.* The fungus parasitized large numbers of *H. avenae* eggs in English cereal fields and played a major role in limiting multiplication of the nematode (Kerry et al. 1982a, b). In a field experiment, effects of root-dip treatment of ornamental plants hollyhock (*Althea rosea*), petunia (*Petunia hybrida*), and poppy (*Papaver rhoeas*) with *P. chlamydosporia*, *P. fluorescens*, and *B. subtilis* were evaluated. The three bioinoculants suppressed galling of *M. incognita* by 37%, 27%, and 24%, respectively (Khan et al. 2005b). Chlamydospores of some biotypes of *P. chlamydosporia* applied to soil significantly reduced (>50%) population densities of *M. hapla* on tomato and of *G. pallida* on potato plants (Siddiqui et al. 2009). In another study, Kumar (2009) reported satisfactory control of root knot of papaya with *P. chlamydosporia.*

Soil application of *Paecilomyces lilacinus* with or without neem leaf powder reduced galling and egg mass production by 24–46% and enhanced yield of okra by 15% (Khan and Ejaz 1997). In another study, soil application or root dip treatment of tomato seedlings with *Bacillus subtilis* or *Pseudomonas stutzeri* controlled root knot of tomato (Khan and Tarannum 1999). Application of *P. fluorescens, T. virens*, or *P. lilacinus* controlled the root knot caused by of *M. incognita* in the presence or absence of wilt fungus, *Fusarium oxysporum* f. sp. *lycopersici* (Khan and Akram 2000; Akram and Khan 2006). A field study conducted to evaluate relative effectiveness of seed treatment with different rhizobacteria (*Azotobacter coccum, Azospirillum lycopirum*, *B. subtilis*, and *Bijrica indica*) and antagonistic fungi (*Arthrobotrys oligospora*, *Cylindrocarpon destructans*, *Pochonia chlamydosporia*, and *P. lilacinus*) on root nodulation and plant growth of green gram revealed that treatment with *B. subtilis* or *B. indica* reduced galling by 33–34% and increased dry weight of shoots by 22–24% (Khan and Kounsar 2000; Khan et al. 2002). Other bioinoculants were also found to be effective. Seed treatment with *P. fluorescens* or *B. subtilis* was effective against root knot of green gram (Khan et al. 2007).

Siddiqui and Shaukat (2004) reported that combined application of *T. harzianum* with *P. fluorescens* in unsterilized sandy loam soil caused significant reduction in *M. javanica* population densities in tomato roots. Application of *P. chlamydosporia* at 20 g/plot (6×10^7 cfu/g substrates) along with *P. lilacinus* and neem cake effectively controlled *M. incognita* and increased yield (58%) of inoculated brinjal plants (Cannayane and Rajendran 2001). Dhawan et al. (2008)

reported that combined effects of *P. chlamydosporia* and *P. fluorescens* significantly managed the root-knot nematode *M. incognita* and increased yield of brinjal in farmer's fields. Bioefficacy and compatibility of formulations of *P. chlamydosporia* (2×10^6 cfu/g) and *P. lilacinus* (2×10^6 cfu/g) were evaluated against the root-knot nematode *M. javanica* infecting nursery of acid lime. Application of 5 or 10 g of each bioinoculant formulation and combined use of *P. lilacinus* and *P. chlamydosporia,* each at 10 g/kg soil, significantly reduced root-galling index and number of nematodes in roots (Rao 2005).

17.1.2 Production Technology of Bioinoculants

For field application of a bioinoculant, an efficient substrate for mass production and an inert immobilizing material are required, which could carry the maximum number of propagules of the organism with minimum volume and necessarily maintain its survival and integrity. An excellent bioinoculant is one that is introduced into an ecosystem, and subsequently survives, proliferates, becomes active, and establishes itself in a new environment (Khan 2005). For preparing a commercial formulation, these attributes must be considered. In addition, the bioinoculant should be mass cultured on an inexpensive substrate in a short period of time. Easy application, effectiveness, and consistent results under a variety of environmental conditions are other desirable features required for production of bioinoculant formulations.

Different techniques of cell immobilization have been developed to devise efficient carrier systems to produce commercial formulations of bioinoculants. A number of carriers for immobilization of microorganisms have been used to develop commercial formulations of biocontrol agents, viz., peat, seeds, meals, kernals, husks, bran, bagasse, farmyard manure, cow dung cake, compost, oil cakes, wood bark, vermiculite, sand, clay, and liquid carriers. Three types of formulations, viz., pellet, granular, and liquid, are widely produced.

17.1.2.1 Pellet Formulations

A small amount of liquid bioinoculant culture encapsulated by some appropriate inert material to hold the suspension and organism intact is termed a pellet. Different materials such as natural polymers (alginate, carrageenan, cellulose, agar, agarose, hen-egg white, gelatin) as well as synthetic polymers (polyacrylamine, photo cross-linkable resins, etc.) can be used to encapsulate liquid suspension of bioinoculants to formulate efficient delivery systems for field application of microorganisms (D'Souza and Melo 1991). The gelant sodium alginate is considered a useful material for encapsulation of liquid preparations of microorganisms. The microbes remained viable for many weeks in alginate pellets. Fravel et al. (1985)

prepared pellet formulation on a comminuted (blended) mixture of sodium alginate and pyrax (pyrophyllite, hydrous aluminum silicate) in a 1:10 ratio. The mixture was amended with bioinoculant liquid suspension in the ratio 9:1. The alginate–pyrax–microorganism mixture was stirred continuously while dripping through a pipette into a solution of 0.25 M $CaCl_2$ or 0.1 M $C_{12}H_{22}CaO_{14}$ (calcium gluconate). The pellets, after drying under sterile air (laminar flow hood), were stored at different temperatures for various durations in a deep freezer to determine viability of the spores. Populations of the microorganism pellets were determined by the dilution plate method. Some pellet formulations are listed in Table 17.3.

Successful encapsulation of liquid suspension of spores and hyphae of *P. chlamydosporia* was conducted with sodium alginate containing 10% (w/v) kaolin or wheat bran (De Leij and Kerry 1991). On soil application, the fungus proliferates in soil from those granules which contained wheat bran as the energy source. In another study, Kerry (1988) estimated approximately 9×10^4 and 4×10^4 cfu of *P. chlamydosporia*/g soil after 1 and 12 weeks of application of granules, respectively.

17.1.2.2 Powder Formulations

Granular or powder carrier systems for microorganisms are more useful than pellets and are compatible with existing farm machinery. Formulations of fungal bioinoculants can be successfully prepared on fermenter biomass in the form of powder with diluents such as cake (semisolid), pyrax, or alginate pellets containing a food base such as bran (Papavizas et al. 1984; Beagle-Ristaino and Papavizas 1985). Papavizas and Lewis (1989) prepared two formulations of *T. virens*, alginate–bran–fermenter biomass pellets and pyrax–fermenter biomass mixture. The formulations were available at low cost as they were developed from inexpensive agriculture/industrial wastes or by-products. A good immobilizing material is one that provides an energy base for the sustenance and multiplication of the bioinoculants. Numerous powder/granular formulations have been prepared and marketed (Table 17.4).

Liquid stillage, a by-product of sorghum fermentation, can be added to granular lignite in a 1:2 ratio and stirred (Jones et al. 1984). The amended granules are dried overnight at 30°C, treated again with the stillage (50% v/v), and autoclaved in conical flasks or polyethylene bags. The sterilized mixture is inoculated with a liquid suspension of fungal bioinoculants such as *T. harzianum* and *T. virens*. Four days after inoculation at 25°C, the flasks/bags are shaken to distribute evenly the sporulating fungus. Populations of the microorganism pergram of granules and their viability with regard to storage temperature and duration are determined by the dilution plate method. The air-dried granules can be prepared and stored at 20°C for up to 4 months with 90% viability of spores.

Various agricultural materials, industrial wastes, and by-products, viz., wheat bran–sand mixture, sawdust–sand–molasses mixture, corn cob–sand–molasses mixture, bagasse–sand–molasses mixture, organic cakes, cow dung–sand mixture, compost/farm manure, inert charcoal, diatomaceous earth, and fly ash can also be used to prepare powder formulations of bioinoculants (Khan 2005).

Table 17.3 Some pellet formulations of fungal bioinoculants

Product name	Bioinoculant	Target pathogen(s)/disease(s)	Crop(s)	Company
AQ10 Biofungicide	*Ampelomyces uisqualis* isolate M-10	Powdery mildew	Various	Ecogen, Inc, USA
Trichopel	*T. harzianum, T. viride*	*Nectia, Phytophthora, Pythium*	Various	Agrimm Technology, New Zealand
SoilGard (GlioGard)	*Gliocladium* (*Trichoderma*) *virens GL-21*	Damping-off, *Pythium*, *Rhizoctonia solani*	Various	Certis, USA
F-Strop	*T. harzianum*	*Pythium, Rhizoctonia*	Various	USA.Reg. No. 68539-3
PlantShield, Planter box	*T. harzianum* T-22 *T. harzianum* KRL_AG2(T-22)	*Pythium, Rhizoctonia, Fusarium*	Various	Bioworks inc, USA
DiTera WDG	*Myrothecium verrucaria*	Parasitic nematodes	Various	Abbott laboratories, USA
Contans WG, Intercept WG	*Coniothyrium verrucaria*	*Slerotinia slerotiorum*, *S. minor*	Many crops	Abbott laboratories, USA
Aspire	*Candida oleophila* I-182	*Botrytis, Penicillium*	Citrus, pome fruit	Ecogen Inc, USA
Rootshield	*Trichoderma* spp.	*Pythium, Rhizoctonia, Phytophthora*	Various	BioWorks, India
T22-Planter Box	*T. harzianum*	*Pythium, Rhizoctonia, Fusarium*	Various	BioWorks, India
Kalasipahi	*Aspergillus niger* AN27	Soil-borne fungi	Various	Cadila Pharmac. Ltd., India

Table 17.4 Some powder formulations of fungal bioinoculants

Product name	Bioinoculant	Target pathogen(s)/disease(s)	Crop(s)	Company
Bio-Fungus	*Trichoderma* spp.	Soil-borne fungi	Various	Grondortsmettingen DeCuester n.v., Belgium
Biowilt-X	*T. harzianum*	*Fusarium* spp.	Pulses	Department of Plant Protection, Aligarh Muslim University, India
Bionem-X	*Pochonia chlamydosporia*	*Meloidogyne* spp.	Pulses, vegetables	Department of Plant Protection, Aligarh Muslim University, India
Trichodowels	*T. harzianum, T. viride*	*Pythium, Rhizoctonia, Fusarium*	Trees, ornamental.	Agrimm Technology, New Zealand
VinevaxTM (formerly Trichoseal)	*T. harzianum*	Various	Vines and trees	Agrimm Technology, New Zealand
Trichodex	*T. harzianum*	*Colletotrichum, Monilin., Plasmopara*	Various	Makhteshim chemical works ltd, USA
Rotstop	*Phlebia gigantean*	*Heterobasidion annosum,*	Trees	Verdera, USA
Shemer	*Metschnikowia fructicola*	*Botrytis, Rhizopus, Aspergillus, Penicillium*	Strawberry, grape, sweet potato, citrus	Minrav, Israel
BINAB T	*T. harzianum/T. polysporum*	Wood decay fungi	Trees	Binab bio-innovation, USA
RootShield	*T. harzianum* T-22	*Pythium, Rhizoctonia, Fusarium*	Various	Bioworks inc, USA
Primastop	*Gliocladium catenulatum*	Soil-borne pathogens	Various	Verdera oy, USA
Polygandron	*Pythium oligsndrum*	*Pythium ultimum*	Sugar beet	
DiTera wp	*Myrothecium verrucaria*	Parasitic nematodes	Various	Abbott laboratories, USA
Plant-Shield	*Trichoderma* spp.	Root diseases	Various	BioWorks, India.
Ecofit	*T. viride*	Root diseases	Various	Hoech. Scher. Agr. Evo. Ltd., India
Sanjeevni	*T. viride*	Seed and soil born diseases	Various	International Panaecea Ltd., India
Bioderma	*T. viride* + *T. harzianum*	*Phytophthora, Pythium, Rhizoctonia*	Various	Biotech International Ltd., India

Bas-derma	*T. viride*	Seed- and soil-borne diseases	Various	Banaras Biocontrol Res. Lab., India
Ecoderma	*T. viride*+*T. harzianum*	*Pythium, Rhizoctonia, Fusarium spp.,* parasitic nematodes	Various	Margo Biocontrol Pvt. Ltd., India
Defense-SF	*T. viride*	Various seed and soil born diseases	Various	Workhard Life Science Ltd., India
Kalisena SL Kalisena SD Pusa Mrida Beej Bandhu	*Aspergillus niger* AN27	*Fusarium oxysporum, F. solani, Macrophomina phaseolina, Pythium aphanidermatum, R. solani, Sclerotinia sclerotiorum* etc.	Various	Cadila Pharmac. Ltd., India
Funginil	*T. viride*	Seed and soil born diseases	Various	Crop Health Biop. Res. Cen. India
Manidharma's	*Paecilomyces lilacinus*	Parasitic nematodes	Various	Mani Dharma Biotech, India
Manidharma's Trichoderma sp	*Trichoderma harzianum, T. viride*	Root rots, Wilts, brown rot, damping-off, charcoal rot, soil born diseases	Various	Mani Dharma Biotech, India
Tricho-X	*T. viride*	Various soil born diseases	Various	Excel Industries Ltd., India
Trieco	*T. viride*	Seed- and soil-borne fungi, nematodes	Various	Ecosense Labs Pvt. Ltd., India
Tri-control	*Trichoderma* spp.	Various seed, soil and foliar diseases	Various	Jepee Biotech. India.
Sun Agro Derma	*Trichoderma viride*	Seed-, soil-borne and foliar diseases	Various	Bio Organic Industries, India
Sun Agro Derma–H	*Trichoderma harzianum*	Seed-, soil-borne and foliar diseases	Various	Bio Organic Industries, India
CHAETO	*Chaetomium globosum*	Spot blotch	Wheat, oats, barely	Bio Organic Industries, India
Sun nema	*Paecilomyces lilacinus*	Plant nematodes	Various	Bio Organic Industries, India
Trichoguard	*T. viride*	Various soil born diseases	Various	Anu Biotech International Ltd., India
Trichorich-WP	*Trichoderma viride*	Seed and soil born diseases	Various	Richgreen Agrochem, India
Vertirich-WP	*Verticillium lecanii*	Seed and soil born diseases	Various	Richgreen Agrochem, India
JaiVJai Bio-Tricure	*Trichoderma viride*	Various fungi	Various	Chaitra Fertilisers & Chemicals, India
Bioguard	*T. viride*	Seed and soil born diseases	Various	Krishi Rasayan Exp., Ltd., India

Backman and Rodriguez-Kabana (1975) prepared a commercial formulation of *T. harzianum* on sterilized granules of diatomaceous earth impregnated in 10% molasses for four days. The bioinoculant remained viable after air-drying for up to 1 month in cold storage. Kelley (1976) used clay granules with additional nutrients to produce *T. harzianum* formulations. Khan et al. (2001) used grains and meals of cereals, corn cob–sand–molasses, compost, leaf litter, bagasse–soil–molasses, and sawdust–sand–molasses to mass-culture *T. harzianum*, *T. virens*, and *P. chlamydosporia*. Highest cfu counts of *Trichoderma* spp. (10^{6-7} cfu/g material) and *P. chlamydosporia* (10^{5-6} cfu/g material) were recorded in bagasse–soil–molasses and leaf litter–sucrose, respectively. Cabanillas and Barkar (1989) tested wheat grains, alginate pellets, and diatomaceous earth granules to produce a commercial formulation of *Paecilomyces lilacinus* for soil application. The formulation contained active propagules of the antagonist in higher number, and the application effectively controlled root-knot disease in tomato and consequently increased yields.

Khan (2005) developed a novel process for production of commercial formulations by bioinoculants, viz., *T. harzianum*, *P. chlamydosporia*, and *P. fluorescens*. The process involved two steps: the first dealt with the preparation of mass culture or stock culture of the microorganisms on sawdust, soil, and 5% molasses mixture in the ratio of 15:5:1. The immobilization of the microorganisms took place on a fly ash-based carrier (fly ash, soil, and 5% molasses, 15:3:1). One part of the stock culture and 20 parts of the carrier were packed in a poly pack and incubated at 25°C for 1 week. Using the process, three commercial formulations of *T. harzianum* and *P. chlamydosporia* were prepared. The bioinoculants were found viable in the formulation up to 32 weeks at 25°C or at room temperature. Seed treatment or soil application of the formulations successfully carried the microorganisms to soil (field) and effectively controlled soil-borne fungi and nematodes on vegetables and pulse crops (Khan 2005).

17.1.2.3 Liquid Formulations

Single-stage liquid fermentation of fungal and bacterial bioinoculants is an attractive process from an industrial point of view, as sometimes it becomes difficult to improve production of conidia (spores) on solid materials (grains, powder, etc.). Several liquid media for fungal bioinoculants such as potato dextrose broth (PDB), Sabouraud dextrose broth with yeast extract (SDYB), Sabouraud maltose broth with yeast extract (SMYB), malt extract broth (MEB), corn meal broth (CMB), jaggery soya broth (JSB), yeast peptone dextrose broth (YPDB), yeast peptone soluble starch broth (YPSS), Czapek–Dox broth (CDB), and yeast peptone soybean oil broth (YPSB) in stationary and shaker culture have been evaluated for mass production of *Beauveria bassiana*, *Metarhizium anisopliae*, *T. harzianum,* and *T. viride*. Maximum biomass production of bioinoculants was observed with JSB in stationary (12.5–20/100 ml wet wt.) and shaker cultures (20–48.8 g/100 ml wet wt.), and highest cfu (5.1 and 9.8×10^8cfu/ml) in stationary and shaker culture were observed, respectively (Rao and Gopalakrishnan 2009). Some liquid formulations are listed in Table 17.5.

Table 17.5 Some liquid formulations of fungal

Product name	Bioinoculant	Target pathogen(s)/disease(s)	Crop(s)	Company
Trichojet	*T. harzianum, T. viride*	*Nectia, Phytophthora, Pythium, Rhizoctonia*	Various	Agrimm Technology, New Zealand
DiTera ES	*Myrothecium verrucaria*	Parasitic nematodes	Cole crops, grapes, ornamental, turf, trees	Valent biosciences corporation, USA
Fusaclean	Nonpathogenic *F. oxysporum*	*F. oxysporum*	Tomato, carnation, basil, cyclamen	Natural Plant Protection, Route d'Artix, France
T22-HC	*T. harzianum*	*Pythium, Rhizoctonia, Fusarium*	Various	BioWorks, India
Enpro-Derma	*Trichoderma viride*			
Filamen AQ	*Ampelomyces quisqualis*	Powdery mildew	Grape, Rose etc	Enpro Bio Sciences, India
Paecilon	*Paecilomyces lilacinus*	Nematode infestation in the soil	Pomegranate	Enpro Bio Sciences, India
Trichorich-L	*Trichoderma viride*	Seed and soil born diseases	Various	Richgreen Agrochem, India
Vertirich-L	*Verticillium lecanii*	Seed and soil born diseases	Various	Do
Bio Chemical Trichoderema	*Trichoderma viride*	Various fungi	Various	Ruchi Biochemicals, India

Peighami-Ashnaei et al. (2009) evaluated combinations of two carbon (sucrose and molasses) and two nitrogen (urea and yeast extract) sources for rapid growth and yield of *P. fluorescens* and *B. subtilis* and found that media containing molasses and yeast extract (MY) in a 1:1 w/w ratio supported rapid growth and high cell yields in both strains. Luna et al. (2002) and Peighami-Ashnaei et al. (2009) showed that maximum growth of the two bioinoculants was obtained when the C/N ratio was 1:1. Molasses is a high quality and inexpensive substrate and can be used for rearing bioinoculants through liquid fermentation. Substantial fungal biomass (spores + mycelia) was formed by incubating *Trichoderma* spp. on molasses in fermenter vessels for 15 days. The biomass was filtered, dried, milled, and mixed with anhydrous aluminum silicate as a diluent to increase volume for application (Papavizas et al. 1984). The filtered microbial biomass may also be formulated with selected liquids.

Bioinoculant formulations are often applied as drenches, spot treatments, or granules, but applying them as foliar sprays creates technical challenges. Use of oils may help to overcome this restriction of foliar application. The intermediate solution is to use more conventional formulations (e.g., wettable powder, WP) or technical materials (e.g., pure, dried fungal conidia) with emulsified oil adjuvants such as "Codacide" (Bateman and Alves 2000). However, as Wraight and Carruthers (1999) point out, oil formulations should be seen as a "silver bullet"; successful development will require a rigorous approach to selection of isolates, delivery system, and deployment in the marketplace.

17.2 Conclusion

Plant diseases are significant constraints on crop production worldwide, and their management is essential to increase food production. In view of the adverse effects of pesticides, fungal bioinoculants offer a potential substitute. Numerous potentially useful microorganisms are available, such as *Trichoderma* spp., *Aspergillus niger*, *Penicillium digitatum*, *P. anatolicum*, *Paecilomyces lilacinus*, *Pochonia chlamydosporia*, or nonpathogenic strains of certain pathogens. These organisms can be applied directly to soil, as a seed treatment or foliar spray to reduce the inoculum level of pathogen or disease severity. Commercial formulations of most bioinoculants are available and provide varied degrees of disease control. Overall performance of phosphate-solubilizing fungi such as *A. niger*, *Trichoderma* spp., *Penicillium* spp., against plant diseases and nematodes is at levels that ensure their commercial exploitation. This necessitates research efforts toward identification of more efficacious and environmentally adaptable strains, development of suitable mass production technologies, and development of efficient immobilization systems.

Bioinoculant formulations can be seen as a tool for developing a more rational pesticide use strategy. Understanding the implications of working with living organisms in agricultural systems is highly desirable. Perhaps more importantly,

biological control/IPM practitioners, organic growers, and other parties willing to promote bioinoculants must understand that they are most likely to succeed as commercial products, available as practicable, stable, efficacious formulations.

17.2.1 Future Recommendations

The use of bioinoculants is likely to become more widespread in the near future, as increasing pressure develops to limit environmental damage from the use of chemicals as well as development of pathogen resistance to pesticides. Environmentally sustainable systems for control of soil-borne pathogens are likely to be developed because the soil environment provides a more favorable habitat for the persistence of antagonists. In addition, the necessity for new systems will increase, requiring greater research efforts to develop technologies and methods for foliar application of bioinoculants. The technology available presently is able to produce liquid, powder, pellet, and granular formulations of bioinoculants, and limited formulations that are compatible for foliar application are available. Moreover, efficient methods are needed for improving multiplication rate of useful bioinoculants, which will enable bulk inoculum production with longer shelf life.

It is likely that genetically engineered microorganisms will be increasingly used in the future because it is often difficult to select, from natural microflora, an organism that both adapts to persist in the environment of roots or shoots of crop plants and possesses a high level of antagonistic activity against pathogens. Introducing a desired antagonistic ability, such as antibiotic or lytic enzyme production, into an organism that is both persistent and an effective colonist of roots or shoots may allow for such difficulties to be overcome. Such development must be combined with risk assessment studies to ensure the safety of the released bioinoculant, to provide adequate food to burgeoning populations, especially in Asia and Africa.

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