MANAGEMENT OF ROOT KNOT NEMATODE IN THIPPALI (*Piper longum* L.)

By

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THESIS

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requirement for the degree of

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DECLARATION

I, Seena R Subhagan (2004-11-15) hereby declare that this thesis entitled **'Management of root knot nematode in thippali (*Piper longum* L.)'** is a bonafide record of research work done by me during the course of research and this thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title of any other University or Society.

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Certified that this thesis, entitled **'Management of root knot nematode in thippali (*Piper longum* L.)'** is a record of research work done independently by **Ms. Seena R Subhagan** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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Seena R Subhagan

Affectionately dedicated to

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&

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

INTRODUCTION

1. INTRODUCTION

India, a treasure house of medicinal plants, has one of the oldest, richest and diverse cultural traditions associated with the use of medicinal plants. In recent years there has been an increased interest in the cultivation of medicinal and aromatic plants. Improvement in agronomic practices and the use of high yielding superior varieties have necessitated the application of plant protection measures, which ensured better yield through the management of pests and diseases especially plant parasitic nematodes which are serious threat to the cultivation of medicinal plants.

Thippali, *Piper longum* is an important medicinal plant belonging to the family Piperaceae. It is the most important species of genus *Piper* after black pepper (*Piper nigrum L.*) and betel vine (*Piper betle L.*) and perhaps the first pepper to reach the Mediterranean and was once regarded superior to black pepper by Greeks and Romans. It is a slender aromatic climber with perennial woody roots and is distributed along the watercourses and over 'shoal' lands in Assam, Kerala and Karnataka.

The unripe female spikes and to a smaller extent, root and thick basal stems constitute the commercial produce. The medicinal use of dry spikes and roots of *Piper longum* in Ayurvedic system of medicine have been described by many workers (Kirtikar and Basu, 1935; Suseelappan, 1991; Hussain *et al*., 1992 and Sivarajan and Indira, 1995). In Ayurveda and Unani medicines, it is used against bronchial asthma, insomnia, jaundice and viral hepatitis. The infusions made from fruits are used as a carminative, a stimulant, alterative and an expectorant and is administered for chronic bronchitis and asthma. In Ayurveda, black pepper, long pepper and ginger are collectively called as '*trikatu*'. The capacity of '*trikatu'* to increase the bioavailability of other drugs was reported by Atal *et al.* (1981), Manavalan (1990) and Johri and Zutshi (1992).

Meloidogyne spp. commonly known as root-knot nematodes, are considered as the most important enemies causing wrecking havoc to agricultural crops in developing nations. They are sedentary endoparasites and produce root knots or galls of various sizes and shapes on wide varieties of plants. The four most common species in the world are *M. incognita, M. javanica, M. arenaria* and *M. hapla.* At present 99 *Meloidogyne* spp. have been reported world over. Among these, seventeen species have been reported from India and over 350 plant species have been recorded as mild or severe hosts of *Meloidogyne* spp.

Root knot nematodes cause aerial symptoms like yellowing, chlorosis, wilting and reduction in growth and underground symptoms like gall formation. The broad-leaved plants may virtually show daytime wilting. Galls are seen through the entire length of roots and may contain many nematodes and as a result the growth of roots are arrested. Formation of galls on roots is the main symptom by which the presence of root knot nematode can be diagnosed.(Plate 1)

The International *Meloidogyne* Project (IMP) had identified four races of *M. incognita* and two races of *M. arenaria*. Races 1, 2 and 3 (out of four races) of *M. incognita* and race 2 (out of two races) of *M. arenaria* are reported to be widely distributed in continent and subcontinents of the world (Sasser, 1982). Among the different *Meloidogyne* spp., *M. arenaria* is widely distributed in various parts of India and it has been reported from Haryana, Gujarat, Bihar, Himachal Pradesh, Uttar Pradesh, Punjab, Delhi, Tamil Nadu and Maharashtra.

A study of plant parasitic nematodes associated with medicinal plants in Kerala revealed the occurrence of root knot, burrowing and spiral nematodes in the rhizosphere of the majority of medicinal plants grown in eight districts viz. Thiruvananthapuram, Kollam, Kottayam, Pathanamthitta, Ernakulam, Thrissur, Malappuram and Kozhikode (Sheela *et al*., 1998). Recently an increase in the root knot nematode attack was observed in the thippali growing plots in the medicinal plants garden of College of Horticulture, Vellanikkara, but the

Thippali (*Piper longum* L.)

Plate 1. Root knots produced by *Meloidogyne arenaria*

nematode species had not been identified. Though nematodes are important pest elements, their pest potential is being underestimated.

The losses caused by nematodes are enormous which necessitates efficient control measures. At present the management practices of nematodes are mainly based on synthetic chemicals like nematicides, which are very costly and have several side effects like residue problems, resurgence of nematode population, environmental pollution and health hazards. Thippali being a medicinal plant, the use of chemical pesticides for nematode management is not advisable. Because of the concealed feeding habits of nematodes, bioagents and organic amendments can substantially suppress its population. In this context, an attempt was made to compare the efficacy of bioagents and organic amendments, which are considered to be ecofriendly with that of carbofuran, the commonly used pesticide for nematode management. The objectives of the present study were

1. To identify the species of root knot nematode infesting thippali
2. To evaluate the effect of organic amendments, bioagents and a chemical for the management of root knot nematode infesting thippali.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

The root knot nematode, *Meloidogyne arenaria* (Neal, 1889) Chitwood, 1949 is an obligate endoparasite having a wide host range. Many workers have reported infestation of *M. incognita* on a number of medicinal plants, but there are no reports on the attack of *M. arenaria* on medicinal plants in Kerala. A study of phytonematodes associated with medicinal plants in Kerala revealed the occurrence of root knot nematode in the rhizosphere of majority of medicinal plants grown in eight districts viz. Thiruvananthapuram, Kollam, Kottayam, Pathanamthitta, Ernakulam, Thrissur, Malappuram and Kozhikode (Sheela *et al*., 1998).

2.1 PEST STATUS

In India, four major species of root knot nematode viz. *Meloidogyne incognita, M. javanica, M. arenaria* and *M. hapla* are known to be widely distributed, attacking a wide range of agricultural crops (Dasgupta and Gaur, 1986; Jain and Hasan 1995; Khan *et al.,* 1994). Sivakumar and Vadivelu (1997) reported presence of phytoparasitic nematodes in forty-six medicinal plants surveyed in Nilgiris and Tamil Nadu.

A survey of nine districts of Bundelkand region of India to determine the presence of root knot nematodes associated with major food and fodder crops of the region revealed 36% incidence of nematode attack. Among the four species of root knot nematode, *M. incognita* was most frequent followed by *M. javanica* and *M. arenaria.* The root knot nematode *M. arenaria* was encountered in four districts i.e. Datia and Guna of Madhya Pradesh and Jalaum and Jhansi of Uttar Pradesh (Hasan and Jain, 1998).

Park *et al.* (1998) collected soil and root samples from the rhizosphere of 11 different medicinal plants to determine the incidence, density and identification of root knot nematode species associated with medicinal herbs in

Korea. Approximately 55 per cent of the medicinal herbs examined were found to be infested with root-knot nematodes. *M. hapla* recorded 43.3 per cent incidence in medicinal herbs, whereas *M. incognita* and *M. arenaria* showed 7.9 per cent and 3.7 per cent respectively.

Different levels of *M. incognita* population (200 to 1000 J_2) had significant adverse effects on the biometric characters and yield of kacholam (Rajani, 1998). A survey was conducted in Flue Cure Virginia (FCV)- tobacco growing areas of Karnataka, India, to assess the incidence and intensity of root-knot nematode and mapping the distribution of *Meloidogyne* sp. Four species, viz. *M. incognita*, *M. arenaria*, *M. javanica* and *M. thamesi* were detected on tobacco and the disease intensity (root knot index) varied from 2.0 to 3.1 in all tobacco growing tracts (Hussaini and Krisnamurthy, 2002).

Crop loss assessment studies indicated that *M. incognita* population at different levels (100, 1000, 10000) adversely affected the biometric characters and yield of *Plumbago rosea*. At the pathogenic level (100 J_2), the percentage reduction in plant height, number of leaves, branches, fresh weight of root, dry weight of shoot and weight of tubers were 14, 26, 20, 18, 28 and 36 per cent respectively over control (Kumar, 2004).

2.2 MANAGEMENT

2.2.1 **Use of plant products**

2.2.1.1 **Mulching**

Singh and Sitaramiah (1967) stated that addition of green leaves of *Azadirachta indica, Melia azadirachta, Cassia fistula, C. occidentalis, Crotalaria juncea, Sesbania aculeata* and saw dust to soil infested with *M. javanica* significantly reduced the incidence of root-knot nematode in okra and tomato in pot experiments.

Hameed (1970) studied the effects of incorporation of organic materials in soil, on the incidence of *Meloidogyne* spp in tomato. It was found that the addition of organic matter generally reduced the incidence of *Meloidogyne* spp. and the addition of *Chrysanthemum coronarium, M. azadirachta* and *Tagetes patula* had reduced the nematode substantially and increased plant growth. Hackney and Dickerson (1975) observed that, root population of *M. incognita* in tomato cultivated simultaneously with marigold and chrysanthemum was significantly lower than in tomato cultivated alone.

Several indigenous plants have been identified for their nematicidal action on root knot nematode. Studies conducted on the use of green leaves like *Calotropis sp*., *Eupatorium sp*., mango and cashew on bhindi, showed reduced root-knot nematode infestation and increased growth of plants (Kumar and Nair, 1976).Kaliram and Gupta (1982) found that combined effect of various treatments like application of chopped neem and datura leaves were significant in reducing the number of galls in the case of chickpea (*Cicer arietinum* L.).

Application of chopped castor leaves (40 g/kg soil) two weeks before transplanting of tomato effectively controlled *M. incognita* (Dutt and Bhatti, 1986). Mansoor *et al*. (1987) reported that incorporation of chopped shoots of latex bearing plants like *Ficus elastica* gave greatest reduction in nematode population, root knot development and showed significant improvement in plant growth.

Jasy and Koshy (1992) reported that chopped leaves of *Glyricidia maculata* 10g/kg soil as green manure was found to decrease the population of *Radopholus similis* and promote the growth of black pepper under pot conditions. Adverse effects of marigold (*Tagetes sp*.) on nematodes were reported by many workers (Dhangar *et al*., 1995; Govindaiah *et al*., 1991; Khan *et al*., 1971).

Application of calotropis leaves @ 80t/ha was found significantly better than neem and castor leaves and was proved as effective as carbofuran granules in reducing the nematode population in betel vine gardens (Subbarao *et al.*, 1996). Nematicidal efficacy of plant mulches against root knot nematode in mulberry was reported by Govindaiah *et al*. (1997).

Prasad *et al.* (1997) reported that chopped leaves of *Calotropis procera* at 10 q/ha, applied 15 days before sowing was the most effective in reducing *M. arenaria* and *Rotylenchulus reniformis* populations and increasing the yield of groundnut over control.

A study conducted by Khanna and Sharma (1998) revealed that application of leaves of *A. indica* and *T. patula* improved plant growth of tomato and reduced nematode count as well as gall index which were at par with that of nematicides (phorate and carbofuran). Application of chopped leaves of *Prosopis juliflora, Catharanthus roseus, Leucaena leucocephala, C. procera* and *A. indica* gave better biomass production than chemical treatment in case of cowpea. The chopped leaves increased the VAM spore production and colonization and reduced nematode population (Santhi and Sundarababu, 1998).

Kumar and Reddy (2001) reported that treatment with marigold waste in sunflower improved the plant growth better over other individual treatment.

2.2.1.2 **Botanical extracts**

It has been known from early days that plant extracts have adverse effects on plant parasitic nematodes. The antihelminthic properties of leaf, seed and flower extracts of various indigenous medicinal plants have been reported as early as 1955 by Singh *et al.*

Desai *et al.* (1973) worked on the nematicidal property of some plant extracts against a mixed population of larvae of *M. incognita* and *M. javanica*. Out of the 26 extracts, 13 were found effective against nematodes.

Plant extracts of *Ocimum basilicum, Asparagus racemosus, Argemone mexicana, Embelia ribis* and *Vinca rosea* (Desai *et al*., 1973) *Curcuma anadalonga* (Pillai *et al*., 1975) *Anona squamosa* and *Tamarindus indica* (Hussain and Masood, 1975) and *M. azadirachta* (Haseeb *et al*., 1978) were reported to be nematicidal against plant parasitic nematodes

Kaliram and Gupta (1980) conducted an experiment to test the efficiency of neem leaf extract against root knot nematode of chickpea. It was found that the best plant growth was obtained at the highest level (leaf extract prepared from 40g neem leaves per kg of soil), which was also comparable with the next lower level (30g neem leaves per kg of soil). There existed a positive correlation between the treatments and reduction in the number of galls.

Mahmood *et al.* (1982) tested different concentrations of leaf and seed extracts of 12 medicinal plants against *R. reniformis* and *M. incognita* and found that *Anagallis arvensis, Linum usitatissimum* and *Sida cordifolia* were highly toxic.

Root dip treatment of egg plant seedlings in margosa and marigold leaf extracts considerably reduced root knot development compared to the treatments with piperazine citrate, chinopodium oil and ground nut cake (Hussain *et al*., 1984). Leaf, stem and root extracts of *Parthenium hysterophorus* were tested for their nematicidal activity on the adults of *M. incognita* by Hasan and Jain (1984). They found 100% mortality in leaf extracts after 24hrs and 48hrs of exposure at 1: 50 concentration.

Mani *et al.* (1986) studied the nematicidal effect of root and leaf extracts of *T. erecta, Brassica campestris, V. rosea* and *A. indica* against citrus nematode

Tylenchulus semipenetrans. They found that juvenile mobility was decreased and neem showed 19.27% mobility at 5% dilution. Tiyagi *et al*. (1986) reported 100% mortality of *M. incognita* after 12hrs exposure to leaf extracts of *Cymbopogon flexuosus.*

Kumari *et al*. (1987) reported 67-100% mortality of *M. javanica* when treated with leaf, stem and bud extracts of *Datura stramonium, Ipomea carnea, T. patula* and *Lawsonia alba.* A study conducted by Subramaniyan and Selvaraj (1988) revealed that leaf extracts of *T. patula* killed or inactivated all the adults of *R. similis* after 4hrs in 1:1 and 1:5 dilutions.

Sasanelli and Vitro (1991) reported that the leaf extracts of *T. erecta* and *T. signata* had no nematicidal effect against the egg stages of *Globodera rostochinensis.* A study conducted by Sharma and Trivedi (1992) demonstrated that the root extracts of *Tagetes* were most nematicidal against the egg stages of *M. incognita* followed by that of *Ocimum sanctum, Artemesia absinthium, Aegle marmelos* and *Euphorbia hirta.*

The aqueous extracts prepared from different plant parts of *T. erecta* (cv. Crackjack) inhibited the egg hatching and larval penetration of *M. javanica* (Dhangar *et al*., 1996). A study on the effect of water soluble leaf extracts of 11 plant species on second stage juveniles and egg masses of *M. incognita, M. javanica* and *M. arenaria* showed that *T. erecta* and *Eucalyptus* at all dilutions were very effective in inhibiting egg hatch of all the three species. *Ageratum conyzoides* and *T. erecta* gave 90% larval mortality at 1:2 dilution (Hussaini *et al.,* 1996).

A study was conducted to screen 20 plant extracts for their efficacy against *R. similis* attacking banana and leaf extracts of *Piper longum* was found to have a high degree of nematicidal effect on the larvae of the nematode (Sreeja and Charles, 1998). Aqueous extracts of leaf, stem and roots of *T. erecta* have

been reported to be nematicidal against *M. incognita* by Mojumder and Mishra (1999).

2.2.2 **Use of neem cake and neem based formulation**

2.2.2.1 **Neem cake**

Singh and Sitaramiah (1966) obtained very significant results when the soil infested with nematodes were amended with oil cakes of margosa, castor and peanut three weeks before planting. The root galls were significantly reduced. They also found that plot experiments with 1600 lb/acre of any of these cakes resulted in better plant growth and reduction in root-knot incidence. Residual effect of these amendments checked root knot of tomato without any further amendment. Roots of plants in amended soils contained lesser number of eggs, larvae and mature females than roots of plants raised in unamended soils. Water extracts of soil amended with oil cakes @ 2 per cent inhibited liberation of egg masses and hatching of larvae.

Anti-nematode effect of neem cake may be attributed to nematotoxic phenolic compounds, which are released during its degradation (Alam *et al*., 1979), apart from its stimulatory effect on root growth and predaceous fungi. Numerous workers have advocated the use of neem and neem products for the management of root knot nematode.

A study conducted by Acharya and Padhi (1988) revealed that, neem oil cake applied @ 1 t/ha in drenches near the root zone of betel vine at the time of planting of vines was most effective in controlling the root knot nematode and increased the yield of betel vine. Pandey and Singh (1990) reported that soil amended with neem cake reduced *M. incognita* significantly on chickpea.

Sundararaju and Sudha (1993) reported the effectiveness of neem oil cake @ 1 kg/palm/year in reducing the nematode population and increasing the yield

significantly in arecanut, banana and black pepper, under arecanut based farming system. Soil amendment with neem cake at 0.1, 0.5 and 1 per cent w/w reduced infection of *M. incognita* on mungbean (*Vigna radiata*) and significantly improved the plant height but reduced root nodulation (Abid *et al*., 1995).

Acid extracts of neem cake at different dilutions enhanced the growth of *Vigna unguiculata* and reduced the population build up of nematode (Alagumalai *et al*., 1995). Neem cake application reduced the population of *M. incognita* and improved the plant growth characters of Japanese mint (Pandey, 1995). Rao *et al*. (1997) reported that use of aqueous extracts of neem cake for seed treatment and soil drenching under field conditions was found to be as effective as application of carbofuran at 2 kg ai/ha for the management of *M. incognita* on okra.

Neem cake and neem dust were found effective in the suppression of root knot nematode, *M. incognita* in tomato (Jacob *et al*., 1998). Rajani (1998) reported the effectiveness of neem cake @ 200 g/m^2 for managing root knot nematode in kacholam, *Kaempferia galanga*.

Application of neem cake was found to reduce fecundity of root knot nematode infecting pointed gourd, *Trichosanthes dioica* cv. *Damodar Kajli* (Chakraborti, 2000). Similar observation has been recorded in case of *M. incognita* infecting onion (Chakraborti, 2003).Neem cake @ 20 g/plant registered minimum root-knot index and was significantly superior to rest of oil cakes against root knot nematode on FCV tobacco (Ravindra *et al*., 2003).

2.2.2.2 **Neem based formulation**

Siddiqui (1986) reported that azadiractin, nimbidic acid and nimbin, when used as bare-root dip treatment, significantly inhibited the penetration of root-knot larvae and the subsequent root galling on tomato and eggplant. A neem pesticide Achook was tested as soil treatment @ 0.5, 1.0, 2.0, 4.0 and 8.0 g kg^{-1}

soil in pots against *M. javanica* on okra. Data recorded 35 days after nematode inoculation indicated that plant growth parameters were improved significantly at 1.0 and 2.0 g kg^{-1} doses over control (Kaushal, 1999).

Sharma (2000) reported significant reduction in the number of galls of *M. incognita* in vegetables, when seeds were soaked in 5 per cent neemark and nimbecidine. Five neem based formulations: Neem Jeevan, Neemark, Neem Gold, Achook and Kranti were tested at 1.0, 0.5 and 0.25 per cent concentrations against *M. incognita*. His findings indicated that these formulations were effective in controlling the nematode population.

Similar observation was reported by Sharma *et al.* (2000), where they found a significant reduction in the number of galls in okra (cv. pusa sawani) when the seeds were soaked in 5% neemark and nimbecidine for 6 and 24hrs.

Javed *et al*. (2001) reported the lowest number of galls when tomato was treated with nimbokil (*A. indica* product extract) compared to control. Randhawa *et al*. (2002) in a pot experiment proved that the growth of okra cv. Punjab 7 was enhanced and the *M. incognita* population was reduced when the botanical extracts and Rakshak Gold (neem based preparation) were applied to seeds at 0.5, 1.0 and 1.5 per cent. Pandey *et al*. (2003) reported that neem compounds were highly useful in suppressing *M. incognita* population and improving herb yield in brahmi, *Bacopa monnieri*.

2.2.3 **Use of bioagents**

Among the non-chemical methods of controlling nematodes, use of biological control agents appears to be the recent strategy gaining more importance. The relevant literature on important bioagents are reviewed and presented.

2.2.3.1 **Bacteria**

Out of the two groups of bacteria as bioagents, the one, which release metabolites that have a killing or inhibitory effect on plant parasitic nematodes, were considered for the study.

2.2.3.1.1 *Pseudomonas fluorescens*

Recently the fluorescent *Pseudomonas* spp. associated with the plant rhizosphere emerged as the largest and most promising biocontrol agent of plant parasitic nematodes (Oostendrop and Sikora, 1989). The effectiveness of *P. fluorescens* as a potential biocontrol agent against root knot nematode, *M. incognita* was due to their ability to envelop or bind the root surface with carbohydrate and lectin thereby interfering with normal host recognition (Oostendrop and Sikora, 1990).

Santhi and Sivakumar (1995) reported the biocontrol potential of *P. fluorescens* (Migula) against root knot nematode on tomato. Thirty isolates of fluorescent pseudomonads, isolated from the rhizosphere of black pepper, were tested for their interaction with *M. incognita*, under green house conditions. The study revealed that the strains of *P. fluorescens* inhibited *M. incognita* (Eapen *et al*., 1997).

Mani *et al*. (1998) reported that the effectiveness of Pf (1) strain of *P. fluorescens* against *M. incognita, T. semipenetrans* and potato cyst nematodes. Application of *P. fluorescens* as seed treatment at a dosage of 10 g kg^{-1} seed was effective in reducing the infestation of *Hirschmaniella gracilis* in rice (Ramakrishna *et al*., 1998).

A talc formulation of *P. fluorescens* containing 15 x 10^8 cfu/g was applied to soil around root knot infested grape vines at 15 cm depth in the basin, at the time of pruning. The bacterial formulation was applied at doses of 1, 2 and

4 g per vine and compared with application of carbofuran at 1.8 g a.i. per vine and an untreated control. Application of *P. fluorescens* at all the three dosage levels significantly reduced the severity of root knot infestation in roots. The extent of colonization by *P. fluorescens* was dosage dependent but not directly proportional to it. The root colonization was significantly better at all dosage levels of *P. fluorescens* (Santhi *et al*., 1998). As reported by Verma *et al*. (1998) application of *P. fluorescens* @ 10 g/kg seed was effective in reducing the menace of root knot nematode, *M. incognita* in tomato.

Seenivasan *et al*. (2000) found that the culture filtrate of *P. fluorescens* had toxic effect on *H. oryzae* population. Devi and Dutta (2002) studied the effect of *P. fluorescens* on root knot nematode (*M. incognita*) of okra plant. They found that *P. fluorescens* improved shoot and root length and reduced root gall number.

A field experiment was conducted by Jyothi *et al*. (2003) to evaluate the efficacy of commercially formulated *P. fluorescens* against root knot nematode *M. incognita* race 3 infesting tomato. *P. fluorescens* treated plants gave the maximum yield (64.3 per cent) and minimum *M. incognita* soil population (56 per cent). Nursery and field experiments were conducted by Mahapatra *et al*. (2003) to determine the efficacy of *P. fluorescens* against *M. incognita* infesting aubergine. *P. fluorescens* at 20 g m^{-2} had the highest reduction (46.4 per cent) in root knot index at transplanting.

A study conducted by Kalaiarasan *et al*. (2006) showed that *P. fluorescens* isolates viz, Pf 1, Pf CBE, Pf POL and Pf BSR were found to protect groundnut from root knot nematode, *M. arenaria*. The plant growth was significantly higher in all the treatments with bacterium and it also reduced the level of infestation by the nematode. The nematode suppression ability of the bacterial isolates was related to its root colonizing ability.

2.2.3.1.2 *Bacillus* spp.

Gokte and Swarup (1988) stressed the larvicidal effect of *B. subtilis* and *B. pumilis* on *Anguina tritici*. Seed treatment of the above isolates in wheat individually and in combination caused reduction in percentage of penetration of juveniles to the roots of wheat seedlings and the viability of larva. The effect of bacteria *B. subtilis, B. pumilis, B. coagulans, B. macerans* and *B. ciculans* was studied on related genera like *Heterodera oryzicola* which revealed that at 1.2 x 10^8 cells per ml of these bacteria caused 70 to 80 per cent larval mortality (Sheela, 1990).

A study conducted by Sheela and Venkitesan (1992) revealed the effectiveness of *B. macerans* against root knot nematode, *M. incognita* in bhindi and pepper. Racke and Sikora (1992) revealed that the plant growth promoting rhizobacterium, *Agrobacterium radiobacter* and *B. sphaeriacus* increased the tuber yield of potato by suppressing the population of *Globodera pallida.*

Various formulations of *B. thuringiensis* were found toxic to eggs and larvae of root knot nematode. Zuckerman *et al*. (1993) found that application of an isolate of *B. thuringiensis* (CR-371) resulted in smaller population of *M. incognita* in tomato. Sheela *et al*. (1999) reported the biocontrol efficiency of *B. subtilis* against *M. incognita* in brinjal.

The effects of biological treatments on root knot nematode *M. incognita* and fruit yield of tomato were evaluated by Reddy *et al*. (1999). *B. subtilis* strain GB 03 plus one additional Plant Growth Promoting Rhizobacteria and a flaked chitosan was used as treatments. Across all categories of fruits, greater yields occurred with biological treatments especially in *B. subtilis* treated plots.

Mahdy *et al*. (2000) tested the ability of the rhizobacterium *B.cereus* S18, to control three species of root knot nematode on tomato: *M. incognita, M.*

javanica and *M. arenaria.* They found that, treatment of tomato plants with *B. cereus* S18 led to an over all reduction in the number of galls and number of egg masses produced by all the three nematode species tested when compared with the non-bacterized plants. The antagonistic rhizobacteria caused only a non-significant reduction in number of galls and number of egg masses of *M. arenaria.*

B. subtilis in combination with either neem cake or *Datura fastuosa* gave better control of the root rot and root knot infection with the enhancement of growth of urdbean compared to use of either component alone (Siddiqui *et al*., 2001). Khan *et al*. (2002) proved that treatment with *B. subtilis* or *Beijerinckia indica* reduced galling by 33-34 per cent and increased the dry weight of shoots by 22-24 per cent in green gram. Egg mass production and soil populations of *M. incognita* were more adversely affected.

2.2.3.2 Fungus

More than 200 fungi have been reported to be antagonistic to nematodes. High efficacy of fungi as bioagents is due to the long co-evolution of these fungi and plant parasitic nematodes in common soil habitat.

2.2.3.2.1 *Trichoderma* sp.

The synergistic effect of fungi *T. viride* along with organic amendment for the enhancement of plant growth by increasing the population of nematode trapping fungus was reported by Reddy *et al*. (1996). Similar observation was recorded by Sundarababu *et al*. (1997). Khan and Saxena (1997) reported that root dip treatment with culture filtrates of *Aspergillus niger, Paecilomyces lilacinus* and *T. viride* was particularly beneficial in reducing *M. incognita* damage on tomato in pot experiments.

The antagonistic effect of *Trichoderma* against root knot nematode had been recorded by Sankaranarayanan *et al*. (1997). Khan (1999) conducted a study on the toxic effects of culture filtrates of fungi, i.e., *Alternaria alternata, Aspergillus clavatus, Rhizoctonia solani, Fusarium solani, Rhizopus stolonifer* and *T. viride* on *M. incognita* larva. Complete larval mortality was observed in *F. solani, T. viride* and *R. solani* filtrates after 24 hrs.

Sankaranarayanan *et al*. (1999) reported that *T. harzianum* isolates were found to be most effective against *M. incognita* on sunflower and had the least number of galls and egg masses on root systems and nematode populations in soil.

Acharya *et al*. (2000) reported good control of root knot nematode in betel vine, *P. betle* by field application of *T. viride*. The fungus showed saprophytic habit when inoculated with suitable oil cake (mustard cake as substrate) and proved to be an effective parasite of root knot nematode. It resulted in decreasing the nematode population in soil and thereby increasing the yield (both number and weight of leaves).

Khan *et al*. (2000) carried out a study by comparing chemicals and biological control treatments against *M. incognita,* on the basis of the number of galls formed on roots per tomato plant. *T. harzianum* and *P. lilacinus* were amended with organic substrates, which resulted in the minimum number of galls per plant.

A study conducted by Ravi *et al*. (2000) established that *T. viride* reduced nematode multiplication and their entry into roots of banana. Parasitization of egg masses of root knot nematode by antagonistic fungus, *T. viride* was observed in tomato treated with bioagents (Reddy *et al*., 2000).

Pot and field trials were conducted by Pandey *et al*. (2003) to study the efficacy of different levels of *T. viride* (1000, 2000, 3000 and 4000 spores per

plant) against root knot nematode *M. incognita* in chickpea. All the treatments of *T. viride* showed significantly higher plant growth parameters over control. The gall development and final nematode population of *M. incognita* decreased with the increasing level of *T. viride* under pot and field conditions.

Pandey and Kalra (2003) reported that highest root knot suppression was noticed, when vermicompost was combined with *T. harzianum*, against root knot disease of Ashwagandha (*Withania somnifera*). A study conducted by Senthilkumar and Rajendran (2004) revealed that *T. viride* reduced final nematode population in grape vine.

2.2.3.2.2 Arbuscular Mycorrhizal Fungi (AMF)

AMF have potential in reducing plant diseases caused by plant parasitic nematodes. Sikora and Schonpeck (1975) in their study established that there is reduction in root penetration and development of *M. incognita* by vesicular arbuscular mycorrhizae (VAM) in tomato. Bagyaraj *et al*. (1979) reported that tomato roots colonized by *Glomus fasciculatum* exhibited fewer and small galls than nematodes (*M. incognita* and *M. hapla*) infested non-mycorrhizal plants.

Kellam and Schenck (1980) showed that gall formation by *M. incognita* in soyabean was reduced only in root portions mycorrhized with *G. macrocarpum* suggesting a direct short range effect and also observed that the yield and root weight were increased in dually inoculated plants and had significantly fewer galls per gram root than inoculating with nematode alone. The number of giant cells formed in mycorrhizal tomato when infected with root knot nematode was significantly low when compared with non-mycorrhizal plants (Suresh *et al*., 1985).

Jain and Hasan (1988) reported that AMF infected plant roots induced tolerance to nematode susceptible plants. AMF and plant parasitic nematodes

occur together in the rhizosphere of the same plant and colonize or infect similar root tissue for their growth and development. Jain and Sethi (1988) concluded that, early establishment of *G. fasciculatum* on cowpea hampered the gall formation by *M. incognita* and their multiplication.

Sivaprasad *et al*. (1990a) observed that deleterious effect of nematodes was made insignificant due to arbuscular mycorrhizal association in cowpea. The root knot index and nematode population were reduced considerably. In pepper (*Piper nigrum*) there was a reduction in nematode population in root and soil, root knot index and an increase in growth of vines when plants were inoculated with *G. fasciculatum* and *G. etunicatum* (Sivaprasad *et al*., 1990b).

A study conducted by Deepthi (1993) indicated that cowpea plants inoculated with *M. incognita* in association with *G. fasciculatum* and *G. mosseae* recorded a root knot index of 1 and 3.16 respectively as against 4.89 observed for control plants. Studies conducted by Sharma *et al*. (1994) indicated that VAM colonization reduced the root knot infestation in tomato. Mycorrhizal tomato seedlings had lesser number of galls, egg masses per plant, eggs and juveniles per egg mass.

Carling *et al*. (1996) determined the individual and combined effects of two arbuscular mycorrhizal fungi (*Gigaspora margarita* and *G. etunicatum*), *M. arenaria* and phosphorus (P) fertilization (0, 25, 75 and 125 µg/g soil) on groundnut plant growth and pod yield under green house condition. Groundnut growth and yield were generally stimulated by AMF development, growth alone was suppressed by *M. arenaria* at 0 and 25 µg P. *M. arenaria* had only a minimal effect on root colonization by AMF and sporulation by the fungi.

A report by Sundarababu *et al*. (1996) showed that when *G. fasciculatum* was inoculated 15 days earlier than nematode inoculation, it enhanced the growth of tomato cv. Co-3 and suppressed *M. incognita*

multiplication in pot experiments. *G. fasciculatum* was very effective in controlling root knot nematode in brinjal (Asha, 1996) and in spices like ginger, turmeric, cardamom, pepper (Sivaprasad and Sheela, 1998) and kacholam (Rajani *et al*., 1998).

Ray and Dalei (1998) reported that, in the case of green gram all plant growth parameters including pod yield, leaf chlorophyll content, bacterial nodulation, leghaemoglobin content of nodules and NPK content of plants showed significant improvement in mycorrhiza inoculated plants.

The effect of an arbuscular mycorrhizal fungus (AMF) and two migratory endoparasitic nematodes on *Musa* plant growth, including root system were examined by Elsen *et al*. (2003). Mycorrhization with *G. mosseae* resulted in a significantly better plant growth even in the presence of nematodes. In the root system, it appeared that the decreased branching caused by nematodes was counterbalanced by the increased branching caused by the AMF.

2.2.4 Use of chemicals

The effect of chemicals in controlling nematodes has been reported by many workers. Sivakumar *et al*. (1973) demonstrated that seed treatment with carbofuran three or six per cent ai can be effectively employed to reduce the severity of root-knot nematode infestation in okra. Prasad *et al*. (1977) suggested that soil drenches of carbofuran at 4 or 8 kg/ha could effectively control *M. incognita* on tomato.

Reddy and Singh (1979) reported that tomato seedlings dipped in carbofuran at 1000 ppm for 20 minutes gave the least root-knot index. Chemicals used as bare root dips were effective in giving adequate initial protection to tomato seedlings from root-knot nematode, which led to better growth, and increased yield

Complete control of *R. similis* was obtained with soil application of phorate @ 25 kg a.i./ha during September, December and May in infested coconut nurseries (Koshy and Nair, 1979; Koshy *et al*., 1985).

Fademi (1984a) conducted a pot culture experiment and reported that carbofuran (1kg ai/ha) was the most effective chemical as seed treatment for control of *M. incognita* in upland rice var. Faro-11. Fademi (1984b) reported that carbofuran 1, 2 or 3 kg a.i./ha, applied after planting, significantly reduced the population of *M. incognita* in upland rice. Early application of 2 kg a.i./ha and late application of 3 kg a.i./ha gave the best results.

Patel *et al*.(1985) observed reduced penetration of *M.arenaria* on groundnut with seed treatment of carbofuran flowable and aldicarb sulfone over control. Carbofuran applied at 1 kg ai/ha increased yield by 68% over control, with a cost benefit ratio of 1:2 (Gunasekharan *et al*., 1987).

A study conducted by Mani *et al*. (1987) revealed that carbofuran @ 4 kg a.i./ha applied in rows to 4 month-old turmeric crop had resulted in 81.6% reduction in root knot nematode population as against 45% increase in untreated plots. Bhagavathi and Phukan (1990) reported that carbofuran reduced the galls and egg masses in roots of pea and increased the yield.

Borah and Phukan (1990) tried carbofuran 3G, phorate 10 G, mocap 10 G and diazinon 10 G each at 1.0, 2.0 and 3.0 per cent as seed treatment for the control of *M. incognita* on green gram and found that increase in concentration of chemicals resulted in the decrease in number of galls and egg masses and increase in plant growth characters and yield. Research findings of Mohan and Mishra (1993) revealed that carbofuran was effective in suppressing *M. incognita* activity and improving plant growth of french bean.

Soil application of carbofuran @ 2 kg ai per ha and seed dressing @ 22 g a.i. per kg seed were highly effective in controlling *M. incognita* larvae and

reduced root knot galls in pea compared to control plants. These treatments also improved the plant growth parameters and yield (Devi, 1993).

A pot experiment conducted by Prasad (1993) for the control of *M. arenaria* in groundnut showed that carbofuran applied to soil at 2kg a.i. /ha before sowing or as foliar spray at 500ppm, 15 days after germination significantly reduced root galls and enhanced growth parameters.

A study was conducted by Shukla and Haseeb (1996) to evaluate the effect of aldicarb, carbofuran and ethoprophos against *Pratylenchus thornei* infesting *Mentha citrate, M. piperita* and *M. spicata* in glass house experiments. All the treatments increased herb weight and oil yield of all the test species of mint and minimized nematode reproduction. Haider *et al*. (1998) reported that application of carbofuran or phorate @ 1 kg a.i. per ha reduced root knot nematode (*M. incognita*) population in turmeric.

Investigations carried out by Ravi *et al*. (2000) revealed that a combined application of neem cake, *T. viride* and carbofuran was the most effective treatment in increasing plant growth parameters (plant height, pseudostem girth, leaf area and number of leaves) and fruit yield and reducing the population of *M. incognita* and *R. similis* both in soil and roots of banana.

In Kerala conditions, application of neem cake @ 1 t ha^{-1} at the time of planting and carbofuran 1 kg a.i. per ha 45 days after planting is recommended for the control of nematodes associated with ginger. Similarly for nematode infested pepper vines, application of phorate or carbofuran @ 1 g a.i. per vine twice a year is recommended (Kerala Agricultural University, 2002).

Tiwari *et al*. (2002) found that tomato nursery bed treated with 0.6 g carbofuran significantly decreased gall index and increased crop yield. Compared to other plots the maximum yield (362 q per ha) was recorded in carbofuran treated nursery beds.

MATERIALS AND METHODS

3. MATERIALS AND METHODS

The objectives of the study entitled 'Management of root knot nematode in thippali' include identification of species of root knot nematode infesting thippali and to evaluate the effect of organic amendments and bioagents for the management of this nematode. The experiments were carried out in the Department of Agricultural Entomology, College of Horticulture, Vellanikkara during June 2005 to July 2006.

3.1 PREPARATION OF DENEMATIZED POTTING MIXTURE

Sieved field soil, sand and well-decomposed farmyard manure was mixed in the ratio 1:1:1 and this potting mixture was filled in earthen pots of size 25cm. Formalin was poured into each pot in the ratio 1:20 and were covered with polythene sheets and tied firmly. After two weeks, the polythene sheets were removed and the mixture in each pot was raked well. Soil samples were taken from each pot to test the presence of nematodes. These pots with denematized potting mixture were used for further pot culture studies.

3.2 MAINTENANCE OF PURE CULTURE OF ROOT KNOT NEMATODE INFESTING THIPPALI

Rooted cuttings of thippali, variety Viswam were used for the maintenance of nematode culture. The cuttings of thippali were planted in pots filled with denematized potting mixture. After identifying the species of root knot nematode on the basis of perineal pattern, pure culture of the nematode was maintained from single egg mass collected from infested thippali roots, which were collected from the medicinal plants garden of the College of Horticulture, Vellanikkara. The second stage juveniles of the nematodes, hatched from the egg mass were inoculated to the potted plants. Repotting and inoculation was repeated periodically for maintaining the pure culture of root knot nematode for different experiments.

3.3 RAISING OF POTTED PLANTS

Earthen pots of size 25 cm diameter, with denematized potting mixture were used for raising potted plants. Two numbers of thippali cuttings with three nodes were planted in each pot. Plants were irrigated periodically to maintain wet condition of soil.

3.4 IDENTIFICATION OF NEMATODES

The species of root knot nematodes were identified by the perenial pattern of the white females. So white females were collected from root galls, in order to identify the species of nematode.

3.4.1. Collection of White Females by Staining Technique

Root samples collected from the culture plants were used for extracting white females. Root samples were washed in a stream of tap water to remove any soil particles adhering to it. Root knots were separated from roots with the help of scissors. It was then placed in a small piece of muslin cloth and was wrapped in it. This small bag containing root galls were plunged into boiling lactophenol containing 0.1 per cent cotton blue till the root tissues become soft. The root knots were removed from muslin cloth and were kept in a Petri plate. It was washed in water to remove excess stain. The root knots were transferred to a microscopic slide containing a drop of lactophenol. It was then placed under a stereomicroscope and was dissected using a needle. The white females, which were stained light blue, came out of the root knots in large numbers and were collected and transferred to a glass vial containing lactophenol. It was then closed tightly, labelled and was sent to the Department of Nematology, Indian Agricultural Research Institute, New Delhi for species identification. (Plate 2)

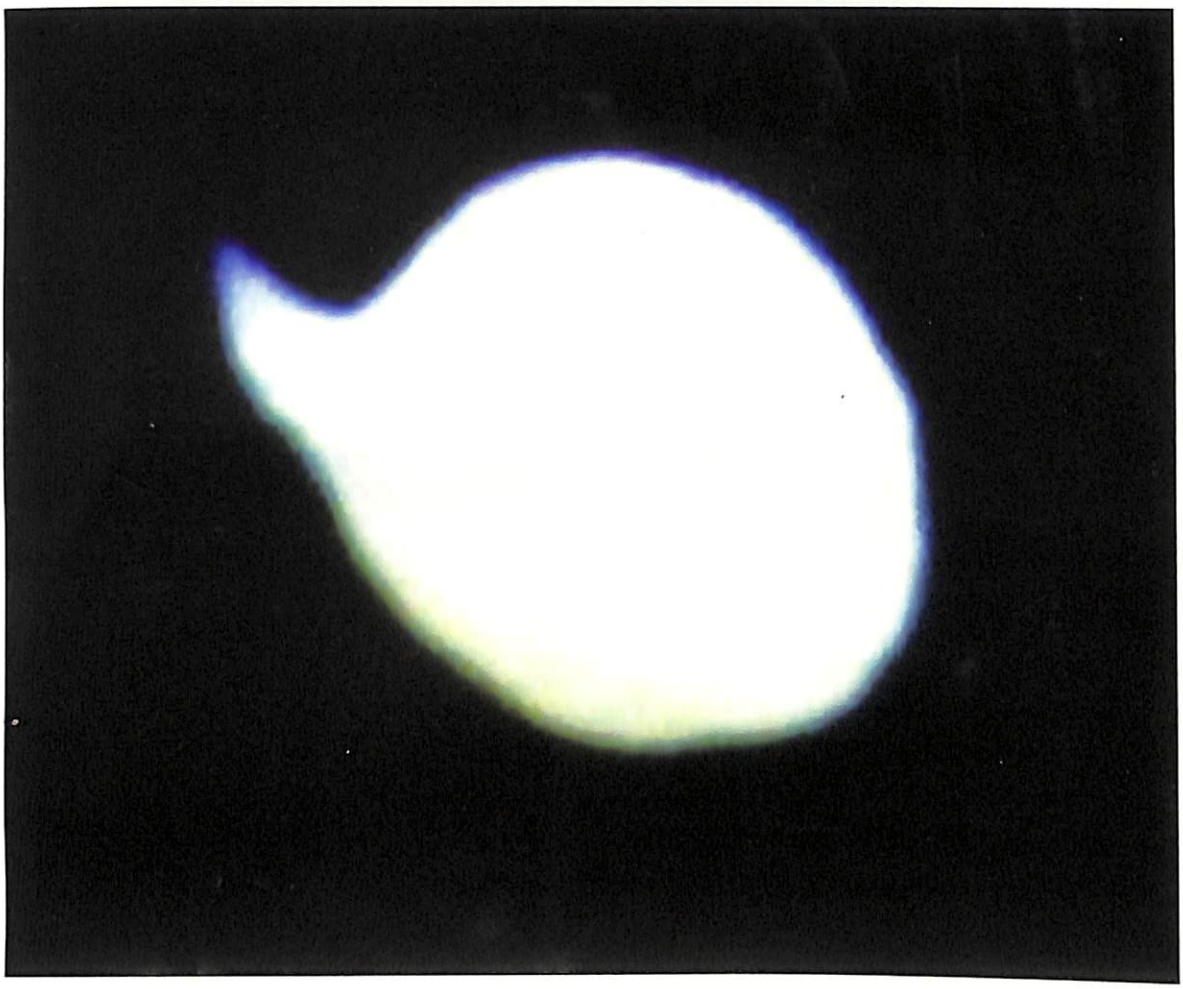

Plate 2. White female of *Meloidogyne arenaria* (40X)

3.5 POT CULTURE STUDIES

Pot culture studies were conducted to determine the efficacy of organic amendments, *Tagetes erecta,* different bioagents and carbofuran in the management for root knot nematode infesting thippali.

Design

The experiments were laid out in Complete Randomized Design with ten treatments and three replications. (Plate 3). The treatments were as follows

T_1 - Neem cake @ 11 g/pot

T_2 - Mulching with *Tagetes* waste @ 250 g/pot

T_3 - Drenching with 5% root extracts of *Tagetes erecta*

T_4 - Soil application of *Pseudomonas fluorescens* @ 10 g/pot

T_5 - Soil application of *Trichoderma viride* @ 10 g/pot

T_6 - Soil application of *Bacillus subtilis* @ 30ml/pot

T_7 - Soil application of Neem granules (amruthguard @ 1 g/pot)

T_8 - Soil application of AMF (50 g/pot)

T_9 - Soil application of carbofuran (0.1 g/pot)

T_{10} - Control

3.5.1 Application of bioagents

P. fluorescens, T. viride, B. subtilis and AMF were applied one week before the inoculation of nematodes. Talc based *P. fluorescens* and *T. viride* suspension was prepared and applied in soil. Required quantity of AMF was added to the pots and covered with a thin layer of denematized potting mixture.

T1 and T2

T3 and T4

T5 and T6

T7 and T8

T9 and T10

Plate 3. Lay out of the experiment

B. subtilis culture suspension having a concentration of 10^{8} cfu/ml was drenched in soil @ 30ml/pot.

3.5.2 Application of other treatments

Required quantities of neem cake, Amruth guard and carbofuran were applied to the soil one week after inoculation of nematodes. Aerial parts of *T. erecta* was cut into small pieces and used for mulching the pots. Root extracts of *T. erecta* were also used for drenching the soil of the respective treatments.

3.5.3 Extraction of Second Stage Juveniles for Inoculation

Modified Baermann funnel technique (Schindler, 1961) was used for extracting second stage juveniles for inoculation. Heavily infested plants from the culture pots were uprooted carefully, washed with water, and egg masses from galled roots were hand-picked using forceps. The second stage juveniles were obtained after keeping the egg masses over two layers of tissue paper supported on a wire mesh, which in turn was placed over a Petri dish with sufficient water just enough to touch the egg masses. Several such sets were kept for getting the required number of second stage juveniles needed for inoculation purpose. Hatched second stage juveniles were transferred to a beaker after every 24hrs. The process was repeated and these hatched juveniles were used for inoculation.

3.5.4 Inoculation of Nematodes

Nematode population in the suspension was assessed. Each pot was inoculated with 10 ml of suspension containing 2000 second stage juveniles of root knot nematode, after the cuttings had established. At the time of inoculation the suspension was thoroughly mixed by blowing air with a pipette, to get uniform distribution of nematodes. This suspension was then poured to the root zone of plants, by making holes of about 5 cm depth in soil using a glass rod.

3.6 OBSERVATION

The following observations were taken during the course of experiment

1. Shoot characters
    a) Number of leaves
    b) Vine length
    c) Number of branches
    d) Fresh weight of shoot
    e) Dry weight of shoot

2. Root characters
    a) Length of root
    b) Fresh weight of root
    c) Dry weight of root

3. Yield characters
    a) Number of fruiting branches
    b) Number of days to spike formation
    c) Number of spikes
    d) Spike length
    e) Spike diameter
    f) Fresh weight of spikes
    g) Dry weight of spikes

4. Nematode population
    a) Nematode population in 200g soil
    b) Nematode population in 10g of root
    c) Number of root-knots in 10g of root
    d) Number of egg mass in 10g of root
    e) Root knot index

3.6.1 Estimation of nematode population from soil

A composite sample of 200g of soil was weighed out from the root zone and processed for extracting the nematodes. Nematodes were extracted from soil samples taken from different treatments, following the Cobb's decanting and sieving technique (Cobb, 1918). The nematode suspension thus obtained was made up to a constant volume (100 ml) by adding water. An aliquot of 1ml was pipetted out into a counting dish and the number of nematodes present were counted under a stereoscopic microscope. The total population of nematodes extracted from 200g soil sample was estimated by multiplying the average population (based on three such counts) by the appropriate factor.

3.6.2 Estimation of number of egg masses from 10g of root

The root system from each pot was carefully lifted by gentle tapping of the pots on all sides and bottom and removing the loose soil, the roots were cleaned of adhering soil particles by gentle washing in water. From this sample 10g of root was weighed and the number of egg masses were counted.

3.6.3 Estimation of root knots from roots

After counting the egg mass, the root sample was pressed gently between folds of blotting paper to remove the excess water and the number of root knots in 10g of root sample were counted.

3.6.4 Root-knot index

Based on the number of galls, the root knot index was rated on a 1-5 scale as follows,

Number of galls	Root knot index
0 - 25	1
26- 50	2
51- 75	3
76-100	4
> 100	5

3.6.5 Estimation of nematode population from root

After counting the number of galls, the root samples were used for extracting nematodes. Modified Baermann funnel technique was used for extracting nematodes from roots (Schindler, 1961). The nematode suspension thus obtained was made to a known volume by adding water and then the population of the nematodes was assessed.

3.7 STATISTICAL ANALYSIS

Data collected during the study was analyzed through statistical method for CRD and ANOVA. Analysis of variance was done using the statistical package, SPSS (Statistical Package for Social Sciences).

RESULTS

4. RESULT

The results of the study entitled 'Management of root knot nematode in thippali (*Piper longum* L.)' are presented in this chapter.

4.1 IDENTIFICATION OF NEMATODE

The white females collected from root knot nematode infested roots of thippali were sent to the Department of Nematology, Indian Agricultural Research Institute, New Delhi for species identification. The species of root knot nematode infesting thippali was identified as *Meloidogyne arenaria*.

4.2 POT CULTURE STUDIES

Pot culture experiments were conducted to study the effect of neem cake, mulching with *Tagetes* waste, drenching with root extracts of *Tagetes erecta*, different bioagents, carbofuran and a granular neem formulation on management of root knot nematode in thippali. Treatments were given as mentioned in 3.5. The effect of different treatments on the shoot characters, yield (upto the time of uprooting), root characters, nematode population, vegetative parametric ratio and nematode relative ratio were observed.

4.2.1 Shoot characters

4.2.1.1 Number of leaves

The results presented in Table 1. showed that there was significant variation in the number of leaves produced in thippali plants by different treatments. Plants treated with *Bacillus subtilis* (T6), recorded the maximum number of leaves (69.83) giving 127.68 per cent increase over control. *Pseudomonas fluorescens* (T4) treated plants also produced 69.33 leaves, which was found equally superior to T6 giving 126.05 per cent increase over control.

Table 1. Effect of different treatments on shoot characters of thippali
(Mean of three replications)

Treatments	Number of leaves	Per cent increase over control	Vine length (cm)	Per cent increase over control	Number of branches	Per cent increase over control	Fresh weight of shoot (g)	Per cent increase over control	Dry weight of shoot (g)	Per cent increase over control
T1	54.50^{b}	77.70	55.00^{b}	31.26	4.67^{bc}	47.32	70.08^{a}	-17.55	15.46^{ab}	20.97
T2	53.33^{b}	73.88	58.50^{bc}	39.62	4.33^{b}	36.59	106.00^{ab}	24.71	19.34^{abcd}	51.33
T3	59.33^{bc}	93.45	58.17^{bc}	38.83	4.17^{ab}	31.55	115.00^{ab}	35.29	16.75^{abc}	31.06
T4	69.33^{d}	126.05	64.00^{c}	52.74	5.67^{c}	78.86	130.00^{b}	52.94	27.17^{cd}	112.60
T5	63.50^{cd}	107.04	64.17^{c}	53.15	5.67^{c}	78.86	110.00^{ab}	29.41	27.88^{d}	118.15
T6	69.83^{d}	127.68	70.92^{d}	69.26	5.50^{c}	73.50	135.00^{b}	58.82	25.34^{bcd}	98.28
T7	57.67^{bc}	88.03	55.15^{b}	31.62	4.33^{b}	36.59	110.00^{ab}	29.41	14.75^{ab}	15.41
T8	64.00^{cd}	108.67	61.07^{bc}	45.75	5.00^{bc}	57.73	104.17^{ab}	22.55	20.12^{abcd}	57.43
T9	62.67^{c}	104.34	57.35^{b}	36.87	4.83^{bc}	52.37	110.00^{ab}	29.41	27.45^{cd}	114.79
T10	30.67^{a}	Nil	41.90^{a}	Nil	3.17^{a}	Nil	85.00^{ab}	Nil	12.78^{a}	Nil

* Values with in the rows and columns followed by the same letters do not differ significantly in DMRT p=(0.05)

Next superior treatment was T8 with 64.00 leaves giving 108.67 per cent increase over control. This was closely followed by T5 (63.50) and T9 (62.67), and was statistically on par with T8, giving 107.04 and 104.34 per cent increase over control respectively. T1 (54.50) and T2 (53.33) were inferior to other treatments and were statistically on par giving 77.70 and 73.88 per cent increase in the number of leaves in the respective treatments. Control plants (T10), produced only 30.67 leaves.

4.2.1.2 Vine length

Statistical analysis of data indicated that there was significant variation in the vine length of different treatments. Maximum vine length of 70.92cm was recorded in plants treated with *B. subtilis* (T6) and gave 69.26 per cent increase over control. The next superior treatment was *T. viride* (T5) with 64.17 cm giving 53.15 per cent increase in vine length over control. The vine length in T4 was 64.00cm, which was found to be statistically on par with T5. There was 52.74 per cent increase in vine length over control in T4. The length of vine in treatments T8 (61.07), T2 (58.50), T3 (58.17), T9 (57.35), T7 (55.15) and T1 (55.00) were statistically on par giving 45.75, 39.62, 38.83, 36.87, 31.62 and 31.26 per cent increase over control respectively. Control plants showed a vine length of only 41.90cm. (Table 1).

4.2.1.3 Number of branches

Total number of branches produced at the time of uprooting under different treatments is given in Table 1. *P. fluorescens* (T4) and *T. viride* (T5) treated plants showed the maximum number of branches and were found to be statistically superior to other treatments. Both these treatments produced 5.67 number of branches giving 78.86 per cent increase over control. T6 also showed superior trend in the number of branches (5.50) giving 73.50 per cent increase over control. Number of branches produced by T8 (5.00), T9 (4.83), T1 (4.67), T2 (4.33) and T7 (4.33) were found to be on par giving 57.73, 52.37, 47.32, and

36.59 per cent increase in the respective treatments. T3 (4.17) was observed to be the inferior treatment, which was statistically on par with control (T10), which produced 3.17 branches.

4.2.1.4. Fresh weight of shoot

The effect of different treatments on the fresh weight of shoot are presented in Table 1. Plants treated with *B. subtilis* (T6) recorded the highest fresh weight of shoot (135.00g) giving 58.82 per cent increase over control. *P. fluorescens* (T4) treated plants with 130.00g fresh weight was ranked statistically as superior as T6 giving 52.94 per cent increase with respect to control. This was followed by T3, T5, T7, T9, T2, T8 and T10. All these treatments were statistically on par and their fresh weight ranged between 85.00g to115.00g. T1 (70.08g) was observed to be inferior to control (T10).

4.2.1.5 Dry weight of shoot

The data presented in Table 1 revealed that there was significant difference in dry weight of shoot under different treatments. The dry weight of shoot was maximum in plants treated with *T. viride* (27.88g). This was closely followed by T9 with 27.45g. There was 118.15 and 114.79 per cent increase in dry weight of shoot over control in the respective treatments. It was observed that T4 (27.17g) and T6 (25.34g) were on par with T9 giving 112.60 and 98.28 per cent increase over control respectively. The dry weight of shoot in T7, T1, T3, T2 and T8 ranged from 14.75 to 20.12. These treatments were observed to be statistically on par with control (T10) giving 12.78g.

4.2.2 Yield characters

The yield data with respect to number of fruiting branches, number of days to spike formation, number of spikes, spike length, spike diameter, fresh weight of spikes and dry weight of spikes were recorded only up to the time of

Table 2. * Effect of different treatments on yield characters of thippali
(Mean of three replications)

Treatments	Number of fruiting branches	Number of days to spike formation	Number of spikes	Spike length (cm)	Spike diameter (cm)	Fresh weight of spikes (g)	Dry weight of spikes (g)
T1	1.75	0.00	0.00	0.00	0.00	0.00	0.00
T2	2.00	214.50	2.25	3.80	0.33	0.97	0.13
T3	1.50	0.00	0.00	0.00	0.00	0.00	0.00
T4	8.50	178.25	9.75	1.95	0.40	1.93	0.44
T5	1.25	171.00	4.00	0.80	0.35	1.71	0.05
T6	3.50	152.00	9.50	2.45	0.70	3.01	0.98
T7	1.00	0.00	0.00	0.00	0.00	0.00	0.00
T8	3.25	190.25	5.25	1.53	0.45	1.90	0.66
T9	2.75	185.00	5.50	1.55	0.65	1.88	0.21
T10	0.00	0.00	0.00	0.00	0.00	0.00	0.00

* Statistically not analysed

uprooting and hence statistical analysis was not carried out. The yield data corresponding to only three harvests are presented in Table 2.

4.2.2.1 Number of fruiting branches

Number of fruiting branches recorded is presented in Table 2. All the treatments except control (T10) produced fruiting branches. Plants treated with *P. fluorescens* (T4) recorded maximum number of fruiting branches (8.50). There was a decreasing trend in the number of fruiting branches in T6 (3.50), T8 (3.25), T9 (2.75), T2 (2.00), T1 (1.75), T3 (1.50), T5 (1.25) and T7 (1.00).

4.2.2.2. Number of days to spike formation

Formation of spikes were noticed only in T6, T5, T4, T8 and T2. There was no spike formation in T1, T3 and T7, eventhough fruiting branches were formed. The number of days to spike formation from the date of planting are presented in Table 2. Plants treated with *B. subtilis* (T6) took only minimum number of days (152.00) for spike formation and was reported to be the best treatment. T5 (171.00) was good which was closely followed by T4 (178.25), T9 (185.00) and T8 (190.25). Maximum number of days for spike formation was taken by T2 (214.50).

4.2.2.3. Number of spikes

Data regarding the number of spikes formed are presented in Table 2. Maximum number of spikes (9.75) were formed on plants treated with *P. fluorescens* (T4) and this was closely followed by *B. subtilis* (T6) which produced 9.50 spikes. Other treatments with spike formation showed a decreasing trend with 5.50, 5.25, 4.00 and 2.25 spikes in T9, T8, T5 and T2 respectively.

4.2.2.4. Spike length

Length of spikes recorded in different treatments is presented in Table 2. Mulching with *Tagetes* (T2) produced the longest spike of 3.80cm and the shortest spike of 0.80cm was produced by plants treated with *T. viride* (T5). In T4, T6, T8 and T9 spike length ranged from 1.00 to 3.00 cm.

4.2.2.5. Spike diameter

Diameter of spikes also showed variation in different treatments (Table 2). Maximum diameter was recorded in T6 (0.70cm), which was closely followed by T9 (0.65cm). Diameter of spikes was minimum in T2 (0.33cm). In T4, T5 and T8 it ranged between 0.35 to 0.45cm.

4.2.2.6 Fresh weight of spikes

Yield assessed in terms of fresh weight of spikes is presented in Table 2. Plants treated with *B. subtilis* (T6) recorded the highest yield (3.01g) from three harvests up to the time of uprooting and was the best treatment. In T4, T5, T8 and T9 fresh weight ranged from 1.00 to 2.00g. T2 recorded the lowest fresh weight of spikes (0.97g).

4.2.2.7 Dry weight of spikes

Dry weight of spikes formed under different treatments is presented in Table 2. T6 recorded the highest dry weight (0.98g). There was a decreasing trend in dry weight of spikes in T8 (0.66g), T4 (0.44g), T9 (0.21), T2 (0.12) and T5 (0.05g).

4.2.3 Root characters

4.2.3.1 Length of root

Root length of thippali showed statistically significant variation in different treatments. There was vigorous root growth in plants treated with *B. subtilis* (T6), which recorded the longest root (49.20cm). The increase in length of root over control was 109.36 per cent. This was followed by T4 (31.52cm), T8 (30.67cm) and T5 (30.17cm) and were found to be statistically on par. The per cent increase in root length over control in the respective treatments were 34.13, 30.51 and 28.38cm.There was a decreasing trend in the root length of plants in T9 (29.07cm), T2 (28.78 cm), T3 (28.00cm), T1 (26.70cm) and T7 (26.47cm) and the treatments were statistically on par with control (T10), which recorded very short roots of 23.50cm. (Table 3).

4.2.3.2. Fresh weight of root

Fresh weight of root at the time of uprooting, under different treatments are given in Table 3. There was statistically significant variation in fresh weight of root. Highest fresh weight of root was observed in T2 (90.00g) giving 140.00 per cent increase over control, and was the best treatment. This treatment was on par with T6 (85.00g) giving 126.67 per cent increase over control. There was a decreasing trend in fresh weight of root in T3 (73.33g), T4 (55.00g), T8 (53.33g), T9 (48.33g), T7 (47.50g), T1 (41.67g) and T5 (39.17g). Even though these treatments were statistically on par with control (T10), they showed 95.55, 46.67, 42.21, 28.88, 26.67, 11.12 and 4.45 per cent increase in fresh weight over control respectively.

4.2.3.3 Dry weight of root

The data regarding the dry weight of roots are presented in Table 3. All treatments were significantly superior to control (T10) giving more than cent per

Table 3. Effect of different treatments on root characters of thippali
(Mean of three replications)

Treatments	Length of root (cm)	Per cent increase over control	Fresh weight of root (g)	Per cent increase over control	Dry weight of root (g)	Per cent increase over control
T1	26.70^{ab}	13.62	41.67^{a}	11.12	19.87^{bc}	221.00
T2	28.78^{ab}	22.47	90.00^{c}	140.00	13.30^{ab}	114.86
T3	28.00^{ab}	19.15	73.33^{abc}	95.55	16.27^{b}	162.84
T4	31.52^{b}	34.13	55.00^{abc}	46.67	27.56^{c}	345.23
T5	30.17^{b}	28.38	39.17^{a}	4.45	17.51^{b}	182.88
T6	49.20^{c}	109.36	85.00^{bc}	126.67	43.32^{d}	599.84
T7	26.47^{ab}	12.64	47.50^{ab}	26.67	18.04^{bc}	191.44
T8	30.67^{b}	30.51	53.33^{abc}	42.21	18.38^{bc}	196.93
T9	29.07^{ab}	23.70	48.33^{ab}	28.88	19.78^{bc}	219.55
T10	23.50^{a}	Nil	37.50^{a}	Nil	6.19^{a}	Nil

* Values with in the rows and columns followed by the same letters do not differ significantly in DMRT p=(0.05)

cent increase over control. T6 was the most superior treatment with respect to dry weight of root giving 43.32g. T4 was ranked as the next superior treatment with 27.56g root weight. It was found that T1 (19.87g), T9 (19.78g), T8 (18.38g) and T7 (18.04g) were on par with T4 Next best treatment was T5 (17.51g), which was statistically on par with T3 (16.27g). T2 was the inferior treatment, which recorded a dry weight of 13.30g and was on par with control (T10), but showed 114.86 per cent increase over control.

4.2.4 Nematode population

4.2.4.1 Nematode population in soil

The results relating to the effect of different treatments on the population build up of nematodes in the rhizosphere of thippali at the time of uprooting are presented in Table 4 and Plate 4.There was a drastic reduction in the mean nematode population in treated pots. The population of nematodes ranged from 1966.67 to 10,677.67 in treated pots against 26,222.33 in control. The best treatment was T6 (1966.67) giving about 92.50 per cent reduction in nematode population over control.

Next superior treatment was T9 (6355.67) giving 75.76 per cent reduction over control and T4, T2 and T5 was statistically on par with T9. The respective number of nematodes and the percentage reduction over control in T4, T2 and T5 were 6522.33 (75.13), 6978.00 (73.39) and 7777.67 (70.34) respectively. Next treatment capable of reducing nematode population was T7 (8033.00) giving 69.37 per cent decrease over control, which was statistically on par with T8 (8466.67) and T1 (8722.00). T3 (10677.67) was found to be inferior to all treatments, but was superior over control by 59.28 per cent.

Plate 4. Effect of different treatments on root characters of thippali

Table 4. Effect of different treatments on nematode population
(Mean of three replications)

Treatments	Nematode population in 200g soil	Per cent decrease over control	Nematode population in 10g root	Per cent decrease over control	Number of egg masses in 10g root	Per cent decrease over control	Number of root knots in 10g root	Per cent decrease over control	Root knot index (1-5 scale)
T1	8722.00^{e}	66.74	21999.33^{f}	70.74	5.00^{d}	44.44	157.92^{d}	26.89	4.83^{e}
T2	6978.00^{bcd}	73.39	16177.33^{cd}	78.48	3.83^{bcd}	57.44	85.50^{bc}	60.42	3.75^{c}
T3	10677.67^{f}	59.28	21622.00^{ef}	71.24	3.50^{bcd}	61.11	116.25^{c}	46.18	4.75^{de}
T4	6522.33^{bc}	75.13	12444.00^{bc}	83.45	1.50^{ab}	83.33	67.58^{b}	68.71	3.17^{b}
T5	7777.67^{bcde}	70.34	15332.67^{bcd}	79.60	0.83^{a}	90.78	97.75^{bc}	54.75	4.33^{d}
T6	1966.67^{a}	92.50	4800.00^{a}	93.62	2.83^{abcd}	68.56	4.67^{a}	97.84	1.00^{a}
T7	8033.00^{cde}	69.37	17110.67^{cde}	77.24	4.33^{cd}	51.89	93.75^{bc}	56.60	3.00^{b}
T8	8466.67^{de}	67.71	17688.00^{def}	76.47	2.17^{abc}	75.89	73.92^{b}	65.78	3.47^{bc}
T9	6355.67^{b}	75.76	10977.33^{b}	85.40	1.67^{ab}	81.44	69.42^{b}	67.86	3.17^{b}
T10	26222.33^{g}	Nil	75177.33^{g}	Nil	9.00^{e}	Nil	216.00^{e}	Nil	5.00^{e}

4.2.4.2. Nematode population in root

The effect of different treatments on nematode population in root of thippali are presented in Table 4. All treatments were significantly superior to control. The mean number of nematodes ranged from 4800.00 to 21,999.33 per 10g of root in various treatments as against a very high population of 75,177.33 in control. Nematode population in root was also observed to very low in T6 (4800.00), which showed 93.62 per cent reduction over control. T9 (10977.33) was ranked as the next best treatment giving 85.40 per cent reduction in nematode population over control. This treatment was statistically on par with T4 (12444.00) recording 83.45 per cent reduction and T5 (15332.67) giving 79.60 per cent reduction over control. It was found that T2, T7, T8 and T3 gave 78.48, 77.24, 76.47 and 71.24 per cent reduction in nematode population over control. The nematode population was very high in T1 (21999.33) and was inferior to all other treatments, but was superior over control by 70.74 per cent.

4.2.4.2. Number of egg masses

The data presented in Table 4 indicated significant difference in the number of egg masses per 10g of root under different treatments.. The most superior treatment was *T. viride* (T5) with 0.83 number of egg mass per 10g of root, giving 90.78 per cent reduction over control. This treatment was on par with T4 (1.50), T9 (1.67), T8 (2.17), and T6 (2.83) giving 83.33, 81.44, 75.89 and 68.56 per cent reduction in number of egg masses over control in the respective treatments. Plants treated with neem cake (T1) was the least effective treatment with 5.00 egg masses, but was superior to control (T10) by 44.44 per cent.

4.2.4.3. Root knot count

The data relating to root knot count revealed the effectiveness of various treatments in reducing gall formation (Table 4). All treatments were significantly superior to control. The mean number of galls ranged from 4.67 to 157.92 per

10g of root in various treatments as against 216.00 in control. T6 (4.67) gave the maximum reduction in number of galls recording 97.84 per cent reduction over control. The next superior treatment was T4 (67.58), which was on par with T9 (69.42) and T8 (73.92), giving 68.71 per cent reduction over control. An increasing trend in number of galls was observed in T2 (85.50), T7 (93.75), T5 (97.75) and T3 (116.25) giving 60.42, 56.60, 54.75 and 46.18 per cent reduction over control respectively. T1 was found statistically inferior to other treatments, but was superior to T10, which produced 216.00 galls per 10g of root.

4.2.4.4. Root knot index

Data regarding root knot index is presented in Table 4. Most superior treatment was *B. subtilis* (T6) with a root knot index of 1.00. Treatments T7 (3.00), T4 (3.17), T9 (3.17), T8 (3.47) and T2 (3.75) were on par. T1 (4.83) was the inferior treatment and was on par with T10 (5.00).

4.2.5 Vegetative parametric ratio

The individual vegetative parametric measurements like dry weight of shoot, fresh weight of shoot, dry weight of root, fresh weight of root, vine length and root length will not give a proper assessment of the impact of the various treatments on the plant growth. It is the relative measurements that are the ultimate indicators of the exact growth of the plant. For this purpose, the relative ratios like ratio of dry weight to fresh weight of shoot, ratio of dry weight to fresh weight of root and ratio of vine length to root length were calculated. Higher the value of ratio, higher will be the impact of these treatments on the plants.

4.2.5.1. Ratio of dry weight to fresh weight of shoot

The results related to the effect of different treatments on the ratio of dry weight to fresh weight of shoot are presented in Table 5. The values ranged from 0.13 to 0.25 for different treatments. Plants treated with *T. viride* (T5) and

Table 5. Effect of different treatments on vegetative parametric ratio (Mean of three replications)

Treatments	Dry weight of shoot / Fresh weight of shoot	Dry weight of root / Fresh weight of root	Vine length / Root length
T1	0.22	0.48	2.06
T2	0.18	0.15	2.03
T3	0.15	0.22	2.08
T4	0.21	0.50	2.03
T5	0.25	0.45	2.13
T6	0.19	0.51	1.44
T7	0.13	0.38	2.08
T8	0.19	0.34	1.99
T9	0.25	0.41	1.97
T10	0.15	0.17	1.78

carbofuran (T9) showed a higher value of 0.25. Least value was for T7 (0.13), which was inferior to control (T10) with a ratio of 0.15.

4.2.5.2. Ratio of dry weight to fresh weight of root

The ratio of dry weight to fresh weight of root is presented in Table 5. Plants treated with *B. subtilis* (T6) recorded highest value (0.51), which was closely followed by T4 (0.50). A decreasing trend was seen in T1, T5, T9, T7 and T8 giving 0.48, 0.45, 0.41, 0.38 and 0.34 respectively. T2 was found to be an inferior treatment with 0.15, which was less than the ratio in T10 (0.17).

4.2.5.3. Ratio of vine length to root length

The ratio of vine length to root length presented in Table 5 revealed that plants treated with *T. viride* (T5) recorded a comparatively higher ratio 2.13. T3, T7, T1, T2 and T4 showed almost equal values ranging from 2.03 to 2.08. The least value for ratio of vine length to root length was observed for T6 (1.44). This treatment was inferior compared to control plants (T10), which showed a value of 1.78.

4.2.6 Nematode relative ratio

As the vegetative parametric ratios alone will not give a measurement of the controlling mechanism of the various treatments, nematode relative ratios were also calculated. The nematode relative ratio gives an assessment of the impact of reduction in nematode population on growth parameters. Thus both the ratios put together will bring out the exact role of the different treatments for the management of nematodes.

Table 6. Effect of different treatments on nematode relative ratio
(Mean of three replications)

Treatments	Vine length Nematode population in root (in 1000's)	Vine length Nematode population in soil (in 1000's)	Root length Nematode population in root (in 1000's)	Root length Nematode population in soil (in 1000's)
T1	2.50	6.31	1.21	3.06
T2	3.62	8.38	1.78	4.12
T3	2.69	5.45	1.29	2.62
T4	5.14	9.81	2.53	4.83
T5	4.19	8.25	1.97	3.88
T6	14.78	36.06	10.25	25.02
T7	3.22	6.87	1.55	3.30
T8	3.45	7.21	1.73	3.62
T9	5.22	9.02	2.65	4.57
T10	0.56	1.60	0.31	0.90

4.2.6.1 Ratio of vine length to nematode population in root (in 1000's)

The influence of different treatments on ratio of vine length to nematode population in root (in 1000's) are presented in Table 6. Maximum value of 14.78 was recorded in plants treated with *B. subtilis* (T6), and was found to be the best treatment. In other treatments, the ratio ranged from 2.50 to 5.22 as against 0.56 in control (T10). Least value for the ratio was recorded in T1 (2.50).

4.2.6.2 Ratio of vine length to nematode population in soil (in 1000's)

The ratio of vine length to nematode population in soil (in 1000's) presented in Table 6 explains the impact of different treatments. The plants treated with *B. subtilis* (T6) recorded the highest ratio of 36.06. The ratio ranged from 5.45 to 9.81 in other treatments as against 1.60 in control (T10). T3 (5.45) was inferior compared to other treatments.

4.2.6.3 Ratio of root length to nematode population in root (in 1000's)

The data related to the influence of different treatments on ratio of root length to nematode population in root (in 1000's) are presented in Table 6. T6 recorded a maximum value of 10.25. In other treatments, ratio ranged from 1.21 to 2.65 as against 0.31 in T10. Least value of 1.21 was recorded in T1 but was better with respect to control.

4.2.6.4 Ratio of root length to nematode population in soil (in 1000's)

The results related to the influence of different treatments on ratio of root length to nematode population in soil (in 1000's) are presented in Table 6. Plants treated with *B. subtilis* (T6) showed a maximum value of 25.02, and was the best treatment. The ratio ranged from 2.62 to 4.83 in other treatments as against 0.90 in control. T3 was recorded to be the inferior treatment with a ratio of 2.62, but was superior compared to control.

DISCUSSION

5. DISCUSSION

The results of the study entitled 'Management of root knot nematode in thippali (*Piper longum* L.)' are discussed in this chapter.

5.1 IDENTIFICATION OF ROOT KNOT NEMATODE IN THIPPALI

The identification report from the Department of Nematology, Indian Agricultural Research Institute, New Delhi indicated that, the root knot nematode in thippali collected from medicinal plants garden at College of Horticulture, Vellanikkara was identified as *Meloidogyne arenaria*. *M. arenaria* is popularly known as groundnut root knot nematode. Infestation of *M. arenaria* in thippali, is reported for the first time in India in this study.

5.2 POT CULTURE EXPERIMENTS

In the present study, the efficacy of organic amendments (neem cake @ 11g/pot), *Tagetes* waste mulching @ 250g/pot, drenching with 5% root extracts of *Tagetes erecta*, bioagents like *Pseudomonas fluorescens* (10g/pot), *Trichoderma viride* (10g/pot), *Bacillus subtilis* (30 ml/pot @ 10^8 cfu/ ml), AMF (50g/pot), a neem formulation (amruthguard @ 1g/pot) and a chemical (carbofuran @ 0.1g/pot) were evaluated by comparing with an untreated control. The results were assessed in terms of shoot characters (number of leaves, vine length, number of branches, fresh weight and dry weight) root characters (length, fresh weight and dry weight) yield characters upto time of uprooting (number of fruiting branches, number of days to spike formation, number of spikes, length and diameter of spike, fresh weight and dry weight of spikes) nematode population build up in soil, nematode population characteristics in root (number of egg masses, number of galls and root knot index) vegetative parametric ratio and nematode relative ratio.

5.2.1 Shoot characters

The influence of different treatments in improving the shoot characters of thippali, in relation to number of leaves, vine length, number of branches, fresh weight and dry weight of shoot are presented in Table 1 and Fig 1. Among the different treatments studied, T4, T5, T6, T8 and T9 were dominant over others. *B. subtilis* (T_6) was the best treatment with maximum number of leaves, vine length and fresh weight which were 69.83, 70.92cm and 135.00g respectively. This finding was in line with that of Govind (2005) in managing root knot nematode, *M. graminicola* on rice. A study conducted by Sheela and Venkitesan (1992) revealed the effectiveness of *B. macerans* against root knot nematode, *M. incognita* in bhindi and pepper. Thus the potential of *B. subtilis* as biocontrol agent in improving shoot growth was established in this study. With respect to the number of branches and dry weight of shoot, *T. viride* (T5) was found to be superior with 5.67 and 27.88g respectively. Similar observation was recorded in a study conducted by Spiegel and Chet (1998), which indicated that improved growth of nematode infested plants and reduction in root galling index and number of egg mass per gram of root were achieved when nematode infested soils were pre exposed to *T. harzianum* preparations. Singh *et al.* (2003) proved that the treatment with *T. viride* significantly improved plant growth characteristics in okra.

Pseudomonas fluorescens (T4) was found to be effective in improving the shoot characters studied. Increase in shoot characters may be due to the influence of certain plant hormones like indole acetic acid (IAA), gibberellin and cytokinin produced by this bacterium. The growth promotion by *P. fluorescens* reported in various crops by many workers is in line with this study. Sanhita *et al*. (2000) reported the growth promotion of tomato plants by the rhizobacteria *P. fluorescens.* This was closely followed by AMF and carbofuran in relation to the number of leaves, vine length and number of branches. The result obtained is in confirmation with those of Ray and Dalei (1998) and Sundarababu *et al.* (1996). The improvement in biometric characters due to reduction in root knot nematode

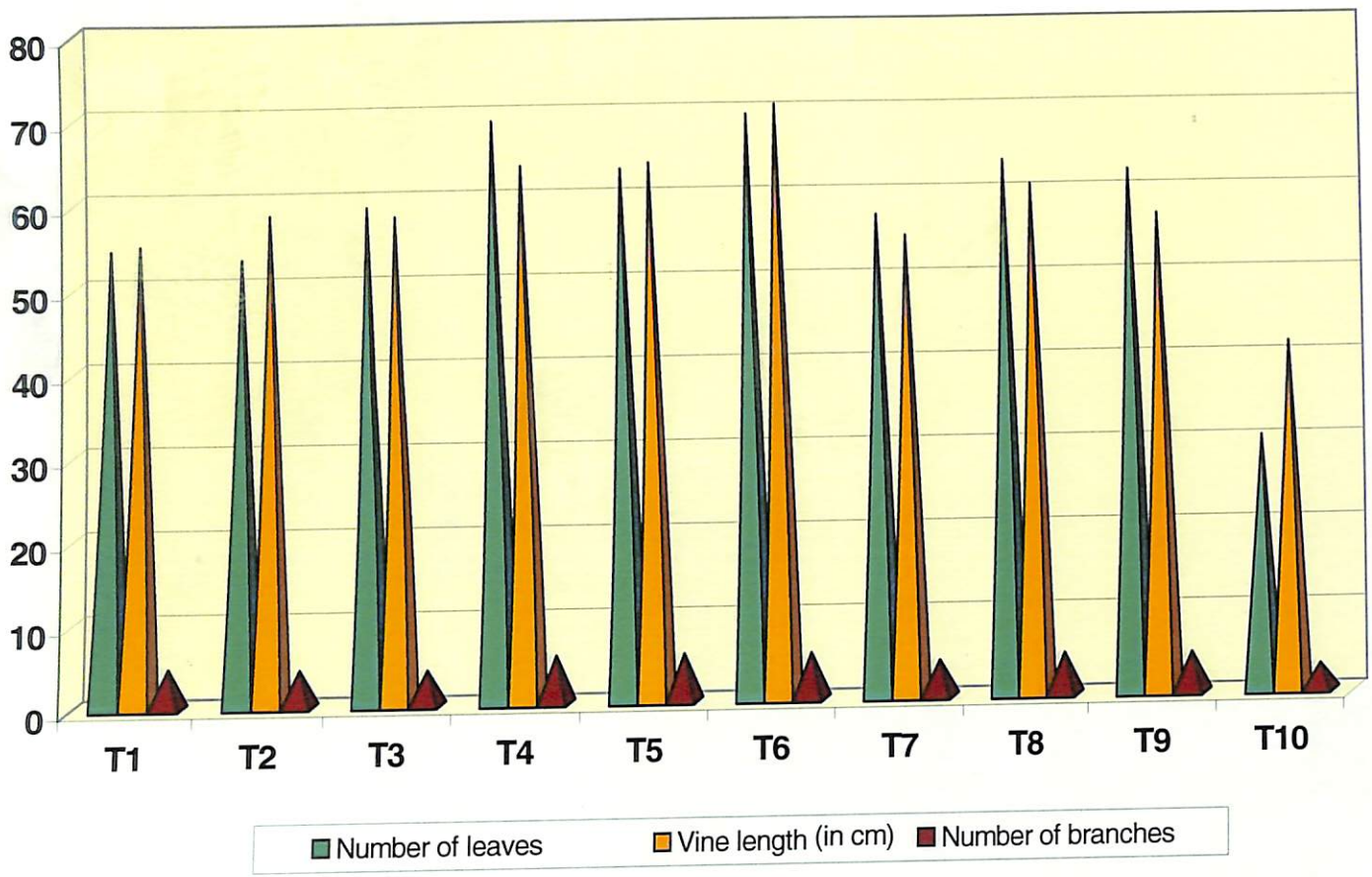

Fig.1. Effect of different treatments on shoot characters of thippali

and burrowing nematodes by the action of AMF in the root zone of various crops were already reported by several workers (Rajani *et al*., 1998; Koshy *et al.,* 1998; Sosamma *et al*., 1998 in kacholam, coconut and banana respectively). The efficacy of carbofuran in suppressing *M. incognita* activity and improving plant growth of french bean was already reported by Mohan and Mishra (1993).

Drenching with root extracts of *T. erecta* (T3) and mulching with wastes of *T. erecta* (T2) were also found to improve the biometric characters of thippali. Plants in control pots showed stunted growth with reduction in number of leaves and branches, which may be due to nematode infestation.

5.2.2 Yield

Yield characters recorded up to the time of uprooting presented in Table 2 revealed that all the treatments except control plants produced fruiting branches. But there was no spike formation in T1, T3 and T7, which indicated that these treatments were not effective in enhancing spike formation up to the time of uprooting.

Out of the treatments with spike formation, the yield attributes with reference to number of days to spike formation, spike diameter, fresh weight of spike and dry weight was superior in T6. It is evident that soil application of *B. subtilis* could effectively improve yield characters. *B. subtilis* is considered as a Plant Growth Promoting Rhizobacteria (PGPR) and was found to induce early spike formation in thippali plants. Reddy *et al.* (1999) reported maximum fruit yield in *B. subtilis* treated tomatoes. Gokte and Swarup (1988) opined that this may be due to the effect of some metabolites produced by the bacteria on the larvae of nematodes. Decrease in nematode population by *B. subtilis* may be due to the effect of lytic enzymes or due to the effect of volatile organic compounds produced by it.

Plants treated with *P. fluorescens* (T4) recorded more number of fruiting branches and spikes. *P. fluorescens* produces certain growth hormones which enhances the growth and vigour of the plants and thereby enhances spike yield. This finding is in line with that of Jyothi *et al*. (2003). They reported that highest yield (64.3%) and lowest *M. incognita* soil population could be obtained in tomato by using *P. fluorescens.* Plants mulched with wastes of *T. erecta* (T2) produced very long spikes of 3.80 cm. Similar observation was reported by Nisha (2001) where green leaf mulching was found effective in reducing the nematode population and increasing the yield of kacholam. Apart from the antihelminthic properties of *T. erecta,* the endothermic reactions which occur during decomposition and organic acids released will improve the physical and chemical properties of the soil, which will also aid the nematode management and boost yield.

5.2.3. Root characters

The results on root characters are presented in Table 3 and Fig. 2. Plants treated with *B. subtilis* (T6) were statistically superior to all other treatments with maximum root length, fresh weight and dry weight of root. Similarly the efficacy of *B. subtilis* in increasing the length and weight of roots in rice plants was observed by Govind (2005). Plant shoot and root fresh weight as well as root length of tomato were significantly increased, when treated with *B. cereus* for three root knot nematode species (viz. *M. incognita, M. javanica, M. arenaria*), when compared with non-bacterized plants (Mahdy *et al.,*2000). Siddiqui *et al*. (2001) reported that *B. subtilis* in combination with either neem cake or *Datura fastuosa* gave better control of the root rot and root knot infection with the enhancement of growth of urd bean compared to individual application.

With regard to the fresh weight of root at the time of uprooting, mulching with wastes of *T. erecta* (T2) was found to be a superior treatment. This was followed by drenching with root extracts of *T. erecta* (T3). Kumar and Reddy (2001) reported that treatment with marigold waste in sunflower improved

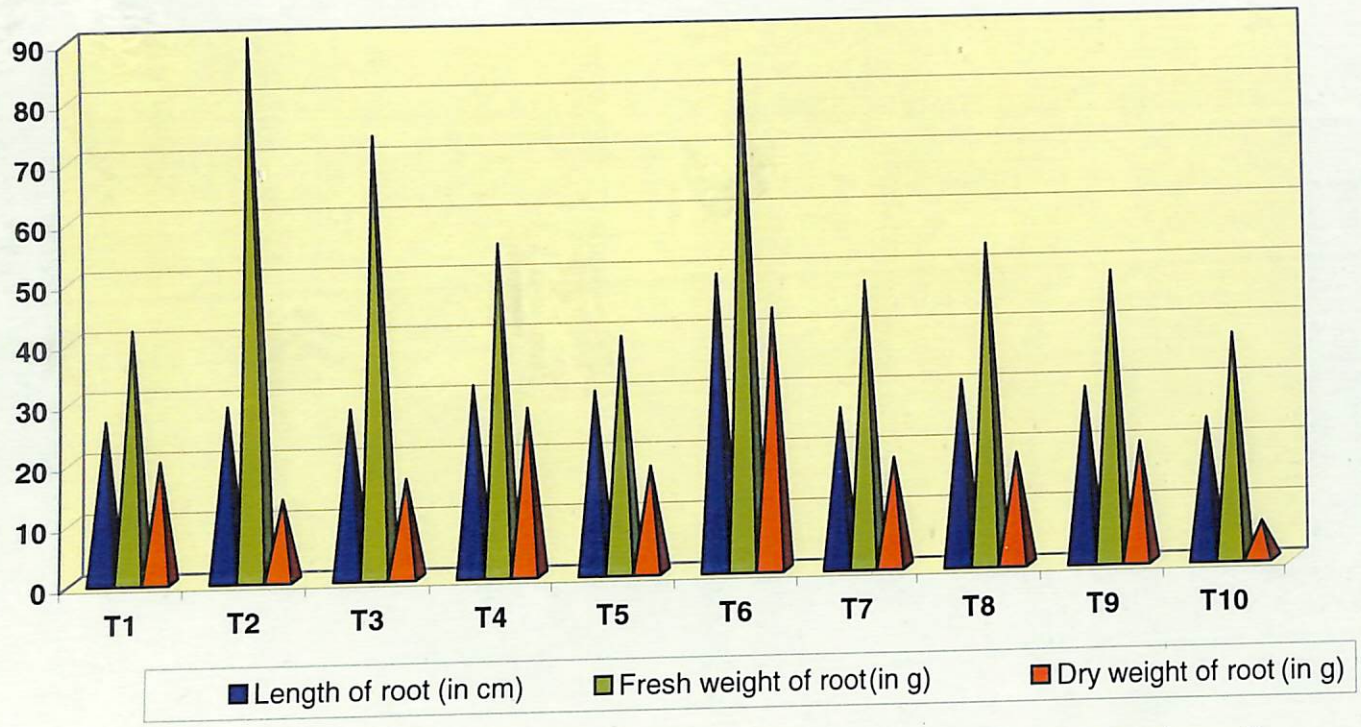

Fig.2. Effect of different treatments on root characters of thippali

the plant growth better over other individual treatment. Mani *et al.* (1986) studied the nematicidal effect of root and leaf extracts of *T. erecta, Brassica campestris, Vinca rosea* and *Azadirachta indica* against citrus nematode *Tylenchulus semipenetrans*. They found that juvenile mobility was decreased and neem showed 19.27% mobility at 5% dilution. The nematicidal property of *T. erecta* may be due to the presence of the chemical α-terthienyl produced by it.

Pseudomonas fluorescens (T4) was reported as the next superior treatment with respect to root length, fresh weight and dry weight of root. The present finding is in conformity with that of Devi and Dutta (2002) on okra. They found that *P. fluorescens* improved shoot and root length and weight, and reduced root gall number. AMF and carbofuran were the next superior treatments in improving root growth. Similar results were reported by Elsen *et al*. (2003), where they found that the decreased branching caused by nematodes were counterbalanced by the increased branching caused by the AMF. The potential of carbofuran in enhancing the root length and weight was already proved by Singh and Kumar (1995). They found that carbofuran 2kg ai. /ha was effective in reducing the population of *M. incognita* and increasing the growth parameters like root length, shoot dry weight, root fresh weight and number of leaves in Japanese mint.

Application of neem cake (T1) did not show any effect in improving the root characters. Control (T10) plants produced only very short roots with more number of multiple galls on it. Poor root growth may be attributed to the damage by second stage juveniles.

5.2.4 Nematode population

Data relating to the nematode population presented in Table 4. and Fig 3, revealed the influence of different treatments in reducing the number of nematodes in soil and root, number of egg masses and the number of galls on roots. Plants treated with *B. subtilis* (T6) recorded maximum reduction in the

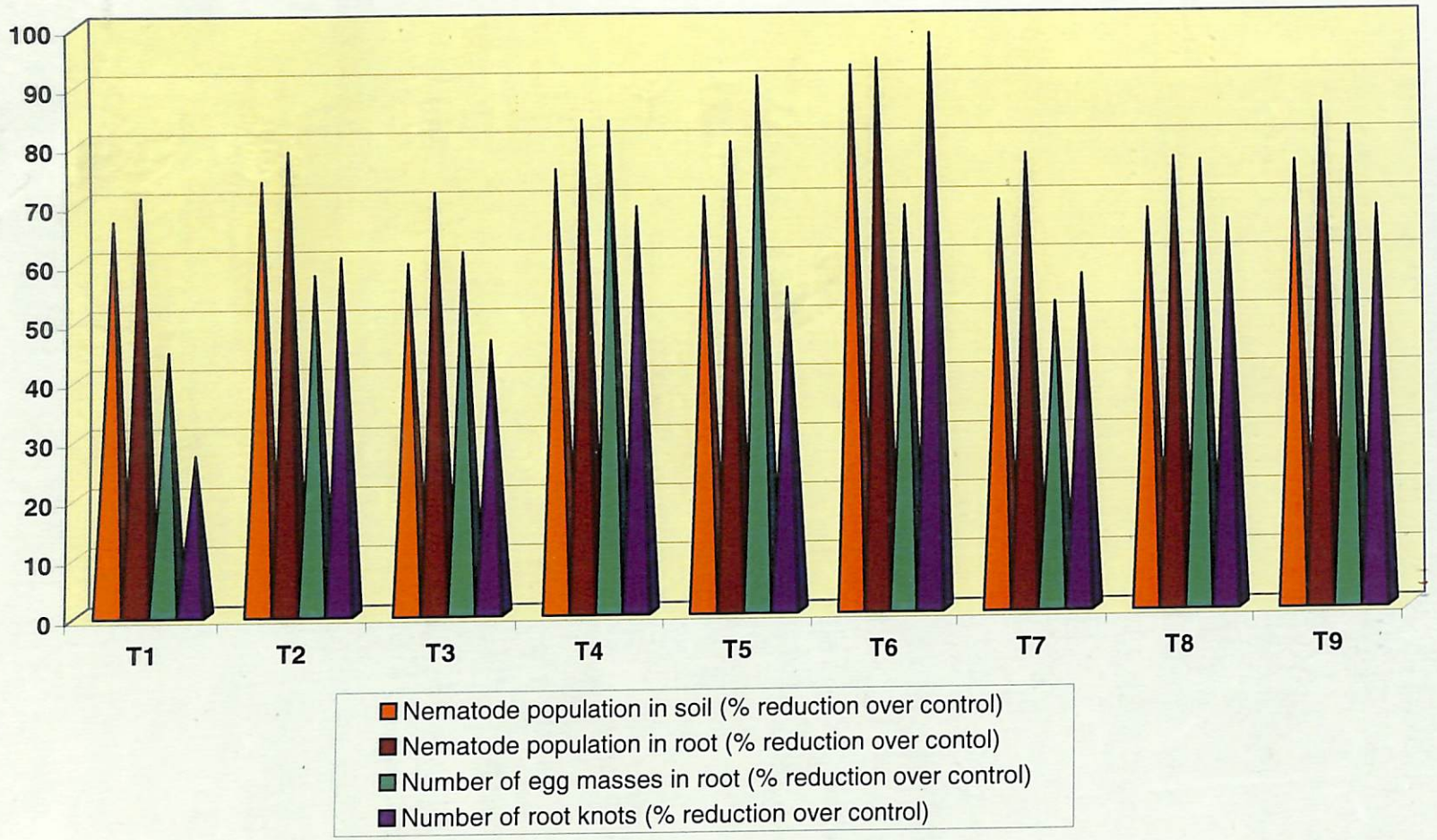

Fig.3. Effect of different treatments on nematode population

number of nematodes in soil, root and gall formation, giving 92.50, 93.62 and 97.84 per cent reduction over control. The least value for root knot index (1.00) was also observed for T6. The reduction in gall number indicated an effect on juvenile penetration. From the result, it is obvious that *B. subtilis* can be considered as an important biocontrol agent for the management of root knot nematode in thippali (*Piper longum* L.). This result confirmed the finding of Govind (2005) with respect to the management of root knot nematode, *M. graminicola* in rice. The inhibitory effect shown by *B. subtilis* may be due to the larvicidal effect of this biocontrol agent as reported by Sheela (1990). The study revealed that at 1.2 x 10^8 cells per ml of these bacteria caused 70 to 80 per cent larval mortality of *Heterodera oryzicola.*

T6 was closely followed by *P. fluorescens* (T4) and carbofuran (T9), giving significant reduction in nematode population in soil and root , gall formation and root knot index. The potentiality of *P. fluorescens* as a biocontrol agent in reducing the nematode population by rhizome treatment was already established by Nisha (2001). Mondal *et al*. (2000) reported the activity of *P. fluorescens,* including competition for space and nutrients, production of antibiotics, volatile and anti-microbial substances and compounds such as iron chelating siderophores and HCN.

A study conducted by Kalaiarasan *et al.* (2006) showed that a higher accumulation of peroxidase and PO1 isoform by Pf isolates might have collectively contributed towards induced resistance in groundnut plants against *M. arenaria.* The plant nematode interaction had also triggered the activities of defense enzymes when the nematode entered into the root. There was evident reduction in nematode population in carbofuran treated plants. This finding is in agreement with Haider *et al*. (1998), where application of carbofuran @ 1 kg ai./ha reduced root knot nematode population in turmeric.

With respect to the reduction in number of egg masses on root, *T. viride* (T5) was found to be the best treatment and was superior to T6. This may

be due to the ovicidal effect of *T. viride.* The reduction in number of egg masses demonstrated a possible effect on nematode development after penetration. The lytic enzyme, chitinase produced by the fungus, damages the eggshell and disrupts the cuticle of developing larvae. The present finding agrees with that of Reddy *et al.* (2000). They reported the parasitization of egg masses of root knot nematode by antagonistic fungus (*T. viride*) in tomato treated with this biocontrol agent.

There was statistically significant reduction in nematode population in all other treatments (AMF, *Tagetes* waste mulching, neem granules and drenching with root extracts of *T. erecta)* except T1 and T10. The efficacy of AMF as a biocontrol agent for reducing the nematode population in thippali as reported in this study is in line with that of Rao *et al*. (1995), Asha (1996), Rajani *et al*.(1998), Sivaprasad and Sheela (1998), Sundarababu *et al*. (1998), Joseph *et al*. (2001) and Sivaprasad *et al.* (2001).They reported the effectiveness of AMF especially *Glomus fasciculatum* in reducing the population of *M. incognita* in tomato, brinjal, kacholam, pepper, okra, ginger and cardamom respectively. Walia and Gupta (1997) reported that plant extracts of *Tagetes* spp. inhibited hatching in *M. javanica.* Above ground plant parts of marigold have been found toxic to nematodes (Siddiqui and Alam, 1998)

Compared to other treatments, soil application of neem cake (T1) was less effective in managing the population of nematodes. T1 showed very high population of nematodes in soil and root and root knot count. Root knot index of T1 was more or less same as that of control (T10) plants.

5.2.5 Vegetative parametric ratio

Vegetative parametric ratios expressed in terms of dry weight to fresh weight of shoot, dry weight to fresh weight of root and vine length to root length presented in Table 5 and Fig 4, reveals the impact of different treatments. It was found that plants treated with *T. viride* (T5) and carbofuran (T9) gave a higher

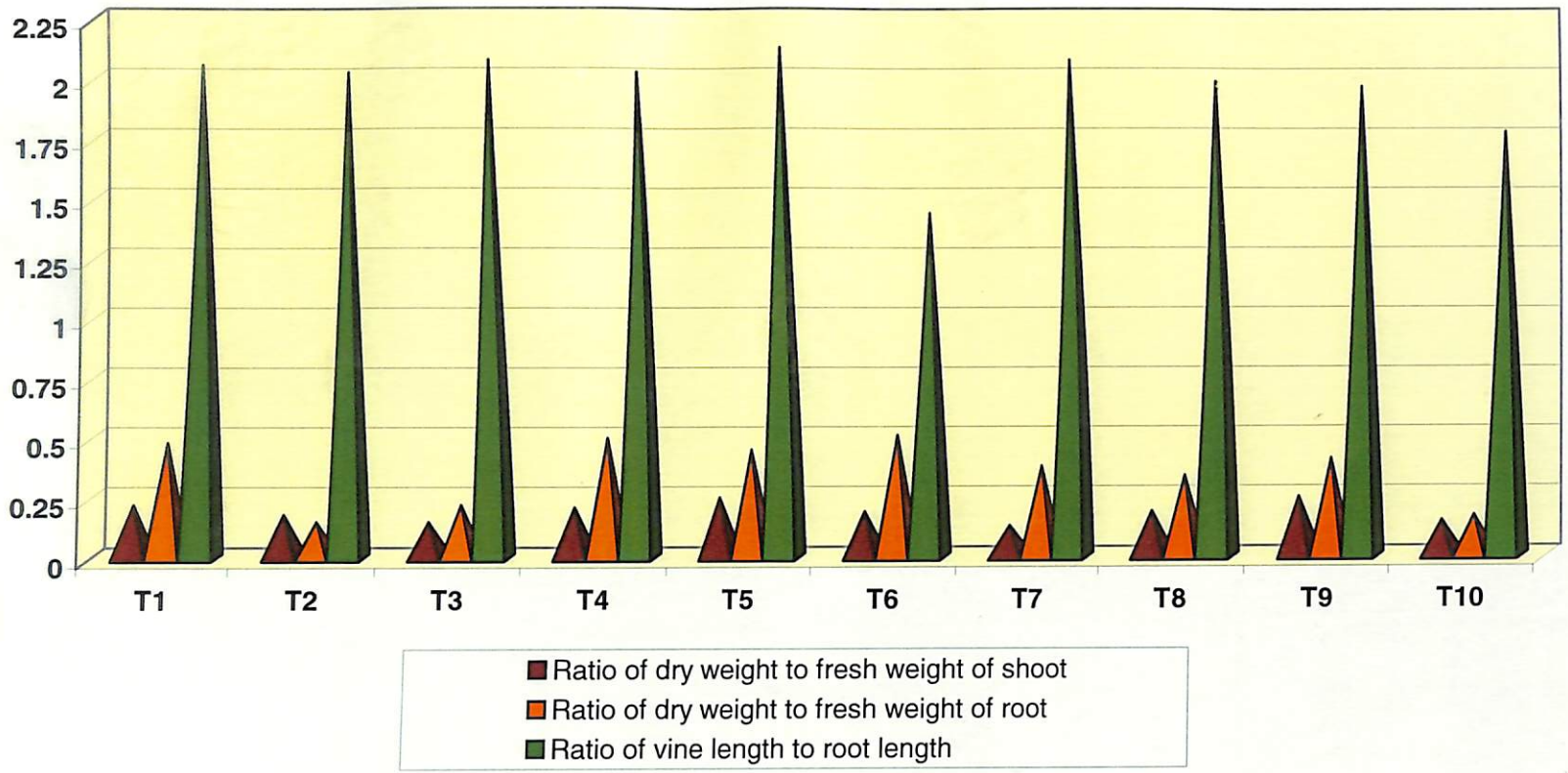

Fig.4. Effect of different treatments on vegetative parametric ratios

ratio of dry weight to fresh weight indicating that dry weight of both root and shoot was more with respect to fresh weight. The direct effect of *T. viride* in increasing the ratio was not reported earlier, but its effect on plant growth was reported by Pandey *et al.* (2003). Pot and field trials conducted by them to study the efficacy of different levels of *T. viride* against root knot nematode (*M. incognita*) in chickpea showed that all the treatments of *T. viride* showed significantly higher plant growth parameters over control. The gall development and final nematode population of *M. incognita* decreased with the increasing level of *T. viride* under pot and field conditions.

Next superior treatments were neem cake (T1), *P. fluorescens* (T4) and neem granules (T7). Numerous workers have advocated the use of neem and neem products for the management of root knot nematode. Anti-nematode effect of neem cake may be attributed to nematotoxic phenolic compounds, which are released during its degradation (Alam *et al*., 1979), apart from its stimulatory effect on root growth and predaceous fungi. The efficacy of *P. fluorescens* in improving plant growth was reported by Sheela *et al*. (1999), where the growth of brinjal seedlings showed improvement due to the reduction in the native population of phytonematodes.

In the present study, all treatments were statistically significant and showed a value greater than 1 for the ratio of vine length to root length, indicating that growth of vine was more compared to that of root. Vine growth is important in the case of thippali as it influences the formation of spikes, which are of medicinal use. Plants treated with *B. subtilis* (T6) were found to be inferior to control (T10) with regard to the ratio of vine length to root length.

5.2.6. Nematode relative ratio

The data regarding nematode relative ratio are presented in Table 6 and Fig 5. Plants treated with *B. subtilis* (T6) were recorded to be the most superior of all the treatments with maximum ratio of vine length to nematode

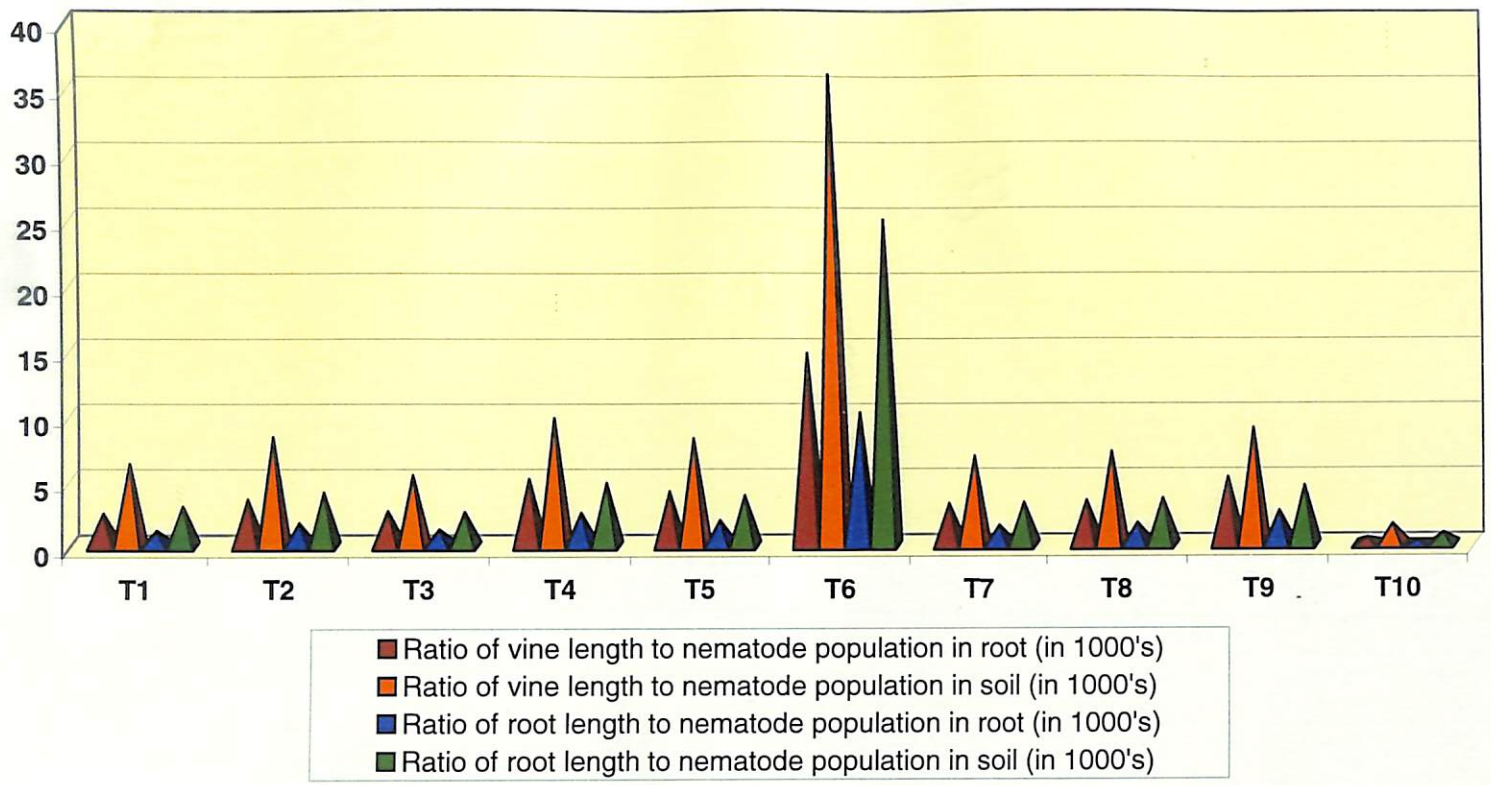

Fig.5. Effect of different treatments on nematode relative ratios

population in root (14.78), ratio of vine length to nematode population in soil (36.06), ratio of root length to nematode population in root (10.25), ratio of root length to nematode population in soil (25.02). This observation clearly indicated that the biocontrol agent *B. subtilis,* was capable of improving the root and shoot length and at the same time could effectively reduce the nematode population in root and soil. *B. subtilis* could improve plant growth as it is a Plant Growth Promoting Rhizobacteria (PGPR). Similar observation was reported by Racke and Sikora (1992). They revealed that the plant growth promoting rhizobacterium, *Agrobacterium radiobacter* and *B. sphaeriacus* increased the tuber yield of potato by suppressing the population of *Globodera pallida.*

Next superior treatments were *P. fluorescens* (T4) and carbofuran (T9). The superiority of *P. fluorescens* in reducing nematode population in root and soil was reported by Nisha (2001). The mechanism responsible for the reduction on nematode population may be due the ability of this bacteria to envelop or bind the root surface with carbohydrate-lectin, thereby interfering with normal host recognition process as reported by Oostendrop and Sikora (1990).

Carbofuran (T9) treated plants were equally superior to *P. fluorescens* in reducing the number of nematodes in soil and root and enhancing plant growth. Investigations carried out by Ravi *et al*. (2000) revealed that a combined application of neem cake, *T. viride* and carbofuran was the most effective treatment in increasing plant growth parameters (plant height, pseudostem girth, leaf area and number of leaves) and fruit yield and reducing the nematode population in both soil and roots of banana. Singh and Kumar (1995) concluded that carbofuran 2 kg a.i. per ha was effective in reducing the population of *M. incognita* and increasing the growth parameters like root length, shoot dry weight, root fresh weight and number of leaves of japanese mint.

There was significant reduction in nematode population in all other treatments (T2, T5, T7 and T8) except T3 and T1. In the case of plants treated with *Tagetes* waste mulch (T2), it was found to improve the vine length and root

length and decrease the nematode population in soil compared to control (T10). These findings are in line with that of Santhi and Sundarababu (1998) and Patel *et al.* (2000) who reported the beneficial effect of mulching in plant growth improvement, better biomass production and yield in the case of cowpea and tomato respectively. Many workers have reported the efficacy of application of green leaves for the management of nematode associated with different crops. Khanna and Sharma (1998) reported minimum galling in tomato roots due to incorporation of green leaves of neem and *Tagetes* with gall index less than or equal to two. The capability of suppression of nematode population may be the inherent quality of AMF. The mechanism of suppression may be either due to AMF induced physiological changes in the host or mycorrhiza induced changes in the root exudates causing fewer nematodes attracted to the host (Ahamed and Alsayed, 1991).

Plants treated with neem cake (T1) and those drenched with root extracts of *Tagetes* sp. (T3) were inferior compared to other treatments. T1 and T3 were found to enhance vine length and reduce nematode population in soil. But not much reduction was observed in the nematode population in root. Numerous workers have advocated the use of neem and neem products for the management of root knot nematode. A study conducted by Acharya and Padhi (1988) revealed that, neem oil cake applied @ 1 t/ha in drenches near the root zone of betel vine at the time of planting of vines was most effective in controlling the root knot nematode and increased the yield of betel vine. In addition to the slight nematicidal and nematostatic properties neem cake improves the soil condition and subsequently the vigour of plants. Aqueous extracts of leaf, stem and roots of *T. erecta* have been reported to be nematicidal against *M. incognita* by Mojumder and Mishra (1999). In the present study, control (T10) plants recorded the lowest value for all ratios. The nematode population in root and soil was very high and their feeding may have led to the reduction in root and vine length.

To sum up the findings, the present study revealed that biocontrol agents namely *B. subtilis, P. fluorescens, T. viride* and AMF showed very promising effect in improving the shoot, yield and root characters as well as reducing the nematode population in root and soil, gall formation and root knot index. The application of carbofuran, the commonly used pesticide for nematode management can be very well replaced by biocontrol agents, as they are ecofriendly and safe. As thippali is a medicinal plant, the use of chemical measures should not be encouraged. Among the biocontrol agents the Plant Growth Promoting Rhizobacteria, *B. subtilis* was found to be superior over other treatments. It could induce early spike formation in thippali, which is very important with regard to its medicinal use. Soil application of *P. fluorescens, T. viride* and AMF was found to improve growth parameters and reduce nematode population. The application of amruthguard also showed effectiveness in managing nematode population to some extent. However, soil application of neem cake was not appropriate to reduce nematode population in soil and also the entry of nematodes into root compared to other treatments.

SUMMARY

6. SUMMARY

A study entitled 'Management of root knot nematode in thippali (*Piper longum* L.)' was carried out at College of Horticulture, Vellanikkara, during June 2005 to August 2006. The objectives of the study included identification of the species of root knot nematode infesting thippali and evaluation of the effect of organic amendments, biocontrol agents, and a chemical for the management of this nematode.

The soil and root samples were collected from thippali plots already infested with root knot nematode at the medicinal plants garden of College of Horticulture, Vellanikkara. The white females collected from root knot nematode infested roots of thippali were sent to the Department of Nematology, Indian Agricultural Research Institute, New Delhi for species identification. The species attacking thippali was identified as *Meloidogyne arenaria* (Neal, 1989) Chitwood, 1949. This is the first report on the attack of *M. arenaria* on thippali from India.

Pot culture experiments were conducted to study the management of root knot nematode in thippali by organic amendment, biocontrol agents, mulching and drenching with *Tagetes erecta,* neem formulation and a chemical. The effect of the treatments on the shoot, yield and root characters, nematode population, vegetative parametric ratio and nematode relative ratio were tested.

Application of *Bacillus subtilis* resulted in an enhancement of shoot characters like number of leaves, vine length and fresh weight of shoot. As far as dry weight of shoot and number of branches are concerned *Trichoderma viride* was more effective. AMF, *Pseudomonas fluorescens* and carbofuran were also found effective in reducing the impact of nematode attack on shoot parameters. Neem cake application was found to have least effect on fresh weight of shoot and was the inferior treatment. Plants in control pots were dwarf with less number of leaves and branches, vine length, fresh weight and dry weight.

All plants except those in control pots produced fruiting branches but spike formation was observed only on plants treated with *B. subtilis, T. viride, P. fluorescens,* AMF, carbofuran and those mulched with *Tagetes waste.* Even though plants treated with *B. subtilis* were the first to produce spikes, an increase in spike number was noticed in *P. fluorescens* treated plants. Plants mulched with wastes of *T. erecta* produced very long spikes.

Among the different treatments studied, application of *B. subtilis* showed a rapid increase in root length, fresh weight and dry weight of root. Drenching with root extracts of *T. erecta* and mulching with *Tagetes* waste showed a significant increase in root characters, which was followed by AMF and carbofuran.

All the different treatments suppressed nematode population compared to control, though the efficacy regime was not alike. The prophylactic application of the biocontrol agents viz. *B. subtilis, P. fluorescens, T. viride* and AMF, produced a soil condition capable enough to suppress the population build up of nematodes in soil and root and kept the infection at a lower level. Maximum reduction in root knot index was observed in plants treated with *B. subtilis. T. viride* was found to be the most effective in reducing the number of egg masses and this may be due to its ovicidal effect. The application of amruthguard also showed effectiveness in managing nematode population.

The dry matter content of a plant is very important. The vegetative parametric ratios revealed that the fresh weight of plants was also pronounced in terms of dry weight. Soil application of *B. subtilis* produced a rapid increase in root length compared to vine length and thus gave a lower ratio.

The relative ratio of nematode population showed that treatment with *B. subtilis* produced a microenvironment most favourable for plant growth and at the same time suppressing nematode multiplication. Even though all treatments

were effective in nematode management compared to control, application of bioagents viz. *B. subtilis, T. viride, P. fluorescens* and AMF were more effective than other treatments.

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MANAGEMENT OF ROOT KNOT NEMATODE IN THIPPALI (*Piper longum* L.)

By

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ABSTRACT OF THE THESIS

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ABSTRACT

The objectives of the study entitled ‘ Management of root knot nematode in thippali (*Piper longum* L.)’ were to identify the species of root knot nematode infesting thippali and to study the management of this nematode using organic amendments, bioagents and a chemical. Soil and root samples were collected from thippali growing plots already infested with root knot nematodes. The species of root knot nematode attacking thippali was identified as *Meloidogyne arenaria* (Neal, 1889) Chitwood, 1949. This is the first report on the attack of *M. arenaria* on thippali from Kerala.

Pot culture experiments were conducted to study the management of root knot nematode infesting thippali using neem cake, mulching with *Tagetes* waste, drenching with root extracts of *Tagetes erecta,* different bioagents, a chemical and a neem formulation. The effect of various treatments on the shoot, yield and root characters, nematode population, vegetative parametric ratio and nematode relative ratio were tested.

Among the various treatments studied the application of bioagents (viz. *B. subtilis, T. viride, P. fluorescens,* AMF) improved the growth of thippali with maximum vine length, number of leaves, number of branches, root length, shoot and root weight and minimum root knot index, gall formation and nematode population in root and soil. Early spike formation and also an increase in number of spikes were observed in plants treated with *B. subtilis* and *P. fluorescens* respectively. The control of root knot nematode achieved as a result of application of biocontrol agents was superior to that due to carbofuran application. The study clearly indicated that the root knot nematode population in *P. longum* can be effectively managed using the bioagents and is a better alternative to nematicide application.