MANAGEMENT OF ROOT-KNOT NEMATODE, *Meloidogyne* sp. (Kofoid and White) IN COLEUS, *Solenostemon rotundifolius* (Poir) Morton

By

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THESIS

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DECLARATION

I, Lakshmy K. Mohan (2011-11-119) hereby declare that this thesis entitled **'Management of root-knot nematode, *Meloidogyne* sp. (Kofoid and White) in coleus, *Solenostemon rotundifolius* (Poir) Morton'** 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 of any degree, diploma, associateship, fellowship or other similar title of any other University or Society.

Vellanikkara
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CERTIFICATE

Certified that this thesis, entitled **'Management of root-knot nematode, *Meloidogyne* sp. (Kofoid and White) in coleus, *Solenostemon rotundifolius* (Poir) Morton'** is a record of research work done independently by **Mrs. Lakshmy K. Mohan** under my guidance and supervision and it has not previously formed the basis for the award of any degree, diploma, associateship or fellowship to her.

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Lakshmy K. Mohan

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Introduction

1. INTRODUCTION

Tuber crops occupy a remarkable position in food security of the developing world due to their high calorific value and carbohydrate content. Coleus, *Solenostemon rotundifolius* (Poir) Morton (Syn. *Coleus parviflorus* Benth.), commonly known as chinese potato, *koorka* (Malayalam) and *koorgan kilangu* (Tamil) is a short duration (120-140 days) under-exploited minor tuber crop. Coleus is grown extensively as a vegetable crop in most of the homestead gardens in Kerala and Tamil Nadu (Hrishi and Mohankumar, 1978). High production potential, low cost of cultivation, consumer preference, good market demand and almost assured high returns make the crop highly popular among vegetable growers in Kerala as evidenced by the steady increase in its area of cultivation over past few years (George, 2008).

Coleus comes up well in the warm humid climatic conditions and well drained medium fertile soil. It is raised purely as a rain-fed crop in Kerala from June to December. Coleus is a small herbaceous bushy annual with succulent stems and aromatic leaves. The plant bears a cluster of heteromorphous tubers with aromatic flavour, which makes it likeable as a delicacy among the vegetables. Coleus tuber contains 14.7 to 20.8 per cent starch, 0.04 to 0.31 per cent protein, 0.57 to 0.96 per cent sugar, minerals like calcium and iron, vitamins like thiamine, riboflavin, niacin and ascorbic acid. Coleus is important from the medicinal perspective also, as the flavanoids present in the tuber are said to lower down the blood cholesterol level (George, 2008).

Coleus plants are relatively free from insect pest and diseases though leaf folders, mealy bugs, tingids, leaf beetles and stem borers were noticed. But one of the major constraints in production of coleus tubers is the damage caused by root-knot nematode (*Meloidogyne* sp.). They are endoparasitic nematodes having extremely wide host range. In India, four major species of root-knot nematode *viz., M. incognita, M javanica, M. arenaria* and *M. hapla* are known to be widely distributed attacking a wide host range of agricultural crops (Dasgupta and Gaur, 1986; Khan *et al.,* 1994; Jain and Hasan, 1995). *M. incognita* has been found associated with 232 host plants like vegetables, pulses, oil seed crops, fruit crops, fiber crops, ornamental and plantation crops (Upadhyay and Dwivedi, 2008).

The root-knot nematode infestations on different tuber crops have been reported by many workers. In Kerala, root-knot nematode infestation in coleus has been reported by Sathyarajan *et al.* (1966). Coleus being a tuber crop, attack of soil inhabiting *Meloidogyne* spp. occurs on roots and tubers of the crop. The crop loss due to *M. incognita* on coleus (*Coleus parviflorus*) was 92 per cent (in terms of fresh weight of tubers) at an inoculum level of 10,000 *M. incognita* juveniles per pot of 10 litre capacity (Sosamma, 1988).

The root-knot nematode infested plants were found to be stunted and symptoms mostly observed as yellow patches in the field which are due to the random distribution of nematodes and these patches grow gradually. The other above-ground symptoms of root-knot nematode injury were wilting, reduced yield and premature death of plants. Severely affected plants will often wilt readily because galled roots have only limited ability to absorb and transport water and nutrients to different parts of the plant, severely infested plants may wilt even in the presence of sufficient soil moisture, especially during the noon. Plants exhibit nutrient deficiency symptoms because of their reduced ability to absorb and transport nutrients from the soil. Additional fertilization will not generally result in remediation of root-knot nematode-induced chlorosis. At high densities, root-knot nematodes can actually kill host plants, particularly if the high populations occur early in the growing season when the plants have minimal root.

The below-ground symptoms are prominent than above-ground symptoms due to the presence of swollen, knotted roots and galled tubers. The second stage juveniles of root-knot nematode enter the roots of host plants near root tips, move through the cortex and remain inside the pericycle of root for their life. As it enters, hypertrophy of cortical cells occurs and after the establishment, they secrete enzymes. The adjacent 8 to 10 cells near the head of nematode larvae enlarge and division of nuclei takes place leading to giant cell formation. In severe cases, rotting of roots and tubers become evident by the third month of planting. Infested plants yield less marketable tubers and the attack of the nematode also affect consumer acceptance of the tubers.

The root-knot nematodes continue to multiply inside the tuber even after harvest, during storage also. Mohandas and Ramakrishnan (1998) reported that coleus tubers heavily infested started rotting, while less infested tubers shrunk and develop more prominent galls during storage. The shrinkage was more pronounced starting from six weeks after storage.

The losses caused by the plant parasitic nematodes are enormous which necessitates efficient control measures. Most of the farmers are ignorant of the nematode problem in coleus. Nematicides are very effective in controlling plant parasitic nematodes. But many nematicides were withdrawn from the market due to their ill-effects like residue problems, environmental pollution and health hazards. Other effective and environment friendly methods have to be adopted and this trend has gained momentum in recent years. So the present study entitled "Management of root-knot nematode, *Meloidogyne* sp. (Kofoid and White) in coleus, *Solenostemon rotundifolius* (Poir) Morton" was proposed to evolve an effective and environmental friendly management strategy utilizing biocontrol agents, organic amendments and a chemical insecticide against the root-knot nematode in coleus with the following objectives.

1.) To assess the population of plant parasitic nematodes infesting coleus in different coleus growing regions of Thrissur District.

2.) To identify the species of *Meloidogyne* infesting coleus.

3.) To evolve the most effective management strategy against root-knot nematode, *Meloidogyne* sp. in coleus utilising biocontrol agents, organic amendments and a chemical insecticide.

Review of literature

2. REVIEW OF LITERATURE

The root-knot nematode, *Meloidogyne* spp. is an obligate endoparasite having a wide host range. More than 80 species of root-knot nematode have been reported from different parts of the world and 13 species from India and more than 2000 plant species have been reported as host plants of *Meloidogyne* spp. (Upadhyay and Dwivedi, 2008). Many workers have reported the infestation of root-knot nematode on different tuber crops. Sathyarajan *et al.* (1966) reported the occurrence of root-knot nematode in coleus from Kerala. Coleus being a tuber crop, attack of soil inhabiting *Meloidogyne* spp. occurs on roots and tubers of the crop. The yield loss due to *M. incognita* on coleus (*Coleus parviflorus*) was 92 per cent at an inoculum level of 10,000 *M. incognita* juveniles per pot (Sosamma, 1988). The nematode continue to multiply within the tuber after harvest during storage. Mohandas and Ramakrishnan (1998) reported that coleus tubers heavily infested started rotting, while less infested tubers shrunk and develop more prominent galls during storage. The shrinkage was more pronounced starting from six weeks after storage. The heavily infested tubers suffered rapid weight loss compared to healthy and less infested tubers.

Literature pertaining to the pest status of root-knot nematode, *Meloidogyne* spp. is presented here. The literature on management of root-knot nematode using organic amendments, bacterial and fungal biocontrol agents and a chemical insecticide are also included.

2.1 PEST STATUS AND CROP LOSS

In India, four major species of root-knot nematode *viz., M. incognita, M. javanica, M. arenaria* and *M. hapla* were known to be widely distributed on a wide host range of agricultural crops (Dasgupta and Gaur, 1986; Khan *et al.,* 1994; Jain and Hasan, 1995).

In turmeric, an avoidable yield loss of 45 per cent was observed due to *M. incognita* infestation (Hebsybai *et al.,* 1995). The avoidable yield loss due to *M. incognita* in ginger was 43.00 per cent at an initial population level of 166 juveniles per 250 g soil sample (Sheela *et al.*, 1995). In potato, *M. incognita* population density @ one J_2 per g of soil was considered as the damage threshold level (Nagesh, 1996).

Mohandas and Ramakrishnan (1997) reported that an initial inoculum level of 100 juveniles of *M. incognita* per plant caused significant reduction in both fresh and dry fibrous root weight and tuber yield of *Dioscorea rotundata* Poir. Makhnotra and Khan (1997) observed yield loss of 20.00 per cent at an initial *M. incognita* population of 200 larvae/ 200 cc soil in ginger.

There was a loss of 0.35 per cent in fruit yield (0.811 g) per tree for every one nematode increase per 5 g root due to *M. incognita* infestation in papaya (Ramakrishnan and Rajendran, 1998). A study of phytonematodes associated with medicinal plants in Kerala revealed the occurrence of root-knot nematodes 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).

Sheela and Rajani (1998) observed that an initial population of 1000 J_2 (*M. incognita*) larvae per kacholam plant reduced the production of leaves, length and weight of rhizome by 43, 24 and 64 per cent respectively. But at low population levels of 200 *M. incognita* larvae/ plant also considerable reduction was noticed with 18, 6 and 15 per cent with regard to number of leaves, length and weight of rhizome respectively.

A survey undertaken in nine districts of Bundelkand region of India to determine the presence of root-knot nematodes associated with major food and fodder crops revealed 36 per cent incidence of root knot nematode attack. Among the four species of root-knot nematode, *M. incognita* was the most frequent followed by *M. javanica* and *M. arenaria* (Hasan and Jain, 1998).

The biometric characters and yield of *Ocimum sanctum* L. was significantly reduced by *M. incognita* compared to uninoculated plants and thus reduction was in accordance with the initial inoculum levels (Haseeb *et al.*, 1999). In red beet, *Beta vulgaris* L. var. Crassa. 50 nematodes per kg soil caused significant decrease in fresh (14.62 per cent) and dry (21.60 per cent) root weight (Pathak and Keshari, 2000).

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).

A survey was undertaken during 2005-06 in six districts of Tamil Nadu to determine the association of plant parasitic nematodes with medicinal coleus. *M. incognita* was the most predominant nematode species in terms of absolute density, relative density and prominence value, followed by *Hoplolaimus indicus* (Seenivasan and Devrajan, 2007). Rao (2007) reported the presence of *M. incognita* on 25-55 per cent of tomato and capsicum seedlings produced in the open conditions in Southern India.

Singh *et al.* (2010) conducted field surveys to study the adverse effect of rice root-knot nematode, *Meloidogyne graminicola* in wheat during 2004–05 and 2005–06 and reported that severe infection of *M. graminicola* reduced height of wheat plants by 50 per cent. The number of grains per ear head was also reduced in the infested plants which were related to number of galls per plant.

The avoidable yield loss in carrot due to *M. hapla* was 35.95 per cent. Symptoms of root-knot nematode damage were severe galling, large proliferation of secondary roots, tap root malformation, forked and stubby carrots resulting in poor quality (Anita and Selvaraj, 2011).

2.2 MANAGEMENT

2.2.1 Organic amendments

The beneficial effects of organic amendments in reducing the plant parasitic nematodes have gained much importance in recent years. The relevant literature pertaining to this study was reviewed and presented.

2.2.1.1 Neem cake

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 or saw dust reduced *M. incognita* population significantly in chickpea.

Sundararaju and Sudha (1993) reported the effectiveness of neem oil cake @ 1kg/ palm/year in reducing the nematode population and significantly increasing the yield in arecanut, banana and black pepper, under arecanut based farming system.

Soil amendment with neem cake at 0.1, 0.5 and 1.0 per cent w/w reduced infection of *M. incognita* on mungbean (*V. radiata*) and significantly improved the plant height, but reduced root nodulation (Abid *et al.*, 1995). Kaul and Bhat (1995) reported that spot application of neem cake @ 30 g per plant was most effective in reducing the larval population of *M. incognita* infesting tomato. It resulted with increase in yield by 30.60 and 40.60 per cent over control in shoot and root length respectively.

Thakur and Darekar (1995) reported that neem cake application @ 35 g per plant reduced root galling by *M incognita*, eggs per egg mass, root-knot index and increased shoot and root weight and yield of aubergine. Soil amended with neem cake and datura powder were effective for the control of *M. incognita* and root-knot disease complex of okra (Haque *et al.*, 1996).

Neem cake application @ 80 q/ha was more effective than carbofuran @ two kg a.i. ha^{-1} in reducing the nematode population and improving the yield of tomato (Jain and Gupta, 1997). Organic amendments *viz.*, sawdust, neem cake and poultry manure each @ 1000 kg ha^{-1} found to be effective against *M. incognita* in reducing the galls and final nematode population with significant increase in the yield of carrot (Devi and Das, 1998).

Neem cake and neem dust were found effective in suppressing root-knot nematode, *M. incognita* infesting tomato plants (Jacob *et al.*, 1998). Amending soil with neem cake was effective in reducing gall formation, *M. incognita* population and increasing the yield in ginger (Vadhera *et al.*, 1998).

Application of neem cake was found to reduce the fecundity of root-knot nematode infesting pointed gourd, *Trichosanthes dioica* cv. Damodar Kajli (Chakraborti, 2000). Neema (2001) reported that in betel vine cv. Bangla Budagar, application of neem cake at two tons ha^{-1} resulted in highest reduction of *M. incognita* population (62.11%) and number of galls (57.00%).

Neem cake application @ 25 g/1000 g of soil significantly reduced the number of galls and number of egg masses per plant in mungbean cv. MNH-92 (Shafique *et al.*, 2001). The fecundity of root-knot nematode, *M. incognita* infesting onion was reduced due to the application of neem cake (Chakraborti, 2003). Neem cake application @ 200 g m^{-2} was very effective in reducing the nematode population in kacholam rhizosphere (Rajani, 1998; Nisha and Sheela, 2003).

Neem cake @ 20 g per 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). Pandey *et al.* (2003) reported that neem compounds were highly useful in suppressing *M. incognita* population and improving the herb yield in brahmi, *Bacopa monnieri*.

Ahmed and Choudhury (2004) conducted field experiment to know the efficacy of four organic amendments *viz.*, saw dust, poultry manure, mustard cake and neem cake against root-knot nematode infesting french bean and found that spot application of neem cake was effective in reducing gall, egg masses and soil population of *M. incognita*. Spot application of all organic amendments was comparatively better than furrow application in reducing the root knot nematode population and increasing the yield. Naidu *et al.*, (2007) found that neem cake extract was better in inhibiting *M. incognita* juvenile emergence at 10 per cent concentration (1.04 per cent emergence) and at 5 per cent concentration (2.84 and 3.43 per cent emergence).

Seenivasan (2010) evaluated the efficacy of locally available organic amendments (at the rate of 500 kg/ha and 400 kg/ha) against *M. incognita* in medicinal coleus, *Coleus forskohlii* and found that neem cake was significantly superior in reducing the nematode population and increasing growth and yield of medicinal coleus than pungam cake, vermicompost and castor cake. Neem cake application reduced *M. incognita* populations by 28.6 -31.2 per cent in soil and coleus plants recorded the gall index of 3.3 - 4.0. It increased the tuber yield by 39.8 - 42.4 per cent under glasshouse condition and 9.0 per cent under field conditions.

Under Kerala conditions, application of neem cake @ 1.0 t ha^{-1} at planting followed by the application of neem cake @ 1.0 t ha^{-1} at 45 days after planting was effective in controlling the nematode infesting ginger in endemic areas (Kerala Agricultural University, 2011) .

An experiment was carried out by Rajvanshi and Bishnoi (2012) on cowpea (variety-RMG 268) and Mungbean (RG 268) with eight treatments against *M. incognita viz*; neem cake @ 5 q/ha (soil application), neem oil at 10 ml/kg seed, neem seed kernel powder (NSKP) at 10 per cent, Neem baan at 10 per cent, T*richoderma viride* @ 2.5 kg/ha (soil application), Carbosulfan at 2 per cent (seed soaking) along with treated check (Carbofuran @ 1.5 kg ai/ha - soil application) and untreated check. They found that the grain yield of carbosulfan treatment (8.64 q/ha) was on par with the neem seed kernel powder treatment (NSKP) (8.50 q/ha) followed by neem cake (7.63 q/ha) in mungbean with reduction in nematode population from 20.90 to 9.77.

2.2.1.2 *Tagetes* sp.

Sweelam (1989) conducted pot experiment to control *M. javanica* on tomatoes and found that root exudates of marigold (*Tagetes erecta*) reduced nematode populations by 69.5 per cent and the egg masses by 68.6 per cent. Agro-wastes of harvested marigold (*T. erecta*) applied at the rate of 25g per kg soil in pots showed highly significant inhibitory effects on the development of *M. incognita* and populations of other plant parasitic nematodes (Akhtar and Alam, 1990).

Addition of dried chopped portions of marigold (*T. erecta*), at the rates of 10, 20, 30 g/pot caused reduction in the eggmass production and gall formation on sunflower plants infested with *M. incognita*. (Abadir *et al.*, 1994). Polthanee and

Yamazaki (1996) found that the marigold grown and incorporated into soil before planting rice suppressed nematode root galling, and increased rice grain yield by 46 per cent over the untreated check. The increase in yield might be attributed to reduction in root knot nematode densities in soil by marigold which also serve as organic manure and provide nutrients for rice growth.

Aqueous extracts from 30 and 60 day old marigold plants (*Tagetes* spp.) inhibited egg hatch of nematode juveniles considerably at all the concentrations compared to control. The juveniles hatched in 60 days old plant extracts were immobile too. The extract without dilution had irreversible toxic effect and no hatching was observed in this treatment. Root exudates without dilution irrespective of the age of the plant, inhibited maximum hatch which decreased with the increase in dilution. Incorporation of chopped *Tagetes* leaves @ 40 and 80 g/kg soil significantly increased tomato and brinjal plant growth and reduced number of galls, egg masses and final juvenile population in soil (Walia and Gupta, 1997).

Marigold cultivars, Polynema and Nema-gone were grown as cover crops and they were chopped and incorporated into the soil reduced larval population of *Meloidogyne* sp. and root galling on lettuce plants. Marigold produced no toxic residues in soil and can provide an alternative means for replacing nematicides (Abawi and Vogel, 2000). Ploeg (2000) reported that amending soil with marigold (*Tagetes patula* cv. Single Gold) increased the weight of tomato plant tops and reduced galling and final nematode population levels.

Ray *et al.* (2000) determined the chemical composition and nematicidal activity of the volatile and non-volatile fractions of *T. erecta*. The non-volatile compounds isolated were dodecanoic acid, myristic acid, mixture of palmitic acid and steric acid and mixture of octaeicosane-8-one and triacontane-1-ol. Essential oils obtained through hydro-distillation of *T. erecta* flowers were alhpa-sesquiphellendrene, beta–sesquiphellendrene, 2-methyl-6-(4-methyl cyclohexadienyl), hept-4-en-2-ol, myristoleic acid and trieicosane. The methanol extract and essential oils of marigold flowers showed maximum nematicidal activity against *M. incognita* juveniles. The ED50 was 852 and 396 micro g ml^{-1} after 24 hrs of exposure to the methanol extract and essential oils, respectively. Among the two purified compounds,

the nematicidal activity of myristic acid was more pronounced than dodecanoic acid against the nematode juveniles.

The marigold extract was more lethal to *M. javanica* juveniles of okra in the mortality test counted after 12, 24, 48 and 96 hrs compared to turmeric and dhalkalmi extracts (Hassan *et al.*, 2003). Cannayane and Rajendran (2004) found that the extracts from freshly collected plant material of *T. erecta* caused mortality of *M. incognita* juveniles within 48 hrs of exposure to extract.

The plant extracts of *Tagetes erecta* caused more than 50 per cent mortality of second stage juvenile (J2) of *M. incognita* in 24 hrs and showed inhibitory effect on egg hatching (Yang-XiuJuan *et al.*, 2004). The water extracts from seed exudates of *T. erecta* cv. Crackerjack and *T. patula* var. *polynema* caused significantly higher mortality to *Heterodera schachtii, Meloidogyne hapla* and *Pratylenchus penetrans* (Riga *et al.*, 2005)

Aggarwal *et al.*, (2005) conducted studies to manage the incidence of bacterial wilt in tomato using either plant refuge or root extract of *Tagetes erecta.* Results showed that bacterial wilt of tomato caused by *Ralstonia solanacearum* was aggravated in soils having combined infection of the bacterium and the root-knot nematode, *M. incognita.* Nematode buildup and incidence were hampered by the plant refuge of *T. erecta* and nematode population was also affected adversely in soils treated with root extract of marigold.

The shoot and root extracts of nine Asteraceae species from Pakistan tested *in vitro* showed that the root extracts were more effective than the corresponding shoot extracts in terms of nematode, *M javanica* inhibition. But the shoot extract of *T. erecta* resulted in the highest nematode juvenile mortality and soil amendments with *T. erecta* increased shoot fresh weight (Siddiqui *et al.*, 2005)

Natarajan *et al.* (2006) tested cold aqueous extracts (20 per cent w/v, 100 ml aliquots) of pre and post flowering whole plants, root and stem portions of *T. erecta* for the ability to control *M. incognita* in infested soil (10 kg) in pots planted with susceptible *Lycopersicon esculentum.* Whole *T. erecta* plant extracts were more effective than stem extracts although both were more effective than root extracts. The extracts from 40-day old plants were more efficacious than those from 70 day old

plants. Plant height, leaf number and fruit yield were significantly greater in *T. erecta* treated tomato plants than the plants grown in untreated infested soils. Root gall indices of *L. esculentum* treated with *T. erecta* plant extracts were significantly lower than untreated checks.

The aqueous plant root extracts of marigold (*T. erecta*), nitta (*Hyptis suaveolens*) and basil (*Ocimum gratissimum*) plants were applied to root-knot nematode infested soil at four concentrations, *viz.* 25000, 500000, 750000 and 1 million/ppm per tomato plant, reduced the root-knot nematode populations in the soil with corresponding increases in plant height, plant leaf and fruit yield over the untreated control treatment (Olabiyi, 2006).

The chopped leaves of marigold applied at 100g/pot has the gall index of 0.64 due to root-knot nematode, *M. incognita* and root galling was also reduced to some extent when the dose was reduced to half (50 g/pot) (Rather *et al.*, 2008). The methanol fractions and the purified compounds from marigold (*T. erecta* L.) flower petal extracts yielded best result against the juveniles of *M. incognita* (Ray *et al.*, 2010).

2.2.1.3 *Chromolaena* sp.

The movement of *M. incognita* larvae was inactivated within 3 hours in 1:1 and 1:5 diluted extracts of *Eupatorium odoratum* and they failed to regain activity when transferred to distilled water. The same effect was observed with 1:10 and 1:20 extracts after 48 hours (Subramaniyan, 1986).

Saxena *et al.* (1990) tested the plant extracts of *Acorus calamus, Millettia ovalifolia, Messua ferrea* and *Eupatorium* sp. for their nematicidal activity against *M. incognita* and found that the *Eupatorium* extract showed less than 100 per cent mortality of second stage juveniles of *M. incognita.*

Ajith and Sheela (1996) observed that the addition of chopped green leaves of neem and *Eupatorium* (15 t/ha) effectively reduced *M. incognita, Rotylenchulus reniformis* and *Helicotylenchus* spp. on bhindi [okra] and cowpea in pot experiments.

The effects of leaf mulches of different plants applied at 5 kg/m^2 at 15 days before planting of Kacholam (*Kaempferia galanga*) were tested and observed that the mulch from *C. odorata* reduced *M. incognita* population by more than 60 per cent with lowest gall index. Highest number of leaves were also obtained by mulching with *C. odorata* (Nisha and Sheela, 2002).

According to Thoden *et al.* (2007), 1,2-dehydropyrrolizidine alkaloids (PAs) from the roots of *C. odorata* have nematicidal effects on the root-knot nematode *M. incognita*, at concentrations of 70-350 ppm *in vitro*. *In vivo* experiments showed that mulch or aqueous crude extracts from *C. odorata* roots reduce the damage by *M. incognita* on lettuce plants.

The root-knot nematode and reniform nematode associated with cowpea can be effectively managed by the application of neem and eupatorium leaves @ 15 t ha^{-1} two weeks before sowing (Kerala Agricultural University, 2011).

Odeyemi *et al.* (2011) conducted pot experiment to determine the effects of organic fertiliser and *C. odorata* residue at 1 per cent w/w on the pathogenicity of *M. incognita* infecting maize. The results showed that both treatments significantly suppressed *M. incognita* root galling, inhibited the nematode fecundity and reduced the number of eggs and juveniles on maize so a remarkable increase in plant characters were observed.

Tobih *et al.* (2011) evaluated the leaves of three plants (*C. odorata, Thevetia peruviana* and *Ocimum viride*) as organic mulch for their potential to protect *Celosia argentea* from damage by nematodes. The study showed that *C. odorata* was more effective against root knot nematodes and the plots treated with *C. odorata* had only moderate damage.

2.2.2 Use of biocontrol agents

Among the nonchemical methods of managing nematodes, use of biocontrol agents appears to be the recent strategy gaining more importance. The relevant literature on the important bioagents are reviewed and presented.

2.2.2.1 Bacterial biocontrol agents

Bacteria are highly potent antagonists giving long lasting effects in controlling plant parasitic nematodes. The group of bacteria which release metabolites that help in killing and inhibiting phytonematodes were considered for the study.

2.2.2.1.1 *Pseudomonas* spp.

The effectiveness of *Pseudomonas 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* against root knot nematode on tomato. Eapen *et al.* (1997) isolated fluorescent *Pseudomonas* from the rhizosphere of black pepper and tested for their interaction on *M. incognita*, under green house conditions and found that the strains of *P. fluorescens* inhibited the population of *M. incognita*.

A talc formulation of *P. fluorescens* containing $15x10^8$ cfu/g was applied to soil around root knot nematode infested grape vine at 15 cm depth in the basin at the time of pruning. The application of *P. fluorescens* at all the dosage levels (1,2, and 4 g per vine) significantly reduced the severity of root knot infestation in roots and the extent of root colonization by *P. fluorescens* and it was dosage dependent according to Santhi *et al.* (1998).

Verma *et al.* (1998) observed that application of *P. fluorescens* @ 10g/kg seed was effective in reducing the menace of root knot nematode, *M. incognita* in tomato. Hanna *et al.* (1999) evaluated *P. fluorescens* for the control of *M. incognita* on tomato plants and found that the per cent gall formation and root gall index were decreased when the bacteria were introduced prior to inoculation with nematodes.

Devi and Dutta (2002) studied the effect of *P. fluorescens* on root-knot nematode (*M. incognita*) of okra plant and found that *P. fluorescens* improved shoot and root length and reduced root gall number. Siddiqui and Shaukat (2002) noted that

P. fluorescens and *P. aeruginosa* reduced *M. javanica* juvenile penetration into tomato plants.

Nursery and field experiments were conducted by Mahapatra *et al.* (2003) to determine the efficacy of *P. fluorescens* against *M. incognita* infesting aubergine and found *P. fluorescens* applied at $20g/m^2$ showed highest reduction (46.4 per cent) in root knot index at transplanting.

In vitro study conducted by El-Hamshary *et al.* (2004) proved that *P. fluorescens* and *P. aeruginosa* affected *M. incognita* juvenile's survival, and the mortality percentages of the nematode which were dependent on the bacterial concentration and exposure time.

Kalaiarasan *et al.* (2006) reported that isolates of *P. fluorescens* (Pf 1, Pf CBE, Pf POL and Pf BSR) were found to protect groundnut from root knot nematode, *M. arenaria* and the treatments with bacteria increased plant growth and reduced the level of infestation by the nematodes. *P. fluorescens* has been reported to be effective against root knot nematode, *M. incognita* in banana (Jonathan *et al.*, 2006).

Senthamarai *et al.* (2006) evaluated biological control agents against management of *M. incognita* in *Coleus forskohlii* and revealed that soil application of *P. fluorescens* at the rate of 2.5 kg/ha showed increased plant growth and reduced root-knot nematode population both in soil and root.

P. fluorescens culture filtrates at 75 per cent tested on *M. incognita* exhibited more than 90 per cent mortality of juveniles and more than 80 per cent inhibition in egg hatching (Cannayane *et al.*, 2007). *P. fluorescens* applied at 2.5 kg/ha recorded significantly higher growth parameters of tropical sugarbeet cv. Indus and lower root knot nematode (*M. incognita)* population and enzyme activity was significantly higher in *P. fluorescens* treated plants (Kavitha *et al.*, 2007). *In vitro* studies conducted by El-Nagdi and Abd-El-Khair (2008) found that the culture filtrates of *P. fluorescens* at 10 per cent concentration caused root-knot nematode mortalities by 99 per cent after 72 hrs exposure to the filtrates.

Integration of strategies such as stem cutting dipping in 0.1 per cent *P. fluorescens*+soil application of neem cake @ 400 kg/ha+growing marigold as intercrop followed by their biomass incorporation increased the yield (22.7-30.0 per cent) and reduced the root-knot nematode population (71.2-73.8 per cent) superiorly, followed by the integration of *P. fluorescens*+marigold intercrop which were almost equally effective against root-knot nematode, *M. incognita* on medicinal coleus (Seenivasan and Devrajan, 2008).

Siddiqui *et al.* (2009) reported that among *Pseudomonas* isolates tested against root-knot nematode (*M. incognita*) on *Pisum sativum*, isolate Pf1 caused greater inhibitory effect on the hatching and penetration of *M. incognita* followed by Pa3, Pa4, Pa2 and Pf5. In greenhouse experiment, isolate Pf1 caused a greater increase in pea growth and higher reduction in galling and nematode multiplication followed by Pa3 and Pa4.

Ramakrishnan and Senthilkumar (2009) evaluated the biocontrol potential of different bioagents, organic amendments and humic acid against root-knot nematode, *M. incognita* infesting Ashwagandha (*Withania somnifera*) and senna (*Cassia angustifolia*). The results showed that the soil application of commercial talc formulation of *P. fluorescens* ($2.6x10^6$ cfu/g) @ 2.5 kg/ha recorded the lowest nematode population with highest economic yield.

Verma (2009) conducted a preliminary green house study to see the efficacy of a bacterial antagonist, *P. fluorescens* (Pf-1) in its aqueous formulation @ 1.0, 2.0 and 3.0 per cent (w/v) dose/dilution against *M. javanica* infesting tomato plants and found that 2.0 and 3.0 per cent dilution was effective in enhancing plant growth and reducing root galling as compared to untreated check.

Anwar-ul-Haq *et al.* (2011) reported that tomato plants treated with *P. fluorescens* significantly suppressed *M. incognita* females per root system (40.52 %), J2/one gm of root (39.80 %), galls per root system (41.50 %) and egg masses per root system (43.23 %) resulting in improved growth over control plants.

Seenivasan (2011) opined that *P. fluorescens* was the most effective biocontrol agent for the management of *M. graminicola* in rice fields when applied as

seed cum soil application and seed treatment alone as it reduced the root invasion and soil population of the nematode. It was found that application of *P. fluorescens* as seed cum soil treatment resulted in higher grain yield (20.6 - 26.9 %) over control.

Khan and Haque (2011) reported that greatest reduction in the numbers of second-stage juveniles of *M. incognita* in soil, root galls and egg mass indices were recorded with the soil application of *P. fluorescens* compared to control and greatest increase in the plant growth and biomass of tobacco (cv. RK-18 P8).

Seed treatment (5 ml/kg seed) with cultures containing 10^{12} colony forming units/ml of *P. fluorescens* and *P. stutzeri* significantly increased yield and root nodulation of chickpea and it suppressed gall formation, reproduction and soil populations of *M. incognita* (Khan *et al.*, 2012). Application of *P. fluorescens* at 20 g kg^{-1} seed of mungbean (*Vigna radiata*) was best in reducing root knot nematode (*M. incognita*) population in field (Sitanshu *et al.*, 2012).

2.2.2.1.2 *Bacillus* spp.

Soil application of *B. subtilis* improved plant growth, leaf pigments and yield of tomato in plots inoculated with *M. incognita* and significant yield enhancement due to root-dip treatments was also recorded with *B. subtilis* (Khan and Tarannum, 1999).

Several studies indicated that rhizobacteria, *B. subtilis* and *P. aeruginosa* not only enhanced plant growth but also suppressed root knot infection and nematode density in the soil (Siddiqui *et al.*, 1999; Siddiqui, 2000).

Application of 10 ml *B. cereus* at 3 x 10^8 cells/ml and 10 ml *B. subtilis* at 3 x 10^8 cells/ml improved the plant growth characters (length and weight of shoot and root) of tomato and inoculation of *B. subtilis* (10 ml at 3 x 10^8 cells/ml) one week after inoculation of *M. incognita* resulted in the significant decrease in the number of galls (from 109.61 to 68.03) and egg masses (from 72.40 to 43.15) per root system. The inoculation of *B. subtilis* (10 ml at 3 x 10^8 cells/ml) one week before infestation resulted in the lowest number of galls and egg masses per root system and nematode population per 200 g soil and highest colonization of bacteria (26.45 x 10^6 cfu/g soil) (Cannayane *et al.*, 2001).

Cannayane and Rajendran (2001) reported that culture filtrates of *B. subtilis* caused 72.51 per cent juvenile mortality at 50 per cent concentration within 48 hours of exposure period on *M. incognita* infesting okra. Root-knot index, egg mass production, eggs/egg mass and soil nematode populations were significantly reduced in plants which received culture filtrates from *B. subtilis* and yield was increased by 48.60 per cent.

Rajendran *et al.* (2001) evaluated *B. cereus* and *B. subtilis* for the control of *M. incognita* infestation on chilli (*Capsicum* sp.) cv. Co1 and brinjal (aubergine) cv. Co1 plants and found that nematode infested plants treated with bacterial suspensions showed better plant growth, lower number of galls and nematode populations compared to those not treated with bacteria.

Siddiqui *et al.* (2001) reported that *B. subtilis* used as seed dressing or as soil drench significantly suppressed root-rot root-knot infection and *M. javanica* population densities under greenhouse and field conditions and thereby enhanced plant growth and yield in mung bean.

Treatment with *B. subtilis* reduced root-knot nematode (*M. incognita*) galling by 33-34 per cent and increased the dry weight of shoots of green gram by 22-24 per cent (Khan *et al.,* 2002). Dhawan *et al.* (2004) evaluated four strains of *B. thuringiensis* and found that the mobility of *M. incognita* juveniles completely ceased after 24 hrs exposure in standard filtrate (S) and S/10 dilutions and all dilutions above S/25 were ineffective. Nagesh *et al.* (2005) confirmed the importance of genus *Bacillus* and the results indicated that, cell-free culture filtrates of *B. cereus* reduced egg hatching (90 %) and caused cent per cent mortality of juveniles.

Subhagan (2006) studied the effectiveness of various biocontrol agents *viz., B. subtilis, P. fluorescence, T. viride* and AMF and organic amendments against root knot nematode, *M. arenaria* infesting thippali. Among the treatments applied, plants treated with *B. subtilis* showed maximum vine length, number of leaves, number of branches, root length, shoot and root weight, early spike formation, minimum root-knot index, gall formation and nematode population in root and soil.

In vitro study conducted by Satyandra and Chaubey (2007) revealed that *B. subtilis* caused high mortality and great suppression in egg hatching at all the

concentrations of culture filtrate (CF), i.e. 100, 75, 50 and 25 per cent . The CF of *B. subtilis* at 100 per cent concentration was highly effective to cause mortality and to suppress egg hatching than low concentrations.

El-Nagdi and Abd-El-Khair (2008) conducted studies to evaluate the effectiveness of bioagents against root-knot (*M. incognita*) and root-rot (*Rhizoctonia solani*) in eggplant. Culture filtrates of *B. subtilis* at 10 per cent concentration caused cent per cent after 72 hrs exposure to the filtrates *in vitro*. Soil drenching of *B. subtilis* at 10 per cent reduced the number of juveniles in soil, galls and egg masses of *M. incognita* on the roots of eggplant cv. Pusa Purple Long by 91.9, 82 and 82.6 per cent, respectively.

Terefe *et al.* (2009) reported that the commercial WP formulation of *Bacillus firmus* (BioNem) applied at 200 and 400 kg ha^{-1} was effective in reducing the number of galls (75–84 %) by *M. incognita*, and increased shoot height (29–31 %) and weight (20–24 %) of tomato plants over the untreated control, 45-days after treatment in the field trial. BioNem applied at 8 g/pot (1200 cc soil) planted with tomato seedlings reduced gall formation by 91 per cent, final nematode populations by 76 per cent and the number of eggs by 45 per cent and it also increased plant height and biomass by 71 per cent and 50 per cent, respectively in the green house trials and treatment of second-stage juveniles with 2.5 per cent and 3 per cent concentration of BioNem, caused cent per cent inhibition of mobility 24 hrs after treatment in the laboratory tests.

Siddiqui *et al.* (2009) reported that among *Bacillus* isolates tested against root-knot nematode (*M. incognita*) on *Pisum sativum*, isolate B2 caused greater inhibitory effect on the hatching and penetration of *M. incognita* followed by B4, B3, B5 and B1. Vetrivelkalai *et al.* (2010) reported that the culture filtrates of endophytic bacterial isolates of *Bacillus* sp. viz., EB16, EB18 and EB19 significantly reduced the number of adult females, egg masses, root and soil infestation of *M. incognita* and the lowest root gall index (1.00) was registered both in EB16 and EB18 isolates.

Singh and Siddiqui (2010) isolated fifteen isolates of *Bacillus* from the root-knot nematode suppressive soils and used for the biocontrol of *M. incognita* on tomato. *Bacillus* isolates B1, B4, B5 and B11 caused greater inhibitory effect on hatching of *M. incognita*, greater colonisation over tomato roots, increased growth of tomato

seedling and reduction in galling and nematode multiplication in green house tests.

Application of talc based formulation of *Bacillus macerans* @ 10g/pepper vine in basins (10^6 cfu/g) at the time of planting of vines or just before the monsoon period in established plantations of Kerala is effective in controlling burrowing nematode (*Radopholus similis*) and root-knot nematode infesting pepper vines (Kerala Agricultural University, 2011).

Radwan *et al.* (2012) evaluated the potential of commercial bioproducts against the root-knot nematode, *M. incognita*, infecting tomato and found that *B. megaterium* (Bioarc ®) at 10 g/kg soil achieved the highest significant reduction in the number of root galling (89.20%) and increased shoot weight at 10 and 20 g/kg soil.

Tong-jian *et al.* (2013) reported that the filtrate of *B. cereus* X5 reduced egg hatching rates of *Meloidogyne sp.* during the incubation period for 14 days and more effectively killed the second-stage juveniles during the incubation period of 24 h than that of *B. thuringiensis* in laboratory. *B. cereus* X5 enhanced effect of biofumigation which resulted in increased plant biomass and reduced nematode counts in the roots and rhizosphere soil of greenhouse tomatoes and field muskmelons.

2.2.2.2 Fungal biocontrol agents

Fungal biocontrol agents have been reported to be antagonistic to plant parasitic nematodes. The high efficacy of fungi as bioagent is due to the long co-evolution of these fungi and phytonematodes in common soil habitat.

2.2.2.2.1 *Trichoderma* spp.

The antagonistic effect of *Trichoderma* against root knot nematode had been recorded by Sankaranarayanan *et al.* (1997). Faruk *et al.* (1999) reported that *Trichoderma* isolates at 5g/ 4 kg soil was the most effective rate for antagonistic activity against *Meloidogyne* spp. Sankaranarayanan *et al.* (1999) found that *T. harzianum* isolates were 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.

A study conducted by Ravi *et al.* (2000) established that *T. viride* reduced nematode multiplication and their entry into roots of banana. Reddy *et al.* (2000) reported on the parasitization of egg masses of root-knot nematode by antagonistic fungus, *T. viride* in tomato.

Pot and field trials were conducted by Pandey *et al.* (2003) to study the efficacy of different levels of *T. viride viz.* 1000, 2000, 3000 and 4000 spores/ plant against *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) observed highest root knot suppression when vermicompost was combined with *T. harzianum*, against root knot disease of Ashwagandha. A study conducted by Sentilkumar and Rajendran (2004) revealed that *T. viride* reduced final nematode population in grape vine. Kumar and Khanna (2006) reported that application of *T. viride* formulation (10^8 spores/g) at 20 g/pot at the time of transplanting of tomato seedlings reduced the root-gall index by *M. incognita.*

T. viride culture filtrates at 75 per cent tested on *M. incognita* exhibited more than 90 per cent mortality of juveniles and more than 80 per cent inhibition in egg hatching (Cannayane *et al.*, 2007). El-Nagdi and Abd-El-Khair (2008) conducted studies to know the effectiveness of bioagents against root-knot (*M. incognita*) and root-rot (*Rhizoctonia solani*) in eggplant. In *in vitro* studies, culture filtrates of *T. harzianum* and *T. viride* at 10 per cent concentration caused nematode mortality to the extent of 98 and 96 per cent, respectively after 72 hrs exposure to the filtrates. *T. harzianum* greatly reduced damping-off and root-rot incidence in brinjal followed by *T. viride, B. subtilis and P. fluorescens.*

The different concentrations of *T. harzianum* at 10^2-10^8 spores/ml decreased *M. javanica* infection and the bioagent was able to penetrate nematode egg mass matrix and significantly decreased egg hatchability of nematode (Sahebani and Hadavi , 2008).

Bokhari (2009) evaluated the efficacy of *T. harzianum*, *T. viride*, *T.*

koningii, *T. reesei* and *T. hamatum* culture filtrates in controlling *R. reniformis* and *M. javanica* on egg plant. The *Trichoderma* species led to the inhibition of the nematode activity and movements *in vitro* during one week exposure. The *Trichoderma* culture filtrate was more significant on *M. javanica* eggs than on larvae and controlled both the nematode genera due to a direct effect of their toxic metabolites and inhibited nematode penetration and development.

Soil drenching of conidial suspension of *T. viride* NRRL 6418 and *T. harzianum* (*Hypocrea lixii*) at $1\times10^{(14)}$ spore mL^{-1} significantly reduced the disease incidence of *M. incognita* in the plant roots and increased the plant growth of foliage plant, *Livistona rotundifolia* (Jegathambigai *et al.*, 2011) .

Ramakrishnan and Deepa (2011) evaluated the effectiveness of bioagents in the experiments for the management of nematode fungal disease complex involving *M. incognita* and *Macrophomina phaseolina* in *Coleus forskohlii* under glasshouse conditions (26+2°C) and revealed that soil application of *T. viride* @ 2.5 kg/ha was the most effective in suppressing the disease incidence and enhanced the tuber yield. The effectiveness of commercially available *T. viride* in talc-formulation (10^6cfu/g) was tested at three different locations in nematode sick field and results confirmed the effectiveness of *T. viride* for the management of nematode fungal disease complex (77.69 per cent) and increased the tuber yield by 68.18 per cent in medicinal coleus.

Freitas *et al.* (2012) evaluated potentiality of *Trichoderma* isolates against *M. incognita* on sugarcane variety RB863129. They found that *Trichoderma* spp. strains 3M, 8M, 17M and 225T reduced gall index and the strains 1M, 3M, 10M, 17M, 311T and 322 decreased nematode reproduction. *In vitro*, all the filtrates of *Trichoderma* spp. were effective for promoting juvenile mortality and in case of nematode eggs, sixteen among twenty-two strains were significant to control parasitism of eggs and strains 8M, 11M, 13M, 15M and 17M were most promising. The strains 4M, 14M, A18 and 4077T showing potential enzymatic action caused mortality of juveniles after hatching.

T. viride highly reduced both the fungal root-rot infection by *Fusarium solani* and root-knot nematode parameters like numbers of J_2 in soil, galls, females and egg-masses on sugar beet. The soil treated with *T. viride* increased the plant weight,

foliage weight, length, diameter and weight of root, survival of plants and root yield (Abd-el-Fattah *et al.*, 2012). Usman and Siddiqui (2012) reported that the strains of *T. harzianum* were found to be the most effective in controlling *M. incognita* when treated at 2 g/egg plant/pot.

Khan and Rizvi (2013) evaluated the effectiveness of three *Trichoderma* spp. *viz.*, *T. harzianum*, *T. virens* and *T. hamatum* through seed and soil application against *M. incognita* in leafy vegetables. *T. harzianum* significantly suppressed the galling by 16-36 per cent and improved the fresh shoot weight of dill soa (17 and 11 %), spinach (18 & 10 %) and fenugreek (20 & 12 %) over control followed by *T. hamatum* and *T. virens* which suppressed the galling by 16-30 and 14-22 per cent and improved the foliage production by 10-14 and 7-13 per cent, respectively. The treatment also reduced the soil population of *M. incognita* by 20-40 per cent at two and four month stage of the crop.

The treatment with *T. harzianum* at 1.25 kg/ha was effective in increasing plant growth parameters and yield of french bean and maximum reduction in disease incidence was recorded in the treatment combining *T. harzianum* @ 1.25 kg/ha along with carbofuran at 1 kg a.i/ha in the management of *M. incognita* and *Rhizoctonia solani* disease complex on French bean (Gogoi and Mahanta, 2013).

2.2.2.2.2 *Paecilomyces lilacinus*

Khan and Khan (1992) tested 15 different fungal filtrates for their nematicidal properties against *M. incognita*. The per cent mortality and inhibition of hatching of nematodes was directly proportional to the concentration of culture filtrates and *Paecilomyces lilacinus* and *Nigrospora sphaerica* showed the highest nematicidal activity.

Noe and Sasser (1995) evaluated the efficacy of *P. lilacinus* in controlling *M. incognita* on four vegetable crops and soyabean under field conditions. Plots treated with *P. lilacinus* had lower *M. incognita* juvenile counts and higher yield of vegetable crops than the control plots.

Hafeez *et al.* (2000) reported that the addition of *P. lilacinus* and *T. harzianum* separately along with organic substrate to infested soil sufficiently retarded

the pathogenic activity of *M. incognita.* Addition of *P. lilacinus* and *T. harzianum* in combination with organic substrate gave effective control of nematode population and thus reduced root knot disease and increased plant vigour of tomato.

Kiewnick and Sikora (2006) evaluated the fungal biocontrol agent, *P. lilacinus* strain 251 (PL251) for its potential to control *M. incognita* on tomato and a pre-planting soil treatment reduced root galling by 66 per cent, number of egg masses by 74 per cent and final nematode population in the roots by 71 per cent compared to the control.

The mode and severity of infection of *M. javanica, H. avenae, R. similis* by *P. lilacinus* was studied under laboratory conditions using microscopy and found that *P. lilacinus* infected the eggs, juveniles and females of *M. javanica* by direct hyphal penetration. The early developed eggs were more susceptible than the eggs containing fully developed juveniles (Khan *et al.*, 2006).

Investigation carried out by Krishnamoorthi and Kumar (2007) revealed the biocontrol potential of *P. lilacinus* at different soil moisture and soil temperature in the management of *M. incognita* in brinjal. Significantly highest plant growth, yield and parasitized nematode and reduced soil and root nematode population was recorded at moisture level of 4 per cent and at temperature of 25 ^{0} C.

A study conducted by Kumar *et al.* (2008) revealed the toxic effect of culture filtrates of *P. lilacinus* on the mortality and hatching of *M. incognita* on vegetables. The isolate P1T3 was found effective among seven isolates to cause both mortality and hatching inhibition of *M. incognita* and the rate of mortality was low in the first 24hrs but it increased with increase in exposure period.

The soil application of *P. lilacinus* at 2.5 kg/ha parasitized the 51.5-71.5 per cent of the nematode eggs of root-knot nematode, *M. incognita*, infesting medicinal coleus, *C. forskohlii* (Seenivasan and Devrajan, 2008).

Azam *et al.* (2009) found that addition of *P. lilacinus* alone into the soil reduced nematode, *M. incognita* population and increased yield of chickpea. The combination of leaf powder of *C. tora* and *P. lilacinus* successfully managed the root knot nematode compared to the combination of two leaf powders.

Oclarit and Cumagun (2009) evaluated the efficacy of *P. lilacinus* strain UP1 as biological control agent of *M.* incognita attacking tomato grown in pots under screen house condition pot experiments and found that number of galls, nematodes and egg masses per one gram root sample were significantly reduced by the application of *P. lilacinus* at all levels and this was comparable with commercial fungicide, Nemacur. The per cent reduction in gall number was the highest at 7.92×10^6 spores of *P. lilacinus* per ml.

Paecilomyces variotii was found in the females and egg masses of *M. javanica* from the root samples collected from three geographical areas of Jordan from fig trees, tomato, aubergine and cucumber and the local isolates of *P. variotii*, as nematode antagonists, resulted in egg parasitism of about 61.4per cent compared to 68.5per cent for *P. lilacinus* (Al-Qasim *et al.*, 2009). The combined use of *P. lilacinus* with cattle manure resulted in maximum reduction in galling and nematode multiplication of *M. incognita* infesting tomato plants (Siddiqui and Futai, 2009) .

Siddiqui and Akhtar (2009) reported that *P. lilacinus* parasitised more females and eggs of *M. incognita* than the other fungi tested and *P. lilacinus* was more effective in reducing galling and improving the growth of nematode-inoculated tomato plants in glasshouse. Simon and Pandey (2010) evaluated the antagonistic efficacy of *P. lilacinus* and *Verticillium chlamydosporium* against *M. incognita* infesting okra. The results showed that the length and weight of root and shoot significantly increased when the plants were treated with *P. lilacinus* and *V. chlamydosporium* in comparison to carbofuran treatment. Among the treatments, maximum plant growth as well as maximum reduction in root galling was observed in plants treated with *P. lilacinus* and *V. chlamydosporium.*

Ganaie and Khan (2010) evaluated the biological potential of *P. lilacinus* on pathogenesis of *M. javanica* infecting tomato plants. *P. lilacinus* significantly improved the growth of tomato plants inoculated with 2000 juveniles of *M. javanica.* The simultaneous inoculation of *P. lilacinus* and *M. javanica* significantly improved plant growth parameters and the sequential inoculation of *P. lilacinus* 10 days prior to nematode inoculation was more effective than sequential inoculation of *M. javanica* 10 days prior to inoculation of *P. lilacinus.*

The application of *B. macerans* or *P. lilacinus* @ 25 g/m^2 in the nursery along with drenching of *B. macerans* or *P. lilacinus* at 3 per cent solution 7 days after sowing is recommended for the control of root- knot nematodes associated with brinjal in Kerala (Kerala Agricultural University, 2011). Joshi *et al.* (2012) reported that *P. lilacinus* @ 2 g/kg soil was found as best treatment in increasing plant growth of tomato plants and in reducing the reproduction of *M. incognita* over other fungal bio-agents.

The field trials conducted by Kannan and Veeravel (2012) revealed that the best application methods of *P. lilacinus* was the combination of seedling (10 g/l water) + soil application (5.0 kg/acre) which documented maximum shoot length at 60 and 90 DAS, shoot weight and root length at 90 DAS and they were positively correlated with fruit yield of okra.

P. lilacinus was studied on different inoculum level of root-knot nematode (500, 1000, 1500, 2000 J2s) infesting *Vigna radiata* and found that *P. lilacinus* could control the root knot nematode population. Best results were achieved when 20 gram of *P. lilacinus* was used to check *M. incognita* infecting *V. radiata* (Sharma and Trivedi, 2012)

P. lilacinus was the best treatment in suppressing *M. incognita* populations infesting tomato plants in the soil (85.2 %) and increased the shoot length and fresh weight of the root system by 22.9 and 47.6 per cent, respectively (Khalil *et al.*, 2012a). Khalil *et al.* (2012b) reported that application of *P. lilacinus* was the most effective treatment to reduce gall index and egg masses *M. incognita* with 88.23 and 76.94 per cent respectively over untreated control.

Soil application of *P. lilacinus* reduced nematode population from 631.8 to 35 nematodes/200 cc with reduction of root galls from 312 to 38 galls/5 g root for the period of 180 days in the experiment on the management of *M. incognita* in pomegranate (Somasekhara *et al.*, 2012). Sitanshu *et al.* (2012) reported that *P. lilacinus* @ 10 g kg^{-1} seed was the most effective in increasing the growth parameters and yield of mungbean infested by *M. incognita.*

2.2.3 Combination of bioagents

2.2.3.1 Combination of *Pseudomonas* spp. and *Bacillus* spp.

Black pepper plants under glasshouse and field conditions, treated with the mixture of native isolates, Pf 123 (*Pseudomonas* isolate) + Bs 214 (*Bacillus* isolate) significantly enhanced the plant growth, yield and reduced nematode infestation both in soil and root. Under *in vitro* studies, greatest reduction in root knot nematode egg hatching and highest juvenile mortality was observed in the culture filtrate of consortia of Pf 123 and Bs 214 at 100 per cent concentration (Devapriyanga *et al.*, 2012).

Soil application of consortia formulation of *P. fluorescens,* Pfbv 22 and *B. subtilis,* Bbv 57 @ 10 g per vine (Pfbv 22+Bbv 57 each @ 5 g) of black pepper cv. Karimunda in the fields naturally infested with *Radopholus similis* and *M. incognita* significantly enhanced the growth and berry yield of vine and reduced the root knot nematode population over untreated control (Jonathan *et al.*, 2012).

Anwar-ul-Haq *et al.* (2012) assessed the efficacy of plant growth promoting rhizobacteria (PGPR) against *M. incognita* infection on roots of tomato in the green house at 30±4°C by applying 20-ml of 5 per cent sugar solution containing 10^7 CFU/ml each of *Bacillus* spp., *Azotobacter* spp., *Pseudomonas putida* and *P. fluorescens* at two days after transplanting. The treatments having *P. putida, Bacillus* spp. and combination of PGPR showed intermediary effects on both nematode reproduction and plant growth.

2.2.3.2 Combination of *Paecilomyces lilacinus* and *Bacillus* spp.

Siddiqui and Mahmood (1993) reported that the combined inoculation of *P. lilacinus* and *B. subtilis* improved dry shoot weight significantly when chickpea plants were simultaneously inoculated either with *M. incognita* or *Macrophomina phaseolina* or with both.

Gautam *et al.* (1995) reported that the combined use of *B. subtilis* and *P. lilacinus* caused an increase in plant growth of tomato plants inoculated with *M. incognita* and use of both biocontrol agents along with green manure of *Eichhornia crassipes* resulted in greatest plant growth of inoculated plants.

Application of *P. lilacinus* and *B. firmus*, singly or together in pot experiments, provided effective control of second-stage juveniles, eggs or egg masses of root-knot nematodes (Anastasiadis *et al.*, 2008).

2.2.3.3 Combination of *Paecilomyces lilacinus* and *Pseudomonas* spp.

Seed treatment with combined formulation of *Pseudomonas fluorescens* (2×10^8 cfu/g) and *Paecilomyces lilacinus* (2×10^6 cfu/g) @ 10 g/kg seed and nursery beds treatment with the formulation at the rate of 50 g and neem cake 500/sq.m increased the vigour of tomato and capsicum seedlings and caused least amount of infestation by root-knot nematodes in the main field (Rao, 2007).

Rao (2008) reported that application of 10 g *P. lilacinus* (10^6 cfu/g), 10 g *P. fluorescens* (10^8 cfu/g) and 250 g of neem seed cake per acid lime tree once in six months, for a period of two years, reduced the population of *M. javanica,* improved root colonization of bio-agents and increased the yield of the crop.

Application of farm yard manure enriched with bio-pesticides (1 kg *P. lilacinus* (10^6 cfu/g) + 1 kg *P. fluorescens* (10^8 cfu/g) +50 kg of neem or pongamia cake +1 ton of farm yard manure) at the rate of 1 kg/papaya seedling at planting and subsequently four more applications at an interval of 6 months reduced the root population of *R. reniformis* and *M. incognita* by 73 and 78 per cent respectively and increased the yield of papaya (Arka Surya) by 26 per cent (Rao, 2010).

The combined application of *P. fluorescens* @ 1 kg/ha along with *P. lilacinus* @ 2.5 kg/ha and carbofuran 3G @ 0.25 kg a.i./ha at the time of sowing enhanced the yield of carrot (43.58 %) compared to untreated control and reduced *M. hapla* population by 66.19 per cent (Anita and Selvaraj, 2011).

Twenty grams of the formulation containing *Pseudomonas putida* and *P. lilacinus* was used as seed (one kg) treatment and five kg for the enrichment of neem cake (200 kg), which was applied to the beds at the rate of 20 g/m^2 as a substrate treatment before sowing and it reduced *M. incognita* (J_2) population in roots by 69 per cent and in soil by 47.6 per cent and *Erwinia carotovora* by 66 per cent, with

significant increase (27.8 %) in the yield of carrot. *P. putida* and *P. lilacinus* co-existed without affecting root colonization (Sowmya *et al.*, 2012).

Rao *et al.* (2012) reported that ten grams of combined formulation of *P. fluorescens* and *P. lilacinus* was used as seed (1 kg) treatment, 5 g for treating 1 kg of substrate (coco peat) and 5 kg for the enrichment of a vermicompost (500 kg) which was applied to the field before transplanting bell pepper seedlings. The application reduced the population of *M. incognita* and increased plant shoot and root length, nematode egg parasitization and crop yield. The colonization of the roots by *P. fluorescens* was not affected by *P. lilacinus*.

2.2.4 Use of Chemicals

The effect of chemicals in controlling nematodes has been reported by many workers. Research findings of Mohan and Mishra (1993) revealed that carbofuran was effective in suppressing *M. incognita* and improving plant growth of french bean.

Soil application of carbofuran @ 2 kg a.i. per ha and seed dressing @ 22 g a.i. per kg seed were highly effective in controlling *M. incognita* larvae and reduced root galls in pea compared to control plants. The treatments also improved 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 2 kg a.i./ha before sowing or as foliar spray at 500 ppm, 15 days after germination significantly reduced root galls and enhanced growth parameters. Haider *et al.* (1998) reported that application of carbofuran or phorate @ 1 kg a.i. per ha reduced root-knot nematode, *M. incognita* in turmeric.

Tiwari *et al.* (2002) found that tomato nursery bed treated with 0.6g carbofuran significantly decreased gall index and increased crop yield. Compared to other plots the maximum yield (362 q ha^{-1}) was reported in carbofuran treated nursery beds. Singh (2006) studied the effect of carbofuran and phorate to manage root-knot nematode infesting cauliflower and to increase cauliflower yield. Microplots were treated separately with carbofuran and phorate @ 1.0 and 1.5 kg a.i./ha. Carbofuran

applied @ 1.5 kg a.i./ha gave 48 per cent control and thus increased the yield up to 46 per cent. Ibrahim *et al.* (2007) reported that the treatment with carbofuran caused reduction (99.4 %) in numbers of root galls and nematode egg masses of *M. incognita* infesting sunflower plants.

A study conducted by Saikia *et al.* (2007) revealed the efficacy of organic amendments *viz.*, neem cake, vermicompost, neem seed kernel, sawdust alone and in combination with carbofuran 3G against *M incognita* in brinjal. All the treatments showed significant effects on plant growth parameters and yield of brinjal with corresponding decrease in the nematode population both in soil and roots. Among all the treatments, the treatment with neem cake + carbofuran 3G, showed superior effect over control. Treatment with carbofuran 3G alone was effective in suppressing *M. incognita* population in soil as well as in roots of brinjal (Saikia and Borah, 2008).

Rajvanshi *et al.* (2008) reported that combination of seed soaking+foliar spray (@ 1000 ppm) with half recommended dose of carbofuran 3G (1.0 kg a.i./ha), gave highest crop yield (84.44 q/ha) in round melon and reduced the number of galls per plant (9.67) and final nematode (*M. incognita*) population of soil (125.20), followed by carbofuran 3G @ 2.0 kg a.i./ha (treated check).

The study conducted by Cabrera *et al.* (2009) revealed that seed treatment with abamectin at concentrations ranging between 0.3 and 1 mg is highly effective against the three species of root-knot nematodes *(M. incognita, M. arenaria and M. javanica)* attacking tomato plants. The highest EC50 was found at 0.2 mg and highest EC80 for the number of egg masses per g root in the three *Meloidogyne* species was attained at 0.51 mg. The chemical retained its efficacy in the soil for 8 weeks.

Adoption of pointed gourd vine dipping in monocrotophos 36SL at 1000 ppm followed by soil inoculation of *T. viride* at 10 g/pit once at planting and second dose at 40 days after planting reduced root galling caused by *M. incognita* and gave fruit yield almost double of the untreated plots (Khan *et al.*, 2009). Kumar *et al.* (2009) reported that maximum inhibition of egg hatching of *M. incognita* was recorded in phorate, followed by bioagents filtrates in 10 days exposure at 80 per cent concentration.

Shendge *et al.* (2010) reported that soil application of carbofuran 3G @ 2 kg a.i./ha was found to be most effective in reducing *M. incognita* population (59.49 %), number of root galls (69.57 %) and gall index (39.33 %) and increasing the length of root (64.28 %) and shoot (71.37 %), fresh weight of root (93.41 %) and shoot (83.67 %), dry weight of root (103.13 %) and shoot (87.58 %) and the fruit yield of okra (32.94 %) in microplots.

Khan and Haque (2011) reported that application of carbofuran resulted in greatest reduction in the numbers of second-stage juveniles of *M. incognita* in soil, host root galls, egg mass indices and 15-30 per cent increase in the plant growth and biomass production was obtained in cv. RK-18 P8 of tobacco. El-Nagdi *et al.* (2011) found that the nematicides, fenamiphos and cadusaphos caused 96-98 per cent mortality of *M. incognita in vitro*.

Application of carbofuran reduced the nematode (*M. incognita)* population from 654.6 to 34.4 with reduction of fresh root galls from 380 to 38 galls/5 g root of pomegranate (Somasekhara *et al.*, 2012). Khalil *et al.* (2012b) reported that the superior treatment that suppressed *M. incognita* populations in tomato plants was oxamyl followed by abamectin.

Treatment with carbofuran @ 2 kg a.i/ha was the best in suppressing the final population of *M. incognita* in soil and carbofuran @ 1 kg a.i/ha was effective in increasing plant growth parameters and yield of french bean. Maximum reduction in disease incidence was recorded in treatment with *T. harzianum* @ 1.25 kg/ha+carbofuran @ 1 kg a.i/ha (Gogoi and Mahanta, 2013) .

Venkatesan and Patel (2013) reported that the soil application of carbofuran at 2 kg a.i/ha (carbofuran at 66.66 kg/ha) resulted in longest vine length (458 cm), highest green biomass (6326 kg/ha) and highest yield (3148 kg/ha) in bitter gourd and reduced *M. incognita* population (313/100 g soil) in soil than seed treatment with carbosulfan at 0.75 per cent (30 g/kg seed) and cartap hydrochloride at 2 kg a.i/ha (50 kg/ha).

Materials and Methods

3. MATERIALS AND METHODS

The study entitled 'Management of root-knot nematode, *Meloidogyne* sp. (Kofoid and White) in coleus, *Solenostemon rotundifolius* (Poir) Morton' was conducted at the College of Horticulture, Kerala Agricultural University, Vellanikkara during 2012-2013 to assess the population of plant parasitic nematodes infesting coleus in different coleus growing regions of Thrissur District and to identify the species of *Meloidogyne*. Pot culture experiments were also carried out to evolve the most effective management strategy using biocontrol agents, organic amendments and a chemical insecticide against root-knot nematode, *Meloidogyne* sp. in coleus.

3.1 ASSESSMENT OF PLANT PARASITIC NEMATODE POPULATION IN COLEUS

A survey was conducted to assess the nematode population at different coleus growing regions of Thrissur District. Soil and tuber samples were collected as a part of survey from eight locations *viz.*, Mundathikode, Wadakkanchery, Varavoor, Thirur, Kolazhi, Vellanikkara, Madakkathara and Vadanapilly. The fields were randomly selected and five samples were taken at random from each location at the time of harvest, making a total of forty soil and tuber samples from the rhizosphere of coleus plants. Each sample was a composite one drawn from five to six spots in a field. The plant parasitic nematodes associated with coleus were recorded from the samples collected during the survey (Plate 1).

The soil samples (200g each) were processed by Cobb's sieving and decanting technique (Cobb, 1918) followed by Modified Baermann funnel technique (Schindler, 1961). The tubers collected were washed free of soil particles, weighed as 10 g, sliced, stained in boiling lacto-phenol-acid fuchsin and macerated to estimate the nematode population. From another 10 g tuber sample, second stage juveniles were extracted by Modified Baermann funnel technique. Species of root-knot nematode infesting coleus plants was also identified from the samples collected.

Kolazhi

Thirur

Madakkathara

Mundathikode

Vellanikkara

Varavoor

Vadanapilly

Wadakkanchery

Plate 1. Sampling from coleus field during survey

3.2 SPECIES IDENTIFICATION OF ROOT-KNOT NEMATODE

Identification of species of root-knot nematode is a prerequisite for their proper management. The species was identified by the perineal pattern present at the vulval anal region (perineal region) of mature females of root-knot nematode after collecting them from root galls (Eisenback *et al.*, 1981).

3.2.1 Collection of mature females by staining technique

White females were extracted from tuber and root samples collected from the coleus plants during the survey. These samples were washed thoroughly in a stream of tap water to remove the soil particles adhering to it. Root-knots separated from the roots were wrapped in muslin cloth, tied properly and the bag was plunged into boiling lacto phenol-acid fuchsin solution till the root tissues became soft. After the solution becomes cool, root-knots were taken out of muslin cloth and washed with water to remove excess stain. The white females were taken out from the stained roots.

3.2.2 Identification of species by perineal pattern

The stained root-knots were dissected to obtain the white females. The white females, which were stained red or pink from the root-knots were collected and transferred to fresh lacto-phenol on a Perspex slide. The posterior end of white female was cut with an optical scalpel. The body tissues were removed by lightly brushing the inner surface of the cuticle with a nylon bristle. Cuticle was carefully trimmed and the perineal end was transferred to a drop of lacto-phenol on a clean glass slide and observed under a stereoscopic microscope and compared with the perineal patterns of different species of *Meloidogyne* (Eisenback *et al.*, 1981).

3.3 PREPARATION OF DENEMATIZED POTTING MIXTURE

The denematized potting mixture was prepared by mixing soil, sand and dried cowdung powder in the proportion of 1:1:1. Potting mixture was spread on polythene sheet in the form of heaps and denematized using 3 per cent formaldehyde at 50 ml for 200 kg potting mixture. Formaldehyde solution was poured into the holes (that touches the bottom of the heap) made on the heap of potting mixture and covered

tightly with polythene sheets. After one week, the polythene sheets were removed and the mixture was raked well and covered again for one more week. Then polythene sheet was removed, mixture was spread to expose it for one week to remove the residues of formaldehyde solution. Soil samples were taken from the treated potting mixture to test the presence of plant parasitic nematodes. This denematized potting mixture was used for pot culture experiment.

3.4 MAINTENANCE OF PURE CULTURE OF ROOT-KNOT NEMATODE

The cuttings of coleus (*S. rotundifolius*) were planted in pots of 22 cm diameter filled with denematised potting mixture and nematodes were inoculated after root establishment of the host plants. Egg masses were collected from the infested coleus tubers and roots from the farmer's field at Madakkathara, Thirur and Wadakkanchery. The pure culture of nematodes was maintained from single egg mass, after identifying the species of root-knot nematode on the basis of perineal pattern.

The egg masses of *M. incognita* were kept on a wire mesh which was placed over a petridish containing water for hatching. One day old second stage juveniles of root-knot nematode hatched from egg masses were inoculated to the plants maintained in the pots. Repotting and multiplication was done periodically for maintaining the pure culture of root-knot nematodes for the experiment.

3.5 POT CULTURE EXPERIMENT

The pot culture experiment was carried out to evolve the most effective management strategy against root-knot nematode, *Meloidogyne incognita* in coleus (variety- Suphala) using biocontrol agents, organic amendments and a chemical insecticide.

3.5.1. Nursery for pot culture

The seed tubers of coleus, variety Suphala released from KAU was planted in the nursery for collecting terminal cuttings. The area for nursery was levelled and made weed free. Beds of 30 cm height were made and soil-sand mixture in the ratio of 1:3 was spread over the beds at 10 cm thickness. The coleus seed tubers

were sowed at a spacing of 15 x 30 cm on the beds and mulched for retaining moisture. After one month of sowing, fresh cowdung slurry and vermicompost was applied for better growth of plants. The terminal cuttings were taken from 45 - 60 days after the planting of seed tubers.

3.5.2. Raising potted plants

Earthen pots of 30 cm diameter filled with denematized potting mixture were used for raising potted plants. Terminal cuttings with four to five nodes were planted at the rate of two per pot. The plants were irrigated periodically and regular weeding was also practised to remove the weeds from pots and interspaces.

3.5.3. EXPERIMENTAL DESIGN AND TREATMENTS

A pot culture experiment was laid out in Complete Randomized Design with twelve treatments and three replications (Plate 2). The treatments were as follows:-

T_1. Soil application of *Trichoderma viride* @ 25g/m^2

T_2. Soil application of *Pseudomonas fluorescens* @ 25g/m^2

T_3. Soil application of *Paecilomyces lilacinus* @ 25g/m^2

T_4. Soil application of *Bacillus subtilis* @ 25g/m^2

T_5. Soil application of *P. fluorescens* + *P. lilacinus* @ 12.5g+12.5g/m^2

T_6. Soil application of *P. lilacinus* + *B. subtilis* @ 12.5g+12.5g/m^2

T_7. Soil application of *B. subtilis* + *P. fluorescens* @ 12.5g+12.5g/m^2

T_8. Incorporation of neem cake @ 100g/m^2

T_9. Incorporation of *Tagetes erecta* (Marigold) as a whole @ 250 g/pot

T_{10}. Incorporation of *Chromolaena odorata* @ 250 g/pot

T_{11}. Cartap hydrochloride 4G @ 1kg ai/ha

T_{12}. Untreated control

Plate 2. View of the experiment

3.5.4. Application of biological agents and nematicide

T. viride (2.25g/pot) was incorporated in the soil prior to planting of terminal cuttings of coleus. Nematodes were inoculated at the rate of one second stage juvenile per g of soil after the establishment of the plant (2 weeks) from the pure culture of root-knot nematodes maintained in the coleus plants. Other treatments like application of *P. lilacinus* (2.25g/pot), *B. subtilis* (2.25g/pot), *P. fluorescens* alone (2.25g/pot) and in combinations (1.125g + 1.125g/pot) were applied two weeks after inoculation at the rhizosphere of the plant. Cartap hydrochloride 4G (0.225 g/pot) was also applied at the rate of 1kg ai/ha, two weeks after inoculation.

3.5.5. Application of organic amendments

Neem cake (9 g/pot) was incorporated in the soil prior to planting of terminal cuttings of coleus. Organic amendments like *T. erecta* (Marigold) and *C. odorata* were incorporated in the experimental pots two weeks after the inoculation of nematodes. The whole plant of *T. erecta* (Marigold) was chopped into pieces, weighed and applied at the rate of 250 g per pot. The leaves of *C. odorata* was also chopped and applied at the rate of 250 g per pot.

3.5.6. Extraction of second stage juveniles for inoculation

Modified Baermann funnel technique (Schindler, 1961) was adopted for extracting second stage juveniles of *M. incognita* for inoculation. Heavily infested plants from the culture pots were uprooted carefully and washed to remove the adhering soil particles. Then the 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. The eggs hatched and the juveniles settled at the bottom of the petriplate. Several such sets were kept for getting the required number of second stage juveniles. The suspension from each petriplate was collected in a beaker. The process was repeated and these hatched juveniles were used for inoculation purposes.

3.5.7. Inoculation of nematodes

Nematode population in the suspension was assessed after the extraction of nematodes. For assessing the population of nematodes, the nematode suspension collected in the beaker was made up to a constant volume by adding water. An aliquot of 1 ml was pipetted out into a counting dish after thoroughly mixing the nematode suspension by blowing air with a pipette and the number of nematodes present was counted under a stereoscopic microscope. Likewise 4-5 aliquots was assessed and the average population per ml was estimated. The total population of nematodes present in the suspension was estimated by multiplying the average population per ml with total volume of nematode suspension. Each pot was inoculated with 100 ml suspension containing 7000 second stage juveniles of root-knot nematode after the plants had established. At the time of inoculation, suspension was thoroughly mixed by blowing air with a pipette to get uniform distribution of nematodes. The suspension was then poured to the root zone region of plants, by making holes of about 5 cm depth covering all sides of plants in soil using a glass rod. After pouring the entire suspension, the holes were covered with a thin layer of soil.

3.6 OBSERVATIONS

The coleus plants were harvested after a period of 4 $^{1}/_{2}$ months of planting. The biometric characters of the plants were recorded at monthly intervals till harvest of the crop. The biometric characters recorded were as follows:

a.) Height of the plant
b.) Number of leaves
c.) Number of branches

At the time of harvest following observations were made:

a.) Height of the plant
b.) Number of leaves
c.) Number of branches
d.) Fresh shoot weight
e.) Fresh root weight
f.) Number of tubers per plant
g.) Weight of the tubers per plant

h.) Population of root-knot nematodes/ 200g soil

i.) Population of root-knot nematodes/ 5g root

j.) Population of root-knot nematodes/ 10g tuber

k.) Number of white females/ 5g root

l.) Number of root-knots/ 5g root

m.) Root-knot index

3.6.1 Estimation of nematode population from soil

A composite sample of 200g soil was collected from the root zone region of coleus plants grown in pots and processed by Cobb's sieving and decanting technique (Cobb, 1918) followed by Modified Baermann funnel technique (Schindler, 1961). The nematode suspension collected in the beaker was made up to a constant volume by adding water and counted as mentioned in 3.5.7.

3.6.2 Estimation of galls

The root system of coleus plant from each pot was carefully lifted by gentle tapping on all sides and bottom of pots. Then loose soil was removed and the roots were cleaned from the adhering soil particles by gentle washing in water. From this, samples of 5g root were randomly taken and they were pressed gently between the folds of blotting paper to remove excess water and the number of root-knots in 5g root samples was counted.

3.6.3 Root-Knot Index

The number of galls in root and tuber samples was counted and root-knot index was worked out by rating on a 1-5 scale (Taylor and Sasser, 1978). The following are the ratings on a 1-5 scale of root-knot index:

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

3.7 STATISTICAL ANALYSIS

Data collected from the experiment were analyzed by statistical method for CRD and ANOVA. Analysis of variance was done using the statistical package, SPSS (Statistical Package for Social Sciences) and the mean values were compared by DMRT (Duncan, 1951).

Results

4. RESULTS

The results of the study entitled 'Management of root-knot nematode, *Meloidogyne* sp. (Kofoid and White) in coleus, *Solenostemon rotundifolius* (Poir) Morton' are presented in this chapter.

4.1. SURVEY OF NEMATODES ASSOCIATED WITH COLEUS *(Solenostemon rotundifolius)*

A survey was conducted to assess the nematode population at different coleus growing regions of Thrissur District namely Mundathikode, Wadakkanchery, Varavoor, Thirur, Kolazhi, Vellanikkara, Madakkathara and Vadanapally. The samples were collected, processed as mentioned in 3.1. and plant parasitic nematodes were recorded from the collected samples. The root-knot nematode population, number of white females and gall count in 10 g tuber and gall index were observed. Species of root-knot nematode infesting coleus plants was also identified from the samples collected.

4.1.1. Occurrence and distribution of plant parasitic nematodes associated with coleus in Thrissur District

The data regarding the occurrence and distribution of plant parasitic nematodes associated with coleus in Thrissur District are presented in Table 1. Six plant parasitic nematode species were found to be associated with the rhizosphere of coleus in the surveyed regions of Thrissur District. All the six plant parasitic nematode species identified belonged to a single Order of Tylenchida under three families namely Heteroderidae, Hoplolaimidae and Pratylenchidae. The plant parasitic nematodes in coleus were identified as root-knot nematode (*Meloidogyne incognita* (Kofoid and White) Chitwood, 1949), reniform nematode (*Rotylenchulus reniformis* Linford and Oliveiria), burrowing nematode (*Radopholus similis* (Cobb, 1893) Thorne, 1949), lance nematode (*Hoplolaimus* sp.), spiral nematode (*Helicotylenchus* sp.) and rice-root nematode (*Hirschmanniella oryzae* Luc and Goodey, 1963).

Meloidogyne incognita was recorded from all the eight regions surveyed. The maximum average root-knot nematode population was recorded in Kolazhi with 450.4 nematodes/ 200g soil, which was followed by Thirur having 428 nematodes/ 200g soil. The next region having maximum average nematode population

Table 1. Occurrence and distribution of plant parasitic nematodes associated with coleus in Thrissur District

Locations	Nematode population/ 200g soil					
	Meloidogyne incognita	*Hirschmanniella oryzae*	*Hoplolaimus* sp.	*Helicotylenchus* sp.	*Rotylenchulus reniformis*	*Radopholus similis*
Wadakkanchery	399.2	6.4	0	0	0	0
Mundathikode	292.8	0	4.8	0	0	0
Kolazhi	450.4	7.2	0	0	0	0
Thrirur	428.0	9	0	0	0	0
Varavoor	45.3	0	0	0	0	0
Vadanapilly	178.8	0	3.6	7.2	47.6	0
Madakkathara	378.0	0	4	7.6	8	4.8
Vellanikkara	136.0	0	0	4	0	0

was Wadakkanchery, followed by Madakkathara, Mundathikode, Vadanapilly and Vellanikkara having 399.2, 378, 292.8, 178.8 and 136 nematodes/ 200g soil respectively. The lowest average nematode population was observed at Varavoor (45.34 nematodes/ 200g soil).

Rotylenchulus reniformis was observed only at two locations. The maximum nematode population was registered at Vadanapilly (47.6 nematodes/ 200g soil) and minimum nematode population at Madakkathara (8.0 nematodes/ 200g soil). *Hirschmanniella* sp. was found in the samples collected from three locations- Wadakkanchery, Kolazhi and Thirur. The maximum nematode population was recorded at Thrirur (9.0 nematodes/ 200g soil), followed by Kolazhi (7.2 nematodes/ 200g soil) and Wadakkanchery (6.4 nematodes/ 200g soil).

The population of *Helicotylenchus* sp. encountered from Madakkathara, Vadanapilly and Vellanikkara was 7.6, 7.2 and 4.0 nematodes/ 200g of soil respectively. The lance nematode, *Hoplolaimus* sp. was observed in few samples collected from Mundathikode (4.8 nematodes/ 200g soil), Madakkathara (4.0 nematodes/ 200g soil) and Vadanapilly (3.6 nematodes/ 200g soil) regions. The burrowing nematode, *Radopholus similis* was found only at Madakkathara and that too only in a few samples with 4.8 mean nematode population/ 200g soil.

4.1.2. Population of root-knot nematode in tubers of coleus surveyed in Thrissur District

Population of root-knot nematode in tubers of coleus surveyed in Thrissur District is presented in Table 2. The root-knot nematode population was maximum at Madakkathara with 204.52 nematodes and minimum at Vellanikkara with 58.7 nematodes per 10 g tuber sample. The other locations, Thirur, Kolazhi, Mundathikode, Wadakkanchery, Varavoor and Vadanapilly had 171.52, 125.52, 115.72, 82.64, 61.44 and 61.00 nematodes per 10 g tuber sample respectively.

The white females of root-knot nematodes recorded was higher at Thirur, Kolazhi and Madakkathara with 146.7, 123.84 and 118.56 nematodes respectively. Mundathikode (79.48), Wadakkanchery (75.44), Vellanikkara (45.33) and Vadanappilly (37.60) had more females than Varavoor (19.67).

Table 2. Population of root-knot nematode in tubers of coleus surveyed in Thrissur District

Locations	Population of root-knot nematode / 10 g tuber			
	Number of second stage juveniles	Number of white females	Number of galls	Gall index
Wadakkanchery	82.64	75.44	39.84	2.4
Mundathikode	115.72	79.48	40.96	2.6
Kolazhi	125.52	123.84	72.88	3.9
Thrirur	171.12	146.70	70.80	3.8
Varavoor	61.44	19.67	7.60	1.1
Vadanapilly	61.00	37.60	12.48	1.6
Madakkathara	204.52	118.56	47.32	2.8
Vellanikkara	58.70	45.33	13.87	1.4

Lowest numbers of galls were observed from Varavoor with 7.6 galls per 10 g tuber and maximum numbers of galls were recorded from Kolazhi (72.88). The other regions, Thirur, Madakkathara, Mundathikode, Wadakkanchery, Vellanikkara and Vadanapilly had 70.80, 47.32, 40.96, 39.84, 13.87 and 12.48 galls per 10 g tuber respectively.

Gall index was minimum at Varavoor (1.1) and maximum at Kolazhi (3.9). Lowest gall index was also recorded from Vellanikkara (1.4) and Vadanapilly (1.6). Wadakkanchery, Mundathikode, Madakkathara and Thirur registered gall index of 2.4, 2.6, 2.8 and 3.8 respectively.

4.1.3. Identification of root- knot nematode species

The white females extracted from root-knot nematode infested roots of coleus collected from various coleus growing locations of Thrissur District were stained and processed for taking sections of perineal region. The species of root-knot nematode infesting coleus was identified as *Meloidogyne incognita* (Kofoid and White) Chitwood, 1949 at the Department of Agricultural Entomology, COH, Vellanikkara. Perineal pattern of *M. incognita* is characterized by the presence of high, squarish dorsal arch that often contains a distinct whorl in the tail terminal area. The striae are smooth to wavy, sometimes zigzagged. Distinct lateral lines are absent but the lateral field was marked by breaks and forks in the striae.

4.2 POT CULTURE EXPERIMENT

The pot culture experiment was carried out to evolve the most effective management strategy using biocontrol agents, organic amendments and a chemical insecticide against root-knot nematode, *Meloidogyne* sp. in coleus *(S. rotundifolius)*. Treatments were given as mentioned in 3.5.3. The effect of different treatments on the biometric characters of coleus plants (number of branches per plant, number of leaves per plant and height of the plants), biometric characters of coleus plants at the time of harvesting (fresh shoot weight of the plant and fresh root weight of the plant), yield of coleus plants (number of tubers per plant and weight of tubers per plant) and root-knot nematode population (nematode population from 200g soil, 5g root and 10 g tuber,

number of white females and root-knots from 5g root and root-knot index) were observed (Plate 3).

4.2.1 Biometric characters of coleus plants

All the treatments showed statistically significant variation in plant height, number of leaves and number of branches at monthly intervals and the results are presented in Tables 3 to 5.

4.2.1.1 Number of leaves per plant

The results presented in Table 3 showed the effect of different treatments on the number of leaves of *S. rotundifolius* at monthly intervals. Plants treated with *Tagetes erecta* recorded maximum number of leaves (792.9) which was on par with incorporation of *Chromolaena odorata* leaves (529.7) and neem cake (495.4). The effect of treatments, application of chemical, *B. subtilis* + *P. fluorescens*, *P. fluorescens* + *P. lilaci*nus, *P. lilacinus* alone. *T. viride* alone and *P. fluorescens* alone were on par having 418.0, 405.3, 361.8, 334.4, 312.0 and 305.6 leaves per plant respectively. The other treatments, application of *B. subtilis* alone (278.6) and *P. lilacinus* + *B. subtilis* (270.3) were on par having more number of leaves than the control with 255.8 leaves per plant.

4.2.1.2 Number of branches per plant

The effect of different treatments on number of branches of *S. rotundifolius* recorded at monthly intervals is presented in Table 4. All the treatments were significantly superior to the control with 26.67 branches per plant. Incorporation of *T. erecta* (69.22) was found to be superior to all other treatments. Both treatments, application of chemical (51.00) and *C. odorata* leaves (51.00) produced similar results and they were on par with incorporation of neem cake (49.22), application of *P. lilacinus* alone (45.78) and *T. viride* alone (43.89). The next best treatments, that are statistically superior to the control were the application of *B. subtilis* + *P. fluorescens* (37.56), *P. fluorescens* alone (34.22), *B. subtilis* alone (33.11), *P. fluorescens* + *P. lilaci*nus (32.78) and *P. lilacinus* + *B. subtilis* (27.44).

Table 3. Effect of treatments on number of leaves of coleus plants

Treatments	Number of leaves of coleus plants *			
	Months after treatment			
	1	2	3	4
T_1 - *T. viride*	193.61 ^{ab}	351.33 ^{cd}	437.20 ^{ef}	312.00 ^{efg}
T_2 - *P. fluorescens* (P.f)	172.60 ^{bcd}	288.70 ^{ef}	391.20 ^{f}	305.61 ^{fgh}
T_3 - *P. lilacinus* (P.l)	159.82 ^{cde}	400.00 ^{bc}	492.90 ^{de}	334.42 ^{ef}
T_4 - *B. subtilis* (B.s)	190.31 ^{ab}	281.30 ^{fg}	387.41 ^{f}	278.61 ^{gh}
T_5 - P.f + P.l	153.35 ^{de}	317.48 ^{def}	477.40 ^{e}	361.82 ^{de}
T_6 - P.l + B.s	172.70 ^{bcd}	230.16 ^{g}	376.00 ^{f}	270.30 ^{gh}
T_7 - B.s + P.f	196.10 ^{ab}	418.40 ^{b}	547.82 ^{cd}	405.32 ^{cd}
T_8 - Neem cake	193.00 ^{ab}	349.00 ^{cd}	612.22 ^{bc}	495.41 ^{b}
T_9 - *Tagetes erecta*	202.70 ^{a}	637.30 ^{a}	889.30 ^{a}	792.90 ^{a}
T_{10} - *Chromolaena odorata*	186.72 ^{ab}	422.70^{b}	648.00 ^{b}	529.70 ^{b}
T_{11} - Cartap hydrochloride 4G	145.00 ^{e}	338.02 ^{de}	550.70 ^{cd}	418.00 ^{e}
T_{12} - Untreated control	184.21 ^{abc}	282.00 ^{fg}	378.01 ^{f}	255.84 ^{h}

In a column, values superscripted by a common letter do not differ significantly in DMRT (P=0.05)

* Mean of three replications

Table 4. Effect of treatments on number of branches of coleus

Treatments	Number of branches of coleus plants*			
	Months after treatment			
	1	2	3	4
T_1 - *T. viride*	11.00 ^{abcd}	25.67 ^{b}	38.67 ^{b}	43.89 ^{cd}
T_2 - *P. fluorescens* (P.f)	9.78 ^{bcde}	15.67 ^{c}	25.78 ^{cd}	34.22 ^{ef}
T_3 - *P. lilacinus* (P.l)	8.11 ^{e}	23.56 ^{b}	38.45 ^{b}	45.78 ^{bc}
T_4 - *B. subtilis* (B.s)	11.00 ^{abcd}	17.44 ^{c}	24.45 ^{cd}	33.11 ^{efg}
T_5 - P.f + P.l	9.11 ^{cde}	14.78 ^{c}	19.56 ^{d}	32.78 ^{efg}
T_6 - P.l + B.s	9.22 ^{bcde}	15.89 ^{c}	27.22 ^{c}	27.44 ^{fg}
T_7 - B.s + P.f	12.00 ^{ab}	16.89 ^{c}	29.56 ^{c}	37.56 ^{de}
T_8 - Neem cake	11.78 ^{abc}	23.44 ^{b}	41.56 ^{b}	49.22 ^{bc}
T_9 - *Tagetes erecta*	12.78 ^{a}	40.22 ^{a}	63.00 ^{a}	69.22 ^{a}
T_{10} - *Chromolaena odorata*	9.56 ^{bcde}	35.67 ^{a}	42.78 ^{b}	51.00 ^{b}
T_{11} - Cartap hydrochloride 4G	8.89 ^{de}	20.11 ^{bc}	43.67 ^{b}	51.00 ^{b}
T_{12} - Untreated control	7.78 ^{e}	14.67 ^{c}	18.89 ^{d}	26.67 ^{g}

In a column, values superscripted by a common letter do not differ significantly in DMRT (P=0.05)

* Mean of three replications

4.2.1.3 Height of coleus plants

The results showing the effect of different treatments on the mean plant height of *S. rotundifolius* at monthly intervals are given in Table 5. Incorporation of *T. erecta* whole plant (45.98 cm) was on par with incorporation of *C. odorata* with a plant height of 44.01 cm. Incorporation of neem cake also increased the plant height with 41.72 cm and the above three treatments were significantly superior to the chemical (39.45 cm) treatment. The other treatments in the order of effectiveness were *P. lilacinus*, *P. fluorescens* + *P. lilacinus*, *B. subtilis* + *P. fluorescens*, *P. fluorescens*, *T. viride*, *P. lilacinus* + *B. subtilis* and *B. subtilis* giving mean plant height to the tune of 38.26 cm to 31.22 cm showed statistically significant increase in plant height over the control with 28.12 cm of plant height.

4.2.2 Biometric characters of coleus plants at the time of harvesting

4.2.2.1 Fresh shoot weight

The effects of different treatments on the fresh weight of shoot per plant are presented in Table 6. Plants treated with *T. erecta* whole plant recorded the highest fresh weight of shoot with 478.4 g and it was statistically superior over the treatments, *C. odorata* and neem cake having 315.7 g and 287.1 g shoot weight per plant respectively. The chemical treatment (222.4 g) increased the shoot weight, which was on par with combination treatment, *B. subtilis* + *P. fluorescens* (216.8 g). The other treatments, *P. fluorescens* + *P. lilacinus* (194.2 g) and *P. lilacinus* alone (176.1 g) also increased the shoot weight of the plant. Soil application of *T. viride* *P. fluorescens*, *P. lilacinus* + *B. subtilis* and *B. subtilis* treatments were on par having 165.5 g, 162.0 g, 147.5 g and 143.4 g of shoot weight respectively and these were found superior over control (142.2 g).

4.2.2.2 Fresh root weight

The fresh root weight per plant observed at the time of harvest under different treatments is given in Table 6. *T. erecta* incorporated plants showed highest fresh weight of root system of plant with 161.80 g. The next superior treatment was the incorporation of *C. odorata* having 126.70 g, which was on par with the treatments, neem cake (121.70 g) and cartap hydrochloride (112.70 g). The other treatments in the order of effectiveness were *B. subtilis* + *P. fluorescens*, *P. fluorescens* + *P. lilacinus*, *P.*

Table 5. Effect of treatments on height of coleus plants

Treatments	Height of coleus plants (cm)*			
	Months after treatment			
	1	2	3	4
T_1 - *T. viride*	24.34 ^{a}	27.98 ^{bcd}	33.85 ^{cd}	34.48 ^{fg}
T_2 - *P. fluorescens* (P.f)	23.06 ^{abc}	27.79 ^{bcd}	35.17 ^{bcd}	36.00 ^{ef}
T_3 - *P. lilacinus* (P.l)	24.23 ^{a}	27.85 ^{bcd}	37.20 ^{bc}	38.26 ^{de}
T_4 - *B. subtilis* (B.s)	22.63 ^{bc}	24.86 ^{d}	30.04 ^{de}	31.22 ^{h}
T_5 - P.f + P.l	23.85 ^{ab}	27.83 ^{bcd}	37.09 ^{bc}	37.90 ^{de}
T_6 - P.l + B.s	22.17 ^{c}	26.55 ^{cd}	31.22 ^{de}	32.29 ^{gh}
T_7 - B.s + P.f	23.65 ^{ab}	26.54 ^{cd}	36.92 ^{bc}	37.42 ^{def}
T_8 - Neem cake	24.32 ^{a}	33.07 ^{a}	39.80 ^{ab}	41.72 ^{bc}
T_9 - *Tagetes erecta*	23.44 ^{abc}	31.46 ^{ab}	42.55 ^{a}	45.98 ^{a}
T_{10} - *Chromolaena odorata*	23.20 ^{abc}	30.22 ^{abc}	37.67 ^{abc}	44.01 ^{ab}
T_{11} - Cartap hydrochloride 4G	23.00 ^{abc}	30.78 ^{ab}	39.00 ^{abc}	39.45 ^{cd}
T_{12} - Untreated control	22.68 ^{bc}	25.41 ^{d}	27.64 ^{e}	28.12 ^{i}

In a column, values superscripted by a common letter do not differ significantly in DMRT (P=0.05)

* Mean of three replications

lilacinus alone, *T. viride* alone, *P. fluorescens* alone, *B. subtilis* alone and *P. lilacinus* + *B. subtilis* with mean fresh root weight to the tune of 108.00, 100.50, 98.54, 90.11, 88.96, 83.33 and 78.98 g showed higher production of root system over the control (53.10 g).

4.2.3 Yield of coleus plants

4.2.3.1 Number of tubers

The data regarding number of tubers per plant at the time of harvest are presented in Table 6. The plants treated with *T. viride* (5.667) produced highest number of tubers per plant and it was on par with the treatments, neem cake, *B. subtilis* + *P. fluorescens*, *P. fluorescens* + *P. lilacinus*, *T. erecta* and chemical having 5.00, 4.78, 4.67, 4.56 and 4.55 tubers per plant. Soil application of *P. lilacinus* alone, *C. odorata* and *P. lilacinus* + *B.subtilis* was statistically on par producing 4.22, 4.22 and 4.11 tubers per plant. The treatments, *P. fluorescens* alone (3.56) and *B. subtilis* (2.89) produced more number of tubers over the control plants (2.00).

4.2.3.2 Weight of tubers

The data on weight of tubers per plant in grams is given in Table 6. The treatments, application of *T. viride* alone, neem cake, chemical, *P. fluorescens* + *P. lilacinus* and *B. subtilis* + *P. fluorescens* were statistically superior treatments having 41.10, 40.54, 39.68, 38.30 and 38.00 g of tubers per plant respectively. The above treatments were on par with combination treatment, *P. lilacinus* + *B. subtilis*, *P. lilacinus* alone and *P. fluorescens* alone with 36.48, 36.30 and 36.10 g tubers respectively. Incorporation of *T. erecta* (27.76 g), application of *B. subtilis* (25.68 g) and *C. odorata* (22.62 g) were inferior to the above treatments but was superior to the control (14.01 g).

4.2.4 Root-knot nematode population

4.2.4.1 Nematode population in soil

The results pertaining to the effect of different treatments on the population build up of nematodes in the root zone of coleus at the time of harvest are presented in Table 7. There was drastic reduction in the mean nematode population in

Table 6. Effect of treatments on plant growth characters and yield of coleus *

Treatments	Fresh shoot weight/plant (g)	Fresh root weight/plant (g)	No. of tubers/plant	Weight of tubers/plant (g)
T_1 - *T. viride*	165.50 ^{gh}	90.11 ^{ef}	5.67 ^{a}	41.10 ^{a}
T_2 - *P. fluorescens* (P.f)	162.00 ^{gh}	88.96 ^{ef}	3.56 ^{cd}	36.10 ^{ab}
T_3 - *P. lilacinus* (P.l)	176.10 ^{fg}	98.54 ^{de}	4.22 ^{bc}	36.30 ^{ab}
T_4 - *B. subtilis* (B.s)	143.40 ^{h}	83.33 ^{f}	2.89 ^{de}	25.68 ^{c}
T_5 - P.f + P.l	194.20 ^{ef}	100.50 ^{de}	4.67 ^{abc}	38.30 ^{a}
T_6 - P.l + B.s	147.50 ^{gh}	78.98 ^{f}	4.11 ^{bc}	36.48 ^{ab}
T_7 - B.s + P.f	216.80 ^{de}	108.00 ^{cd}	4.78 ^{ab}	38.00 ^{a}
T_8 - Neem cake	287.10 ^{c}	121.70 ^{bc}	5.00 ^{ab}	40.54 ^{a}
T_9 - *Tagetes erecta*	478.40 ^{a}	161.80 ^{a}	4.56 ^{abc}	27.76 ^{b}
T_{10} - *Chromolaena odorata*	315.70 ^{b}	126.70 ^{b}	4.22 ^{bc}	22.62 ^{c}
T_{11} - Cartap hydrochloride 4G	222.40 ^{d}	112.70 ^{bcd}	4.55 ^{abc}	39.68 ^{a}
T_{12} - Untreated control	142.20 ^{h}	53.10 ^{g}	2.00 ^{e}	14.01 ^{d}

In a column, values superscripted by a common letter do not differ significantly in DMRT (P=0.05)

* Mean of three replications

treated pots compared to the untreated control. The population of nematode in soil ranged from 31.83 to 105.80 in treated pots against 157.80 nematodes in control.

The application of *P. lilacinus* alone (31.83) and *P. fluorescens* + *P. lilacinus* (41.00) recorded lowest nematode population giving about 79.83 and 74.02 per cent reduction in nematode population respectively over control. Next best treatment was the application of chemical giving 61.66 per cent reduction over control, which was on par with the incorporation of neem cake and *T. erecta* having 60.50, 63.67 & 65.67 nematodes respectively. The other treatments, incorporation of *C. odorata* (81), application of *P. fluorescens* alone (82.50), *B. subtilis* + *P. fluorescens* (91.17), *P. lilacinus* + *B. subtilis* (96.17), *B. subtilis* alone (98.17) and *T. viride* alone (105.80) were on par and these treatments had less number of nematodes than the control (157.8) giving 48.67, 47.72, 42.22, 39.06, 37.79 and 32.95 per cent respective reduction in nematode population over control.

4.2.4.2 Nematode population in root

The effect of different treatments on nematode population in 5g root of coleus is presented in Table 7. All the treatments were significantly superior to control. The mean number of nematodes ranged from 109.20 to 232.80 per 5 g of root in various treatments as against a high population of 316.8 in control.

Nematode population in root was observed to be very low in the pots treated with Cartap (109.20) which showed 65.53 per cent reduction over control and it was on par with the treatments, *P. lilacinus* alone (114.00), *P. fluorescens* + *P. lilacinus* (131.20), neem cake (134.80), *T. erecta* (139.50), *C. odorata* (159.50), *P. fluorescens* alone (163.20) and *P. lilacinus* + *B. subtilis* (178.20) giving 64.02, 58.55, 57.45, 55.97, 49.65, 48.48 and 43.75 per cent reduction over control respectively. The next best treatment was the application of *B. subtilis* alone (213.50) having 32.61 per cent reduction over control, which was on par with the treatments, soil application of *T. viride* alone (217.80) and combination of *B. subtilis* + *P. fluorescens* (232.80) with 31.25 and 26.52 per cent reduction over control. All the treated plants were superior to the control (316.80) having less population of nematodes in 5 g root.

Table 7. Effect of treatments on root-knot nematode population

Treatments	Root-knot nematode population *					
	Soil (200g)	Per cent decrease over control	Root (5g)	Per cent decrease over control	Tuber (10g)	Per cent decrease over control
T_1 - *T. viride*	105.80 ^{b} (2.02)	32.95	217.80 ^{b} (2.34)	31.25	139.00 ^{b} (2.15)	33.75
T_2 - *P. fluorescens* (P.f)	82.5 ^{cd} (1.92)	47.72	163.20 ^{de} (2.22)	48.48	104.30 ^{cde} (2.02)	50.29
T_3 - *P. lilacinus* (P.l)	31.83 ^{f} (1.52)	79.83	114.00 ^{f} (2.06)	64.02	71.50 ^{fg} (1.86)	65.92
T_4 - *B. subtilis* (B.s)	98.17 ^{bc} (2.00)	37.79	213.50 ^{bc} (2.33)	32.61	137.70 ^{b} (2.14)	34.37
T_5 - P.f + P.l	41.00 ^{f} (1.62)	74.02	131.30 ^{ef} (2.12)	58.55	81.17 ^{ef} (1.91)	61.31
T_6 - P.l + B.s	96.17 ^{bc} (1.99)	39.06	178.20 ^{cd} (2.25)	43.75	114.80 ^{c} (2.06)	45.28
T_7 - B.s + P.f	91.17 ^{bc} (1.96)	42.22	232.80 ^{b} (2.37)	26.52	106.50 ^{cd} (2.03)	49.24
T_8 - Neem cake	63.67 ^{de} (1.81)	59.65	134.80 ^{ef} (2.13)	57.45	84.33 ^{def} (1.93)	59.80
T_9 - *Tagetes erecta*	65.67 ^{de} (1.82)	58.38	139.50 ^{def} (2.14)	55.97	53.83 ^{g} (1.74)	74.34
T_{10} - *Chromolaena odorata*	81.00 ^{cd} (1.91)	48.67	159.50 ^{de} (2.20)	49.65	83.50 ^{def} (1.93)	60.20
T_{11} - Cartap hydrochloride 4G	60.50 ^{e} (1.79)	61.66	109.20 ^{f} (2.04)	65.53	93.17 ^{cdef} (1.97)	55.59
T_{12} - Untreated control	157.80 ^{a} (2.20)	-	316.80 ^{a} (2.50)	-	209.80 ^{a} (2.32)	-

In a column, values superscripted by a common letter do not differ significantly in DMRT (P=0.05)

Figures in parenthesis are log (x+1) transformed values

* Mean of three replications

4.2.4.3 Nematode population in tubers

Statistical analysis of data regarding the effect of different treatments on the nematode population in 10g tuber sample was presented in Table 7. The population of nematode in tuber sample ranged from 53.83 to 139.0 in treated pots against 209.8 in control. The superior treatment was incorporation of *T. erecta* (53.83) and it was on par with *P. lilacinus* alone (71.50) having 74.34 and 65.92 per cent reduction in nematode population over control respectively. These treatments were on par with *P. fluorescens* + *P. lilacinus* (81.17), *C. odorata* (83.50), neem cake (84.33), chemical (93.17), *P. fluorescens* alone (104.30) and *B. subtilis* + *P. fluorescens* (106.50) giving 61.31, 60.20, 59.80, 55.59, 50.29 and 49.24 per cent reduction in the number of nematodes over control in respective treatments. Application of *P. lilacinus* + *B. subtilis* (114.80) was significantly different with *B. subtilis* alone (137.70) and *T. viride* alone (139.00) giving 45.28, 34.37 and 33.75 per cent reduction over control respectively. The above treatments were least effective but were superior to the control.

4.2.4.4 Number of white females in root

The mean number of white females ranged from 49.67 to 104.00 in 5g root sample of various treatments as against 181.0 in control (Table 8).

Incorporation of *T. erecta* (49.67) gave the highest reduction in number of white females recording 72.56 per cent reduction over control and it was on par with the treatments, application of *P. fluorescens* + *P. lilacinus* (57.67), *C. odorata* (61.33), *P. lilacinus* alone (65.67), neem cake (68.00), chemical (70.33), *B. subtilis* + *P. fluorescens* (74.33) and *P. lilacinus* + *B. subtilis* (76.67) giving 68.14, 66.12, 63.72, 62.43, 61.14, 58.93 and 57.64 per cent reduction over control respectively. The application of *P. fluorescens* alone (84.33) and *B. subtilis* alone (89.00) were on par having 53.41 and 50.83 per cent reduction in respective treatments over control. The soil application of *T. viride* alone (104.00) with 42.54 per cent reduction over control, was found inferior to other treatments, but was superior to the control (181.00).

4.2.4.5 Number of root-knots

The data relating to the number of root-knots in 5g root are presented in Table 8. All the treatments were superior to the control. The mean number of galls

Table 8. Effect of treatments on the number of white females, root-knots and root-knot index *

Treatments	Number of white females in 5g root	Per cent decrease over control	Number of root knots in 5 g root	Per cent decrease over control	Root-knot Index (1-5 scale)
T_1 - *T. viride*	104.00 ^{b} (2.02)	42.54	76.33 ^{bc}	54.83	3.67 ^{b}
T_2 - *P. fluorescens* (P.f)	84.33 ^{bc} (1.93)	53.41	54.67 ^{bcd}	67.65	3.33 ^{bc}
T_3 - *P. lilacinus* (P.l)	65.67 ^{cd} (1.82)	63.72	48.00 ^{cd}	71.60	2.43 ^{cd}
T_4 - *B. subtilis* (B.s)	89.00 ^{bc} (1.95)	50.83	84.33 ^{b}	50.10	3.89 ^{b}
T_5 - P.f + P.l	57.67 ^{cd} (1.77)	68.14	49.67 ^{cd}	70.61	2.45 ^{cd}
T_6 - P.l + B.s	76.67 ^{bcd} (1.89)	57.64	70.67 ^{bc}	58.18	2.97 ^{bcd}
T_7 - B.s + P.f	74.33 ^{bcd} (1.88)	58.93	62.33 ^{bcd}	63.12	2.73 ^{bcd}
T_8 - Neem cake	68.00 ^{cd} (1.84)	62.43	51.33 ^{cd}	69.63	2.33 ^{cd}
T_9 - *Tagetes erecta*	49.67 ^{d} (1.70)	72.56	33.33 ^{d}	80.28	2.00 ^{d}
T_{10} - *Chromolaena odorata*	61.33 ^{cd} (1.79)	66.12	55.33 ^{bcd}	67.26	2.37 ^{cd}
T_{11} - Cartap hydrochloride 4G	70.33 ^{cd} (1.85)	61.14	59.67 ^{bcd}	64.69	2.67 ^{bcd}
T_{12} - Untreated control	181.00 ^{a} (2.26)	-	169.00 ^{a}	-	5.00 ^{a}

In a column, values superscripted by a common letter do not differ significantly in DMRT (P=0.05)

Figures in parenthesis are log (x+1) transformed values

* Mean of three replications

ranged from 33.33 to 169.00 per 5g root in various treatments. Incorporation of *T. erecta* (33.33) gave maximum reduction in number of galls recording 80.28 per cent reduction over control. The next superior treatments were application of *P. lilacinus* alone (48.00), *P. fluorescens* + *P. lilacinus* (49.67) and neem cake (51.33) giving 71.60, 70.61 and 69.63 per cent respective reduction in number of galls over control. Slight increasing trend in number of galls was observed in *P. fluorescens* alone (54.67), *C. odorata* (55.33), chemical (59.67), *B. subtilis* + *P. fluorescens* (62.33), *P. lilacinus* + *B. subtilis* (70.67) and *T. viride* alone (76.33) giving 67.65, 67.26, 64.69, 63.12, 58.18 and 54.83 per cent respective reduction over control. Application of *B. subtilis* alone (84.33) having 50.10 per cent reduction over control, was found to be inferior to all other treatments, but was superior to the control having 169 galls per 5g root sample.

4.2.4.6 Root-knot index (RKI)

Data presented in Table 8 indicate the results regarding the effect of different treatments on root-knot index. Most superior treatment was incorporation of *T. erecta* with root-knot index of 2.00. The treatments, application of neem cake, *C. odorata*, *P. lilacinus* alone and *P. fluorescens* + *P. lilacinus* were on par having the root knot index of 2.33, 2.37, 2.43 and 2.45 respectively. Next best treatments were the application of chemical insecticide, *B. subtilis* + *P. fluorescens* and *P. lilacinus* + *B. subtilis* producing the similar results with slight variation in root-knot index of 2.67, 2.73 and 2.97 respectively. Application of *P. fluorescens* alone with 3.33 root-knot index was on par with the application of *T. viride* alone (3.67) and *B. subtilis* alone (3.89) and these treatments were superior to the control having root-knot index of 5.00.

T_1 - *T. viride*

T_2 - *P. fluorescens*

T_3 - *P. lilacinus*

T_4 - *B. subtilis*

T_5 - *P. fluorescens* + *P. lilacinus*

T_6 - *P. lilacinus* + *B. subtilis*

Plate 3. Effect of treatments on coleus plants

T_7 - *B. subtilis* + *P. fluorescens*

T_8 - Neem cake

T_9 - *Tagetes erecta*

T_{10} - *Chromolaena odorata*

T_{11} - Cartap hydrochloride 4G

T_{12} - Untreated control

Plate 3. Effect of treatments on coleus plants

Discussion

5. DISCUSSION

The results of the present study entitled 'Management of root-knot nematode, *Meloidogyne* sp. (Kofoid and White) in coleus, *Solenostemon rotundifolius* (Poir) Morton' discussed hereunder.

5.1. ASSESSMENT OF PLANT PARASITIC NEMATODE POPULATION IN COLEUS

A survey was conducted to assess the nematode population at different coleus growing regions of Thrissur District. Soil and tuber samples were collected from the major coleus growing locations namely Mundathikode, Wadakkanchery, Varavoor, Thirur, Kolazhi, Vellanikkara, Madakkathara and Vadanapilly. The plant parasitic nematodes associated with coleus were recorded from the soil samples collected during the survey. Further the root-knot nematode population, number of white females and gall index in 10 g tuber were observed. Species of root-knot nematode infesting coleus plants was also identified from the samples collected.

The data regarding plant parasitic nematodes associated with major coleus growing regions of Thrissur District are presented in Fig. 1 and the root-knot nematode populations in 10 g tuber are presented in Fig. 2. Six plant parasitic nematodes were observed from the rhizosphere of coleus in the surveyed regions of Thrissur District. The plant parasitic nematodes recorded were root-knot nematode (*Meloidogyne incognita* (Kofoid and White) Chitwood, 1949), reniform nematode (*Rotylenchulus reniformis* Linford and Oliveiria), burrowing nematode (*Radopholus similis* (Cobb, 1893) Thorne, 1949), lance nematode (*Hoplolaimus* sp.), spiral nematode (*Helicotylenchus* sp.) and rice-root nematode (*Hirschmanniella oryzae* Luc and Goodey, 1963)

Meloidogyne incognita was recorded from all the eight locations surveyed. The maximum average root-knot nematode population of 450.4 nematodes/ 200g soil, 72.88 galls/ 10 g tuber and gall index of 3.9 was recorded from the samples collected from Kolazhi followed by Thirur and Madakkathara (Fig. 3). The higher population of nematodes in these areas will be due to the intense cultivation of

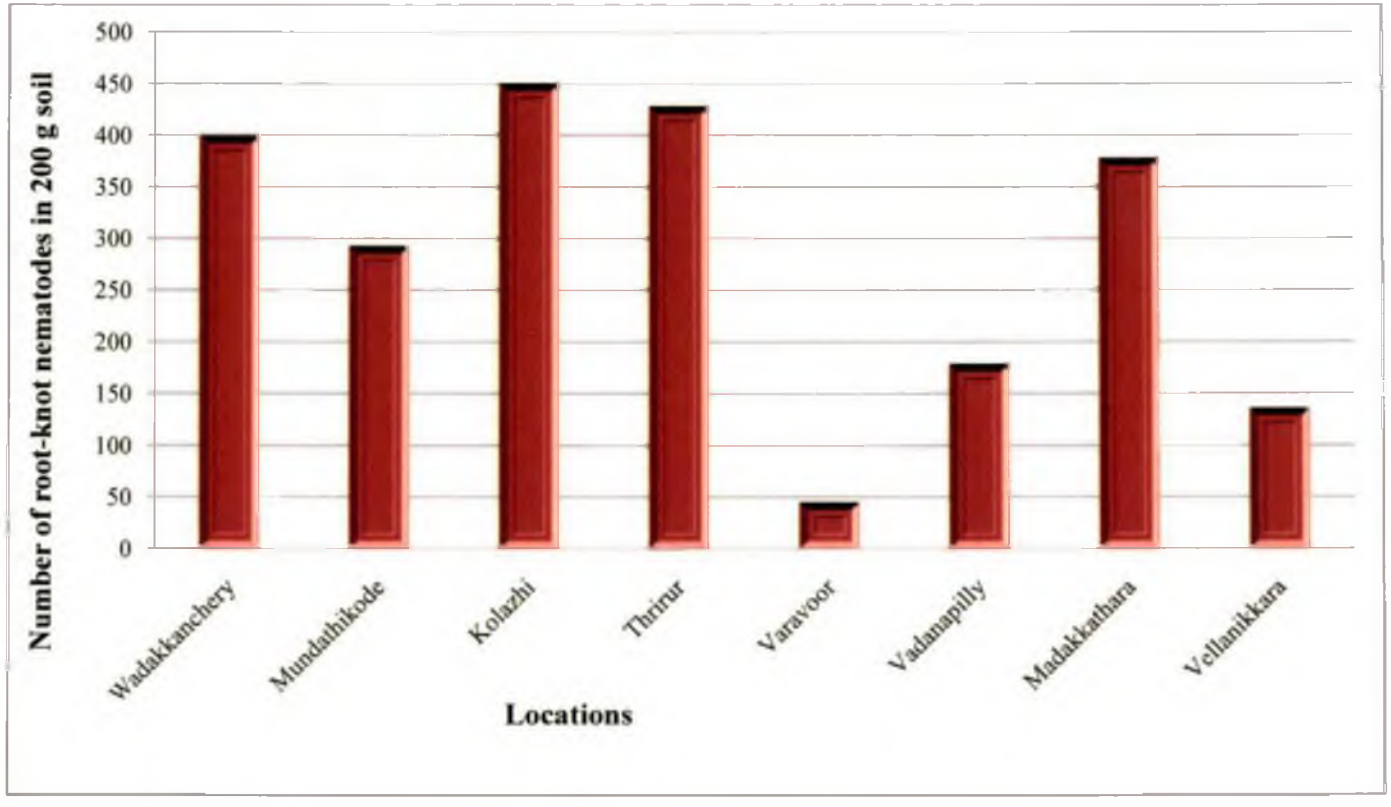


Fig. 1. Population of root-knot nematodes in soil

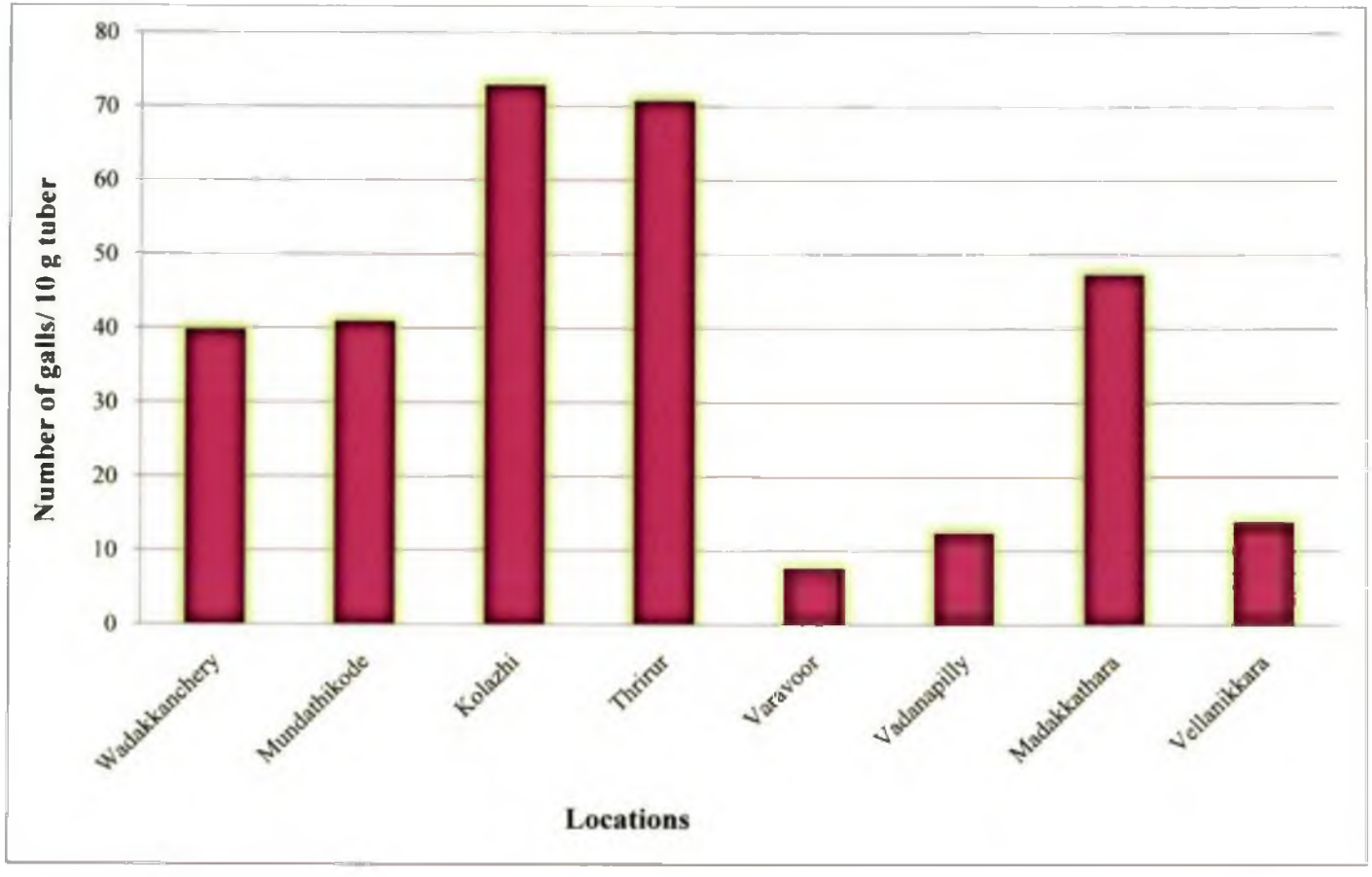


Fig.2. Number of galls per 10 g tuber

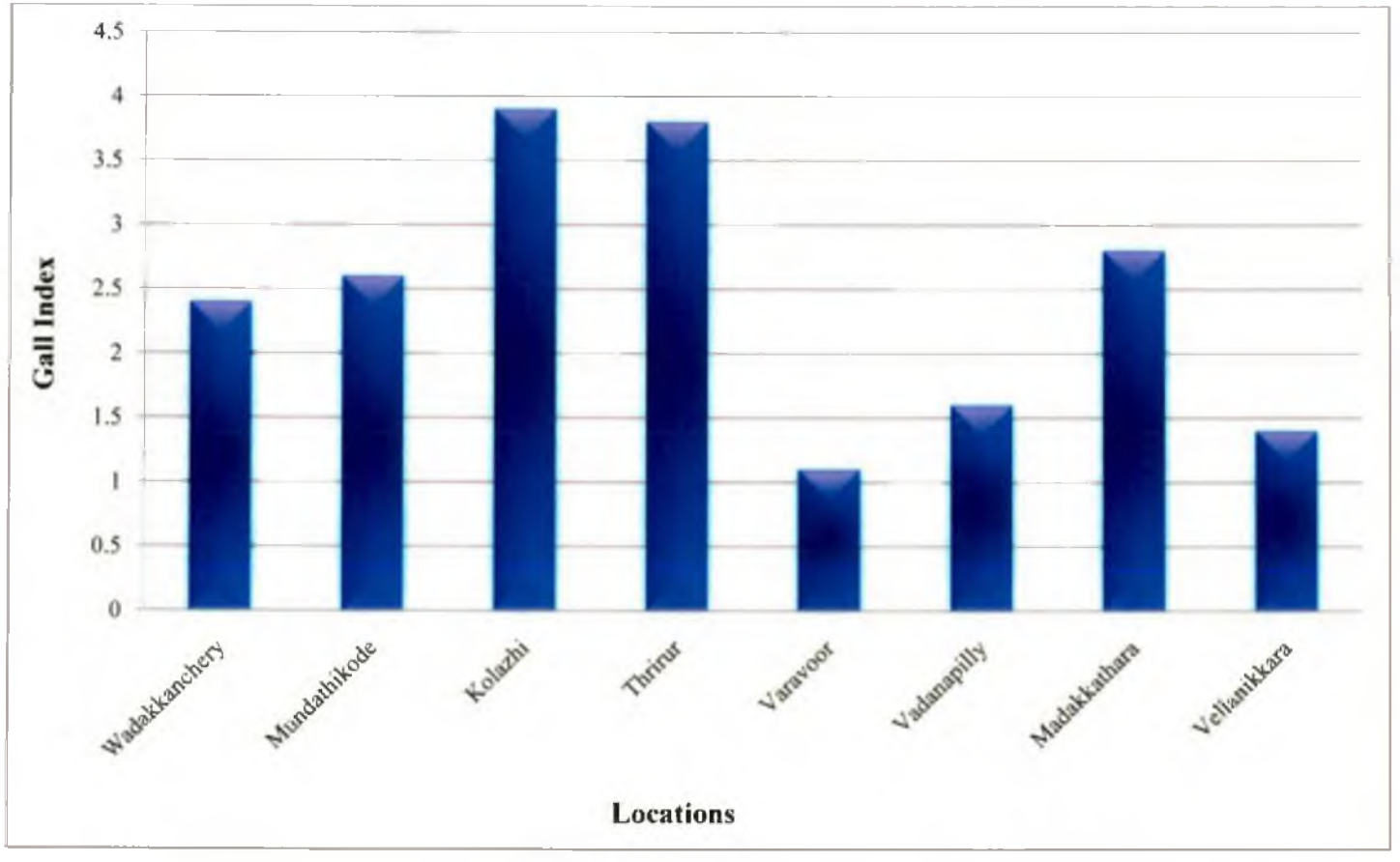


Fig. 3. Incidence of root-knot nematode in different locations of Thrissur District

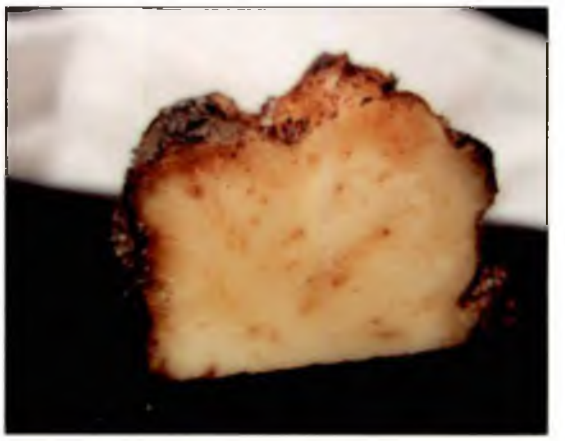
vegetables like tomato, brinjal and okra, which are all root-knot nematode loving crops grown prior to coleus. The lowest population of root-knot nematode was recorded from Varavoor location as the growers have kept the field fallow for the previous seasons. Other nematodes were relatively few in numbers and they are not harmful to coleus tubers. Therefore, it can be concluded that *M. incognita* is the important plant parasitic nematode associated with coleus in major coleus growing regions of Thrissur District. The species of root-knot nematode infesting coleus plants was also identified as *Meloidogyne incognita* (Kofoid and White) Chitwood, 1949. Sathyarajan *et al.* (1966) reported on the root-knot nematode infestation in coleus from Kerala. The association of *M. incognita* with coleus, *S. rotundifolius* was also reported earlier (Sosamma, 1988; Nisha and Sheela, 2006).

During the survey, the root-knot nematode infested plants were found to be stunted and symptoms mostly observed in patches. Stunted plants with small, pale green leaves, yellowing, wilting, reduced yield, and premature death of plants were the symptoms observed above ground due to root-knot nematode infestation. Severely infested plants were also seen to wilt even in the presence of sufficient soil moisture, especially during the noon. The underground symptoms were more prominent than above ground symptoms due to the presence of swollen knotted roots and swollen galled tubers. In severe cases of infestations, rotting of the galled roots and tubers were observed (Plate 4). Sosamma (1988) reported that the crop loss due to *M. incognita* on coleus (*Coleus parviflorus*) was 92 per cent in terms of fresh weight of tubers at an inoculum level of 10,000 *M. incognita* juveniles per pot of 10 litre capacity.

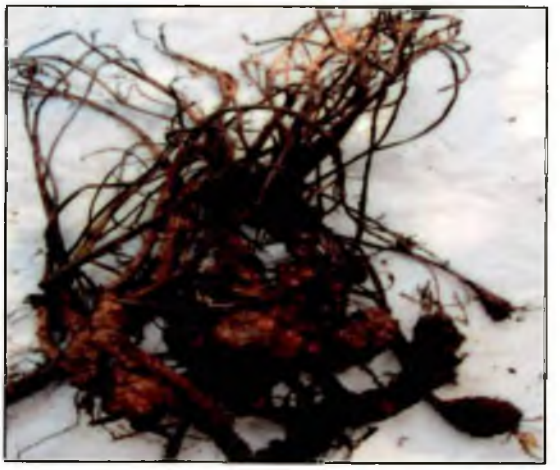
The losses caused by these nematodes are enormous and this problem has to be viewed very seriously. So an eco-friendly and sustainable management strategy against the root knot nematode has to be adopted at the earliest. Most of the farmers are ignorant of the damage caused by plant parasitic nematodes on coleus in the field and storage. Mohandas and Ramakrishnan (1998) observed that the root-knot nematodes continue to multiply inside the tuber after harvest during the storage of tubers. So there must be adequate awareness programmes among the farmers to familiarise with the attack of nematodes and to adopt management measures against root-knot nematode to increase the marketable yield of the crop.

Swollen, knotted roots

Galled tubers



Cross section of infested tuber



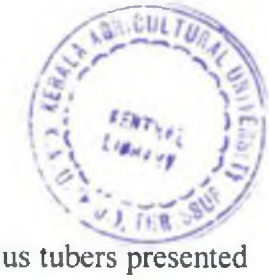
Rotten tubers

Plate 4. Coleus roots and tubers infested with *Meloidogyne incognita*

5.2 POT CULTURE EXPERIMENT

In the present study, the efficacy of biocontrol agents (*Trichoderma viride* @ 25g/m^2, *Pseudomonas fluorescens* @ 25g/m^2, *Paecilomyces lilacinus* @ 25g/m^2 , *Bacillus subtilis* @ 25g/m^2 , *P. fluorescens* + *P. lilacinus* @ 12.5g+12.5g/m^2 , *P. lilacinus* + *B. subtilis* @ 12.5g+12.5g/m^2 and *B. subtilis* + *P. fluorescens* @ 12.5g+12.5g/m^2), organic amendments (neem cake @ 100g/m^2, incorporation of *Tagetes erecta* as a whole @ 250 g/pot and *Chromolaena odorata* leaves @ 250 g/pot and a chemical insecticide (cartap hydrochloride @ 4G 1kg ai/ha) were evaluated by comparing with an untreated control.

The results were assessed in terms of biometric characters of coleus plants during growth and at the time of harvest, yield and root-knot nematode population in soil, root and tuber.



5.2.1 Biometric characters of coleus plants

5.2.1.1 Number of leaves per plant

Number of leaves recorded till the harvesting of coleus tubers presented in Fig. 4 revealed that the plants incorporated with whole plants of *Tagetes erecta* (Marigold) produced maximum number of leaves than the other treatments with 792.9 leaves per plant. This is in line with the findings of Natarajan *et al.* (2006) who reported that the plant height, leaf number and fruit yield were significantly greater in tomato plants infested with *M. incognita* when treated with whole plant extracts of *T. erecta* (20 per cent w/v 100 ml aliquots). Apart from the antihelminthic properties of *T. erecta*, the exothermic reactions which occur during decomposition and the organic acids released during decomposition will improve physical and chemical properties of the soil are explained as possible reason for the reduction of the nematode population and enhancement in the plant growth parameters.

Incorporation of *Chromolaena odorata* leaves and neem cake were reported as the next best treatments having 529.7 and 495.4 leaves per plant respectively. Similar observation in relation to the incorporation of *C. odorata* leaves was reported in the study conducted by Nisha and Sheela (2002), whose work revealed that the highest number of leaves were obtained by mulching *C. odorata* leaves @ 5 kg/m^2 at 15 days before planting of kacholam. Seenivasan (2010) evaluated

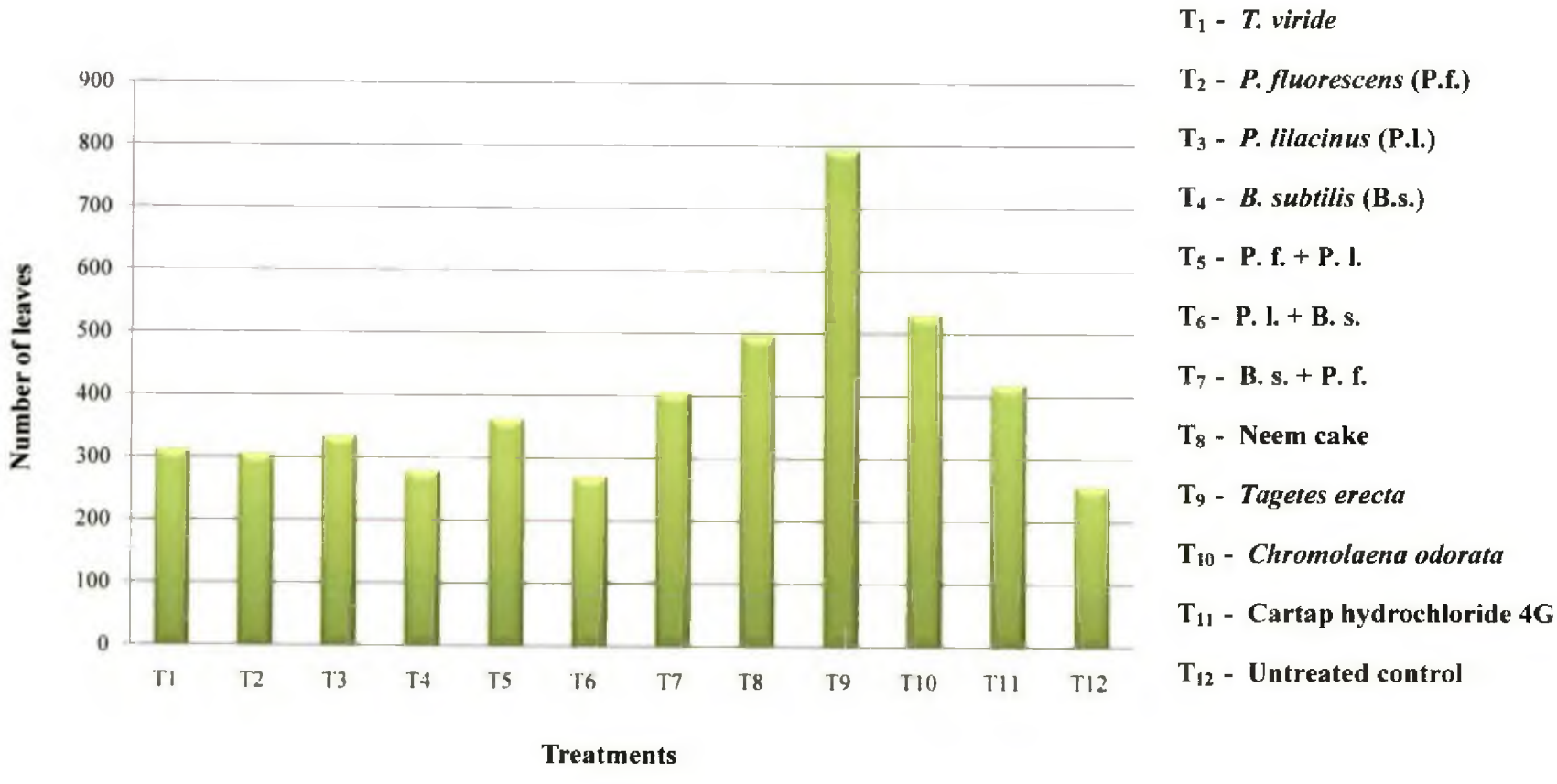


Fig.4. Effect of treatments on number of leaves of coleus plants

the efficacy of locally available organic amendments (at the rate of 500 kg/ha and 400 kg/ha) against *M. incognita* in medicinal coleus, *Coleus forskohlii* and found that neem cake was significantly superior in reducing the nematode population and increasing growth and yield of medicinal coleus than other treatments.

5.2.1.2 Number of branches per plant

The influence of different treatments on number of branches of coleus plants are presented in Fig. 5. Among the different treatments, plants treated with *T. erecta*, Cartap hydrochloride and *C. odorata* leaves produced more number of branches giving 69.22, 51.00 and 51.00 respectively compared to other treatments.

Ploeg (2000) reported that amending soil with marigold (*Tagetes patula* cv. Single Gold) increased the weight of tomato plant tops, reduced galling and final nematode population levels. Soil application of cartap hydrochloride not only reduced the nematode population but also improved the plant growth parameters of coleus plants. Tobih *et al.* (2011) reported that *C. odorata* applied as organic mulch (10 tons/ha) to protect *Celosia argentea* from damage by nematodes was more effective against root knot nematodes and the application also improved the plant growth characters.

5.2.1.3 Height of coleus plants

The results on plant height of coleus are presented in Fig. 6. Plants incorporated with *T. erecta* were statistically superior to all other treatments with 45.98 cm of plant height. This result agrees with the findings of Kumar and Reddy (2001) as they reported that the treatment with marigold waste in sunflower improved the plant growth better over other individual treatments. Olabiyi (2006) also observed an increase in plant height, leaf number and fruit yield of tomato plants with reduction of nematode population in soil when treated with the root extracts of marigold plants.

The next best treatments that increased plant height were incorporation of *C. odorata* leaves and neem cake application. Incorporation of *C. odorata* leaves to the infested soil reduced the nematode population and improved the plant growth. Odeyemi *et al.* (2011) reported that *C. odorata* residue at 1% w/w along with organic fertiliser significantly suppressed *M. incognita* galling, inhibited the nematode

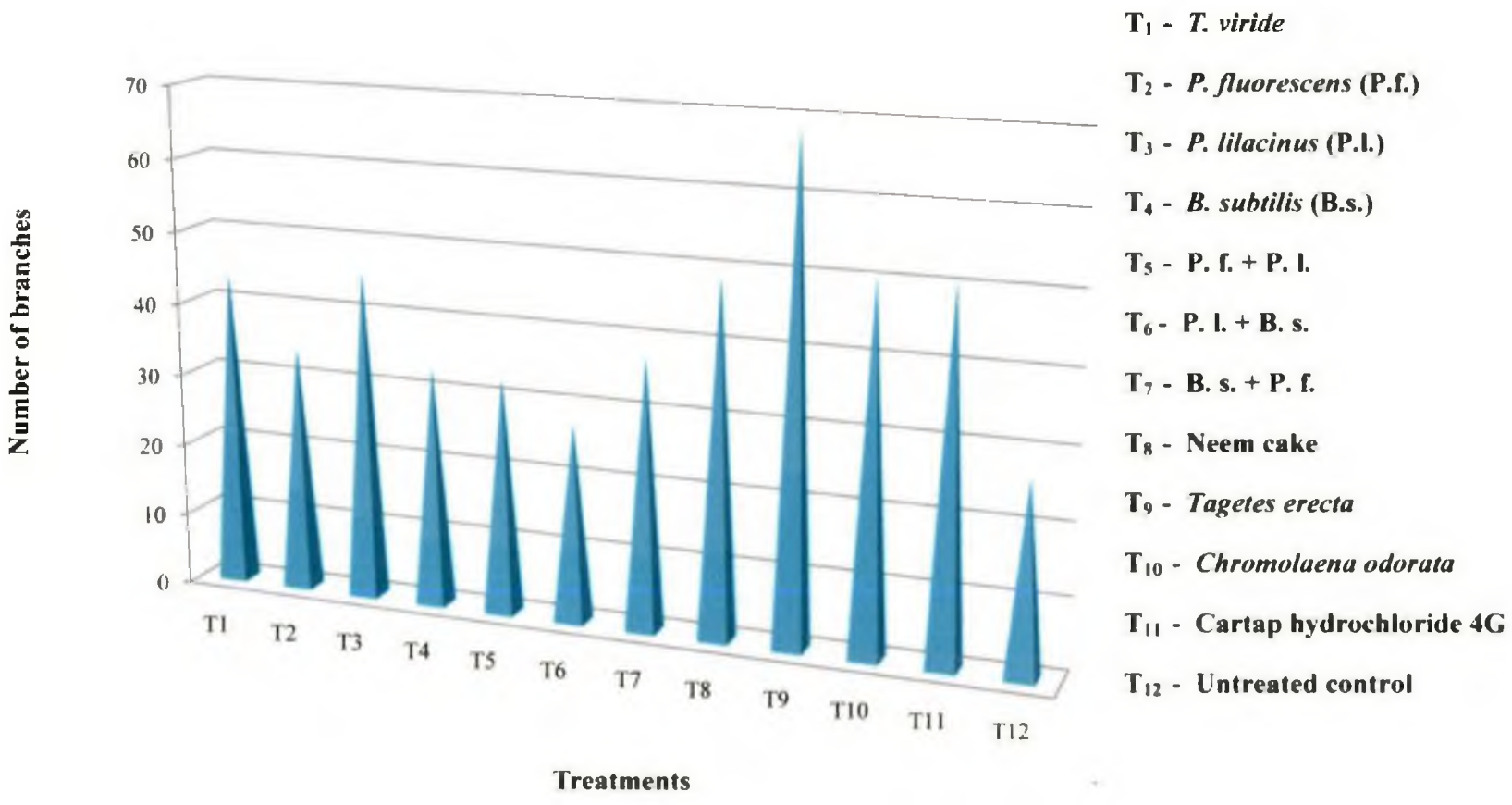
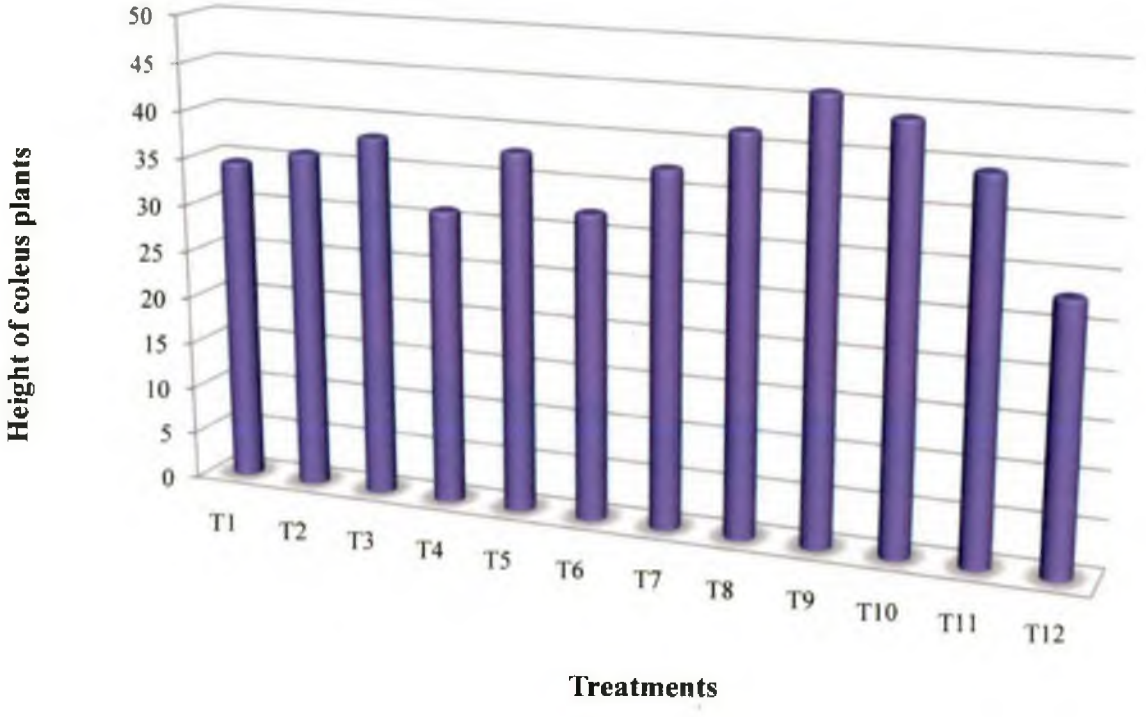


Fig. 5. Effect of treatments on number of branches of coleus plants



T_1 - *T. viride*

T_2 - *P. fluorescens* (P.f.)

T_3 - *P. lilacinus* (P.l.)

T_4 - *B. subtilis* (B.s.)

T_5 - P. f. + P. l.

T_6 - P. l. + B. s.

T_7 - B. s. + P. f.

T_8 - Neem cake

T_9 - *Tagetes erecta*

T_{10} - *Chromolaena odorata*

T_{11} - Cartap hydrochloride 4G

T_{12} - Untreated control

Fig.6. Effect of treatments on height of coleus plants

fecundity and reduced the number of eggs and juveniles and a remarkable increase in plant height, number of leaves per plant, leaf area, cob weight and grain yield were also observed on maize plants when treated with the mixture. Soil amendment with neem cake reduced the attack of *M. incognita* on mungbean, *Vigna radiata* and significantly improved the plant height also (Abid *et al.*, 1995).

5.2.2 Biometric characters of coleus plants at the time of harvest

The influence of different treatments in improving the biometric characters of coleus in relation to fresh weight of shoot and root at the time of harvest is presented in Fig. 7. Among different treatments studied, incorporation of *T. erecta*, *C. odorata* leaves, neem cake, cartap hydrochloride 4 G application and combination of *B. subtilis* + *P. fluorescens* were more effective over other treatments.

Incorporation of *T. erecta* was the best treatment with maximum fresh shoot and root weight at the time of harvest, which were 478.4 g and 161.8 g respectively. This finding was in line with that of Siddiqui *et al.* (2005), as he opined that the soil amendments with *T. erecta* increased shoot fresh weight and was very effective in controlling *M. javanica.* Similar observation was found in the study conducted by Polthanee and Yamazaki (1996) which indicated that marigold treatment (grown and incorporated into soil before planting rice) suppressed root knot nematode population, increased the yield and provided nutrients for rice growth as they serve as organic manure also.

Incorporation of *C. odorata* leaves was found to be effective in improving the fresh shoot and root weight of coleus plants and this finding was in agreement with that of Nisha and Sheela (2002) in managing root-knot nematode in kacholam. This treatment was closely followed by the neem cake application and application of cartap hydrochloride. Thoden *et al.* (2007) found that 1,2-dehydropyrrolizidine alkaloids (PAs) from the roots of *C. odorata* have nematicidal effects on the root-knot nematode *M. incognita*, at concentrations of 70-350 ppm under *in vitro* studies. *In vivo* experiments showed that mulch or aqueous crude extracts of *C. odorata* roots reduced the damage by *M. incognita* on lettuce plants.

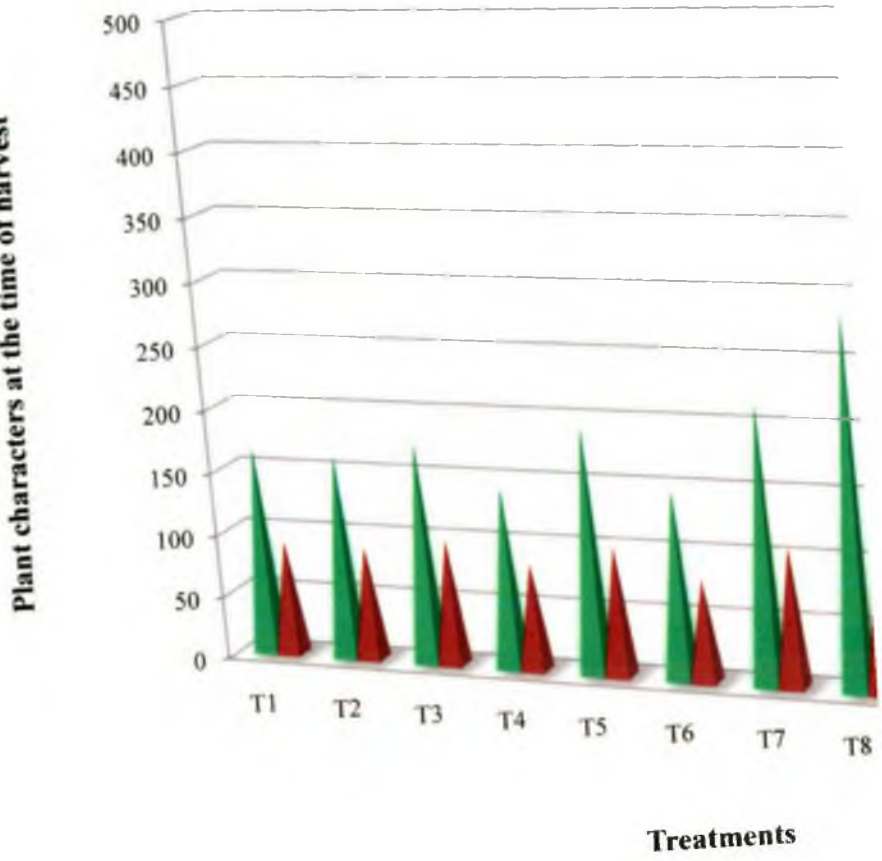
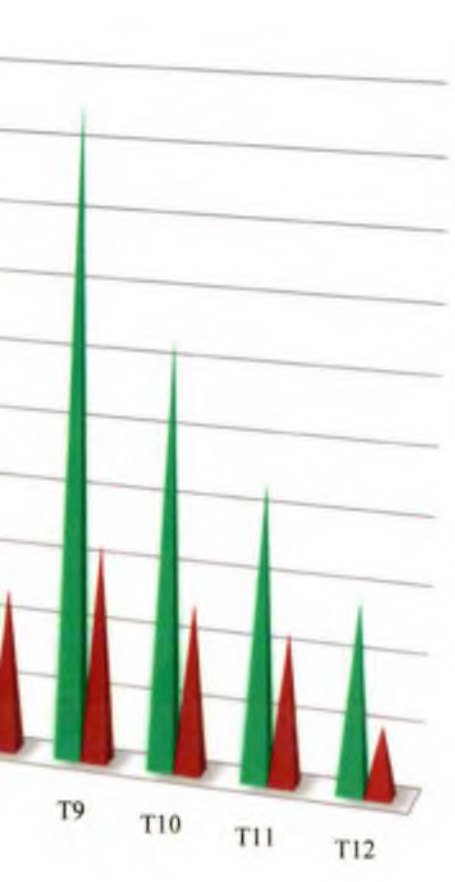


Fig.7. Effect of treatments on plant biomass of coleus



T_1 - *T. viride*

T_2 - *P. fluorescens* (P.f.)

T_3 - *P. lilacinus* (P.l.)

T_4 - *B. subtilis* (B.s.)

T_5 - P. f. + P. l.

T_6 - P. l. + B. s.

T_7 - B. s. + P. f.

T_8 - Neem cake

T_9 - *Tagetes erecta*

T_{10} - *Chromolaena odorata*

T_{11} - Cartap hydrochloride 4G

T_{12} - Untreated control

■ Fresh shoot weight/plant (g)

■ Fresh root weight/plant (g)

Thakur and Darekar (1995) reported that neem cake application @ 35 g per plant reduced root galling by *M. incognita*, eggs per egg mass, root-knot index and increased shoot and root weight and yield of brinjal. Organic amendments, in addition to their suppressive effects on nematode densities, improved soil structure and water holding capacity which improved growth and yield (Singh and Sitaramaiah, 1973).

Soil application of cartap hydrochloride 4G was also observed to boost the plant growth of coleus plants. Kumar *et al.* (2010; 2011) opined that the growth parameters of cowpea plants were higher and root–knot nematode (*M. incognita*) population were reduced when compared to inoculated control by seed treatment of cowpea with cartap hydrochloride 50 (SP) @ 1.5% (w/w) before sowing.

The application of mixture of *B. subtilis* and *P. fluorescens* to the soil was very effective in enhancing the plant growth by reducing the nematode population. Such increase in plant growth may be due to production of gibberellins, cytokinins and IAA by plant growth promoting rhizobacteria, *P. fluorescens* as reported by Lifshits *et al.* (1987). Jonathan *et al.* (2012) proved that the soil application of mixtures of bioagents (*P. fluorescens*, Pfbv 22 and *B. subtilis*, Bbv 57 each at 5 g) at 10 g per black pepper vine significantly enhanced the growth and berry yield, and reduced the population of *M. incognita* and *Radopholus similis* over untreated control. Jonathan *et al.* (2000, 2004) also reported that *P. fluorescens* and *B. subtilis* were observed to induce profuse root development in banana, tomato and betelvine and reduced the infestation of *M. incognita*.

5.2.3 Yield

The results on the yield character (weight of tubers per plant) recorded at the time of harvest are presented in Fig. 8.

The plants treated with *T. viride* alone were statistically superior to all other treatments in terms of number of tubers and weight of tubers per plant having 5.667 tubers and 205.5g respectively. The present finding was in conformity with that of Ramakrishnan and Deepa (2011) on medicinal coleus *(Coleus forskohlii)*. They found that soil application of *T. viride* @ 2.5 kg/ha were most effective in suppressing the complex disease incidence by *M. incognita* and *Macrophomina phaseolina* and the application also enhanced the tuber yield. They also tested the effectiveness of

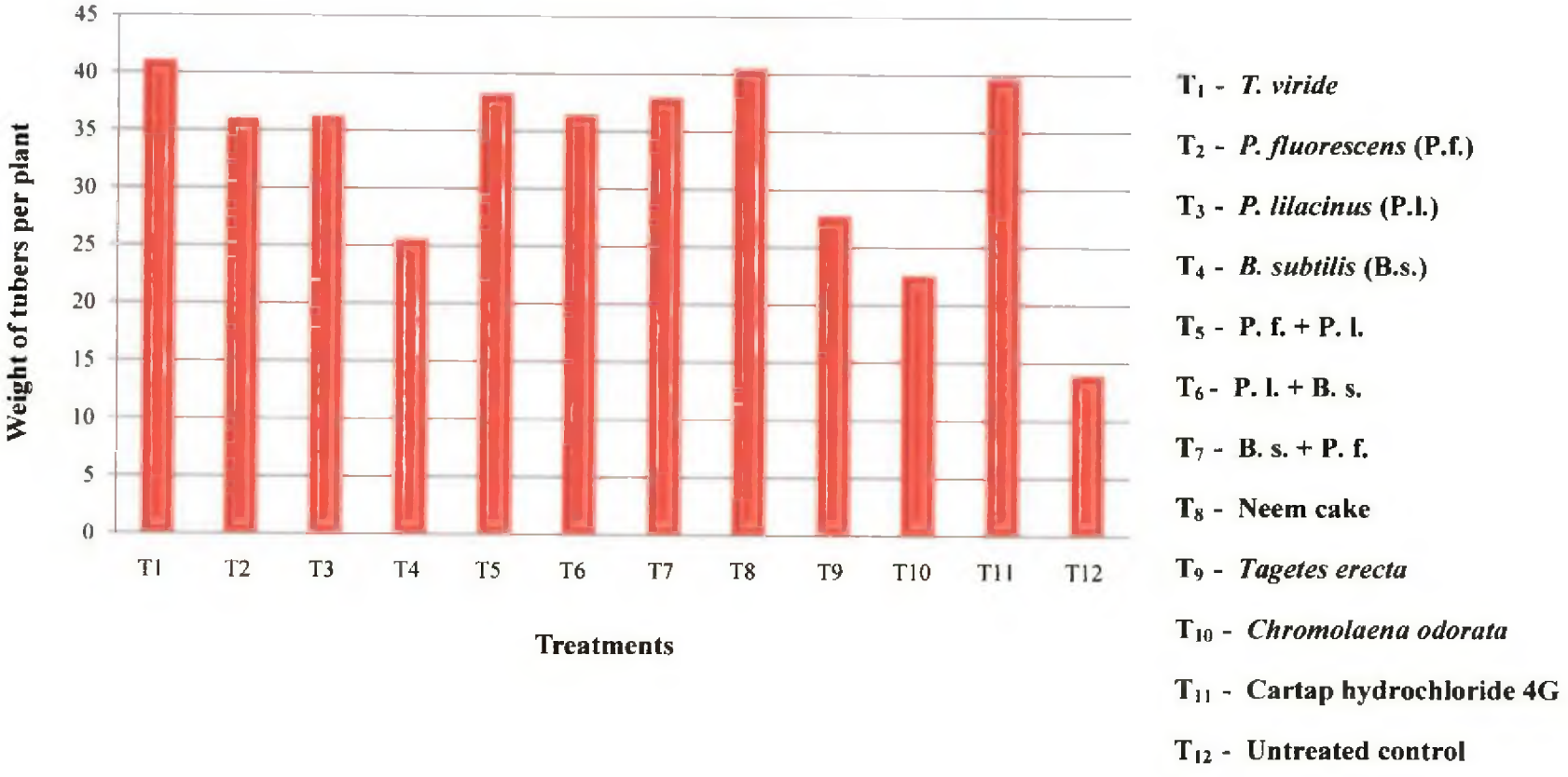


Fig. 8. Effect of treatments on weight of tubers of coleus

commercial talc-formulation of *T. viride* (10^6cfu/g) in nematode sick field and the results confirmed the effectiveness of *T. viride* for the management of nematode fungal disease complex (77.69 per cent) and increased the tuber yield by 68.18 per cent in medicinal coleus. Abd-el-Fattah *et al.* (2012) reported that soil treated with *T. viride* increased the biometric parameters of sugarbeet, reduced root-knot nematode population and increased the root yield. Similar observation was reported by Gogoi and Mahanta (2013) where the treatment with *T. harzianum* at 1.25 kg/ha was effective in increasing plant growth parameters and yield of french bean.

Trichoderma spp. is highly rhizosphere competent. They are able to colonize on roots as they develop and promote the plant growth. They may also exert several other mechanisms such as tolerance to stress through enhanced root and plant development, induced resistance, inactivation of pathogen's enzymes in promoting plant growth and suppressing plant pathogens (Weeder *et al.*, 2008). The possible mechanism involved in *Trichoderma* antagonism against root-knot nematode has been studied intensively by Sharon *et al.* (2001). They reported two mechanisms - *Trichoderma* produced metabolites with antinematode activity that immobilized J_2 thus reduced root penetration and direct parasitism by the antagonist. Guohong *et al.*, (2007) reported that *Trichoderma* releases compounds like viridian and gliotoxin which are antifungal and nematicidal in nature. It is also known to parasitize the eggs of root-knot nematode (Sahebani and Hadavi, 2008) causing reduction in nematode population.

Application of neem cake (T_8), *B. subtilis* + *P. fluorescens* (T_7) and *P. fluorescens* + *P. lilacinus* (T_5) were the other superior treatments which enhanced the yield of coleus plants. The yield enhancement by neem cake application was in confirmation with that of Seenivasan (2010) and the results revealed that neem cake application (at the rate of 500 kg/ha and 400 kg/ha) reduced *M. incognita* populations and increased the tuber yield of medicinal coleus by 39.8 - 42.4 per cent under glasshouse condition and 9.0 per cent under field conditions. Devapriyanga *et al.* (2012) reported that treatment with the mixture of *Pseudomonas* isolate and *Bacillus* isolate (10 g per vine) significantly enhanced the plant growth, yield and reduced root-knot nematode infestation both in soil and root of black pepper plants. Reduction of nematode population in *Pseudomonas* and *Bacillus* applied field might be due to the production of toxic metabolites like antibiotics and cyanide (Voisard *et al.*, 1989).

The combination treatment with bioagents, *P. lilacinus* and *P. fluorescens* also improved the yield of coleus plants. The results are in line with the findings of Rao (2010). He found that the application of farm yard manure enriched with bio-pesticides (I kg *P. lilacinus* (10^6 cfu/g) + 1 kg *P. fluorescens* (10^8 cfu/g) along with neem or pongamia cake at the rate of 1kg per papaya seedling at planting and subsequently four more applications at an interval of 6 months reduced the root population of *R. reniformis* and *M. incognita* by 73 and 78 per cent respectively and increased the yield of papaya (Arka Surya) crop by 26 per cent. Rao *et al.* (2012) reported that ten grams of combined formulation of *P. fluorescens* and *P. lilacinus* was used for the seed (1 kg) treatment, 5 g for treating 1 kg of substrate (coco peat) and 5 kg for the enrichment of a vermi-compost (500 kg) which was applied to the field before transplanting bell pepper seedlings. The results revealed that the application reduced the population of *M. incognita* and increased plant growth components (shoot and root length), nematode egg parasitization and crop yield.

5.2.4 Root-knot nematode Population

Data relating to the nematode population presented in Fig. 9, Fig 10 and 11 indicated the influence of different treatments in reducing the number of root-knot nematodes in soil, root and tuber, number of white females and root-knots index.

Application of *P. lilacinus* alone was the best treatment in reducing the nematode population in soil and it was the second best treatment in reducing the nematode population in root and coleus tubers with 31.83 nematodes/ 200g soil, 114 nematodes/ 5g root and 71.5 nematodes/ 10g tuber. The mode and severity of infection of nematodes (*M. javanica, H. avenae, R. similis*) by *P. lilacinus* was studied under laboratory conditions using microscopy and found that *P. lilacinus* infected the eggs, juveniles and females of *M. javanica* by direct hyphal penetration (Khan *et al.*, 2006). The hyphal penetration into *M. incognita* eggs occurs within 3-5 days of fungal inoculation of culture filtrates of *P. lilacinus* isolates (Khan and Goswami, 2000). Besides oviparasitism, mortality of second stage juveniles in the culture filtrates of *P. lilacinus* has been observed (Khan and Khan, 1992). A study conducted by Kumar *et al.* (2008) revealed the toxic effect of culture filtrates of *P. lilacinus* on the mortality and hatching of *M. incognita* on vegetables. *P. lilacinus* has been reported to produce

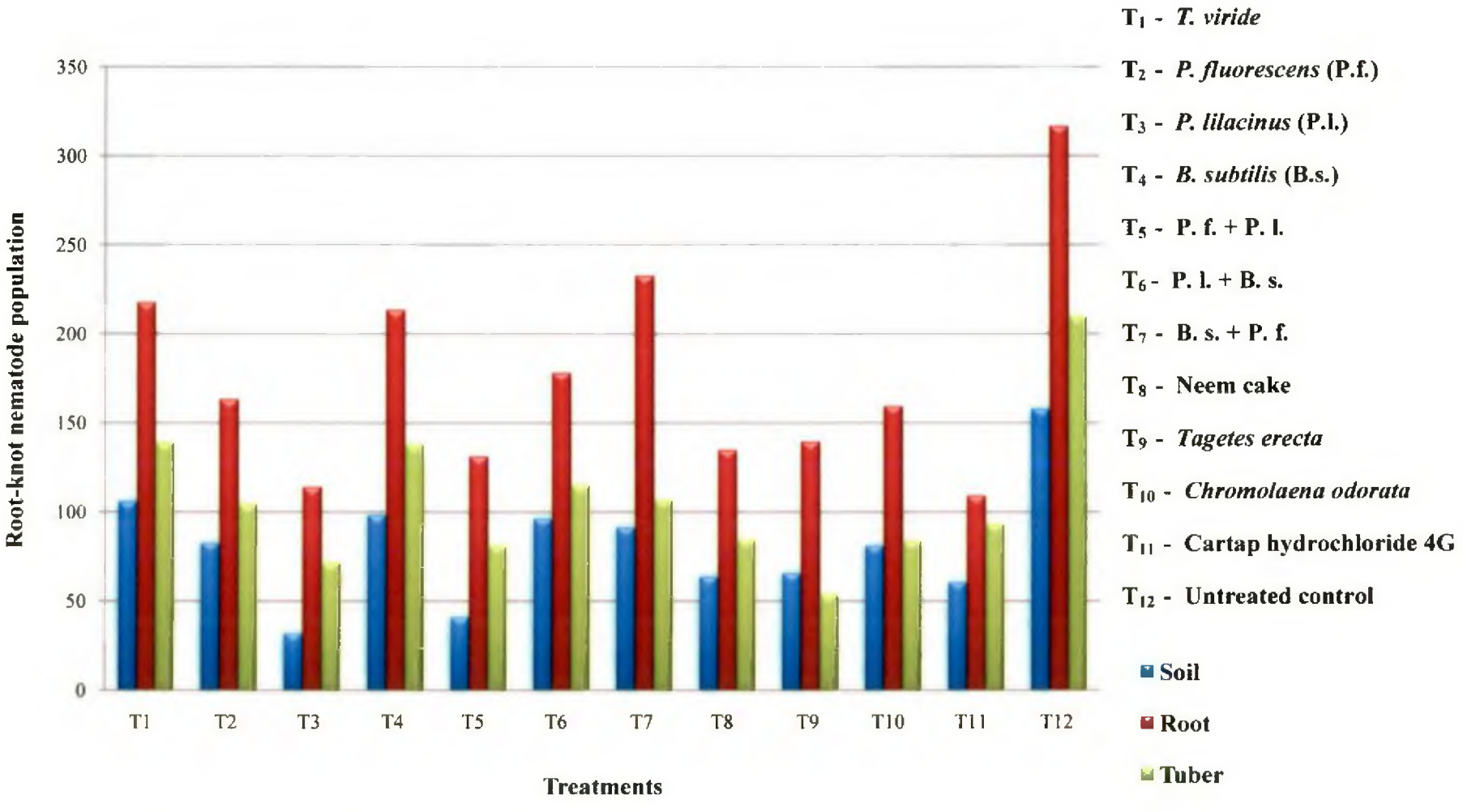


Fig. 9. Effect of treatments on nematode population in soil, root and tuber

peptidal antibiotics like lilacinin, leucinostatin and paecilotoxin (Mikami *et al.*, 1989). Acetic acid was also identified from culture filtrates of this fungus which affect the movement of nematode (Djian *et al.*, 1991).

Seenivasan and Devrajan (2008) reported that soil application of *P. lilacinus* at 2.5 kg/ha, parasitized about 51.5-71.5 per cent nematode eggs of root-knot nematode, *M. incognita*, infecting medicinal coleus, *C. forskohlii*. Siddiqui and Akhtar (2009) opined that *P. lilacinus* parasitised more females and eggs of the nematode, *M. incognita* than the other fungi tested and *P. lilacinus* was more effective in reducing galling and improving the growth of nematode-inoculated tomato plants in glasshouse. The soil application of *P. lilacinus* reduced root-knot nematode population from 631.8 to 35 nematodes/200 cc with reduction of root galls from 312 to 38 galls per 5g root for the period of 180 days in pomegranate (Somasekhara *et al.*, 2012). Khalil *et al.* (2012) reported that application of *P. lilacinus* was the most effective treatment on reducing number of galls and egg masses of *M. incognita* on the tomato plants achieving 88.23 and 76.94 per cent reduction over control respectively.

The combination treatment of *P. fluorescens* and *P. lilacinus* (T_5) was also found to be very effective in reducing the nematode population in soil, root and tuber. The effectiveness of bioagents might be increased when they were applied together. The mechanism responsible for the reduction of nematode population may be due to the ability of *P. fluorescens* to envelop and bind the root surface with carbohydrate – lecithin, thereby interfering with normal host recognition process as reported by Oostendrop and Sikora (1990). 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. Kavitha *et al.* (2013) reported that *P. fluorescens* isolates induce defence enzymes (peroxidase, polyphenol oxidase and phenylalanine ammonia lyase) in tomato against root-knot nematode, *M. incognita*. *P. lilacinus* causes egg shell perforation of *M. incognita* eventually destroying the embryo within 5-12 days (Cabanillas *et al.*, 1989).

The results of the present study agree with the findings of Anita and Selvaraj (2011). They observed that the combined application of *P. fluorescens* @ 1 kg/ha along with *P. lilacinus* @ 2.5 kg/ha and carbofuran 3G @ 0.25 kg a.i./ha at the

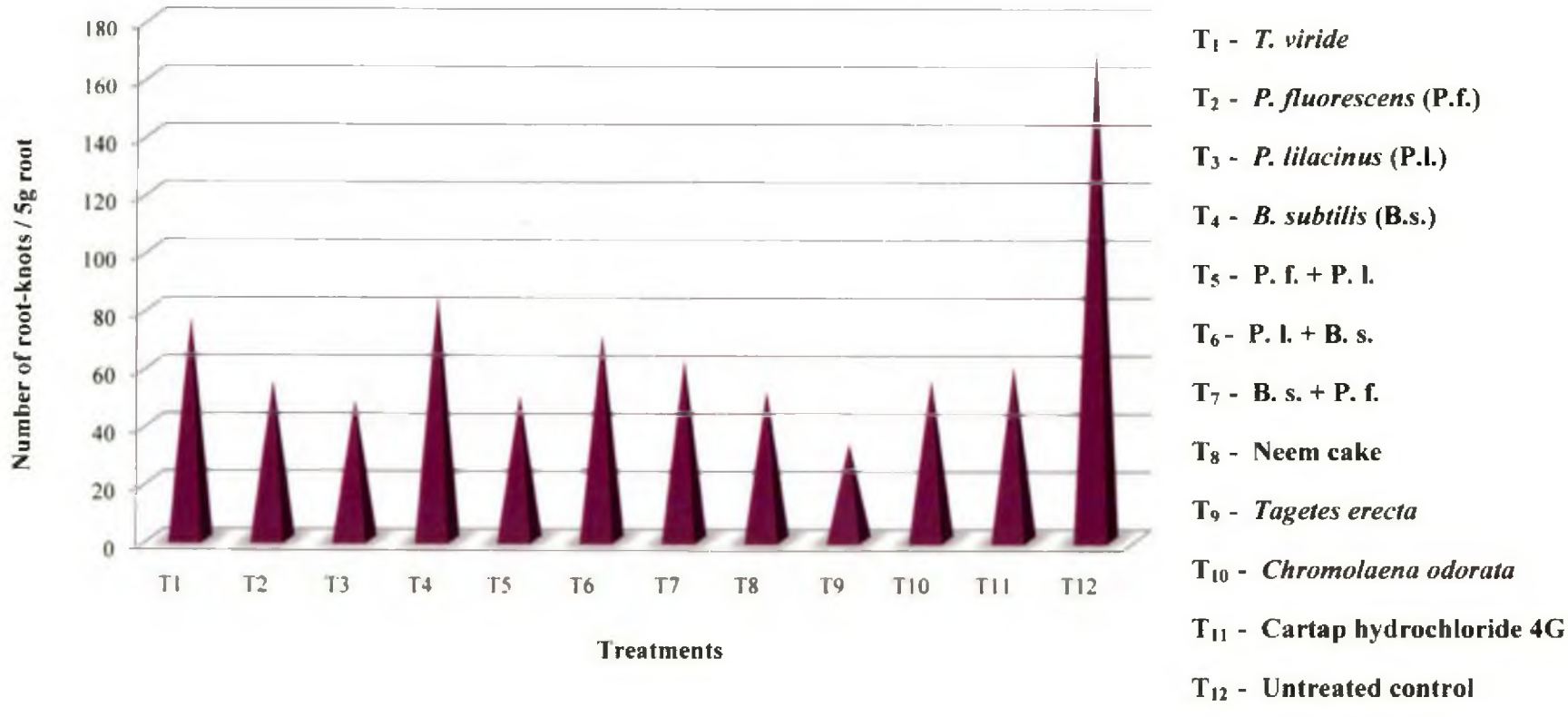


Fig.10. Effect of treatments on number of root-knots

time of sowing enhanced the yield of carrot (43.58 per cent) compared to untreated control and reduced the soil root-knot nematode (*M. hapla*) population by 66.19 per cent. Sowmya *et al.* (2012) opined that *M. incognita* (J_2) population was reduced in roots by 69 per cent and in soil by 47.6 per cent, when treated with twenty grams mixture containing *P. putida* and *P. lilacinus* in carrot.

Incorporation of *T. erecta* whole plants to the soil was effective in reducing the nematode population in tuber (53.83 nematodes/ 10 g tuber), number of females (49.67females/ 5g root) and number of galls (33.33 galls/ 5g root) with a Root-Knot Index of 2.0. The results of the study agree with the findings of Abadir *et al.* (1994). They reported that addition of dried chopped portions of marigold (*T. erecta*), at the rates of 10, 20, 30 g/pot caused reduction in the egg mass production and gall formation on sunflower plants infected with *M. incognita*. Incorporation of chopped *Tagetes* leaves applied @ 40 and 80 g/kg soil significantly increased tomato and brinjal plant growth and reduced number of galls, egg masses and final juvenile population in soil (Walia and Gupta, 1997). Similar observation was reported by Khanna and Sharma (1998) that the incorporation of green leaves of neem and *Tagetes* reduced gall formation on tomato roots and achieved gall index less than or equal to two. Rather *et al.*, (2008) also opined that chopped leaves of marigold applied at 100g/pot has the gall index of 0.64 due to *M. incognita* and the root galling was also reduced to some extent when the dose was reduced to half (50 g/pot).

The incorporation of marigold biomass releases nematoxic organic acids such as dodecanoic acid, myristic acid, palmitic acid and steric acid (Debprasad *et al.*, 2000). Ray *et al.* (2000) determined the chemical composition and nematicidal activity of the volatile and non-volatile fractions of *T. erecta* and found that the methanol extract and essential oils of marigold flowers showed maximum nematicidal activity against *M. incognita* juveniles. The ED50 was 852 and 396 micro g ml^{-1} after 24 hrs of exposure to the methanol extract and essential oils, respectively. Among the two purified compounds, the nematicidal activity of myristic acid was more pronounced than dodecanoic acid against the nematode juveniles. Siddiqui and Alam (1988) reported that alpha terthienyl compound present in the roots of *Tagetes* inhibit the hatching of nematode eggs. Aqueous extracts of leaf, stem and roots of *T. erecta* have been reported to be nematicidal against *M. incognita* by Mojumder and Mishra (1999). The plant extracts of *T. erecta* caused more than 50 per cent mortality of

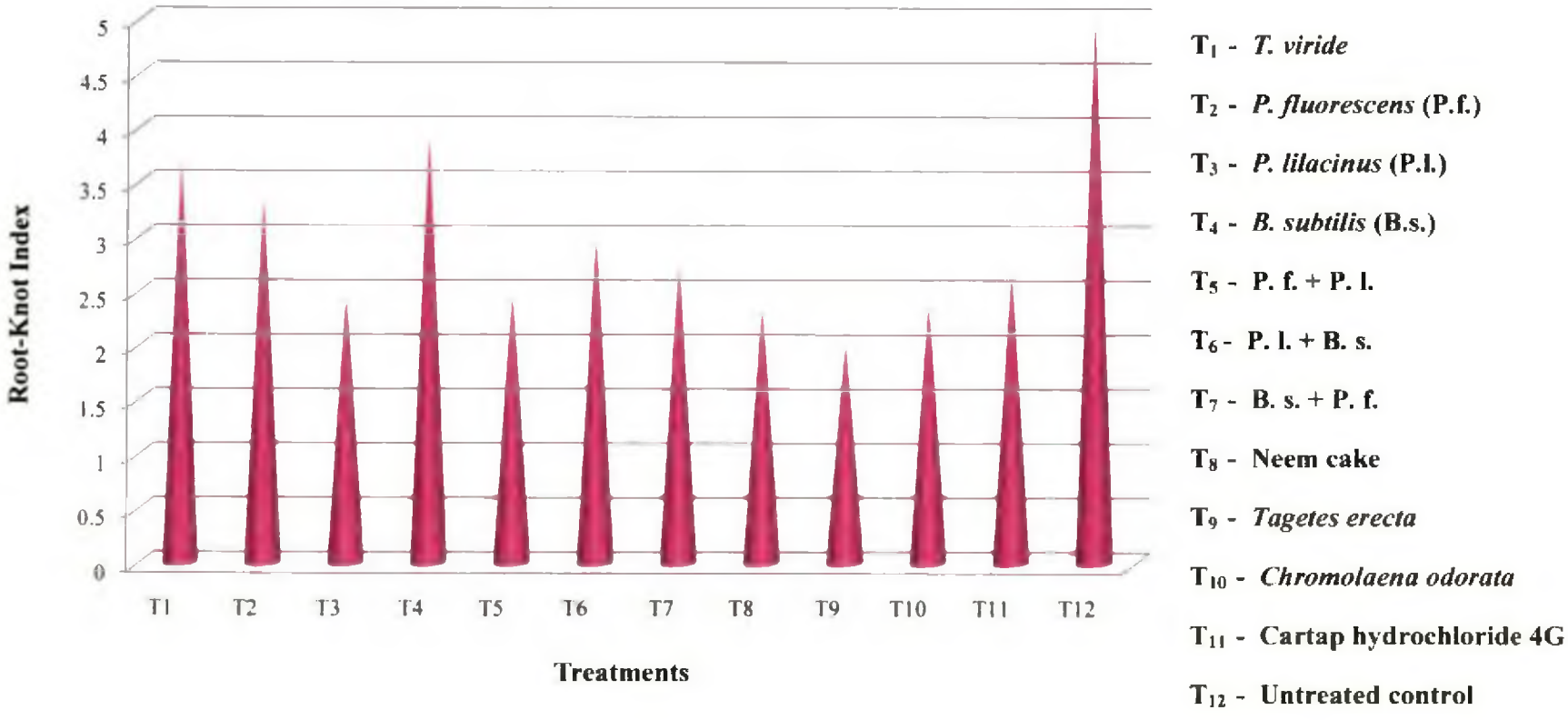


Fig.11. Effect of treatments on Root-Knot Index

second stage juvenile (J_2) of *M. incognita* in 24 hrs and showed inhibitory effect on egg hatching (Yang-XiuJuan *et al.*, 2004).

The application of chemical insecticide (cartap hydrochloride) was found to reduce the nematode population in soil and root of coleus plants. Yeh *et al.* (2012) reported that cartap hydrochloride had the strongest nematicidal effects on *M. incognita* juveniles as it decreased the penetration rate of the nematode and showed inhibition to *M. incognita* egg hatching rates.

Incorporation of neem cake to the soil also reduced the nematode population in soil, root and tubers, number of females and number of galls. The result of the present study is in conformity with that of Vadhera *et al.* (1998). They found that amending soil with neem cake was effective in reducing gall formation, *M. incognita* population and increasing the yield in ginger. Neem cake application @ 200 gm^{-2} was very effective in reducing the root-knot nematode population in kacholam rhizosphere (Rajani, 1998; Nisha and Sheela, 2003). Ravindra *et al.* (2003) evaluated the effects of different oil cakes and observed that neem cake applied at 20 g per plant registered minimum root-knot index and was significantly superior to other oil cakes used against root-knot nematode on FCV tobacco. Seenivasan (2010) found that application neem cake reduced *M. incognita* populations by 28.6 -31.2 per cent in soil and coleus plants recorded the gall index of 3.3 - 4.0.

Singh and Sitaramaiah (1973) and Khan (1974) reported that the nematostatic and nematicidal properties of neem products might be due to active principles and toxic chemicals present in them. During the decomposition of neem cake, many nematicidal compounds like ammonia, phenols, aldehydes, amino acids and fatty acids are released which are highly deleterious to nematodes (Reddy *et al.*, 1997).

To sum up the findings, the present study revealed that the organic amendments namely whole plant of *T. erecta*, leaves of *C. odorata* and neem cake gave good results in increasing the biometric characters of coleus plants and they were also effective in reducing the nematode population. The bio control agents namely *T. viride, P. fluorescens, P. lilacinus* and *B. subtilis* showed promising effects in improving the yield characters and reducing the nematode population in soil, root and tuber. Among the treatments, using combination of bio control agents, soil application of mixture of *P. fluorescens* and *P. lilacinus* was found to be more effective in

reducing the nematode population of soil, root and tuber, number of females and number of root-knots. The chemical insecticide, cartap hydrochloride 4G (a substitute chemical for carbofuran) was also effective in increasing the plant growth parameters and reducing the nematode population.

In the present study, the application of organic amendments and biocontrol agents were found to be effective as cartap hydrochloride 4G. So the application of chemical can be very well replaced by the use of organic amendments and biocontrol agents, as they are very effective and environment friendly alternatives for managing the nematodes. Though the nematicides are effective against nematodes, they are hazardous for health, soil and environment. The application of chemicals adversely affects the soil environment due to its toxic residues. Modern agriculture tends to adopt more environment friendly practices. There is increasing interest in the use of biopesticides that are pest specific and non toxic to human and beneficial organism. Moreover biocontrol of nematodes is important in view of long-term advantage of management. So a sustainable management strategy reducing the risk of health and environmental hazards adopting the use of bio control agents, their combinations, organic amendments and other non chemical measures has to be given more emphasis to maintain the root-knot nematode population below the threshold levels and to increase the yield of the crop.

Summary

6. SUMMARY

The study entitled 'Management of root-knot nematode, *Meloidogyne* sp. (Kofoid and White) in coleus, *Solenostemon rotundifolius* (Poir) Morton' was conducted at College of Horticulture, Vellanikkara during 2012-2013. The objectives of the study were to assess the population of plant parasitic nematodes infesting coleus at different coleus growing regions of Thrissur District and to identify the species of *Meloidogyne* infesting coleus. Pot culture experiments were also carried out to evolve the most effective management strategy using biocontrol agents, organic amendments and a chemical insecticide against root- knot nematode, *Meloidogyne* sp. in coleus.

The survey was conducted to assess the nematode population at eight locations namely Mundathikode, Wadakkanchery, Varavoor, Thirur, Kolazhi, Vellanikkara, Madakkathara and Vadanapilly which are the major coleus growing areas of Thrissur District. The fields were randomly selected and five soil and tuber samples of each were taken at random from each location at the time of harvest, making a total of forty soil and tuber samples from the rhizosphere of coleus plants. Each sample was a composite one drawn from five to six spots in a field. The plant parasitic nematodes associated with coleus were recorded from the samples collected during the survey. Further the root-knot nematode population, number of white females and number of galls in 10 g tuber and gall index were observed. Species of root-knot nematode infesting coleus plants was also identified based on the perineal pattern of white females collected from the root and tuber samples.

Six plant parasitic nematode species were found associated with the rhizosphere of coleus in the surveyed regions of Thrissur District. The plant parasitic nematodes recorded were root-knot nematode (*Meloidogyne incognita* (Kofoid and White) Chitwood, 1949), reniform nematode (*Rotylenchulus reniformis* Linford and Oliveiria), burrowing nematode (*Radopholus similis* (Cobb, 1893) Thorne, 1949), lance nematode (*Hoplolaimus* sp.), spiral nematode (*Helicotylenchus* sp.) and rice-root nematode (*Hirschmanniella oryzae* Luc & Goodey, 1963).

Among the 40 fields surveyed, all the fields were infested with root-knot nematode and *Meloidogyne incognita* was the only root-knot nematode species present in all the fields. The maximum average root knot nematode population was

recorded in Kolazhi with 450.4 nematodes/ 200g soil, which was followed by Thirur, Wadakkanchery, Madakkathara, Mundathikode, Vadanapilly, Vellanikkara and Varavoor. The root-knot nematode population from 10 g tuber was recorded maximum at Madakkathara with 204.52 nematodes. The highest Gall Index was observed at Kolazhi (3.9) and the lowest at Varavoor (1.1). Other nematode species were relatively few in numbers compared to the population of *M. incognita* and they were not harmful to the coleus tubers.

The pot culture experiment was carried out to evolve the most effective management strategy using biocontrol agents, organic amendments and a chemical insecticide against root-knot nematode, *Meloidogyne* sp. in coleus *(S. rotundifolius)*. The results were assessed in terms of biometric characters of coleus plants during growth and at the time of harvest, yield and root-knot nematode population in soil, root and tuber.

Treatment with organic amendments namely whole plant of *T. erecta*, leaves of *C. odorata* and neem cake resulted in the enhancement of biometric characters like number of leaves, number of branches and height of the plant. Among the organic amendments applied incorporation of *T. erecta* whole plant was the superior treatment in increasing the biometric characters of coleus plants. Application of cartap hydrochloride and *P. lilacinus* alone was found effective in improving the plant height and number of branches. The combination of *B.subtilis* and *P. fluorescens* and the application of cartap hydrochloride also increased the production of leaves on coleus plants.

Incorporation of *T. erecta* whole plant showed a significant superiority on the biometric characters at the time of harvest like fresh shoot and root weight. Application of *C. odorata* leaves, neem cake, cartap hydrochloride and combination of *B.subtilis* and *P. fluorescens* were also effective in improving the fresh shoot and root weight of the plants. Plants treated with *T. viride* produced more number of tubers and increased the weight of the tubers. Neem cake application and combination treatments like *B.subtilis* + *P. fluorescens* and *P. fluorescens* + *P. lilacinus* also improved the yield of coleus plants.

All the treatments were effective in reducing root-knot nematode population compared to control, though the efficacy regime was not alike. Soil

application of *P. lilacinus* alone, cartap hydrochloride and incorporation of *T. erecta* suppressed the nematode population in soil, root and tuber respectively. The combination treatment with *P. fluorescens* + *P. lilacinus* and application of neem cake also reduced population build up of nematode and kept the infestation at a lower level.

Among the different treatments studied, incorporation of *T. erecta* whole plants was most effective in reducing the number of females, number of root-knots producing lowest root-knot index. Maximum reduction in number of females, number of root-knots and root-knot index was also observed in the treatment with *P. lilacinus* alone, *P. fluorescens* + *P. lilacinus*, application of neem cake and incorporation of *C. odorata* leaves.

Even though all the treatments were effective in managing root-knot nematode compared to control, application of organic amendments like whole plants of *T. erecta*, *C. odorata* leaves and neem cake improved the biometric characters of plants and reduced the nematode population. The application of bio control agents (*T. viride*, *P. fluorescens*, *P. lilacinus* and *B. subtilis*) and their combinations (*P. fluorescens* + *P. lilacinus*, *P. lilacinus* + *B. subtilis* and *B. subtilis* + *P. fluorescens*) were effective in increasing the yield characters and were also good at suppressing the root-knot nematode build up. The study clearly indicated that application of organic amendments and biocontrol agents were found to be as effective as cartap hydrochloride 4G in managing the root-knot nematode population in coleus and can be considered as alternative to nematicide application.

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MANAGEMENT OF ROOT-KNOT NEMATODE, *Meloidogyne* sp. (Kofoid and White) IN COLEUS, *Solenostemon rotundifolius* (Poir) Morton

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ABSTRACT OF THE THESIS

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ABSTRACT

The study entitled 'Management of root-knot nematode, *Meloidogyne* sp. in coleus, *Solenostemon rotundifolius* (Poir) Morton' was conducted at College of Horticulture, Vellanikkara during 2012-2013 with the objectives of assessment of the population of plant parasitic nematodes infesting coleus at different coleus growing regions of Thrissur District, identification of the species of *Meloidogyne* infesting coleus and evaluation of biocontrol agents, organic amendments and a chemical insecticide against this root- knot nematode in coleus.

Survey was conducted in the major coleus growing areas of Thrissur District, namely Mundathikode, Wadakkanchery, Varavoor, Thirur, Kolazhi, Vellanikkara, Madakkathara and Vadanapilly. Soil and tuber samples were collected from these localities and the nematodes were extracted to assess the population of plant parasitic nematodes associated with coleus. It was found that root-knot nematode was the major problem in all the eight locations and the species of root-knot nematode was identified as *Meloidogyne incognita* (Kofoid and White) Chitwood, 1949 on the basis of perineal pattern of white females. Other plant parasitic nematodes recorded from soil samples were reniform nematode (*Rotylenchulus reniformis* Linford and Oliveiria), burrowing nematode (*Radopholus similis* (Cobb, 1893) Thorne, 1949), lance nematode (*Hoplolaimus* sp.), spiral nematode (*Helicotylenchus* sp.) and rice-root nematode (*Hirschmanniella oryzae* Luc and Goodey, 1963) which were not harmful to the tubers of coleus. The highest average root-knot nematode population was recorded from Kolazhi with 450.4 nematodes/ 200g soil with a gall index of 3.9.

Pot culture experiment was carried out to evaluate the efficacy of biocontrol agents, organic amendments and a chemical insecticide against root-knot nematode infesting coleus. The results showed that application of organic amendments namely whole plant of *Tagetes erecta*, leaves of *Chromolaena odorata* and neem cake resulted in the enhancement of plant growth characters and resulted in a reduction of root-knot nematode population. Incorporation of *T. erecta* whole plant ranked first among the organic amendments. Neem cake, cartap hydrochloride 4G and combination treatments of *Bacillus subtilis* + *Pseudomonas fluorescens* and *P. fluorescens* + *Paecilomyces lilacinus* improved the yield of coleus plants. Soil application of *P.*

lilacinus, cartap hydrochloride 4G and incorporation of *T. erecta* suppressed the nematode population in soil, root and tuber respectively. Combination treatment of *P. fluorescens* + *P. lilacinus* and application of neem cake also reduced population buildup of nematode and kept the infestation at a lower level. Highest reduction in number of females, number of root knots and root knot index was also observed in *T. erecta* and was followed by *P. lilacinus*, *P. fluorescens* + *P. lilacinus*, neem cake and *C. odorata*.

The study clearly indicated that application of organic amendments and biocontrol agents were found to be as effective as cartap hydrochloride 4G in managing the root-knot nematode population in coleus and can be considered as alternative to nematicide application.