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5.2. NEMATODE INDUCED DISEASES OF BLACK PEPPER

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Black pepper is cultivated as a monocrop in most of the pepper growing countries like Indonesia, Malaysia, Brazil etc. while in Kerala, India, it is mostly grown as a mixed crop in homesteads, trailed either on arecanut or coconut palms or other trees, or as a companion crop in coffee or cocoa plantations. Crops like banana, elephant foot yam, colocasia, ginger, turmeric and a variety of vegetables are also grown along with pepper in homestead gardens in India. Such crop combinations play an important role in building up populations of certain polyphagous pests and pathogens, and they are major constraints limiting production and productivity of pepper.

Among such constraints, the most serious ones are diseases induced by fungal pathogens like *Phytophthora* sp., *Fusarium solani* and plant parasitic nematodes viz., *Radopholus similis* and *Meloidogyne incognita*. Interactions among these organisms lead to disease complexes in all major pepper growing countries. Abiotic factors like soil moisture, temperature and nutritional status of the soil also have definite roles in the onset, progression and severity of the diseases.

PLANT PARASITIC NEMATODES OF PEPPER

Plant parasitic nematodes are recognized as a serious constraint to crop productivity in almost all countries. Out of the 15,000 nematode species described, 2200 are plant parasites. The nematodes may be major pests in their own right, in addition they can cause damage to crops when they interact with other disease causing organisms. Nematode damages often go unnoticed or are overlooked as the damages caused by them are not easily identifiable, and often confused with nutrient deficiency, moisture stress, etc. Roots damaged by nematodes cannot absorb water and nutrients effectively. Nematodes spend their lives either in host tissues or in the rhizosphere. The most severe nematode problems occur when susceptible host crops are grown continuously or too frequently on the same land.

Several plant parasitic nematodes belonging to different groups are reported in association with pepper (Table 5.2.1). Based on their parasitic habits they can be classified as ectoparasites, endoparasites and semi-endoparasites. Further, they can be grouped as migratory or sedentary on the basis of their movement in host plant tissues. The compilation of plant parasitic nematodes associated with pepper in the



Ectoparasites

Acontylus sp. Aglenchus sp. Aphelenchoides sp. A. dactylocerus Aphelenchus sp. A. avenae, A. isomerus Basiroliaimus columbus, B. indicus, B. seinhorstii Criconemoides sp. Dipitherophora sp. Discocriconemella limitanea Dolichodorus sp. Helicotylenchus sp., H. abunaamai, H. dihystera, H. erythrinae, H. paracanalis, H. pseudorobustus Hemicriconemoides gaddi, H. mangiferae Hemicycliophora sp. Hoplolaimus sp. Longidorus sp. Macroposthonia onoensis, M. ornata Neolobocriconema braziliense

Rotylenchoides variocaudatus

Rotylenchus sp.

Scutellonema sp., S. siamens

Trichodorus sp. Tylenchorhynchus sp., T. clarus, T. mashhoodi

Xiphinema sp., X. elongatus, X. radicicola, X. vulgare

Semi endoparasites

Paratylenchus sp., P. leptos Rotylenchulus reniformis Trophotylenchulus piperis Tylenchulus semipenetrans

Sedentary endoparasites

Heterodera sp., H. marioni Meloidogyne sp., M. arenaria, M. incognita, M. javanica

Migratory endoparasites

Pratylenchus sp., P. coffeae Radopholus similis

major growing countries by Sundararaju et al. (1979) listed 48 species belonging to 29 genera, while Ramana and Eapen (1998) listed 30 genera and 54 species on pepper. In India, 17 genera of nematodes were recorded in Kerala and Karnataka, the two major pepper growing states (Sundararaju et al. 1980). Plant parasitic nematodes belonging to 14 genera in association with pepper were reported in the

detailed surveys conducted during 1980's in Kerala and two districts in Karnataka. (Ramana and Mohandas 1987, 1989). A new species of a semi-endoparasitic nematode, Trophotylenchulus piperis, was reported on pepper from India (Mohandas et al. 1985). The occurrence of this nematode on pepper has not been reported from any other country. In Indonesia, 14 genera of plant parasitic nematodes were associated with pepper (Mustika and Zainuddin 1978, Bridge 1978). Among them Meloidogyne spp., Radopholus similis, Trophotylenchulus piperis, Helicotylenchus sp. and Rotylenchulus reniformis are predominant in India (Jacob and Kuriyan 1979b, 1980, Ramana and Mohandas 1987, 1989), while in Indonesia and Malaysia only Meloidogyne sp. and R. similis are predominant (Mustika 1990). According to Sher et al. (1969), Meloidogyne sp., Tylenchulus semipenetrans and R. reniformis are more prevalent in black pepper plantations in Thailand. In Para, Brazil, M. incognita, Xiphinema sp., Helicotylenchus sp. and Macroposthonia onoensis are commonly associated with black pepper (Freire and Monteiro 1978). Similarly in Sri Lanka, root knot and burrowing nematodes are of common occurrence in pepper (Lamberti et al. 1983, Gnanapragasam et al. 1985). Other plant parasitic nematodes like Hoplolaimus seinhorsti and Xiphinema ifacolum are also known to affect the growth of pepper adversely in Sri Lanka (Lamberti et al. 1983). The economic damages caused by many of these species are yet to be established. However, Meloidogyne spp. and R. similis are of much economic significance as they cause severe damage to pepper and are implicated in the slow decline/yellows disease, a major production constraint in all pepper growing countries (Fig. 5.2.1). Though T. piperis is very much prevalent with high infestation levels, its impact on pepper cultivation is yet to be evaluated and research in this direction is in progress in India.

Root Knot Nematodes (Meloidogyne spp.)

On a global scale, root knot nematodes (Meloidogyne spp.) are the most destructive of all the nematode parasites of crop plants. They belong to the family Meloidogynidae which are sedentary endoparasites with specialized and complex relationships with the host plants. The genus Meloidogyne is one of the most intensively studied among different genera of plant parasitic nematodes. Out of several species identified in the genus, the most prevalent ones are M. incognita (47%), M. javanica (40%), M. arenaria (7%) and M. hapla (6%). Of these, M. incognita and M. javanica are widely distributed in tropical, subtropical and warm temperate regions.

Survey and distribution

The first record of root-knot nematode infestation on pepper was from Cochin-China (presently a part of Vietnam) by Delacroix (1902). Almost during the same period, Barber (cited by Ridley 1912) observed root-knot nematode infestation on pepper in Wynad, Kerala, India. He described a series of tumours (root knots) on plant tissues due to the eelworm (Heterodera radicicola = Meloidogyne incognita)

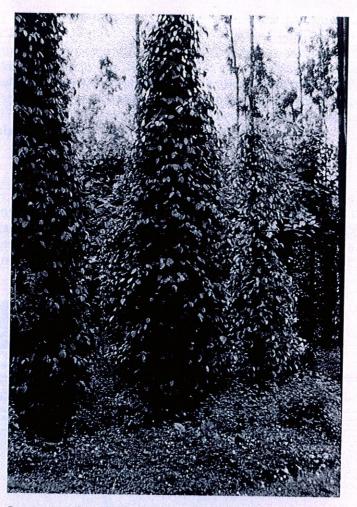


Figure 5.2.1 Pepper vine showing slow decline symptom (yellowing).

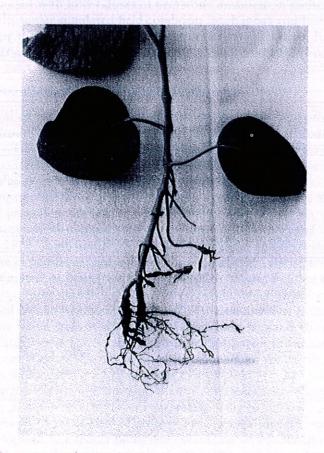
and that when these tumours decay it is not easy to detect the remains of the eelworms. Butler (1906) in his further investigations on the disease in Wynad, also reported the association of root-knot nematodes with the diseased plants. Later Ayyar (1926) reported the wide spread occurrence of root-knot nematodes on pepper in Wynad. Root-knot nematode infestations were also reported from many pepper growing countries like Malaysia (Holliday and Mowat 1963, Kueh 1975, Ting 1975, Razak 1981), Indonesia (Ichinohe 1976, Bridge 1978), Brazil (Sharma and Loof 1974, Ichinohe 1975), Thailand (Sher et al. 1969), Fiji (Swaine 1971), Guyana (Biessar 1969) and Sri Lanka (Lamberti et al. 1983).

Among the four major species of Meloidogyne, M. incognita is a major parasite on pepper. Three species namely, M. incognita, M. javanica and M. arenaria were reported on pepper in Sarawak (Ruen 19/3), the first two are widely distributed

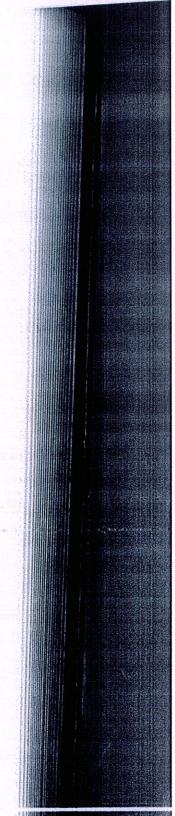
(Kueh and Sim 1992). However, Siti Hajijah (1993) found that only *M. incognita* is widely distributed in all the plantations surveyed in Sarawak and both healthy as well as diseased plants (pepper plants showing foliar yellowing) harboured the nematode. In Sri Lanka, *M. arenaria* was also observed to affect the growth of black pepper (Lamberti *et al.* 1983). In Kerala and Karnataka about 70 per cent and 54 per cent of plants, respectively, were found infested with *M.incognita* (Ramana and Mohandas 1987, 1989) and both apparently healthy and slow decline affected vines harboured high populations of the nematode (Ramana *et al.* 1987).

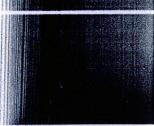
Symptoms

Root knot nematodes are sedentary obligate endoparasites. They have a specialized and complex relationship with the host plants. Infestation by them leads to the development of elongated swellings on the thick primary roots due to multiple infections and typical knots or galls on secondary/fibrous roots due to hypertrophy and hyperplasia of the infested tissues (Fig. 5.2.2). In thick primary roots a number of



rigure 5.2.2 Koot system of pepper plant showing root knot nematode infestation.





adult females with egg masses are situated deep below the epidermis and the whole length of the root turns in to a gall and hence appear almost smooth with occasional swellings here and there (Mohandas and Ramana 1987). Nematodes feed on vascular tissues and cause disruption in the arrangement and continuity of vascular tissues affecting absorption and translocation of water and nutrients. The galled roots decay in due course and considerable amount of root is lost under severe infestation (Mohandas and Ramana 1991, Siti Hajijah 1993).

Pepper plants infested with root knot nematodes generally exhibit foliar yellowing, poor growth and gradual decline in health and vigour. Sometimes leaves of infested vines show dense yellowing of interveinal areas making the leaf veins quite prominent with deep green colour (Ramana 1992, Ramana et al. 1994). Kueh (1979, 1990) reported that in the plants infested with root knot nematodes, leaves were held inward and upward followed by defoliation. In the pathogenicity trials with M. incognita and Fusarium solani, Mustika (1990, 1992) could not reproduce the symptoms like stiff droop and yellowing of leaves in plants inoculated with M. incognita alone. Similarly, severe foliar yellowing could not be observed in the plants inoculated with lower doses of nematode inoculum in pathogenicity tests conducted in India under simulated field condition (Mohandas and Ramana 1991). Nematodes occupy the stelar portion of roots and feed on giant cells. In due course many giant cells coalesce and stelar portion is completely destroyed (Mustika 1990).

Pathogenicity and economic significance

Several experiments were conducted to establish the pathogenicity of root knot nematodes on pepper. Winoto (1972) found significant reduction in the growth of cv. Kuching when inoculated with M. incognita and M. javanica. Ferraz and Sharma (1979) found significant reduction in shoot and root dry weight in the same cultivar inoculated with M. incognita. Freire and Bridge (1985c) found M. incognita highly pathogenic to black pepper seedlings at an inoculum level of 100-1000 second stage juveniles. Similar effects on the growth of pepper plants inoculated with M. incognita were reported from Sri Lanka (Lamberti et al. 1983) and India (Koshy et al. 1979, Jacob and Kuriyan 1980, Mohandas and Ramana 1983). In all these pot culture experiments under green house conditions, the actual loss in yield could not be estimated, as plants were not exposed to natural weather conditions to reproduce the symptoms caused by nematode damage under field conditions. This gap was bridged by the large scale pathogenicity tests conducted under simulated field conditions using grown up plants. These tests showed that foliar yellowing and defoliation were low in the plants inoculated with lower inoculum levels (100 and 1000 nematodes). Characteristic interveinal chlorosis of the leaves was observed in plants which received higher doses of inoculum (10,000 and 100,000 nematodes). The reduction in yield was significant in the plants inoculated with higher inoculum levels, 37 per cent and 46 per cent, respectively (Mohandas and Ramana 1991).

Certain physiological changes were also observed in plants infested with M. incognita, like reduction in absorption and translocation of P, K, Zn, Mn, Cu, Ca and Mg and these elements accumulated in the leaves (Ferraz et al. 1266). Total

chlorophyll content of leaves was significantly low (Ferraz et al. 1989) resulting in growth retardation. Plants inoculated with M. incognita accumulated high concentration of total phenols but without expression of any resistance to the pest (Ferraz et al. 1984). The changes in host physiology and nutrient absorption capacity may account for reduction in leaf chlorophyll content in the diseased plants. Several changes in the levels of amino acids, organic acids and sugars were also observed in the plants infested with M. incognita (Freire and Bridge 1985b).

The Burrowing Nematode (Radopholus similis)

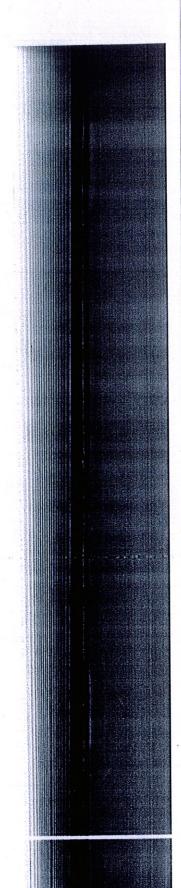
The burrowing nematode is an obligate migratory endoparasite. It belongs to the family Pratylenchinae. It is widely prevalent in most of the tropical and sub tropical regions of the world, has a host range of about 370 plant species and is a major constraint in agricultural production (Peachey 1969). This nematode is a serious problem to citrus, avocado, coffee, tea, banana, pepper, ginger, several palms and indoor decorative plants (Holdeman 1986). R. similis was first reported in Kerala, India on banana by Nair et al. (1966).

Survey and distribution

Pepper as a host of R. similis (= Angiullulina oryzae) was first reported by Goodey (1936). During 1950's pepper plantations in the islands of Bangka, Indonesia were affected by a devastating disease known as 'yellows', resulting in the death of several million pepper plants that led to a major economic disaster for the island inhabitants (Christie 1957, 1959). van der Vecht (1950), after thorough investigations, found that R. similis is responsible for the yellows disease in pepper. Due to this disease the life span of new plantations was reduced to 3 to 5 years (Thorne 1961). Association of this nematode with pepper in India was first reported by D'Souza et al. (1970) and subsequently its wide spread occurrence in pepper plantations in South India was confirmed (Kumar et al. 1971, Venkitesan 1972, Koshy et al. 1978, Jacob and Kurian 1979b, Ramana and Mohandas 1987, 1989). R. similis is also recognized as a major pest of pepper in Malaysia (Reddy 1977), Thailand (Sher et al. 1969) and Sri Lanka (Gnanapragasam et al. 1985).

Symptoms

R. similis invades any succulent underground plant part but favours the area near the root tip. Nematodes take feeding position inter and intra cellularly and the cortical cells immediately around the nematode turn necrotic and further feeding and movement of the nematode in the root tissues lead to the development of large necrotic lesions throughout the root cortex (Fig. 5.2.3). Under artificial inoculation the nematodes penetrated the pepper roots within 24 hours (Venkitesan and Setty 1977). The nematodes starve to death in less than 6 months in the absence of a host piant. Aii stages of the nematode after hatching from egg, except the adult males, are



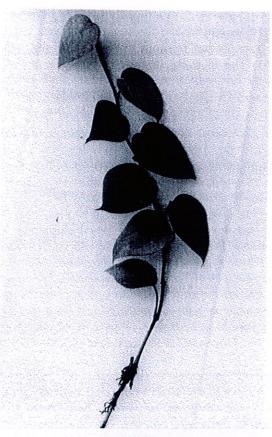


Figure 5.2.3 Pepper plant infested with burrowing nematode showing total destruction of roots.

infective. R. similis feeds on cortical tissues and produces elongated dark brown necrotic lesions on the roots at the infection sites. After draining the cell contents, nematode pushes through the cell wall to the next cell, thus destruction of successive cells results in the formation of tunnels or burrows in the root tissues. When the infestation is severe many lesions coalesce and encircle the root cortex. Due to damage to cortical tissues, root portion distal to these lesions gradually disintegrates. Plants tend to produce new roots which are also in turn infected resulting in a bunch of decayed root mass (Mohandas and Ramana 1987). R. similis do not invade stelar portions of the root, but plugging of the xylem vessels with 'gum like' substance has been reported (Freire and Bridge 1985a).

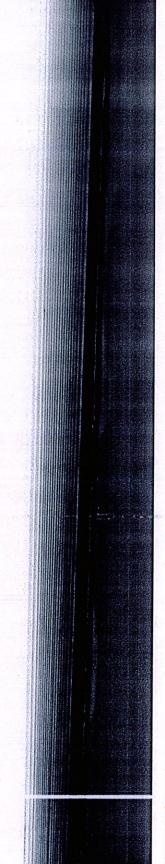
Pepper plants infested with R. similis express through above ground symptoms like foliar yellowing, defoliation, lack of vigour and retardation in growth. van der Vecht (1950) correlated the occurrence of 'yellows disease' characterized by foliar yellowing with R. similis infestation in Bangka, Indonesia. Similarly in India also a high correlation was noticed between the foliar yellowing and intestation with

R.simils in pepper plantations (Ramana et al. 1987). Freire (1982) found that R. similis predisposed pepper seedlings to a weak pathogenic isolate of F. solani and root rot was more severe.

Pathogenicity and economic significance

Pathogenicity tests conducted under greenhouse conditions established the pathogenic effect of the nematode on pepper. Mustika (1990) observed yellow leaves with stiff droop in the plants inoculated with R.similis. However, Venkitesan and Setty (1977) could not observe yellowing of leaves under artificial inoculations, but the nematodes caused considerable reduction in all growth parameters like height, number of leaves and nodes, leaf area, dry weight of shoot and root and the severity increased with the increase in the inoculum level in these tests. Further, plants inoculated with higher nematode levels lacked feeder roots and the roots present were black and almost decayed. The pathogenic tests of R. similis on pepper, conducted under simulated field conditions on adult plants, showed that R. similis is highly pathogenic to pepper (Mohandas and Ramana 1991). Nematode infestations caused foliar yellowing, defoliation and intensity of these symptoms increased with the increase in the inoculum level and with time, typical of slow decline disease. All growth characters namely height (0.2-20%), number of primary shoots (13-57%), dry weight of shoot (25-61%), leaf (25-77%), root (33.5-82%) and yield (0.3-59%) were significantly reduced in the pepper plants inoculated with R. similis @ 100 to 10,000 nematodes/plant (Mohandas and Ramana 1991). The burrowing nematodes could alter the nutrient balance of the infested plants. Leaf Ca, N, and Mg increased in plants infested by R. similis, M. incognita and F. solani and this is attributed to be due to reduction in leaf size and alteration in the overall nutrient balance within the plant system (Mustika 1990).

R. similis populations from arecanut and pepper in Kerala complete their life cycle in 25-30 days at temperature of 21-23° C (Koshy 1986, Geetha 1991). This nematode species has two morphologically indistinguishable races, 'banana race' infecting banana but not citrus and 'citrus race' which infects both citrus and banana (Du Charme and Birchfield 1956). The 'banana race' is identified from most of the banana growing regions of the world, whereas 'citrus race' is known to occur in Florida, USA. The banana and citrus races have chromosome numbers of n = 4 and n = 5, respectively (Huettel and Dickson 1981). However, a number of biological differences, including karyotypes, isozyme and pheromone mediated behaviour were detected between these two races (Huettel 1982, Huettel and Dickson 1981, Huettel et al. 1982, 1983a & b). The lack of common isozyme bands at seven loci indicated that gene flow does not occur between these two races and therefore reproductive isolation is complete, even though they are sympatric. R. similis population of pepper from Indonesia and Kerala, India have haploid chromosomes of n = 4 (Huettel 1982, Huettel et al. 1984a, Koshy 1986). Further, pepper race from Kerala did not infest 16 varieties of citrus and it was confirmed as banana race (Ramana 1992). Huettel et al. (1984b) separated citrus race and described it as a new species, Radophoius citrophilus.



The Pepper Nematode (Trophotylenchulus piperis)

The pepper nematode is a sedentary, semi-endoparasite, belonging to the family Tylenchulidae. The nematode was described from the roots of pepper in Kerala, India (Mohandas et al. 1985). The adult females on the root surface are covered with hard dark brown cases. This nematode is widely prevalent in all major pepper growing areas in Kerala and Karnataka (Ramana and Mohandas 1987, 1989, Ramana and Eapen 1997) but so far not reported from any other pepper growing country.

The second stage juvenile of the nematode is infective. After penetration, the head portion only is embedded in the root tissues with the distal portion of the body remaining outside the root. The nematode starts feeding once it takes a position on the root and development of the nematode progresses. The protective covering (case) starts developing at 30-40 days after infection. This developing cover looks like jelly initially which becomes hard and the whole nematode is covered with the case in about 40-50 days after infection. Once the case is fully formed, the nematode lays about 25-35 eggs. Each case contains eggs in different stages of development, second stage juveniles and an adult female. At 50-55 days, most of the cases are empty indicating the second stage juveniles had emerged out of the cases for further infesting the host roots (Sundararaju et al. 1995). The nematode is able to infest and develop on thick main roots as well as on tender fibrous roots, though more of them occur in fibrous roots (IISR 1995, Ramana and Eapen 1997). The studies conducted at Indian Institute of Spices Research have shown that the cases have developed from the secretions of excretory glands in the developing nematodes. The nematode penetrates only 3-4 cell layers deep in the roots and necrotic lesions develop at the feeding sites. The infested roots also show shrinkage and drying at the site of infection. Besides pepper, Glyricidia sepium and Artocarpus heterophyllus, which are used as live standards for trailing pepper, are also hosts of this nematode (Ramana and Eapen 1997).

SLOW DECLINE DISEASE

Slow decline disease of pepper, otherwise known as 'yellows' disease is a major problem for pepper cultivation that has upset the economy of Bangka islands in Indonesia where millions of pepper plants died during 1950's (Christie 1959). About 30 per cent of the plants are damaged by this disease in Guyana (Biessar 1969). The disease is prevalent in most pepper growing countries like India (Nambiar and Sarma 1977), Malaysia (Kueh 1979, 1990), Brazil (Sharma and Loof 1974, Ichinohe 1975), Thailand (Sher et al. 1969, Bridge 1978). The disease, was first observed in Bangka (van der Vecht 1950), spread to other areas in Indonesia and in the islands of Bangka and Belantung, almost all plantations were affected. The annual loss of production was estimated to be up to 32 per cent (Sitepu and Kasim 1991). Yellows disease is one of the reasons for low productivity of pepper in Indonesia (Mustika 1990). This disease is also widely prevalent in Johore and Sarawak in Malaysia, and yield losses range from 25–90 per cent and the life span of the vine is reduced to

8–10 years (Varughese and Anuar 1992). In the Kampot region of Cambodia, the pepper industry suffered heavily due to a nematode-fungal complex disease and pepper population was reduced from 2.5 millions in 1942 to 0.5 millions in 1953 (Hubert 1957). Crop loss estimates due to this disease in India are not available though Menon (1949) reported about 10 per cent mortality of pepper plants in Kerala. Wahid and Sitepu (1987) reported that annual loss may reach up to 10–32 per cent as almost all plantations in Indonesia are affected by this disease. According to them the symptoms of this disease are foliar yellowing and leaf fall in both young and older plants. The yellowing of leaf starts from the bottom of the plant and spreads to the top, covering the whole plant at later stages of the disease. They are also of the opinion that the disease is mainly due to plant parasitic nematodes, *R. similis*, *M. incognita* and the fungus *Fusarium* sp. combined with agronomic disorders.

Slow decline is a debilitating disease over a period of time. The above ground symptoms of the disease are yellowing of leaves, defoliation, die-back, loss in vigour and productivity, leading to slow death (Fig. 5.2.1). On roots, nematode infestation results in the formation of galls due to root-knot nematodes, necrotic lesions and rotting caused by *R. similis* resulting in total loss of feeder roots (Fig. 5.2.2 & 5.2.3). Infested plants sometimes recover with the onset of monsoon when the plants put forth new roots and leaves. However, the plants succumb to the disease as the root regeneration cannot compensate the root loss due to nematode damage (Mohandas and Ramana 1987).

Barber (Ridley 1912) observed that in pepper plantations in Wynad, Kerala, many pepper plants died due to nematode disease after a period of phenomenal success in pepper cultivation. He also found a series of tumours (root galls) in plant tissues due to eelworms (Heterodera radicicola = Meloidogyne incognita). Butler (1906) in his investigation on the disease in Wynad, observed drooping of leaves as the first aerial symptom of the disease followed by yellowing and leaf shedding. The diseased plants could not be recovered once leaf drooping commenced and the disease came to be known as 'slow wilt'.

Similarly during 1930s in the Indonesian island, Bangka, pepper plants with foliar yellowing and defoliation were observed and the disease was termed as 'yellows' by Bregman (1940). Further detailed investigation on the disease by van der Vecht (1950), showed that plant parasitic nematode, *R. similis* is responsible for the disease. Now this nematode disease of pepper with characteristic symptoms of foliar yellowing and defoliation is known as 'slow decline' for the sake of uniform terminology.

The disease is primarily attributed to R. similis or Meloidogyne spp. in all the pepper growing countries (Christie 1957, Ting 1975, Ichinohe 1976, Nambiar and Sarma 1977, Venkitesan and Setty 1977, Mustika 1978, Ramana et al. 1987, 1992). However, there are different opinions on the etiology of the disease. Hubert (1957) and Bridge (1978) were of the view that though R. similis is primarily responsible for the disease, combined infestation of Fusarium solani along with the nematode results in the yellows disease. Infestation by the nematode and fungus together enhanced the root damage and severity of foliar yellowing (Lopes and Lordello 1979, Freire 1982, Hamada et al. 1985, Sheela and Venkitesan 1990). Mustika

(1990), in a pot culture test, observed that R. similis alone can cause yellowing of leaves with stiff droop but these symptoms were more severe when plants were inoculated with R. similis along with M. incognita or F. solani thus indicating that pepper is more affected by R. similis than M. incognita causing more root damage and thereby severe growth inhibition. Pathogenicity trials conducted in micro plots under simulated field conditions in India confirmed that R. similis causes more damage to pepper than M. incognita (Mohandas and Ramana 1991). The possible role of Fusarium sp. in the disease complex is not elucidated in the large scale field trials conducted in India (Ramana et al. 1992). It was also reported that both R. similis and M. incognita were mutually suppressive under greenhouse experiments (Eisenback 1985). Pythium sp. (Nambiar and Sarma 1977, Varughese and Anuar 1992) and Rhizoctonia bataticola (Nambiar and Sarma 1977) were also reported in association with the roots of diseased pepper plants. However, no further attempts were made to understand their role in the disease incidence.

Phytophthora sp. is a major fungal pathogen of pepper and causes the foot rot disease. Winoto (1972) observed that plants infested with root-knot nematodes were more susceptible to Phytophthora infection, but the relation between, M. javanica infestation and the occurrence of foot rot caused by P. palmivora (the species infecting pepper is now known as P. capsici) could not be established (Holliday and Mowat 1963). In India, P. capsici is a major constraint in black pepper cultivation. Roots of diseased plants show infestation of R. similis, M. incognita and P. capsici either alone or in combination and there is no spatial segregation under field condition. Feeder root damage caused by P. capsici was reported to lead to slow decline symptoms (Anandaraj et al. 1991a, 1996b,c; Ramana et al. 1992). Their role in the disease development was assessed under simulated field condition in micro plots and the results showed that all of them are highly pathogenic. The plants inoculated with either R. similis or P. capsici alone or together showed foliar yellowing and defoliation and root rotting, typical of slow decline disease. Further, these trials showed that even though enough soil moisture and nutrients were available, the plants exhibited declining symptoms due to damage of feeder roots. When the fungal infection reaches the collar region through roots, it results in foot rot disease (Anandaraj et al. 1991a, 1996b). In another study by the same authors, P. capsici and R. similis together caused rapid damage to root system leading to faster disease development. The damage caused by M. incognita alone is less but in combination with R. similis and P. capsici the damage is synergistic. So an integrated approach to check all the three pathogens is essential for the management of slow decline disease (Anandaraj et al. 1996c).

MANAGEMENT OF NEMATODE DISEASES

Soil borne diseases, in general, are elusive to management as the chemicals or other agents employed for the control do not reach in sufficient quantity/concentration to the target pathogen/pest in the soil. In nematode diseases, the visible symptoms are noticeable only after severe damage to roots. The symptoms can be confused with

those caused by soil factors, nutritional deficiency or drought and they are not exclusively diagnostic of nematode damage. It is well known that nematodes cannot be eliminated from agricultural soils in any given situation particularly in perennial cropping systems. Hence, the concept "live with the nematodes" by managing their populations below economic threshold levels is appropriate. In India, pepper is cultivated in homestead gardens along with a variety of crops. This type of agroecosystem adds further dimensions to the nematode management. The roots weakened by nematode infestation are prone to attack by otherwise weak pathogens particularly fungi like *Fusarium* spp. Frequent monitoring of nematode populations and diagnostic services are essential to bring the nematode population down to non-injurious levels by adopting appropriate management technology.

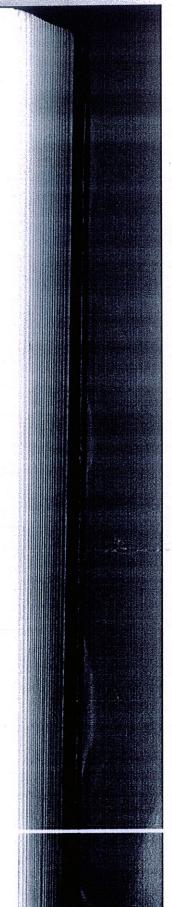
The nematode management programmes should be in line with the present day concepts of organic farming, eco-friendly management of pests and diseases and demand for pesticide residue free produce. Considering the complex nature of slow decline disease, the aim should be to develop an integrated disease management schedule to reduce the nematode population and associated fungal pathogens below threshold levels and providing favourable conditions for growth of pepper. Cultural practices, host resistance and biocontrol agents can be profitably used with minimal use of chemicals (Ramana and Eapen 1995).

Cultural Methods

Nematode management through cultural methods are generally less expensive, but have to be modified and adopted depending on the situation. They are primarily preventive and aim at suppressing the nematode population by providing favourable conditions for the growth of the plants and associated rhizosphere microorganisms antagonistic to pathogens. Crop rotation, mulching with organics, soil amendments, flooding, fallowing, phytosanitation, planting nematode free plants are some of the cultural practices recommended for nematode management. Pepper being a perennial crop, many of these practices cannot be adopted, and many require further modification.

In nurseries

Nurseries are the main source for the spread of insect pests and pathogens. The conditions provided in the nurseries for plant growth are also highly congenial for nematode multiplication. Phytosanitation and hygiene in nurseries are essential in nematode disease control. Pepper is propagated through stem cuttings. Runner shoots or stolons from the base of the vine are also used as planting material. Three node cuttings are planted in polythene bags containing nursery mixture for rooting. In the rapid multiplication system the planting materials are generated from single nodes rooted on bamboo splits (NRCS 1993). Planting materials are produced throughout the year in this method and are retained in the nurseries for longer periods before planting in the fields than in the conventional method. The nodal roots of runner shoots collected from field should be removed before planting in



nursery. Planting rooted cuttings infested with nematodes leads to slow decline disease in course of time. Denematization of nursery mixture either through solarization or fumigation with chemicals is highly effective in reducing the initial

nematode load and for production of healthy rooted cuttings.

Incorporation of biocontrol agents like vesicular arbuscular mycorrhizal fungi (VAM) suppressive to nematodes and fungal pathogens to solarized soil mixture is recommended (Anandaraj and Sarma 1994a,b, Sarma et al. 1996). VAM fungi also enhance growth and vigour of pepper rooted cuttings besides suppressing nematodes and fungi (Anandaraj et al. 1996a). Trichoderma species in combination with soil solarization suppresses M. incognita in cardamom nurseries (Eapen 1995). These fungi suppressed the hatching of M. incognita eggs from pepper in laboratory assays (IISR 1995). Paecilomyces lilacinus also suppress the root knot nematodes in cardamom and pepper (Eapen and Venugopal 1995, Ramana 1994). Hence, incorporation of these fungi in the nursery mixture would be advantageous.

In plantations

Phytosanitation, nutrient management, mulching, proper drainage, addition of soil amendments like oil cakes would help to suppress the nematode populations in pepper plantations. To reduce the source of inoculum and the spread of the pathogens, destruction of the diseased plants along with root mass is very essential. Barber (cited by Ridley 1912) suggested that all the galled roots and underground plant parts should be collected and burned and on no account replanting should be done in these pits for many years. DeWaard (1979) reported that application of fertilizers at 400 kg N, 180 kg P, 480 kg K, 425 kg Ca and 112 kg Mg/ha/year effectively controlled the disease in Bangka, Indonesia. Mustika (1990) also was of the opinion that the disease could be controlled by application of fertilizers along with mulch and chemicals.

Green manuring with legumes is a traditional practice with Indian farmers to improve the organic matter and nitrogen status of the soils. Addition of organic matter (mulch) to the soil increases water holding capacity of the soil, besides enhancing the fertility status leading to improved growth of plants that can withstand nematode attack. Addition of organic matter and soil amendments enhances the activity of natural enemies particularly the antagonistic fungi suppressive to plant parasitic nematodes. The chemicals released during organic decomposition like azadirichtin (neem), ricin (castor), ammonia, nitrates, hydrogen sulphide, organic acids are all toxic to nematodes (Stirling 1991). DeWaard (1979) observed that the pepper yellows appeared early in the plots without mulching compared to the plots applied with mulch. Mulching with green manure plants reduced root knot nematode population and the incidence of the disease in pepper plantations in Indonesia and Amazonian region (Wahid 1976, deWaard 1979, Ichinohe 1980, 1985). Hubert (1957) was of the opinion that the nematode-fungal complex disease can be controlled by mulching the plants with Eupatorium. The effect of mulching pepper plants with Chromolaena odorata (Eupatorium odoratum) in controlling the nematodes and vellows disease in Cambodia was also reported (Litzenberger and Lip

1961). However, this was found not effective in pot culture tests in India (Ramana 1992). Barber (cited by Ridley 1912) suggested planting of goat weed (Ageratum conyzoides) in the abandoned pits to get rid of the root-knot nematodes as these weeds attract the nematodes and after a time the weeds can be pulled out and burnt. By repeating this process the nematodes may be eliminated. However, this method involves the risk of increasing the nematode populations, if the weeds are not

properly removed and destroyed in time.

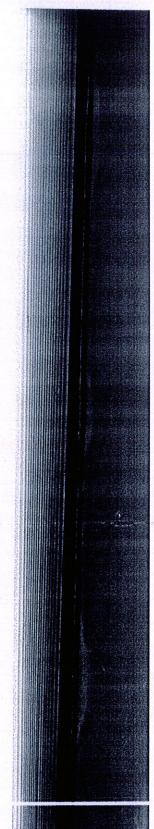
Addition of chopped leaves of Glyricidia maculata (10g/kg soil) under pot culture tests, reduced R. similis populations in pepper and increased plant growth (Jasy and Koshy 1992). Kueh and Sim (1992) suggested in addition to adequate fertilization, growing a leguminous cover crop in pepper plantation for improving the growth and vigour of plants as increased root regeneration compensated the root damage caused by nematodes. Growing non-host plants like Macroptillium atropurpureum, Centrosema pubescens, Clitoria ternatea, Cajanas cajan, Arachis hypogea and Crotalaria sp. as cover crops in pepper plantations in Brazil reduced M.incognita populations. Derris elliptica and Indigofera hirsuta are also detrimental to nematode multiplication (Mustika1991). Nematode antagonistic plants such as Tagetes patula is also useful in suppressing root knot nematode populations in black pepper (Mustika 1991, Ramana 1992). However, the feasibility of cultivating such crops in pepper plantations is doubtful.

Addition of organic amendments like organic manures, crop residues, green manures, oilseed cakes, plant extracts etc. improves the soil texture and also enhances the growth of useful microorganisms suppressive to pests and pathogens. Certain oilseed cakes like neem, karanj (Pongamia glabra), mahua (Madhuca indica), castor (Ricinus communis), mustard (Brassica spp.) are commonly used in India (Mishra and Majumdar 1995). Nematicidal properties of neem oil cake is well known and it is attributed to several chemicals occurring in neem such as nimbidin, thionemone, azadirachtin, nimbin, nimbidic acid, etc. However, not much information is available on the effectiveness of these oil cakes for nematode management in pepper. Application of neem cake @ 2 kg/vine twice a year along with fertilizers was found highly effective in suppressing the populations of M.incognita, but its effect was not that encouraging against R.similis (Ramana et al.

1992).

Plant Resistance

Plant resistance is one of the potential, probably the most economical and practical solution. But resistance to nematode pests are not available in many crop plants. Polygenic-horizontal resistance to nematodes is more desirable than vertical monogenic resistance (Fassuliotis and Bhatt 1982). Existence of physiological races or pathotypes in nematodes particularly in *Meloidogyne* species is another factor to be considered in breeding programmes for resistance. The present trend is to evolve varieties tolerant to nematodes rather than aiming at absolute resistance. The tolerant varieties have the ability to grow and yield in the presence of nematodes. In pepper no serious efforts were made in these lines except screening the available





cultivars for their reaction to nematodes. Degree of resistance/susceptibility of various cultivars of pepper to nematodes has been reported from India, Malaysia and Sri Lanka.

In Sarawak, Kueh (1986) found cv. Uthirancotta as most susceptible, while cultivars like Balancotta, Belantung, Cheriakaniakkadan, Jambi and Kalluvally are less susceptible to root knot nematodes under field conditions. Among the cultivars tested by Mustika (1990, 1991), cv. Kuching appeared to be tolerant to M. incognita and R. similis compared to Kalluvally, Jambi and Cunuk. In India, none of the cultivars tested was found resistant to M. incognita (Koshy and Sundararaju 1979, Jacob and Kuriyan 1979a, Ramana and Mohandas 1986) and to R. similis (Venkitesan and Setty 1978, Ramana et al. 1987b). However, Ramana and Mohandas (1986) reported that cv. Pournami, a selection from the germplasm, is tolerant to M. incognita and field evaluation trials also showed that this cultivar supported less population of nematodes compared to other susceptible cultivars. This is having an average yield of 4.7 kg/plant and a potential yield of 10.8 kg/plant. (Ravindran et al. 1992). Paulus et al. (1993) screened 45 pepper lines under greenhouse conditions and reported that cultivars like Balancotta, Jambi and Hybrid-10 (F1 hybrid of Balancotta and Kuching) were less susceptible to M. incognita and none was resistant to nematode infestation. Though no resistance was reported to R. similis from India, Indonesia and Malaysia, in Sri Lanka a variety (Pw 14) was reported resistant to this nematode (Gnanapragasam 1989).

Sources of resistance to plant parasitic nematodes in wild and related species of crop plants is also important which can be incorporated to cultivated species. Attempts made in this direction showed *Piper colubrinum* and *P. aduncum* as highly resistant to *M. incognita* (Ramana and Mohandas 1986, Paulus *et al.* 1993). Wild species, *P. hymenophyllum* and *P. attenuatum*, recorded least root reduction (less than 30%) and minimum nematode multiplication on testing with *R. similis* (Venkitesan and Setty 1978). *P. colubrinum* was immune to *R. similis* also (Ramana *et al.* 1994). Crosses between *P. nigrum* and *P. colubrinum* have not succeeded so far. Hence efforts should be made to identify the gene(s) responsible for the resistance in these species and transfer them to cultivated species through biotechnological approaches to solve the major nematode problem in pepper.

Biological Control

According to Sewell (1965) biological control is "the induced or natural, direct or indirect limitations of a harmful organism or its effects by another organism or group of organisms". A variety of microorganisms, inhabit soil, some of which are either predatory or antagonistic to plant parasitic nematodes. Perennial cropping systems are sources of potentially useful antagonists of nematodes and they possibly are providing natural suppression of nematodes to some extent (Stirling and West 1991). However, the efficacy of biological control as the sole means of nematode management is still debatable and the present consensus is that this approach can be

an integral part of the overall management schedule (Ramana et al. 1993). Though several organisms possess antagonistic potential against nematodes, a few fungi and bacteria only are generally considered as biocontrol agents. Very limited research efforts have gone into this area for pepper nematode management. Eapen and Ramana (1996) reviewed the status of biological control of plant parasitic nematodes of spice crops.

Paecilomyces lilacinus

This is an opportunistic fungus present in many types of agricultural soils and has been tested for its potential against root knot nematodes and cyst nematodes of several crops. Sedentary plant parasitic nematodes such as root knot and cyst nematodes are more vulnerable to attack by this fungus. On contact with the egg masses of nematodes, it colonizes and grows rapidly on the eggs that are in the early stages of embryonic development. The chitinolytic enzymes produced by this fungus help in penetration of eggs. Apart from the embryonic disruption, it can also cause suppression through production of diffusible toxic metabolites. Jatala (1986) in his review stated that this fungus has proved its efficacy in suppressing root knot nematodes in various crops. This has the ability to readily colonize the reproductive structures of the nematodes, and it can survive under host free conditions in the soil and is easy to culture in the laboratory.

Inoculation of *P. lilacinus* to rooted cuttings/seedlings of pepper significantly suppressed *M.incognita* populations as evidenced by less damage to roots and increase in total root mass (Friere and Bridge 1985d, Ramana 1994, Sosamma and Koshy 1995). The efficacy was low against *R. similis* (Geetha 1991, Ramana 1994). Freire and Bridge (1985d) also reported from Brazil that this fungus parasitized root knot nematode eggs and suppressed the infestation on pepper. These studies suggest a wider scope for using this fungus as a biocontrol agent against root knot nematode in pepper, but requires large scale field evaluation before inclusion as a component in the integrated nematode management schedule.

Trichoderma species

Trichoderma belongs to hypomycetous group and members of this genus are being used against several pathogenic fungi. Recently they were tested for suppression of root knot nematodes in spice crops particularly pepper and cardamom in India (Eapen and Ramana 1996). In preliminary in vitro tests, several isolates of Trichoderma harzianum, T. hamatum, T. viridae, T. aureoviridae, T. polysporium, T. longibracheatum, T. koningi, T. pseudokoningi and Gliocladium virens, though not colonized, destroyed the root knot nematodes collected from pepper by preventing their embryonic development. Culture filtrates of these fungi also showed nematicidal property (IISR 1995). Further, they are also effective in suppressing Phytophthora capsicii (Anandaraj and Sarma 1994b, Sarma et al. 1996). Hence they are useful in checking the slow decline disease particularly in India where nematodes and P. capsici are involved in the disease.

Verticillium chlamydosporium

This fungus is a biocontrol agent for root knot and cyst nematodes (Kerry 1990). It readily colonizes rhizosphere and rhizoplane, infects adult females and egg masses and reduces nematode multiplication by inhibiting egg hatching. Freire and Bridge (1985d) reported from Brazil that root knot nematode eggs were parasitized by V. chlamydosporium. The occurrence of this fungus in pepper plantations of India was reported for the first time in association with Trophotylenchulus piperis (Sreeja et al. 1996). This suppressed hatching of M. incognita eggs by 41.4 per cent within 5 days and the egg masses were heavily colonized by the fungus in an in vitro test. This fungus needs further evaluation as a bicontrol agent for use in pepper.

Vesicular arbuscular mycorrhizal (VAM) fungi

VAM fungi are obligate dependants on plants for their nourishment. Their symbiotic association with roots of crop plants increase the plants' ability to absorb water and nutrients, particularly phosphorus from soil. This increases the tolerance to nematode damage due to higher P status of the plants or by the competitive or antagonistic effect (Smith 1987). The beneficial effects of VAM fungi in several crops are now well established. Glomus fasciculatum, G. microcarpum and Gigaspora gigantea are associated with the roots of pepper (Sarma et al. 1996). The occurrence of VAM fungi on pepper roots was reported by Manjunath and Bagyaraj (1982). Addition of these fungi to the nursery mixture enhanced the growth and root production in pepper rooted cuttings (Anandaraj and Sarma 1994a). In pot culture trials, significant increase in growth and reduction in M. incognita infestation and their multiplication were observed when pepper plants were inoculated with VAM fungi, Glomus mossae, G. fasciculatum, G. etunicatum, Acaulospora laevis and Gigaspora margarita (Anandaraj et al. 1991b, Sivaprasad et al. 1990, 1992). G. fasciculatum was also reported to suppress R. similis and P. capsici in black pepper (NRCS 1991). These fungi are obligates and need the host plants for their multiplication, so alternative methods for mass production have to be explored.

Pasteuria penetrans

Bacteria as biological control agents against plant parasitic nematodes have not been fully investigated in pepper except for a couple of studies. *Pasteuria penetrans* is an obligate parasite of some nematode species particularly the second stage juveniles of root knot nematodes. It infects the nematode by direct penetration through the cuticle by germinating spores sticking to the body surface of the nematodes. Studies on its efficacy for suppressing the nematodes showed that inoculation with this bacterium suppressed *M. incognita* and *R. similis* infestations and their multiplication. It also improved the growth of the plants (Geetha 1991, Sosamma and Koshy 1995). This bacterium colonized heavily the second stage juveniles of *M. mcognita* in laboratory bioassays (IISR 1996).

Other Bacteria

Bacillus pumilis, B. macerans and B. circulans also suppressed M. incognita populations on pepper and also increased the growth of the plants (Sheela et al. 1993).

Attempts were also made to test the efficacy of fluorescent pseudomonads especially *Pseudomonas fluorescens* on root knot nematodes. Several isolates of the bacterium showed inhibitory action on the multiplication of *M. incognita* in pot culture studies (Eapen et al. 1997).

Biological control of plant parasitic nematodes in black pepper is only in the initial stages. Many of these potential biocontrol agents have to be evaluated under field conditions before adoption. Methods have to be developed for large scale multiplication and delivery systems particularly for highly potential microorganisms like VAM fungi, *P. penetrans* etc. Attempts have to be made in potentiating these biocontrol agents through biotechnological techniques.

Chemical Control

Nematicides are chemicals used to kill or stall the activity of nematodes and consist of two groups, soil fumigants and systemic nematicides. Most of the nematicides are applied to soil and are translocated from roots to shoots. Use of nematicides was a major component in the management programmes of plant parasitic nematodes in several crops before the importance of the biological control was realized. However, high cost and environmental hazards due to their indiscriminate use are limiting factors in chemical control. But no effective alternative methods are available for large scale adoption so far. Fumigants like DD, Methyl Bromide, Ethylene di Bromide, etc. are highly effective in eliminating nematode populations in the nursery mixture.

Various nematicides like phenamiphos (Nambiar and Sarma 1980), aldicarbsulphone (Venkitesan and Setty 1979), phorate, DBCP (Venkitesan and Charles 1980) were found effective in controlling nematodes. The granular nematicides namely, Temik 10 G (aldicarb), Thimet 10 G (phorate), Furadan 3 G (carbofuran), applied @ 3 g. a.i./plant, twice a year significantly reduced the populations of M. incognita and R. similis and improved the health of the plants (Mohandas and Ramana 1987, Ramana et al. 1994). In Malaysia carbofuran @ 114 g/plant (Kueh and Teo 1978), phenamiphos and oxamyl (Kueh 1979) were found effective. In Indonesia, Shell DD, Vapam EC, Nemagon 75 EC, Temik 10 G, Furadan 3 G, Nemacur 5 G, Mocap 10 G, Hostathion 5 G, Dasanit 5 G and Basudin 60 EC were all effective in controlling the nematodes of pepper (Mustika and Zainuddin 1978). Leong (1986) reported that phenamiphos was the most effective nematicide in controlling nematodes in Sarawak. Similarly, in Brazil, Ichinohe (1980) found application of Temik 10 G @ 12.5 g or Furadan 5 G @ 50 g/plant twice a year reduced nematode population and improved the growth of the plants. However, in recent years many of these chemicals have been banned for use in agricultural crops due to several reasons. Demand for "pesticide residue free" produce also limits the usage of these chemicals. Hence, research should continue to identify safer chemicals with high potential for nematode control.

CONCLUSIONS

Plant parasitic nematodes, particularly Radopholus similis and Meloidogyne spp., are major economic constraints in pepper cultivation in all major pepper growing countries. They either on their own or in association with fungi like Phytophthora sp. or Fusarium spp. cause severe damage to roots leading to slow decline disease. Though a number of chemicals were found effective to manage nematode damage, they are seldom used due to several reasons. An integrated approach with priority to plant resistance and biocontrol agents has to be formulated. Several biocontrol agents suppressive to plant parasitic nematodes have been identified, but their potentials under field conditions on a large scale have to be worked out. Biotechnological approaches for incorporating resistance from wild species to the cultivated and potentiating the biocontrol agents would help in the management of plant parasitic nematodes and in increasing the production and productivity of pepper.

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