BIOLOGICAL CONTROL OF ROOT-KNOT NEMATODE, *Meloidogyne incognita* (Kofoid and White, 1919) IN BANANA, *Musa* (AAA) Var. ROBUSTA

By

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THESIS

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DECLARATION

I hereby declare that this thesis entitled "Biological control of root-knot nematode, *Meloidogyne incognita* (Kofoid and White, 1919) in banana, *Musa* (AAA) var. Robusta" is a bonafide record of research work done by me during the course of research and that this thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title of any other University or Society.

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CERTIFICATE

Certified that this thesis, entitled "Biological control of root-knot nematode, *Meloidogyne incognita* (Kofoid and White, 1919) in banana, *Musa* (AAA) var. Robusta " is a record of research work done independently by **Miss. Lini, G.** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to her.

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Lida, G.

DEDICATED TO ACHAN, AMMA AND ETTAN

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Introduction

1. INTRODUCTION

Banana (*Musa* spp.) is a fruit crop of global importance in terms of income security to millions of farmers throughout the developing countries. It is the world's fourth most important commodity after rice, wheat and maize and is produced in tropical and subtropical regions of developing countries. India is the largest producer of banana in the world, contributing 19.71 per cent to the global production with a total production of 19.19 million tonnes from an area of 5.5 lakh ha (Singh, 2008). Among the different banana growing states of India, though Kerala ranks third in area, the production and productivity is low. This is due to polyclonal system of cultivation that too, mostly under homestead and perennial conditions. This provides a favourable environment for pests and diseases to sustain throughout the year affecting the productivity. Banana is known to adapt very quickly and produce high yields under favourable conditions. It is, however, prone to attack by different pests *viz*., fungi, viruses, bacteria, insects and nematodes. Among these, nematodes constitute one of the major limiting factors to banana production causing extensive root damage resulting in serious economic losses. Plant parasitic nematodes, as pests of different horticultural and field crops, are responsible for an annual monetary loss of Rs. 300 crores in India. Crop losses caused by nematodes to banana are very high, with an average annual yield losses estimated at about 20 per cent worldwide (Sundararaju, 2011).

More than 134 species of nematodes belonging to 54 genera have been associated with the rhizosphere of banana in the world. Out of these, 71 species of nematodes belonging to 33 genera are recorded from banana in various parts of India and are mainly responsible for limiting banana production to a greater extent. The root-knot nematode, *Meloidogyne incognita* (Kofoid and White, 1919) is considered

to be the most economically important nematode pests of banana widely distributed in South India (Sundararaju, 1996). Hebsybai *et al.* (1996) reported that *M. incognita* equally preferred the five varieties of banana namely, Nendran, Palayankodan, Red banana, Robusta and Poovan. Crop losses caused due to *M. incognita* in 'Poovan' was reported to be 30.9 per cent (Jonathan and Rajendran, 2000). Other economically important nematode pests of banana having regional significance are burrowing nematode, *Radopholus similis* (Cobb) Thorne, root-lesion nematode, *Pratylenchus coffeae* Filipjev, spiral nematode, *Helicotylenchus multicinctus* Cobb and *H. dihystera* (Cobb) Sher, cyst nematode, *Heterodera oryzicola* Schmidt and reniform nematode, *Rotylenchulus reniformis* Liniford and Oliveria.

Root-knot nematodes are worldwide in distribution. They are essentially hot weather organisms and are most important in regions where summers are long and winter is short and mild. These parasites are confined to the tropics and subtropics. More than 100 species of *Meloidogyne* have been described. Among them four species *viz., M. incognita, M. javanica, M. arenaria* and *M. hapla* are very important and widely distributed throughout the world. *M. incognita, M. javanica* and *M. arenaria* are mostly confined to tropical and sub-tropical conditions attacking several vegetables and fruit crops, whereas *M. hapla* is confined to temperate regions where the temperature ranges between 10 to 15°C (Sundararaju, 2011). Among these, *M. incognita* and *M. javanica* are common and widely distributed root-knot nematodes in banana plantations. In India, the root-knot nematode, *M. incognita* is widely distributed in major banana growing regions in the country whereas *M. javanica* is confined mainly to mid hills and plains, where the temperature is higher. The nematode easily gets introduced into virgin land through infested banana suckers usually transported from old infested plantations.

The root-knot nematode, *M. incognita* is predominantly observed in tissue culture plants even at the primary and secondary stage itself. Heavy nematode infested seedlings on transplantation to main field fail to establish as the newly

formed roots get infected. It was observed that more nematode infestation was recorded in tissue culture plants than in conventional planting materials (Sundararaju, 2011). The National Research Centre for Banana, Thiruchirapalli, Tamil Nadu carried out a preliminary survey among various banana tissue culture companies and it revealed the occurrence of heavy root-knot nematode infestation at secondary hardening stage. About 25 to 45 per cent of infestation was noticed during a survey conducted in the tissue culture banana field in certain parts of Tamil Nadu.

The root-knot nematode infested banana plants exhibit general decline, stunting, premature defoliation, unthriftiness and bear only small bunches and fruits. However, symptoms on the roots and corms are more specific exhibiting characteristic galls varying in size and mostly occur in root tips (Plate 1). The entire inner content of the roots were damaged due to the penetration and multiplication of nematodes. The white females of root-knot nematode, *Meloidogyne* spp. lays several hundred eggs within an egg sac outside its body. The root-knot nematode egg and juvenile populations declined by 70 to 90 per cent during winter, making them dependent on subsequent spring and summer conditions for survival. In all cases, galling of roots interferes with the plants ability to deliver water and nutrients to above ground portions of the plant by blocking the conducting vessels for the transport of major and minor nutrients. In addition, openings created in the roots increase the plant's susceptibility to harmful bacterial and fungal organisms, creating a secondary detrimental effect on the plant finally. The duration of the crop was prolonged to 13 months in *M. incognita* infested plants, whereas the plant protected with nematicides produced mature bunches in 12 months period.

The losses caused by nematodes are enormous which necessitates efficient control measures. Although the chemical nematicides are effective in providing rapid kill of nematodes, their use has been questioned in the recent years because of increasing concern about environmental contamination and human health risks. With an increase in general awareness of harmful effects of chemical pesticides and the

Plate 1. Banana roots infested with *Meloidogyne incognita*

changing public attitude towards environmental pollution, chemical nematicides are losing their popularity among farmers for protecting their crop from nematode infestations. So environmentally compatible and economically viable new strategies should be developed for nematode management. Biological control has shown to be an effective alternative that can be combined with other measures.

In this context, an attempt was made to compare the efficacy of bioagents which are considered to be ecofriendly, with that of carbofuran, hitherto used pesticide for nematode management. The objective of the study includes the identification of an effective biocontrol agent for the management of root-knot nematode (*M. incognita*) infesting banana.

Review of literature

2. REVIEW OF LITERATURE

The root-knot nematode, *Meloidogyne incognita* (Kofoid and White, 1919) is an obligate endoparasite having a wide host range. More than 2000 plant species have been reported as host plants of *Meloidogyne* spp. (Upadhyay and Dwivedi, 2008). They are considered to be a serious problem on important fruit crops *viz.,* banana, papaya and grapes. Survey of nematodes associated with banana undertaken by the Banana Research Station, Kannara, Thrissur, under the All India Co-ordinated Fruit Improvement Project in Palakkad, Thrissur, Ernakulam, Idukki, Alleppey, Kollam and Thiruvananthapuram districts revealed the presence of *M. incognita* in addition to other species of nematodes. Severe infestation of *M. incognita* was observed in banana plants grown along with vegetables (Sheela *et al.*, 1990; Sheela, 1995). Hebsybai *et al.* (1996) reported that *M. incognita* equally preferred the five varieties of banana *viz.*, Nendran, Palayankodan, Red banana, Robusta and Poovan. The literature on different aspects of root-knot nematodes are given below.

2.1 PEST STATUS

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 range of agricultural crops (Dasgupta and Gaur, 1986; Khan *et al.*, 1994; Jain and Hasan, 1995). The most pathogenic and widely distributed species of root-knot nematode from India on cowpea is *M. incognita.* (Khan *et al.*, 1996).

A study conducted by Sheela *et al.* (1998) revealed the occurrence of root-knot nematode in the rhizosphere of majority of medicinal plants grown in eight districts of Kerala *viz.*, Thiruvananthapuram, Kollam, Kottayam, Pathanamthitta, Ernakulam, Thrissur, Malappuram and Kozhikode.

A survey of nine districts of Bundelkand region of India to determine the presence of root-knot nematodes associated with major food and fodder crops of the region revealed 36 per cent incidence of nematode attack. Among the four species of root- knot nematode, *M. incognita* was most frequent followed by *M. javanica* and *M. arenaria* (Hasan and Jain, 1998). Different levels of *M. incognita* population (200 to 1000 J_2) had significant adverse effects on the biometric characters and yield of kacholam (Rajani, 1998).

Jonathan and Rajendran (2000) reported that crop losses caused due to *M. incognita* in banana var. Poovan was 30.9 per cent. Significant reduction in plant height, pseudostem girth, number of leaves and leaf area was observed due to the root-knot nematode infestation. The nematode infestation also deteriorated the edible qualities of the fruit by reducing the carbohydrates, reducing and non-reducing sugars, total soluble solids and ascorbic acid. The root-knot nematode infestation delayed the duration of the crop by 42 days. A survey was conducted in Flue Cure Virginia (FCV) tobacco growing areas of Karnataka, India to assess the incidence and intensity of root-knot nematode and mapping the distribution of *Meloidogyne* sp. Four species, *viz.*, *M. incognita, M. javanica, M. arenaria* and *M. thamesi* were detected on tobacco and disease intensity (root knot index) varied from 2.0 to 3.1 in all tobacco growing tracts (Hussaini and Krishnamurthy, 2002).

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

Singh *et al.* (2010) conducted surveys to study the adverse effect of rice root nematode, *M. graminicola* in wheat during 2004-05 and 2005-06. They found that

severe infestation of wheat by *M. graminicola* reduced the plant height by 50 per cent. The number of grains per head was also reduced in the infected plants which were related to the number of galls per plant.

2.2 MANAGEMENT

2.2.1 Plant Products

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

A study was conducted to screen 20 plant extracts for their efficacy against *Radopholus similis* affecting banana and the leaf extracts of *Piper longum* was found to have a high degree of nematicidal effect on the larvae of the nematode (Sreeja and Charles, 1998). Aqueous extracts of leaf, stem and roots of *T. erecta* have been reported to be nematicidal against *M. incognita* (Mojumder and Mishra, 1999).

Sharma (2000) reported significant reduction in the number of galls of *M. incognita* in vegetables, when seeds were soaked in 5 per cent Neemark and nimbecidine. Five neem based formulations *viz.*, Neem Jeevan, Neemark, Neem Gold, Achook and Kranti were tested at 1.0, 0.5 and 0.25 per cent concentrations against *M. incognita*. His findings indicated that these formulations were effective in controlling the nematode population. Similar observation was reported by Sharma *et al.* (2000) where they found a significant reduction in the number of galls in okra cv.

Pusa Sawani when the seeds were soaked in 5 per cent neemark and nimbecidine for 6 and 24 hrs.

Application of neem cake was found to reduce fecundity of root-knot nematode infesting pointed gourd, *Trichosanthes dioica* cv. Damodar Kajli (Chakraborti, 2000). Similar observation has been recorded in case of *M. incognita* infesting onion (Chakraborti, 2003). Neem cake @ 20g/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.*

The field experiment conducted with four organic amendments, *viz.*, saw dust, poultry manure, mustard cake and neem cake to manage root-knot nematode, *M. incognita* in french bean showed that poultry manure applied as spot application was found to be effective in increasing plant growth characters whereas neem cake as spot application was found effective in reducing gall, egg masses and soil population of *M. incognita.* Spot application of all the organic amendments was comparatively better than furrow application in reducing nematode population and increasing yield (Ahmed and Choudhury, 2004)

An *in vitro* experiment was carried out to test the efficacy of seven plant extracts *viz.*, leaf extracts of *T. erecta, Azadirachta indica, Calotropis gigantea,* seed extracts of *Citrullus lanatus, Areca catechu,* latex of *Carica papaya* and *C. gigantea* on the juveniles of *M. incognita.* Leaf extract of *C. gigantea* (17.7%) and latex of *C. gigantea* (32.6%) were found to be the best at 0.1 and 1.0 per cent concentrations respectively and latex of *C. papaya* recorded cent per cent juvenile mortality after 72h of exposure both at 5.0 and 10.0 per cent concentrations. At 10.0 per cent concentration, seed extract of *C. lanatus* and latex of *C. gigantea* caused cent per cent

mortality after 72 h and 120 h of exposure respectively (Saravanapriya and Sivakumar, 2004).

A pot culture experiment carried out by Bhosle *et al.* (2006) revealed the efficacy of different organic amendments in the management of root-knot nematode, *M. incognita* on okra. Different organic amendments *viz.*, groundnut cake, sunflower cake, cotton cake and safflower cake each @ 250 kg/ha and sawdust and coal ash @ 5000 kg/ha were applied to okra cv. Parbhani Kranti. Results indicated that spot application of groundnut cake, sawdust, coal ash and sunflower cake significantly reduced the root-knot nematode population.

Patel *et al.* (2009) conducted an experiment to evaluate the efficacy of different organic amendments *viz.*, mustard cake, neem cake and dry azolla each @ 2 t per ha, press mud and poultry manure @ 3 t per ha against root-knot nematode, *M. incognita* during kharif season of the year 2004 using bitter gourd variety Surati. Carbofuran was applied @ 1 kg a.i. per ha in spots at the time of sowing. Statistical analysis of the data indicated that mustard cake and poultry manure were most effective and were on par with each other with an average yield of 10.188 kg/ha and 9.597 kg/ha respectively.

Meena *et al.* (2010) tested the efficacy of acetone extracts of leaf, flower, root and stem of five varieties of *Tagetes viz.*, *T. patula*, *T. erecta* c.v. Atlantis Orange, *T. erecta* c.v. Single Orange, *T. erecta* c.v. Indian Yellow and *T. minuta* against egg hatching and mortality of second stage juveniles of *Meloidogyne incognita*. Among the plant parts, flower extract of *T. erecta* c.v. Indian Yellow was found to be highly effective in causing mortality of juveniles and inhibition of egg hatching followed by root extracts.

2.2.2 Use of Biocontrol Agents

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

2.2.2.1 Bacterial biocontrol agents

Bacteria are present in the rhizosphere of crop plants and are very effective in controlling plant parasitic nematodes. They are highly potent antagonists giving long lasting effects in controlling plant parasitic nematodes.

2.2.2.1.1 *Pseudomonas* spp.

Recently the fluorescent *Pseudomonas* spp. associated with the plant rhizosphere emerged as the most promising biocontrol agent of the plant parasitic nematodes (Oostendrop and Sikora, 1989). The effectiveness of *P. fluorescens* (Migula) 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. Thirty isolates of fluorescent pseudomonads, isolated from the rhizosphere of black pepper were tested for their interaction on *M. incognita*, under green house conditions. The study revealed that the strains of *P. fluorescens* inhibited the population of *M. incognita* (Eapen *et al.*, 1997).

Mani *et al.* (1998) reported the effectiveness of Pf (1) strain of *P. fluorescens* against *M. incognita, Tylenchulus semipenetrans* and *Globodera rostochiensis*. Application of *P. fluorescens* as seed treatment at a dosage of 10g kg^{-1} seed was

effective in reducing the infestation of *Hirschmaniella gracilis* in rice (Ramakrishnan *et al.*, 1998).

A talc formulation of *P. fluorescens* containing 15×10^8 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 bacterial formulation was applied at doses of 1, 2 and 4 g per vine and compared with the application of carbofuran @ 1.8g a.i. per vine and an untreated control. Application of *P. fluorescens* at all the three dosage levels significantly reduced the severity of root knot infestation in roots. The extent of colonization by *P. fluorescens* was dosage dependent but not directly proportional to it. The root colonization was significantly better at all dosage levels of *P. fluorescens* (Santhi *et al.*, 1998).

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. They found that the per cent of gall formation and root gall index were decreased when the bacteria were introduced prior to inoculation with nematodes.

Seenivasan *et al.* (2000) found that the culture filtrate of *P. fluorescens* had toxic effect on *Heterodera oryzae* population. Devi and Dutta (2002) studied the effect of *P. fluorescens* on root knot nematode (*M. incognita*) of okra plant. They found that *P. fluorescens* improved shoot and root length and reduced root gall number. Siddiqui and Shaukat (2002) noted that *P. fluorescens* and *P. aeruginosa* reduced *M. javanica* juvenile penetration into tomato plants.

A field experiment was conducted by Jyothi *et al.* (2003) to evaluate the efficacy of commercially formulated *P. fluorescens* against root knot nematode, *M. incognita* race 3 infesting tomato. *P. fluorescens* treated plants gave the maximum

yield (64.3 %) and minimum *M. incognita* soil population (56 %). Nursery and field experiments were conducted by Mahapatra *et al.* (2003) to determine the efficacy of *P. fluorescens* against *M. incognita* infesting aubergine. *P. fluorescens* at 20 g m^{-2} had the highest reduction (46.4 per cent) in root knot index at transplanting.

El-Hamshary *et al.* (2004) found in an *in vitro* study that *P. fluorescens* and *P. aeruginosa* affected *M. incognita* juveniles survival, and the mortality percentages of the nematode were dependent on the bacterial concentration and exposure time. Siddiqui and Shaukat (2004) concluded that fluorescent pseudomonads induce systemic resistance against *M. javanica* via. a signal transduction pathway which is independent of salicylic acid accumulation in roots.

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

Senthamarai *et al.* (2008) reported the effectiveness of *P. fluorescens* against *M. incognita* infesting medicinal coleus, *Coleus forskohlii*. Soil application of *P. fluorescens* @ 2.5 kg per ha increased plant growth and reduced root-knot nematode population both in soil and root.

Kalaiarasan *et al.* (2008) revealed the influence of *P. fluorescens* on histopathological changes caused by *M. arenaria* in groundnut. *M. arenaria* caused formation of typical multinucleate giant cell and associated abnormalities and the

seed treatment of *P. fluorescens* @10g/kg seed resisted the nematode infection in groundnut by reduced and poor development of giant cells.

Strains of *P. fluorescens* native to India were isolated from the rhizosphere of healthy banana and tested for their efficacy to manage *R. similis* infesting banana. The most promising isolates PfB 13, PfB 19, PfB 24, PfB 32, PfB 35 and PfB 39 caused significant mortality of *R. similis* female, with the largest mortality of 82 per cent occurring after 72 hr exposure to 100 per cent culture filtrate of PfB 13. Root and soil population and root lesion index are significantly lower with PfB 13 and these were on par with Pf1 and carbofuran treatment compared to control (Senthilkumar *et al.*, 2008).

Channappa *et al.* (2008) investigated the effect of cell concentrations of four strains (Pf 1-12, Pf 1-20, Pf 2-1 and Pf-ws) of *P. fluorescens* on haching and mortality of root-knot nematode, *M. incognita*. Results indicated that the bacterial cell concentrations of all the four strains significantly reduced hatching and increased mortality compared to control (distilled water). However, strain Pf 2-1 caused relatively higher inhibition of hatching and higher mortality of second stage juveniles of *M. incognita*.

Prakob *et al.* (2009) studied the effects of *P. aeruginosa, B. subtilis* and antagonistic fungus *Paecilomyces lilacinus* (provitan) on the growth and gall development of lettuce infected by root knot nematodes, *Meloidogyne* spp. under green house and field environments. The results showed that the weight of lettuce planted in nematode infested soil containing these three test organisms, was higher than those cultivated in nematode infested soil with no control agents. *B. subtilis. P. aeruginosa* and *P. lilacinus* also decreased nematode population densities and suppressed nematode infection. As a result fewer galls were developed within the roots.

A pot culture study was conducted by Bhagawati *et al.* (2009) during kharif, 2007 in a net house at Assam Agricultural University, Jorhat, Assam to manage the disease complex caused by *M. incognita* and *Rhizoctonia solani* on okra (var. Parbhani Kranti) using *P. fluorescens* and *T. harzianum*. The efficacy of bioagents were tested both as seed treatments as well as soil application singly and in combinations and compared with a known nematicide (carbosulfan 25 ST) and a fungicide (carbendazim 50% WP). Both the bioagents were found to be significantly effective in reducing the damage and increasing the growth parameters of okra as compared to the treatment inoculated with *M. incognita* and *R. solani*

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*). Results showed that the use of plant growth promoting rhizobacterium, *P. fluorescens* available commercially in talc formulation (2.6×10^6 cfu g^{-1}) @ 2.5 kg ha^{-1} as soil application recorded the lowest nematode population accompanied 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.

2.2.2.1.2 *Bacillus* spp.

Gokte and Swarup (1988) stressed the larvicidal effect of *B. subtilis* and *B. pumilus* on *Anguina tritici*. Seed treatment of the above isolates in wheat individually and in combination caused reduction in percentage of penetration of juveniles to the roots of wheat seedlings and the viability of larva. The effect of bacteria *B. subtilis,*

B. pumilus, B. coagulans, B. macerans and *B. ciculans* were studied on related genera like *H. oryzicola* which revealed that at 1.2×10^8 cells per ml of these bacteria caused 70 to 80 per cent larval mortality (Sheela, 1990).

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

Various formulations of *B. thuringiensis* were found toxic to eggs and larvae of root knot nematode. Zuckerman *et al.* (1993) found that application of an isolate of *B. thuringiensis* (CR-371) resulted in smaller population of *M. incognita* in tomato. The effects of biocontrol treatments on root knot nematode, *M. incognita* and fruit yield of tomato were evaluated by Reddy *et al.* (1999). *B. subtilis* strain GB 03 plus one additional Plant Growth Promoting Rhizobacteria and a flaked chitosan was used as treatments. Across all categories of fruits, greater yields occurred with biological treatments especially in *B. subtilis* treated plots.

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

Mahdy *et al.* (2000) tested the ability of the rhizobacterium, *B. cereus* S18, to control three species of root knot nematode on tomato *viz.*, *M. incognita, M. javanica* and *M. arenaria.* They found that treatment of tomato plants with *B. cereus* S18 led to an overall reduction in the number of galls and number of egg masses produced by all the three nematode species tested when compared with the non- bacterized plants. The antagonistic rhizobacteria caused only a non significant reduction in number of galls and number of egg masses of *M. arenaria.*

B. subtilis in combination with either neem cake or *Datura fastuosa* gave better control of the root rot and root knot infection with the enhancement of growth of urd bean compared to use of either component alone (Siddiqui *et al.*, 2001).

Dhawan *et al.* (2004) evaluated four strains of *B. thuringiensis* and they found that the mobility of *M. incognita* juveniles completely ceased after 24h exposure in standard filtrate (S) and S/10 dilutions. However, all dilutions above S/25 were ineffective. Nagesh *et al.* (2005) confirmed the importance of genus *Bacillus.* Their results indicated that, cell-free culture filtrates of *B. cereus* reduced egg hatching (90%) and caused 100 per cent mortality of juveniles.

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

Endophytic bacteria, *viz., Pseudomonas, Bacillus, Agrobacterium, Stenotrophomonas* and *Enterobacter* were isolated from coffee roots in Ethiopea by Mekete *et al.* (2009) and they revealed that the nematicidal effects of culture filtrates of the bacterial isolates ranged between 38 and 98 per cent. They also proved the role of *B. pumilus* and *B. mycoides* in reducing the number of galls and egg masses by 33 and 39 per cent respectively in an *in vivo* experiment.

2.2.2.1.3 *Pasteuria penetrans*

Daudi *et al.* (1990) applied *P. penetrans* to sterilized soil in microplots in Pakistan. Inoculations with *M. javanica* led to less galling and fewer egg masses on tomato roots than in similar soil without *P. penetrans*; shoot growth was also greater. These differences were accentuated in a second cycle after incorporating the roots of the first crop in the soil. In field plots in Malawi, *P. penetrans* was mixed with soil at two sites where there was an indigenous population of nematodes. Root galling was decreased where roots were re-incorporated into the second crop regardless of whether *P. penetrans* was artificially applied or not.

Tateishi (1999) found that the population of second-stage juveniles of *M. incognita* in soil samples at harvest was significantly lower and marketable yield was significantly higher in plots to which *P. penetrans* was applied compared with controls. *P. penetrans* suppresses *Meloidogyne* spp. root attack by limiting egg production after the nematode has parasitized the roots. High densities of *P. penetrans* spores may also suppress root invasion by *Meloidogyne* spp., because heavily encumbered second stage juveniles are less motile although they are not infected while in the soil.

Rangaswamy *et al.* (2000) evaluated the efficacy of *P. penetrans* and *T. viride* and botanicals (neem and castor cake) in controlling the root knot nematode, *M. incognita* in tomato. *P. penetrans* alone or in combination with neem cake parasitized nematode juveniles and adults, whereas *T. viride* alone or in combination with either neem or castor cake, was most effective in parasitizing the egg masses of the nematodes.

Rao *et al.* (2000) conducted glass house and field experiments during August-November 1999 and February-June 2000 in Bangalore to evaluate the efficacy of *P. penetrans* and *Glomus mosseae* in controlling the root knot nematode *M. incognita* in tomato. Treatment with both the organisms significantly reduced root galling,

nematode population and fecundity of the nematode. Incorporation of *G. mosseae* in nursery beds resulted in a significant increase in the growth of tomato seedlings. The combination of *P. penetrans* and *G. mosseae* resulted in higher parasitism of female nematodes compared with *P. penetrans* alone.

Matching root-knot nematode populations with aggressive *P. penetrans* populations is possible and use can also be made of plant resistance and cultural treatments to diminish nematode levels without influencing residual *P. penetrans* spore densities (Gowen and Pembroke, 2004). Kumari and Sivakumar (2005) described that application of *P. penetrans* against root-knot nematode, *M. incognta* in brinjal resulted in 63, 78 and 89 per cent reduction in egg mass production as compared to that of control at 35 (seedling), 100 (flowering) and 160 (fruiting) days after sowing respectively.

Pasteuria penetrans induced soil suppressiveness is dependent on endospore concentrations and is manifested at the level of root penetration by second stage juveniles and loss of nematode fecundity (Kariuki *et al.,* 2006).

Ahmad and Mukhtar (2007) studied the effects of *P. penetrans* isolates and their blend on the production of galls and egg masses by *M. javanica* and number of juveniles per 500 g of soil over three crop cycles of aubergine. They found that the three subsequent crops have affected the numbers of galls and egg-masses. After each crop, the numbers of galls and egg-masses were significantly reduced, but a marked reduction was found there after the third crop.

A field study was conducted by Ravichandra and Reddy (2008) to evaluate the efficacy of *P. penetrans* in the management of *M. incognita* infesting tomato. *P. penetrans* @ 1×10^9 spores per m^2 was found to be effective in reducing nematode population in the main field by 72.3 per cent over incubated check. Improved plant growth and nutritional status of fruits and plants were also observed with highest yield of 68 q/ ha compared to incubated check (33.25q/ha).

. 2.2.2.2 Fungal Biocontrol Agents

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

2.2.2.2.1 *Paecilomyces lilacinus*

Dube and Smart (1987) observed that the root-knot nematode, *M. incognita* was controlled more effectively and yields of host plants were greater when *Paecilomyces lilacinus* and *Pasteuria penetrans* were applied together in field microplots than when either of them was applied alone.

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. *P. lilacinus* and *Nigrospora sphaerica* had the highest nematicidal activity and *Theilavia terricola* the least.

Noe and Sasser (1995) evaluated the efficacy of *P. lilacinus* in controlling *M. incognita* on four vegetable crops and soybean under field conditions. The yield of vegetable crops in plots treated with *P.lilacinus* was higher than untreated plots. Similarly, *M. incognita* juvenile counts were lower in treated plots than in control plots. The fungus provided same level of nematode suppression as the nematicide.

Bhat *et al.* (2000) reported that the root knot nematode, *M. incognita* was controlled more effectively when *P. lilacinus* and *G. mosseae* were applied together in a pot culture experiment than either was applied alone. Inoculation of tomato plants with *G. mosseae* did not markedly increase the growth of plants infected with *M. incognita*. Inoculation of plants with *G. mosseae* and *P. lilacinus* together or alone

resulted in a similar shoot and plant height. The highest root development was achieved when mycorrhizal plants were inoculated with *P. lilacinus* to combat root knot nematode. Inoculation of tomato plants with *P. lilacinus* suppressed gall/ root system and egg/ egg masses, compared to seedlings inoculated with *M. incognita* alone. Mycorrhizal colonization was not affected by inoculation of *P. lilacinus*.

Hafeez *et al.* (2000) reported that the addition of *P. lilacinus* and *T. harzianum* as nematophagous fungi separately along with organic substrate to the infested soil sufficiently retarded the pathogenic activity of *M. incognita*. Addition of *P. lilacinus* and *T. harzianum* in combination amended with organic substrate gave the effective control of knot nematode population and thus reduced the root knot disease and increased plant vigour.

Khan *et al.* (2001) revealed that egg hatching percentage of root-knot nematode differed with different fungi. Different dilutions of fungal filtrates significantly inhibited hatching of *M. incognita*. Larval emergence was, however, inversely proportional to filtrate dilutions. *P. lilacinus, T. harzianum* and *Verticillium chlamydosporium* were more toxic than other fungi as least egg hatching were observed at standard and S/2 dilutions.

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

An *in vitro* experiment carried out to study the effect of culture filtrates of four opportunistic fungi (*P. lilacinus, Cladosporium oxysporum, Gliocladium virens* and *Talaromyces flavus*) on the larval hatching and mortality of *M. javanica*, showed that *P. lilacinus* was most effective against *M. javanica* (Ashraf and Khan, 2005).

Khan *et al.* (2006a) tested two fungi, *P. lilacinus* and *Monacrosporium lysipagum* individually and in combination against root knot nematode (*M. javanica*), cereal cyst nematode (*H. avenae*) and burrowing nematode (*R. similis*) on tomatoes, barley and tissue cultured banana plants respectively. In all cases, nematode populations were substantially reduced by both individual and combined application of the fungi. The mode and severity of infection of nematodes by *P. lilacinus* was studied under laboratory conditions using microscopy. *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.*, 2006b).

Goswami *et al.* (2006) studied the effect of two fungal bioagents along with mustard oil cake and furadan against root-knot nematode *M. incognita* infesting tomato under green house condition. Bioagents *viz.*, *P. lilacinus* and *T. viride* alone or in combination with mustard cake and furadan promoted plant growth, reduced number of galls per plant, egg masses per root system and eggs per egg mass. The fungal bioagents along with mustard cake and nematicide showed least nematode reproduction factor as compared to untreated infested soil.

Investigation carried out by Krishnamoorthi and Kumar (2007) revealed the biocontrol potential of *P. lilacinus* at different soil moisture and soil temperature for 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 25° C temperature.

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 PlT3 was found to be more effective among seven isolates in both mortality and hatching inhibition of *M. incognita*. Percentage mortality and hatching

inhibition of *M. incognita* were directly proportional to the concentration of culture filtrates and exposure period to each filtrate. Rate of mortality was low in the first 24h but it appreciably increased with the increase in exposure period.

A pot culture experiment was conducted to study the efficacy of leaf power of *Cassia tora* and *Morus alba* (20g each per kg soil) along with *P. lilacinus* alone and in combination against *M. incognita* on chickpea (Azam *et al.*, 2009). They concluded that a combination of leaf powder of *C. tora* and *P. lilacinus* successfully managed the root-knot nematode compared to a combination of two leaf powders.

Sundararaju and Kiruthika (2009) reported the effect of biocontrol agent (*P.lilacinus*), neem cake and botanicals (*T. erecta* and *Solanum torvum)* against root-knot nematode, *M. incognita* on banana var. Robusta. Among the treatments, the combined application of *P. lilacinus* + neem cake and *P. lilacinus* + *T. erecta* (flower extracts) resulted in maximum increase of plant height (5.3cm each), number of leaves (26.6 and 25.0), pseudostem girth (11.7 and 11.0cm), root length (36 and 26cm), number of healthy roots (42 and 43), root weight (45 each), root gall index (1) and nematode population from soil (30 and 50/250cc) and roots (110 and 115 /5g) compared to the maximum root gall index (5) with the nematode population from soil (1020/250cc) and roots (1760 /5g) in nematode inoculated control plants.

Simon and Pandey (2010) evaluated the antagonistic efficacy of *P. lilacinus* and *V. chlamydosporium* against *M. incognita* infesting okra. The results showed that the length and weight of root and shoot significantly increased where 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 plant. *P. lilacinus* significantly improved the growth of tomato plants inoculated with 2000 juveniles of *M. javanica*. The plant height, fresh weight, dry weight and number of leaves per plant were significantly improved, whereas number of galls, egg masses, eggs per egg mass and final nematode population were greatly reduced on simultaneous and sequential inoculation of *P. lilacinus* and *M. javanica*. The efficacy of *P. lilacinus* significantly varied with inoculation time. The simultaneous inoculation of *P. lilacinus* and *M. javanica* significantly improved plant growth parameters. However, sequential inoculation of *P. lilacinus* 10 days prior to nematode inoculation was more effective than sequential inoculation of *M. javanica* 10 days prior to the inoculation of *P. lilacinus*.

2.2.2.2.2 Arbuscular Mycorrhizal Fungi (AMF)

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

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

Jain and Hasan (1988) reported that AMF infected plant roots induced tolerance to nematode susceptible plants. AMF and plant parasitic nematodes occur together in the rhizosphere of same plant and colonize or infect similar root tissue for their growth and development. Jain and Sethi (1988) concluded that, early establishment of *G. fasciculatum* on cowpea hampered the gall formation by *M. incognita* and their multiplication.

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

A study conducted by Deepthi (1993) indicated that cowpea plants inoculated with *M. incognita* in association with G. *fasciculatum* and *G. mosseae* recorded root knot index of 1 and 3.16 respectively as against 4.89 observed for control plants. Establishment of *G. fasciculatum* two weeks prior to the inoculation of *M. incognita* resulted in increased fresh and dry shoot weight of okra and reduced larval penetration, development, number of galls and egg masses per plant (Sharma and Trivedi, 1994). Studies conducted by Sharma *et al.* (1994) indicated that VAM colonization reduced the root-knot nematode infestation in tomato. Mycorrhizal tomato seedlings had lesser number of galls, egg masses per plant, eggs and juveniles per egg mass.

Carling *et al.* (1996) determined the individual and combined effects of two Arbuscular Mycorrhizal Fungi (*Gigaspora margarita* and *G. etunicatum),* on *M. arenaria* and phosphorus (P) fertilization (0, 25, 75 and 125 µg/soil) on groundnut plant growth and pod yield under green house condition. Groundnut growth and yield were generally stimulated by AMF development, growth alone was suppressed by *M.*

arenaria at 0 and 25 µg phosphorus. *M. arenaria* had only a minimal effect on root colonization by AMF and sporulation by the fungi.

A report by Sundarababu *et al.* (1996) showed that when *G. fasciculatum* was inoculated 15 days earlier than nematode inoculation, it enhanced the growth of tomato cv. Co-3 and suppressed *M. incognita* multiplication in pot experiments. *G. fasciculatum* was very effective in controlling root knot nematode in brinjal (Asha, 1996) and in spices like ginger, turmeric, cardamom, pepper (Sivaprasad and Sheela, 1998) and kacholam (Rajani *et al.*, 1998).

Ray and Dalei (1998) reported that, in case of green gram all plant growth parameters including pod yield, leaf chlorophyll content, bacterial nodulation, leghaemoglobin content of nodules and NPK content of plants showed significant improvement in mycorrhiza nodulated plants. Four species of Arbuscular Mycorrhizal Fungi *viz., Aucalospora laevis, G. fasciculatum, G. intraradices* and *G. mosseae* in combination with neem cake were evaluated for their comparative efficacy to colonise crossandra roots and suppress *M. incognita* infection. *G. fasciculatum* and *G. mosseae* colonised crossandra roots better compared to *G. intraradices* and *A. laevis* and resulted in comparatively lower nematode infection and multiplication, thus promoting better root health and plant growth in former species. Although all the four species of AMF in combination with neem cake gave better control of nematodes over carbofuran treatment, two species *viz., G. fasciculatum* and *G. mosseae* were found to be more efficient (Nagesh *et al.,* 1999).

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

John *et al.* (2004) examined the influence of six isolates of AMF *viz., G. fasciculatum, G. etunicatum, G. constrictum, G. mosseae, G. monosporum* and *Acaulospora morroweae* on the root-knot infestation and biomass production in amaranthus. Plants treated with *G. monosporum, G. etunicatum* and *G. mosseae* resulted in a significant increase in fresh weight of plants and reduction in nematode population in root and soil. John and Bhai (2004) reported the biocontrol efficacy of AMF for the management of root-knot nematode in brinjal.

Masadeh *et al.* (2004) investigated the effects of the combination of the Arbuscular Mycorrhizal Fungus (AMF), *G. intraradices* and the fungus *T. viride* on the control of root knot nematode, *M. hapla,* in green house experiments on the tomato cultivars 'Hildares' and 'Tiptop' (less suitable as host for root-knot nematode, showing retarded development of the giant cell system, retarded growth of the nematode and consequently reduced production of egg sacs). Neither of the beneficial fungi, inoculated singly or together, changed general susceptibility of the cultivars. In Hildares, application of the beneficials reduced the number of galls and egg sacs. However, a combination of *G. intraradices* and *T. viride* did not result in synergism. In Tiptop, biocontrol of root knot nematode was not achieved.

Prakob *et al.* (2007) investigated the effects of mixed Arbuscular Mycorrhizal Fungi (AMF), rhizobacteria *P. aeruginosa, B. subtilis,* and antagonistic fungus *P. lilacinus* (provitan) on growth and gall development of tomatoes infected by root-knot nematodes *Meloidogyne* spp. under green house conditions.

Oyekanmi *et al.* (2007) studied the effects of soybean inoculation with Arbuscular Mycorrhizal Fungus *G. mosseae* (200 spores/plant), the nodulating bacterium *Bradyrhizobium japonicum* (10^6 cells/plant) and the nematode antagonistic fungus *T. pseudokoningii* (6.8×10^7 spores/plant). Application of the microorganisms separately, in dual or in triple combinations were assessed in the presence of the plant parasitic nematode, *M. incognita* under green house (1000 second stage juvenile/

plant) and field (1500 eggs/ plant) conditions, with two soybean genotypes. The microorganism treatments were compared with application of a synthetic nematicide.

Saikia and Borah (2008) conducted a study to find out the comparative efficacy of *G. fasciculatum, P. penetrans* and *T. harzianum* with carbofuran against *M. incognita* on brinjal. Among the treatments *G. fasciculatum* + carbofuran 3G combination was found to be the best in increasing plant growth characters and yield and decreasing the number of galls and egg masses followed by *T. harzianum*+ carbofuran 3G.

2.2.2.2.3 Use of Other Fungal Biocontrol Agents

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

The efficacy of microorganisms *viz., T. viride and P. fluorescens* for nematode management in banana was studied under field conditions (Sheela *et al.*, 1999). They observed that maximum yield was obtained in application of *T. viride* @ 2.5g/plant at the time of planting followed by 2.5g/plant at 45 DAP in m^2 basin area, was on par with paring + hot water treatment + neem cake @ 1kg /plant + carbofuran @16.6 g/plant.

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

Sankaranarayanan *et al.* (2000) tested the efficacy of *V. chlamydosporium* cultured on different substrates such as sorghum grain, rice grain, broken wheat, maize grain and wheat bran against *M. incognita* with tomato cv, Pusa Ruby under

potted condition. All the substrates favoured the multiplication of *V. chlamydosporium* and enabled the fungus to suppress galls, egg masses and nematode population. The degree of suppression of nematode by *V. chlamydosporium* varied with the substrate used and the percentage parasitism of egg masses and eggs of *M. incognita* ranged from 39 to 70 and 51 to 89 respectively. *V. chlamydosporium* cultured on sorghum grains applied at 10 g per plant was superior to other substrates in terms of parasitism of egg masses (70 and 89.3 respectively) and eggs (63 and 69 % respectively). A significant increase in growth of tomato plants was observed with *V. chlamydosporium* treated plants.

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

Pot and field trials were conducted by Pandey *et al.* (2003) to study the efficacy of different levels of *T. viride* (1000, 2000, 3000 and 4000 spores per plant) against root-knot nematode *M. incognita* in chickpea. All the treatments of *T. viride* showed significantly higher plant growth parameters over control. The gall development and final nematode population of *M. incognita* decreased with the increasing level of *T. viride* under pot and field conditions.

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

Radwan (2007) studied the comparative effects of culture filtrate of soil-borne fungi *viz.*, *Aspergillus niger, Pythium debarianum, Fusarium oxysporum* f. sp. *lycopersici, F. solani* and *F. moniliforme* on the mortality and infectivity of second

stage juveniles of root-knot nematode, *M. incognita*. The data clearly established that the culture filtrates of *A. niger* were most effective against second stage juveniles followed by *F. moniliforme, F. solani, F. oxysporum* and *P. debarianum.*

A green house study was undertaken to see the efficacy of fungal antagonists *viz.*, *T. viride* and *G. virens* as seed treatment against root-knot nematode, *M. incognita* infesting cowpea (Verma *et al.*, 2009). The seeds of a susceptible cowpea variety Pusa Barsati were treated with laboratory propagated fungal cultures @ 5 and 10g/kg seed before sowing and the results indicated that the growth parameters of cowpea plants, i.e. shoot length, fresh and dry shoot and root weight were maximum and significantly higher in plants treated with *T. viride* and *G. virens* @ 10g/kg seed.

Dhawan and Singh (2010) conducted a study to determine the biocontrol efficacy of an antagonistic fungus, *Pochonia chlamydosporia* against root-knot nematode, *M. incognita* infesting okra. Effect of seed treatment and soil application of *P. chlamydosporia* was studied at various doses under *in-vivo* and microplots. The results showed that highest recovery in plant vigour with maximum reduction in nematode multiplication including final soil population was recorded in treatment that received soil application @ 30g/plot at sowing time coupled with seed treatment @ 3% W/W over control.

2.2.2.3 Entomopathogenic Nematode (EPN)

Entomopathogenic nematodes of family *Steinernematidae* and *Heterorhabditidae* are of considerable value as biological control agents and serve as a measure alternative to chemical control (Poinar, 1986). They are closely associated with symbiotic bacteria of the genera *Xenorhabdus* and *Photorhabdus.*

Antagonistic interactions between EPN and Plant Parasitic Nematodes (PPN) were first shown by Bird and Bird (1986), who showed that a reduction of the

infection of *M. javanica* in tomato plants was caused by *Steinernema glaseri* (Steiner) in greenhouse pot tests. Similarly, *S. glaseri* DD-136 and *S. feltiae* (Filipjev) reduced populations of PPN and increased the populations of bacteriophagous rhabditid nematodes (Ishibashi and Kondo, 1986).

EPNs have shown potential as antagonists to PPN in several green house and field trials (Lewis *et al.*, 2001). They demonstrated the suppressive effect of EPN on PPN. Suppressive effects of *S. riobrave* on *M. incognita* and *M. javanica* on okra and groundnut was reported by Maghodia *et al.* (2003) and Vyas *et al.* (2004).

Lewis and Grewal (2006) reviewed the literature describing the interactions and have found that, while antagonism exists in many cases, the amount of PPN reduction caused is rarely to a level that would be considered acceptable in most agricultural settings.

Application of *S. abbasi, S. feltiae* and *S. carpocapsae* to tomato plants reduced invasion, development and reproduction of *M. incognita* (Hussaini *et al.*, 2008). Application of *Heterorhabditis indica, S. abbasi* and *S. carpocapsae* reduced *M. incognita* population in soil and increased the growth of tomato plants (Hussaini and Kirankumar, 2009; Hussaini *et al.*, 2009).

Vyas *et al.* (2009) conducted a field experiment to study the interaction of entomopathogenic nematode and their bacterial toxins with plant parasitic nematodes (*Melodogyne* spp.) in *kharif* 2004. Nine treatments *viz.*, infective juveniles(IJ_s) of *S. riobrave* isolates M and A, undiluted exotoxins of symbiotic bacteria *Xenorhabdus* A and M as seed soaking and soil application, along with treated checks, Tricho X-P (*Paecilomyces* and *Trichoderma*) with an untreated control were tried in okra GOH-1.

Results proved that seed soaking treatment of toxins was more effective than soil application of toxins and *S. riobrave* IJ_s application.

2.2.2.4 Use of Chemicals

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

Soil application of carbofuran @ 2 kg 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 the 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.

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

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

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

Tiwari *et al.* (2002) found that tomato nursery bed treated with 0.6 g carbofuran significantly decreased gall index and increased crop yield. Compared to other plots the maximum yield (362 q 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. per ha. Carbofuran applied @ 1.5 kg a.i. per ha gave 48 per cent control and thus increased the yield up to 46 per cent.

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.

A green house experiment conducted by Srivastava and Lal (2007) showed that soil application of cadusafos @ 1 kg a.i. per ha either at the time of sowing or in

split dose was most effective than carbofuran in enhancing plant growth parameters of maize cv. Decan-103 and reducing final population of maize cyst nematode, *H. zeae.*

Rajvanshi *et al.*(2008) investigated the efficacy of seed soaking and foliar application of carbosulfan on root-knot nematode, *M. incognita* infesting round melon. The 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) and reduced the number of galls per plant (9.67) and final nematode population of soil (125.20), followed by carbofuran 3G @ 2.0 kg a.i. / ha with yield 71.67 q/ha, number of galls /plant(12.80) and final nematode population of soil (135.50).

Patel and Patel (2009) carried out an experiment for the management of nematodes through chemicals in bidi tobacco nursery for four years. Eight treatments *viz.*, drenching of carbosulfan (Marshal 25EC) @ 2.5 l/ha one day prior to seeding (DPS) + 25 days after seeding (DAS), carbosulfan @ 5 l/ha (drenching), isoazophos, carbofuran, sebuphos @ 5kg/ha as broadcasting 1 DPS, diazomet @ 294 kg/ha 20DPS, soil solarization for 15 days and control were tried. Pooled results revealed that drenching of carbosulfan @ 2.5 l/ha DPS + 25 DAS and soil solarization significantly reduced root-knot disease and increased number of transplants of bidi tobacco as compared to control.

Materials and Methods

3. MATERIALS AND METHODS

The objective of the study entitled 'Biological control of root-knot nematode, *Meloidogyne incognita* (Kofoid and White, 1919) in banana, *Musa* (AAA) var. Robusta' includes the identification of an effective biocontrol agent for the management of root-knot nematode in banana. The experiment was carried out in the Department of Agricultural Entomology, College of Horticulture, Vellanikkara and Banana Research Station, Kannara during April 2010 to June 2011. The study was undertaken with tissue culture banana plants in pot culture experiment.

3.1 PREPARATION OF DENEMATIZED POTTING MIXTURE

Potting mixture was prepared by mixing sieved field soil, sand and well decomposed farmyard manure in the ratio 1:1:1. Potting mixture was denematized using 3 per cent formaldehyde @ 50 ml for 200 kg potting mixture. Formaldehyde was thoroughly mixed with potting mixture and were made into small heaps and covered tightly with polythene sheets. After four days, polythene sheets were opened and the mixture was raked well and covered again for another one week. After the specified period the sheets were removed and the mixture was spread on the floor to remove the formaldehyde residues. From the treated potting mixture samples were taken randomly to test the presence of nematodes. This denematized potting mixture was used for further pot culture studies.

3.2 MAINTENANCE OF PURE CULTURE OF ROOT-KNOT NEMATODE INFESTING BANANA

Rooted cuttings of coleus plants were used for the maintenance of nematode culture. The cuttings of coleus were planted in pots of size 25 cm diameter filled with denematized potting mixture. After identifying the species of root-knot nematode on the basis of perineal pattern, pure culture of nematode was maintained

from single egg mass collected from infested banana roots collected from the fields of Banana Research Station, Kannara. The second stage juveniles of the nematodes, hatched from the egg masses were inoculated to the potted coleus plants. Repotting and inoculation was repeated periodically for maintaining the pure culture of root-knot nematode, *M. incognita* for the experiments.

3.3 IDENTIFICATION OF ALTERNATE WEED HOSTS OF *M. incognita* FROM BANANA PLOTS

Weed plants present in the banana plots of Banana Research Station, Kannara were examined for root-knot infection. The weeds were carefully uprooted and brought to the laboratory in polythene bags. Then the roots of the weeds were thoroughly washed under running tap water to remove the adhering soil particles and examined for root-knot infection.

3.4 TAXONOMIC IDENTIFICATION OF NEMATODES

The species of root-knot nematodes infesting banana plants were identified by the perineal pattern of the white females. In order to identify the species of nematode, white females were collected from root galls.

3.4.1 Collection of White Females by Staining Technique

Root samples collected from the infested banana plants were used for extracting white females. Root samples were washed in a stream of tap water to remove any soil particles adhering to it. Root knots were separated from roots with the help of scissors. It was then wrapped in a small piece of muslin cloth. This small bag containing root galls were immersed into boiling lactophenol containing 0.1 per cent cotton blue till the root tissues become soft. The root knots were removed from muslin cloth and kept in a Petri plate. It was washed in water to remove excess stain. The root knots were transferred to a microscopic slide containing a drop of lactophenol. It was then placed under a stereomicroscope and dissected using a

needle. The white females, which were stained light blue, were collected and transferred to fresh lactophenol on a perspex slide. The posterior end of the 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 lactophenol on a clean glass slide and observed under a stereoscopic microscope and compared with the perineal pattern of *M. incognita*.

3.5 RAISING POTTED PLANTS

The experimental area for keeping the potted plants was levelled and made weed free. Potting bags of size 80×35 cm with denematized potting mixture were used for raising potted plants. Tissue culture banana plants of uniform age were used for pot culture studies. These tissue culture plants were collected from Banana Research Station, Kannara. Plants were irrigated periodically to maintain wet condition of soil and the fertilizer recommendation as per Package of Practices of Kerala Agricultural University was followed. Regular weeding was practiced to make the interspaces weed free.

3.6 POT CULTURE EXPERIMENT

Pot culture study was conducted to determine the efficacy of different biocontrol agents and carbofuran 3G in the management of root-knot nematode, *M. incognita* infesting banana.

DESIGN AND TREATMENTS

The experiment was laid out in a Completely Randomized Design with ten treatments and three replications. (Plate 2). The treatments were as follows:

T1 - Soil application of Arbuscular Mycorrhizal Fungi (AMF) @ 250 g/ plant

T2 - Soil application of *Pseudomonas fluorescens* @ 25 g/m^2

T3 - Soil application of *Paecilomyces lilacinus* @ 25 g/m^2

T4 - Soil application of *Bacillus subtilis* @ 25 g/ m^2

T5 - Entomopathogenic Nematode (EPN)- *Heterorhabditis indica* @ 1× 10^6 IJs/m^2

T6 - Soil application of *P. fluorescens* + *P. lilacinus* @ (12.5 g + 12.5 g) /m^2

T7 - Soil application of *P. lilacinus* + *B. subtilis* @ (12.5 g + 12.5 g) /m^2

T8 - Soil application of *B. subtilis* + *P. fluorescens* @ (12.5 g + 12.5 g) /m^2

T9 - carbofuran 3G @ 1.2 g a.i./ plant

T10 - Untreated control

3.6.1 Application of Biocontrol Agents

Required quantity of AMF, collected from the Tamil Nadu Agricultural University, Coimbatore, was added to the potting mixture at the time of planting of tissue culture banana Var. Robusta. The tissue culture plants were established within two to four weeks with well developed root system. Then the second stage juveniles of the root-knot nematode, *M. incognita* was inoculated to the banana plants @ one second stage juvenile per gram of soil. One month after the inoculation of nematodes required quantity of *P. fluorescens*, *P. lilacinus* and *B. subtilis* alone and in different combinations were incorporated in the pots. Talc based formulations of the above bioagents were mixed thoroughly with the soil and immediately after the application of biocontrol agents, the plants were irrigated gently to maintain the moisture level required for the action of these biocontrol agents. Similarly EPN was also inoculated to the potted plants after assessing their population in the suspension. Carbofuran 3G

Plate 2. Lay out of the experiment

@ 40g per plant was also applied one month after the inoculation of root-knot nematodes.

3.6.2 Maintenance of EPN Culture on *Galleria mellonella*

The entomopathogenic nematode, *H. indica* used in the study was procured from the stock maintained at the Department of Nematology, Sugarcane Breeding Institute, Coimbatore. Laboratory culture of EPN was mass multiplied and maintained on the final instar larvae of greater wax moth, *Galleria mellonella*. Nematodes were multiplied using the methods of Dutky *et al.* (1964). The nematode suspension was applied to a single layer of Whatman No. 1 filter paper in a Petri dish. The larvae were introduced into the EPN treated Petri dish and these Petri dishes were sealed and incubated at 24°C for 3-4 days. The infected larvae became soft, flaccid and the colour changed to brick red that faintly luminesce in the dark. These infected larvae were transferred to a clean and dry Petri dish containing a single layer of tissue paper and kept for 5-6 days.

3.6.3 Extraction of EPN

Extraction of EPN was done by the Modified White trap method prepared in a Petri dish (110 × 25mm) with an inverted watch glass (52mm) to serve as a stage. A single filter paper (Whatman No. 1, dia. 90 mm) was used to absorb and provide moisture for the nematodes. Infected larvae were transferred to the stage of the White trap @ 10-15 cadaver per watch glass and incubated at 23°C for 4-5 days. When the nematodes were observed in the trap they were extracted in a beaker and cleaned two or three times with distilled water by sedimentation, followed by decantation. Several such White traps were kept for getting the required number of entomopathogenic nematodes. The extracted nematodes were stored at 24°C in distilled water in a B.O.D. incubator for future use. (Plate 3)

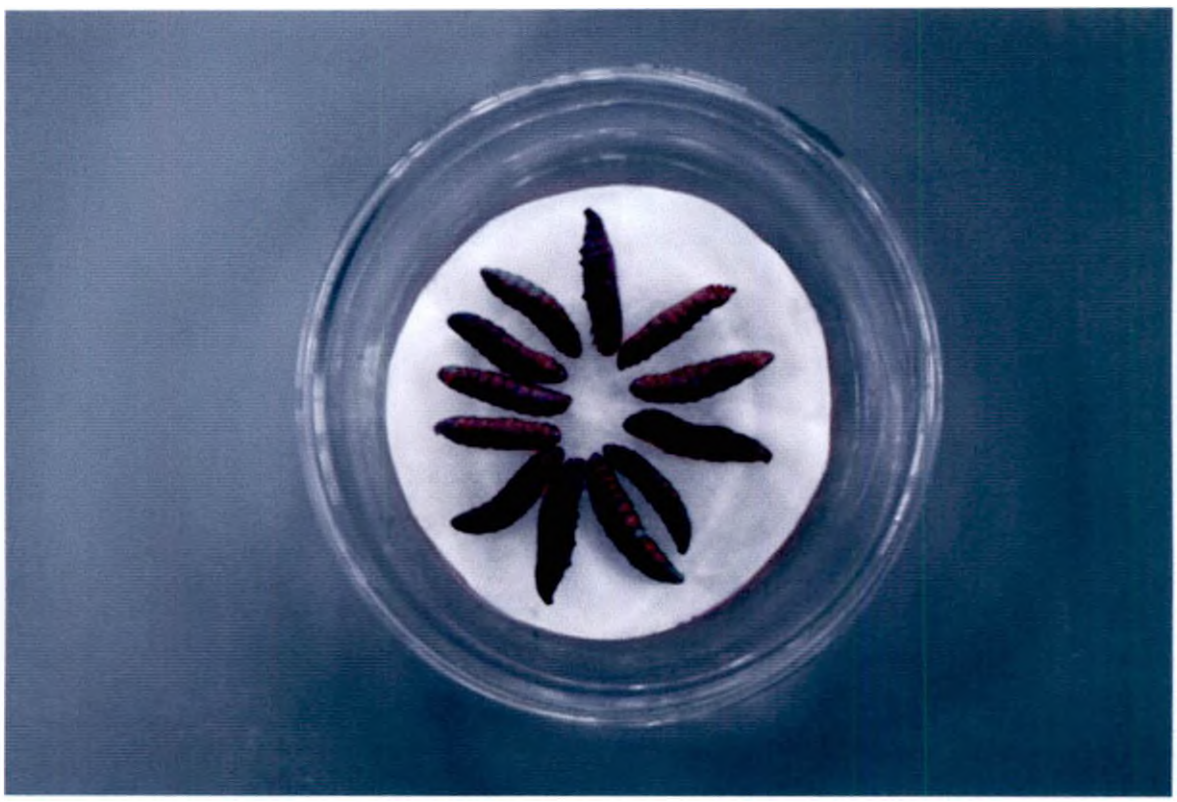

Plate 3. Extraction of EPN using Modified White trap

3.6.4 Inoculation of EPN

The EPN suspension obtained from Modified White trap, was made up to a constant volume by adding water. An aliquot of 50µl was pipetted out into a counting dish after thoroughly mixing the nematode suspension by blowing air with the pipette and the number of nematodes present were counted under a stereoscopic microscope. The total population of nematodes present in the suspension was estimated by multiplying the average population (based on five such counts) by the appropriate factor. At the time of inoculation the 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 5cm depth in soil using a glass rod. After draining the entire suspension, the holes were covered with a thin layer of soil.

3.6.4 Extraction of Second Stage Juveniles of *M. incognita* for Inoculation

Modified Baermann funnel technique (Schindler, 1961) was used for extracting the second stage juveniles of root-knot nematode, *M. incognita* for inoculation. Heavily infested plants from the culture pots were uprooted carefully and washed with water to remove the soil particles. Then the egg masses from the galled roots were collected using forceps. The second stage juveniles were extracted by keeping the egg masses over two layers of tissue paper supported on a wire mesh, which in turn was placed over a Petri dish containing water just enough to touch the egg masses. Several such sets were kept for getting the required number of second stage juveniles needed for inoculation purpose. Hatched second stage juveniles were collected in a beaker after every 12h. This nematode suspension was used for inoculation.

3.6.5 Inoculation of Second Stage Juveniles of *M. incognita* to Banana Plants

Nematode population in the suspension was assessed before inoculating to the banana plants. For assessing the population of nematodes, the nematode suspension collected in the beaker was made upto a constant volume by adding water. An aliquot of 1ml 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 were counted under a stereoscopic microscope. The total population of nematodes present in the suspension was estimated by multiplying the average population (based on five such counts) by the appropriate factor. Each pot containing banana plant was inoculated with 100 ml suspension containing 15,000 second stage juveniles of root-knot nematode after the plants had established. At the time of inoculation also, the 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 5cm 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.

3.7 OBSERVATIONS

The banana plants were allowed to grow for a period of seven months and the following observations were recorded. Biometric characters recorded at monthly intervals during the course of experiment include:

a) Height of the plant
b) Pseudostem girth
c) Number of leaves

When the plants were about to form bunches, plants were uprooted and the following observations were taken:

a) Fresh weight of the whole plant

b) Fresh weight of the corm
c) Fresh weight of the roots
d) Nematode population in 250g soil
e) Nematode population in 20g of root
f) Number of root-knots in 20g of root
g) Root-knot index

3.7.1 Estimation of Nematode Population from Soil

A composite sample of 250g of soil was collected from the root zone region of banana plants grown in pots and processed for extracting the nematodes. Nematodes were extracted from soil samples taken from different treatments, following the Cobb's decanting and sieving technique (Cobb, 1918). The residues obtained on 100, 200 and 325 mesh sieve were collected in a 100 ml beaker. The residue thus collected was cleared by adopting the modified Baermann funnel technique (Schindler, 1961). The nematode suspension was collected in a beaker after 12 h and volume was made up to a known level (100 ml) by adding water. An aliquot of 1 ml was pipetted out into a counting dish and the number of nematodes present were counted under a stereoscopic microscope. The total population of nematodes extracted from 250 g soil sample was estimated by multiplying the average population (based on five such counts) by the appropriate factor.

3.7.2 Estimation of Root Knots from 20g of Roots

The root system of banana plant from each pot was carefully lifted by gentle tapping the potting bags on all sides and bottom and removing the loose soil, the roots were cleaned of adhering soil particles by gentle washing in water. From this, sample of 20g root was randomly taken and the root sample was pressed gently between the folds of blotting paper to remove excess water and the number of root knots in 20g of root sample were counted.

3.7.3 Root-knot Index

Based on the number of galls counted, the root knot index was worked out by rating on a 1-5 scale as follows

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

3.7.4 Estimation of Nematode Population from Root

After counting the number of galls, the same root samples were used for extracting the nematodes. Modified Baermann funnel technique was used for extracting nematodes from roots (Schindler, 1961). The root samples were cut into small pieces and placed in a mixer grinder with a little quantity of water. After crushing, the root sample was transferred over two layers of tissue paper supported on a wire mesh, which in turn was placed over a Petri dish with sufficient water. After every 24 hrs, the nematode suspension was drawn out from the Petri dish. This was continued (five days) till no nematode was obtained. The nematode suspensions thus obtained were pooled together and made to a known volume by adding water and then the population of the nematodes were assessed under a stereoscopic microscope.

3.8 STATISTICAL ANALYSIS

Data collected from the study were analysed 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 'Biological control of root-knot nematode, *Meloidogyne incognita* (Kofoid and White, 1919) in banana, *Musa* (AAA) var. Robusta' are presented in this chapter.

4.1 IDENTIFICATION OF ALTERNATE WEED HOSTS OF *M. incognita* FROM BANANA PLOTS

Weed plants present in the banana plots of Banana Research Station, Kannara were examined for root-knot infection. The weeds were carefully uprooted and brought to the Laboratory in polythene bags. Then the roots of the weeds were thoroughly washed under running tap water to remove the adhering soil particles and examined for root-knot infection. The important weed hosts identified were *Ageratum conyzoides* L. and *Vernonia cineria* L.

4.2 POT CULTURE STUDY

Pot culture experiment was conducted to study the effect of different biocontrol agents *viz.*, Arbuscular Mycorrhizal Fungi (AMF), *Pseudomonas fluorescens*, *Paecilomyces lilacinus*, *Bacillus subtilis* and Entomopathogenic Nematode (EPN), *Heterorhabditis indica* alone and in different combinations with carbofuran as the standard nematicide check for the management of root-knot nematode in banana. Treatments were given as mentioned in 3.6. The effect of different treatments on the biometric characters of banana *viz.*, height of the plant, girth of pseudostem and number of leaves were observed at monthly intervals. When the tissue culture banana plants were about to form bunches (seven months after planting) the plants were uprooted and the effect of different treatments on following characters were also calculated.

a) Fresh weight of the whole plant
b) Fresh weight of the corm
c) Fresh weight of the roots
d) Nematode population in 250g soil
e) Nematode population in 20g of root
f) Number of root-knots in 20g of root
g) Root-knot index

4.2.1 Biometric Characters of Banana

4.2.1.1 Height of the Plant

Height of the banana plants were recorded at monthly intervals, till seven month after planting. The results presented in Table 1 showed that there was significant variation in the height of banana plants by different treatments. The mean height of the plants at the time of uprooting (seven months after planting) ranged from 107.22cm to 131.22 cm. Plants treated with a combination of *P. fluorescens* + *P. lilacinus* (T6) recorded maximum plant height of 131.22 cm and it was considered as the superior treatment over all other treatments. Carbofuran (T9) and *P. lilacinus* (T3) treated plants produced an average plant height of 126.55 cm and 121.66 cm respectively and were found statistically on par with T6. Next better treatment was T1 with a mean height of 118.55 cm which was on par with T10 (118.22 cm). The treatments *viz.*, T4 (118.11cm), T7 (115.89 cm), T8 (114.88 cm), T2 (112.55 cm) and T5 (107.22 cm) were observed statistically inferior to control (T10) and were found on par with each other. (Plate 4).

4.2.1.2 Pseudostem Girth

Statistical analysis of the data indicated that there was significant variation in the girth of pseudostem of banana in different treatments. Highest pseudostem girth

Table 1. Effect of different bioagents on the height of banana plants

Treatments	Height of the banana plants (cm) (Mean of three replications)				
	Months after treatment				
	1	2	3	4	5
T1 - AMF	44.66^{cd}	54.77^{bc}	65.77^{de}	82.11^{bc}	118.55^{bc}
T2 - *P. fluorescens* (P.f)	44.00^{d}	53.77^{c}	64.00^{e}	78.11^{c}	112.55^{cd}
T3 - *P. lilacinus* (P.l.)	40.66^{e}	54.11^{c}	66.00^{cde}	83.22^{bc}	121.66^{abc}
T4 - *B. subtilis* (B.s)	47.33^{a}	57.00^{ab}	68.22^{ab}	80.33^{c}	118.11^{bc}
T5 - EPN	46.44 ^{ab}	56.22^{abc}	65.22^{e}	77.66^{c}	107.22^{d}
T6 - P.f. +P.l.	47.00^{a}	57.55^{a}	69.44^{ab}	96.00^{a}	131.22^{a}
T7 - P.l.+ B.s.	46.33^{ab}	57.00 ^{ab}	68.00^{abc}	97.22^{a}	115.89^{bcd}
T8 - B.s.+ P.f	47.00^{a}	57.11^{ab}	70.00^{a}	101.66^{a}	114.88^{cd}
T9 - carbofuran 3G	46.11 ^{abc}	57.44^{a}	69.87^{a}	101.00^{a}	126.55^{ab}
T10 -Untreated control	44.99^{bcd}	56.89^{ab}	67.55^{bcd}	87.55^{b}	118.22^{bc}

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

Table 2. Effect of different bioagents on the pseudostem girth of banana plants

Treatments	Pseudostem girth of banana plants (cm) (Mean of three replications)				
	Months after treatment				
	1	2	3	4	5
T1 - AMF	14.30^{bc}	15.74^{c}	17.45^{bcde}	19.89^{c}	35.35^{ab}
T2 - *P. fluorescens* (P.f)	14.45^{bc}	15.55^{c}	17.03^{cde}	19.83^{c}	31.98^{b}
T3 - *P. lilacinus* (P.l.)	14.74^{abc}	15.97^{bc}	17.41^{bcde}	19.98^{c}	34.40^{ab}
T4 - *B. subtilis* (B.s)	14.83^{ab}	15.76^{bc}	16.94^{de}	19.01^{c}	33.10^{ab}
T5 - EPN	14.52^{abc}	15.62^{c}	16.64^{e}	18.90^{c}	33.77^{ab}
T6 - P.f. +P.l.	14.76^{abc}	15.99^{bc}	17.57^{bcd}	21.91^{b}	37.51^{a}
T7 - P.l.+ B.s.	14.60^{abc}	16.36^{ab}	17.89^{bc}	22.95^{ab}	34.53^{ab}
T8 - B.s.+ P.f	14.14^{bc}	15.96^{bc}	17.94^{b}	22.51^{b}	34.05^{ab}
T9 - carbofuran 3G	15.09^{a}	16.60^{a}	19.02^{a}	23.81^{a}	36.65^{ab}
T10 -Untreated control	14.03^{c}	15.72^{c}	17.24^{bcde}	22.13^{b}	34.57^{ab}

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

Table 3. Effect of different bioagents on the number of leaves of banana plants

Treatments	Number of leaves of banana plants (Mean of three replications)				
	Months after treatment				
	1	2	3	4	5
T1 - AMF	9.44^{a}	9.44^{b}	9.11^{d}	11.77^{ab}	15.66^{abc}
T2 - *P. fluorescens* (P.f)	9.33^{a}	9.55^{b}	9.55^{cd}	12.33^{ab}	15.99^{a}
T3 - *P. lilacinus* (P.l.)	9.66^{a}	9.44^{b}	9.00^{d}	11.66^{ab}	15.66^{abc}
T4 - *B. subtilis* (B.s)	10.00^{a}	10.00^{ab}	10.44^{ab}	11.99^{ab}	15.23^{bc}
T5 - EPN	9.22^{a}	9.55^{b}	9.22^{cd}	11.22^{b}	15.11^{c}
T6 - P.f. +P.l.	9.55^{a}	9.66^{b}	9.66^{cd}	12.66^{a}	16.33^{a}
T7 - P.l.+ B.s.	9.66^{a}	9.99^{ab}	10.00^{bc}	12.22^{ab}	15.89^{ab}
T8 - B.s.+ P.f	9.89^{a}	9.66^{b}	9.55^{cd}	11.55^{ab}	15.89^{ab}
T9 - carbofuran 3G	10.00^{a}	10.55^{a}	11.11^{a}	12.22^{ab}	15.99^{a}
T10 -Untreated control	9.55^{a}	9.66^{a}	9.44^{cd}	11.22^{b}	15.22^{bc}

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

of 37.51 cm was recorded in plants treated with *P. fluorescens* + *P. lilacinus* (T6). *Pseudomonas fluorescens* alone (T2) recorded the minimum girth of 31. 98 cm and was found significantly inferior to T6. But T6 and T2 were statistically on par with control. All other treatments *viz.*, T9, T1, T7, T3, T8, T5 and T4 recorded a mean pseudostem girth of 36.65 cm, 35.35 cm, 34.53 cm, 34.40 cm, 34.05 cm, 33.77 cm and 33.10 cm respectively and were found statistically on par with T10. Application of *P. fluorescens* alone (T2) was considered to be the least effective treatment.

4.2.1.3. Number of Leaves

Total number of leaves produced one to five months after the application of different treatments are given in Table 3. Plants treated with *P.fluorescens* + *P. lilacinus* (T6), carbofuran (T9) and *P. fluorescens* (T2) produced higher number of leaves and were found to be statistically superior to seven other treatments. At five months after treatment, T6 produced 16.33 number of leaves and both T9 and T2 produced 15.99 number of leaves and these were found highly superior over control (T10). Number of leaves produced by T7 (15.89), T8 (15.89), T1 (15.66), T3 (15.66), T4 (15.23) and T5 (15.11) were found to be on par and T5 was found inferior over control and was reported as the least effective treatment.

4.2.2. Nematode Population

4.2.2.1. Nematode Population in Soil

The effect of different treatments on the population build up of root-knot nematodes in the rhizosphere of banana at the time of uprooting are presented in the Table 4. There was a drastic reduction with regard to the mean nematode population in treated plants. The mean population of nematodes ranged from 312.16 to 3697.90 in treated plots against 8892.00 in control. The best treatment was carbofuran (T9) with 312.16 nematodes in 250g soil giving about 96.49 per cent reduction in nematode population over control.

Table 4. Effect of different bioagents on nematode population in soil and roots

Treatments	Nematode population (Mean of three replications)			
	250g soil	Per cent decrease over control	20g root	Per cent decrease over control
T1 - AMF	3697. 90^{f} (3.57)	58.41	5292.20^{c} (3.72)	68.00
T2 - *P. fluorescens* (P.f)	931.11^{cd} (2.97)	89.53	1522.30^{b} (3.15)	90.80
T3 - *P. lilacinus* (P.l.)	731.05^{c} (2.86)	91.78	1290.90^{b} (3.10)	92.19
T4 - *B. subtilis* (B.s)	1388.20^{e} (3.14)	84.39	1685.00^{b} (3.23)	89.81
T5 - EPN	3675.80^{f} (3.56)	58.66	4465.30^{c} (3.64)	73.00
T6 - P.f. +P.l.	448.05^{b} (2.64)	94.96	564.59^{a} (2.72)	96.59
T7 - P.l.+ B.s.	1294.40^{de} (3.11)	85.44	1827.60^{b} (3.25)	88.95
T8 - B.s.+ P.f	1617.30^{e} (3.20)	81.81	1818.70^{b} (3.26)	89.00
T9 - carbofuran 3G	312.16^{a} (2.49)	96.49	364.16^{a} (2.56)	97.80
T10 -Untreated control	8892.00^{g} (3.95)	-	16538.00^{d} (4.22)	-

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

Next superior treatment was T6 ie. a combination of *P. fluorescens* and *P. lilacinus* (448.05) giving 94.96 per cent reduction over control. The mean number of nematodes and the per cent reduction over control in T3 (*P. lilacinus*) and T2 (*P. fluorescens*) were 731.05 (91.78) and 931.11(89.53) and were found to statistically on par with each other. The treatments, T7 (*P. lilacinus* + *B. subtilis*), T4 (*B. subtilis*) and T8 (*B. subtilis* + *P. fluorescens*) recorded a mean nematode population and per cent reduction of 1294.40 (85.44), 1388.20 (84.39) and 1617.30 (81.81) respectively. T1 (AMF) and T5 (EPN) were found to be inferior to all other treatments with mean population of nematodes 3697.90 and 3675.80 respectively, but were superior over control by 58.41 and 58.66 per cent respectively.

4.2.2.2. Nematode Population in Root

The effects of different treatments on nematode population in roots of banana are presented in Table 4. All the treatments were significantly superior to control. The mean number of nematodes ranged from 364.16 to 5292.20 per 20g of root in various treatments as against a high population of 16538.00 in control. The nematode population in root was also observed very low in T9 (364.16), which showed 97.80 per cent reduction over control. T6 (564.59) was ranked as the next best treatment giving 96.59 per cent reduction over control. T6 was found equally superior to T9 and this treatment was statistically on par with T9. The other treatments *viz.*, T3 (1290.90), T2 (1522.30), T4 (1685.00), T8 (1818.7) and T7 (1827.60) recorded 92.19, 90.80, 89.81, 89.00 and 88.95 per cent reduction in nematode population over control. The nematode population was high in T1 (5292.20) and T5 (4465.30) and were inferior to all other treatments , but were superior over control by 68.00 and 73.00 per cent respectively.

4.2.2.3 Root-knot Count

The data relating to root knot count revealed the effectiveness of various treatments in reducing gall formation in banana plants. All the treatments were

Table 5. Effect of different bioagents on root gall formation and root knot index (Mean of three replications)

Treatments	Number of root knots in 20g root	Per cent decrease over control	Root knot index (1-5 scale)
T1 - AMF	101.78[e]	9.59	4.45[e]
T2 - *P. fluorescens* (P.f)	66.33[c]	44.36	3.22[c]
T3 - *P. lilacinus* (P.l.)	49.00[b]	58.90	2.45[b]
T4 - *B. subtilis* (B.s)	84.00[d]	29.54	3.89[d]
T5 - EPN	101.33[e]	15.22	4.56[e]
T6 - P.f. +P.l.	16.11[a]	86.48	1.00[a]
T7 - P.l.+ B.s.	41.66[b]	65.06	2.11[b]
T8 - B.s.+ P.f	43.55[b]	63.47	2.11[b]
T9 - carbofuran 3G	16.66[a]	86.03	1.10[a]
T10 -Untreated control	119.22[f]	-	4.89[e]

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

significantly superior to control. The mean number of galls ranged from 16.11 to 101.78 per 20g of roots in various treatments. T6 (16.11) gave the maximum reduction in number of galls recording 86.48 per cent reduction over control. The next superior treatment was T9 (16.66) which was on par with T6 giving 86.03 per cent reduction in number of root galls over control. An increasing trend in number of galls was observed in T7 (41.66), T8 (43.55), T3 (49.00), T2 (66.33) and T4 (84.00) giving 65.06, 63.47, 58.90, 44.36 and 29.54 per cent respective reduction over control. T1 and T5 was found statistically inferior to all other treatments, but both were superior to T10 and produced 101.78 and 101.33 galls per 20 g of root respectively. (Plate 5).

4.2.2.4. Root-knot Index

Data regarding root knot index is presented in Table 5. Most superior treatment was T6 with a root knot index of 1.00. T9 was equally superior to T6 with a slightly lower root knot index of 1.10. T7 and T8 are found statistically on par with a root knot index of 2.11 which in turn was on par with T3 (2.45). The next best treatments were T2 and T4 with a root knot index of 3.22 and 3.89 respectively. Here also T5 (4.56) and T1 (4.45) were found inferior to all other treatments and were on par with T10 (4.89).

4.2.3 Biometric Characters of Banana at the Time of Uprooting

4.2.3.1 Fresh Weight of Whole Plant

Statistical analysis of data presented in Table 6 indicated that there was significant variation in the fresh weight of whole plant in different treatments. Fresh weight of whole plant varied from 11.19 kg to 5.44kg with a fresh weight of 5.13kg in control. All the treatments were found significantly superior to control (T10). The most superior treatment was T6 (11.19kg) which gave 118.13 per cent increase of fresh weight over control. The next superior treatment was T9 (9.67kg) which was on

Table 6. Effect of different bioagents on pseudostem, corm and root characters of banana (Mean of three replications)

Treatments	Fresh weight of total plant (kg)	Per cent increase over control	Fresh weight of corm (kg)	Per cent increase over control	Fresh weight of roots (kg)	Per cent increase over control
T1 - AMF	6.54^{c}	27.49	3.23^{abc}	35.71	0.68^{a}	126.67
T2 - *P. fluorescens* (P.f)	6.96^{bc}	35.67	2.60^{bc}	9.24	0.36^{b}	20.00
T3 - *P. lilacinus* (P.l.)	7.98^{bc}	55.56	3.14^{abc}	31.51	0.43^{b}	43.33
T4 - *B. subtilis* (B.s)	6.79^{bc}	32.36	2.33^{bc}	-2.10	0.43^{b}	43.33
T5 - EPN	5.44^{c}	6.04	2.20^{c}	-7.56	0.30^{b}	0.00
T6 - P.f. +P.l.	11.19^{a}	118.13	4.11^{a}	72.69	0.85^{a}	183.33
T7 - P.l.+ B.s.	5.83^{c}	13.65	2.28^{c}	-4.20	0.44^{b}	46.67
T8 - B.s.+ P.f	6.43^{c}	25.34	2.40^{bc}	0.84	0.41^{b}	36.67
T9 - carbofuran 3G	9.67^{ab}	88.49	3.65^{ab}	53.36	0.73^{a}	143.33
T10 -Untreated control	5.13^{c}	-	2.38^{bc}	-	0.30^{b}	-

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

T_1 - **AMF**

T_2 - *P. fluorescens*

T_3 - *P. lilacinus*

T_4 - *B. subtilis*

T_5 - **EPN**

Plate 4. Effect of different bioagents on root characters of banana

T_6- *P. fluorescens* + *P. lilacinus*

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

T_8- *B. subtilis* + *P. fluorescens*

T_9- carbofuran 3G

T_{10}- Untreated control

Plate 4. Effect of different bioagents on root characters of banana

par with T6 and gave 88.49 per cent increase over control. The next best treatments were T3 (7.98kg), T2(6.96kg) and T4 (6.79kg) which were found statistically on par giving 55.56, 35.67 and 32.36 per cent increase of fresh weight over control. It was observed that the treatments T1 (6.54kg), T8 (6.43kg), T7 (5.83kg) and T5 (5.44kg) gave 27.49, 25.34, 13.65 and 6.04 per cent increase of fresh weight of whole plant over control respectively. The treatment with entomopathogenic nematode, *Heterorhabditis indica* (T5) was found inferior to all other treatments.

4.2.3.2 Fresh Weight of Corm

Fresh weight of corm at the time of uprooting of banana under different treatments was given in Table 6. There was statistically significant variation in the fresh weight of corm in different treatments. Highest fresh weight of corm was observed in plants which received a treatment combination of *P. fluorescens* + *P. lilacinus* (4.11 kg) which gave 72.69 per cent increase of fresh weight over control (2.38 kg). The next best treatments were T9 (3.65 kg), T1 (3.23 kg) and T3 (3.14 kg) which were statistically on par with T6 and gave 53.36, 35.71 and 31.51 per cent increase in fresh weight of corm over control. T2 and T8 recorded a fresh weight 2.60 kg and 2.40 kg respectively and were found superior over control by 9.24 and 0.84 per cent. It was also observed that three other treatments *viz.*, T4 (2.33 kg), T7 (2.28 kg) and T5 (2.20 kg) were statistically inferior to control.

4.2.3.3 Fresh Weight of Root

Fresh weight of root of banana showed statistically significant variation in different treatments. Highest fresh weight of root was observed in T6 (0.85kg) giving 183.33 per cent increase over control and was the best treatment. This treatment was on par with T9 (0.73 kg) and T1 (0.68 kg) giving 143.33 and 126.67 per cent increase over control. The six other treatments like T7 (0.44 kg), T3 (0.43 kg), T4 (0.43 kg), T8 (0.41 kg), T2 (0.36 kg) and T5 (0.30 kg) were on par with control giving 46.67,

43.33, 43.33, 36.67, 20.00 and zero per cent increase over control respectively. The least superior treatment was T5.

Discussion

5. DISCUSSION

Plant parasitic nematodes are one of the important group of crop pests requiring concerted efforts on their management. During the last decade, there has been a reorientation of management strategies for nematodes with a shift towards eco-friendly and sustainable techniques. Research on biological control and its related aspects, as a component in management of phytoparasitic nematodes is relatively new and less explored, both in India and elsewhere in the world. In this context, an attempt was made to compare the efficacy of bioagents which are considered to be ecofriendly, with that of carbofuran, hitherto used pesticide for nematode management. The results of the present study entitled 'Biological control of root-knot nematode, *Meloidogyne incognita* (Kofoid and White, 1919) in banana, *Musa* (AAA) var. Robusta' are discussed in this chapter.

5.1 POT CULTURE EXPERIMENT

Much progress has not been made in the identification of an effective biocontrol agent for the management of root-knot nematode problems in banana in Kerala. From the intial studies seven nematophagous fungi *viz., Alternaria alternata, Drechslera tetramera, Trichoderma viride, Syncephalastrum racemosum, Curvularia lunata, Paecilomyces lilacinus* and *Beauveria bassiana* were identified as highly pathogenic to the cyst nematode, *Heterodera oryzicola* under in vitro condition (KAU, 1999). The efficiency of micro organisms *viz., T. viride* and *Pseudomonas fluorescens* for nematode management was studied under field conditions (Sheela *et al.,* 1999).

5.1.1 Biometric characters of banana

5.1.1.1 Height of the plant

The influence of different treatments on the height of banana plant was presented in Table 1 and Fig. 1. Among the different treatments studied plants treated with a combination of *P. fluorescens and P. lilacinus* (T6) recorded highest plant height of 131.22cm and it was considered as the best treatment over all other treatments. Increase in plant height may be due to the influence of certain plant hormones like indole acetic acid (IAA), gibberllin and cytokinin produced by the bacterium *P. fluorescens*. The growth promotion by *P. fluorescens* reported in various crops by many workers is in agreement with the present study. *P. fluorescens* is capable of surviving and colonizing in the rhizosphere of all field crops and is reported to promote plant growth by secreting auxins, gibberllins and cytokinins (Vidhyasekaran, 1988). The egg parasite, *P. lilacinus* also contributed to the improvement in height of the plant along with *P. fluorescens* by reducing the nematode population by parasitizing the nematode eggs. Similar observation was recorded in a study conducted by Simon and Pandey (2010), whose work revealed that maximum plant growth was observed in plants treated with *P. lilacinus* in combination with *Verticillium chlamydosporium.*

Carbofuran (T9) and *P. lilacinus* alone (T3) were reported as the next best treatments with a plant height of 126.55 cm and 121.66 cm respectively. The result obtained was in confirmation with that of Ravi *et al.* (2000). They revealed that a combined application of neem cake , *Trichoderma viride* and carbofuran was the most effective treatment in increasing the plant growth parameters and fruit yield and reducing the population of *M. incognita* and *Radopholus similis* in banana. Effectiveness of *P. lilacinus* and other antagonistic fungi against root-knot nematode, *M. incognita* on ground nut raised in pots has been reported by Vyas *et al.,* 1997. Similarly, Sivakumar *et al.* (1993) and Saikia and Roy (1994) have also reported the

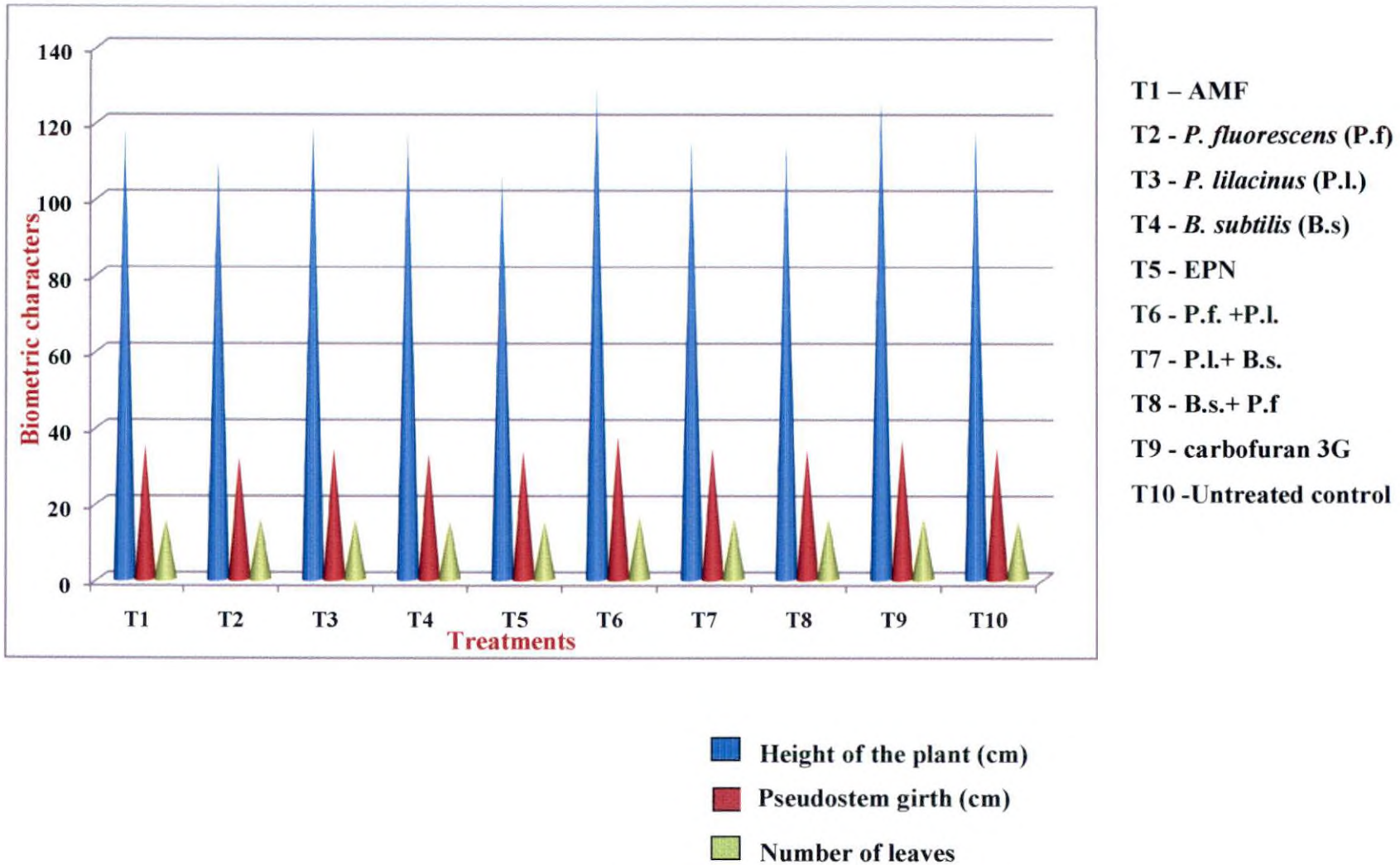

Fig. 1. Effect of different bioagents on biometric characters of banana (5 Months After Treatment)

efficacy of *P. lilacinus* against root-knot nematodes on vegetable crops, brinjal and okra.

5.1.1.2 Pseudostem girth

The effect of different treatments on the pseudostem girth of banana are presented in Table 2. Plants treated with *P. fluorescens* in combination with *P. lilacinus* (T6) were observed to be the most superior of all the other treatments with a highest pseudostem girth of 37.51cm. This observation clearly indicated that the combined application of *P. fluorescens* and *P. lilacinus* was highly effective in improving the plant growth parameters of banana. Sundararaju and Kiruthika (2009) reported the effect of *P. lilacinus*, neem cake and botanicals against root-knot nematode, *M. incognita* on banana var. Robusta. Among the treatments, the combined application of *P. lilacinus* + neem cake and *P. lilacinus* + *Tagetes erecta* (flower extracts) resulted in maximum increase of plant height, number of leaves, pseudostem girth, root length, number of healthy roots, root weight, root gall index and nematode population from soil and roots compared to nematode alone inoculated control plants.

Carbofuran (T9) was recorded as the next superior treatment followed by T6. The superiority of carbofuran in improving the plant growth parameters in brinjal was reported by Saikia *et al.* (2007). They found that plants treated with neem cake + carbofuran 3G showed significant effects on plant growth parameters and yield of brinjal and with corresponding decrease in the nematode population both in soil and roots. The efficacy of carbofuran in suppressing *M. incognita* activity and improving plant growth of french bean was already reported by Mohan and Mishra (1993). This was closely followed by Arbuscular Mycorrhizal Fungus (T_1). The result is in confirmation with those of Ray and Dalei (1998) and Sundarababu *et al.* (1996). The improvement in biometric characters due to the reduction in root-knot nematode and burrowing nematodes by the action of AMF in the root zone of various crops were

already reported by several workers (Rajani *et al.,* 1998; Koshy *et al.,* 1998; Sosamma *et al.,* 1998 in kacholam, coconut and banana respectively).

5.1.1.3 Number of leaves

Number of leaves recorded up to the time of uprooting of banana plants presented in Table 3 revealed that plants treated with *P. fluorescens* + *P. lilacinus* (T6), carbofuran (T9) and *P. fluorescens* (T2) produced more number of leaves giving 16.33, 15.99 and 15.99 respectively. The present study showed that these three treatments were highly effective in controlling root-knot nematode in banana. *P. fluorescens* produces certain growth hormones which enhances the growth and vigour of the plants and thereby increases the number of leaves. *P. fluorescens* and *Bacillus subtilis* were reported to induce systemic resistance (ISR) in banana against lesion nematode (Santhi and Rajendran, 2006). Similarly carbofuran has got phytotonic effect which enhances the plant growth parameters along with *P. fluorescens*. A pot culture experiment conducted by Bhagawati *et al.* (2009) to manage the disease complex caused by *M. incognita* and *Rhizoctonia solani* on okra using *P. fluorescens* and *T. harzianum* proved that both the bioagents were effective in reducing the damage and increasing the growth parameters of okra.

A combination of *P. fluorescens and P. lilacinus* was ascertained to be the most effective treatment in managing the root-knot nematode in banana compared to other treatments.

5.1.2 Nematode Population

The nematode population presented in Table 4, Fig. 2 and 3. indicated the influence of different treatments in reducing the number of nematodes in soil and roots. Plants treated with the chemical nematicide carbofuran (T9) recorded the highest reduction in the number of the nematodes in soil and roots giving 96.49 and 97.80 per cent reduction respectively over control. The lowest value for nematode

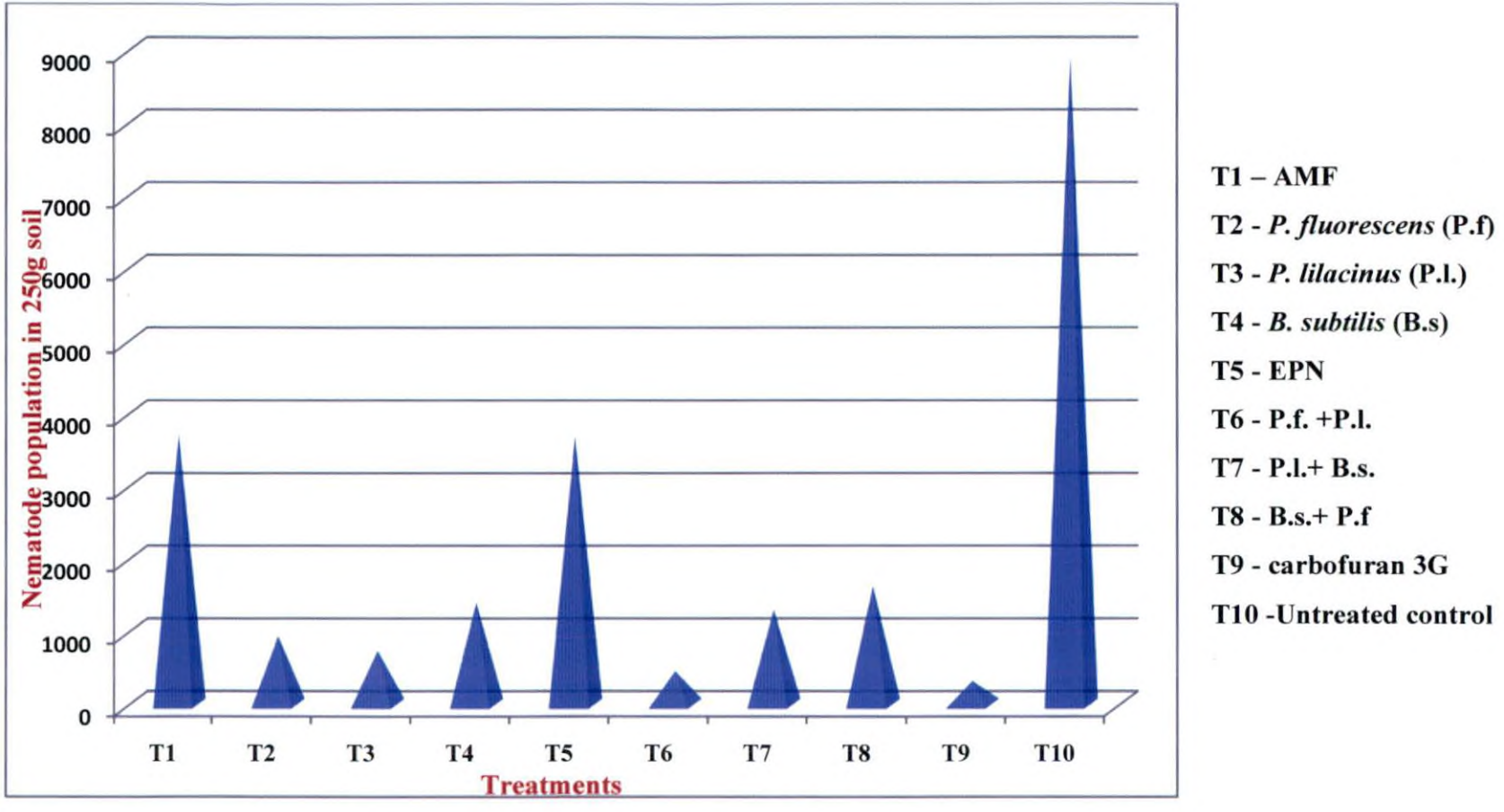

Fig. 2. Effect of different bioagents on nematode population in soil

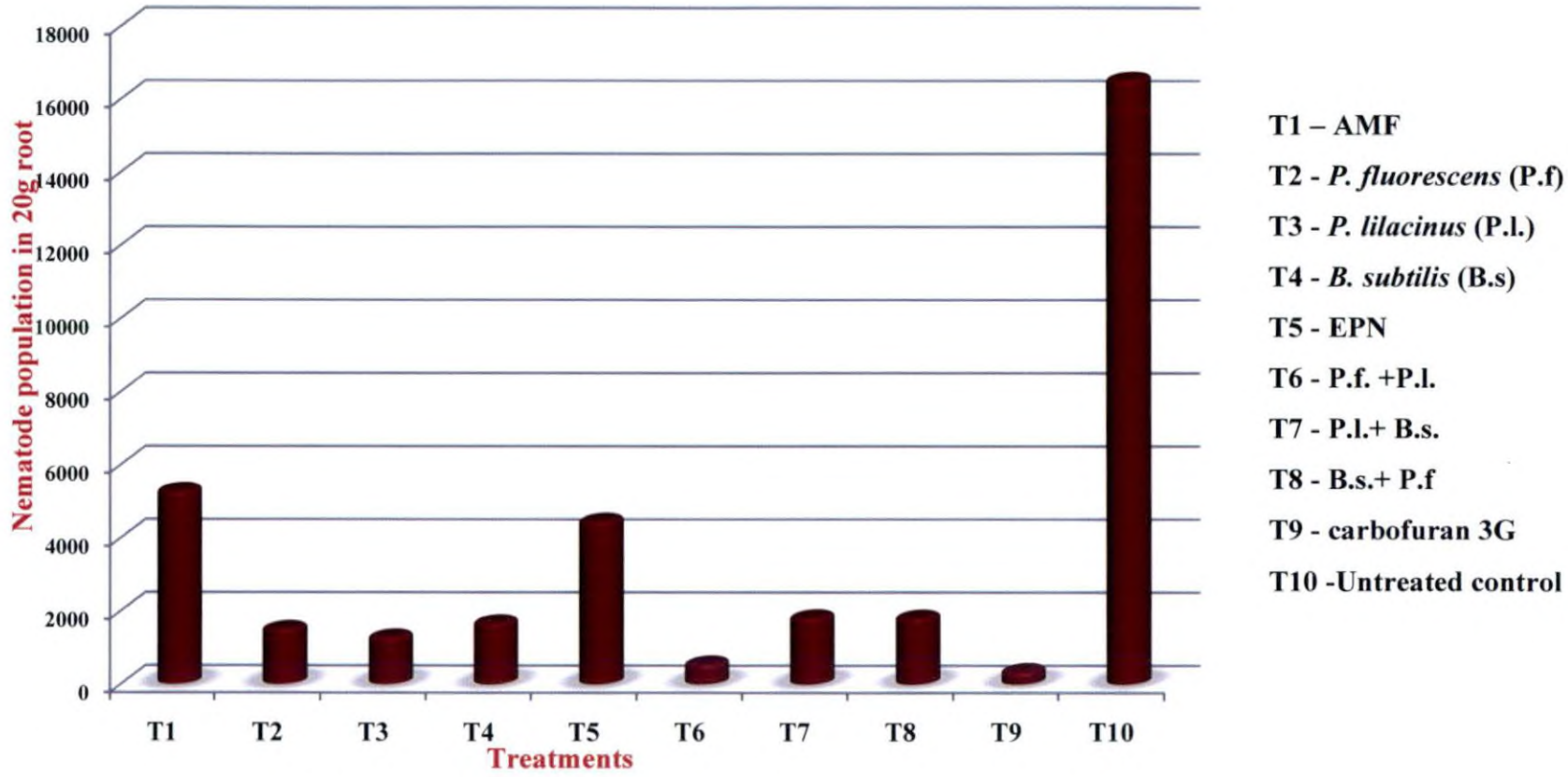

Fig. 3. Effect of different bioagents on nematode population in root

population in soil and roots of banana plants treated with carbofuran was 312.6 and 364.16. From the result, it is obvious that carbofuran can be considered as an important control measure for the management of root-knot nematode in banana (*Musa* AAA var. Robusta). This finding is in agreement with that of Haider *et al.* (1998) where the application of carbofuran @1 kg ai. per ha reduced root-knot nematode population in turmeric. Although carbofuran was effective in providing rapid kill of nematodes, its use has been questioned and banned in the recent years because of increasing concern about environmental contamination and human health risks. With an increase in general awareness of harmful effects of chemical pesticides and the changing public attitude towards environmental pollution, chemical nematicides are losing their popularity among farmers for protecting their crop from nematode infestations. So environmentally compatible and economically viable new strategies should be developed for nematode management. Biological control has shown to be an effective alternative that can be combined with other measures.

Among the different biocontrol agents, plants treated with a combination of *P. fluorescens and P. lilacinus* (T6) recorded the highest reduction in the number of nematodes in soil, root and gall formation, giving 94.96, 96.59 and 86.48 per cent reduction over control (T10). The lowest value for root-knot index (1.00) was also observed for T6. The reduction in gall number indicated an adverse effect on juvenile penetration. The potentiality of *P. fluorescens* as a biocontrol agent in reducing nematode population by rhizome treatment was already established by Nisha (2001). The mechanism responsible for the reduction on nematode population may be due to the ability of this bacteria to envelop and bind the root surface with carbohydrate-lectin, 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. The results of the present study agree with Krishnaveni

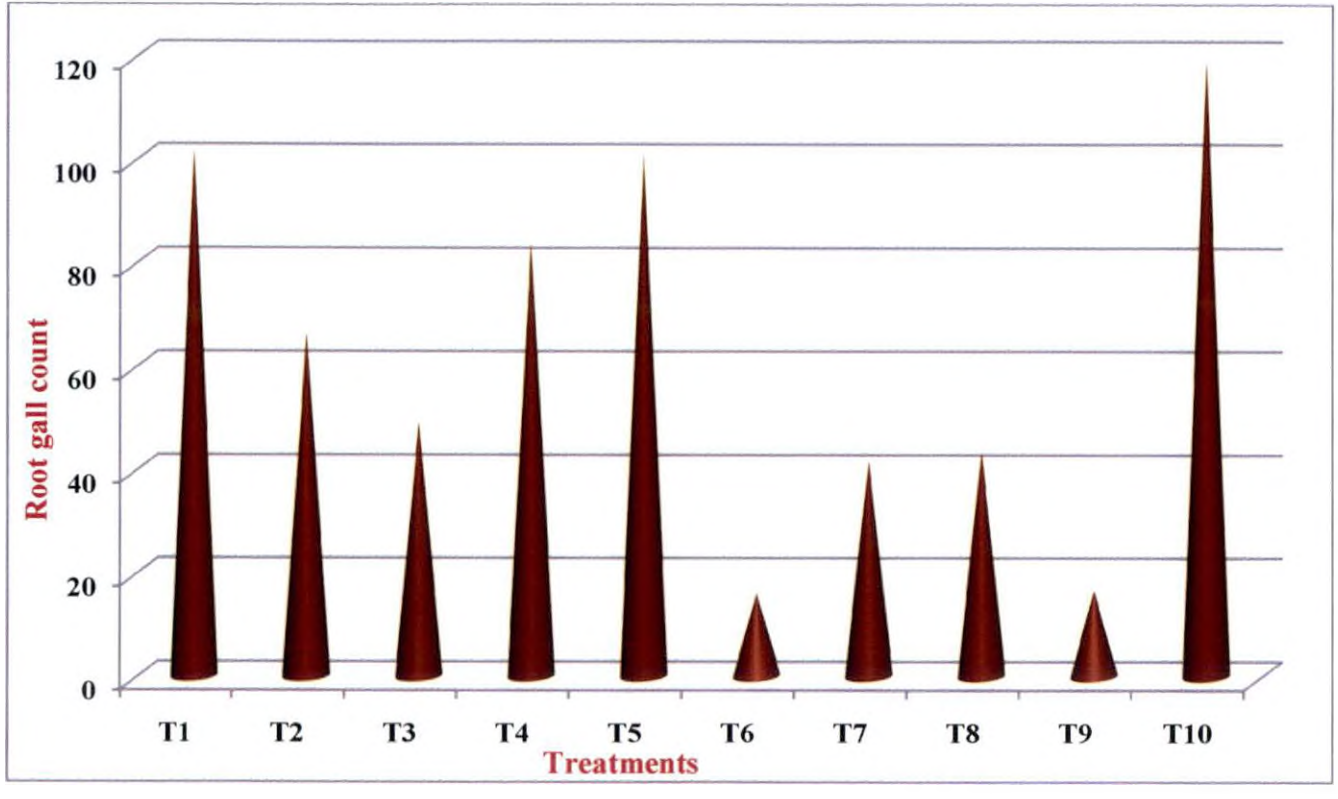

Fig. 4. Effect of different bioagents on root gall count

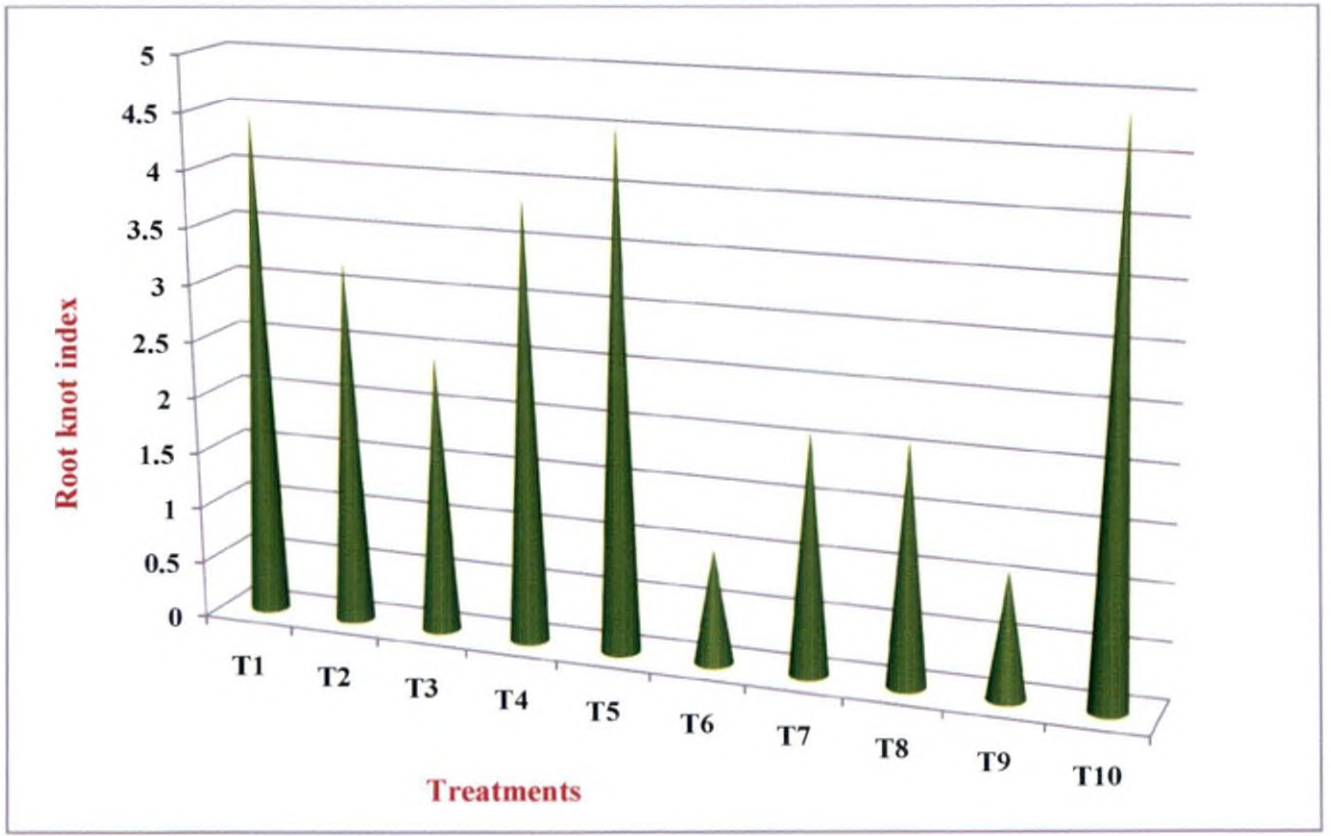

Fig. 5. Effect of different bioagents on root knot index

(2005) who observed the toxic effects of the native *P. fluorescens* isolate PfB 34, isolated from banana field against the spiral nematode, *Helicotylenchus multicinctus*. The toxic property of *P. fluorescens* culture filtrates had also been reported on the juveniles of *M. incognita* and *Heterodera cajani* (Gokte and Swarup, 1988).

A study conducted by Kalaiarasan *et al.* (2006) showed that a higher accumulation of peroxidase and PO1 isoform by Pf isolates might have collectively contributed towards induced resistance in groundnut plants against *M. arenaria*. The plant nematode interaction had also triggered the activities of defence enzymes when the nematode enters into the plant.

Simon and Pandey (2010) reported the antagonistic efficacy of *P. lilacinus* and *Verticillium chlamydosporium* against *M. incognita* infesting okra. Maximum reduction in root galling was recorded in plants treated with *P. lilacinus* in combination with *V. chlamydosporium*. These two bioagents are egg parasites of plant parasitic nematodes and hence they are more effective in reducing the nematode populations. Nematode eggs of the group *Heteroderidae* and those deposited in gelatinous matrix are more vulnerable to attack by these organisms than those of migratory parasites. Once in contact with cysts or egg masses, the fungus grows rapidly and eventually parasitizes all the eggs that are in the early embryonic developmental stages.

The next best treatment with regard to reduction in nematode population in soil and roots of banana was *P. lilacinus* alone (T3) giving 91.78 and 92.19 per cent reduction in nematode population over control respectively. Khan and Goswami (1999) reported the nematicidal action of culture filtrate of *P. lilacinus* on *M. incognita*. Priya and Kumar (2006) reported significant reduction in root galling where soil was treated with *P. lilacinus*. A replicated field trial was carried out by Patel *et al.* (1998) to manage *M. javanica* in groundnut cv. GG-2 during Kharif 1994 to 1997 and three years pooled data indicated that the lowest root knot index (1.33)

was recorded in *P.lilacinus* @ 50kg /ha treatment with significantly higher dry pod yield and fodder yield. Carbofuran and *P. lilacinus* at lower dosage (25 kg/ha) remained as the second best effective treatment.

There was statistically significant reduction in nematode population in all other treatments *viz.*, T7 (*P. lilacinus* + *B. subtilis)*, T8 (*B. subtilis* + *P. fluorescens)*, T4 (*B. subtilis*) and T2 *(P. fluorescens*) except T10 (control), T1(AMF) and T5 (EPN). The inhibitory effect shown by *B. subtilis* may be due to the larvicidal effect of this biocontrol agent as reported by Sheela (1990). The study revealed that at 1.2 × 10^8 cells per ml of these bacteria caused 70 to 80 per cent larval mortality of *H. oryzicola* in rice. *B. subtilis* colonizes the roots and expresses their antagonism to plant parasitic nematodes through the production of repellent metabolites.

Compared to other treatments, soil application of EPN (T5) and AMF(T1) was found to be least effective in managing the population of nematodes showing very high population of nematodes in soil and root. The root knot count and the root knot index of T1 and T5 was observed to be on par with control (T10) plants.

5.1.3 Biometric characters at the time of uprooting

5.1.3.1 Fresh weight of whole plant

The influence of different treatments in improving the biometric characters of banana in relation to fresh weight of total plant, fresh weight of corm and fresh weight of roots are presented in Table 8 and Fig. 6. Fresh weight of whole plant varied from 5.44 to 11.19 kg with a fresh weight of 5.13 kg in control. All the treatments were found significantly superior than the plants treated with nematode alone (T10). The treatment T6 was observed statistically superior to all other treatments with maximum fresh weight of whole plant giving 118.13 per cent increase over control. Prakob *et al.* (2009) proved that *P. aeruginosa, P. lilacinus* and *B. subtilis* were highly effective in improving the growth and reducing the gall

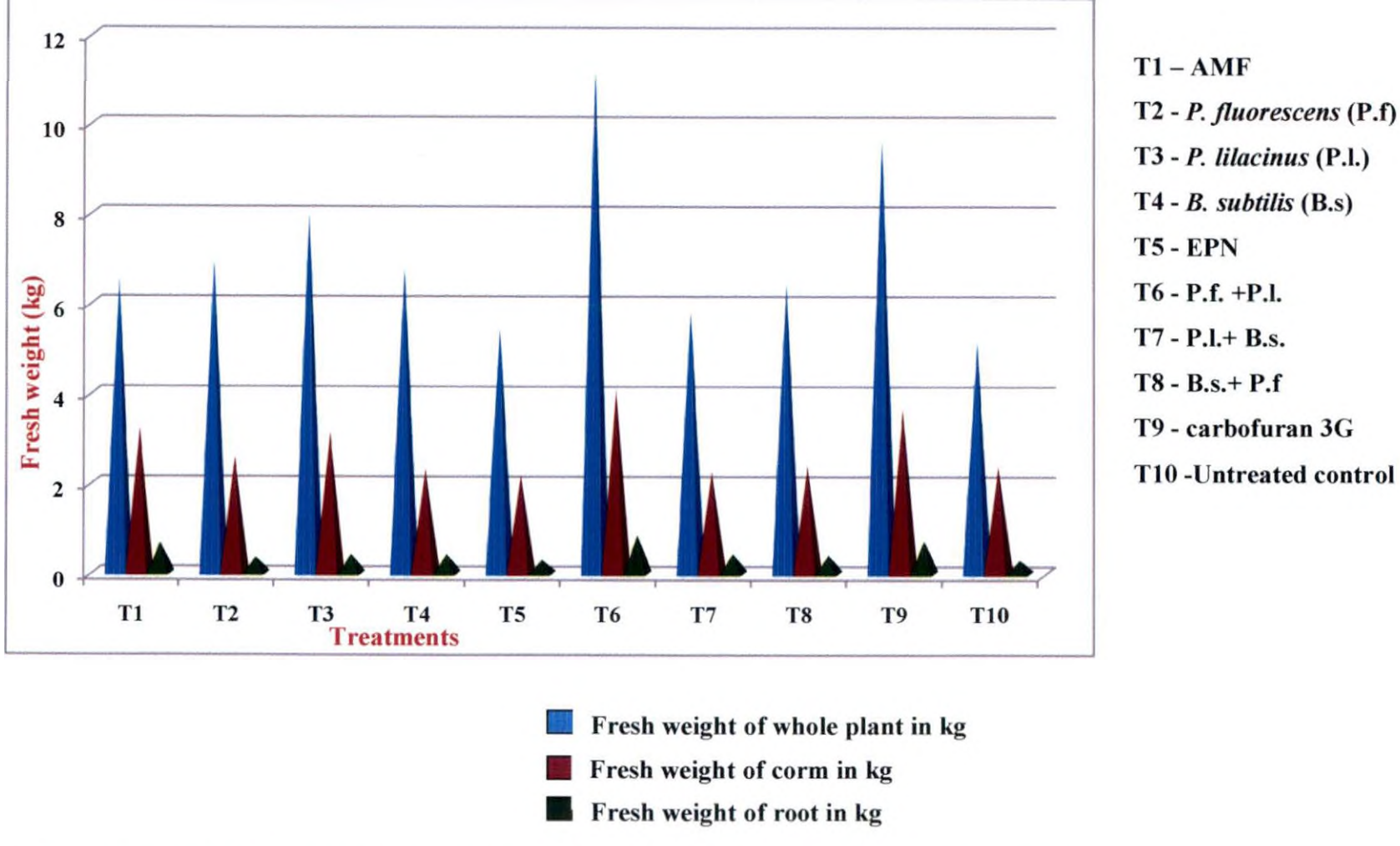

Fig. 6. Effect of different bioagents on fresh weight of whole plant, corm and root of banana

development of lettuce infected by root-knot nematode under green house and field environments. The results showed that the weight of lettuce planted in nematode infested soil containing these three tested organisms, was higher than those cultivated in nematode infested soil with no control agents. Carbofuran (T9) treated plants were equally superior to T6 in improving the growth parameters of banana by reducing root-knot nematode population. Singh and Kumar (1995) concluded that carbofuran 2 kg ai. per ha was effective in reducing the population of *M. incognita* and increasing the growth parameters like root length, shoot dry weight, root fresh weight and number of leaves of Japanese mint.

5.1.3.2 Fresh weight of corm

Fresh weight of corm at the time of uprooting of banana under different treatments was given in Table 6. Here also the treatment T6 was recorded as the best treatment giving the highest corm weight of 4.11kg with 72.69 per cent increase of fresh weight of corm over control (T10). The plants treated with EPN (T5) was recorded as the least effective treatment in managing root-knot nematode, *M. incognita* in banana. The non significant effect of EPN against root-knot nematode as observed in the present study is in conformity with the findings of Grewal *et al.* (1999). They found that there is no suppression of plant parasitic nematodes by living entomopathogenic nematodes, but the application of dead *Steinernema feltiae* and *S. riobrave* temporarily suppressed root penetration by *M. incognita*.

5.1.3.3 Fresh weight of roots

Out of the ten treatments, the combined application of *P. fluorescens and P. lilacinus* (T6) recorded the highest fresh weight of root (0.85 kg). It is evident that soil application of *P. fluorescens* and *P.lilacinus* together could effectively improve the root characteristics of banana. The present finding is in conformity with that of Devi and Dutta (2002) on okra. They found that *P. fluorescens* improved shoot and root length and weight, and reduced root gall number in okra. Carbofuran (0.73kg)

and AMF (0.68kg) were the next superior treatments in improving the root weight giving 143.33 and 126.67 per cent increase over control. Similar results were reported by Elsen *et al.* (2003) where they found that the decreased branching caused by nematodes were counter balanced by the increased branching caused by the AMF. The VAM colonization of roots mechanically may prevent nematode penetration and establishment, increased nutritional status, increased sugar content of roots (Smith, 1987), increased levels of P in roots or increased host tolerance. Although most of the research conducted under green house conditions indicated that mycorrhizal fungi have the potential to nullify the damage due to phytonematodes, but intensive investigations need to be carried out under field conditions to assess their potential utilization as biocontrol agents. Since VAM fungi are obligate parasites, they cannot be cultured on large scale which hinders their effective utilization in integrated nematode management programmes under field conditions.The effect of carbofuran in enhancing the root length and weight was already proved by Singh and Kumar (1995). They found that carbofuran 2kg ai. per ha was effective in reducing the the population of *M. incognita* and increasing the growth parameters like root length, shoot dry weight, root fresh weight and number of leaves in Japanese mint.

To sum up the findings, the present study revealed that the biocontrol agents namely *P. fluorescens*, *P. lilacinus* and *B. subtilis* showed very promising effects in improving the biometric characters as well as reducing the nematode population in root and soil, gall formation and root-knot index. Among these biocontrol agents, combined application of *P. fluorescens* and *P. lilacinus* in soil was found to be the most effective in increasing the plant growth parameters and thereby managing root-knot nematode, *M. incognita* in banana. In our investigation, these biocontrol agents are found equally effective as carbofuran, the commonly used pesticide for nematode management. So the application of carbofuran, can be very well replaced by the biocontrol agents and more over they are ecofriendly and safe too. However, soil application of entomopathogenic nematodes and AMF was not effective in reducing

the nematode population in soil and also the entry of nematodes into root compared to other treatments in banana. As *P. fluorescens* and *P. lilacinus* can be easily mass produced, it appears very promising in the management of root-knot nematode and hence it can be recommended as bioagents for consideration in an integrated management programme of *M. incognita* in banana.

Summary

6. SUMMARY

The study entitled 'Biological control of root-knot nematode, *Meloidogyne incognita* (Kofoid and White, 1919) in banana, *Musa* (AAA) var. Robusta' was carried out in the Department of Agricultural Entomology, College of Horticulture, Vellanikkara and Banana Research Station, Kannara during April 2010 to June 2011. The objective of the study included the identification of an effective biocontrol agent for the management of root-knot nematode in banana.

The soil and root samples were collected from banana plots already infested with root-knot nematode at the Banana Research Station, Kannara. The white females were collected from root-knot infested banana roots and the species of root-knot nematode was identified and confirmed as *M. incognita* by the perineal pattern of the white females. Weed plants seen in banana plots were also examined for root-knot infection and *Ageratum conyzoides* and *Vernonia cineria* were identified as the important alternate weed hosts of root-knot nematode.

Pot culture experiment was conducted to study the effect of different biocontrol agents *viz.*, Arbuscular Mycorrhizal Fungi (AMF), *Pseudomonas fluorescens*, *Paecilomyces lilacinus*, *Bacillus subtilis* and EPN (*Heterorhabditis indica*) alone and in different combinations with carbofuran as the standard nematicide check for the management of root-knot nematode in banana. The effect of the treatments on the biometric characters of banana *viz.*, height of the plant, girth of pseudostem and number of leaves were observed at monthly intervals. When the plants were about to form bunches (seven months after planting) tissue culture banana plants were uprooted and the effect of different treatments on various parameters

viz., fresh weight of the whole plant, fresh weight of the corm, fresh weight of the roots, gall formation on roots and nematode population in soil and roots were recorded.

The combined application of *P. fluorescens and P. lilacinus* resulted in an enhancement of biometric characters of banana like height of plant, girth of pseudostem and number of leaves. This treatment combination was found to be equally good as the most commonly used chemical nematicide, carbofuran. *P. lilacinus* and AMF were also found effective in reducing the impact of nematode attack on biometric characters of banana. Application of EPN (*H. indica*) was found to have least effect on height of the plant and number of leaves whereas *P. fluorescens* application alone was noticed as the inferior treatment regarding the pseudostem girth of banana.

All the treatments suppressed root-knot nematode population compared to control, though the efficacy regime was not alike. The curative application of carbofuran and biocontrol agents *viz.*, *P. fluorescens* + *P. lilacinus, P. liacinus, P. fluorescens* and *B. subtilis*, produced a soil condition capable enough to suppress the further population build up of nematodes in soil and root and lowered the infection to a tolerable level. Maximum reduction in nematode population was noticed in carbofuran treated plants. This was closely followed by *P. fluorescens* + *P. lilacinus* treatment and was found to be equally superior to carbofuran application in reducing the nematode population in soil and roots. Application of AMF and EPN were regarded as the least effective treatments and recorded maximum nematode population both in soil and roots. Highest reduction in root knot index and minimum gall formation was observed in plants treated with *P. fluorescens* + *P. lilacinus.* Application of carbofuran, *P. lilacinus* + *B. subtilis, B. subtilis* + *P. fluorescens* and *P. lilacinus* also showed significantly low values for root knot count and root knot index.

Among the different treatments studied, the combined application of *P. fluorescens* and *P. lilacinus* showed highest values for fresh weight of whole plant, corm and roots. Soil application of carbofuran, *P. lilacinus, P.fluorescens* and AMF also resulted in an improvement in the above characters.

The present study indicated that the combined application of *P. fluorescens* and *P. lilacinus* produced a microenvironment most favourable for plant growth and at the same time suppressing nematode population. Even though all the treatments were effective in nematode management compared to control, application of bioagents *viz.*, *P. fluorescens* + *P. lilacinus, P. lilacinus, B. subtilis* and *P. fluorescens* were most effective than other treatments.

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*Originals not seen

BIOLOGICAL CONTROL OF ROOT-KNOT NEMATODE, *Meloidogyne incognita* (Kofoid and White, 1919) IN BANANA, *Musa* (AAA) Var. ROBUSTA

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ABSTRACT OF THE THESIS

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ABSTRACT

A study entitled 'Biological control of root-knot nematode, *Meloidogyne incognita* (Kofoid and White, 1919) in banana, *Musa* (AAA) var. Robusta' was carried out in the Department of Agricultural Entomology, College of Horticulture, Vellanikkara and Banana Research Station, Kannara during April 2010 to June 2011. The objective of the study was to identify an effective biocontrol agent for the management of root-knot nematode in banana.

The species of root-knot nematode was identified and confirmed as *M. incognita* by the perineal pattern of the white females collected from the infested roots of banana plants from BRS, Kannara.

Pot culture experiment was conducted to study the effect of different biocontrol agents *viz.*, Arbuscular Mycorrhizal Fungi (AMF), *Pseudomonas fluorescens*, *Paecilomyces lilacinus*, *Bacillus subtilis* and *Heterorhabditis indica* (EPN) alone and in different combinations in comparison with the commonly used chemical nematicide, carbofuran on the management of root-knot nematode in banana. The effect of the treatments on the biometric characters of banana *viz.*, height of the plant, girth of pseudostem and number of leaves were observed at monthly intervals. When the plants were about to form bunches (seven months after planting) these plants were uprooted and the effects of different treatments on various parameters *viz.*, fresh weight of the whole plant, corm, roots, gall formation on roots and the nematode population in soil and roots were recorded.

Among the various treatments tried, the combined application of *P. fluorescens and P. lilacinus* was found to be very effective in enhancing the biometric characters of banana which was on par with that of carbofuran, followed by *P. lilacinus* and AMF when treated alone, whereas EPN was found to be the least effective one

With regard to nematode population in soil and roots, though carbofuran was found to be the best treatment, this was closely followed by *P. fluorescens* and *P. lilacinus* treatment. Same trend was noticed in the case of root knot index, gall formation, fresh weight of whole plant, corm and roots. Application of AMF and EPN were observed as the least effective treatments and recorded maximum nematode population both in soil and roots. .

Considering the above results, the present study indicated that the combined application of *P. fluorescens* and *P. lilacinus* was found to be the most effective substitute for the chemical nematicide carbofuran for the management of root-knot nematode in banana.