Chapter 17
Potency of Three Cruciferous Plants Extracts as Agro-Phyto-Remidiator Against Root Knot Nematode *Meloidogyne spp.* in *Daucus carota* (Carrot) Under Climate Stress Conditions

Baby Tabassum, Mohammad Hashim, and Jagriti Madan Dhingra

Abstract Carrot, *Daucas carota* is another important crop that is most cultivated throughout India and consumed by human beings and animals. The root knot nematode (RKN) *Meloidogyne incognita* infestation significantly reduces the yield of carrot at initial inoculums of 230–2300 J_2/g soil. One strategy to address these concerns is to develop an effective agro-phyto-remediator to these tiny enemies that have zero toxicity to non-target organisms and can be applied at very low cost. Biochemical studies reveals that in certain cruciferous plants like *Brassica rapa*, *Brassica botrytis* and *Raphanus sativous* having nematicidal principle as α tetraethylene and 5-1-3-butenyl 2,2 bithienyl, polyacetylene compounds like trans 3,11-trideca-1-3,11-triene 5,7,9 trizene etc. targeted the percent mortality of *Meloidogyne incognita* juveniles increased almost equally from higher 100% upto 6.25% dilution after 24, 48, and 72 h exposure period of *Raphanus sativus* leaf extract, while *Brassica botrytis* caused significant percent mortality of *Meloidogyne* juveniles i.e. 100% was observed within 24 h exposure with leaf extract in its 100 and 50% concentrations whereas leaves extract of turnip was most effective and showed 100% J_2 killed followed by 85.67–96.75% mortality with 50–6.25% dilation after 72 h exposure. Histo-pathological and molecular studies show infection of *Meloidogyne* incognita increased transpiration, photosynthesis or water content and decreased the level of sugars, ascorbic acid and fruit quality. In present study, observed high metabolic activities with intense cytoplasm and nuclei in giant cells produced by nematodes in the carrot.

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Introduction

Nematodes show tremendous structural diversities and occur in almost all kinds of biotypes in enormous numbers. An acre of cultivable land contains 3,000,000,000 nematodes while marine beach sand may contain approximately 11–18,000 or sometimes even 90,000 specimens of *Anguina tritici* Jairajpuri [1]. Upto 1930 approximately 4,500 species of nematodes had been described which rose upto 9,000 by 1950. The latter day numbers of investigated species are almost 15,000 but the estimates of subsist species are around more or less 500,000 or more. It reminds the remark of the late eminent nematologist Dr. N. A. Cob of the US department of Agriculture, "If all the matter in the universe except the nematodes were swept away, our world would still be recognizable, we would find its mountains, hills, valleys, rivers, lakes and ocean represented by a film of nematodes".

Evaluated comprehensive average easy yield loss is 12.3% by plant parasitic nematodes in total prime crops. Annual 14% yield loss evaluated in 20 crops and average deprivations for 42 crops in advanced countries are reported almost 8.9% when contrast to 15.7% of developing countries. Uttar Pradesh is one of the most fertile states of India, where almost all types of crops and vegetables are extensively grown. The state leads in total production of a variety of crops though in many cases yield per acre is rather low but the farmers who are mostly ignorant of these microscopic nematode pests inhabiting the soil and attacking their crops, fail to understand the reason for crop failure. 102 known species belonging to 33 genera of *Tylenchida* and 59 species belonging to *Dorylimids* had been noticed in Utter Pradesh Sehgal et al. [2]. Moens and Wesemael [3] also reported that carrots (*Daucus carota L.*) great loss occurred by the RK nematode *Meloidogyne chitwoodi.*

Carrot, *Daucus carota* is another important crop which is most cultivated throughout India and consumed by human beings as well as by animals. It is rich in carotene and is used in various ways of coloring butter and other food articles. Out of the major groups of carrot, Asiatic and Temperate groups are rich in carotenoids which contain appreciable quantity riboflavin and thiamine while the Asiatic types have more anthocyanin pigments and less of carotenoid pigments Gill and Kataria [4]; Rebecca et al. [5]; Raees-ul and Prasad [6].

In temperate regions carrot is seriously affected by *Heterodera carotae*. Greco and Brandonisio [7]; Moens and Wesemael [3] estimated 100% crop loss by the nematode. Other important nematode pests found on carrots are carrot cyst nematode and RKN, *M. javanica* and *M. incognita* respectively. *M. javanica* on carrot exhibits constriction, digitation and cracking in the tap root system. The RKN infection significantly reduces the yield loss in carrot at initial inoculum of 230–2300 J/g soil. Ribonuclease activity also decreases in carrot plants, tolerant to *Meloidogyne hapla*, whereas, increase in the secondary phloem and xylem tissues of susceptible plants have been noticed by Krypl and Janas [8]; Phillips [9] resulting in reduced functional metabolism.

Nowadays, crop scientists are searching for simple, eco-healthy, economically low tactics which integrate into an overall nematode management system. In the current

study also an endeavor has been made to calculate the impact of attract various parts of the cruciferous plants like *Raphanus sativus*, *Brassica rapa* and *Brassica botrytis* against *Meloidogyne* second stage juvenilies in in vitro and in vivo on *D. carota* plants.

Histo-Pathological and Molecular Studies

Histopathological and molecular studies reveal that there is an increase of total DNA and RNA in *M. incognita* infected regions of the host plant Masood and Saxena [10]; Phani et al. [11]; Bayani et al. [12]. Infection of *M. incognita* increased transpiration, photosynthesis or water content and decreased the level of sugars, ascorbic acid and fruit quality Tyagi and Rehman [13]; Ahmed et al. [14] recorded increased protein and ascorbic acid devoid of lignin in giant cells because of the *M. incognita* infection. Chlorophyll content also become low because of *M. incognita* Ganguly and Dasgupta [15]; Lu et al. [16] estimated low protein and Indole acetic acid activity and high auxin peroxidase activity in RKN infected gall than healthy roots.

Bruno et al. [17]; Meidani et al. [18] observed high metabolic activity with intense cytoplasm and nuclei of giant cells produced due to nematodes in carrots. The cultivars susceptibility of *M. javanica* on carrot in terms of penetration development was observed by Debia et al. [19]. Also noticed that the symptoms produced by *M. javanica* on carrot include constriction, digitation and cracking in the tap root system. The RKN infection significantly yields loss of carrot at initial inoculum of 230–2300 J_2/g soil Huang and Charchar [20]. Such symptoms on root; as well quantitative estimation of yield loss was studied by Hay et al. [21] at different: inoculum levels and indicated 50 yield loss at 10 J_2/g soil and no yield at 30–50 J_2/g soil.

Singh et al. [22] observed the physio-biochemical changes in carrot root caused by *M. hapla* and also reported reduction in protein synthesis and Protein Amino Lipid (PAL) levels in susceptible plants and increased level of RNA. The phenol level also increased in infected plants but was more resistant than susceptible plants. However, studies on tolerance and resistance of carrot to RKN by Meidani et al. [18] indicated a link, with high number of foliage and low soluble polysaccharide. The acidic fraction of pectins obtained from carrot contains 74% galacturonic acid. The oligogalacturonides containing fraction with the lower molecular weight turned out to be the most active in blocking the adherence of bacteria and epithelial cells in a biological test system by preparing oligo-galacturonic acids Therefore, two oligo-galacturonoides, produced by partial hydrolysis of carrot pectin in stomach are responsible for the anti-diarrheal activity of carrot soup by blocking the adherence of bacteria to epithelial cell Follrich et al. [23]. Agarwal and Ghosh [24] reported, carrot juice contains an alkaloid, pyrolidine, and daucine and is a refrigerant, a tonic and useful in the kitchen in many other ways.

Krishnamurthy and Murthy [25] and Dhaliwal and Arora [26] reported economic losses due to pets between 6,000 and 29,000 crores, while Van Burkum and Sheshadri [27] probably for the first time accounted annual losses of *Anguina tritici* caused about

10 million in wheat, 3 million in coffee by *pratylenchus coffeae* and *Heterodera* caused disease of Molya 8 million annually in Rajasthan, India. It is estimated that in South Asia 89,000 tonnes of chickpea are lost due to nematode infestation Cunha et al. [28].

Nematode damage is so insidious that it is highly devastating to crop. More than 2000 plant parasitic species of nematodes are recorded and they tenanted every possible métier the plant offers. Thus, all the parts of the plants over and beneath the ground seem to be attacked by nematodes, which may be specifically ectoparasitic or endoparasitic.

Chemical Control

Chemical control of nematodes in soil dates back to 1881 when Kuhn applied carbon di-sulphide (CS_2) to control sugar-beet cyst nematodes in Germany. After that, Bessey [29] also observed its efficacy against RKN. Then Mathews [30] found nematicidal qualities of chloropicrin (tear gas) and surplus chloropicrin of World War I was used in greenhouses, seed beds and special crops. With the commencement of World War II its use was deflected into war efforts and commercial soil fumigation terminated until Taylor and McBeth [31] manifested nematode control by methyl-bromide (MBr), a broad spectrum biocide. The introduction of 1,2-Dichloropropane 1,3-Dichloropropane and Ethylene Di-Bromide Haydock et al. [32] led to the acceptance and verify of the significance of phyto pathogenic nematodes in yield losses which increased nematode management options. This catalyzed the development of the phyto-nematology and fumigation industry as well. The problem of phyto-toxicity of DD and EDB was overcome by the development of 1,2-Di Bromo,3 Chloro-propane D'errico et al. [33]. In (2020) Talavera-Rubia et al. [34] reported nematicidal efficacy of milbemectin sodium.

Nematode Control by Fumigants

The rapidity and extent of the use of fumigant were the most interesting and surprising responses in the history of pesticides. Widespread use of fumigants started somewhere in 1950 as crop insurance and after having dominated an era of two decades, the fumigant nematicides gave way to nonvolatile non-fumigants organophosphates and carbamates in 1970s in due course of programs designed for insecticides. The non-fumigants were advantageous over fumigants being less phytotoxic Van Burkum and Hoestra [35] VC-13 (dichlofenthion), the first organophosphate nematicide was used to protect ornamental and turfs Perry et al. [36]; Gad [37]. Thionazin was the next important: nematicide used by Jenkin and Guengerich [38].

Other environmental impacts include phytotoxic effects to non-target organisms and residues in soil and crops. Some of them are carcinogenic and also produce

suppressing effects on nitrifying bacteria Castro and Beiser [39] and Mckerny [40]. There is also risk to livestock in consumption of produce from pesticide treated soil Young et al. [41]. However, despite of their well-founded concerns about their impacts on unwanted elements, usable water, air quality, and food safety measurement of the crop protection chemicals are very likely to important contrivance in agriculture well into 21st because of the pivotal role in modern global food production Beyer [42], One strategy to inscription these concerns to develop practically effectual agro-phyto-chemicals remediator that have minimum mephitic to non-target organisms and can be applied at economic rate.

Agro-Phytoremediator

Pest control agents from natural sources had evolved eco-healthy, economic as well as suppressed pest populations reported by many workers like Waterfield and Zilberman [43] and Zaki and Bhatti [44]. As plant products being naturally evolved ingredients, they preserved the natural equilibrium in the ecosystem.

There are also several reports that cellulose when integrated in the soil reduced the percentage of plant parasitic nematodes (PPN). The population of *P. penetrans* and *Heterodera tobacum* was considerably inhibited by the application of chopped paper and white pine saw dust as reported by Miller and Edgington [45] and Miller and Weihrmenn [46] respectively. Mankau and Das [47] observed that addition of pure chitin to the soil inhibited the percentage of *M. incognita* and also the development of knot in root. Soil amended with the hydrated extract of sawdust reduced the salvation of eggs in *M. javanica* Sitaramiah [48]. In the various parts of the world there is a common use of oil cake as fertilizers. Lear [49] had reported reduction in *M. javanica* and *Heterodera schactii* by amending with Castor pomace. Hundred percent reductions of *T. semi-penetrans* were reported by Szczygłowska et al. [50]. In India exhaustive work had been done by Singh and Sitaramiah [51] who found oil cakes of *Azadirachta indica*, *Ricinus communicus*, *Brassica*, *peanuts linseed*, *Madhuca indica* etc. capable of reducing *Meloidogyne* population in field/plots. Tarla et al. [52] found oil cakes and its extracts harmful to the nematodes. Many other unusual amendments had been shown to reduce nematode percentage, however the related function is poorly defined till date but some of them may offer an effective means of nematode control only in small plots.

Many weeds like *Catharanthus, Chenopodium, Argemone, Datura, Ricinus* and many more having phyto-therapeutic value had been reported by Abid and Maqbod [53]; Vats and Nandal [54] reported that the percentage loss of carrots *(Daucus carota L.)* damaged by the RKN *Meloidogyme chitwoodi*. Various effect of chemicals and their mode of action had also been studied in detail by many workers Douda et al. [55]; Pinheiro et al. [56]; Ahmad et al. [14]; Cunha et al. [28] from plants and had been proved toxic to nematodes.

Green synthesis of silver nanoparticles by *Cnidoscolus aconitifolius* extract was experimented by Fabiyi [57] in plants of carrots infected by *M. incognita* juveniles

in soil as reducing elements, whereas silver nitrate is the metal precursor. AgNPs treated carrot plants showed higher yield and inhibition of *M. incognita* as well.

Management of nematodes by modern techniques

Traditionally, physical, chemical, biological, cultural and regulatory methods are adopted for the management of these tiny nematodes. But modern biology is influenced by ultra-modern techniques like gene cloning, genetic engineering; gene splicing and recombinant DNA used as resistance factors against RKN in egg plants. Another unusual approach to the plant genetic transformation is introducing foreign DNA by micro-projectile bombardment. Enormous amount of work is done in the identification of gene loci in nematodes pests in numbers of crops. Pireda et al. [58] derived head towards the location of chromosomal resistance to *G. rostochiensis* in the potato crops with RKN, *Maloidogyne spp.* (Klein) Anna et al. [59]; Rybczyński et al. [60]. The single dimensional Polyacrylamide Gel Electrophoresis (PAGE) on a fixed pH or pH gradient gel is more commonly used for characterization of nematodes. The pH gradient gels obtained with the incorporation of suitable ampholytes are used for isoelectric focusing of proteins Michael et al. [61].

The techniques of hybridization using specific primers, DNA polymerase enzymes, thermal cycling leading to Polymerase Chain Reaction (PCR) for DNA synthesis and use of Restriction Fragment Length Polymorphism (RFLP) among different population/species of a nematode taxon is gaining popularity in nematology these days. RFLP of mtDNA has been performed to convert several *Meloidogyne* species and genetic divergence in mt DNA was observed by Powers and Harris [62]. Castagnone-Sereno et al. [63]; Bairwa et al. [64] studied the phylogenetic relationships between the amphimictic and pathogenetic species of Meloidogyne using DNA analysis.

Material and Methods

Cobb's Technique Modified by Barker [65]

The extractions of nematodes from the soil or roots were held by "Cobb's sieving and decanting method" water was mixed in the soil by passing supernatant through 100, 200 and 400 mesh sieves. Nematode suspensions thus collected were used to study the population dynamics and rate of infection.

Calculations for population dynamics and rate of infection have been done by using following formulae:

Norton's Formulae [66]**:**

1. Relative Abundance (RA)

$$RA = \frac{Number\ of\ samples\ containing\ different\ species}{Number\ of\ samples\ collected} \times 100$$

2. Relative Density (RD)

$$RD = \frac{Number\ of\ individual\ of\ a\ species\ in\ a\ sample}{Total\ number\ of\ all\ individuals\ in\ a\ sample} \times 100$$

3. Relative Frequency (RF)

$$RF = \frac{Frequency\ of\ one\ species}{Total\ number\ of\ all\ individuals\ in\ a\ sample} \times 100$$

4. Dominance Value Index (DVI)

$$DVI = \frac{RA + RF + RD}{3}$$

Johnson [67]**:**

a. **For Histopathology**

Selected root parts of host plant 1–2 cm long washed properly then bleached in NaOCl (sodium hypochlorite) for one-two minute. After proper washing root parts were transferred into acid fuchsin stain then heated upto boiling point and cooled down at room temperature. Finally root parts were mounted in glycerin and microphotographs were taken for histo-pathological studies.

b. **For Histology**

Galled roots were preserved in 4% formalin for histology of *M. incognita* female by following procedure—Took the infected root parts of the carrot. Passed through ethanol series:

50%—3 changes (30 min each)
60%—for 30 min
70%—overnight
80%—for 30 min
90%—for 30 min
90%—15 min (2 changes)

- Cleared the material in methyl-benzoate (50–60) min and transferred to 20% celloidin solution in methyl-benzoate for at least 3 days.
- Passed through three changes of benzene, 10 min each.
- Passed through two changes, paraffin warmed at 70 °C, 10 min each.
- Embedded in clean paraffin.
- The ribbons were made with the help of a microtome and kept for all night at 35–40 °C in the incubator.
- Mounted in DPX.

- Sections were studied under microscope and suitable microphotographs had been taken.

Details of Experimental Plants (In-Vitro)

Fresh parts: of ten experimental plants categorized into three parts were taken.

Cruciferous Plants:

In India substantial study perform to inhibit the nematodes (Plant Parasitic) by the use of several cruciferous plants. Stahmann et al. [68] found the presence of antinemic phenyl isothiocyanate in crucifers.

i. ***Brassica rapa*** **(Turnip)**: It is a well-known vegetable which belongs to Family Cruciferae. It is largely cultivated for the sake of its leaves as well as the thickened roots Mathur [69].
ii. ***Brassica botrytis*** **(Cauliflower)**: The cauliflower belongs to Family Cruciferae and is eaten for its inflorescence. The leaves are applied in gout and rheumatism Mathur [69].
iii. ***Raphanus sativus*** **(Radish)**: Another member of Family Cruciferae is annual or biennial plants mostly cultivated during winter months for the fleshy tuberous roots. The juice of the fresh leaves is diuretic and laxative. The seeds are carminative and also yield an essential oil. The roots are used as drugs for urinary complaints, piles and gastrodynia pains Mathur [69].

A. **Reddy et al.** [70]: It is used for the Mean Gall Index value **(MGI) Scale**
1 = 1–25 galls without egg masses
2 = 26–50 galls without egg masses
3 = with numerous egg masses

$$\mathbf{MGI} = \frac{Number\ of\ total\ galls\ counted\ in\ each\ replicate}{3}$$

B. **Atwal and Balraj** [71]: It is used for in vivo yield loss of *D. carota.*
C. **Statistical Calculation**: Statistical calculation like minimum value, maximum value, average median value, standard deviation, correlation coefficient and root squared value were taken with the help of a computer package.

Results and Discussion

Histology of M. incognita *Female*

Cross section of female body of *Meloidogyne incognita* through the anterior side showed esophageal gland lobe and intestine Whereas, in the posterior end sections

showed various organs like ovary, oviduct, oviduct with oocytes, spermatheca, and uterus without eggs while, in some cases uterus with eggs as well as rectal gland had also been noticed (Figs. 17.1, 17.2, and 17.3).

Almost in all cases ovaries, uterus and rectal glands had been noticed from the posterior end. Eisenback [72] observed that in the sections of female *Meloidogyne*, a large portion of the body cavity is filled by a pair of tubular, highly convoluted gonads. Approximately 60% of the gonad was occupied by ovaries. Spermatheca was located

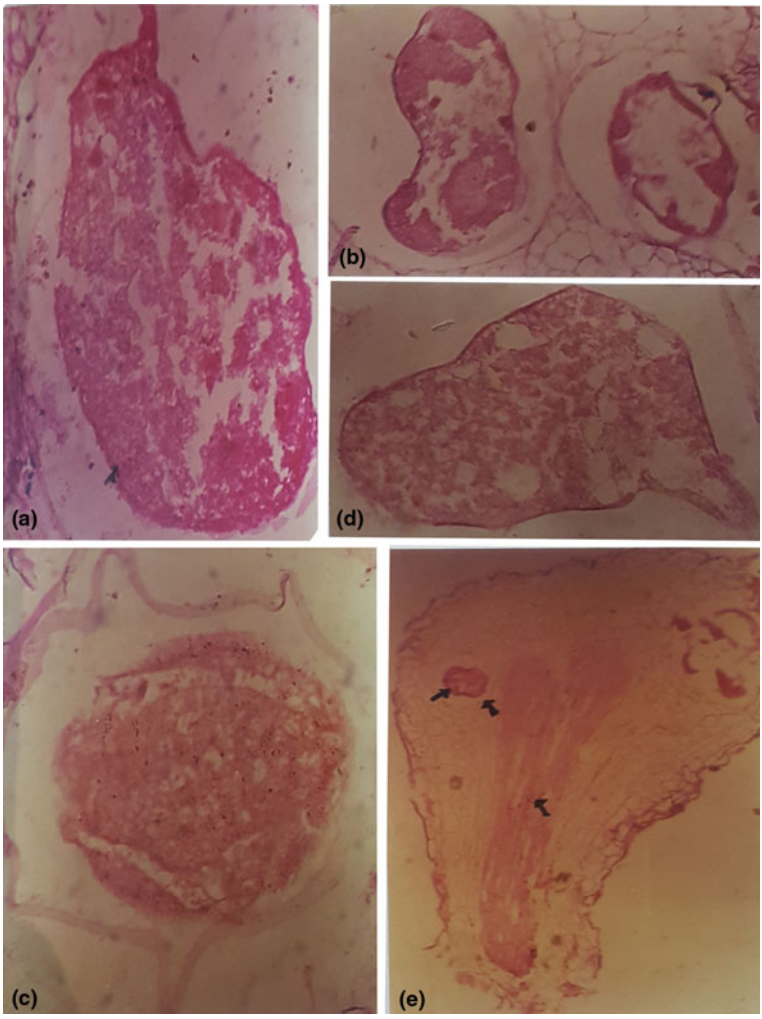

Fig. 17.1 **a** Anterior posterior region of female *M. incognita* (L.S.), **b** female *M. incognita* through neck region (C.S.), **c** anterior lateral region of egg laying female *M. incognita* (C.S.), **d** posterior lateral region of female *M. incognita (L.S.).* **e** *D. carota* showing giant cell adjacent to vascular bundles and abnormal growth of tissues after 26 days inoculation of *M. incognita* J_2 (L.S.)

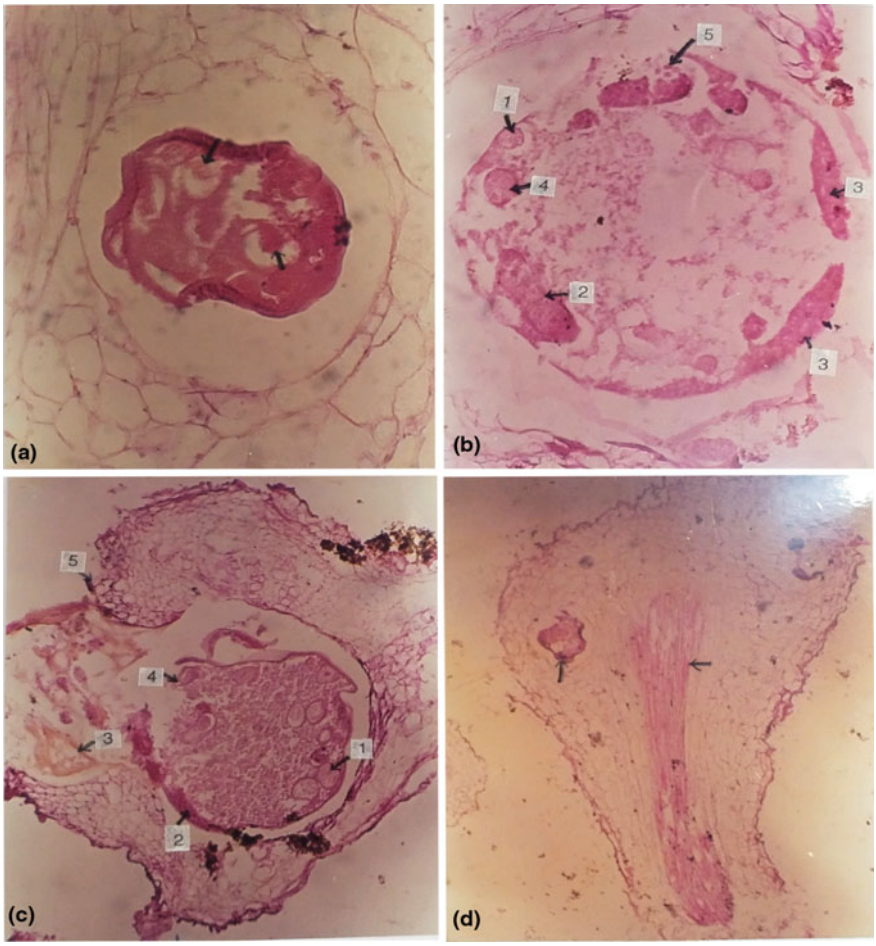

Fig. 17.2 **a** Anterior part of female *M. incognit*a showing esophageal lobe and intestine (C.S.). **b** Posterior region of egg laying female of *M. incognit*a showing (C.S.): (1) ovary, (2) oviduct with oocyte, (4) uterus, (3) rectal gland, (5) spermatheca. **c** Posterior region of female *M. incognita* showing (C.S.): (1) uterus, (2) rectal gland, (3) hyaline portion of the gelatinous sheath, (4) ruptured cell wall and cortical cells of *D. carot*a, (5) abnormal vascular bundle of infected *D. carota.* **d** Hyperplasia and hypertrophy in infected *D. carota* after 15 days of the inoculation of J_2 (L.S.)

posterior to the oviduct. Posterior to the spermatheca, the uterus was differentiated. The two uteri of the female reproductive tract fuse to form one common duct posterior to which laid a large rectal gland. The present observations were in confirmation of Viglierchio [73]; Nguyen and Duong [74] findings.

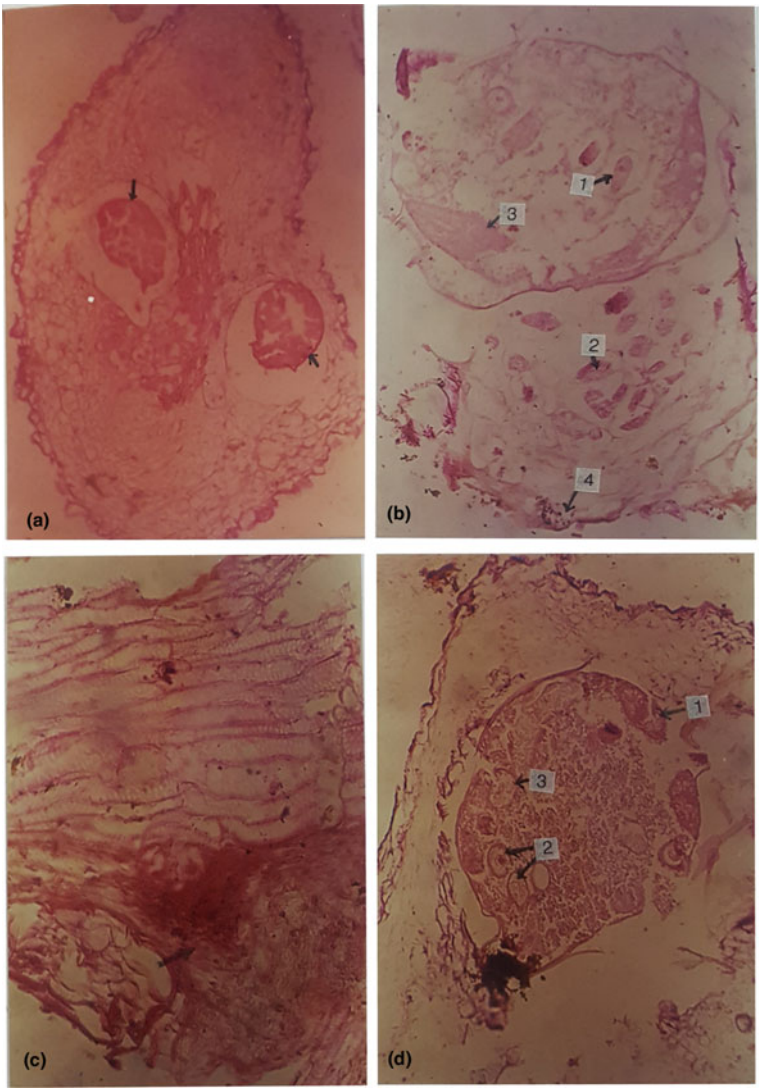

Fig. 17.3 **a** Giant cells in the infected root of *D. carota* (20 days) after inoculation with *M. incognita* J_2 sections through the posterior part of body. **b** Posterior end of female *M. incognita* showing (C.S.): (1) uterus with few eggs, (2) spermatheca and ovary, (3) rectal gland, (4) hyaline sheath with few eggs. **c** Infected root of *D. carota* showing feeding side in cortical cells and thick dense granular protoplasm in phloem and nurse cells (L.S.). **d** Posterior end of egg laying female of *M. incognita* showing (C.S.): (1) rectal gland, (3) uterus, (2) ovary and oviduct

Histopathology of Carrot

No visual symptoms were generally observed above the ground parts of the carrot. However, the nematode infestation resulted in the formation of cracks on tubers, forking of tap root accompanied by beads like galls on secondary roots and extensively reduced plant size.

After gaining entry through the root cap epidermis, the second stage juveniles penetrate secondary roots inter-cellular as well as intra-cellular through the cortex, endodermis and pericycle and reach the phloem. Soon-after penetration, J_2 began to feed, increased in size and became oriented perpendicularly towards the longitudinal perpendicular axis of the root having the posterior portion outside from the root (Fig. 17.4a, b). A slightly wider passage than the nematode bodies with thick walls was formed by the destruction of cortical cells. Soon after the infection, the juveniles were found to enlarge in size perhaps due to the pressure exerted on the cortical cells. The nematodes feed in the cortex as well as in the phloem. In the cortex, the cells at the feeding site stain pink red with lactophenol. The giant cells formed by *M. incognita* in carrot differed from those in roots of other susceptible crops, like tomato, by their thin walls and smaller size. Characteristic wound healing responses i.e. formation of callus like tissues or wound periderm and their precipitated constituents had also been observed. The proliferation of phloem cells at the feeding site was not so marked, though these cells had thick and dense granular protoplasm. In several sections hyperplasia of cortical and hypertrophy of pericycle cells and nurse cells had also been observed (Figs. 17.5 and 17.6).

Highly infected roots reveal histo-pathological changes which conduct to the element conjation as reported by Sudha and Prabhoo [75]. Whereas, in the histopathological studies Charles and Venkitesan [76] reported rupturing of cortical cells and formation of syncytial cells with thick end walls in the stellar region. Khan and Khan [77] observed reduced plant growth due to low and small size of stomata and trachoma. Procinai and Ambroguini [78] observed high metabolic activity with intense cytoplasm and nuclei in giant cells, produced by nematodes in carrots. Abnormal xylem and parenchyma with thickened cell walls were observed in all root knot nematode infected tissues except in rhizome meristems Routaray et al. [79]. Lanjewar and Shukla [80] found *M. incognita* was entering the cortex and stellar regions converting into giant cells. These giant cells showed karyotin nuclear divisions and had thickened cell walls. Sasser and Carter [81] presumed that giant cells produced by parasitic activity were chiefly nurse cells in the vascular tissues, which had cell wall impressions to soak nutrients from nearby cells. These were produced by mitosis without cytokinesis Dropkin [82]. Haseeb et al. [83] observed greater oxidase and peroxidase activity in vascular bundles which might be responsible for delaying lignification. Corky wounds were found at infection sites in differentiated rhizomes and fresh roots Shah and Raju [84]. Whereby, characteristic wound healing responses like formation of callus like tissues or wound periderm at the wound site observed in present study had also been reported by Stobbe [85] in yam tubers who presumed that it might be due to the production of resin, gum, latex or callose and

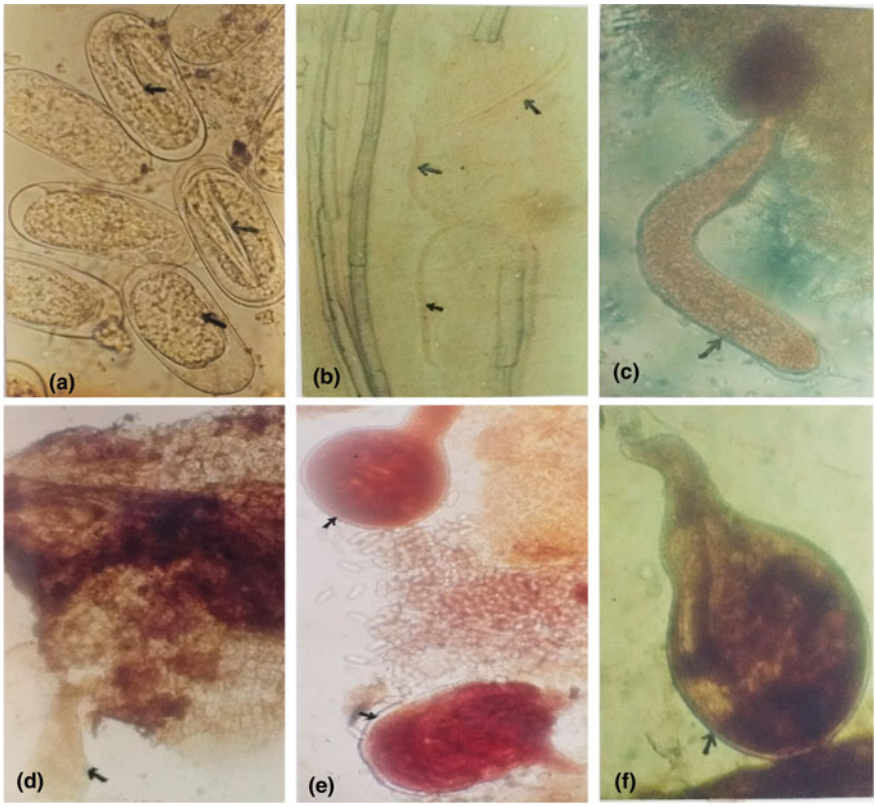

Fig. 17.4 **a** Embryogenesis in the *M. incognita* eggs and juvenile of the previous stage just before hatching. **b** Second stage juveniles (J_2) of *M. incognita.* **c** Spike tailed or sausage shaped J, larvae of *M. incognita.* **d** Fourth stage of female *M. incognita.* **e** Developing female of *M. incognita.* **f** Mature female of *M. incognita*

intense suberization in the wound area. Vilsoni et al. [86]; Valette et al. [87]; Alamgir [88] reported that the burrowing nematodes migrate intra-cellular which leads into the giant galleries in rhizomes by infestation of nematodes.

Hence, the infestation of nematodes somehow, disturbed the metabolic activities of infected plants and in infected plants stellar regions of roots were occupied by developing females. Cortical cells in areas where females occurred showed rupture. The epidermis disintegrated, thus, allowing the body of the female to protrude out of the roots. The damage caused to the root tissues may suppress the flow of food materials to various parts of the plants. Moreover, the nutritive value of the tubers was lowered to a considerable extent. Additionally, dwarf tap root, constriction and formation of crack on tap root affected the yield reducing the market value of carrot.

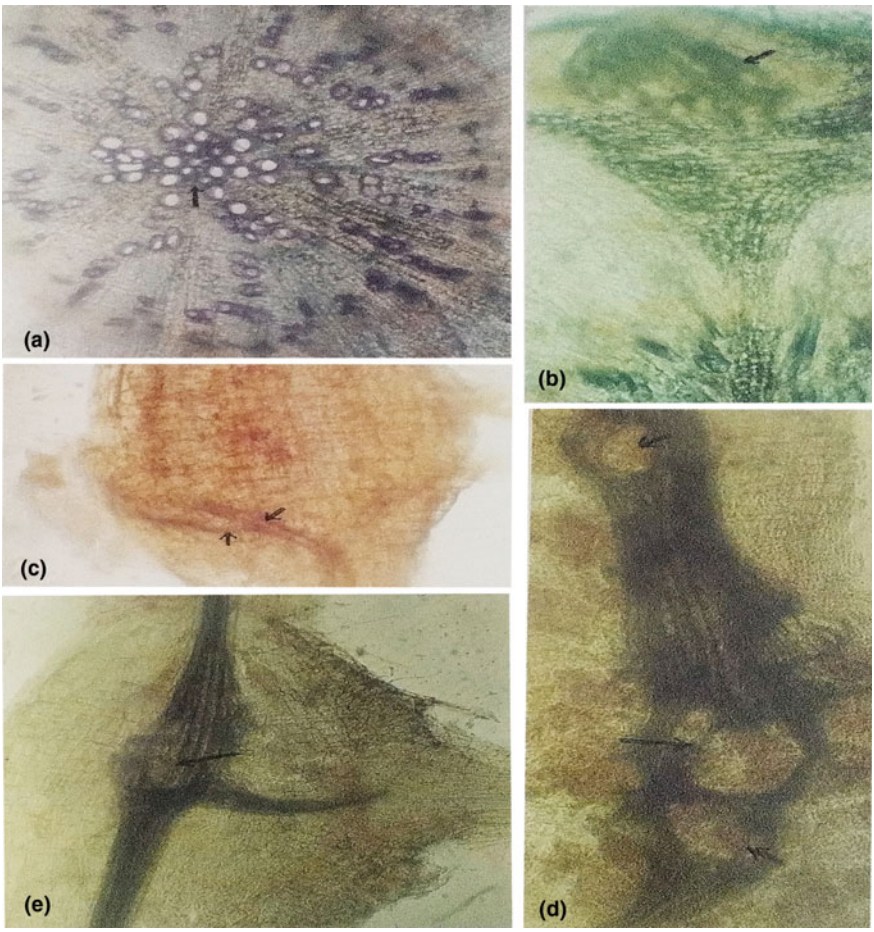

Fig. 17.5 **a** Normal root of *D. carota* showing exarch type of xylem (T.S.). **b** Infected root of *D. carota* showing characteristic "Callus" like tissue formation (T.S.). **c** Gall with J_2 in secondary root of infected *D. carota* (W.M.). **d** Abnormal xylem and phloem cells in infected root of *D. carota* (W.M.). **e** Abnormal growth of vascular bundle in infected root of *D. carota* (W.M.)

Brassica rapa *(Turnip)*

The nematostatic effect of *Brassica rapa* leaves, petiole and roots extracts on juvenile mortality showed in Tables 17.1 and 17.2. Leaves extract of turnip was most effective and showed 100% J_2 killed followed by 85.67–96.75% mortality with 50–6.25% dilutions after 72 h exposure. In all the cases at lower concentrations the nematicidal activity started diminishing as less percent of juveniles' mortality in root extract had been noticed. However, the efficacy of the stock solution of petiole and root extract was noticed to be 51.10–89.17%. The percent mortality increased from 31.10 to

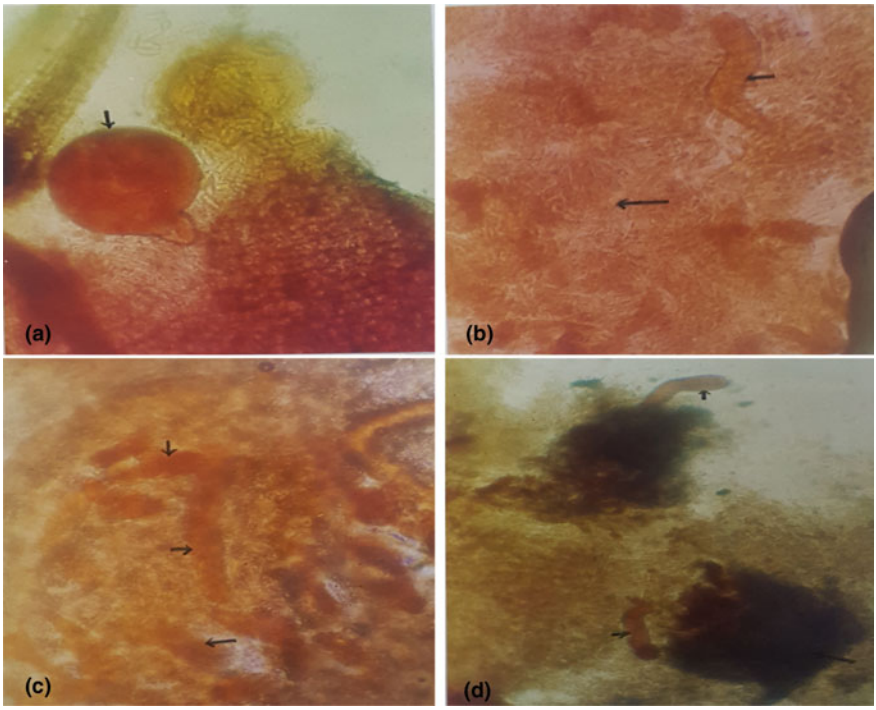

Fig. 17.6 **a** Developing female of M. incognita in the root of *D. carota* plant tissues (W.M.). **b** Infected secondary root with third stage larvae of *M. incognita* and abrupted stelar region of infected *D. carota* (W.M.). **c** Infected root of *D. carota* showing dense granular protoplasm and abundance of larvae of *M. incognita* in cortical region (T.S.). **d** Sausage shaped larvae of *M. incognita* along with damaged plant cells and precipitated constituents of infected *D. carota* (W.M.)

40.58% after 72 h exposure with 12.5% and 25% dose respectively but remained remarkably lower than the leaf extract in which the mortality also showed an upward trend with exposure timing.

Rao et al. [89] recorded 42.80% J_2 mortality of *M. incognita* with root exudates and 55.07% with leaf extract of *Brassica campestris* after 48 h respectively which supported present findings. On the other hand *Brassica rapa* showed least susceptibility to *M. incognita* among ten different vegetables examined by Kihika-Opanda et al. [90]. In India, Ahuja and Mukhopadhyay [91] also reported least 10–25% susceptibility of *Brassica rapa* against *M. incognita* in field and micro-plots experiments among twenty three vegetables examined. Hence, may presume *Brassica rapa* to possess some antagonistic properties against *M. incognita*. Further proved by seedlings of tomato roots were dipped in the water extracts of oil seed cakes of *Brassica rapa* by Vijayalakshmi and Goswami [92] and then disclosed to *M. incognita* (1000 J_2/pot). After 45 days, the most significant enhancement in plant growth and marked inhibition in nematode infestation had been recorded with *Brassica rapa* aqueous extract. Feyisa et al. [93] also tested *Brassica campestris* aqueous leaf extract

Table 17.1 In-vitro experiments of plant extracts

Plant extract	Exposure time (h)	Doses %						
Barassica rapa		100	50	25	12.50	6.50	3.13	1
Leaf	24	68.27	61.9	78.2	56.79	48.78	10.98	
	48	92.39	76.75	65.22	68.83	63.43	24.39	4.45
	72	100	93.33	96.75	93.18	85.67	42.86	5.7
Petiole	24	58.89	37.66	28.8	27.23	12.98	0	0
	48	83.37	49.38	34.44	29.52	12.55	2.18	0
	72	89.17	71.74	64.18	40.58	23.77	2.9	0
Root	24	51.1	22.25	13.23	0	0	0	0
	48	75.83	48.16	17.16	6.16	0	0	0
	72	88.12	84.5	50.2	31.1	2.2	0	0
Barassica botrytis								
Leaf	24	100	100	68.51	58.8	35.57	26.69	2.2
	48	100	100	100	93.35	86.5	28.53	6.65
	72	100	100	100	100	93.42	31.5	8.91
Petiole	24	100	77.71	35.55	22.21	13.33	0	0
	48	100	78.5	42.56	26.68	17.77	2.22	0
	72	100	100	57.8	37.41	26.6	4.45	0
Shoot	24	86.67	80	64.42	44.45	28.85	0	0
	48	100	82.14	73.3	46.2	37.59	0	0
	72	100	100	75.17	55.5	48.8	0	0
Root	24	51.1	22.25	13.23	0	0	0	0
	48	75.83	48.16	17.16	6.16	0	0	0
	72	88.12	84.5	50.2	31.1	2.2	0	0
Raphanus sativus								
Leaf	24	100	78.69	52.94	47.71	40.02	34	20.85
	48	100	87.89	65.22	65.1	56.54	44.54	24.78
	72	100	93.35	91.84	86.36	73.91	59.74	29.44
Petiole	24	100	78.62	41.44	32.75	30.3	8.09	2.1
	48	100	85.89	53.21	50.54	48.83	11.22	3.5
	72	100	100	75.4	64.59	62	14.73	3.5
Root	24	91.11	77.75	66.06	55.5	37.73	24.4	0
	48	100	88.51	71.01	64.44	44.2	35.55	6.1
	72	100	100	84.2	73.57	55.55	40	17.7

Table 17.2 Statistical analysis of in-vitro experiments of *Cruciferous* plant extract with exposure hrs and doses against J_2 of *Meloidogyne*

Statistical analysis of in-vitro experiment								
Plant extracts	Exposure time (h)	Minimum value	Maximum value	Average	Median value	Standard deviation	Correlation coefficient	R-squared
Brassica rapa								
Leaf	24	2	68.27	48.84	56.79	25.0583	−0.9133	0.8341
	48	4.45	92.39	58.34	68.83	29.4846	−0.9321	0.8688
	72	5.07	100	73.92	93.15	33.1787	−0.8501	0.7228
Petiole	24	0	58.89	23.65	27.23	19.6758	−0.9122	0.9452
	48	0	84.17	41.76	40.58	32.134	−0.9904	0.981
	72	0	87.17	41.76	40.58	32.134	−0.9904	0.981
Root	24	0	51.1	12.36	0	17.7731	−0.8425	0.7195
	48	0	75.83	21.04	6.16	27.5212	−0.8849	0.7831
	72	0	88.12	36.58	31.1	35.9094	−0.9574	0.9167
Brassica botrytis								
Leaf	24	2.2	100	55.96	58.8	34.2412	−0.9866	0.9734
	48	6.65	100	73.57	93.35	36.176	−0.8618	0.7427
	72	8.91	100	76.26	100	36.0323	−0.8263	0.6828
Petiole	24	0	100	35.54	22.21	36.1159	−0.9446	0.8923
	48	0	100	37.81	26.68	34.9129	−0.9643	0.9299
	72	0	100	46.49	37.41	38.2544	−0.9737	0.9481
Shoot	24	0	86.67	43.48	44.45	33.0228	−0.9854	0.971
	48	0	100	48.46	46.2	36.3077	−0.9836	0.9675
	72	0	100	54.21	55.5	38.8056	−0.9688	0.9387
Root	24	0	51.1	12.36	0	17.77	−0.8482	0.7195
	48	0	75.83	21.04	6.16	27.5212	−0.8849	0.7831
	72	0	88.12	36.58	31.1	35.9094	−0.9574	0.9167
Raphanus sativus								
Leaf	24	20.85	100	53.45	47.71	25.2377	−0.9615	0.9246
	48	24.78	100	63.43	65.1	23.4209	−0.9791	0.9586
	72	29.44	100	76.37	86.36	22.8999	−0.9258	0.8572
Petiole	24	2.1	100	41.5	32.75	33.1007	−0.9622	0.9258
	48	3.33	100	50.43	50.54	32.6906	−0.9695	0.94
	72	3.5	100	60.03	64.59	35.2619	−0.959	0.9197
Root	24	0	91.11	50.36	55.5	29.4066	−0.9919	0.9838
	48	6.1	100	58.54	64.44	29.9799	−0.9873	0.9749
	72	17.7	100	67.28	73.57	28.8023	−0.9809	0.9699

and found marked reduction in the hatching of egg in *M. incognita.* Aqueous extracts of *Brassica rapa* inhibition of hatching from mass eggs and penetration of juveniles of *M. incognita* into *V. radiata* as reported by Majumdar and Mishra [94].

Brassica botrytis *(Cauliflower)*

The data present in Tables 17.1 and 17.2 showed that leaf extract of *Brassica botrytis* caused significant percent mortality of *M. incognita* juveniles than the other tested parts of this plant. Highest mortality i.e. 100% was observed within 24 h exposure with leaf extract in its 100 and 50% concentrations while with petiole extract in stock solution the same observation had been noticed. With 100 and 50% shoot extract 100% mortality occurred after 48 h exposure. Higher concentration of root extract of *B. botrytis* suppressed mortality in comparison to other part's extract, whereas, lowest 1% dilution of petiole, shoot and root extract was totally a failure to cause mortality. It was also proved statistically (Tables 17.1 and 17.2). Percent mortality in the leaf extract was less at 3.125 and 1% doses and in the petiole extract at 6.25 and 3.125% dilution while in root extract at 12.5 and 6.25% doses when compared to higher concentrations.

These findings confirmed the observations by Abid et al. [95] who noticed ethanol extract of *Brassica botrytis* causing 4, 10, and 23% juveniles' mortality of *M. javanica* after 24, 48, and 72 h of exposure respectively in lower concentrations. Alam [96] reported that minced leaves of cauliflower inhibited the percentage of plant parasitic nematodes. Aisha et al. [97] reported seeds and oil cakes of *Brassica* species to be extremely nematicidal against *Heterodera schachtii*. The chopped floral parts of *Brassica botrytis* against *Tylenchids* had been reported very effective by Haseeb and Alam [98] while Chandravadana et al. [99] and Abid et al. [95] had also reported *Brassica botrytis* possessing nematicidal potential against *M. incognita.* Ahuja and Mukhopadhyay [91] reported *Brassica botrytis* to be resistant against *M. incognita* infestation. Whereby, Thies [100] studies that marked inhibition in root gall index of *M. incognita* with the treatment of oil cakes of Brassica species infecting different vegetables in the field trials.

Raphanus sativus *(Radish)*

The percent mortality of *M. incognita* juveniles increased almost equally from highest 100% upto 6.25% dilutions after the exposure 24, 48, and 72 h. Root extract was interestingly more effective than petiole extract except the initial low mortality after 24 h exposures was 91.11% instead of 100% mortality like petiole extract 100% dose. It was discernible that in the juveniles, exposed to 3.125 and 1% concentrations, mortality occurred from 8.09 to 59.74% and 2.10 to 29.44% respectively for 24, 48, and 72 h exposure in all the leaves, petiole and root extracts. In all cases a marked

increment in percent mortality with the increment of treatment time (Tables 17.1 and 17.2). No J_2 mortality had been recorded in root extract at 24 h.

The above findings support the work of Nandal and Bhatti [101] also confirming the result of root extract of seven alkaloids bearing plants including *Raphanus, Brassica botrytis, B. compestris and Mentha* etc. were more inhibitory than shoot extracts for hatching of juveniles of *M. incognita* observed by Haseeb et al. [102]. According to Kerakalamatti et al. [103] experimented aqueous extracts of oil cakes R. sativus against *Hoplolaimus indicus* reported nematicidal efficacy. Gardner and Caswell-Chen [104] tested cultivars of *R. sativus* against *M. javanica* and *M. incognita* finding all vascular plants to be exposed. On the contrary Belair [105] did not find *R. sativus* as the host for *M. hapla.* Muller [106] also reported that *R. sativus* was implicated in inhibiting the percentage of *M. incognita* studied under greenhouse and micro-plot conditions.

Some of the workers detected certain toxic principles like ricinine ($C_8H_8M_2O_2$), sinigrin (Glycoside), quercetin ($C_{15}H_{10}O_7$), arachin and conarchin, nimbidin and thiniomone which had been isolated from castor, mustard, mahua, groundnut and neem oil cakes respectively. On the other hand menthol and menthone were extracted from *M. arvensis* and certain alkaloids like ajmalicine, serpentine and reserpine from *C. roseus.* Agarwal and Ghosh [24] observed that all these compounds had inhibited nematode percentage. Decline in nematode percentage population probably appears due to production of fatty acids as suggested by Johnson [107]; Klemens and Gerard [108]. Whereas, Khan [109] proposed that probably the position of the "OH" group present in hydroquinone, arbutin, phloroglucinol, orcinol and resorcinol and in some pyrogallol, catechol and gallic acid, some precursor and compounds evinced in plants determine the toxicity against nematodes.

Conclusions

As far as the mechanism is concerned this is understood that the efficacy of plant extract is governed by composition of the compound present in plant parts and the degree of decomposition as influenced by the biological and physical environment of the soil. By and large, the following explanations have been given by different workers: The products from decomposition of plant matter are directly toxic to nematodes Ntalli et al. [110]. Organic matter present in plants on decomposition brings changes in the abiotic and biotic factor of plants surrounding it which results in the host-parasitic equation Vander Laan [111]. Organic amendments facilitate the soil array for higher root length, resulting in more absorption of the nutrients of the soil, and minimizing the distraction of nematodes. Widmer et al. [112]; Ansari et al. [113] suggested that biological management, uses of botanical and topsoil modification techniques record high among others practices of nematode control in environmental safety point of view. As almost 2400 plant species around the World known as pesticidal or nematicidal tendency, but now a days caution should be taken

because many report shows phytochemicals contain many toxicants which may cause eco-toxicity, hepatotoxicity, cytotoxicity and even cause carcinogenicity.

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