HOST PARASITE RELATIONSHIP AND MANAGEMENT OF IMPORTANT NEMATODES ASSOCIATED WITH CHETHIKODUVELI, *Plumbago rosea* L.

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Dedicated to my

Beloved Parents, Brothers,

my Wife & Children

DECLARATION

I hereby declare that this thesis entitled **"Host parasite relationship and management of important nematodes associated with chethikoduveli, *Plumbago rosea* L."** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar title, of any other university or society.

Vellayani,
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CERTIFICATE

Certified that this thesis entitled **"Host parasite relationship and management of important nematodes associated with chethikoduveli, *Plumbago rosea* L."** is a record of research work done independently by Mr. Santhosh Kumar T. (99-21-12) under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to him.

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Introduction

1. INTRODUCTION

Plants have been used as medicinal agents from the earliest days of man's existence. The ancient Indian system of medicine is predominantly a plant based materia medica, making use of our native plants. It caters to almost the entire rural population of our country. A perusal of literature showed that Indian medicinal plants attracted the attention of various scholars both from within the country and abroad.

Indian plants have always provided a base for upgradation and synthesis of biologically active drugs. Several isolates from plant parts are now utilized as effective medicines against cancer, cardiac diseases, leucoderma, liver cirrhosis, blood pressure etc. During the last decade there has been an ever increasing demand from the developed countries for more and more therapeutically active alkaloids *viz*., quinones, steroids, glycocides and terpenoid derivatives. This has necessitated the cultivation of important medicinal plants on commercial scale and development of knowledge in the plant protection aspects.

'Chethikoduveli', *Plumbago rosea* L. belonging to the family Plumbaginaceae is a highly valued perennial shrub distributed throughout the plains of India. Its main use is in chemotherapeutics. The roots of this plant contain an acrid crystalline principle called 'plumbagin' 2-methyl 4 hydroxy 1-4 napthoquinone which is extensively used in the treatment of the skin disease leucoderma, as an abortifacient and for lowering blood pressure. It has also antifungal, antimicrobial and anticancerous properties. Apart from its medicinal and antimicrobial properties, plumbagin can also be used as preservative for non alcoholic drinks and vine.

The commercial cultivation of this crop is gaining importance owing to its varied uses and amenable to cultivation in tropics. Even though several such properties are attributed to this medicinal plant they are prone to attack by several fungal leaf diseases and nematode pests. The occurrence and distribution of *Radopholus similis* (Cobb, 1893) Thorne, 1949 and *Meloidogyne incognita* (Kofoid and White, 1919) Chittwood, 1949 on this crop were already reported from Kerala (KAU, 1993). These nematode pests were found to be a major problem for the commercial cultivation of the crop and is found to cause considerable yield loss. Since tubers are the economically and medically important plant part of this crop, the feeding by nematodes on these underground plant parts directly affect the quality and quantity of the produce.

Studies on the pathogenicity due to *M. incognita* and *R. similis*, two major nematode pests infesting this economically important medicinal plant extensively grown all over the state of Kerala will give basic information for developing effective and economic management strategy. Nematode being an underground plant part feeder, the information regarding the histopathological and biochemical changes will be very useful for assessing the quality and quantity of the produce (Pharmacological preparation). In *P. rosea* the tubers are directly utilized for the pharmacological preparations. Hence a non chemical ecofriendly nematode management is very much essential for keeping the quality of the produce. The available literature on 'Chethikoduveli' does not reveal any information regarding these aspects and hence the study was taken up with the following objectives :

1) To workout the pathogenicity and crop loss due to root-knot nematode (*M. incognita*) and burrowing nematode (*R. similis*) in Chethikoduveli

2) To study the histopathological and biochemical changes caused by these nematodes

3) To evolve a suitable agro-ecofriendly integrated management strategy for controlling these nematodes using botanical pesticides / plant products, bioagents and organic amendments.

Review of Literature

2. REVIEW OF LITERATURE

Literature pertaining to the pathogenicity, crop loss, histopathological and biochemical changes caused by root-knot nematode *Meloidogyne incognita* (Kofoid and White, 1919) Chittwood, 1949 and *Radopholus similis* (Cobb, 1893) Thorne 1949 on Chethikoduveli *Plumbago rosea* L. were presented here. The relevant literature on crop loss incurred by these nematodes under field conditions and their management is also included. The relevant literature pertaining to medicinal plants was scanty, hence some related plants, these aspects were reviewed and presented here.

2.1 PATHOGENICITY

2.1.1 *M. incognita*

The pathogenicity studies of root-knot nematode in various host plants were conducted by several workers. Charles (1978) studied the pathogenicity of *M. incognita* in ginger var. Rio-de-Janeiro and found that the extent of damage done by the nematode progressively increased with graded inoculum levels of 10, 100, 500, 1000 and 5000 larvae per plant. Significant reduction in the growth characteristics and yield were recorded at an initial inoculum level of 500 larvae onwards. Routaray *et al*. (1987) reported that in ginger significant pathogenic effects due to *M. incognita* were noticeable at 1-10 nematode/gram of soil onwards and the fibrous roots were very much reduced at the highest inoculum level.

Prasad and Reddy (1984) studied the pathogenic effect of *M. incognita* in Patchouli *Pogostemon cablin* at inoculum levels of 100, 1000 and 10000 nematodes / plant under pot culture conditions and reported that at 10000 level there was significant reduction in root (27.9 per cent) and shoot weight (15.97 per cent).

Pathogenicity of *M. incognita* on garlic revealed that increasing population has a positive correlation with reduction in plant growth (Midha and Trivedi, 1988).

Eapen (1994) studied the pathogenic effect of *M. incognita* on small cardamom, *Elettaria cardamomum* with logarithmic series of five initial densities (P_1) (0 to 400 nematodes / 100 cm^3 soil) and reported that maximum growth suppression and yield loss (46 %) were noticed at Pi = 4 nematodes / 100 cm^3 soil followed by Pi = 6/100 cm^3 soil.

Mohanty and Das (1994) conducted studies on the effect of *M. incognita* on tube rose plant var. Single using different inoculum levels of 0, 10, 100, 1000, 5000, 10,000 and 20,000 nematodes per plant. They found significant reduction in plant growth characters over control plants at an initial inoculum level of 100 nematodes onwards. In betel plants infestation of 1000 larvae of *M. incognita* per plant significantly reduced the chewable leaves (Nalinakumari *et al*., 1995).

Singh and Nath (1996) found that in papaya, an initial population of 1000 *M. incognita* per 500 g sandy loam soil was pathogenic. An initial inoculum level of 100 juveniles of *M. incognita* per plant caused significant reduction in fresh and dry fibrous root weight and tuber yield of *Dioscorea rotundata* (Mohandas and Ramakrishnan, 1997).

Kumar and Singh (1997) studied the influence of different initial densities (0, 10, 100, 1000 and 10000 J_2 per pot) on the growth of *Mentha arvensis* cv. Shivalik under pot culture condition and found that there was progressive decrease in length and weight of shoot and root with the increase in the initial nematode inoculum. Pathogenicity studies of root-knot nematode *M. incognita* associated with three medicinal plants *viz*., *Ammi visnaga, Costus speciosus* and *Solanum indicum* revealed that growth parameters of the plants were inversely correlated with initial population densities of *M. incognita* (Pandey and Haseeb, 1997).

Haider *et al*. (1998) reported that an initial inoculum level of 100 J_2 per plant was pathogenic and cause considerable yield reduction in turmeric.

Sharma *et al*. (1999) reported that an initial inoculum level of 1000 and 10000 J_2 significantly reduced plant growth and yield in groundnut. Haseeb *et al*. (1999) studied the effect of different inoculum (500, 1000, 2000, 4000, 8000, 16000) levels of *M. incognita* on growth, physiology and oil yield of *Ocimum sanctum* L. and reported that there was an inversely proportional relation with initial inoculum densities and growth and oil yield, total chlorophyll, sugar, phenol and oil content. Significant reduction in growth, physiological characters and yield was recorded from lowest inoculum level onwards.

Significant plant growth reduction over control was observed with an initial population of 1000 nematodes per 500 g soil which was established as potential pathogenic level of *M. incognita* in cowpea (Singh and Goswami, 2000).

According to Kumar (2000) plant growth and flower yield of tube rose was significantly reduced at an initial inoculum level of 10 J_2 / g of soil.

Khan (2003a) reported that plant growth of balsam (*Impatiens balsamina* L.) was significantly reduced at 1000 J_2 of *M. incognita*/kg of soil. Effect of different inoculum levels of *M. incognita* Race 2 on onion (*Allium cepa*) revealed that 10 juveniles/500 cc soil could cause significant reduction in weight of onion bulbs (Khan, 2003b).

2.1.2 *R. similis*

The review pertaining to *R. similis* on medicinal plants was scanty and hence effect of *R. similis* on other host plants were reviewed here.

Venkitesan and Sethi (1977) studied the pathogenicity of *R. similis* to black pepper (*Piper nigrum*) with four initial inoculum levels of 10,

100, 1000 and 10000 on 55 days old rooted cuttings and reported that there was decline in growth of plants with an increase in inoculum level.

Sundararaju *et al*. (1979) reported that reduced plant growth, intensity of root lesions and rotting were directly proportional to the increase in nematode population in ginger. Significant reduction was recorded in root and rhizome weight as well as in the length of root and shoot and there was 73.6 per cent reduction in the rhizome weight at an initial inoculum level of 10,000 nematodes over a period of six months.

According to Sosamma *et al*. (1979) an initial inoculum level of ten nematodes caused 35 per cent reduction of rhizome weight of turmeric after four months and 46 per cent at the end of the season (8 months).

Jasy and Koshy (1992a) studied the pathogenicity of *R. similis* on avocado with different inoculum levels 0, 10, 100, 1000 and 10000 per plant in earthen pots and found that an initial inoculum density of 10,000 nematodes per plant caused 10, 10, 17, 46 and 62 per cent reduction over control in length and weight of shoot, number of leaves, weight and volume of root respectively over a period of four months. The pathogenic threshold level of *R. similis* was 100 nematodes per plant or one nematode in 12 cm^3 of sandy loam soil for root growth characters.

2.2 HISTOPATHOLOGY

2.2.1 *M. incognita*

Entry of nematode into the host tissue brings about several histomorphological changes. Second stage juveniles of root-knot nematode penetrate the root epidermis and make their way through the root cortex to the infection site (Shetty and Rudramuniyappa, 1992).

Feeding site of nematode was found as the vascular region. Histopathological changes due to *M. incognita* infection is characterised by hypertrophy and hyperplasia (Hasan and Jain, 1985). Characteristic giant cell formation in susceptible hosts has been reported by many

workers (Siddiqui *et al*., 1974; Jacob, 1977; Shah and Raju, 1977; Sudha and Prabhoo, 1983; Molina and Nelson, 1983; Pasha *et al*., 1987; Fawole, 1988; Sosamma, 1988; Shetty and Rudra Muniyappa, 1992; Das and Barman, 1995).

There is controversy regarding the tissue from which giant cells originate. Siddiqui *et al*. (1974) reported that the giant cells are produced from the phloem or the interfasicular region while some others reported giant cell formation from the xylem (Jacob, 1977; Shah and Raju, 1977; Charles, 1978; Fawole, 1988). Molina and Nelson (1983) found giant cell formation from xylem and phloem. According to Shetty and Rudramuniyappa (1992), giant cells originate from the phloem and ray parenchyma.

The number of giant cells produced as a result of *M. incognita* infection vary in different crops. The number of giant cells formed was four to six in pepper (Jacob, 1977), two to six in brinjal (Pasha *et al*., 1987), four in *Coleus parviflorus* (Sosamma, 1988) and seven in greengram var. AAU-34 (Das and Barman, 1995). The giant cells are multinucleate (Molina and Nelson, 1983; Sudha and Prabhoo, 1983; Pasha *et al*., 1987; Fawole, 1988; Das and Barman, 1995) polygonal or roughly quadrangular in shape (Sosamma, 1988) and thick walled with dense cytoplasm (Pasha *et al*., 1987; Das and Barman, 1995). Fawole (1988) observed thin walled giant cells in white yam, *Dioscorea rotundata* tubers. Rajani (1998) studied the histopathology of Kacholam roots and observed similar changes.

Abnormal xylem elements of variable size and shape have been reported by Siddiqui *et al*., 1974; Pasha *et al*., 1987; and Shetty and Rudramuniyappa, 1992. Siddiqui and Ghouse (1975) found that roots of *Lagenaria leucantha* when infested with *M. incognita*, at the early stage of infestation phloem first partially and later completely got destroyed due to pressure of accumulating piles of undifferentiated tissues in the induced

cambial zone. Advanced stage of infection revealed that after the destruction of normal phloem, new phloem developed out of the outer derivatives of the newly formed mass of cells. Charles (1978) observed that in ginger the affected portion of the root get decayed and pre-disposed the roots for attack by other micro organisms. Necrosis of the roots due to *M. incognita* has been reported by Pasha *et al*. (1987) and Sosamma (1988).

2.2.2 *R. similis*

Sundararaju and Koshy (1988) reported that longitudinal burrows developed underneath the outer cortical cell layer and nematodes and eggs could be located here in arecanut roots. Nematodes were also seen both inter and intracellular portions although intercellular orientation was more common. Necrotic changes occurred around the head of the nematodes and the burrows harbouring them. The nematode feeding disintegrated the cytoplasm and cell wall of the host and coalescence of these led to the formation of cavities or burrows in which the nematodes bred and multiplied. According to Venkitesan and Sethi (1977) the nematode penetrated the roots within 24 hours producing dark brown lesions within 72 hours of inoculation. They were observed to feed on cortical parenchyma cells.

2.3 BIOCHEMICAL CHANGES

2.3.1 Phenol

Sing *et al*. (1978) reported an increase in phenol content in infected roots of brinjal compared to their healthy control plants. But there was reduction in total phenolic content in shoots and roots of rice with infestation of rice root nematode *Hirschmaniella oryzae* (Jayaprakas *et al*., 1981).

Thakar and Yadad (1991) reported that high total phenol content was present in the reniform nematode resistant varieties of pigeon pea.

Pankaj *et al*. (1992) reported that there was high total phenol content in the shoots of healthy and resistant barley cultivars compared to the susceptible cultivars and in the presence of nematodes the phenol content in resistant cultivars significantly increased compared to the susceptible.

Sharma and Trivedi (1996) reported that there was an increase in the phenol content in susceptible bhindi varieties (Pusa Sawani).

Haseeb *et al*. (1999) reported that there was a decreasing trend in total phenols as the initial inoculum level increased in Tulsi, *Ocimum sactum* infected with *M. incognita*.

Naidu *et al*. (2000) reported that there was high phenolic content in *Tylenchorynchus brevilineatus* resistant groundnut varieties.

Devarajan and Rajendran (2002) reported higher quantity of phenol in roots of banana inoculated with *R. similis* and the accumulation of phenols increased with infestation by *R. similis*.

2.3.2 Amino Acids

Singh *et al*. (1978) reported an increase in amino acid content in roots of brinjal infected with *M. incognita*.

Chromatographic analysis of free amino acids of blackgram (variety –T_9) root infected with *M. incognita* revealed that L-glutamic acid was absent, but 4 more amino acids *viz*., L-cystine, L-lysine, L-proline and L-leucine were present (Sahu and Mohanty, 1987).

Mohanthy and Pradhan (1989) studied the quantitative estimation of free amino acids and amides in resistant and susceptible greengram varieties inoculated with *M. incognita*. Nine amino acids *viz*., L-cystine, L-arginine, L-histidine, L-lysine, L-serine, L-tyrosine, L-Tryptophan, L-aspartic acid and L-isoleucine were found to be common in the cultivars Pusa Baisakhi and K 851 to both healthy and inoculated root extracts except L-arginine which was not detected in inoculated samples of Pusa Baisakhi. In addition to this, the inoculated roots of both the cultivars contained 4 amino

acids and one amide such as L-threonine, L-proline, L-valine, L-leucine and L-glutamine.

Swain and Prasad (1991) studied the biochemical changes in rice due to *M. incognita* and the results indicated that the amino acids alanine, aspartic acid, serine and methionine increased in inoculated plants over the uninoculated ones and also in resistant varieties than in susceptible ones. In the uninoculated plants, alanine and aspartic acids were higher in resistant varieties than the susceptible but serine and methionine content were higher in susceptible variety than the resistant ones.

Sharma and Trivedi (1996) reported an increase in total free amino acids in *M. incognita* infested roots of bhindi cultivars over their healthy counter parts.

Biochemical alternations in cowpea (Pusa Kamal) roots infected by reniform nematode, *Rotylenchulus reniformis* were determined by Mohanty *et al*. (1999). Among various amino acids detected, ten were common in both healthy and infected plants and four amino acids, L-glutamine, L-serine, L-proline and L-Phenyl alanine were present only in infested cowpea, whereas L-asparagine and L-tyrosine disappeared.

As a result of nematode infection, the quantity of most amino acids and amides increased during post infection period in the susceptible as well as resistant cultivars.

2.4 CROP LOSS

2.4.1 *M. incognita*

Bhatti and Jain (1977) reported that the losses in yield of lady's finger, tomato and brinjal were 90.9, 46.2 and 27.3 per cent respectively in a field infested with *M. incognita* @ 2800-3460 juvenile/kg soil.

Charles (1978) reported that in ginger the reduction in rhizome weight varied from 9.4 to 46.4 per cent as the inoculum level increased from 10 to 5000. The avoidable yield loss due to *M. incognita* in ginger was 43 per cent at an initial population level of 166-juvenile/250 g soil sample (Sheela *et al*., 1995). Makhnotra and Khan (1997) reported that in Himachal Pradesh 20 per cent loss in ginger yield was caused due to *M. incognita* with an initial population of 200 larvae per 200 cc of the soil.

Patel *et al*. (1981) reported heavy galling of roots and reduced size of rhizomes in turmeric infected with *M. incognita*.

Meloidogyne spp. had incurred 32.66 per cent yield loss in tobacco in Pinar del Rio Province, Cuba (Garcia and Espinosa, 1982). Under field condition, *M. incognita* caused 47 and 86.7 per cent reduction respectively in top weight and shade dried leaf yield of Patchouli, *Pogostemon cablin* at 10,000 J_2 level/plant (Prasad and Reddy, 1984).

Eapen (1994) reported that 46.1 per cent yield loss was observed in small cardamom at four nematodes / 100 cm^3 soil. Nalinakumari *et al*. (1995) reported 56.9 and 67.7 per cent reduction of chewable leaves of betel vine at an initial inoculum level of 4000 and 5000 *M. incognita* larvae/plant.

The biometric characters and yield of oil of *Ocimum sanctum* was significantly reduced by *M. incognita* compared to uninoculated plants and this reduction was in accordance with the initial inoculum levels (Haseeb *et al*., 1999).

2.4.2 R. similis

Sosamma *et al*. (1979) reported that an initial inoculum level of ten nematodes caused 35 per cent reduction of rhizome weight after four months and 46 per cent at the end of the season (8 months) in turmeric.

Sundararaju *et al*. (1979) reported 73.6 per cent reduction in the rhizome weight of ginger at an initial inoculum level of 10,000 nematodes over a period of six months.

Geetha *et al*. (1995) reported that 100 *R. similis* in 2500 cm^3 fumigated Sandy loam soil caused 38.67 per cent reduction in the number of leaves of betelvine and maximum damage was encountered in plants that received 1000 nematodes.

In banana, *R. similis* caused a loss in fruit yield of 31.61 per cent at a population of 344/10 g root (Reddy *et al*., 1996).

In *Anthurium andreanum* the total yield loss due to *R. similis* was estimated to be 18–33 per cent (number), 15.35 per cent (weight), 9–197 (stem length) and 6–15 per cent (width) of the flowers (Amsing *et al*., 2002).

2.5 MANAGEMENT

In recent years, nematode management strategy was diverted to non-chemical ecofriendly approaches rather than chemical control. This ecofriendly integrated approaches include application of plant products, organic amendments and microbial agents alone or in combination. The important literature relevant to these aspects are reviewed.

2.5.1 Botanical Pesticides / Plant Products

2.5.1.1 Green Leaves

Several indigenous plants have been identified for their nematicidal action on root-knot nematode. Studies conducted on the use of green leaves like *Calotropis* sp., *Eupatorium* sp., mango and cashew on okra showed reduced root-knot nematode infestation and increased growth of plants (Kumar and Nair, 1976).

Sudha and Sundararaju (2001) reported that application of Glyricidia leaves @ 5 kg $plant^{-1}$ significantly reduced the *R. similis*

population in arecanut based cropping system (arecanut, banana and black pepper).

Kaliram and Gupta (1982) found that combined effect of various treatments like application of chopped neem and datura leaves were significant in reducing the number of galls in the case of chickpea (*Cicer arietinum* L.). Application of chopped castor leaves (40 g kg^{-1} of soil) two weeks before transplanting of tomato effectively controlled *Meloidogyne javanica* (Dutt and Bhatti, 1986).

Sundarababu *et al*. (1993) reported that chopped leaves of bougainvillea, *Ocimum*, onion, *Prosopis*, *Calotropis* and subabul enhanced the growth of tomato and greengram and suppressed the final population of root-knot nematode in tomato and reniform nematode in greengram. Among them, prosopis was superior followed by subabul, calotropis and bougainvillea. Application of calotropis leaves @ 80 t ha^{-1} was found significantly better than neem and castor leaves which proved as effective as carbofuran granules in reducing the nematode population in betel vine gardens (Subbarao *et al*., 1996). Ramakrishna *et al.* (1997) reported that application of *Azadirachta indica* leaves (80 g/pot) resulted in maximum reduction in root-knot index and nematode population in okra.

A study conducted by Khanna and Sharma (1998) revealed that application of leaves of *Azadirachta indica* and *Tagetes patula* improved plant growth of tomato and reduced nematode count as well as gall index and the effect was on par with that of nematicides (Phorate and carbofuran). Application of chopped leaves of *Prosopis juliflora, Catharanthus roseus, Leucaena leucocephala, Calotropis procera* and *Azadirachta indica* gave better biomass production than chemical treatment in cowpea. The chopped leaves increased the VAM spore production and colonization and reduced nematode population (Santhi and Sundarababu, 1998). In tomato, plant materials like lemongrass and neem leaves significantly improved plant growth and reduced root-knot disease,

producing significantly more number of transplantable and total seedlings over control (Patel *et al*., 2000).

Jasy and Koshy (1992b) reported that chopped leaves of *Glyricidia maculata* 10 g kg^{-1} soil when applied as green manure reduced the population of *R. similis* and promoted the growth of black pepper under pot conditions. Ajith *et al*. (1993) reported that application of chopped neem leaves @ 7.5 t ha^{-1}, 15 days prior to sowing of cowpea seeds significantly reduced the pathogenic nematodes like *M. incognita*, *R. reniformis* and *Helicotylenchus* sp. Application of chopped green leaves of neem and *Chromolaena odorata* on soil before sowing significantly reduced the nematode population and improved the yield in okra and cowpea (Sheela *et al*., 1999).

2.5.1.2 Dry Leaf Powder

Soil amendment with dried flowers, leaves, stems and roots of *Calotropis procera* significantly improved plant growth by reducing the root-knot nematode population in egg plant (Ahmad *et al*., 1996).

Minimum galling and increase in growth of okra after the application of *Clerodendron inerme* @ 1.5 per cent w/w was observed by Patel *et al*. (1985). Organic amendment with powdered leaves of the plants like Curry leaf, *Murraya koenigii;* Jasmine, *Jasminum sambac*; sour orange, *Citrus aurantifolia;* Patal garuda, *Rauwolfia serpentina;* Ber, *Zizyphus jujuba;* China rose, *Hibiscus rosachinensis;* and Justicia, *Justicia gandurusa;* were effective in reducing population of *M. incognita* (Padhi and Behera, 2000).

2.5.1.3 Leaf Extracts

Root dip treatment of egg plant seedlings in margosa and marigold leaf extracts considerably reduced root-knot development compared to treatment with piperazine citrate, chinopodium oil and groundnut cake (Hussain *et al*., 1984). Leaf extracts (10 to 0.1 per cent dilution) of *Euphorbia caducifolia* and *Calotropis procera* were highly effective

against *M. javanica* on tomato and brinjal showing improved growth (Maqbool *et al*., 1987).

The extract of *Argemone mexicana* acted as a nematicide to *M. javanica* in okra raised in micro plots (Nath *et al*., 1982). Similarly Haseeb *et al*. (1982) identified the nematicidal properties of *Mentha viridis, Cassia fistula* L.*, Cordia myxa* L.*, Carissa carandas* L.*, Colocasia arniquorum* against *R. reniformis*. Extracts of *Andrographis paniculata, Calendula officianalis, Enhydra fluctuam* and *Solanum khasianum* reduced root galling by *M. incognita* on tomato transplants (Goswamy and Vijayalakshmi, 1986a). Aqueous leaf extracts of *Erythrina indica* at 1 to 1.5 per cent concentration proved highly toxic to *M. incognita* and *Tylenchorhynchus mashhoodi* and per cent mortality of both the nematodes increased with exposure period (Mohanty and Das, 1988). Extracts of leaves of citronella (*Cymbopogan winterianus)* reduced root galls and populations of *M. incognita* in soil and roots at concentrations of S/20, S/10 and S/5 on tomato (Mahapatra and Swain, 1993). Fresh leaf extracts of Eucalyptus and neem plants (40 per cent w/v) with a dip duration of six hours were found to be highly effective in improving the growth parameters and suppressing the population of *M. javanica* on tomato (Vats and Nandal, 1994). Gupta and Sharma (1995) reported the nematicidal activity of aqueous extracts of *Allium sativum* L. against the juveniles of *M. incognita*.

The aqueous extracts prepared from different plant parts of *Tagetes erecta* (cv. Crackjack) inhibited the egg hatching and larval penetration of *M. javanica* (Dhangar *et al*., 1996). Exposure of larvae of *Pratylenchus thornei* to aqueous rhizome extract of *Acorus calamus* L. resulted in 100 per cent mortality (Romabati *et al*., 1999). Walia and Dalal (2000) observed an inhibition in the emergence of larvae from *Heterodera avenae* cysts in treatments with the extracts of two *Chenopodium* spp., *C. album* and *C. murale*. The effect decreased with dilution of the extract but increased with exposure time.

Maheswari and Sundarababu (2001a) reported the soil application of 15 per cent neem leaf extract recorded 90 and 90.3 per cent reduction respectively in soil and root population of *M. incognita* infecting cowpea over the control plants and also recorded minimum gall index and increased plant growth and yield. They also reported that soil application of 15 per cent leaf extract of calotropis reduced the *M. incognita* population in soil and roots of cowpea and also increased the growth and pod yield (Maheswari and Sundarababu, 2001b).

Study conducted by Sosamma *et al*. (1998) revealed that leaf extracts of *Moringa pterigosperma, Momordica charantia* and *Leucas aspera* have nematicidal and nematostatic properties against the root-knot nematode, *M. incognita*. Sundararaju *et al*. (1999) reported that the leaf extracts of *Chromolaena odorata* and *Ananas comosus* exhibited a high degree of nematicidal action against the adults and larvae of *R. similis* under lab conditions.

2.5.1.4 Shoots

Mansoor *et al.* (1987) reported that incorporation of chopped shoots of latex bearing plants like *Ficus elastica* gave greatest reduction in nematode population, root-knot development and showed significant improvement in plant growth. Soil amendment with chopped shoots of latex bearing plants *viz*., *Carica papaya, Artocarpus heterophyllus, Ficus carica, F. elastica, F. glomerata, Ipomea fistulosa, Nerium odorum* and *Tabarnaemontana coronaria* were effective in reducing the populations of *Helicotylenchus indicus, Rotylenchulus reniformis*, *Tylenchorynchus brassicae* and *Tylenchus filiformis* infecting tomato and egg plant. *F. glomerata* and *F. elastica* significantly improved the growth of plants also (Siddiqui *et al*., 1992).

2.5.1.5 Oil Dipping

Significant reduction of root-knot nematode on tomato by seed coating with Achook and neem oil was reported by Akhtar and Mahmood (1995).

Pradhan *et al*. (1989) showed that seedling root dip in oils of chalmogra, neem and karanj at half to one eighth dilution was highly effective in preventing larval penetration and gall production by root-knot nematode in the roots of tomato. Higher concentration of organic oils of karanj, neem, mahua and castor proved effective on preventing larval penetration and gall production in the roots of tomato (Poornima and Vadivelu, 1997).

2.5.1.6 Commercial Formulations

Naik *et al*. (1998) reported that the extracts of neem products had no adverse effect on the growth of tomato plants, but significantly decreased the nematode development and reproduction. Maximum reduction in galls, egg masses and egg production was recorded in nimbecidine treated plants and found superior over seed kernel and cake extracts. Among neem based formulations (achook, neemark, nimbecidine), achook was found to be most effective in reducing the penetration, number of root-knots and final soil population (Mojumder and Basu, 1999). Sharma *et al*. (2000) reported that significant reduction in number of galls and improvement in plant growth were observed when okra seeds were soaked in neemark or nimbecidine five per cent for six hours.

2.5.2 Organic Amendments

The beneficial effects of organic amendments and plant residues in reducing the plant parasitic nematodes have gained much importance in recent years.

2.5.2.1 Neem Cake

Neem products (neem kernel, neem cake, neem bark etc.) are known to possess nematicidal activity against nematode population (Zaki and Bhatti, 1989; Darekar *et al*., 1990). Soil amended with neem cake and datura powder were effective for the control of root-knot and root-knot

disease complex of okra (Haque *et al*., 1996). Neem cake and neem dust were found effective in the suppression of root-knot nematode, *M. incognita* in tomato (Jacob and Haque, 1998).

Neem oil cake applied @ 1 t ha^{-1} in trenches near the root zone of betel vine at the time of planting of vines was most effective in controlling the root-knot and increasing the yield of betel vine (Acharya and Padhi, 1988). Acid extracts of neem cake at different dilutions also enhanced the growth of *V. unguiculata* and reduced the population build up of nematode (Alagumalai *et al*., 1995). Rao *et al*. (1997) reported that use of aqueous extract of neem cake for seed treatment and soil drenching under field conditions was as effective as application of carbofuran at 2 kg ai ha^{-1} or neem cake at 2 t ha^{-1} for the management of *M. incognita* on okra. Neem cake was found very effective for the management of root-knot nematode in chickpea (Patel and Patel, 1998). Neem cake was the best in improving growth parameters and in reducing nematode infection and multiplication (Vats *et al*., 1998).

Kamalakshiamma (1986) reported that soil application of neem cake @ 240 g $plot^{-1}$ significantly reduced the population of root-knot nematode in brinjal.

Sundararaju and Sudha (1993) reported the effectiveness of neem oil cake @ 1 kg/palm/year in reducing the nematode population and increasing the yield significantly in arecanut, banana and black pepper under arecanut based farming system. Rajani (1998) reported the effectiveness of neem cake (@ 200 g m^{-2}) for managing root-knot nematode in kacholam. According to Nisha and Sheela (2003) neem cake at the rate of 200 g m^2 was very effective in reducing the nematode population in kacholam rhizosphere.

2.5.2.2 Other Oil Cakes

Coconut oil cake reduced the infestation of root-knot nematode in okra and increased the growth of plants (Kumar and Nair, 1976).

Kamalakshiamma (1986) reported that soil application of groundnut cake, castor cake and mustard cake significantly reduced the population of root-knot nematode in brinjal rhizosphere.

Soil amended with cakes of *Shorea robusta* and *Calophyllum inophyllum* resulted in slow hatching of *M. incognita* from egg masses (Goswamy and Vijayalakshmi, 1986b). Bhattacharya and Goswamy (1987) studied the role of micro organisms in the decomposition of neem and ground nut cakes and their effect on nematode build up and penetration and established that oil cakes have significant role in reducing nematode population. Alam (1989) reported that soil amendment with horn meal, bone meal and oil cakes of mahua, castor, mustard, neem and pea nut were effective in inhibiting the root-knot development and population build up of *T. brassicae* on egg plant, chilli, okra, cabbage and cauliflower and improving the plant growth.

The effectiveness of organic amendments (oil seed cakes of castor, mustard and linseed) for the management of root-knot nematodes in *Hyoscyamus albus* was reported by Haseeb and Butool (1994). Kumar and Vadivelu (1996) reported that application of organic amendments viz., neem cake, castor cake and mahua cake each at 500 kg ha^{-1} were effective in increasing plant growth parameters and reducing root-knot and reniform nematode population in brinjal. Mustard cakes were found to be very effective in controlling root-knot nematode in chickpea (Patel and Patel, 1998). Amendment of soil with mustard cake was found superior and effective than poultry manure with regard to shoot length and root weight in okra and bottle gourd (Dahiya *et al*., 1998).

2.5.2.3 Organic Wastes and Soil Conditioners

Application of poultry manure at higher dose reduced the infestation of root-knot nematode and increased fruit yield of tomato (Chindo and Khan, 1990). Organic amendments like sawdust, neem cake and poultry manure each @ 1000 kg ha^{-1} have been reported to be

effective against *M. incognita* in reducing the galls and increasing the yield of carrot (Devi and Das, 1998). Press mud was found effective as neem cake and mustard cake in managing the root-knot nematode and subsequently increasing the yield in bottlegourd crop under field conditions (Patel *et al*., 1998). A study conducted by Vats *et al*. (1998) revealed that application of organic manures like poultry manure, spent compost, FYM and biogas slurry improved plant growth of cotton and led to reduction in root-knot nematode populations. Patel and Patel (1998) found soil application of organic amendment, press mud (3 t ha^{-1}) and poultry manure (3 t ha^{-1}) significantly improved plant growth attributes and increased grain and fodder yields with reduction in nematode population in chick pea. Vemana *et al*. (1999) observed that among the organic amendments, the highest reduction in the nematode population was in sawdust (25 q ha^{-1}) treatment supplemented with nitrogen (30 kg ha^{-1}), phosphorus (40 kg ha^{-1}) and potassium (50 kg ha^{-1}) followed by neem cake (10 q ha^{-1}), poultry manure (50 q ha^{-1}), farmyard manure (100 q ha^{-1}), press mud (25 q ha^{-1}) and castor cake (10 q ha^{-1}) in groundnut.

Organic wastes like coconut husk powder, paddy husk, lemon grass waste, cashew shell powder etc. @ 2500 kg ha^{-1} significantly reduced root-knot nematode population and increased the yield of brinjal (Kamalakshiamma, 1986).

2.5.3 Bioagents

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

2.5.3.1 Bacteria

There are two groups of bacteria, one which release metabolites that have a killing or inhibitory effect on phytonematodes (species of *Bacillus*, *Clostridum*, *Pseudomonas*, *Azotobacter* etc.) and the other which

parasitize directly on nematodes, thereby affecting the entry, penetration, reproduction, egg hatching and larval mortality of nematodes (*Pasteuria penetrans*).

2.5.3.1.1 *Pseudomonas fluorescens*

Recently the fluorescent *Pseudomonas* spp. associated with the plant rhizosphere emerged as the largest and most promising biocontrol agent of plant parasitic nematodes (Oostendrop and Sikora, 1989). The effectiveness of *P. fluorescens* as a potential biocontrol agent against *M. incognita* 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).

Pseudomonas sp. 1 and 2 were most effective against the nematodes viz., *H. cajani, H. zeae, H. avenae* and *M. incognita* under *in vitro* condition (Gokte and Swarup, 1988). Santhi and Sivakumar (1995) reported the biocontrol potential of *P. fluorescens* (Migula) against root-knot nematode on tomato. Application of *P. fluorescens* @ 10 g kg^{-1} seed was effective in reducing the menace of root-knot nematode, *M. incognita* in tomato (Verma *et al*., 1998) and grape vine (Santhi *et al*., 1998). Mani *et al*. (1998) reported the effectiveness of pf (1) strain of *P. fluorescens* against *M. incognita*, *Tylenchulus semipenetrans* and potato cyst nematode. Application of *P. fluorescens* as seed treatment at a dosage of 10 g kg^{-1} seed was effective in reducing the infestation of *Hirschmaniella gracilis* (Ramakrishna *et al*., 1998). Sheela *et al*. (1999) also reported the biocontrol efficiency of this bacteria in brinjal. Seenivasan *et al*. (2000) found that the culture filtrate of *P. fluorescens* have toxic effect on *H. oryzae* population. Sirohi *et al*. (2000) reported that culture filtrates of *Bacillus* and *Pseudomonas* can cause 80 to 90 per cent mortality of *M. incognita* juveniles. Application of *P. fluorescens* in kacholam rhizosphere increased the yield substantially and found effective as chemical treatment (Nisha and Sheela, 2003).

2.5.3.1.2 *Bacillus* spp.

Study conducted by Racke and Sikora (1992) revealed that the plant growth promoting rhizobacteria, *Agrobacterium radiobacter* and *Bacillus sphaeriacus* increased the tuber yield of potato by suppressing the population of *Globodera pallida*. Roots from the plants infested with the nematode and bacteria had lower root indices and fewer Meloidogyne larvae and eggs than those infested with the nematode only (Vargas *et al.*, 1992). Oka *et al*. (1993) opined that exposure of *Meloidogyne* juveniles to *Bacillus cereus* inhibited the penetration of the nematodes into the tomato roots. The various formulations of *Bacillus thuringiensis* were found toxic to eggs and larvae of *Meloidogyne* sp. Zuckerman *et al*. (1993) found that application of an isolate of *Bacillus thuringiensis* (CR-371) resulted in smaller population of *M. incognita* in tomato and *P. penetrans* in strawberry.

Sharma (1994) reported that bacterial nematicides, *Bacillus thuringiensis* var. *thuringiensis* (Btt) and *B. thuringiensis* var. israelensis (Bti) were effective in controlling *M. incognita* on barley.

Bacillus macerans was found effective against root-knot nematode in bhindi and pepper (Sheela and Venkitesan, 1992).

2.5.3.1.3 *Pasteuria penetrans*

P. penetrans, a mycelial endospore forming bacterium, is an obligate parasite of large number of nematodes and it completes its life cycle in second stage juveniles. Ahmad *et al*. (1994) reported that *P. penetrans* applied at 2.5 cm soil depth was the most effective method for the control of *M. incognita* on tomato.

P. penetrans application significantly reduced the population of root-knot nematode and gall index in okra (Walia and Mehta, 2000).

2.5.3.2 Fungi

2.5.3.2.1 Arbuscular Mycorrhizal Fungi (AMF)

Arbuscular mycorrhizal fungi have potential in reducing plant diseases caused by plant parasitic nematodes. Increased resistance against nematodes and suppression of nematode population have been observed in mycorrhizal soybean (Franil and Dropkin, 1985), tomato and white clover (Cooper and Grandison, 1986). Development and reproduction of nematodes are often inhibited by mycorrhizal association (Cooper and Grandison, 1986; Jain and Sethi, 1988). Mycorrhizal inoculation enhanced the growth parameters significantly in white clover (Habte *et al*., 1999).

Bhagyaraj *et al*. (1979) reported that tomato roots colonized by *G. fasciculatum* exhibited fewer and small galls than nematode (*M. incognita* and *M. hapla*) infested non mycorrhizal plants. The number of giant cells formed in mycorrhizal tomato when infected with the root-knot nematode was significantly low when compared with non-mycorrhizal plants (Suresh *et al*., 1985). The gall formation by *M. incognita* and their multiplication were hampered by the early establishment of *G. fasciculatum* on cowpea (Jain and Sethi, 1988). Sharma *et al*. (1994) reported that AMF colonization reduced the root-knot infestation in tomato. Mycorrhizal tomato seedlings had lesser number of galls, egg masses per plant, eggs and juveniles per egg mass. They studied the effect of arbuscular mycorrhizal fungus, *G. fasciculatum* in the survival, penetration and development of root-knot nematode *M. incognita* in tomato under glasshouse condition and found that the symbiont caused a reduction of 30 per cent in galls and egg masses per plant. Sundarababu *et al*. (1996) reported that when *G. fasciculatum* was inoculated 15 days earlier than nematode inoculation, enhanced the growth of tomato cv. Co-3 and suppressed *M. incognita* multiplication in pot experiments. Study conducted by Nageswari and Sundarababu (1998) revealed that *G. fasciculatum* can be effectively used as a biocontrol agent for *H. cajani*

in cowpea. Ray and Dalei (1998) reported that in the case of greengram all plant growth parameters including pod yield, leaf chlorophyll content, bacterial nodulation, leghaemoglobin content of nodules and NPK contents of plants showed significant improvement in mycorrhiza inoculated plants. Application of *G. fasciculatum* @ 10 g kg^{-1} soil was sufficient for the effective management of root-knot nematode infesting tomato and okra (Sundarababu *et al*., 1998). Mycorrhizal inoculation enhanced significantly oil yield in *Mentha arvensis* cv. Himalaya (Pandey *et al*., 1998), flower yield in *Crossandra undulaefolia* (Nagesh *et al*., 1999).

Sivaprasad *et al*. (1990) observed that deleterious effect of nematodes was made insignificant due to arbuscular mycorrhizal associations in cowpea. The root-knot index and nematode population were reduced considerably. In pepper there was reduction in nematode population in root and soil, root-knot index and increased growth of vines when plants were inoculated with *G. fasciculatum* and *G. etunicatum* (Sivaprasad *et al*., 1990). Cowpea plants inoculated with *M. incognita* in association with *G. fasciculatum*, *G. mosseae* and *A. morroweae* recorded a root-knot index of 1, 3.16 and 3.43 respectively as against 4.89 observed for control plants (Deepthi, 1993). *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). *G. mosseae* was found effective in reducing burrowing nematode population in coconut (Koshy *et al*., 1998) and banana (Sosamma *et al*., 1998). Application of AMF @ 10 g m^{-2} was very effective in reducing the nematode population in kacholam and increasing the yield significantly (Nisha and Sheela, 2003).

2.5.3.2.2 *Paecilomyces lilacinus*

P. lilacinus has widely been recognized as an effective egg parasite of many sedentary plant parasitic nematodes including *M. incognita*. Khan and Goswami (2000a) reported that 8 g (57.92 x 10^8 spores) fungus

(*P. lilacinus*) infested rice per kg soil was considered to be optimum for suppression of *M. incognita* in tomato. *P. lilacinus* isolate 6 showed highest percentage (75 per cent) of egg parasitism of *M. incognita* (Khan and Goswami, 2000b).

Sudha *et al*. (2000) reported that *P. lilacinus* significantly reduced the population of *R. similis* in soil and roots of arecanut. Granular application of *P. lilacinus* reported 34.5 per cent egg parasitization of *M. incognita* on tomato (Sankaranarayanan *et al*., 2000). Application of *P. lilacinus* at the time of planting in banana was very effective in reducing *R. similis* population in soil and roots (Devarajan and Rajendran, 2001).

2.5.3.2.3 *Trichoderma viride*

Trichoderma and *Gliocladium* have been reported as potential fungal biocontrol agents against several plant pathogens and plant parasitic nematodes (Papavizas, 1985).

T. harzianum is a saprophytic fungus often found in the soil rich in organic matter. The parasitic fungus, *T. harzianum* has been reported to be one of the promising biocontrol agent against plant parasitic nematodes (Reddy *et al*., 1996 and Rao *et al*., 1998). Exposure of second stage juveniles of rice root nematode to the *T. harzianum* culture filtrate also caused significant nematode mortality. The synergistic effect of fungi *T. viride* along with the organic amendment for the enhancement of plant growth by increasing the population of nematode trapping fungus was reported by Reddy *et al*. (1996) and Sundarababu *et al*. (1997). Sankaranarayanan *et al*. (1997) reported the nematicidal effect of fungal filtrates (*T. harzianum*) against root-knot nematode in tomato. Khan and Saxena (1997b) reported that root dip treatment with culture filtrates of *A. niger, P. lilacinus* and *T. viride* was particularly beneficial in reducing *M. incognita* damage on tomato in pot experiments.

Sankaranarayanan *et al*. (1998) studied the antagonistic effect of *Trichoderma* and *Gliocladium* sp. against the root-knot nematode (*M. incognita*) on sunflower and found that these fungi were effective in reducing the number of galls and egg masses on the root system and nematode population in soil. Application of plant based formulations of *T. harzianum* to nursery beds of aubergine was effective in producing vigorous seedlings with least root galling, increased root colonization and parasitization of *M. incognita* females by *T. harzianum* (Rao *et al*., 1998). Acharya *et al*. (2000) reported good control of root-knot nematode in betel vine by field application of *T. viride*. The fungus showing saprophytic habit when inoculated with suitable oil cake (mustard cake as substrate) proved to be an effective parasite of root-knot nematode, reducing the nematode population in soil and thereby increasing the yield (both number and weight of leaves).

According to Ravi *et al*. (2001) combination of neem cake + *Trichoderma viride* + carbofuran was found to be very effective in reducing the nematode population in soil as well as in roots of banana and increased the plant growth parameters and yield.

2.5.4 Chemicals

The effect of chemicals for managing root-knot nematode in various crops are reviewed here. Mahajan (1978) observed reduced root-knot index after four weeks by the application of carbofuran flowable paste at different concentrations in okra seeds. Spot application of chemical was superior than row or broadcast in reducing root-knot incidence (Sitaramaiah and Vishwakarma, 1978).

Carbofuran at 2 kg ai ha^{-1} effectively controlled *M. incognita* on ginger and improved plant growth in pot culture experiments (Parihar and Yadav, 1986). Jain *et al*. (1988) reported that aldicarb and aldicarb based chemicals are most effective in increasing tomato yield in *M. javanica* infested plots. Carbofuran reduced the galls and egg mass in roots of pea

and increased the yield (Bhagavati and Phukan, 1990). Borah and Phukan (1990) tried carbofuran 3 G, phorate 10 G, Mocap 10 G and diazinon 10 G each at one, two and three per cent as seed treatment for the control of *M. incognita* on greengram and revealed 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. In another study, carbofuran was found effective in suppressing *M. incognita* activity and improving plant growth of French bean (Mohan and Mishra, 1993).

Soil application of carbofuran @ 2 kg ai ha^{-1} and seed dressing @ 2 g ai kg^{-1} were highly effective in controlling *M. incognita* larvae and reduced root-knot galls in pea compared to control plants. This treatment also improved the plant growth parameters and yield (Devi, 1993).

Joshy and Patel (1996) tried seed treatment and soil application of nematicides for management of *M. javanica* on groundnut. Results revealed that soil application of phorate was most effective followed by carbofuran in improving plant growth, while phenamiphos was found most effective in reducing host infection.

Bare root dip treatment of brinjal seedlings with monocrotophos, triazophos or carbosulfan @ 0.05 per cent was beneficial in reducing the root-knot nematode population significantly and increasing the yield between 34 to 80 per cent over the check (Reddy *et al*., 1997).

Pareek *et al*. (1998) reported that in gram, application of carbofuran @ 2 kg ai ha^{-1} was better in improving the plant growth characters like shoot-root weight and length and in reducing number of galls, egg/egg mass and final soil population in comparison to other chemicals like sebufos and phorate.

Patel *et al*. (1998) reported that soil application of carbofuran @ 3 kg ai ha^{-1} increased dry pod yield by 42.8 per cent and dry fodder yield by 28.8 per cent and reduced root-knot disease by 45.2 per cent over control in the case of groundnut.

Vadhera *et al*. (2000) reported that carbofuran @ 0.6 g ai m^{-2} in nursery bed treatment improved the germination, seedling vigour, weight and significantly reduced the gall index from 5 to 3.6 and increased the yield by 36 per cent.

2.5.5 Combination of Bioagents and Organic Amendments

Verticillium lecani in combination with the oilcakes viz., castor, karanj and neem at 200 g $plant^{-1}$ was effective in increasing the plant growth and reducing the citrus nematode population in soil and root of acid lime (Reddy *et al*., 1996).

Rao *et al*. (1997) reported that integration of *G. fasciculatum* (500 g m^{-2}) with castor cake (400 g m^{-2}) resulted significant reduction in root galling and fecundity of *M. incognita* and an increase in root colonization by endomycorrhiza in tomato plants.

Reddy *et al*. (1997) reported that by integrating eco-friendly components like endomycorrhiza *G. mosseae* along with oil cakes like karanj or neem cake, *R. similis* population in banana can be effectively managed.

Investigations on interaction of endomycorrhiza with organic amendments revealed that neem and castor cake encourage the multiplication of *G. mosseae* and *G. fasciculatum* respectively. Integration of these oil cakes with endomycorrhiza in the nursery protected the seedlings of tomato from the attack of *M. incognita* (Reddy *et al*., 1998).

Ayuso (2002) reported that VAM (*Glomus* sp.) + chicken manure was very effective in reducing *R. similis* population in banana.

2.5.6 Combination of Bioagents and Plant Products

G. mosseae in combination with neem leaf or neem leaf extract proved very effective in increasing the plant growth parameters of egg plant seedlings in the nursery beds and reducing nematode infestation, indicating combined and complimentary interacting effect of both components on the

management of root-knot nematode due to their synergistic actions (Rao *et al*., 1993). Bhagavati *et al*. (2000) reported that *G. etunicatum* and mustard cake were equally effective in reducing the damage caused by the nematodes when applied individually and the combined effect of these two was much more than that of single application.

2.5.7 Combination of Bioagents and Chemicals

Treatment with a combination of either *Trichoderma* – Vydate (oxamyl) or *Trichoderma*-Nemacur (fenamiphos) significantly decreased disease and root-gall index and improved plant height and shoot dry weight of tomato plants (Stephan *et al*., 1996).

The application of *P. fluorescens* and carbofuran in combination enhanced the yield and improved the vigour of plants in the case of tomato by suppressing the root-knot nematode (Khan, 2000) and cyst nematode, *Heterodera cajani* in soil and root system (Sujata *et al*., 2000).

2.5.8 Combination of Organic Amendments and Chemicals

Singh *et al*. (1980) recommended combined application of oil cakes and nematicides for effective control of nematode population, since it was found superior to oil cakes alone and only a low concentration of nematicide was required when mixed with oil cakes. Gaur and Mishra (1990) reported that combined application of organic amendment (neem cake 1 t ha^{-1}) and phorate 10 G @ 1 per cent (w/w) as seed treatment and basal application of aldicarb 10 G @ 40 kg ha^{-1} significantly reduced the plant parasitic nematodes and increased the yield of greengram. Neem cake followed by carbofuran treated plots showed minimum gall index in ginger (Mohanty *et al*., 1992).

Seed treatment with carbofuran 3 G @ three per cent (w/w) and organic amendments *viz*., neem cake, poultry manure and mustard oil cake each at 2 t ha^{-1} alone and combined application of seed dressing followed by organic amendments @ 1 t ha^{-1} each were found effective in improving

plant growth characters and yield of greengram (Barman and Das, 1996).

Neem cake and carbofuran treated plants supported minimum nematode population and root-knot index in papaya plants (Mohanty *et al*., 2000). Keshari and Pathak (2000) reported neem cake, mustard cake, press mud @ 25 g kg^{-1} soil and carbofuran 20 and 10 mg ai kg^{-1} soil reduced root-gall formation and increased the yield (root-weight) and shoot length of red beet.

In ginger, neem cake @ 2.5 t ha^{-1} at the time of planting and carbofuran 1 kg ai ha^{-1} forty five days after planting were effective in reducing *M. incognita* population in soil and root and also the root-knot index and increasing the yield (Sheela *et al*., 1995). Sudha and Sundararaju (2001) reported that phorate @ 25 g $plant^{-1}$ alone or in combination with neem cake @ 1 kg $plant^{-1}$ was effective in reducing the *R. similis* population in arecanut based cropping system (arecanut, banana and black pepper).

2.5.9 Others

The combination of endomycorrhizal fungus *G. deserticola* and *P. penetrans* was beneficial for sustainable management of *M. incognita* in tomato (Rao and Gowen, 1998). The combination significantly reduced the number of egg masses in root system and increased the parasitization of females by *P. penetrans*. Root colonization by *G. deserticola* was not affected by *P. penetrans*.

Integration of calotropis leaf and *G. fasciculatum* (Rao *et al*., 1996) and oil cakes, bone and horn meals with *P. lilacinus* (Khan and Saxena, 1997a) resulted in increased plant growth and reduced population build up of nematodes and root gallings in the case of tomato. The combined effect of the mycorrhizal fungi and *P. lilacinus* gave maximum reduction of nematode galls in okra (Sharma and Trivedi, 1997).

According to Vidya and Reddy (1998) combined application of neemark + carbofuran + *P. penetrans* + *G. fasciculatum* was most effective in enhancing the plant growth and yield of banana and reducing the nematode population in soil (95.16 per cent) and in roots (89.27 per cent) over control.

Integration of *P. penetrans, T. viride* and neem cake in nursery and rotation of tomato with marigold in the field gave significant reduction in root galling, egg mass production and final nematode population in soil which resulted in increased tomato yield (Reddy *et al*., 2000).

Application of neem cake + carbofuran + *T. viride* was the most effective treatment among different combinations of organic amendments with bioagents and chemicals for the management of *R. similis* in banana and improving the plant growth and yield (Harish and Gowda, 2001; Ravi *et al*., 2001).

Materials and Methods

3. MATERIALS AND METHODS

Studies were conducted to workout the host parasite relationship and management of important nematodes associated with Chethikoduveli, *Plumbago rosea* L. Since the root-knot nematode, *Meloidogyne incognita* (Kofoid and White, 1919) Chittwood, 1949 and the burrowing nematode, *Radopholus similis* (Cobb, 1893) Thorne, 1949 were the important nematodes associated with this medicinal plant causing economic yield losses, pathogenicity and crop losses, histopathological and biochemical changes caused by these nematodes were studied. A suitable management strategy was also worked out by incorporating bioagents, organic amendments and green leaves. The crop loss assessment and management study were conducted in microplot conditions and the pathogenicity, histopathological and biochemical changes were conducted in pot culture condition.

3.1 PATHOGENICITY

3.1.1 Preparation of Denematised Potting Mixture and Raising Pure Culture of Nematodes

3.1.1.1 Preparation of Denematised Potting Mixture

Sieved field soil, sand and well decomposed farmyard manure were mixed in the ratio, 2 : 1 : 1 and the mixture was spread on the ground in the form of beds of 15 cm thickness. The beds were divided into blocks of one square feet which were moistened and drenched uniformly with 10 per cent formaldehyde solution. The beds were then covered with polythene sheet for two weeks. The sheets were removed and the mixture raked well and exposed after two weeks. The denematized potting mixture was used for pot culture studies.

3.1.1.2 Raising Pure Culture of M. incognita

Egg masses of *M. incognita* collected from infested coleus roots, were used for raising pure culture of nematodes on Chethikoduveli cutting maintained in denematized soil. Subculturing was done periodically to ensure availability of sufficient larval population for inoculation purposes for the experiments.

For obtaining one day old juveniles of the nematode for experiments, viable egg masses were hand picked from the infested roots of culture plants and kept in cavity blocks containing sterile water. Every 24 hours the larvae hatched were collected. The number of larvae per ml of suspension was determined with the help of stereoscopic microscope using counting dish. The larval concentration was adjusted to required number of larvae per ml of suspension by adding required quantity of sterile water.

3.1.1.3 Raising Pure Culture of R. similis

Larvae and adults of *R. similis* collected from the heavily infested banana plants were used for raising pure culture of nematodes on Chethikoduveli maintained in denematized soil. Subculturing was done periodically to ensure availability of sufficient nematodes for inoculation purposes for the experiments.

For obtaining the nematodes for inoculation purposes infested Chethikoduveli roots were extracted by root incubation techniques in room temperature. The number of nematodes per ml of suspension was estimated with the help of a stereoscopic microscope using counting dish.

3.1.2 Inoculation of Nematodes

The nematodes were inoculated to the root zone of the plants by making five holes in the soil around the root zone about 4 cm deep with a glass rod, 1.5 cm away from the base of the plants and required quantity of

inoculum was pipetted out and poured equally in the bore holes. The holes were closed immediately with sterile moist sand and then the pots were moistened gently to keep the soil just wet.

3.1.2.1 M. incognita

Rooted cuttings of Chethikoduveli were planted in pots containing denematized potting mixture. One month after planting, the plants were inoculated with different levels of second stage juveniles (J_2) of *M. incognita*. The levels studied were 0, 10,100, 1000 and 10000 J_2 per plant. The experiment was laid out in completely randomised design with five replication.

3.1.2.2 R. similis

Rooted cuttings of Chethikoduveli were planted in pots containing denematized potting mixture and one month after planting the plants were inoculated with different inoculum levels of *R. similis* (0, 10, 100, 1000 and 10000).

Design : CRD

Replication : 5

The main items of observations were :

1) biometric characters at monthly intervals
2) fresh root weight and shoot weight
3) leaf area
4) dry weight of shoot and root
5) fresh dry weight ratio of shoot and roots
6) nematode population characteristics (nematode population in soil, root, root-knot / lesion index).

3.2 HISTOPATHOLOGICAL CHANGES DUE TO *M. INCOGNITA* AND *R. SIMILIS* IN CHETHIKODUVELI

Rooted cuttings of Chethikoduveli were planted in pots filled with denematized potting mixture. One month after planting the plants were inoculated with 1000 J_2 in the case of *M. incognita* and 1000 nematodes (larva + adult) in the case of *R. similis*. The changes were studied by uprooting the plants from the fifth to 45^{th} day after inoculation at five days interval and the roots were fixed in FAA fixative (formalin acetate and alcohol fixative). The fixed roots were then processed for microtomy using safranin as described by Johanson (1940) Hand sections were also taken for studying the histopathological changes. Pattern of entry of nematodes, development and expression of symptoms on roots at different intervals after inoculation were studied.

3.3 BIOCHEMICAL CHANGES

Rooted cuttings of Chethikoduveli were planted in pots filled with denematized potting mixture. One month after planting different levels of *M. incognita* and *R. similis* were inoculated as mentioned in para 3.1.2. After 12 months the changes in plumbagin, phenol and total free amino acids were estimated at various levels of *M. incognita* and *R. similis* inoculum.

3.3.1 *M. incognita*

3.3.1.1 Plumbagin

Plumbagin content was estimated as per Gupta *et al*. (1993) with suitable modifications.

Preparation of Extract

One gram of dried powdered root material of 10, 100, 1000 and 10000 J_2 inoculated plants and uninoculated plants were taken in stoppered test tubes. Ten ml of acetone was added to each tube, shake

well and kept for 48 hours with intermittent shaking. The tubes were washed repeatedly with acetone, filtered and quantitatively transferred to 50 ml standard flasks.

Estimation of plumbagin in the extract

The extraction was by a high pressure liquid chromatograph of Shimadzu Instrument Corporation, Japan make, Model LC8A. The detector used was spectrophotometer detector model SPD10A (wavelength 415 nm). SHIM-PAK-CLC-SIL 250 x 48 mm column was used for detection. Solvent system used was four per cent propanol in n-hexane with a flow rate of 1 ml/min.

One ml extract of each sample was injected into the column and the peak area at 415 nm was measured and the plumbagin content was estimated from the standard curve prepared by using different concentrations of standard plumbagin.

3.3.1.2 Phenol

The estimation of phenol was carried out by following the methods of Sadasivam and Manikam (1992). The root materials of 10, 100, 1000 and 10,000 J_2 inoculated plants and uninoculated plants were taken, dried and powdered. An aliquot of 0.5 g each was taken in different tubes and five ml of 80 per cent ethanol was added to it. The homogenates were centrifuged at 10,000 rpm for 20 minutes. The supernatants were collected and the residue re-extracted with 2.5 ml, 80 per cent ethanol, centrifuged and the supernatants were pooled. The supernatants were then evaporated to dryness and the residues dissolved in five ml distilled water. Then samples (0.2 ml each) were pipetted out into test tubes and the volume was made up in each tube to 3 ml with water. To this 0.5 ml of Folin-Ciocaltean reagent was added and after three minutes two ml of 20 per cent sodium carbonate solution was added to each tube. The solutions were mixed thoroughly and the tubes were placed in boiling water for

exactly one minute, cooled and the absorbance measured at 650 nm against a reagent blank using spectrophotometer. This absorbance was compared with standard curve prepared by using different concentrations of catechol. From this the concentration of phenol was obtained and expressed in mg g^{-1} of material.

3.3.1.3 Total Free Amino Acids

Total free aminoacids in the roots of *P. rosea* were estimated according to Sadasivam and Manikam (1992).

Preparation of extract

Five hundred milligram of 0, 10, 100, 1000 and 10,000 *M. incognita* infested root materials and root materials from uninoculated plants were taken ground in a mortar with pestle using small quantity of acid washed sand. Five to ten ml of 80 per cent ethanol was added to this homogenate and centrifuged. The supernatant was collected and the procedure was repeated once again for complete recovery of the free amino acids.

Estimation

One ml of ninhydrin solution was added to 0.1 ml of the extract. The volume was made up to 2 ml with distilled water and the tubes were heated in boiling water bath for 20 minutes. Five ml of the diluent (equal volume of n'-propanol and water) was added to this and the contents were mixed. After 15 minutes, the intensity of the purple colour was read against a reagent blank in a colorimeter at 570 nm. The reagent blank was prepared by taking 0.1 ml of 80 per cent ethanol instead of the extract.

Ninhydrin was prepared by dissolving 0.8 g stannous chloride ($SnCl_2$. $2H_2O$) in 500 ml of 0.2 M citrate buffer (pH 5.0). Twenty g of ninhydrin was added to this solution in 500 ml methyl cellosolve (2 methoxy ethanol). The standard was prepared by dissolving 50 mg

leucine in 50 ml of distilled water in a volumetric flask. Ten 'ml' of this stock standard was taken and diluted to 100 ml in another volumetric flask for working standard solution. A series of volume from 0.1 to 1 ml of this standard was prepared and the colour was read at 570 nm against the reagent blank. A standard curve was drawn using the absorbance versus concentration. From this curve the concentration of the total free amino acids in the sample was found and expressed as micro gram equivalent of leucine.

3.3.2 *R. similis*

3.3.2.1 Plumbagin

One gram of dried powdered root material of 10, 100, 1000 and 10000 nematode inoculated plants and uninoculated plants were taken in stoppered test tubes and the estimation of plumbagin was done as described in para 3.1.4.1.1.

3.3.2.2 Phenol

The root materials of 10, 100, 1000 and 10,000 nematodes inoculated plants and uninoculated plants were taken, dried and powdered and the estimation of phenol was done as mentioned in 3.1.4.1.2.

3.3.2.3 Total Free Amino Acids

Preparation of extract

Five hundred mg of root materials from 10, 100, 1000 and 10,000 *R. similis* inoculated plants and uninoculated plants were taken and ground in a mortar with pestle using a small quantity of acid washed sand as described in para 3.3.1.3.

3.4 CROP LOSS ASSESSMENT

3.4.1 Crop Loss Assessment due to *M. incognita* and *R. similis*

A microplot study was conducted to assess the crop loss incurred by *M. incognita* and *R. similis* on Chethikoduveli. The experiment was

conducted at College of Agriculture, Vellayani by raising rooted cuttings of Chethikoduveli in microplots as mentioned below.

3.4.1.1 Raising Rooted Cuttings of Chethikoduveli

The microplots were filled with garden soil which was made into fine tilth and rooted cuttings were planted at a spacing of 15 x 15 cm.

Inoculation method

Newly hatched second stage larvae (juveniles) of *M. incognita* were inoculated to the root zone of the transplanted rooted cuttings as per the method suggested by Venkitesan and Sethi (1977). Inoculation was done by making four holes in the soil around the root zone 1.5 cm away from the base of the plant about 4 cm deep with a glass rod and required quantity of inoculum was pipetted out and poured equally into the bore holes. The holes were closed immediately with sterile moist sand. Then the plants were irrigated gently to keep the soil just moist. In the case of *R. similis* the same method was followed but, nematode suspension used was a mixture of larvae and adults.

Plants were maintained as per the Package of Practices of Kerala Agricultural University (KAU, 1996).

The experimental details were as follows.

Plot size : 1 x 1 m

Design : RBD

Replication : 5

Treatments : 5

3.4.1.1.1 *M. incognita*

T_1 – level 0

T_2 – 100 J_2

T_3 – 1000 J_2

T_4 – 10000 J_2

T_5 – 1000 J_2 + carbosulfan 1 kg ai ha^{-1}

3.4.1.1.2 *R. similis*

T_1 – 0

T_2 – 100 nematodes

T_3 – 1000 nematodes

T_4 – 10000 nematodes

T_5 – 1000 nematodes + carbosulfan 1 kg ai ha^{-1}

The main items of observations recorded were :

(1) biometric character at monthly intervals

(2) fresh root weight and shoot weight

(3) leaf area

(4) dry weight of shoot and root

(5) fresh-dry weight ratio of shoots and roots

(6) nematode population characteristics (nematode population in soil, root, root-knot index and lesion index)

(7) computation of loss in terms of leaf and root production.

3.5 MANAGEMENT STUDIES

A field experiment was conducted in sick plots to evaluate the efficiency of different treatments *viz*., bioagents, organic amendments, botanicals and were compared with the chemical carbosulfan and wood ash (farmers practice) for the management of root-knot and burrowing nematodes in Chethikoduveli. Initially a buffer crop was raised in the plots to boost the population of *M. incognita* and *R. similis* to the required inoculum level. The pre-treatment nematode population was estimated by

standard method. The experimental details of the management trials were as follows :

Design : RBD

Replication : 3

Plot size : 2 x 2 m

Treatments

T_1 – *Glomus fasciculatum* (20 g m^{-2} having 100 chlamydospores per gram of media)

T_2 – *Pseudomonas fluorescens* (10 g m^{-2} x 10^6 cells per gram)

T_3 – Neem cake (1 t ha^{-1})

T_4 – Groundnut cake (1 t ha^{-1})

T_5 – *Glyricidia maculata* (5 kg m^{-2})

T_6 – *Clerodendron infortunatum* (5 kg m^{-2})

T_7 – Carbosulfan (1 kg ai ha^{-1})

T_8 – Wood ash (Farmer's practice)

T_9 – Nematodes alone

T_{10} – Check (nematode free condition by denematization and solarization)

T_{11} – *Paecilomyces lilacinus* (10 g m^{-2} having 10^6 spores per g of media)

Nematode population in soil was estimated at 2, 4, 6, 8 and 10 months after treatment. Biometric characters of plant *viz*., plant height, number of branches and number of leaves were taken at monthly intervals and also at the time of harvest. The plants were uprooted 18 months after planting. Fresh weight of roots (yield) and shoots, dry weight of roots and shoots, nematode population characteristics (root-knot count, number of females in five gram root, nematode population in five gram root sample)

were recorded at the time of harvest. Ten gram of soil sample was taken for estimation of bioagents in the rhizosphere.

The main items of observations made in management studies were :

1) pre-treatment population
2) biometric characters at monthly intervals
3) nematode population from soil at 60 days interval
4) biometric characters (fresh and dry weight of shoots and roots) just before harvest
5) yield
6) NPK content of leaf (promising treatments only)
7) nematode population characteristics (nematode population in soil and roots)

Root-knot nematodes

1. root-knot count
2. root-knot index
3. number of root-knots with and without egg mass
4. number of viable eggs/egg mass

Burrowing nematode

1. number of larvae per gram of root
2. number of adults per gram of root
3. number of lesions and lesion index

8) Re-isolation of bioagents

3.5.1 Preparation and Application of Different Treatments for Management Studies

Neem and ground nut cake were applied directly in the microplots at the rate of 1 t ha^{-1}.

Glyricidia maculata L. and *Clerodendron inforutnatum* were collected and chopped into small bits and applied in microplots at the rate of five kg m^{-2}.

3.5.2 Preparation of *P. fluorescens*

Talc based formulations of *P. fluorescens* obtained from the Department of Plant Pathology, College of Agriculture, Vellayani was used for *in situ* application. *P. fluorescens* formulation (10^6 cells per g) @ 10 g m^{-2} was applied directly as water suspension in the soil at the time of planting near the plant base (drenching).

3.5.3 Preparation of AMF

Pure culture of AMF was maintained on guinea grass *Panicum maximum* in pots containing sterile sand : soil mixture in the Department of Plant Pathology, College of Agriculture, Vellayani. Root segments of *P. maximum* colonized with mycorrhizal fungi and chlamydospores in the soil : sand mixture on which the grass was grown were mixed thoroughly and it served as the mycorrhizal inoculum. 20 g m^{-2} having 100 spores per gram of media was mixed with the top soil and rooted cuttings were planted over it.

3.5.4 Establishment of Bioagents in the Roots and Rhizosphere

3.5.4.1 Mycorrhizal Colonization Percentage and Intensity

The percentage of mycorrhizal colonization in root was estimated following the procedure of Phillips and Hayman (1970). Cleaned root samples free of soil particles were cut into 1 cm sized bits and fixed in FAA (formalin : acetic acid : ethanol 5 : 5 : 90) for three hours. The root bits were softened by simmering in 10 per cent potassium hydroxide at 90^0C for one hour. After cooling, the excess of alkali was removed by repeated rinsing in tap water and then acidified with two per cent hydrochloric acid. Staining was done by keeping the root bits in 0.05 per

cent Trypan blue solution (Trypan blue (Romali) 50 mg, Lactophenol 100 ml) in lactophenol reagent (lactic acid 20 ml, phenol 20 ml, glycerol 40 ml, distilled water 40 ml)at 90^0C for three minutes. The excess stain from the root tissue was removed by clearing overnight in fresh lactophenol. The root bits were examined at a time for the typical arbuscular mycorrhizal infection under a light microscope. Each root bit was divided into four equal segments for recording the presence or absence of mycorrhiza and based on this, different grades from zero to four were given depending on the extent of mycorrhizal infection. The average value thus obtained for 100 root bits examined was taken as mycorrhizal index.

Percentage of mycorrhizal infection

$$= \frac{\text{Number of root bits having infection}}{\text{Number of root bits subjected for observation}} \times 100$$

3.5.4.2 Estimation of Mycorrhizal Spore Count

The number of spores in the soil was estimated by adopting wet sieving and decanting technique of Gerdemann and Nicolson (1963). For this, 50 g of soil collected from the rhizosphere of Chethikoduveli was initially suspended in 100 ml of tap water in a measuring cylinder. After the heavier particles had settled, the supernatant was passed through a set of sieves of BSS No. 60 (250 microns), 150 (150 microns) and 350 (45 microns). The residue left behind in the measuring cylinder was resuspended in 100 ml of fresh tap water and passed through the same set of sieves. The procedure was repeated three to four times in order to collect maximum number of spores from the soil. Finally the material present on each sieve was transferred to 100 ml beakers in small volume of water and spread over Whatman No. 1 filter paper. The contents of each filter paper were carefully examined under a stereromicroscope for Arbuscular Mycorrhizal Fungal (AMF) spores and the spore count was recorded.

3.5.4.3 Estimation of Population of P. fluorescens and P. lilacinus

The population of *P. fluorescens* and *P. lilacinus* in the rhizosphere soil was estimated by the serial dilution plate technique (Johnson and Curl, 1972).

One gram of rhizosphere soil was taken along with the root and transferred to 100 ml sterile water blank and shaken for 5-10 minutes on a shaker. From this stock suspension, different dilutions of 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} were prepared. The population of fungi and bacteria were estimated at 10^{-3} and 10^{-6} dilutions respectively. King's B medium; Martins Rose Bengal Agar medium and potato dextrose agar medium were used for plating *P. fluorescens* and *P. lilacinus* respectively. The composition of media used were as follows :

King's B media

1) Peptone – 20g
2) Dipotassium hydrogen phosphate – 1.5 g
3) Magnesium sulphate – 1.5 g
4) Glycerol – 10 ml
5) Agar agar – 20 g

Martins Rose Bengal Agar Medium

1) Peptone – 5 g
2) Potassium di hydrongen phosphate – 1 g
3) Magnesium sulphate – 0.5 g
4) Dextrose – 10 g
5) Rose Bengal – 33 mg
6) Agar agar – 15 g
7) Distilled water – 1 litre

pH – 6 – 6.5

Potato Dextrose Agar

Potato : 200 g

Agar : 20 g

Dextrose : 20 g

Distilled water : 1 litre

After sterilization one per cent streptomycin sulphate solution 3 ml l^{-1} was added.

One ml aliquots from the dilutions 10^{-3} and 10^{-6} were transferred to sterile petriplates. Melted and cooled media at 45°C was poured at the rate of 20 ml per dish and rotated gently for thorough mixing. The petridishes were incubated at 28 ± 1°C for 96 hours. Observations were recorded as number of colony forming units (cfu) per g of soil.

3.5.5 Assessment of Results

3.5.5.1 Estimation of Nematode Population in Soil

Soil samples from each plots were collected before planting of rooted cuttings of 'Chethikoduveli' for estimating the pre-treatment population. Soil samples were also collected 2, 4, 6, 8 and 10 months after planting and nematodes were extracted from the representative soil samples following the method of Cobb's sieving and decanting technique (Cobb, 1918) and modified Baermann's method. The nematodes thus extracted were counted under a stereoscopic microscope using counting dish and fully counted.

3.5.5.2 Estimation of Nematode Population in Roots

Nematode population in root samples were estimated by modified Baermann funnel technique and root incubation technique as follows.

Modified Baermann funnel method

Root samples collected were washed thoroughly in a stream of tap water. Five gram of root was weighed and cut into small bits of 2-3 cm length and placed above the tissue paper supported by the wire guaze placed on petriplate. After 24 hours, the nematode suspension were collected, pooled and counted under a stereoscopic microscope.

Root incubation technique

Root samples collected were washed thoroughly in tap water. Five gram of root was weighed and cut into small bits of 2-3 cm length and were put in 200 gauge polythene cover containing small quantity of sterile water and incubated. The suspension was collected every 24 hours upto three days and each collected suspension was filtered through a set of sieves *viz.*, 20, 60, 100 and 400 mesh sieves. The residues left on the 400 mesh sieves were collected and the nematodes were counted by using a tally counter under a stereoscopic microscope.

3.5.5.3 Root-knot Index

The number of galls per gram of root was counted and the root-knot indexing was calculated as detailed below.

Number of galls/g of root	Root-knot index
1 – 5 galls	1
6 – 10 galls	2
11 – 15 galls	3
16 – 20 galls	4
Above 20 galls	5

3.5.5.4 Lesion Index

The number of lesions per gram of root were counted and the lesion index was worked out as detailed below:

Number of lesions /g of root	Lesion index
1 – 4 lesions	1
5 – 8 lesions	2
More than 8 lesions	3

3.5.5.5 Number of Females

Five gram root sample from the root system of 'Chethikoduveli' was cut into small bits of 2 – 3 cm length and stained by differential staining method using acid fuschsin–lactophenol mixture. The processed roots were pressed between glass slides and then teased with a needle and examined under a microscope to count the number of females.

3.5.5.6 Number of Eggs per Egg Mass

Four fixed number of egg masses were hand picked and kept in sterile water in a petridish. The total number of freshly hatched larvae was counted and from that the number of viable eggs per egg mass was determined. Then the egg mass is kept between two microscopic slides (glass slides), crushed thoroughly, stained and examined under a microscope to count the remaining eggs. The number of viable eggs plus the eggs remaining in the egg sac gave the total number of eggs present per egg mass.

3.5.5.7 NPK Content of Leaves

3.5.5.7.1 Total Nitrogen

Total nitrogen was estimated by Microkjeldahl digestion method (Jackson, 1973).

3.5.5.7.2 Total Phosphorus

Total phosphorus content was estimated by Vanado molybdophosphoric yellow colour method after extraction with triple acid (9 : 2 : 1 of HNO_3, H_2SO_4 and $HClO_4$ respectively). The intensity of yellow colour developed was read in Klett Summerson Photoelectric Colorimeter at 660 nm (Jackson, 1973).

3.5.5.7.3 Total Potassium

The same extract used for phosphorus estimation was used for the estimation of total potassium using the EEL Flame Photometer method (Jackson, 1973).

3.6 STATISTICAL ANALYSIS

The data generated from the experiments were subjected to Analysis of Variance (ANOVA) technique (Cochran and Cox, 1965). The variables which did not satisfy the basic assumption of ANOVA were transformed to square roots and then analysed.

Results

4. RESULTS

4.1 PATHOGENICITY

Pathogenicity of *Meloidogyne incognita* and *Radopholus similis* in Chethikoduveli was studied in pot culture conditions and the results are presented in Table 1 to 6.

The pathogenic effect of different levels of *M. incognita* and *R. similis* were assessed in terms of the effect on the reduction in plant height (Table 1), number of leaves (Table 2), number of branches (Table 3) and yield of tubers. The yield was in terms of the number, length, width and weight of tubers (Table 4). The fresh and dry weight of roots and shoots were also recorded (Table 5). The nematode population in soil and root were estimated using standard procedures and presented in Table 6.

4.1.1 Effect on Plant Height

4.1.1.1 M. incognita

The mean plant height (cm) at different intervals after inoculation of various levels of *M. incognita* are presented in Table 1. There was no significant difference in the height of the plants upto six months after inoculation.

Seven months after inoculation onwards there was statistically significant variation between different treatments and the untreated control. The plants inoculated with 10,000 and 1,000 second stage juveniles (J_2) of *M. incognita* recorded minimum height being 41 and 44 cm respectively. The effect of these two were statistically on par and showed significant difference from the 10 J_2 level and untreated control.

Table 1 Effect of different levels of *M. incognita* juveniles and *R. similis* on the height of *P. rosea* at different intervals after inoculation

Levels of inoculum	Mean height of plants (cm) at monthly intervals [months after inoculation (MAI)]										
a. *M. incognita*	1 MAI	2 MAI	3 MAI	4 MAI	5 MAI	6 MAI	7 MAI	8 MAI	9 MAI	10 MAI	11 MAI
0	39.00	46.00	48.00	54.80	56.60	61.60	57.00	56.80	56.40	54.20	54.80
10 J_2	38.40	46.20	49.60	53.40	56.60	56.60	53.00	54.60	48.60	47.80	47.80
100 J_2	36.60	44.40	48.40	50.60	54.60	55.60	51.00	50.60	45.80	47.20	47.20
1000 J_2	34.80	40.80	46.00	49.80	53.80	54.60	44.00	43.00	43.20	43.40	43.20
10000 J_2	34.00	40.20	41.40	45.60	53.80	53.40	41.00	40.20	40.80	39.60	39.60
CD (0.05)	NS	NS	NS	NS	NS	NS	11.268	9.582	9.241	7.982	6.521
b. *R. similis*											
0	41.80	44.00	56.00	56.80	57.40	62.20	62.60	63.60	64.60	65.20	65.00
10	41.40	44.60	54.20	55.00	57.00	62.40	63.00	63.60	65.00	64.00	63.40
100	44.20	51.00	53.00	55.00	56.00	56.60	57.80	57.40	60.60	61.60	62.00
1000	43.80	49.20	52.80	53.60	53.60	54.20	55.20	55.20	55.40	55.00	54.60
10000	44.00	46.00	50.60	50.60	50.60	51.40	52.20	52.20	51.60	50.80	50.00
CD (0.05)	NS	NS	NS	NS	NS	8.382	8.718	8.392	9.136	8.138	8.216

J_2 – Second stage juveniles of *M. incognita* NS – Not significant

The lower levels (10 and 100 J_2) also recorded reduction in plant height over the control but the reduction was not enough to get statistical significance.

The plant height recorded at eight months after inoculation also showed statistical significance. Minimum height was recorded by 10,000 J_2 inoculated plants (40.20 cm) and it was statistically on par with 1000 J_2 level (43.00 cm). These two were significantly different from 10 J_2 level and untreated control. Maximum height was recorded by untreated control (56.80 cm) followed by 10 J_2 (54.60 cm) and 100 J_2 (50.60 cm) levels respectively. Nine months after inoculation there was statistically significant variation between different treatments and untreated control. Minimum height was recorded by 10,000 J_2 level (40.80 cm) and was statistically on par with 1000 J_2 (43.20 cm) and 100 J_2 (45.80 cm). The 10 J_2 level also recorded reduction in height over control but was on par with untreated control (56.40 cm).

The plants inoculated with 10,000 and 1000 second stage juveniles of *M. incognita* (J_2) recorded minimum height of plants (39.60 and 43.40 cm respectively) after 10 months of inoculation. The effect of these two were statistically on par and significantly different from other lower levels (100 and 10 J_2) and untreated control. The lower inoculum levels (10 and 100 J_2) also reduced the plant height but the reduction was not enough to get statistical significance. The plant height recorded at 11 months after inoculation also showed statistical significance. Minimum height of the plants was recorded by 10,000 J_2 level (39.60 cm) and was statistically on par with 1000 J_2 level (43.20 cm). The effect of 10,000 J_2 was significantly different from the other two lower levels and untreated control. The inoculum levels 1000, 100 and 10 J_2 were also statistically on par showing significant variation from the untreated plants. Maximum plant height was recorded by untreated plants (54.8 cm) followed by 100

J_2 (47.2 cm) and 10 J_2 (47.8 cm) levels. At eleven months after inoculation the lowest inoculum level (10 J_2) also showed its pathogenic effect.

4.1.1.2 R. similis

The mean plant height (cm) at different intervals after inoculation of various levels of *R. similis* are presented in Table 1. There was no statistical significance in the height of plants upto five months after inoculation. Six months after inoculation onwards there was statistically significant variation between different treatments and the untreated control. At 10,000 level there was significant variation in the height of *P. rosea* over untreated control and recorded minimum height of 51.40 cm. The effect of this level was statistically on par with 1000 and 100 levels of *R. similis* and these levels recorded 54.20 and 56.60 cm respectively. The untreated control recorded a height of 62.60 cm.

Seven months after inoculation 10,000 level of *R. similis* recorded minimum height (52.20 cm) and showed statistically significant variation from the 10 level (62.40 cm) of *R. similis* and untreated control. The untreated control recorded a height of 62.60 cm. All other levels were statistically on par with the untreated control. At 10,000 level there was significant variation in the height of *P. rosea* over the untreated control (63.60 cm) and recorded a height of 52.20 cm after eight months of inoculation. All other levels were statistically on par with the untreated control.

Plants inoculated with 1000 and 10,000 *R. similis* recorded statistically significant variation over the untreated plants after nine months of inoculation and these two treatments were statistically on par. Plants inoculated with 10,000 and 1000 *R. similis* recorded 51.60 and 55.40 cm respectively and the untreated recorded 64.60 cm. The effect of other two levels (100 and 10) were statistically on par with the untreated plants.

Ten months after inoculation 10,000 and 1000 *R. similis* inoculated plants recorded 50.80 and 55.00 cm height respectively and these two treatments were statistically on par and significantly different from other two lower levels. The effect of 100 and 10 *R. similis* per plant on the height of *P. rosea* (61.60 and 64.00 respectively) were on par with the untreated plants (65.20 cm).

Eleven months after inoculation also the same trend was recorded. The effect of 1000 and 10,000 levels of *R. similis* were on par and recorded minimum height of 54.6 and 50.0 cm respectively.

4.1.2 Number of Leaves

4.1.2.1 M. incognita

The mean number of leaves recorded at different intervals after inoculation of various levels of *M. incognita* are presented in Table 2. There was no significant variation in the number of leaves of *P. rosea* recorded upto six months after inoculation. When the observations were taken seven months after inoculation, there was statistically significant variation in the number of leaves between different treatments and untreated control. Plants inoculated with 10,000 J_2 recorded minimum number of leaves (28.40) and was statistically on par with 1000 J_2 (33.6) and showed significant variation from the other lower levels and untreated control. Maximum number of leaves was recorded in untreated control (50). Eight months after inoculation minimum number of leaves was recorded in 10,000 J_2 level (28) and was statistically on par with 1,000 J_2 (32.4) and showed statistically significant variation from the other levels and untreated control. The effect of 1000 J_2 and 100 J_2 were statistically on par. Maximum number of leaves was recorded in untreated control (49.8).

Table 2 Effect of different levels of *M. incognita* juveniles and *R. similis* on the number of leaves of *P. rosea* at different intervals after inoculation

Levels of inoculum	Mean number of leaves at monthly intervals [months after inoculation (MAI)]										
a. *M. incognita*	1 MAI	2 MAI	3 MAI	4 MAI	5 MAI	6 MAI	7 MAI	8 MAI	9 MAI	10 MAI	11 MAI
0	19.40	27.20	31.40	29.40	33.80	37.60	50.00	49.80	54.20	54.80	53.80
10 J_2	22.40	28.60	31.80	33.80	35.00	36.60	45.00	47.60	47.20	47.20	47.20
100 J_2	21.00	28.40	27.60	26.60	30.20	32.60	44.20	43.80	39.40	40.80	38.60
1000 J_2	20.60	23.20	28.60	28.60	26.40	26.40	33.60	32.40	33.40	34.40	35.00
10000 J_2	22.60	24.00	26.40	26.40	29.60	29.60	28.40	28.00	27.20	26.60	25.00
CD (0.05)	NS	NS	NS	NS	NS	NS	13.685	13.472	12.423	8.741	7.442
b. *R. similis*											
0	47.40	63.00	80.20	79.20	83.40	95.60	102.20	104.00	104.40	104.40	101.60
10	44.00	63.60	77.60	79.60	73.60	84.00	85.40	91.20	93.00	94.00	95.40
100	44.80	62.00	77.40	80.40	82.60	91.60	88.60	89.60	90.40	90.60	86.00
1000	46.20	69.60	75.20	74.20	74.00	74.00	71.80	71.00	69.00	68.20	65.60
10000	46.60	68.00	65.40	69.80	68.80	59.80	59.20	60.00	58.40	57.00	56.00
CD (0.05)	NS	NS	NS	NS	NS	20.42	22.68	22.82	22.08	20.44	18.03

J_2 – Second stage juveniles of *M. incognita* NS – Not significant

Plants inoculated with 10,000, 1000 and 100 J_2 showed maximum reduction in number of leaves after nine months of inoculation and the effect of these levels were statistically on par and significantly different from the lowest level (10 J_2) and untreated control. Minimum number of leaves was recorded by 10,000 J_2 inoculated plants (27) followed by 1000 J_2 (33) and 100 J_2 (39). The uninoculated plants recorded maximum number of leaves (54). The plants inoculated with 10 J_2 level was statistically on par with 100 J_2 and showed reduction in the number of leaves (47 leaves as against 54 in control).

Ten months after inoculation of *M. incognita* juveniles there was statistically significant variation in the number of leaves. The effect of inoculation with 10,000 and 1000 J_2 were statistically on par and they recorded minimum number of leaves (26.6 and 34.4 respectively). The effect of inoculation with 1000 and 100 J_2 were also statistically on par. At 100 J_2 level there was slight reduction in the number of leaves from the untreated. Plants inoculated with 10 J_2 also showed numerical reduction (7.6) in production of leaves but the effect was statistically on par with untreated.

Plants inoculated with 10,000 J_2 recorded minimum number of leaves (25) after eleven months of inoculation and was significantly different from all other treatments. The effect of 1000 and 100 J_2 levels were statistically on par and recorded 35 and 38.6 leaves respectively. The plants inoculated with 10 J_2 also showed slight reduction in number of leaves over untreated plants but the effect was statistically on par with the untreated revealing that pathogenic effect initiated from 100 J_2 onwards.

4.1.2.2 R. similis

The mean number of leaves at different intervals after inoculation of *R. similis* are presented in Table 2. There was no significant difference in the number of leaves of *P. rosea* upto five months after inoculation.

Six months after inoculation there was significant variation in the number of leaves between different levels of *R. similis* inoculated plants and untreated. At levels of 1000 and 10,000 *R. similis*, *P. rosea* plants recorded minimum number of leaves (74 and 59.8 respectively) and the two levels were statistically on par. Plants inoculated with 10,000 *R. similis* showed significant variation from other two lower levels and untreated plants. The effect of 1000, 100 and 10 *R. similis* on *P. rosea* leaf number also was statistically on par. But 1000 *R. similis* inoculated plants only recorded statistically significant variation from the untreated plants. The levels of 100 and 10 *R. similis* were on par with untreated control plants revealing that they are not pathogenic at this level. After seven and eight months of inoculation also results showed the same trend.

Nine months after inoculation minimum number of leaves was recorded by 10,000 *R. similis* inoculated plants (58.4) followed by 1000 level (69.0) and these two levels were statistically on par and significantly different from the other two levels and untreated plants. The levels 100 and 10 *R. similis* also showed numerical reduction but were statistically on par with the untreated plants. The same trend was noticed ten and eleven months after inoculation. The plants inoculated with 10,000 *R. similis* recorded minimum number of leaves (56) after eleven months followed by 1000 *R. similis* (65.6) and these two levels were statistically on par and significantly different from the other two levels and untreated control plants. The levels 100 and 10 *R. similis* also showed numerical reduction in number of leaves (16 and 6 respectively) over the untreated but these two levels were statistically on par with the untreated plants.

4.1.3 Number of Branches

4.1.3.1 M. incognita

The mean number of branches at different intervals after inoculation of various levels of *M. incognita* juveniles (J_2) are presented

Table 3 Effect of different levels of *M. incognita* juveniles and *R. similis* on the number of branches of *P. rosea* at different intervals after inoculation

Levels of inoculum **a. *M. incognita***	Mean number of branches at monthly intervals [months after inoculation (MAI)]										
	1 MAI	2 MAI	3 MAI	4 MAI	5 MAI	6 MAI	7 MAI	8 MAI	9 MAI	10 MAI	11 MAI
0	3.60	4.40	4.60	5.40	6.60	7.00	6.40	6.60	6.60	6.80	7.00
10 J_2	3.00	3.80	4.00	4.40	5.80	5.80	5.40	5.40	6.00	6.00	7.00
100 J_2	3.20	4.20	4.20	5.60	6.00	6.20	6.20	6.20	5.80	5.80	5.80
1000 J_2	3.00	3.80	3.60	4.00	5.80	4.80	4.80	4.80	4.80	4.80	5.00
10000 J_2	3.20	3.60	3.40	4.80	5.60	4.40	4.40	4.40	4.40	4.40	3.60
CD (0.05)	NS	NS	NS	NS	NS	1.252	1.186	1.196	1.074	1.152	1.073
b. *R. similis* 0	5.80	8.00	8.60	9.00	9.40	10.20	10.40	11.20	11.20	10.80	10.80
10	4.80	7.80	8.00	8.60	9.00	10.00	10.00	10.40	10.60	10.60	10.40
100	5.60	8.00	8.40	8.60	8.80	9.60	10.20	10.40	10.20	10.00	9.80
1000	6.60	8.00	8.40	8.40	8.60	9.20	9.00	8.60	8.60	8.40	8.20
10000	5.80	7.20	7.80	8.40	8.50	9.00	9.20	9.20	8.80	8.40	8.20
CD (0.05)	NS	NS	NS	NS	NS	NS	NS	1.33	1.52	1.87	1.68

J_2 – Second stage juveniles of *M. incognita* NS – Not significant

in Table 3. There was no statistically significant variation in the number of branches of Chethikoduveli plants upto five months after inoculation eventhough there was numerical difference. The effect of inoculation on the production of branches was observed six months after inoculation onwards and showed statistically significant variation. Minimum number of branches was recorded by 10,000 J_2 (4.40) and the effect was statistically on par with 1000 J_2 (4.80) and showed significant variation from the untreated control. Maximum number of branches (7) were recorded in untreated control and this trend was continued upto eight months after inoculation. After nine months of inoculation minimum number of branches were recorded by 10,000 J_2 inoculated plants (4.4) followed by 1000 J_2 (4.8) and these two treatments were statistically on par. Plants inoculated with 100 and 10 J_2 showed numerical reduction in number of branches over the untreated but their effect was not enough to get statistical significance over the untreated revealing that these inoculum levels were not pathogenic to *P. rosea* regarding the production of branches. Ten months after inoculation also the result showed the same trend.

When the observations were recorded at eleven months after inoculation, the plants inoculated with 10,000 J_2 recorded minimum number of branches (3.6) and was significantly different from the rest of the treatments revealing that 10,000 J_2 level was highly pathogenic to these plants when compared to the lower 1000 J_2. The effect of 1000 and 100 J_2 were statistically on par and recorded 5 and 5.8 branches respectively. Plants inoculated with 10 J_2 and untreated plants showed same number of branches (7) revealing that this inoculum level was not pathogenic.

4.1.3.2 R. similis

The mean number of branches at different intervals after inoculation of *R. similis* are presented in Table 3. There was no statistically significant variation in the number of branches of *P. rosea* upto seven months after inoculation. After eight months of inoculation there was statistically significant variation.

Eight months after inoculation of *R. similis* minimum number of branches was recorded by 1000 *R. similis* inoculated plants (8.6) followed by 10,000 *R. similis* (9.2) and these two levels were statistically on par and showed significant variation from the untreated control plants. The levels 10,000 was on par with 100 and 10 levels also.

Nine months after inoculation of *R. similis*, minimum branch number was recorded by 1000 *R. similis* inoculated plants (8.6) followed by 10,000 *R. similis* (8.8) and these two levels were statistically on par and significantly different from untreated control plants. Maximum number of branches were recorded in untreated plants (10.8).

Plants inoculated with 10,000 and 1000 *R. similis* recorded minimum number of branches (8.4) at 10 months after inoculation and they showed significant variation from the untreated plants and the lowest inoculum level of 10. The levels 100 and 10 *R. similis* were statistically on par with the untreated plants which recorded 10.8 branches.

Eleven months after inoculation minimum number of branches was recorded by 10,000 and 1000 levels (8.2) and showed statistically significant variation from the level '10' and untreated plants. These two higher levels of *R. similis* were statistically on par with next lower level 100 (9.8). The levels 100 and 10 were statistically on par with untreated plants which recorded maximum number of branches (10.8).

Table 4 Effect of different levels of *M. incognita* juveniles and *R. similis* on the biometric characters of *P. rosea* at the time of harvest (twelve months after inoculation)

Levels of inoculum **a. *M. incognita***	Plant height (cm)	Number of branches	Number of leaves	Leaf area (mm^2)
0	53.4	7.6	50.2	1524.5
10 J_2	49.4	7.0	46.4	1362.8
100 J_2	47.0	5.8	37.8	1215.0
1000 J_2	43.0	5.0	34.0	1147.0
10000 J_2	38.6	3.6	23.6	991.0
CD (0.05)	5.53	1.59	9.84	122.63
b. *R. similis* 0 level	64.0	10.8	98.2	1510.2
10 *R. similis*	62.0	10.4	93.4	1401.2
100 *R. similis*	61.6	9.6	87.0	1314.6
1000 *R. similis*	53.0	8.2	63.2	1261.0
10000 *R. similis*	47.8	8.0	53.8	1090
CD (0.05)	7.87	1.49	15.88	116.84

J_2 – Second stage juveniles of *M. incognita*

4.1.4 Biometric Characters at the Time of Harvest (Twelve months after inoculation)

4.1.4.1 M. incognita

The results presented in Table 4 revealed that minimum plant height was recorded by 10,000 J_2 inoculated plants (38.6 cm) followed by 1000 J_2 inoculated plants (43 cm) and these two treatments were statistically on par. The pathogenic effect of 1000 and 100 J_2 inoculated plants were also statistically on par. Plant inoculated with 10 J_2 also showed numerical reduction in plant height (4 cm) over untreated but was statistically on par with untreated.

Regarding the number of branches, minimum was recorded by 10,000 J_2 inoculated plants followed by 1000 J_2 and these two treatments were statistically on par. Plants inoculated with 100 J_2 recorded 5.8 branches and was statistically on par with 10 J_2 (7) and untreated plants (7.6).

In the case of number of leaves maximum reduction was recorded in 10,000 J_2 inoculated plants (26.6) and was statistically on par with 1000 J_2 inoculated plants (16.2). The treatments 1000 J_2 and 100 J_2 were also statistically on par and these treatments were significantly different from untreated control. Maximum number of leaves was recorded by uninoculated plants (50.2) followed by 10 J_2 inoculated plants (46.4) and these two treatments were statistically on par.

Regarding the leaf area of *P. rosea*, minimum was recorded by 10,000 J_2 inoculated plants (991 mm^2) followed by 1000 J_2 (1147 mm^2). Plants inoculated with 10,000 J_2 showed statistically significant variation in leaf area over the rest of the treatments. Treatments 1000 J_2 and 100 J_2 were statistically on par. Maximum leaf area was recorded in control plants (1524.5 mm^2) followed by 10 J_2 (1362.8 mm^2). But 10 J_2 level was

significantly different from untreated plants revealing its pathogenic effect on *P. rosea*.

4.1.4.2 R. similis

Data presented in Table 4 revealed the following results. After twelve months of inoculation, minimum height of *P. rosea* (47.80 cm) was recorded in 10,000 *R. similis* inoculated plants followed by 1000 *R. similis* (53.00 cm) and these two levels were statistically on par and significantly different from the other two lower levels and untreated control. The other two levels (100 and 10) and untreated recorded 61.60, 62.00 and 64.00 cm respectively.

Regarding the number of branches minimum number of branches were recorded by 10,000 *R. similis* inoculated plants (8.0) followed by 1000 *R. similis* (8.2) and 100 *R. similis* (9.6) and these three levels were statistically on par. The higher levels 10,000 and 1000 showed significant variation from the lower levels and untreated plants. The levels 100 *R. similis* (9.6) and 10 *R. similis* (10.4) were statistically on par with untreated plants.

In the case of number of leaves minimum was recorded by 10,000 *R. similis* inoculated plants (53.8) and was on par with 1000 level (63.2) and these two levels showed statistically significant variation from the other two levels and untreated plants. The levels 100 *R. similis* (87) and 10 *R. similis* (93.4) were also statistically on par with untreated plants with 98 leaves per plant.

The leaf area was minimum in plants inoculated with 10,000 *R. similis* (1090 mm^2) and showed statistically significant variation from all other levels and untreated plants. The leaf area of plant inoculated with 1000 and 100 *R. similis* were statistically on par and recorded 1314.6 mm^2 and 1261 mm^2 leaf area respectively. The effect of inoculation of

Table 5 Effect of different levels of *M. incognita* and *R. similis* on the fresh and dry weight of shoots and roots of *P. rosea* at the time of harvest (twelve months after inoculation)

Levels of inoculum **a.** *M. incognita*	Fresh weight of shoot (g)	Fresh weight of root (g)	Dry weight of shoot (g)	Dry weight of root (g)	Mean weight Fresh : Dry	
					Shoot	Root
0	67.0	46.0	23.0	12.4	2.91 : 1	3.7 : 1
10 J_2	56.4	41.0	21.8	11.8	2.58 : 1	3.47 : 1
100 J_2	47.6	37.4	17.2	11.6	2.76 : 1	3.22 : 1
1000 J_2	30.0	33.0	9.6	9.6	3.12 : 1	3.43 : 1
10000 J_2	18.0	29.0	6.2	8.8	2.90 : 1	3.29 : 1
CD (0.05)	9.83	6.65	4.53	2.42		
b. *R. similis* 0	117.0	99.0	34.0	26.0	3.44 : 1	3.81 : 1
10	78.0	77.0	22.0	20.0	3.54 : 1	3.85 : 1
100	64.0	65.0	21.0	19.0	3.04 : 1	3.42 : 1
1000	53.0	49.0	15.0	14.0	3.53 : 1	3.50 : 1
10000	42.0	34.0	11.0	9.2	3.82 : 1	3.69 : 1
CD (0.05)	8.52	15.35	4.55	5.32		

J_2 – Second stage juveniles of *M. incognita*

100 and 10 *R. similis* were on par and also the effect of 10 *R. similis* (1401.2 mm^2) was on par with untreated plants (1510.2 mm^2).

4.1.5 Fresh and Dry Weight of Shoots and Roots

4.1.5.1 M. incognita

The effect of different levels of *M. incognita* on the fresh and dry weight of shoots and roots of *P. rosea* at the time of harvest are presented in Table 5.

The data presented in Table 5 showed statistically significant variation in the shoot weight (fresh) of *P. rosea* between various levels of *M. incognita*.

Regarding fresh weight of shoot, minimum was recorded in 10,000 J_2 inoculated plants (18 g) and was significantly different from all other treatments. Plants inoculated with 1000 J_2 of *M. incognita* recorded 30 g fresh weight of shoot which was also showed significant variation from all other lower levels and untreated control. At 10 J_2 level also there was statistically significant variation over the untreated which recorded 56.4 g and the effect was on par with 100 J_2 level (47.6 g) revealing that 10 J_2 level onwards *M. incognita* is pathogenic. Maximum fresh shoot weight was recorded by uninoculated plants (67 g).

The fresh weight of roots also showed significant variation between different levels of *M. incognita* inoculated plants and untreated control plants and the results are presented in Table 5.

Minimum fresh weight of root was recorded by 10,000 J_2 inoculated plants (29 g) and was statistically on par with 1000 J_2 inoculated plants (33 g). The effect of 1000 J_2 and 100 J_2 were also on par. Maximum fresh weight of root was recorded by uninoculated plants (46 g) and was on par with lowest inoculum level of 10 J_2 (41 g), thus 10 J_2 level was not pathogenic.

In the case of dry weight of shoot minimum weight was recorded in 10,000 J_2 inoculated plants (6.2 g) and was on par with 1000 J_2 (9.69 g). Plants inoculated with 100 J_2 level recorded 17.2 g dry weight which showed statistically significant variation from the rest of the treatments. Maximum dry shoot weight was recorded by uninoculated plants (23.8 g) followed by the lowest level, 10 J_2 (21.8 g) and the effects were statistically on par revealing that 10 J_2 level is not pathogenic.

Regarding the dry weight of root, minimum (8.8 g) was recorded by 10,000 J_2 inoculated plants and was statistically on par with 1000 J_2 inoculated plants (9.6 g). The effect of 1000 J_2 and 100 J_2 inoculated plants were also statistically on par. Maximum dry weight of root was recorded in uninoculated plants (12.4 g). The effect of 10 J_2 and 100 J_2 were on par with untreated revealing that these two levels were not pathogenic. In the case of the fresh dry weight ratio of roots and shoots there was no statistically significant variation between different treatments and the fresh dry weight ratio ranged between 2.58 : 1 to 3.12 : 1 for shoots and 3.22 : 1 to 3.7 : 1 for roots.

4.1.5.2 R. similis

The effect of different levels of *R. similis* on the fresh and dry weight of shoots and roots of *P. rosea* at the time of harvest are presented in Table 5.

The result showed statistically significant variation in the fresh weight of *P. rosea* shoot between various levels of *R. similis* inoculated plants. Minimum was recorded in 10,000 *R. similis* inoculated plants (42 g) followed by 1000 (53. g) and 100 (64 g). Maximum fresh shoot weight was recorded by untreated plants (117 g) followed by 10 *R. similis* inoculated plants (78 g). All the levels showed statistically significant variation between each other and also from the untreated plants.

The fresh weight of roots showed significant variation between different levels of *R. similis* inoculated plants and untreated plants. Minimum fresh weight of root was recorded by 10,000 *R. similis* inoculated plants (34 g) and was statistically on par with 1000 *R. similis* inoculated plants (49 g). The effect of 100 and 10 *R. similis* inoculation was on par. Maximum fresh weight of root was recorded by uninoculated plants (99 g).

In the case of dry weight of shoot, minimum was recorded in plants inoculated with 10,000 *R. similis* (11 g) and was statistically significant from other levels and untreated. Next lower level 1000 *R. similis* inoculation (15.0 g) was also significantly different from all other lower and higher levels and untreated plants. The dry weight of shoot (21 and 22 g) recorded in 100 and 10 *R. similis* inoculated plants respectively were statistically on par and significantly different from untreated control plants (34 g) indicating that pathogenic effect initiated from 10 *R. similis* inoculation onwards.

Regarding the dry weight of root minimum was recorded by 10,000 *R. similis* inoculated plants (9.2 g) and was statistically on par with 1000 *R. similis* inoculated plants (14 g). Statistically the effect of 1000 and 100 *R. similis* inoculated plants were also on par. Maximum dry weight of roots was recorded by uninoculated plants (26 g). The effect of 100 and 10 *R. similis* were on par and showed significant variation from the untreated plants revealing the pathogenic effect of *R. similis* from lowest level onwards.

In the case of fresh dry weight ratio of roots and shoots there was no statistically significant variation between different levels of *R. similis* and fresh–dry weight ratio ranged from 3.04:1 to 3.82 : 1 for shoots and from 3.42 : 1 to 3.85 : 1 for roots.

Table 6 Effect of different levels of *M. incognita* and *R. similis* on the yield of *P. rosea* and the recovery of nematodes at the time of harvest (twelve months after inoculation)

Levels of inoculum **a. *M. incognita***	Length of tubers (cm)	Width of tubers (cm)	Number of tubers	Yield per plant (g)	Soil population (100g)	Root population (10 g root)	Root knot count (one gram)	Root knot index
0	36.00	0.64	7.00	46.00	0 (1)	0 (1)	0 (1)	0
10 J_2	34.20	0.48	6.40	41.00	12.26 (4.718)	13.6 (3.794)	0.18 (1.086)	0
100 J_2	28.00	0.46	5.00	37.40	28.96 (5.476)	24.60 (5.044)	2.66 (1.913)	1
1000 J_2	25.60	0.38	4.20	33.00	55.40 (7.51)	114.00 (10.70)	24.05 (50.004)	5
10000 J_2	22.20	0.32	3.00	29.00	72.79 (8.588)	521.6 (22.836)	33.36 (5.667)	5
CD (0.05)	4.23	0.15	0.98	6.65	0.70	0.98	0.25	
b. *R. similis*							**Number of lesions**	**Lesion index**
0	34.00	0.84	7.80	99.00	0 (1)	0 (1)	0 (1)	0
10	29.80	0.76	6.20	77.00	17.6 (4.48)	13.2 (3.76)	2.0 (1.72)	1
100	25.40	0.64	5.60	65.00	34.4 (5.94)	23.8 (4.97)	5.6 (2.57)	2
1000	23.00	0.54	3.60	49.00	50.6 (7.17)	31.0 (5.65)	7.6 (2.93)	2
10000	20.20	0.44	3.20	34.00	67.0 (8.24)	40.4 (6.43)	9.2 (3.49)	3
CD (0.05)	5.22	0.194	1.35	15.35	(0.73)	(0.35)	(0.85)	

J_2 – Second stage juveniles of *M. incognita* Figures in parenthesis are $\sqrt{x + 1}$ transformed values

4.1.6 Yield

4.1.6.1 M. incognita

The effect of different levels of *M. incognita* on yield and yield characteristics of *P. rosea* were presented in Table 6.

Lowest yield in terms of weight of tubers was recorded by 10,000 J_2 inoculated plants (29 g) and was statistically on par with 1000 J_2 inoculated plant. The effect of 100 J_2 and 1000 J_2 inoculated plants were statistically on par giving 37.4 g and 33 g tuber per plant. Maximum yield was recorded by uninoculated plants with 46 g tuber per plant (Plate 1) followed by 10 J_2 (41 g) inoculated plants.

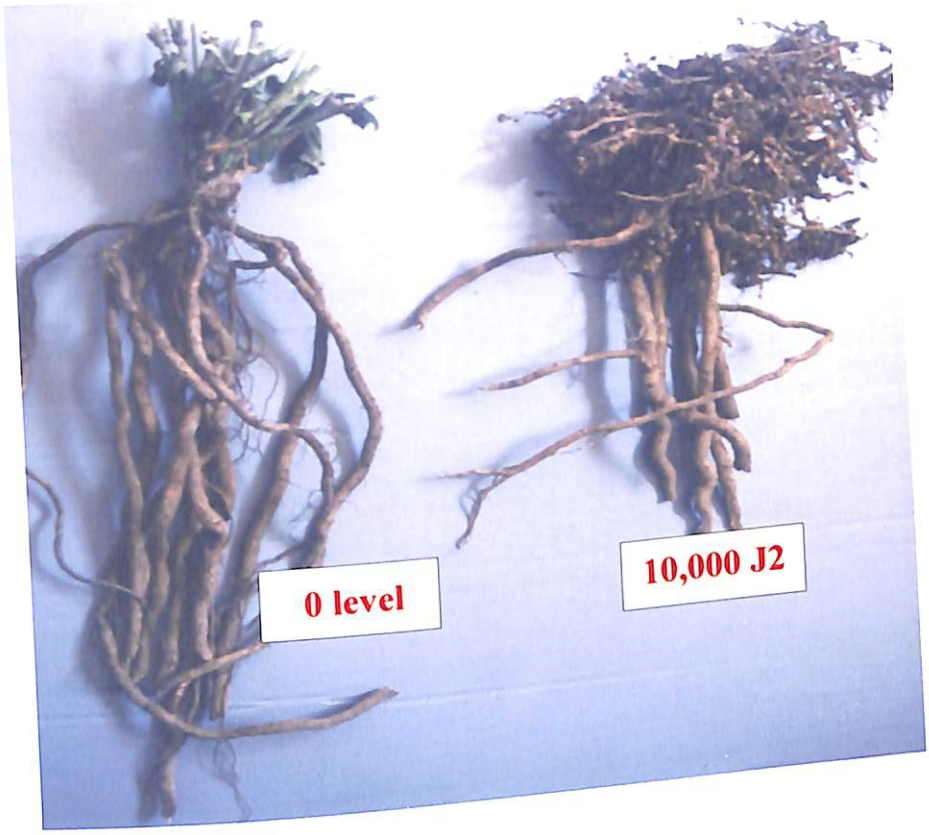
Regarding the length of tubers minimum was recorded in 10,000 J_2 inoculated plants (22.2 cm) and was on par with 1000 and 100 J_2 inoculated plants which recorded 25.6 and 28 cm respectively. Maximum tuber length was recorded by uninoculated plants (36 cm) followed by 10 J_2 (34.2 cm) and the effect was on statistically par, recording that 10 J_2 is not sufficient to reduce the tuber length of *P. rosea*.

The tuber width was minimum in 10,000 J_2 inoculated plants (0.32 cm) and was statistically on par with 1000 J_2 (0.38 cm) and 100 J_2 levels (0.46 cm). Maximum tuber width was recorded by uninoculated plants (0.64 cm) followed by 10 J_2 (0.48 cm). The plants inoculated with 10 J_2 showed statistically significant reduction in width of tubers from the untreated plants giving an indication that 10 J_2 onwards *M. incognita* is pathogenic to *P. rosea*.

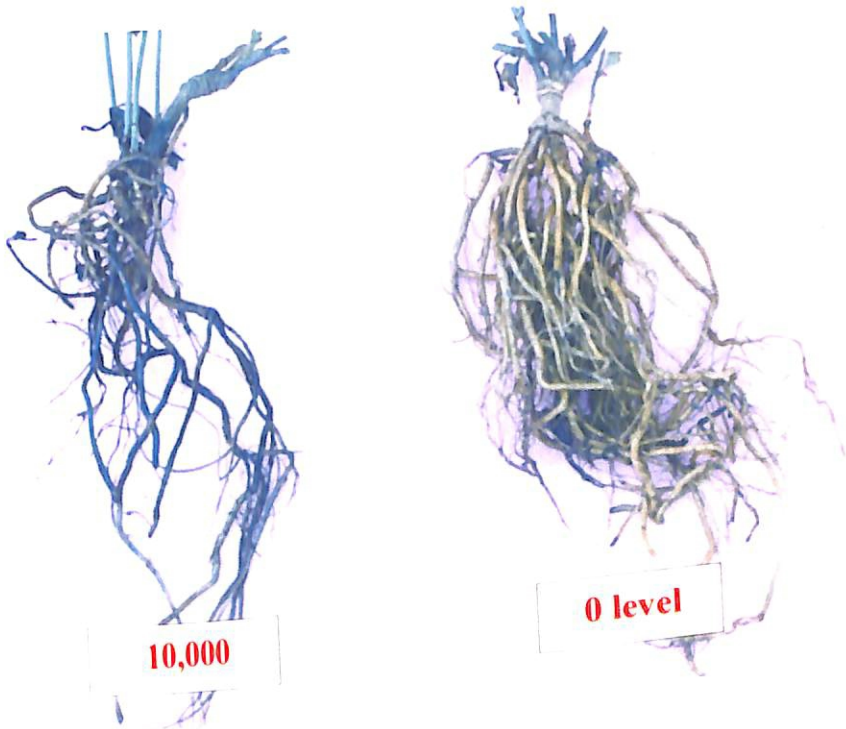
Regarding the number of tubers, minimum number was recorded by 10,000 J_2 inoculated plants (3.0) and was significantly different from rest of the treatments. The effect of 1000 and 100 J_2 inoculated plants were statistically on par giving an average tuber number of 4.2 and 5.0 respectively and was significantly different from the lower and higher inoculum levels and untreated plants. There was no significant variation

Plate 1

a. Effect of different levels of *Meloidogyne incognita* on the tuber yield of Chethikoduveli, *Plumbago rosea*



b. Effect of different levels of *Radopholus similis* on the tuber yield of Chethikoduveli, *Plumbago rosea*



in 10 J_2 and untreated plants indicating that 10 J_2 level was not pathogenic to *P. rosea* in the case of tuber number.

4.1.6.2 R. similis

The effect of different levels of *R. similis* on the yield attributing characters and yield of *P. rosea* were presented in Table 6.

Minimum yield was recorded by 10,000 *R. similis* inoculated plants (34 g) and was statistically on par with 1000 *R. similis* inoculated plants (49 g) and these two levels showed statistically significant variation from other two levels and untreated plants. The effect of 100 *R. similis* and 10 *R. similis* were statistically on par giving 65 g and 77 g tuber per plant. Maximum yield was recorded by uninoculated plants with 99 g tuber per plant (Plate 1).

Regarding the length of tuber minimum tuber length was recorded in 10,000 *R. similis* inoculated plants (20.2 cm) and was on par with 1000 *R. similis* (23 cm) and 100 *R. similis* (25.4 cm). The levels 10,000 and 1000 showed significant variation from other two levels and untreated control. Maximum tuber length was recorded by untreated plants (34 cm) followed by 10 *R. similis* (29.8 cm).

The tuber width was minimum in 10,000 *R. similis* inoculated plants (0.44 cm) and was statistically on par with 1000 *R. similis* inoculated plants (0.54) and the level 10,000 was significantly different from other two lower levels and untreated control plants. The levels 1000 *R. similis* and 100 *R. similis* were statistically on par and level 100 recorded a tuber width of 0.64 cm. The level 1000 was significantly different from 10 *R. similis* and untreated control plants. Maximum tuber width was recorded by uninoculated plants (0.84 cm) followed by the level 10 *R. similis* (0.76 cm) and these two were statistically on par.

Minimum mean number of tubers was recorded by 10,000 *R. similis* inoculated plants (3.2) and was statistically significant from other two

levels and untreated control plants. The levels 100 and 10 *R. similis* were statistically on par and recorded a mean tuber number of 5.6 and 6.2 respectively. These two levels also showed significant variation from the untreated plants (7.8).

4.1.7 Nematode Population

4.1.7.1 M. incognita

Data presented in Table 6 revealed statistically significant variation in *M. incognita* population in soil at different levels of inoculation at the time of harvest (12 months after inoculation). Regarding population of *M. incognita* in soil, maximum nematode recovery was recorded in 10,000 J_2 inoculated pots (73 per 100 g soil) and it showed a statistically significant superiority over the lower levels. Plants inoculated with 1000 J_2 recorded 55 nematodes per 100 g soil and was significantly different from rest of the treatments. At 100 J_2 level the mean nematode recovery from 100 g soil was 29 and at 10 J_2 it was only 14 per 100 g soil. The recovery of nematode from soil showed statistically significant variation in lowest level of (10 J_2) inoculation also.

Regarding the root population all the treatments were significantly different from others and maximum root population was recorded by 10,000 J_2 inoculated plants (521.6 / 10 g root sample) and was significantly different from all other inoculum levels. This was followed by 1000 J_2 (114.0), 100 J_2 (24.6) and 10 J_2 (13.6) inoculated plants respectively.

Data presented in the Table 6 showed that there was statistically significant variation in root-knot count of different treatments. Root-knot count was maximum in 10,000 J_2 inoculated plants (33.36 / g of root) followed by 1000 J_2 (24.05 / g of root). At 100 J_2 level the root-knot count was 2.66 / g of root and at 10 J_2 level it was only 0.18. Maximum root-knot index of five was recorded in 1000 and 10,000 J_2 level.

4.1.7.2 R. similis

Data presented in Table 6 revealed statistically significant variation in *R. similis* population in soil at different levels of inoculation at the time of harvest of *P. rosea* (12 months after inoculation).

Regarding the population of *R. similis* in soil, maximum nematode recovery was obtained at an initial inoculum of 10,000 level (67.0). This showed a statistically significant superiority over other levels. Plants inoculated with 1000 *R. similis* recorded an average of 50.6 nematodes per 100 g soil sample and was significantly different from all other levels and untreated control. At 100 *R. similis* level the mean nematode recovery from soil was 34.4 per 100 g sample and at 10 *R. similis* level it was only 13.2 per 100 g soil. The reduction in nematode population was noticed in accordance with the initial inoculum level.

Regarding the nematode population in root, all the treatments were significantly different from others and maximum population was recorded in 10,000 *R. similis* inoculated plants (40.4) and was significantly different from all other inoculum levels. This was followed by 1000 (31), 100 (23.8) and 10 *R. similis* (13.2) inoculated plants respectively.

Data presented in Table 6 showed that there was statistically significant variation in the number of lesions at different inoculum levels of *R. similis* and was maximum in 10,000 level (9.2 / g of root) followed by 1000, 100 and 10 levels with a mean lesions of 7.6, 5.6 and 2.0 per g of root respectively.

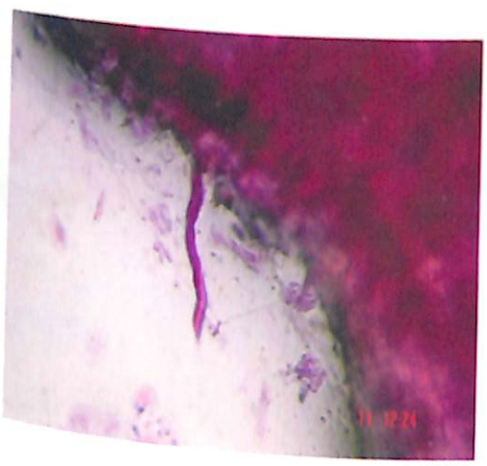
Regarding the lesion index maximum was recorded at 10,000 level (3) followed by 1000 (2), 100 (2) and 10 (1) levels.

4.2 HISTOPATHOLOGY

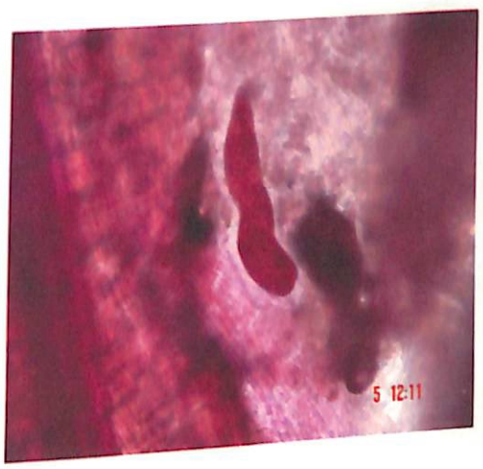
The nematode (*M. incognita* and *R. similis*) infested roots of Chethikoduveli were taken at five days interval after inoculation, for 45 days and these roots were cut into small bits and processed for microtomy

Plate 2

Histopathological changes due to *Meloidogyne incognita* on Chethikoduveli, *Plumbago rosea*

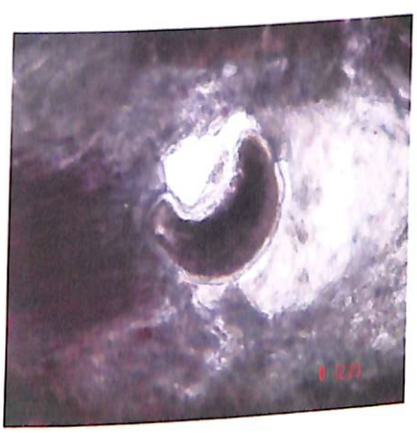


A. Entry of J_2

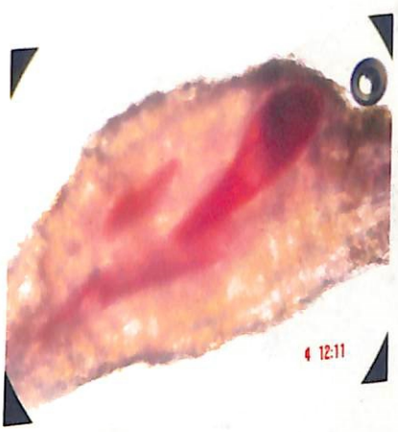


B. Sausage stage

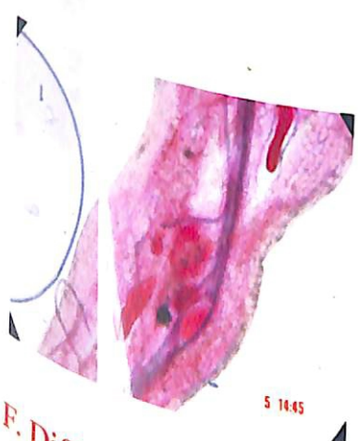
C. Feeding on vascular bundles



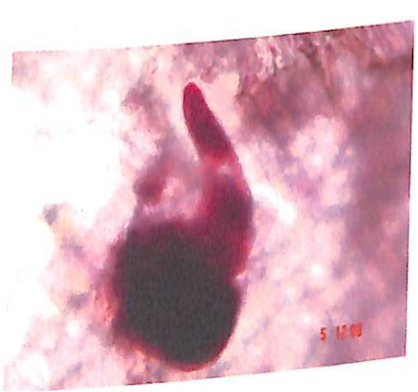
D. Giant cell formation



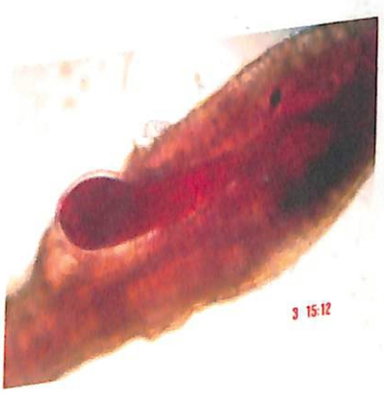
E. Gall formation



F. Differ stages



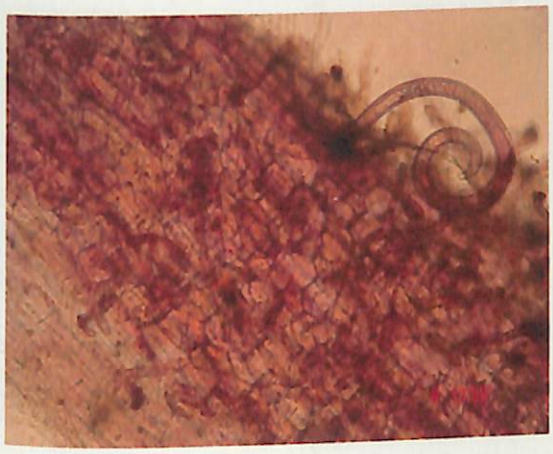
G. Pre-adult stage



H. Adult female

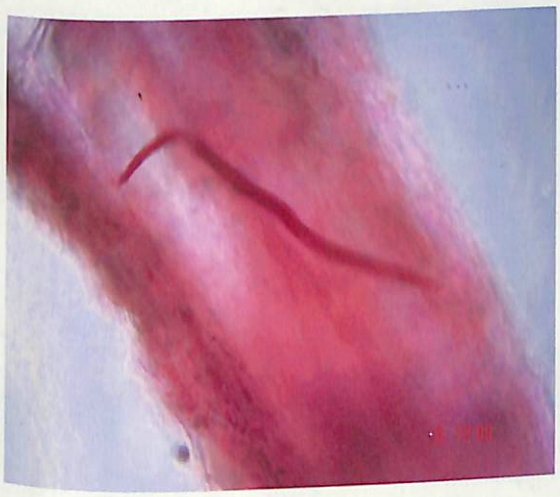
Plate 3

Histopathological changes due to *Radopholus similis* in Chethikoduveli, *Plumbago rosea*

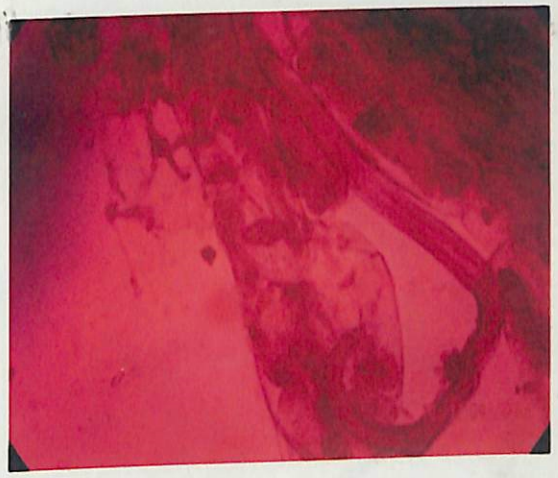


A. Entry of nematode

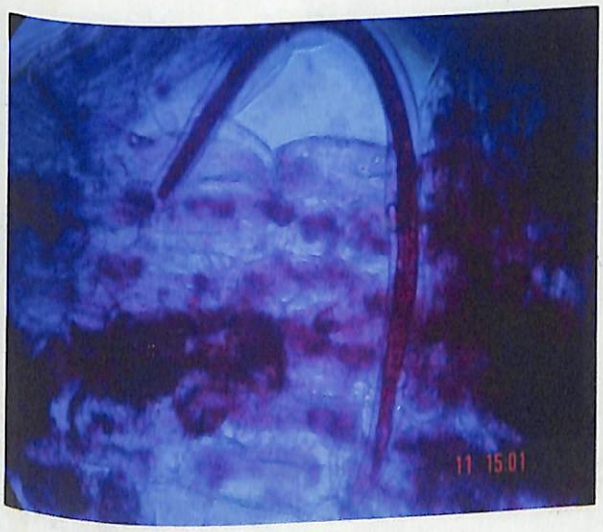
B. Migration



C. Intracellular orientation



D. Cavity formation



E Cellular disintegration.

E. Burrow formation with necrosis

as mentioned in para 3.1.3. Transverse and longitudinal sections were taken and examined under the microscope.

4.2.1 *M. incognita*

After five days of inoculation, it was observed that the second stage juveniles of *M. incognita* penetrated the root tissues and established its feeding cite on the vascular parenchyma (Plate 2). The head of the nematode was seen directed towards the vascular region. The endodermal, pericycle, xylem and phloem cells in the vicinity of nematode head undergone hypertrophy and hyperplasia during 10 to 15 days. The tissues around the nematode head became enlarged giant cell and was noticed during 15 to 25 days (Plate 2). During this period roots grew axially and formed galls due to hypertrophy and hyperplacia. The adult females become sac like and the back portion of the nematode touched the surface of the root and they produced egg mass in a gelatinous matrix which protruded out from the root surface.

4.2.2 *R. similis*

Longitudinal and transverse sections of *R. similis* infested *Plumbago* roots revealed considerable damage to root tissues. After five days of inoculation, the nematodes entered the root and started feeding in the cortical region. After ten days, longitudinal burrows (Plate 3 D) were developed underneath the outer cortical cell layer. The presence of nematodes in the inter and intra-cellular positions were also observed (Plate 3 E). After fifteen days necrotic regions were formed around the head of the nematode and burrows harbouring them. Burrows formed due to the disintegration of cytoplasm and coalescence of cells were also observed after fifteen days. The cavities coalesce and breakdown leading to tunnel formation (Plate 3F).

Table 7 Effect of different levels of *M. incognita* and *R. similis* on plumbagin, phenol and total free amino acid content of roots of *P. rosea* at the time of harvest (12 months after inoculation)

Level of inoculum **a. *M. incognita***	Plumbagin (ppm)	Percentage changes over control	Phenol (mg / g)	Percentage changes over control	Total free amino acids (µg equivalent of leucine)	Percentage changes over control
0	3977.66	-	0.193	-	0.25	
10 J_2	3960.33	-0.43	0.266	+37.82	0.28	12.00
100 J_2	3867.00	-2.78	0.320	+65.80	0.36	44.00
1000 J_2	3672.33	-7.67	0.318	+64.76	0.49	96.00
10000 J_2	3966.66	-0.28	0.296	+53.36	0.79	216.00
b. *R. similis*						
0	3977.66	-	0.082	-	0.25	-
10	3977.00	-	0.087	+6.09	0.27	+8.00
100	4084.00	+2.69	0.118	+43.90	0.31	+24.00
1000	3944.33	-0.82	0.153	+86.58	0.51	+104.00
10000	4121.00	+3.62	0.130	+58.53	0.85	+240.00

J_2 – Second stage juveniles of *M. incognita*
+ indicates percentage increase over control
- indicates percentage reduction over control

4.3 BIOCHEMICAL CHANGES

Biochemical changes caused by various levels of *M. incognita* and *R. similis* are presented in Table 7. The changes in phenol, plumbagin and total free amino acids due to infestation by the above nematodes were studied and presented here. The percentage changes in plumbagin, phenol and total free amino acids are also presented.

4.3.1 Plumbagin

4.3.1.1 M. incognita

The effect of different levels of *M. incognita* on the plumbagin content of *P. rosea* revealed that there was no significant variation between different levels. But slight reduction in the plumbagin content was noticed in different levels of *M. incognita* inoculated plants. Maximum reduction of 7.67 per cent was recorded in 1000 J_2 level. This was followed by the 100 J_2 level with 2.78 per cent. The reduction in plumbagin content in the levels of 10 and 10,000 J_2 inoculated plants was negligible (less than one per cent).

4.3.1.2 R. similis

The results showed that there was no significant variation in the plumbagin content of *P. rosea* at different levels of inoculation. The levels 100 and 10,000 recorded slight increase in the plumbagin content of *P. rosea* over the control and the percentage increase was only 2.69 and 3.62 respectively. The other two levels showed slight variation (less than one per cent).

4.3.2 Phenol

4.3.2.1 M. incognita

The effect of different levels of *M. incognita* on the phenol content of *P. rosea* revealed that maximum content was recorded in 100 J_2 inoculated plants (0.320 mg/g of root) followed by 1000 J_2 (0.318 mg g^{-1})

and 10,000 J_2 (0.296 mg g^{-1}). Minimum phenol content was recorded in uninoculated plants (0.193 mg g^{-1}) and 10 J_2 inoculated plants (0.266 mg g^{-1}). The phenol production was increased with increase in initial inoculum level.

The percentage increase in the phenol content of *P. rosea* over uninoculated were 37.82, 65.8, 64.76 and 53.36 for 10, 100, 1000 and 10,000 J_2 inoculated plants respectively.

4.3.2.2 R. similis

In the case of *R. similis* maximum phenol content was recorded by 1000 level inoculated plants (0.153 mg g^{-1} of root) followed by 10,000 and 100 level with 0.130 mg g^{-1} and 0.118 mg g^{-1} respectively. Minimum was recorded by uninoculated plants (0.082 mg g^{-1}).

The percentage increase in the phenol content of *P. rosea* at different levels of *R. similis* over uninoculated plants were 6.09, 43.9, 86.58 and 58.53 for 10, 100, 1000 and 10,000 *R. similis* inoculated plants respectively. In 10,000 level there was slight reduction in phenol production due to *R. similis* infestation.

4.3.3 Total Free Amino Acids

4.3.3.1 M. incognita

The variation in the total free amino acid content of *P. rosea* at different levels of *M. incognita* were presented in Table 7. There was considerable increase in the free amino acid content as the level of inoculation increase from 0 to 10,000 J_2. Maximum free amino acid was present in 10,000 J_2 inoculated plants (0.79 µg equivalent of leucine) followed by 1000 J_2 (0.49 µg), 100 J_2 (0.36 µg) and 10 J_2 (0.28 µg) respectively.

The percentage increase in the total free amino acids at different levels of inoculation were 12, 44, 96 and 216 for 10, 100, 1000 and 10,000 J_2 respectively.

4.3.3.2 R. similis

The data presented in Table 7 revealed that there was considerable increase in the total free amino acid content of *P. rosea* at various inoculum levels of *R. similis*. Maximum production was recorded at 10,000 level with 0.85 µg followed by 1000 level (0.51 µg), 100 level (0.31 µg) and 10 level (0.27 µg). Uninoculated plants recorded minimum total free amino acids (0.25 µg).

The plants inoculated with 10,000 *R. similis* showed more than cent per cent (240) increase over the uninoculated and this was followed by 1000 level (104 per cent increase) and 100 level (24 per cent increase). Minimum increase in total free amino acids was recorded at 10 *R. similis* per plant (8 per cent).

The rate of production of phenol and total free amino acids were high in *R. similis* infection while the rate of plumbagin production was less in *R. similis* when compared to *M. incognita*.

4.4 CROP LOSS ASSESSMENT

Crop loss incurred by root-knot nematode, *M. incognita* and burrowing nematode, *R. similis* in Chethikoduveli, *P. rosea* were studied in micro plot conditions and the results were presented in Tables 8 to 14.

The crop loss due to different levels of *M. incognita* and *R. similis* (100, 1000, 10,000, 1000 J_2 + carbosulfan and untreated) on *P. rosea* were studied and presented in Tables 8 to 14. The effect on the loss in plant height, number of leaves, branches, leaf area, number, length and width of tubers, tuber yield in terms of fresh weight and fresh and dry weight of shoots and roots were studied. The nematode population in soil and root were also estimated using standard procedures and presented in Table 14.

Table 8 Effect of different levels of *M. incognita* and *R. similis* on the height of *P. rosea* at different intervals after inoculation

Levels of inoculum	Mean height of plants (cm) at monthly intervals [months after inoculation (MAI)]											
a. *M. incognita*	1 MAI	2 MAI	3 MAI	4 MAI	5 MAI	6 MAI	7 MAI	8 MAI	9 MAI	10 MAI	11 MAI	CD (0.05)
0	30.00	35.60	41.60	46.80	53.40	59.80	61.00	61.20	60.60	59.00	60.60	7.021
100 J_2	30.40	38.20	37.60	44.00	47.40	54.00	54.00	54.20	54.00	52.00	55.40	8.152
1000 J_2	26.60	33.60	40.20	44.60	47.60	51.60	49.80	50.00	51.60	48.60	50.00	6.182
10000 J_2	30.60	32.80	35.00	41.50	45.60	48.00	49.60	47.40	46.50	44.20	45.80	10.821
1000 J_2 + Carbosulfan	29.40	34.20	40.20	46.40	49.20	56.00	57.40	57.00	59.40	57.40	62.20	6.601
CD (0.05)	NS	NS	NS	NS	NS	6.326	6.582	6.672	6.521	7.962	7.334	
b. *R. similis*												
0	28.40	33.40	38.60	40.00	41.20	42.00	43.80	45.80	47.20	48.60	50.00	2.062
100	25.20	26.40	29.60	29.60	30.80	31.80	33.40	33.80	35.80	36.80	38.40	1.341
1000	24.60	26.00	23.80	25.00	26.20	27.00	28.60	30.80	32.80	34.00	35.60	1.213
10000	22.80	22.00	24.60	24.40	24.80	22.60	24.40	26.40	27.20	28.20	30.00	1.224
1000 + Carbosulfan	29.40	35.00	43.00	45.40	46.60	50.40	52.20	53.00	54.20	55.00	56.20	3.215
CD (0.05)	NS	6.782	6.816	6.162	6.002	6.362	5.441	5.332	5.392	5.174	4.805	

J_2 – Second stage juveniles of *M. incognita* NS – Not significant

4.4.1 Plant Height

4.4.1.1 M. incognita

The mean plant height (cm) of *P. rosea* at different intervals of *M. incognita* infestation are presented in Table 8. There was no significant difference in the height of the plant upto five months after inoculation but there was numerical variation between different treatments.

Six months after inoculation onwards there was statistically significant variation in plant height in different levels of *M. incognita* inoculated plants and uninoculated plants. Plants inoculated with 10,000 J_2 of *M. incognita* recorded minimum height (48.00 cm) and was statistically on par with 1000 J_2 (51.60 cm) and showed statistically significant variation from control and check plants. Maximum plant height was recorded in uninoculated plants (59.80 cm) followed by 1000 J_2 + carbosulfan treated control plants (56.00 cm).

After seven months, plants inoculated with 10,000 J_2 of *M. incognita* recorded minimum plant height (49.60 cm) and was statistically on par with 1000 J_2 (49.80 cm). These two levels showed statistically significant variation from control and check plants. The level 100 J_2 also showed statistically significant variation from uninoculated check plants. Maximum plant height was recorded by uninoculated plants (61.00 cm) followed by 1000 J_2 + carbosulfan treated control plants (57.40 cm).

Plants inoculated with 10,000 juveniles of *M. incognita* (J_2) recorded minimum plant height (47.40 cm) and was statistically on par with 1000 J_2 (50.00 cm) and showed significant variation from other levels and untreated plants after eight months of inoculation. The height of plants inoculated with 100 J_2 (54.2 cm) was on par with 1000 J_2 + carbosulfan treated plants. Maximum plant height was recorded by

uninoculated plants (61.20 cm) followed by 1000 J_2 + carbosulfan treated plants (57.00 cm) and these two treatments were statistically on par.

After nine months, plants inoculated with 10,000 J_2 *M. incognita* recorded minimum plant height (46.5 cm) and was statistically on par with 1000 J_2 (51.6 cm) and these two levels showed significant reduction from the uninoculated plants (60.6 cm) and 1000 J_2 + carbosulfan treated plants (59.4 cm). The 1000 J_2 level was statistically on par with 100 J_2 level and the 100 J_2 level was on par with uninoculated plants and 1000 J_2 + carbosulfan treated plants.

The height of plants recorded at ten months after treatment was minimum in 10,000 J_2 inoculated plants (44.20 cm) and it was statistically on par with 1000 J_2 and 100 J_2 levels and showed significant variation from the control (1000 J_2 + carbosulfan) and check (0 level). This was followed by 1000 J_2 (48.6 cm) and 100 J_2 (52 cm) levels. The plants inoculated with 1000 J_2 + carbosulfan showed 57.4 cm height and was statistically on par with uninoculated plants and 100 J_2 level.

Eleven months after treatment minimum height was recorded by 10,000 J_2 level (45.8 cm) and was statistically on par with 1000 J_2 inoculated plants (50 cm) and showed significant variation from other treatments. The height of plants inoculated with 1000 J_2 were statistically on par with the 100 J_2 level and showed significant variation from the control and check. Whereas the height of 100 J_2 inoculated plant were on par with the control (1000 J_2 + carbosulfan) and check (0 level). Maximum plant height was recorded by 1000 J_2 + carbosulfan treated plants (62.2 cm) followed by uninoculated plants (60.6 cm) and 100 J_2 inoculated plants (55.4 cm).

4.4.1.2 R. similis

The mean plant height (cm) at different intervals after inoculation with various levels of *R. similis* are presented in Table 8. There was no

significant difference in the height of *P. rosea* after one month. From two months onwards there was significant difference.

Two months after inoculation the height was minimum in 10,000 level of *R. similis* inoculated plants (22 cm) and was on par with 1000 level (26 cm). These two levels of inoculum showed significant reduction in plant height over other treatments. The levels 1000 and 100 were statistically on par and showed significantly lower height over untreated check and control (1000 *R. similis* + carbosulfan). The height of uninoculated check (0 level) and control (1000 *R. similis* + carbosulfan) were statistically on par and recorded a height of 33.4 cm and 35 cm respectively. Same trend was noticed upto five months after inoculation.

After six months plants inoculated with 10,000 *R. similis* recorded minimum plant height (22.6 cm). This was statistically on par with 1000 level (27 cm). The level 1000 and 100 were also statistically on par and these three levels showed statistically significant reduction in plant height over control and absolute check. The carbosulfan treated control plants recorded maximum height of 50.4 which showed significant variation over all the levels of inoculum. The uninoculated plants (check) recorded 41.2 cm height. Upto eight months after inoculation the same trend was noticed.

The effect of 10,000 levels of *R. similis* on height of *P. rosea* at nine months after inoculation (MAI) showed statistically significant reduction from all other treatments. This level recorded 27.2 cm height. This was followed by 1000 and 100 levels and recorded 32.8 and 35.4 cm respectively. The effect of these two levels were statistically on par and showed significant reduction over control and check. The treatment 1000 *R. similis* + carbosulfan recorded maximum height (54.2 cm) and was significantly superior to uninoculated check which recorded only 47.2 cm. After ten and eleven months of inoculation same trend was recorded.

Table 9 Effect of different levels of *M. incognita* and *R. similis* on the number of leaves of *P. rosea* at different intervals after inoculation

Levels of inoculum **a. *M. incognita***	Mean number of leaves at monthly intervals [months after inoculation (MAI)]											
	1 MAI	2 MAI	3 MAI	4 MAI	5 MAI	6 MAI	7 MAI	8 MAI	9 MAI	10 MAI	11 MAI	CD (0.05)
0	29.00	36.80	47.80	61.80	78.00	91.00	117.40	120.80	116.60	106.80	112.20	19.852
100 J_2	24.00	32.40	51.00	65.60	73.20	85..20	97.60	107.60	104.00	89.20	77.80	26.293
1000 J_2	19.40	29.80	43.80	61.00	70.60	76.67	94.00	96.60	89.00	81.00	75.20	19.552
10000 J_2	21.00	27.00	38.60	46.60	58.40	70.00	76.40	72.60	71.60	66.00	69.20	24.034
1000 J_2 + Carbosulfan	29.20	37.40	50.60	59.00	70.20	90.80	100.80	109.60	104.40	101.80	103.20	19.431
CD (0.05)	NS	NS	NS	NS	NS	NS	22.234	26.523	25.252	30.044	28.173	
b. *R. similis* 0 level	34.20	42.80	52.60	52.80	54.20	55.00	58.40	61.20	63.80	65.80	67.20	4.772
100	26.60	29.20	29.00	28.60	30.00	32.00	34.20	35.00	38.20	44.60	53.20	3.534
1000	25.20	25.20	25.00	24.60	26.60	27.60	30.00	31.20	36.80	44.20	50.20	3.883
10000	24.40	22.80	22.60	21.00	21.60	21.20	24.20	27.80	31.60	38.20	44.20	2.705
1000 + Carbosulfan	35.60	42.40	56.80	70.00	71.00	76.80	84.60	87.80	89.20	90.00	91.20	11.012
CD (0.05)	NS	NS	19.534	19.972	19.123	17.852	17.533	16.654	17.026	18.152	17.981	

J_2 – Second stage juveniles of *M. incognita* NS – Not significant

Eleven months after inoculation of *R. similis* minimum plant height was recorded in 10,000 level (30 cm) and showed significant reduction over other two levels, control and absolute check. The effect of the levels 1000 and 100 were statistically on par and recorded 30 and 35.6 cm. These two levels also showed significant decrease from the control and check. Maximum plant height was showed by 1000 *R. similis* + carbosulfan (control) and recorded 56.2 cm. The next treatment recorded maximum height was uninoculated check (50 cm).

4.4.2 Number of Leaves

4.4.2.1 M. incognita

The mean number of leaves of *P. rosea* at different intervals after inoculation of various levels of *M. incognita* juveniles were presented in Table 9. There was no significant difference in the mean number of leaves of *P. rosea* upto six months after inoculation though there was numerical reduction. Seven months onwards there was significant variation in the mean number of leaves at different levels after inoculation over the control plants (1000 J_2 + carbosulfan) and uninoculated check (0 level).

Seven months after inoculation minimum number of leaves was recorded by 10,000 J_2 inoculated plants (76.4) which was statistically on par with 1000 J_2 inoculated plants (94.0) and showed statistically significant variation from other treatments. The effect of 1000 J_2 inoculated plants was statistically on par with other lower levels, though there was numerical variations. Maximum number of leaves was recorded by uninoculated plants (117.4) followed by 1000 J_2 + carbosulfan treated plants (100.8) but these two treatments showed statistically significant variation.

Plants inoculated with 10,000 J_2 recorded minimum mean number of leaves (72.6) at eight months after inoculation and was on par with

1000 J_2 level (96.6) and showed statistically significant variation from control and check. The mean number of leaves at 1000 J_2 level was statistically on par with 100 J_2. Maximum number of leaves was recorded in uninoculated plants (120.8) followed by control (1000 J_2 + carbosulfan treated plants) and 100 J_2 with a mean value of 109.6 and 107.6 respectively.

The number of leaves recorded at nine months after inoculation showed minimum number in 10,000 J_2 level (71.6) and was statistically on par with 1000 J_2 (89.0) and this level showed significant variation from all other treatments. The number of leaves of 1000 J_2 inoculated plants were statistically on par with all other levels except the uninoculated plants (116.6).

Ten months after inoculation, minimum mean number of leaves was recorded in 10,000 J_2 inoculated plants (66.4) which was statistically on par with 1000 and 100 J_2 inoculated plants and showed statistically significant variation from the control and check plants. The mean number of leaves in 1000 J_2 inoculated plants was 81.0 and it was statistically on par with all other treatments. Maximum number of leaves were recorded by uninoculated plants (106.8) followed by 1000 J_2 + carbosulfan (101.8) and 100 J_2 level (89.2) respectively.

The mean number of leaves at eleven months recorded minimum values of 10,000 J_2 level (69.2) and was statistically on par with 1000 J_2 (75.2) and 100 J_2 (77.8) levels. The mean number of leaves in 1000 J_2 level showed significant variation from the control and check plants. Maximum leaf number was recorded by uninoculated plants (112.2) followed by 1000 J_2 + carbosulfan treated plants (105.2) and 100 J_2 inoculated plants (77.8) respectively.

4.4.2.2 R. similis

The mean number of leaves at different intervals after inoculation of various levels of *R. similis* are presented in Table 9. There was no significant variation in the number of leaves of *P. rosea* upto two months after inoculation. After three months onwards there was significant variation.

P. rosea recorded minimum mean number of leaves at 10,000 level of *R. similis* which was statistically on par with 1000 and 100 levels. The number of leaves recorded in these levels were 22.6, 25 and 29 respectively. All these levels showed statistically significant reduction in the number of leaves over control and check. Maximum number of leaves was recorded in control (56.80) and was statistically on par with check. Same trend was noticed upto five months after inoculation.

Six months after inoculation the effect of 10,000, 1000 and 100 levels of inoculum of *R. similis* were statistically on par and these treatments recorded 21.2, 27.6 and 32 leaves respectively. All these levels were significantly inferior to uninoculated check and control. The check plants recorded 55 leaves which was statistically inferior to control (76.8 leaves). The observation at seven eight, nine, ten and eleven months after treatment recorded the same trend.

The number of leaves of *P. rosea* inoculated with 10,000 level of *R. similis* recorded minimum (44.2) after eleven months and showed significant reduction from the control and check. This level was found to be statistically on par with 1000 and 100 levels. These levels recorded 50.2 and 53.2 leaves respectively. The effect of 1000 and 100 levels were statistically on par with check which recorded 67.2 leaves. All these three showed significant reduction from the control plants which recorded maximum number of leaves (91.2).

4.4.3 Number of Branches

4.4.3.1 M. incognita

The mean number of branches at different intervals after inoculation with various levels of *M. incognita* were presented in Table 10. There was no statistically significant difference in the number of branches of *P. rosea* upto six months after inoculation even though there was numerical variation. Seven months after inoculation onwards there was statistically significant variation.

The number of branches recorded in 10,000 J_2 level inoculated plants was minimum (12.8) at seven months after inoculation. This level was statistically on par with 1000 J_2 and 100 J_2 levels. This level showed significant variation from the control plants (1000 J_2 + carbosulfan) and check plants (uninoculated). Maximum number of branches were recorded by uninoculated plants (check) and recorded 16.6 mean branch number followed by 1000 J_2 + carbosulfan treated plants (15.0). The same trend was recorded in eight and nine months after inoculation.

Ten months after treatment minimum number of branches were recorded by 10,000 J_2 inoculated plants (10.8) which was on par with 1000 J_2 (12.00) and 100 J_2 (13.2) inoculated plants. These treatments were statistically significant from the uninoculated plants (check) and 1000 J_2 + carbosulfan treated plants (control). Maximum number of branches were recorded in uninoculated plants (16.6) followed by 1000 J_2 + carbosulfan treated plants (16.0). The same trend was noticed eleven months after inoculation also.

4.4.3.2 R. similis

The mean number of branches of *P. rosea* at different intervals after inoculation with various levels of *R. similis* are presented in Table 10. There was no significant difference in the number of branches of

Table 10 Effect of different levels of *M. incognita* and *R. similis* on the number of branches of *P. rosea* at different intervals after inoculation

Levels of inoculum **a. *M. incognita***	Mean number of branches at monthly intervals [months after inoculation (MAI)]											
	1 MAI	2 MAI	3 MAI	4 MAI	5 MAI	6 MAI	7 MAI	8 MAI	9 MAI	10 MAI	11 MAI	CD (0.05)
0	5.40	7.20	9.40	11.60	13.80	15.20	16.60	16.60	16.40	16.60	16.40	1.892
100 J_2	4.40	6.80	7.60	9.20	10.80	12.40	13.80	14.00	13.40	13.20	13.20	2.014
1000 J_2	4.25	7.25	9.00	11.25	12.50	13.25	13.80	14.25	14.00	12.00	12.00	2.234
10000 J_2	4.60	6.20	7.40	8.60	10.20	12.40	12.80	13.20	12.80	10.80	10.80	2.261
1000 J_2 + Carbosulfan	5.80	8.00	9.20	11.80	12.60	14.60	15.00	15.60	15.60	16.00	16.80	1.961
CD (0.05)	NS	NS	NS	NS	NS	NS	2.152	2.234	2.332	2.512	3.480	
b. *R. similis*												
0 level	6.00	7.00	6.80	7.00	7.00	7.80	8.80	9.00	9.40	10.40	10.40	0.682
100	5.00	4.80	5.00	5.20	5.80	6.20	7.20	8.00	8.80	9.20	10.00	0.621
1000	4.20	4.00	4.20	4.80	4.80	5.60	6.00	6.40	7.20	8.20	8.80	0.634
10000	3.80	3.80	3.80	4.60	4.80	4.80	6.00	6.20	6.60	7.40	8.00	0.582
1000 + Carbosulfan	5.80	6.20	6.40	10.40	10.60	11.40	12.20	12.20	12.20	12.40	12.40	1.442
CD (0.05)	NS	NS	NS	2.734	2.682	2.491	2.354	2.372	2.462	2.422	2.205	

J_2 – Second stage juveniles of *M. incognita* NS – Not significant

P. rosea upto three months. After four months onwards there was significant difference in the number of branches of *P. rosea*.

Four months after inoculation minimum mean number of branches of 4.6 was recorded in 10,000 *R. similis* inoculated plants. This level showed statistically significant reduction from the control and check plants. The effect of level 10,000 was found to be statistically on par with 1000 and 100 levels. The level 1000 recorded 4.8 mean number of branches and showed significant reduction over the control and check. Maximum number of branches were recorded in 1000 *R. similis* + carbosulfan treated plants (10.4). Same trend was noticed upto eight months after inoculation.

After nine months minimum mean branch number was recorded at 10,000 level (6.6) and this showed statistically significant reduction from the control and check. The effect of 1000 and 100 level was statistically on par with 10,000 level and showed significant reduction from the control. Maximum number of branches were recorded in control (12.2) followed by check and 100 level (9.4 and 8.8 branches) respectively. The same trend was observed upto 11 months after inoculation.

The number of branches recorded was minimum in 10,000 level (8) after eleven months of inoculation. This showed significant reduction from the control and check. The effect of 1000 and 100 level showed 8.8 and 10 mean number of branches and was found to be statistically on par with 10,000 level and showed statistically significant reduction from the control. Maximum number of branches were recorded by control (12.4) and was found to be on par with check (10.4).

4.4.4 Soil Population

4.4.4.1 M. incognita

The mean populations of *M. incognita* at different intervals after inoculation of various levels of *M. incognita* were presented in Table 11.

Table 11 Effect of different levels of *M. incognita* and *R. similis* on the soil population at different intervals after inoculation

Levels of inoculum **a.** *M. incognita*	Mean population of nematodes at monthly intervals [months after inoculation (MAI)]						
	Initial population	2 MAI	4 MAI	6 MAI	8 MAI	10 MAI	CD (0.05)
0	0 (1)	0 (1)	0 (1)	0 (1)	0 (1)	0 (1)	-
100 J_2	0 (1)	17.20 (4.23)	31.60 (5.706)	43.60 (6.674)	46.60 (6.94)	46.60 (6.94)	0.433
1000 J_2	0 (1)	20.40 (4.162)	32.00 (5.74)	45.40 (6.836)	51.80 (7.264)	58.00 (7.74)	0.422
10000 J_2	0 (1)	17.80 (4.35)	39.40 (6.35)	48.40 (7.012)	55.80 (7.528)	60.20 (7.82)	0.462
1000 J_2 + Carbosulfan	0 (1)	0 (1)	0 (1)	0 (1)	0 (1)	0 (1)	0.283
CD (0.05)	-	0.432	0.312	0.394	0.291	0.302	
b. *R. similis* 0 level	0 (1)	0 (1)	0 (1)	0 (1)	0 (1)	0 (1)	
100	0 (1)	10.40 (3.334)	15.40 (4.028)	21.60 (4.738)	23.60 (4.646)	22.00 (4.828)	0.421
1000	0 (1)	16.40 (4.158)	23.80 (4.360)	31.40 (5.684)	42.00 (6.544)	43.60 (6.25)	0.992
10000	0 (1)	17.80 (4.316)	24.00 (4.986)	41.00 (6.468)	53.20 (7.360)	62.20 (7.932)	0.335
1000 + Carbosulfan	0 (1)	0 (1)	0 (1)	0 (1)	0 (1)	0 (1)	
CD (0.05)		0.342	0.426	0.362	0.325	0.721	

J_2 – Second stage juveniles of *M. incognita* Figures in parenthesis denotes $\sqrt{x + 1}$ transformed values

There was statistically significant variation in the mean population of various levels of *M. incognita* at different intervals. The plants treated with carbosulfan and uninoculated plants recorded zero population at all the intervals. Whereas all the inoculum levels showed statistically significant variation in population of *M. incognita* after four months onwards.

The soil population revealed at 2 MAI showed that there was no statistically significant variation between different levels. After four months the population of 10,000 J_2 level showed statistically significant variation from other two levels. The levels 1000 and 100 were statistically on par and recorded 32 and 31 *M. incognita* / 100 g soil respectively. Maximum population was recorded at 10,000 level (39.4 / 100 g soil).

The population of *M. incognita* recorded maximum (48.4 / 100 g soil) at 10,000 level after six months and was statistically on par with 1000 level (45.4 / 100 g soil). The levels 1000 and 100 were also statistically on par.

Eight months after inoculation the population recorded maximum at 10,000 level (55.8 / 100 g soil) and was followed by other two levels. The population of levels 10,000 and 1000 were statistically on par and showed significant variation from the level 100 J_2.

Ten months after treatment maximum recovery of *M. incognita* from 100 g soil was from 10,000 J_2 treated plots (60.2) which was statistically on par with 1000 level and these two showed significant variation from the 100 level.

4.4.4.2 R. similis

The mean population of *R. similis* in soil at different intervals after inoculation were presented in Table 11. The data revealed significant variation in the recovery of nematodes from 100 g soil sample of various levels of inoculation.

Table 12 Effect of different levels of *M. incognita* juveniles and *R. similis* on the biometric characters of *P. rosea* at the time of harvest (twelve months after inoculation)

Levels of inoculum **a. *M. incognita***	Plant height	Leaf number	Branch number	Leaf area (mm^2)
0	74.00	134.80	17.60	1637.08
100 J_2	62.80	94.00	15.00	1559.18
1000 J_2	59.80	90.00	14.60	1345.42
10000 J_2	51.20	66.60	13.20	1060.00
1000 J_2 + Carbosulfan	73.00	127.00	18.20	1741.25
CD (0.05)	6.741	20.881	3.192	80.712
b. *R. similis*				
0 level	52.60	78.40	12.20	1678.18
100	42.60	62.20	10.60	1554.50
1000	38.00	57.20	9.80	1378.70
10000	33.00	52.80	8.60	1167.05
1000 + Carbosulfan	58.40	105.20	15.60	1741.15
CD (0.05)	3.864	19.192	2.662	58.943

J_2 – Second stage juveniles of *M. incognita*

Two months after inoculation maximum recovery of *R. similis* was recorded in 10,000 levels inoculated plots (17.8) and was statistically on par with 1000 level (16.4). These two levels showed significant variation from the lower level (100 *R. similis*), control and check. The control and check plots recorded zero recovery of nematodes.

After four months the recovery of nematodes from 100 g soil was maximum in 10,000 level (24). This was statistically on par with 1000 levels (23.8) and showed significant increase in the population of *R. similis* from all other treatments. The nematode recovery from 100 level recorded 15.4 nematode per 100 g soil and showed significant increase over control and check. The control and check plots recorded zero recovery of *R. similis*.

The recovery of *R. similis* was maximum in 10,000 level (41 / 100 g soil) after six months and showed significant increase over other levels, control and check. This was followed by levels 1000 and 100. These levels recorded 31.4 and 21.6 nematodes in 100 g soil sample respectively and showed significant variation between them and from control and check. The control and check plots recorded zero recovery. After eight and ten months same trend was noticed.

4.4.5 Biometric Characters at the Time of Harvest (Twelve months after inoculation)

4.4.5.1 M. incognita

Data presented in Table 12 revealed the following results. Twelve months after inoculation minimum height of the plants (mean) was recorded by 10,000 J_2 inoculated plants (51 cm) and it was significantly different from the other levels. The plants inoculated with 1000 J_2 and 100 J_2 were statistically on par and recorded a height of 59.8 cm and 62.8 cm respectively. Maximum plant height was recorded by uninoculated plants (74 cm) followed by 1000 J_2 + carbosulfan treated plants (73 cm).

In the case of leaf number 10,000 J_2 inoculated plants showed statistically significant variation from all other treatments and recorded 66.4 leaves. The levels of 1000 and 100 J_2 were statistically on par and also showed significant variation from the control and check. Plants inoculated with 1000 J_2 recorded 90 leaves and 100 J_2 inoculated plants recorded 33 leaves. Maximum leaf number (134.8) was recorded by uninoculated plants and this was followed by 1000 J_2 + carbosulfan treated plants (control) (127).

Regarding branch number minimum was observed in 10,000 J_2 inoculated plants (13.2 branches) and was statistically on par with 1000 J_2 and 100 J_2 levels. The levels 1000 and 100 J_2 were statistically on par with uninoculated plants and showed significant variation from the control (1000 J_2 + carbosulfan). Maximum branch number was recorded by 1000 J_2 + carbosulfan treated plants (18.2) followed by uninoculated plants (17.6).

The leaf area was minimum for 10,000 J_2 inoculated plants (1060 mm^2) and showed statistically significant variation from the other treatments. The leaf area of plants inoculated with 1000 J_2 recorded 1345.42 mm^2 and 100 J_2 recorded 1559.18 mm^2. These two levels also showed significant variation each other and also from all other treatments. Maximum leaf area was recorded in 1000 J_2 + carbosulfan treated plants (1741.25 mm^2) followed by uninoculated plants (1637.08 mm^2).

The fresh and dry weight of shoot and root were presented in Table 13. The data reveals statistically significant variation between different treatments. Regarding fresh weight of shoot, minimum was recorded in 10,000 J_2 inoculated plants (73.4 g) and showed statistically significant variation from all other treatments. Plants inoculated with 1000 J_2 recorded 70 g reduction over control and 60 g reduction over check. The effect of 1000 J_2 inoculated plant was statistically on par with 100 J_2 level in the case of fresh weight of shoot and recorded 152 g and 182 g

respectively. Maximum fresh weight of shoot was recorded in control plants (222 g) followed by check (212 g). Same trend was noticed in the case of dry weight of shoots also. Minimum dry weight was recorded by 10,000 J_2 inoculated plants (22.2 g) and showed significant variation from all other treatments. The levels 1000 J_2 and 100 J_2 were statistically on par and recorded 43 g and 51.6 g respectively. Maximum dry weight of shoot (11) was recorded by 1000 J_2 + carbosulfan treated plants (71.6 g) followed by uninoculated plants (71.0 g).

Fresh - dry weight ratio of shoot was ranged between 2.98 : 1 to 3.63 : 1.

Fresh weight of roots recorded minimum in 10,000 J_2 inoculated plants (108 g) and was statistically on par with 1000 J_2 (116 g) and 100 J_2 (132 g) levels. All these three levels recorded statistically significant variation from the uninoculated plants and 1000 J_2 + carbosulfan treated plants. Maximum fresh root weight was recorded by 1000 J_2 + carbosulfan treated plants (207 g) followed by uninoculated plants (160 g). Regarding the dry weight of roots minimum dry weight of root was recorded in 10,000 J_2 inoculated plants (26 g) and showed significant variation from all other levels. The dry root weight of the f 1000 and 100 J_2 inoculated plants were statistically on par and recorded 36 and 45 g respectively.

The fresh-dry weight ratio of roots recorded maximum in 10,000 J_2 inoculated plants (4.15:1) and all other treatments ranged between 2.86:1 to 3.23:1.

4.4.5.2 R. similis

The effect of different levels of *R. similis* on the mean biometric characters of *P. rosea* are presented in Table 12. The data revealed significant variation in the biometric characters of *P. rosea* at different levels of inoculation.

Table 13 Effect of different levels of *M. incognita* and *R. similis* on the fresh and dry weight of shoots and roots of *P. rosea* at the time of harvest (twelve months after inoculation)

Levels of inoculum **a. *M. incognita***	Fresh weight of shoot (g)	Dry weight of shoot (g)	Fresh- dry weight ratio (Shoot)	Fresh weight of root (g)	Dry weight of root (g)	Fresh- dry weight ratio of roots
0	212.00	71.00	2.98 : 1	160.00	56.00	2.86 : 1
100 J_2	182.00	51.60	3.63 : 1	132.00	45.00	2.93 : 1
1000 J_2	152.00	43.00	3.53 : 1	116.00	36.00	3.22 : 1
10000 J_2	73.40	22.20	3.31 : 1	108.00	26.00	4.15 : 1
1000 J_2 + Carbosulfan	222.00	71.60	3.10 : 1	207.00	64.00	3.23 : 1
CD (0.05)	41.764	9.762	-	37.332	8.774	-
b. *R. similis*						
0 level	183.00	45.00	4.06 : 1	194.00	48.00	4.04 : 1
100	161.00	41.00	3.93 : 1	154.00	36.00	4.27 : 1
1000	143.00	38.00	3.76 : 1	141.00	33.00	4.27 : 1
10000	131.00	32.00	4.09 : 1	122.00	29.00	4.20 : 1
1000 + Carbosulfan	224.00	57.00	3.93 : 1	218.00	56.00	3.89 : 1
CD (0.05)	18.416	6.032	-	27.203	6.484	-

J_2 – Second stage juveniles of *M. incognita*

Regarding the plant height, 10,000 level recorded minimum (33 cm) and showed a maximum reduction of 19.6 cm over check and 25.4 cm over control. This level showed statistically significant reduction from all other levels, control and check. The levels 1000 and 100 also showed significant reduction over control and check. These two levels recorded 38 and 42.6 cms respectively. The check plants also showed significant reduction over control. Maximum height was recorded by control plants (58.4 cm). The uninoculated check recorded 52.6 cm height.

Number of leaves recorded minimum in10,000 level (52.8) and was statistically on par with 1000 level and showed significant reduction over control and check. The level 1000 recorded 57.8 mean leaves and 100 recorded 62.2 leaves. The control plants recorded maximum number of leaves (105.2) and showed significant increase over the check.

In the case of number of branches minimum was recorded by 10,000 level (8.6) and was statistically on par with 1000 and 100 levels. These levels recorded 9.8 and 10.6 branches respectively. All these levels showed significant reduction in mean number of branches over the control. The uninoculated plants also showed significant reduction over the control. Maximum number of branches were recorded in control plants (15.6). The uninoculated plants (check) recorded an average of 12.2 branches.

Regarding the leaf area all the treatments showed statistically significant variation. Maximum reduction was noticed in 10,000 level followed by 1000 and 100 level and recorded 1167.05, 1378.7 and 1554.5 mm^2 respectively. All these levels showed statistically significant reduction over control and untreated check. Maximum leaf area was recorded in control (1741.15 m^2) and was followed by untreated check (1678.18 mm^2).

The fresh and dry weight of shoot and roots were presented in Table 13. The data revealed statistically significant variation between different treatments. In the case of fresh shoot weight minimum was

recorded in 10,000 level (131 g). This was statistically on par with 1000 level (143 g) and showed significant reduction from 100 level, control and untreated check. The 1000 level was statistically on par with 100 level. All these levels showed statistically significant reduction over control and untreated check. Maximum fresh shoot weight was recorded in control (224 g) and this was followed by untreated check (183 g). The untreated check showed statistically significant reduction over control.

The fresh root weight was minimum in10,000 level (122 g) and was statistically on par with 1000 level. This level showed significant reduction from all other treatments. The level 1000 was statistically on par with 100 and these levels recorded 143 and 161 g respectively. These two levels showed significant reduction from control and untreated check. Maximum fresh root weight was recorded in control (218 g) followed by untreated check (194 g).

Regarding the dry shoot weight maximum reduction from the control and check plants was noticed in 10,000 level and this was followed by 1000 and 100 levels. These treatments recorded 32, 38 and 41 grams respectively. The levels 10,000 and 1000 were statistically on par and the levels 1000 and 100 were also statistically on par. All these levels showed significant reduction from the control (57 g) and untreated check (45 g).

In the case of dry root weight minimum was recorded in 10,000 level (29 g) and was statistically on par with 1000 level (33 g). This level showed significant reduction from all other treatments. The level 1000 was statistically on par with level 100 and these two levels showed statistically significant reduction from the control and check. The control plants recorded maximum (56 g) and this was followed by untreated check (48 g) and 100 level (36 g).

The fresh dry weight ratio of shoot and root ranged between 3.76 : 1 to 4.09 : 1 and 3.89:1 to 4.27:1 respectively at various levels of inoculation.

Table 14 Effect of different levels of *M. incognita* and *R. similis* on the yield of *P. rosea* and population of nematodes at the time of harvest (twelve months after inoculation)

Levels of inoculum **a. *M. incognita***	Yield (g) / plant	Tuber length	Tuber width (cm)	Tuber number	Population in soil (100 g)	Population in root (10 g)	Root knot count (1 g)	Root knot index
0	160.00	39.14	0.85	7.05	0 (1)	0 (1)	0 (1)	0
100 J_2	132.00	37.15	0.57	6.30	47.94 (6.996)	27.6 (5.347)	4.88 (2.425)	1
1000 J_2	116.00	33.30	0.47	4.85	56.57 (7.588)	127.6 (11.340)	22.12 (4.808)	5
10000 J_2	108.00	21.15	0.30	3.05	62.93 (7.996)	508.60 (22.574)	38.05 (6.248)	5
1000 J_2 + Carbosulfan	207.00	49.60	0.89	10.80	0 (1)	0 (1)	0 (1)	0
CD (0.05)	37.531	4.911	0.114	0.772	0.321	0.952	0.291	
b. *R. similis*							Number of lesions (1 g)	Lesion index
0	194.00	39.80	0.90	13.00	0 (0)	0 (1)	0 (1)	0
100	154.00	34.40	0.66	9.40	22.60 (4.806)	25.40 (5.12)	10.00 (3.30)	2
1000	141.00	29.60	0.54	8.00	46.00 (6.924)	36.00 (6.06)	15.80 (4.09)	2
10000	122.00	21.20	0.50	6.60	64.80 (8.098)	45.40 (6.79)	19.20 (4.48)	3
1000 + Carbosulfan	218.00	43.80	0.96	15.40	0 (1)	0 (1)	0 (1)	0
CD (0.05)	27.212	4.271	0.135	2.282	0.522	0.872	0.364	

J_2 – Second stage juveniles of *M. incognita* Figures in parenthesis denotes $\sqrt{x + 1}$ transformed values

4.4.6 Yield Attributing Characters and Yield

4.4.6.1 M. incognita

The effect of different levels of *M. incognita* on the yield and yield attributing characters of *P. rosea* were presented in Table 14.

Regarding the tuber length, minimum length was recorded by 10,000 J_2 inoculated plants (21.15 cm) and showed statistically significant variation from all other treatments. The tuber length of plants inoculated with 1000 and 100 J_2 were statistically on par and recorded 33.30 and 3.7.15 cm respectively. These two treatments showed statistically significant variation from the control plants. Maximum tuber length was recorded in 1000 J_2 + carbosulfan (49.60 cm) and the uninoculated plants recorded 39.14 cm.

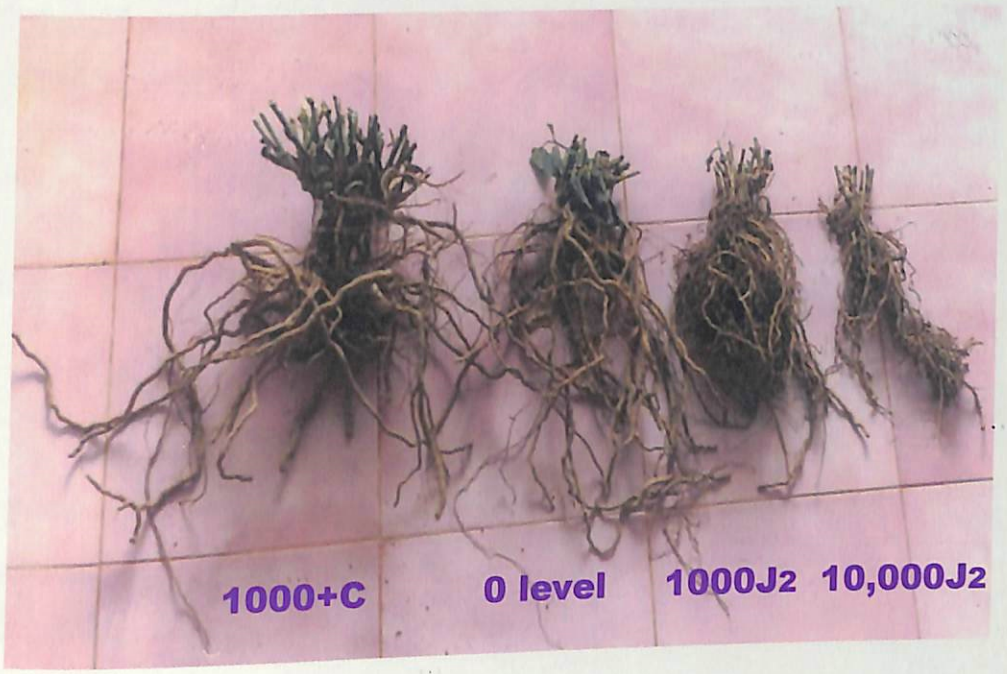
The tuber width was minimum in 10,000 J_2 inoculated plants (0.3 cm) and was significantly different from all other treatments. The effect of the levels 1000 J_2 and 100 J_2 were statistically on par and recorded tuber width of 0.47 cm and 0.57 cm respectively. Maximum tuber width was recorded by control plants (0.89 cm) followed by check (0.85 cm).

Regarding the mean tuber number minimum was recorded in 10,000 J_2 inoculated plants (3.05) and showed a reduction of 7.75 tubers over control and 4 tubers over the check and also showed significant variations from other two levels. Plants inoculated with 1000 J_2 recorded 4.85 tubers and was significantly different from all other treatments. The tuber number of 100 J_2 inoculated plants and uninoculated plants were statistically on par. The uninoculated plants also showed significant variation from control plants Maximum tuber number (10.8) was recorded by control plants (1000 J_2 + carbosulfan).

Maximum reduction in yield in terms of weight of tubers was recorded by 10,000 J_2 inoculated plants and was statistically on par with

Plate 4

a. Effect of different levels of *Meloidogyne incognita* on the yield of Chethikoduveli, *Plumbago rosea*



b. Effect of different levels of *Radopholus similis* on the biometric characters and yield of Chethikoduveli, *Plumbago rosea*

1000 J_2 and 100 J_2 inoculated plants. Plants inoculated with 10,000 juveniles of *M. incognita* recorded minimum weight (108 g) and showing a reduction of 99 and 52 g over control and check respectively. The level 1000 J_2 recorded 116 g tuber weight. The tuber weight recorded by 100 J_2 inoculated plants was 132 g. Maximum tuber weight (207 g) was recorded in control plants (1000 J_2 + carbosulfan) and followed by uninoculated check (160 g) (Plate 4a).

4.4.6.2 R. similis

The effect of different levels of burrowing nematode on the yield and yield attributing characters of *P. rosea* were presented in Table 14.

Regarding the tuber number minimum was recorded in 10,000 level (6.6 tubers / plant). This level showed statistically significant reduction from all other treatments except 1000 level which recorded a mean number of eight tubers per plant. The level 1000 was statistically on par with level 100 and all these levels showed significant reduction from the control and check. Maximum number of tubers was recorded in control (15.4 tubers / plant). This was followed by untreated check and 100 level and these two recorded 13 and 9.4 tubers / plant respectively. The untreated check also showed significant reduction from the control (Plate 4b).

In the case of tuber length there was statistically significant variation between different levels and these levels showed significant reduction from the control and check. The tuber length was minimum in 10,000 level (21.2 cm) and this was followed by 1000 and 100 levels and these levels recorded 29.6 and 34.4 cm respectively. The tuber length of control and check were statistically on par. Maximum tuber length was recorded in control (43.8 cm). The untreated check recorded 39.8 cm tuber length.

The width of the tuber recorded was minimum in 10,000 level (0.5 cm) and this showed statistically significant reduction from all other

treatments except 1000 level (0.54 cm). The level 1000 was statistically on par with level 100. These two levels also showed statistically significant reduction from the control and check. The tuber width of untreated check showed significant reduction from the control plants. Maximum tuber width was recorded in control (0.96). The untreated check recorded 0.9 cm tuber width.

Regarding the yield per plant in terms of weight of tubers, minimum was recorded in 10,000 level (122 g). This was statistically on par with 1000 level and showed statistically significant reduction from all other treatments. The levels 1000 and 100 were statistically on par and these levels showed significant reduction in yield from control and check. Maximum yield per plant was recorded in control (218 g) and the untreated check recorded 194 g. These two were statistically on par.

4.4.7 Nematode Population

4.4.7.1 M. incognita

The data presented in Table 14 revealed statistically significant variation between different treatments in the case of nematode population in soil and root, root-knot count and root-knot index.

Maximum mean population of nematode in soil was recorded by 10,000 J_2 inoculated plants (62.93/ 100 g soil) and showed significant variation from other treatments. This was followed by 1000 J_2 level (56.57 *M. incognita* / 100 g soil). At 100 J_2 level also the nematode population in soil showed statistically significant variation from all other treatments. The recovery of *M. incognita* from various levels of inoculation revealed a progressive increase in the population in response to the increase in initial inoculum level.

The population of *M. incognita* in roots of *P. rosea* was maximum in 10,000 J_2 inoculated plants and showed a recovery of 508.6 nematodes from 5 g roots and was statistically significant from all other treatments.

The nematode recovery was 128 and 28 per 5 g roots in 1000 and 100 J_2 levels respectively. All the treatments showed statistically significant variation. The uninoculated check plants and 1000 J_2 + carbosulfan treated control plants recorded 'zero' population of *M. incognita*.

Regarding root-knot count and root-knot index, maximum was recorded by 10,000 J_2 inoculated plants (count 38 / g root and an index of 5) and was statistically significant from all other treatments. The 1000 J_2 inoculated plants also recorded 22 root-knots / g of root with an index of five. At level, 100 J_2 inoculated mean root-knot count was only 4.88 per gram of root sample with a root-knot index 'one'.

4.4.7.2 R. similis

The data presented in Table 14 revealed statistically significant variation between different treatments in the case of nematode population in soil, root, number of lesions and lesion index.

Maximum mean population of nematodes in soil was recorded by 10,000 level (64.8 / 100 g soil) and showed significant variation from other treatments. This was followed by 1000 level and 100 level and recorded 46 and 23 *R. similis* / 100 g soil respectively. The recovery of *R. similis* from various levels of inoculation revealed a progressive increase in population in response to the increase in inoculum level.

The mean population of *R. similis* in the roots of *P. rosea* was maximum in 10,000 *R. similis* inoculated plants and showed a recovery of 45 nematodes from 5 g roots. This was on par with the recovery of *R. similis* from 1000 level inoculated plants. The recovery of *R. similis* from 1000 level and 100 levels were also statistically on par and recorded 36 and 25 *R. similis*/ 5 g roots. The control and check plants recorded zero population.

In the case of mean number of lesions and lesion index maximum was recorded in the roots of 10,000 level inoculated plants (9.55 lesions /

Table 15 Effect of different treatments on the height of *P. rosea* at different intervals after treatment

Treatments	Mean height of plants (cm) at different intervals [months after treatment (MAT)]												
	Initial	1 MAT	2 MAT	3 MAT	4 MAT	5 MAT	6 MAT	7 MAT	8 MAT	9 MAT	10 MAT	11 MAT	CD (0.05)
Gl. f.	11.66	13.66	16.00	18.66	21.00	24.00	28.33	33.33	39.33	43.33	47.33	52.66	2.675
P.fl.	11.66	13.66	15.66	18.33	20.33	23.33	27.00	31.66	36.66	40.33	45.33	51.00	2.012
NC	10.00	12.00	13.66	15.66	16.66	19.66	23.66	26.33	30.00	33.00	37.66	43.00	2.291
Gn.c.	11.33	13.00	14.33	16.00	17.33	19.66	23.66	26.33	31.66	33.33	38.00	43.66	2.034
gly.m.	11.33	12.00	13.66	15.00	17.66	19.66	22.00	25.66	29.33	31.00	34.66	40.00	1.502
Cle	11.33	13.33	14.66	16.33	17.66	20.33	23.66	26.33	30.66	33.00	38.00	44.66	2.413
Carb	11.66	13.33	15.33	17.00	19.33	22.66	26.00	30.33	35.66	38.00	43.66	52.33	0.894
WA	11.33	12.66	14.33	15.33	17.00	19.00	20.66	23.33	27.66	30.33	34.33	39.66	1.452
NA	12.00	13.00	13.66	15.33	15.33	16.66	18.33	20.33	24.00	25.33	27.00	31.66	1.886
NF	11.33	13.00	15.00	16.33	17.66	20.66	25.00	28.00	35.00	37.33	43.00	46.66	1.405
P	11.66	13.33	15.33	17.66	19.33	23.00	26.66	32.00	37.33	40.33	45.00	49.66	2.032
CD (0.05)	NS	NS	NS	2.052	1.613	1.725	2.154	2.153	3.274	2.845	4.632	3.615	

Gl.f. – *Glomus fasciculatum*, P.fl. – *Pseudomonas fluorescens,* NC – Neem cake, Gnc – Groundnut cake, Gly.m. – *Glyricidia maculata*, Cle – *Clerodendron infortunatum*, Carb – Carbosulfan, WA – Wood ash, NA – Nematode alone, NF – nematode free, P – *Paecilomyces lilacinus*

g of root and an index of 3). This was statistically on par with 1000 level which recorded 7.9 lesions per gram of root with an index '2'. These two levels showed statistically significant variation from the 100 level which recorded only 5 lesions / g of root and the lesion index was 2.

4.5 MANAGEMENT

The effect of different treatments on the management of nematodes (*M. incognita* and *R. similis*) infesting *P. rosea* were evaluated in sick plots (2 x 2 m) having *M. incognita* and *R. similis* infestation. The effect of treatment was assessed in terms of improvement in biometric characters and yield and reduction in population of nematodes at different intervals and NPK content of leaves. The results on the above are presented in Table 15-22.

4.5.1 Plant Height

The data presented in Table 15 revealed the effect of different treatments on the mean plant height of *P. rosea* at different intervals. There was no significant variation in height of *P. rosea* upto two months after treatment. The plant height recorded at three months after treatment onwards showed statistically significant variation.

The height of *P. rosea* was maximum in *Glomus fasciculatum* followed by *Pseudomonas fluorescens*, *Paecilomyces lilacinus* and carbosulfan with 18.7, 18.3, 17.7 and 17 cm respectively and all these treatments were statistically on par and superior to all other treatments and untreated (absolute check). However there was no statistically significant variation between other treatments having organic amendments and green leaves. After 4 and 5 MAT maximum height was recorded by *G. fasciculatum* (24 cm) and this was statistically on par with *P. fluorescens*, *P. lilacinus* and Carbosulfan and all these treatments showed statistically significant variation from other treatments and recorded a plant height of above 20 cms. Plants in nematode free

(absolute check) treatment and application of leaves of *Clerodendron* also recorded a plant height of above 20 cm and these two treatments were statistically on par. These two are statistically on par with neem cake, groundnut cake, *Glyricidia* and wood ash. Minimum plant height was recorded in untreated plants.

When plant height was recorded six months after treatment, maximum mean plant height was recorded by *G. fasciculatum* (23.33 cm) and this was statistically on par with *P. fluorescens* (27 cm) and *P. lilacinus* (26.66 cm). These treatments were superior to all other treatments and absolute check. But the treatments *P. fluorescens*, *P. lilacinus*, Carbosulfan and check were also statistically on par. The mean minimum plant height (18.33 cm) was recorded in nematode alone treated plants. When observations were recorded at seven months after treatments, the above treatments showed the same trend.

Eight months after treatment (8 MAT) there was significant variation in plant height between different treatments and control. Maximum mean plant height was recorded by *G. fasciculatum* (39.33 cm) and was on par with *P. fluorescens* (36.66 cm) and *P. lilacinus* (37.33 cm). Chethikoduveli plants treated with *G. fasciculatum* showed maximum plant height (43.33 cm) at 9 MAT and was significantly different from all other treatments. The effect of the treatments *P. fluorescens*, *P. lilacinus* and Carbosulfan were statistically on par and superior to all other treatments. Minimum plant height was recorded by treatment 'nematode alone' (25.33 cm).

The treatment *G. fasciculatum* recorded plant height of 47.33 cm at 10 MAT and was statistically on par with *P. fluorescens*, Carbosulfan, *P. lilacinus* and nematode free and these treatments showed significant variation from all other treatments. Treatments neem cake, groundnut cake, *Glyricidia*, *Clerodendron* and wood ash were also statistically

Table 16 Effect of different treatments on the number of leaves of *P. rosea* at different intervals after treatment

Treatments	Mean number of leaves at different intervals [months after treatment (MAT)]												
	Initial	1 MAT	2 MAT	3 MAT	4 MAT	5 MAT	6 MAT	7 MAT	8 MAT	9 MAT	10 MAT	11 MAT	CD (0.05)
Gl. f.	5.33	6.33	8.66	16.66	19.00	21.00	26.00	38.66	45.00	49.00	55.33	65.33	2.472
P.fl.	5.66	6.66	8.66	15.00	18.33	20.00	24.33	36.33	43.66	46.00	57.00	60.66	2.671
NC	5.33	6.00	7.33	11.66	14.33	16.33	21.00	25.33	32.33	36.33	39.66	47.66	3.410
Gn.c.	6.00	7.00	8.33	13.00	14.33	16.33	19.66	26.33	33.33	38.66	43.00	49.00	2.470
gly.m.	5.33	5.66	7.33	11.66	14.00	16.66	18.00	24.66	29.33	36.00	38.66	47.33	2.214
Cle	6.00	6.66	8.66	12.66	14.66	17.00	20.33	25.66	31.00	35.66	40.00	50.00	3.032
Carb	6.66	7.33	8.33	13.33	16.66	19.33	22.66	32.66	41.66	45.00	50.66	59.33	4.914
WA	5.33	6.33	8.66	12.00	14.00	16.33	17.00	24.00	27.66	34.33	38.00	44.33	2.596
NA	5.66	5.66	7.33	11.33	12.33	12.66	14.66	17.33	21.66	25.66	28.33	33.66	2.112
NF	4.66	5.33	7.33	11.33	13.00	18.00	22.66	32.00	35.66	43.00	48.66	54.00	4.346
P	6.00	6.66	8.66	14.33	16.66	20.00	25.00	35.00	41.66	47.33	52.33	59.66	2.948
CD (0.05)	NS	NS	NS	1.832	1.569	1.658	3.154	5.623	5.165	4.858	4.732	4.154	

Gl.f. – *Glomus fasciculatum*, P.fl. – *Pseudomonas fluorescens,* NC – Neem cake, Gnc – Groundnut cake, Gly.m. – *Glyricidia maculata*, Cle – *Clerodendron infortunatum*, Carb – Carbosulfan, WA – Wood ash, NA – Nematode alone, NF – nematode free, P – *Paecilomyces lilacinus*

superior to treatment 'nematode alone' which recorded minimum plant height (27 cm).

At 11 MAT maximum plant height was recorded by *G. fasciculatum* (52.66 cm) and was statistically on par with *P. fluorescens*, Carbosulfan and *P. lilacinus* and were significantly superior to all other treatments. The height of the plant treated with *P. lilacinus* was not effective as above treatments and was statistically on par with nematode free and showed significant variation from other treatments.

Regarding monthly increase in plant height maximum variation in all monthly intervals were recorded by *G. fasciculatum* treated plants. At all the MAT's it recorded statistically significant increase in plant height. The treatment *P. fluorescens* also recorded statistically significant monthly increase in height except between initial and 1 MAT and also between 1 MAT and 2 MAT. The plant height at 4 MAT and 5 MAT were also statistically on par.

Plants treated with carbosulfan also recorded statistically significant monthly increase in height at all the periods. The height of the plant treated with *P. lilacinus* recorded significant increase in all monthly observations except between height at initial and 1 MAT, 1 MAT and 2 MAT and also between 3 MAT and 4 MAT. All other treatments also recorded statistically significant increase in height of plants from the previous MAT's whereas in treatment nematode alone there was no significant increase in plant height except between 10 and 11 MAT.

4.5.2 Number of Leaves

The data related to the number of leaves revealing the effect of different treatments on the mean number of leaves of *P. rosea* at different intervals are presented in Table 16. There was no significant variation in the number of leaves of *P. rosea* upto two months after the treatment (2 MAT). The number of leaves recorded at three months after treatment

showed statistically significant variation. The number of leaves of *P. rosea* was maximum in *G. fasciculatum* treated plants which recorded on average of 16.66 leaves and was statistically on par with *P. fluorescens*. The treatment, *G. fasciculatum* was statistically superior to all the treatments. The treatments *P. fluorescens*, *P. lilacinus* and Carbosulfan were statistically on par and recorded 15, 14.33 and 13.33 mean number of leaves respectively. Organic amendments (Groundnut cake, Neem cake), green leaves (*Glyricidia* and *Clerodendron*) and wood ash treated plants were also statistically on par in the case of number of leaves. Minimum number of leaves was recorded by nematode alone (11.33 leaves).

Maximum number of leaves at 4 MAT was recorded by *G. fasciculatum* (19) and was statistically on par with *P. fluorescens* (18.33). These two showed statistically significant superiority over other treatments. Treatments Carbosulfan and *P. lilacinus* were also statistically on par and showed significant increase in number of leaves over other treatments whereas inferior to *G. fasciculatum* and *P. fluorescens*. The treatments receiving organic amendments and green leaves were statistically on par and significantly superior to untreated check and nematode alone. Minimum number was recorded in nematode alone (12.33 leaves).

The effect recorded at 5 MAT showed that *G. fasciculatum*, *P. fluorescens* and *P. lilacinus* were statistically on par and recorded mean number of 21, 20 and 20 leaves respectively. These treatments were significantly superior to other treatments. The treatments Carbosulfan and nematode free were statistically on par and recorded an average of 19.33 and 18 leaves respectively. These two treatments were significantly superior to other treatments *viz*., neem cake, groundnut cake, wood ash and nematode alone. There was no statistically significant variation between organic amendments and green leaves. Minimum mean leaf number was recorded by nematode alone (12.66).

At 6 MAT maximum mean number of leaves (26) was recorded by *G. fasciculatum* which was statistically on par with *P. lilacinus* and *P. fluorescens* and was significantly superior to all other treatments. The effect of organic amendments and green leaves were also statistically on par and were significantly superior to 'nematode alone'. Minimum mean leaf number of 14.66 was recorded by nematode alone.

Seven months after treatment *G. fasciculatum* (38.66) showed significant superiority over other treatments except *P. fluorescens* and *P. lilacinus* and these three were statistically on par. There was no significant variation in the number of leaves of plants receiving organic amendments and green leaves and these showed significant superiority over nematode alone which recorded the minimum mean number of leaves (17.33).

The effect of the treatments on the number of leaves of *P. rosea* at 8 MAT showed statistically significant variation. Maximum number was given by *G. fasciculatum* and this was followed by *P. fluorescens*, Carbosulfan and *P. lilacinus* with 45, 43.66, 41.66 and 41.66 leaves respectively. All these treatments were statistically on par and showed significant superiority over other treatments. The effect of neem cake and *Clerodendron* on the number of leaves of *P. rosea* were statistically on par and significantly superior to other treatments except the bioagents and carbosulfan. The effect of *Glyricidia* and wood ash also showed significant superiority over nematode alone in the production of number of leaves. Minimum mean number of leaves was recorded in nematode alone (21.66).

The mean number of leaves recorded at 9 months after treatment showed maximum values in *G. fasciculatum* (49) and was followed by *P. lilacinus*, *P. fluorescens* and Carbosulfan with 47.33, 46 and 45 leaves respectively. These treatments were statistically on par and recorded significant superiority over other treatments. The effect of treatments

neem cake, groundnut cake and *Glyricidia* were also statistically on par and showed marked increase in the number of leaves over the nematode alone (25.66).

At 10 MAT maximum mean number of leaves was recorded by *P. fluorescens* (57) and was statistically on par with *G. fasciculatum* (55.33) and *P. lilacinus* (52.33). The effect of the treatments *G. fasciculatum* and *P. lilacinus* were statistically on par with the effect of organic amendments and green leaves and significantly superior to nematode alone. Minimum number of leaves was recorded in nematode alone (28.33).

Maximum mean leaf number of 65.33 was recorded by *G. fasciculatum* after eleven months of treatment and showed statistically significant superiority over all other treatments. This was followed by the treatments *P. fluorescens*, *P. lilacinus* and Carbosulfan and these were statistically on par with 60.66, 59.66 and 59.33 mean leaves respectively. These treatments also recorded significant superiority over all other treatments. The effect of the treatments organic amendments and green leaves were statistically on par and superior to 'nematode alone'. Minimum leaf number of 33.66 was recorded by 'nematode alone'.

Regarding the monthly variation in the number of leaves maximum variation was recorded in treatments of biocontrol agents (*G. fasciculatum*, *P. fluorescens* and *P. lilacinus*).

In *G. fasciculatum* the initial leaf number and leaf number at 1 MAT and between 1 MAT and 2 MAT were statistically on par and after that there was significant increase in the number of leaves at every MAT. The same trend was noticed in *P. lilacinus* also.

In the case of *P. fluorescens* the same trend was noticed but the leaf number at 4 and 5 MAT were also statistically on par.

Table 17 Effect of different treatments on the number of branches of *P. rosea* at different intervals after treatment

Treatments	Mean number of branches at different intervals [months after treatment (MAT)]												
	Initial	1 MAT	2 MAT	3 MAT	4 MAT	5 MAT	6 MAT	7 MAT	8 MAT	9 MAT	10 MAT	11 MAT	CD (0.05)
Gl. f.	1.33	1.33	1.66	3.33	5.66	6.33	7.66	11.66	12.00	12.00	14.00	15.66	1.312
P.fl.	1.33	1.33	1.66	3.00	5.00	6.33	7.66	9.66	10.66	10.66	12.66	14.66	1.494
NC	.33	.33	1.33	2.00	3.33	4.00	4.66	5.33	7.33	7.33	8.33	10.33	0.924
Gn.c.	1.0	1.0	1.33	2.00	3.66	3.66	4.00	5.66	7.33	7.33	8.66	10.00	0.924
gly.m.	1.33	1.33	1.33	2.00	3.33	4.00	4.33	5.33	6.00	6.00	6.66	9.66	0.756
Cle	1.0	1.0	1.66	2.00	3.66	3.66	4.33	5.33	6.66	6.66	7.66	10.00	0.667
Carb	1.00	1.00	1.33	2.66	4.33	5.00	6.00	8.33	9.66	9.66	11.33	13.33	1.168
WA	1.00	1.00	1.33	2.00	3.66	3.66	4.66	5.33	6.00	6.00	7.33	8.66	0.925
NA	1.00	1.00	1.33	1.66	2.33	2.66	3.00	3.33	4.33	4.33	5.00	6.66	0.825
NF	1.33	1.33	1.33	2.66	3.33	4.66	5.66	7.66	8.33	8.33	9.66	12.33	1.215
P	1.00	1.00	1.33	3.33	5.33	6.00	7.33	8.66	9.33	9.33	12.33	14.33	0.934
CD (0.05)	NS	NS	NS	0.623	0.924	0.935	1.146	1.698	1.374	2.784	1.567	1.526	

Gl.f. – *Glomus fasciculatum*, P.fl. – *Pseudomonas fluorescens,* NC – Neem cake, Gnc – Groundnut cake, Gly.m. – *Glyricidia maculata*, Cle – *Clerodendron infortunatum*, Carb – Carbosulfan, WA – Wood ash, NA – Nematode alone, NF – nematode free, P – *Paecilomyces lilacinus*

Carbosulfan treated plants showed significant increase in the number of leaves from 6 MAT. Almost same trend was noticed in the case of nematode free (absolute check). But there was significant variation in the number of leaves between 4 MAT and 5 MAT. At 7 and 8 MAT the number of leaves were statistically on par in nematode free condition. In the case of treatment nematode alone there was no significant increase in the monthly leaf number except at some intervals. There was significant variation in the number of leaves between 2 MAT and 3 MAT, 6 MAT and 7 MAT and after 7 months there was significant variation in the number of leaves. All other treatments showed significant increase in the number of leaves except between first two or three monthly intervals.

4.5.3 Number of Branches

The effect of different treatments on the mean number of branches at different intervals were presented in Table 17. The data revealed that there was no statistically significant variation upto two months. The number of branches recorded three months after treatment onwards showed statistically significant variation.

The mean number of branches of *P. rosea* was maximum in *G. fasciculatum* and *P. lilacinus* with 3.33 branches and was statistically on par with *P. fluorescens* (3.0). All these treatments showed significant superiority over organic amendments, green leaves and nematode alone.

Four months after treatment maximum branches were recorded by *G. fasciculatum* (5.66) and was significantly superior to all other treatments except *P. fluorescens* (5.00). The effect of *P. fluorescens* and Carbosulfan were statistically on par and Carbosulfan, groundnut cake, *Clerodendron* and wood ash were also statistically on par. All these treatments showed statistically significant variation. The minimum branch number was recorded in nematode alone (2.33).

The mean number of branches at 5 MAT was maximum in *G. fasciculatum* and *P. fluorescens* with 6.33 branches and were statistically on par with *P. lilacinus* (6.00). All these treatments showed significant superiority over other treatments and nematode alone. There was no significant variation in the mean branch number in organic amendments and green leaves treated plants. These treatments also showed significant superiority over nematode alone. Minimum branch number was recorded in nematode alone with 2.66 branches.

Six months after treatment maximum number of branches recorded by treatments *G. fasciculatum* and *P. fluorescens* with 6.33 branches and were statistically on par with *P. lilacinus*. These three showed statistically significant increase in branch number over other treatments and nematode alone. There was no significant variation in the number of branches in treatments with organic amendments and green leaves but they showed significant superiority over nematode alone. Minimum branch number of 3.0 was recorded in nematode alone.

The effect of the treatment *G. fasciculatum* showed statistically significant superiority over all other treatments at seven months after treatment and recorded a mean branch number of 11.66. This was followed by treatments, *P. fluorescens*, *P. lilacinus* and Carbosulfan which recorded 9.66, 8.66 and 8.33 branches respectively and all these three were statistically on par. The effect of nematode free (absolute check) was on par with Carbosulfan and P. There was no significant variation in the number of branches of *P. rosea* plants received organic amendments and green leaves. These treatments also showed significant superiority over nematode alone and inferiority over all other treatments. The minimum mean branches was recorded in nematode alone (3.33).

Eight months after treatment the effect of *G. fasciculatum* and *P. fluorescens* were statistically on par and recorded maximum mean branch numbers (12 and 10.66 respectively). The treatment *G. fasciculatum*

showed statistically significant superiority over other treatments. The effect of *P. fluorescens*, Carbosulfan and *P. lilacinus* were statistically on par and significantly superior to organic amendments, green leaves and nematode alone. The effect of groundnut cake was on par with neem cake. Minimum number of branches was recorded in nematode alone (4.33).

When the number of branches were recorded at 9 MAT, maximum branches (12) were recorded in treatment *G. fasciculatum* and it was statistically on par with *P. fluorescens* (10.66) and significantly superior to all other treatments. The effect of *P. fluorescens* was on par with *Carbosulfan* and *P. lilacinus*. The effect of organic amendments and green leaves were statistically on par and significantly superior to nematode alone. Minimum branch number of 4.33 was recorded in nematode alone.

Ten months after treatment maximum mean number of branches (14) were recorded in *G. fasciculatum*. This was significantly superior to all other treatments except *P. fluorescens* (12.66). The effect of *P. fluorescens*, *P. lilacinus* and Carbosulfan were statistically on par and showed statistically significant increase in branch number over other treatments. The lowest branch number was recorded in nematode alone (5.0).

The effect of treatments *G. fasciculatum*, *P. fluorescens* and *P. lilacinus* were statistically on par when the number of branches recorded eleven months after treatment and they gave 15.66, 14.66 and 14.33 branches respectively. Carbosulfan was statistically on par with *P. fluorescens* and *P. lilacinus*. Minimum number of branches were recorded in nematode alone (6.66).

Regarding the monthly variation in the number of branches there was statistically significant increase in all treatments except in control plants (nematode alone). There was no significant increase in branch number in nematode alone upto nine months and after that there was

Table 18 Effect of different treatments on the soil population of nematodes in soil at different intervals

Treatments	Mean population of nematodes at different intervals [months after treatment (MAT)]									
	Initial		2 MAT		4 MAT		6 MAT	8 MAT	10 MAT	CD (0.05)
	MI	RS	MI	RS	MI	RS	MI	MI	MI	MI
Gl. f.	113.60	53.00	47.33	17.66	48.66	9.00	47.00	42.00	36.33	7.584
P.fl.	107.00	55.66	45.00	17.33	57.33	8.33	57.00	47.00	39.00	6.412
NC	108.33	52.66	42.33	12.33	51.66	3.33	67.33	56.00	42.33	8.346
Gn.c.	106.00	46.00	42.33	11.33	70.66	5.66	61.66	54.33	50.33	18.613
gly.m.	106.33	47.66	35.66	10.00	70.66	4.00	59.66	55.66	53.66	12.378
Cle	98.66	48.66	46.00	15.33	78.00	6.66	99.66	78.33	74.00	13.635
Carb	107.66	48.00	23.66	13.00	38.00	4.33	57.66	47.00	38.33	5.965
WA	104.33	49.66	35.33	14.00	56.33	7.00	58.33	55.33	48.66	5.458
NA	103.33	45.33	121.33	24.00	162.66	40.00	270.33	304.00	319.00	19.426
NF	-	-	-	-	-	-	-	-	-	-
P	106.66	50.00	55.00	17.00	64.33	7.00	61.33	45.33	40.00	6.712
CD (0.05)	NS	-	8.358	-	8.992	-	8.314	7.326	7.135	

Gl.f. – *Glomus fasciculatum*, P.fl. – *Pseudomonas fluorescens,* NC – Neem cake, Gnc – Groundnut cake, Gly.m. – *Glyricidia maculata*, Cle – *Clerodendron infortunatum*, Carb – Carbosulfan, WA – Wood ash, NA – Nematode alone, NF – nematode free, P – *Paecilomyces lilacinus* MI – *M. incognita*, RS – *R. similis*

significant increase revealing that nematode infestation was vulnerable in the initial stage of infestation in *P. rosea*, *i.e.*, upto 11 months.

4.5.4 Nematode Population in Soil

The mean population of nematodes (*M. incognita* and *R. similis*) estimated at bimonthly intervals after the application of different treatment were presented in Table 18. The data showed that there was significant variation in the population of nematodes in different treatments and also between different intervals. The initial population of nematodes in all the treatments were almost uniform and recorded 98-108 *M. incognita* and 45-55 *R. similis* per 100 g soil of sick plots. After two months onwards there was significant variation in the nematode population between different treatments and the treatment *G. fasciculatum* showed maximum reduction in nematode population in the soil throughout the crop period. The next best treatments were *P. fluorescens*, *P. lilacinus* and neem cake and these treatments were statistically on par with Carbosulfan in most of the intervals.

At two months after treatment (MAT) Carbosulfan recorded minimum population of *M. incognita* (23.66) and showed significant nematode suppression from all other treatments. The effect of wood ash, *Glyricidia*, groundnut cake and neem cake were statistically on par and recorded 35.33, 35.66, 42.33 and 42.33 *M. incognita* per 100 g soil and these treatments showed significant reduction in nematode population over the other treatments and control. Maximum nematode recovery was from nematode alone treated plots (121.33 per 100 g soil). There was no statistical significance in the population of *R. similis* after two months.

The population of *M. incognita* recorded minimum in Carbosulfan (38 / 100 g soil) and was significantly superior to other treatment in suppressing *M. incognita* in soil at 4 months after treatment. This was followed by *G. fasciculatum*, neem cake, wood ash and *P. fluorescens*. These treatments recorded 48.66, 51.66, 56.33 and 57.33 *M. incognita* per

100 g soil and the effect was statistically on par. The effect of *P. lilacinus* was on par with *P. fluorescens* and wood ash. All other treatments recorded significant reduction in nematode population over the control. Maximum population build up of *M. incognita* was recorded in nematode alone (162.66). The mean population of *R. similis* was significantly low in all the treatments.

After six months *G. fasciculatum* recorded minimum population of *M. incognita* (47 / 100 g soil). This was significantly superior in reducing nematode population to all other treatments. This was followed by treatments *P. fluorescens*, Carbosulfan, wood ash, *P. lilacinus*, *Glyricidia* and groundnut cake (57, 57.66, 58.33, 67.33, 61.66, 61.66 *M. incognita* per 100 g soil respectively) and these treatments were statistically on par and significantly superior to control. Maximum population of *M. incognita* was recorded in control (270.33),

Eight months after treatment, the population of *M. incognita* was found to be minimum in *G. fasciculatum* (42) treatment and it was statistically on par with treatments *P. lilacinus* (45.33), Carbosulfan (47) and *P. fluorescens* (47) and significantly superior to other treatments and control. The effect of treatments groundnut cake, wood ash, *Glyricidia* and neem cake were superior to control. Minimum nematode population was recorded in control (304 / 100 g soil).

The population of *M. incognita* was minimum in *G. fasciculatum* (36.33) at 10 MAT and was statistically on par with Carbosulfan, *P. fluorescens*, *P. lilacinus* and neem cake with 38.33, 39, 40 and 42.33 nematodes / 100 g soil. All these treatments were superior in controlling nematodes over other treatments. Maximum nematode population of 319 / 100 g was recorded in control (nematode alone).

At the time of harvest the lowest nematode population was recorded by *G. fasciculatum* (28.66/100 g soil) and was followed by *P. fluorescens* (31.33), *P. lilacinus* (32.66), Carbosulfan (33), neem cake (36), groundnut

Table 19 Effect of different treatments on the biometric characters of *P. rosea* at the time of harvest (twelve months after treatment)

Treatments	Plant height (cm)	Number of leaves	Number of branches	Leaf area (mm^2)	Fresh weight shoot (g)	Dry weight shoot (g)	Fresh weight root (g)	Dry weight root (g)
Gl. f.	62.66	80.33	18.33	1721.66	335.00	86.66	231.66	60.00
P.fl.	60.33	71.66	17.00	1676.66	310.00	80.00	220.00	50.00
NC	49.00	55.66	10.66	1538.33	241.66	61.66	175.00	43.33
Gn.c.	46.00	52.00	11.00	1481.00	211.66	53.33	170.00	41.66
gly.m.	48.66	56.00	11.33	1523.33	226.66	50.00	170.00	41.66
Cle	49.33	57.00	11.66	1475.00	213.33	51.66	153.33	41.66
Carb	59.33	71.00	15.00	1715.00	335.00	86.00	211.66	55.00
WA	44.33	52.33	10.00	1435.00	206.33	50.00	141.66	36.00
NA	34.66	42.33	7.33	1123.00	145.00	43.33	101.66	28.33
NF	51.00	63.33	13.66	1679.00	258.33	63.33	193.33	48.33
P	57.66	71.66	16.00	1671.00	306.66	81.66	201.66	53.33
CD (0.05)	4.155	7.472	2.021	137.94	20.000	7.542	22.853	6.576

Gl.f. – *Glomus fasciculatum*, P.fl. – *Pseudomonas fluorescens,* NC – Neem cake, Gnc – Groundnut cake, Gly.m. – *Glyricidia maculata*, Cle – *Clerodendron infortunatum*, Carb – Carbosulfan, WA – Wood ash, NA – Nematode alone, NF – nematode free, P – *Paecilomyces lilacinus*

cake (38.33) and wood ash (41.66) and these treatments were statistically on par and superior to all other treatments. The treatment nematode alone recorded the highest population (346.66).

Regarding the monthly variation in population all the treatments recorded statistically significant reduction in population at 2 MAT from the initial level and after that the variation was minimum. In *G. fasciculatum* treatment the population was statistically on par at 2, 4, 6 and 8 MAT. At 10 MAT the population was on par with 8 MAT but showed variation from the population of previous MAT's and also from population at harvest.

In *P. fluorescens* the population at 4 MAT showed significant increase from population at 2 MAT and also 10 MAT showed reduction from 8 MAT. The mean population at 4, 6, 8 MAT were statistically on par. There was also significant decrease in the population at 10 MAT and at harvest. The effect of neem cake showed variation in population at every MAT from the previous level. In groundnut cake and *Glyricidia* the population at different MAT's were on par with previous population from 4 MAT to 10 MAT.

4.5.5 Biometric Characters at the Time of Harvest

The effect of different treatments on the biometric characters of *P. rosea* at the time of harvest are presented in Table 19. The data revealed significant variation in the biometric characters (plant height, number of leaves, number of branches and leaf area) and fresh and dry weight of shoots between different treatments.

4.5.5.1 Plant Height

Te plant height recorded maximum in *G. fasciculatum* treated plants (62.66 cm) and was found statistically on par with *P. fluorescens* (60.33 cm) and Carbosulfan (59.33 cm) and was significantly superior to all other treatments. The fungus, *P. lilacinus* treatment (57.66 cm) was

statistically on par with *P. fluorescens* and Carbosulfan. The nematode free plants recorded 51 cm height and was statistically on par with neem cake. *Glyricidia* was significantly superior to nematode alone (control plants) and inferior to the above mentioned treatments. Minimum height was recorded by nematode alone treated control plants (34.66 cm).

4.5.5.2 Number of Leaves

Regarding the number of leaves, the best treatment was *G. fasciculatum* with an average of 80.33 leaves and was significantly superior to all other treatments. The number of leaves of plants received treatments *P. fluorescens* (71.66), Carbosulfan (71) and *P. lilacinus* (71.66) were statistically on par and significantly superior to all other treatment. The treatments neem cake, groundnut cake, *Glyricidia*, *Clerodendron* and wood ash were statistically on par and significantly superior to control plants and inferior to the bioagents and carbosulfan. Minimum number of leaves was recorded in control (42.44).

4.5.5.3 Number of Branches

Maximum number of branches (18.33) was recorded in *G. fasciculatum* and was statistically on par with *P. fluorescens* (17) and these two treatments showed significant superiority over other treatments. The treatments organic cakes, green leaves and wood ash were statistically on par and was significantly superior to control plants and inferior to all other treatments.

4.5.5.4 Leaf Area

Maximum leaf area was recorded in *G. fasciculatum* treated plants (1721.66 mm^2) and it was statistically on par with *P. fluorescens*, Carbosulfan, nematode free and *P. lilacinus* and these treatments were superior to all other treatments. The treatments organic cakes, green leaves and wood ash were statistically on par and showed significant

superiority over control plants. The control plants recorded the lowest leaf area (1123.00 mm^2).

4.5.5.5 Fresh Weight of Shoot

Maximum fresh weight of shoots (335 g) was found in *G. fasciculatum* and Carbosulfan and was significantly superior over all other treatments. The next best treatment was *P. fluorescens* (310 g) followed by *P. lilacinus* (306 g) and these two treatments were statistically on par and statistically superior to all other treatments. The treatment neem cake (241.66 g) and nematode free (258.33 g) were also statistically on par and superior to the next treatments and control. All other treatments were also found to be significantly superior to control plants and the control plants recorded minimum fresh shoot weight (145 g).

4.5.5.6 Dry Weight of Shoots

This also recorded the same result of fresh weight of shoots.

4.5.5.7 Fresh Weight of Roots

Fresh weight of roots was maximum in *G. fasciculatum* treated plants (231.66 g) and was found significantly superior to all other treatments except *P. fluorescens* (220 g) and these two treatments were statistically on par. This was followed by Carbosulfan (211 g), *P. lilacinus* (201.6 g) and nematode free (193.33 g). The treatments like organic cakes, wood ash and green leaves also recorded significant superiority over control plants (nematode alone) and inferior to the above treatments. The control plants recorded 101.66 g.

4.5.5.8 Dry Weight of Roots

Regarding the dry weight of roots maximum was recorded by *G. fasciculatum* (60 g) which showed significant superiority over other treatments. This was followed by Carbosulfan (55 g), *P. lilacinus* (53.33 g) and *P. fluorescens* (50 g) and these treatments were statistically on par

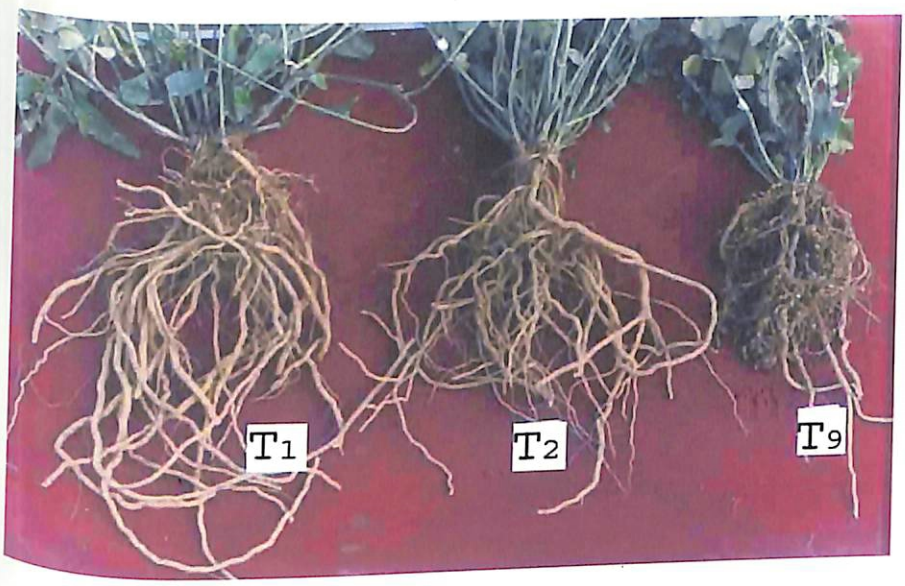
Table 20 Effect of different treatments on the yield of *P. rosea* and yield attributing characters at the time of harvest (twelve months after treatment)

Treatments	Number of tuber / plant	Length of tuber	Width of tuber	Yield / plant (g)	Yield / plot (g)
Gl. f.	23.33	60.00	1.23	231.66	2085.00
P.fl.	20.33	54.00	1.20	220.00	1980.00
NC	12.33	45.33	0.90	175.00	1575.00
Gn.c.	10.33	37.66	0.70	170.00	1530.00
gly.m.	12.33	40.33	0.80	170.00	1530.00
Cle	10.33	41.66	0.76	153.33	1380.00
Carb	17.33	52.33	1.23	211.66	1905.00
WA	8.33	36.00	0.76	141.66	1275.00
NA	6.00	25.00	0.50	101.66	915.00
NF	14.00	48.33	1.13	193.33	1740.00
P	16.00	52.33	1.06	201.66	1815.00
CD (0.05)	2.048	5.462	0.160	19.62	176.154

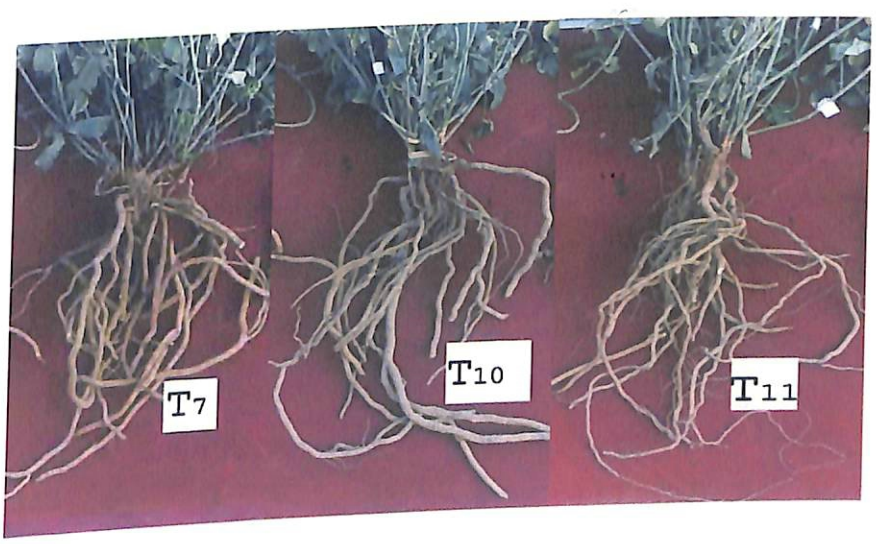
Gl.f. – *Glomus fasciculatum*, P.fl. – *Pseudomonas fluorescens,* NC – Neem cake, Gnc – Groundnut cake, Gly.m. – *Glyricidia maculata*, Cle – *Clerodendron infortunatum*, Carb – Carbosulfan, WA – Wood ash, NA – Nematode alone, NF – nematode free, P – *Paecilomyces lilacinus*

Plate 5

Effect of different management practices on the biometric characters and yield of Chethikoduveli, *Plumbago rosea*



T1 -*Glomus fasciculatum*
T2 -*Pseudomonas fluorescens*
T9 -*Nematode alone*



T7-Carbosulfan
T10-Nematode free
T11-*Paecilomyces lilacinus*

and significantly superior over other treatments. Control treatments (nematode alone) showed the minimum dry root weight (28.33 g).

4.5.6 Yield

The data presented in Table 20 revealed significant variation in yield and yield attributing characters like length, width and number of tubers in different treatments.

4.5.6.1 Number of Tubers

The treatment *G. fasciculatum* recorded an average of 23.33 tubers which showed significant superiority overall other treatments (Plate 5). The next best treatment was *P. fluorescens* (20.33) which also showed significant superiority over all other treatments. This was followed by Carbosulfan (17.33 tubers) and *P. lilacinus* (16 tubers) and these two treatments were statistically on par and was superior to rest of the treatments and inferior to the above two treatments.

4.5.6.2 Length of Tubers

In the case of tuber length the best treatment was *G. fasciculatum* (60 cm) which showed significantly higher tuber length over other treatments. This was followed by treatments *P. fluorescens* (54 cm), Carbosulfan (52.33 cm) and *P. lilacinus* (52.33 cm) and all these treatments were statistically on par and showed significant superiority over all other treatments except nematode free (48.33 cm). All other treatments were significantly superior to nematode alone and inferior to the above mentioned treatments. The nematode alone treated control plants recorded 25 cm tuber length which was significantly inferior to all other treatments.

4.5.6.3 Width of Tubers

The width of the tubers was maximum (1.23 cm) in *G. fasciculatum* and Carbosulfan treatments and were significantly superior to other

Table 21 Effect of different treatments on the soil population and nematode population characters (twelve months after treatment)

Treatments	Population in soil (100 g)	Number of larvae/ 5 g root sample	Number of females/ 5 g root sample	Root-knot count per gram of root sample	Root-knot index	Root-knot with egg mass	Root-knot without egg mass	Number of viable eggs per egg mass	Number of larvae per egg mass	Percentage of root-knot with egg mass
Gl. f.	28.66	10.00 (3.32)	7.33 (2.88)	4.66 (2.38)	1	2.00	2.66	71.66	71.66	42.91
P.fl.	31.3	16.33 (4.16)	12.33 (3.64)	8.66 (3.11)	2	3.66	5.00	76.00	76.00	42.26
NC	36.00	15.00 (4.00)	13.33 (3.78)	4.00 (2.23)	1	2.00	2.00	77.33	77.33	50.00
Gn.c.	38.33	21.66 (4.76)	17.66 (4.32)	9.66 (3.31)	2	5.33	4.33	98.33	98.33	55.17
gly.m.	48.33	22.33 (4.83)	20.00 (4.58)	9.66 (3.31)	2	5.66	4.00	113.66	113.66	58.59
Cle	65.66	24.00 (5.00)	21.00 (4.69)	11.00 (3.46)	3	5.66	5.33	116.33	116.33	51.45
Carb	33.00	10.33 (3.36)	3.33 (2.08)	2.33 (1.82)	1	0.66	1.66	69.33	69.33	28.33
WA	41.66	22.66 (4.86)	16.66 (4.20)	10.00 (3.32)	2	6.00	4.00	107.33	107.33	60.00
NA	346.66	93.66 (9.76)	61.66 (7.92)	33.66 (5.87)	5	23.66	10.00	127.00	127.00	70.29
NF	-	0.00 (1)	0.00 (1)	0.00 (1)	0	0	0	-	-	-
P	32.66	17.66 (4.32)	16.33 (4.16	9.33 (3.31)	2	4.33	5.00	78.00	78.00	46.40
CD (0.05)	14.486	0.492	0.462	0.413	-	-	-	21.625	-	-

Gl.f. – *Glomus fasciculatum*, P.fl. – *Pseudomonas fluorescens,* NC – Neem cake, Gnc – Groundnut cake, Gly.m. – *Glyricidia maculata*, Cle – *Clerodendron infortunatum*, Carb – Carbosulfan, WA – Wood ash, NA – Nematode alone, NF – nematode free, P – *Paecilomyces lilacinus*
Figures in parenthesis are $\sqrt{x + 1}$ transformed values

treatments and statistically on par with *P. fluorescens* (1.2 cm) and nematode free (1.13 cm). The next best treatment was *P. lilacinus* (1.06 cm) and was statistically on par with neem cake (0.9 cm). All other treatments were significantly superior to control but inferior to the above mentioned treatments. The control plant recorded 0.5 cm tuber width which was significantly lower compared to other treatments.

4.5.6.4 Weight of Tubers

Tuber yield in terms of weight was maximum in *G. fasciculatum* (231.66 g / plant) and the effect was on par with the treatment *P. fluorescens* (220 g /plant). The next best treatment was Carbosulfan (211.66 g/plant) and was on par with *P. fluorescens* and *P. lilacinus* (201.66 g/plant) and showed significant superiority over other treatments except nematode free which record tuber yield of 193.33 g/plant (Plate 5). All other treatments were significantly superior to control plants and the treatments neem cake, groundnut cake and *Glyricidia* were statistically on par. The nematode alone treatment (control) recorded significantly lower yield (101.66 g/plant) compared to all other treatments. The tuber yield per plot also showed the same trend.

4.5.7 Nematode Population Characteristics

The effect of different treatments on the population of nematode in soil, root and population characteristics at the time of harvest are presented in Table 21.

4.5.7.1 Nematode Population in Soil

The best treatment for managing the nematodes was found to be *G. fasciculatum* which recorded 28.66 nematodes / 100 g soil sample and this was followed by *P. fluorescens* (31.33), *P. lilacinus* (32.66), carbosulfan (33.00) neem cake (36.00), groundnut cake (38.33) and wood ash (41.66) respectively and all these treatments were statistically on par and significantly superior to control plants. The treatment *Glyricidia* was

found to be statistically on par with neem cake, groundnut cake and wood ash and was significantly superior to control. The control treatment recorded minimum recovery of nematodes from soil (346.66 in 100 soil surface).

4.5.7.2 Nematode Population in Root

Minimum nematode recovery was recorded in treatment *G. fasciculatum* (10) which was statistically on par with the treatment Carbosulfan (10.33) and these two treatments were significantly superior to other treatments. The next best treatment was neem cake (15) followed by *P. fluorescens* (16.33) and *P. lilacinus* (17.66) and these treatments were also statistically on par and significantly superior to control in suppressing the nematode population in roots. All other treatments also showed significant variation from the control plants but inferior to the above mentioned treatments. The maximum nematode recovery was recorded in untreated control (93.66 / 5 g of root).

4.5.7.3 Number of Females

The number of females in the roots was minimum in Carbosulfan (3.33/5 g of root) and was statistically lower than other treatments. The next best treatment was *G. fasciculatum* recorded an average of 7.33 females per 5 g root and this was also significantly superior to other treatments in nematode suppression. This was followed by *P. fluorescens* (12.33), neem cake (13.33), *P. lilacinus* (16.33), wood ash (16.66) and groundnut cake (17.66) respectively. All these treatments showed significant reduction over the control (61.66). The effect of the treatments groundnut cake, *Clerodendron*, wood ash and *Glyricidia* were statistically on par.

4.5.7.4 Root-knot Count

Root-knot count was minimum in the treatment Carbosulfan (2.33 / g) which showed statistically significant variation from all other treatments.

The next best treatment was neem cake (4 / g of root) and was on par with *G. fasciculatum* (4.66 /g of root) and these two were also significantly superior to other treatments in reducing the root-knots. The treatments *P. fluorescens* (8.66/g root) and *P. lilacinus* (9.33/g root) were also found statistically on par and significantly superior to other treatments in reducing the root-knots but inferior to the above mentioned treatments. Rest of the treatments were also found statistically on par and significantly superior in reducing the root-knots over the control and the control plants recorded maximum root-knots (33.66/g of root).

4.5.7.5 Root-knot Index

Regarding the root-knot index minimum index 'one' was recorded in treatments *G. fasciculatum*, neem cake and Carbosulfan and these three treatments were found to be the best in reducing the root-knots. This was followed by *P. fluorescens*, *P. lilacinus*, groundnut cake, wood ash and *Glyricidia* and these treatments recorded an index of '2'. The next best treatment was *Clerodendron* which recorded an index '3' and maximum root-knot index '5' was recorded by the nematode alone treated plants.

4.5.7.6 Number of Root-knots with Egg Mass

The minimum number of root-knots with egg mass was recorded in treatment Carbosulfan (0.66) and was followed by treatments *G. fasciculatum* (2) and neem cake (2) and these three treatments were superior to other treatments in reducing the nematode population. All other treatments recorded 3-6 root-knot with egg mass / g of root which were also significantly superior to control plants and the control plants recorded 23.66 root-knots with egg mass and this was 70.29 per cent of the total rot-knots. The treatment Carbosulfan recorded only 28.33 per cent root-knots having egg mass and other treatments like *G. fasciculatum*, *P. lilacinus* and *P. fluorescens* recorded root-knots having egg mass below 50 percentage. The treatments like groundnut cake, *Glyricidia* and *Clerodendron* recorded 50-60 per cent root-knots having egg mass.

Table 22 Effect of different treatments on NPK content of leaves at the time of harvest (twelve months after treatment)

Treatments	N (%)	P (%)	K (%)
Glomus fasciculatum	1.23	0.149	1.68
Pseudomonas fluorescens	1.20	0.125	1.92
Neem cake	1.06	0.150	1.56
Clerodendron	1.12	0.138	1.38
Carbosulfan	1.06	0.150	1.56
Nematode alone	0.63	0.082	0.96
Nematode free	1.16	0.132	1.48
Paecilomyces lilacinus	1.43	0.158	1.44
CD (0.05)	0.323	0.041	0.352

4.5.7.7 Number of Viable Eggs/Egg Mass

Minimum number of viable eggs / egg mass was found in treatment Carbosulfan (69.33) and this was followed by *G. fasciculatum* (71.66), *P. fluorescens* (76.00), neem cake (77.33) and *P. lilacinus* (78) and all these treatments were found to be best over other treatments and control. The treatments groundnut cake, *Glyricidia*, *Clerodendron* and wood ash recorded 98 to 116 viable eggs/egg mass and maximum viable eggs per egg mass was recorded in the root-knots of nematode alone (127).

4.5.7.8 Number of Larvae per Egg Mass

In the case of number of larvae per egg mass the same result was obtained. Number of larvae per egg mass was minimum in treatment Carbosulfan (69.33) and this was followed by *G. fasciculatum* (71.66), *P. fluorescens* (76.00), neem cake (77.33) and *P. lilacinus* (78) and all these treatments were found to be best over control. Maximum larvae per egg mass was recorded in nematode alone (127).

4.5.8 NPK Content of Leaves

The NPK content of leaves of *P. rosea* are presented in Table 22. The data revealed that there was significant variation in the NPK content of leaves in different treatments.

The percentage of nitrogen content was maximum in the leaves of *P. lilacinus* treated plants (1.43) and was statistically on par with treatments *G. fasciculatum* (1.23) and *P. fluorescens* (1.20). Control plants recorded the minimum N content (0.63 %).

Regarding the phosphorus content in the leaves (percentage) maximum was recorded by *P. lilacinus* treated plants (0.158 %) which was statistically on par with neem cake (0.150), Carbosulfan (0.150) and *G. fasciculatum* (0.149) and was significantly superior to the phosphorus content of nematode alone treated plants (0.082 %).

Potassium content in the leaves of *P. rosea* was maximum in *P. fluorescens* treated plants (1.92 %) and was statistically on par with *G. fasciculatum* (1.68 %), neem cake (1.56 %) and Carbosulfan (1.56 %). All the treatments tested were significantly superior in 'K' content of leaves over the control plants (0.96 %).

4.5. 9 Re-isolation of Bioagents from Roots and Rhizosphere

Re-isolation of *P. fluorescens* from the rhizosphere recorded 22 colony forming units in 10^{-6} dilution. In the case of *P. lilacinus* there was 48 colony forming units (cfu) in Rose Bengal agar and 35 cfu in potato dextrose agar at 10^{-3} dilution. In the case of AMF as main field treatment, the colonization percentage in root was 72 and spore count in soil was 70 per 25 g soil.

Discussion

5. DISCUSSION

Studies were conducted to evolve an ecofriendly management strategy for controlling nematodes (*Meloidogyne incognita* and *Radopholus similis*) associated with Chethikoduveli (*Plumbago rosea* L.) using biocontrol agents (*Glomus fasciculatum*, *Pseudomonas fluorescens* and *Paecilomyces lilacinus*) and organic amendments (neem and groundnut cake). The effect of green leaves (*Glyricidia maculata* and *Clerodendron infortunatum*) having antihelminthic properties and available in farmer's field was also studied as a low cost method.

Chethikoduveli (*P. rosea*) being a newly domesticated medicinal plant, information on the pathogenic level of nematodes and their loss in yield due to various pests and diseases are lacking. Hence pathogenicity and crop loss due to the above two major nematodes were studied in detail. The histopathological changes due to the infestation by *M. incognita* and *R. similis* were also investigated. These nematode infestation also induce biochemical changes in roots during feeding. Since the root portion of the crop is used for pharmacological preparations, these aspects were also studied.

5.1 PATHOGENICITY

The results on the biometric characters of *P. rosea* presented in para 4.1.1 revealed that there was no significant reduction in the height of the plants upto six months after inoculation (MAI) of *R. similis* and 7 MAI in the case of *M. incognita*. The height of plant was reduced from 12 to 13 per cent by 10,000 J_2 level at varying intervals (2 to 6 months). From seven months onwards the levels 1000 and 10,000 J_2 showed statistically significant variation from the untreated control. *M. incognita* @ 1000 J_2 per plant showed pathogenic effect giving 23, 24, 23 and 27 per cent

reduction in plant height at 7, 8, 9 and 10 MAI respectively revealing that 7 to 8 months time is required for exhibiting growth retardation in *P. rosea*. But at the time of harvest (12 MAI) the rate of reduction in plant height was lowered slightly (20 per cent) (Fig. 1). Though at 100 J_2 level the reduction in height ranged from 10 to 12 per cent at 7 to 12 MAI (harvest), the effect was not enough to get statistically significant variation over control. Thus significant reduction in plant height was observed from 1000 J_2 level onwards. This finding is in agreement with Kumar and Singh (1997). They reported significant reduction in the height of *Mentha arvensis* at an initial inoculum level of 1000 J_2 onwards.

In the case of *R. similis*, statistically significant reduction in plant height was observed from the sixth months onwards. But there was a height reduction of 10 per cent at 3 MAI in 10,000 level of *R. similis* and the rate of reduction at 5 MAI was 12 per cent. *R. similis* @ 10,000 nematodes per plant only showed statistically significant reduction from the untreated control from six MAI onwards. But the effect of 10,000 and 1000 *R. similis* per plant were statistically on par. From 9 MAI onwards 1000 level of *R. similis* also showed statistically significant variation from the untreated control. *R. similis* @ 1000 per plant showed a reduction of 13 to 17 per cent from 6 MAI to 12 MAI. At 10,000 level a height reduction of 17 to 25 per cent was observed from 6 to 12 MAI revealing that in *P. rosea* 10,000 level of *R. similis* per plant is required to cause substantial reduction in plant height (Fig. 2). There was scanty reports on the infestation of *R. similis* on medicinal plants and no reports on *P. rosea*. Thus the study on this is reported for the first time. But there are similar reports on the reduction in height of plants in many other crops due to *R. similis* at same inoculum level as well as lower levels at different periods. Reduction in vine length of black pepper with the same level of *R. similis* (10,000 per vine), 55 days after inoculation was reported by

Venkitesan and Sethi (1977) while Geetha and Koshy (1995) reported a lower level of 100 *R. similis* per plant as pathogenic to sugarcane.

Comparing the effect of these two nematodes on height of *P. rosea*, *M. incognita* is exhibiting more damage at 1000 J_2 level.

The results presented in para 4.1.2 revealed that there was statistically significant reduction in the number of leaves of *P. rosea* at 7 and 6 MAI of *M. incognita* and *R. similis* respectively. But the reduction in number of leaves of *P. rosea* ranged from 12 to 21 per cent from 2 to 6 MAI at 10,000 J_2 level of *M. incognita*. Seven months after inoculation onwards the effect of 10,000 and 1000 J_2 level were statistically on par and showed significant variation from untreated control. *M. incognita* @ 10,000 J_2 level showed significant variation from the levels 100 and 10 J_2 from seven months after inoculation till harvest. From 7 MAI onwards 1000 J_2 level showed an increasing trend in the percentage reduction in number of leaves over control. There was 24, 25 32 and 38 per cent reduction at 7, 8, 9 and 10 MAI respectively. At the time of harvest (12 MAI) the reduction in number of leaves was only 32 per cent at 1000 J_2 level (Fig. 1). These results are similar to the findings of Nalinakumari *et al*. (1995) in betelvine. They reported 56.9 and 67.7 per cent reduction in number of leaves at a higher level of 4000 and 5000 J_2 of *M. incognita* per plant.

In *P. rosea*, *R. similis* inoculation also reduced the number of leaves. The effect of the levels 1000 and 10,000 were statistically on par and showed significant variation from the untreated control from 7 MAI onwards. There was 23, 30, 32, 34 and 35 per cent reduction at 6, 7, 8, 9 and 10 MAI respectively when the plants were inoculated with *R. similis* @ 1000 per plant. At the time of harvest (12 MAI), this level of inoculum showed 36 per cent reduction in the number of leaves (Fig. 2). However at lower level (100 *R. similis*) also, same reduction in the number of

leaves of betelvine was reported by Geetha *et al*. (1995). They reported 38.70 per cent reduction in number of leaves of betelvine at 100 *R. similis* per plant, whereas Jasy and Koshy (1992) reported 17 per cent reduction in the number of leaves of avocado over a period of four months at an initial inoculum level of 10,000 *R. similis* per plant.

The comparative study of these two nematodes revealed that the pathogenic effect of *M. incognita* is more than that of *R. similis* at 10,000 level where as at 1000 level the effect of *R. similis* was slightly higher than that of *M. incognita*. At lower level (100) the effect of *M. incognita* and *R. similis* showed 25 and 11 per cent reduction in number leaves respectively revealing that *M. incognita* induce more reduction. This type of findings are lacking in medicinal plants.

The results presented in para 4.1.3 revealed that there was statistically significant reduction in the number of branches of *P. rosea*, six and eight months after inoculation of *M. incognita* and *R. similis* respectively. Though infestation of the above nematodes showed reduction in the rate of production of branches from 2 MAI onwards the effect was insignificant. *M. incognita* @ 1000 J_2 per plant showed 37, 31, 33, 33, 35, 29 and 34 per cent reduction in number of branches of *P. rosea* at 6, 7, 8, 9, 10, 11 and 12 MAI respectively. Plants inoculated with 100 J_2 level also recorded statistically significant reduction in the number of branches over untreated, 11 months after inoculation onwards. At the time of harvest this level showed 24 per cent reduction indicating that 100 J_2 of *M. incognita* became pathogenic to *P. rosea* after one year of inoculation (Fig. 1). The progressive multiplication of the nematode may be responsible for the pathogenic effect of the lower level.

In the case of *R. similis* the levels 1000 and 10,000 only showed statistically significant reduction from the untreated control from seven months after inoculation. The reduction being 23, 23, 22, 24 and 24 per cent

at 8, 9, 10, 11 and 12 MAI respectively at 1000 level of *R. similis*. Number of branches recorded maximum reduction in 1000 and 10,000 levels for both the nematodes at the time of harvest and it was 34 and 52 per cent in *M. incognita* and 24 and 25 per cent in *R. similis* respectively (Fig. 1 & 2). Thus an inoculum level of 1000 and above of the nematodes were pathogenic to *P. rosea*.

Comparing the effect of these two nematodes, *M. incognita* established its pathogenicity within short period (6 MAI) when compared to *R. similis* (8 MAI). The percentage reduction was also high in *M. incognita* (34) when compared to *R. similis* (24) at 1000 level. The inoculum required for getting the above effect was also lower in *M. incognita* (100 J_2) than *R. similis* (1000). In both the cases pathogenic effect increased with increase in time.

Regarding the leaf area (para 4.1.4) the effect of *M. incognita* was more than that of *R. similis*. All the levels of *M. incognita* showed statistically significant reduction in leaf area whereas in the case of *R. similis* from 100 level onwards only there was statistically significant reduction from the untreated control (Fig. 1 & 2). There was 25 and 35 per cent reduction in leaf area at 1000 and 10,000 J_2 levels of *M. incognita* whereas in the case of *R. similis* these levels recorded 17 and 28 per cent reduction respectively revealing that 10,000 level of *R. similis* was pathogenic in reducing leaf area. But in *M. incognita* even 100 J_2 level there was 20 per cent reduction and this level onwards *M. incognita* is pathogenic. These findings are in agreement with that of Haider *et al*. (1998). They reported statistically significant reduction in leaf area of turmeric at an initial inoculum level of 1000 and 10,000 J_2 of *M. incognita*. There were no reports on the reduction in leaf area due to *R. similis* in medicinal plants.

The data presented in para 4.1.5 revealed that there was statistically significant variation in the fresh weight of shoots and roots of *P. rosea* infested by various levels of *M. incognita* and *R. similis*. The reduction in plant height, number of leaves and branches directly contributed to the reduction in weight of shoots. All the levels (10, 100, 1000 and 10,000) showed statistically significant reduction in fresh weight of shoot over control plants in both the nematode inoculated plants. There was a progressive reduction in weight of shoots in response to increase in inoculum levels of *M. incognita*, the percentage being 16, 29, 55 and 73 for 10, 100, 1000 and 10000 J_2 levels respectively. These findings are in agreement with that of Kumar and Singh (1997). They reported a progressive reduction in weight of shoots of *M. arvensis* with increase in the initial nematode inoculum (10, 100, 1000 and 10,000 J_2 of *M. incognita* per pot). In *R. similis* inoculated plants, the same levels showed 16, 45, 54 and 61 per cent reduction in shoot weight. Similar results were reported by Jasy and Koshy (1992). They reported 10 per cent reduction in the fresh shoot weight of avocado when the plants were inoculated with 10000 *R. similis* per plant. But in the parent study this level recorded higher amount of reduction (61 per cent) in *P. rosea* and this may be due to difference in the physiology of the plant.

The levels 1000 and 10,000 J_2 of *M. incognita* exhibited maximum reduction than *R. similis* whereas at 100 level the effect of *R. similis* was more than that of *M. incognita*. The dry weight of shoot also recorded the same trend. Similar results were reported by Prasad and Reddy (1984) in Patcholi, *Pogostemon cablin*. They reported 15.97 per cent reduction in shoot weight (dry) at an initial inoculum level of 10,000 J_2 of *M. incognita* per plant.

The data on the fresh weight of root revealed that both the nematodes recorded significant reduction over the control from 100 level onwards. In *M. incognita* inoculated plants the reduction in fresh root

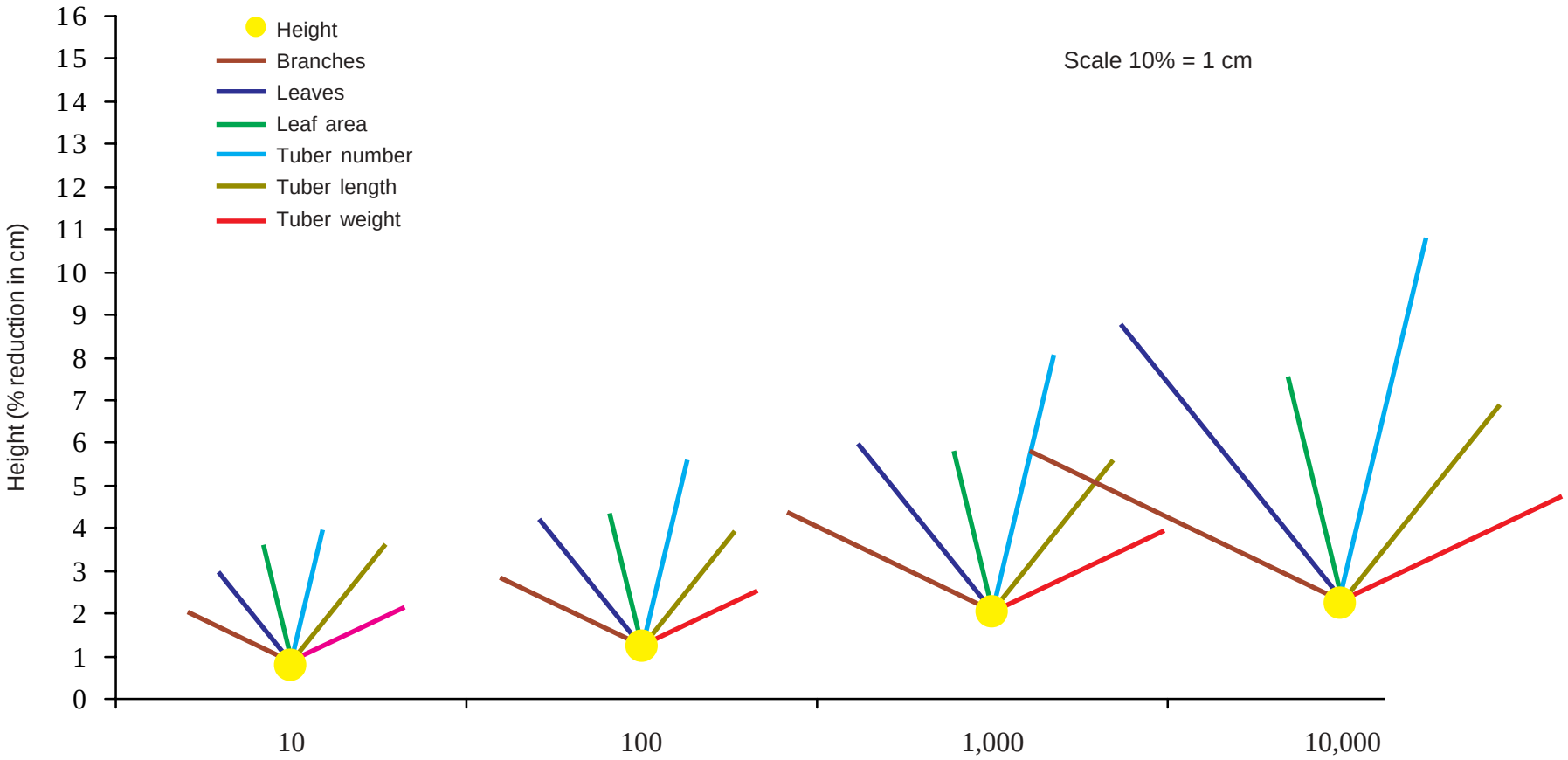


Fig. 1. Pathogenicity - Effect of different levels of *M. incognita* on the biometric characters and yield of *P. rosea* at the time of harvest (Percentage reduction over control)

weight was 11, 19, 28 and 37 per cent for 10, 100, 1000 and 10,000 J_2 levels whereas in *R. similis* inoculated plants it was 12, 34, 51 and 66 per cent for the same level of inoculum. The rate of reduction of fresh root weight was more in *R. similis* than in *M. incognita* at the same inoculum levels. The pathogenic effect was established by *R. similis* at 100 level whereas it was established only in 1000 level in *M. incognita*. Similar results are reported by Jasy and Koshy (1992a) in Avocado. They reported significant reduction in root weight of avocado at 100 level of *R. similis* onwards and 46 per cent reduction was recorded at an initial inoculum level of 10,000 *R. similis* per plant.

The yield of *P. rosea* presented in para 4.1.6 revealed that there was significant reduction in yield and yield attributing characters of *P. rosea* as initial inoculum level increased from 0 to 10,000 for both the nematodes. There was 21, 28, 54 and 59 per cent reduction in the number of tubers of *P. rosea* at 10, 100, 1000 and 10,000 level respectively in the case of *R. similis* where as in *M. incognita* inoculated plants the reduction in number of tuber was 9, 29, 40 and 57 per cent at 10, 100, 1000 and 10,000 J_2 levels respectively (Fig. 1 & 2). Maximum reduction in the number of tubers was recorded in *R. similis* inoculated plants in all the levels compared to *M. incognita*. Thus *M. incognita* was found pathogenic from 100 level onwards whereas *R. similis* from 10 level onwards.

Regarding the length of tubers, statistically significant reduction was observed at 100 level onwards in both nematode treated plants. *R. similis* caused 12, 25, 32 and 40 per cent reduction in tuber length at 10, 100, 1000 and 10,000 levels respectively. At the same levels of *M. incognita* there was 5, 22, 29 and 38 per cent reduction. The pathogenic effect of *R. similis* was more than that of *M. incognita.* There was statistically significant reduction in tuber width at 10 J_2 level onwards in the case of *M. incognita* and 100 level onwards in *R. similis*. The percentage reduction in tuber width due to different levels of *M. incognita*

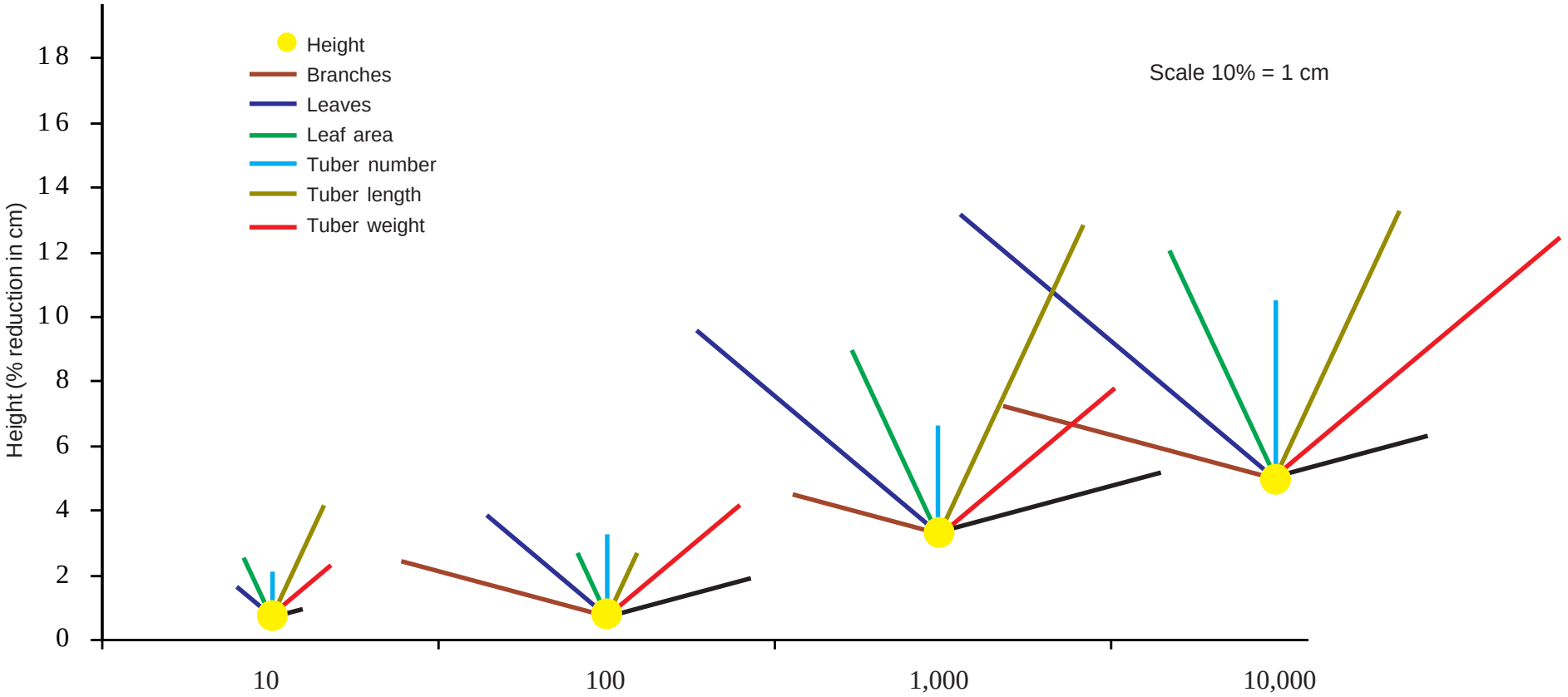


Fig. 2. Pathogenicity - Effect of different levels of *R. similis* on the biometric characters and yield of *P. rosea* at the time of harvest (Percentage reduction over control)

was 25, 28, 41 and 50 while it was 10, 24, 36 and 48 for *R. similis*. Thus it can be concluded that *M. incognita* and *R. similis* were found pathogenic from 10 and 100 levels respectively.

Yield in terms of weight of tubers recorded statistically significant variation from 100 J_2 level onwards in *M. incognita* and 10 level onwards in *R. similis*. Maximum reduction was recorded by 10,000 *R. similis* (66 per cent) whereas in *M. incognita* it was only 37 per cent at the same level (Fig. 1 & 2). At 100 and 1000 level of *R. similis* there was 34 and 51 per cent reduction in tuber weight over control. But the same levels of *M. incognita* showed only 19 and 28 per cent reduction revealing that *R. similis* is more pathogenic than *M. incognita* and the pathogenic effect was exhibited from 100 level onwards. But in *M. incognita* pathogenic effect was observed at 1000 J_2 and above. This yield (weight) reduction is a reflection of reduction in tuber number, tuber length and width. This may be due to the formation of lesion and necrosis of cortical cells and roots and finally the decay of feeder roots. This type of yield reduction due to *M. incognita* and *R. similis* was reported by several authors in various crops. Haider *et al*. (1998) reported significant reduction in yield in turmeric, *Curcuma longa* at an initial inoculum level of 100 J_2 of *M. incognita* per plant. But Sosamma *et al*. (1979) reported an yield reduction of 35 per cent in turmeric at a lower initial inoculum density of 10 *R. similis* per plant. Sundararaju *et al*. (1979) reported an yield reduction of 70 per cent in ginger at an initial inoculum level of 10,000 *R. similis*. But in this study the same level produced 60 per cent reduction in yield of *P. rosea* in terms of weight of tubers. It may be due to difference in susceptibility of *P. rosea* to *R. similis* compared to ginger.

Based on the pathogenic trial, initial inoculum levels 100 and 10,000 J_2 of *M. incognita* and *R. similis* respectively were fixed as pathogenic to *P. rosea*.

Regarding the population of nematodes in soil and roots, there was a progressive increase in accordance with the increase in initial inoculum level. *M. incognita* @ 10,000 J_2 produced maximum root-knot and recorded a root-knot index of five. In the case of *R. similis* also the level 10,000 recorded maximum number of lesions and lesion index of '3'.

5.2 HISTOPATHOLOGY

The histopathological changes due to infestation by *M. incognita* and *R. similis* were studied in *P. rosea* and the results revealed that *M. incognita* second stage juvenile penetrated the root tissues and established its feeding site in vascular parenchyma (Plate 1) within five days. This finding has been established by many workers (Siddiqui *et al*., 1974; Jacob, 1977; Shah and Raju, 1977; Sudha and Prabhoo, 1983; Molina and Nelson, 1983; Pasha *et al*., 1987; Fawole, 1988; Sosamma, 1988; Shetty and Rudra Muniyappa, 1992; Das and Barman, 1995). The formation of hypertrophy and hyperplasia in *P. rosea* and the pattern of giant cell formation is in agreement with the findings of Hassan and Jain (1985). But in *P. rosea* this is the first report of the above phenomenon. Rajani (1998) studied the histopathology of kacholam root infested by *M. incognita* and reported hypertrophy and giant cell formation in kacholam.

In the case of *R. similis* the nematode entered the root and started feeding in the cortical region (Plate 2b). Lesions and darkening of root took place five to seven days after the entry of the nematode. Longitudinal burrows were developed underneath the outer vertical cell layer. Burrows were formed due to the disintegration of cytoplasm and coalescence of cells (Plate 2d). After 20 days of inoculation, the disintegration and coalescence leading to tunnel formation were observed (Plate 2e). Similar findings were reported in arecanut roots by Sundararaju and Koshy (1988). According to Venkitesan and Sethi (1977) *R. similis*

penetrated the roots within 24 hours producing dark brown lesions within 72 hours of inoculation in pepper roots and they also observed that the point of feeding is the cortical parenchyma as seen in *P. rosea*.

5.3 BIOCHEMICAL CHANGES

Results presented in para 4.3 revealed that there was considerable variation in the phenol and total free amino acid contents consequent to infestation by *M. incognita* and *R. similis*. The phenol production was increased with increase in initial inoculum level in both the nematodes. This result is in agreement with that of Devarajan and Rajendran (2002). They reported higher quantity of phenol in banana roots infested with *R. similis* and the accumulation of phenol increased with infestation of *R. similis*. Variation in total free amino acids content expressed in terms of 0.79 µg equivalent of leucine revealed that the percentage increase in different levels of inoculum ranged from 12 to 216 for *M. incognita* and 8 to 240 in *R. similis*. These increase in production of phenol and total free amino acids may be due to the operation of the defence mechanism of the plant and the rate of production depends up on the mode of parasitism of the nematodes. The results are in conformity with the findings of Singh *et al*. (1978). They reported an increase in amino acid concentration in the roots of brinjal infested with *M. incognita*. Sharma and Trivedi (1996) also reported similar findings in bhindi roots due to *M. incognita* inoculation.

The rate of production of plumbagin was less in *R. similis* infested plants when compared to *M. incognita*. Maximum reduction of 7.76 per cent was recorded in 1000 J_2 level while it as 3.62 per cent in *R. similis*.

Roots of *P. rosea* contain a number of alkaloids but plumbagin is the major one. This type of finding is reported for the first time in *P. rosea*. No work on production of plumbagin content on same or other crops are available in the literature.

5.4 CROP LOSS ASSESSMENT

The assessment of losses and avoidable losses due to root-knot and burrowing nematode in *P. rosea* in terms of biometric characters and yield are presented in para 4.4. The results showed that there was significant variation in the biometric characters and yield of *P. rosea* due to infestation by different initial inoculum levels.

The variation in height of the plants showed statistical significance from two and six months after inoculation onwards in *R. similis* and *M. incognita* respectively (Para 4.4.1). The higher level (10,000 J_2) of *M. incognita* only showed statistically significant variation after six months. Eight months after inoculation onwards 1000 J_2 level also showed statistically significant variation from the control and check. There was 14 to 18 per cent reduction over control (1000 J_2 + Carbosulfan) from 8 to 12 MAI of *M. incognita* @ 1000 J_2 per plant. Thus the results revealed that 1000 J_2 of *M. incognita* attributed an avoidable height reduction of 14-18 per cent over a period of 6 to 12 months (Fig. 3). Similar findings with lower inoculum level of (500 J_2) was reported by Haseeb *et al*. (1999). They reported significant reduction in plant height of *Ocimum sanctum* at an initial inoculum level of 500 J_2 *M. incognita* and above. The higher level of inoculum required for getting significant reduction in plant height in *P. rosea* may be due to the sturdy nature of the plant and peculiar coating in roots.

In *R. similis* inoculated plants all the three levels (100, 1000 and 10,000) showed statistically significant reduction in plant height over control and check from 2 MAI onwards. There was 26 to 35 per cent reduction over control (1000 J_2 + Carbosulfan) from 2 to 12 MAI of *R. similis* @ 1000 nematodes per plant revealing that there was an avoidable reduction of 26 to 35 per cent in plant height over a period of 2 to 12 months (Fig. 4). The reports on the effect of *R. similis* on height

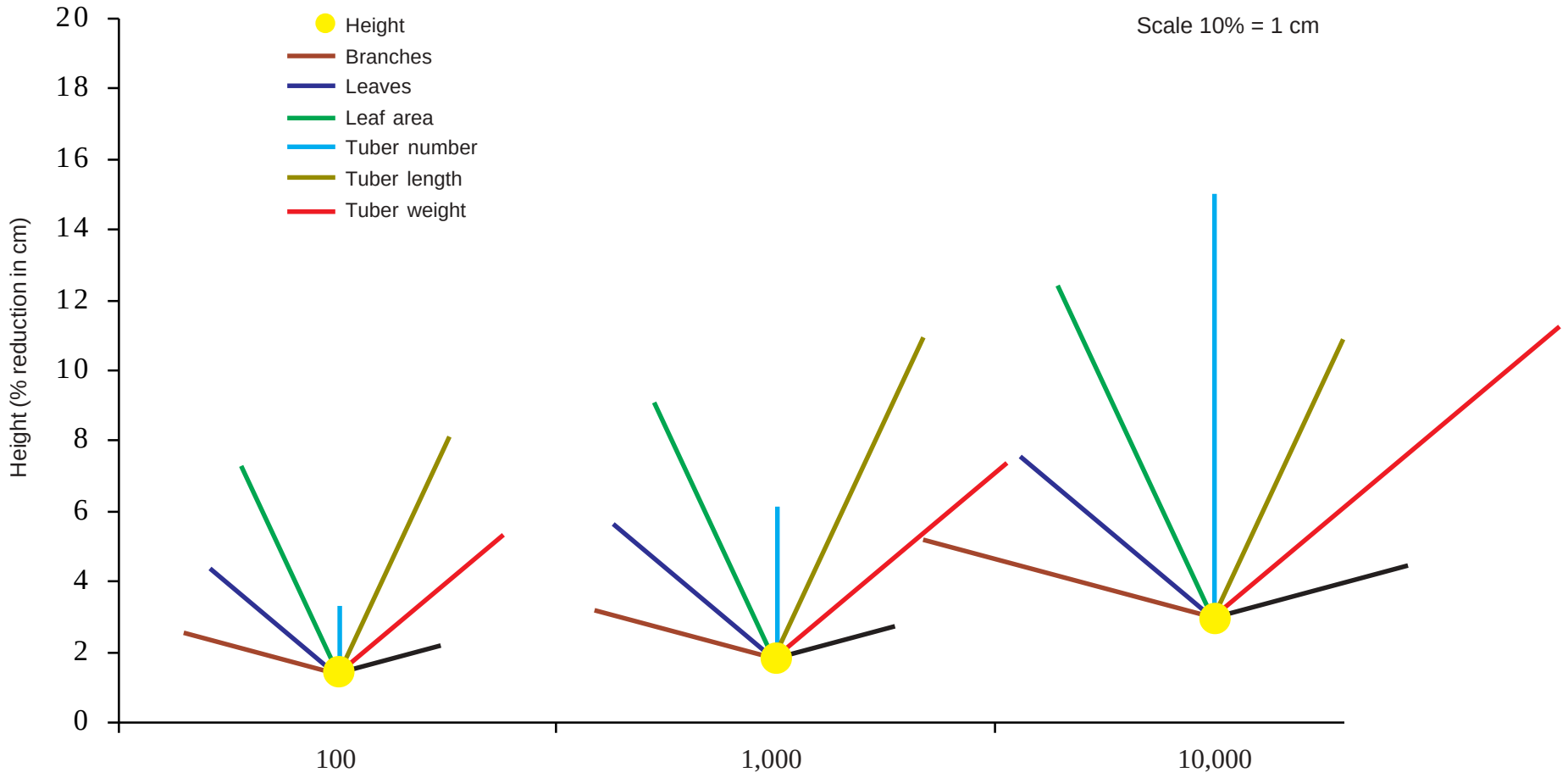


Fig. 3. Crop loss - Effect of different levels of *M. incognita* on the biometric characters and yield of *P. rosea* at the time of harvest (Percentage reduction over control)

reduction in medicinal plants is scanty but similar results in avocado was reported by Jasy and Koshy (1992). They found 10 per cent reduction in length of shoot at an initial inoculum density of 10,000 *R. similis* per plant. But in our study the lower level (100 nematodes per plant) also recorded significant reduction in plant height of *P. rosea*.

At 100 level the reduction over untreated was 18 to 23 per cent over a period of two to twelve months in *R. similis* whereas, in *M. incognita* the height reduction at the same level was 5 to 15 per cent over a period of 8-12 months. At 10,000 level the reduction over untreated was 34 to 37 per cent in *R. similis* and 11 to 31 per cent in *M. incognita* inoculated plants.

Comparing the effect of *M. incognita* and *R. similis* at different levels, *R. similis* caused more reduction than *M. incognita*. *R. similis* caused significant reduction from two months after inoculation while in *M. incognita* significant effect was obtained only from eight months after inoculation. The increased reduction in height of *P. rosea* due to *R. similis* may be due to the increased susceptibility in early periods of the crop and difference in the nature of parasitism of *M. incognita* and *R. similis*.

The effect of various levels of *M. incognita* and *R. similis* on the number of leaves presented in para 4.4.2 revealed that there was statistically significant reduction in the number of leaves over control and check. The level 10,000 J_2 of *M. incognita* only showed significant difference over the control from 7 MAI onwards. At the time of harvest all the levels showed significant difference over control (Fig. 3). However, 1000 J_2 level reduced the leaf number at the rate of 16 to 29 per cent over the control over a period of 7 to 12 MAI. Thus it can be concluded that 1000 J_2 of *M. incognita* produced an avoidable loss of 16 to 29 per cent in the number of leaves. Even higher rate of reduction of leaf number due to

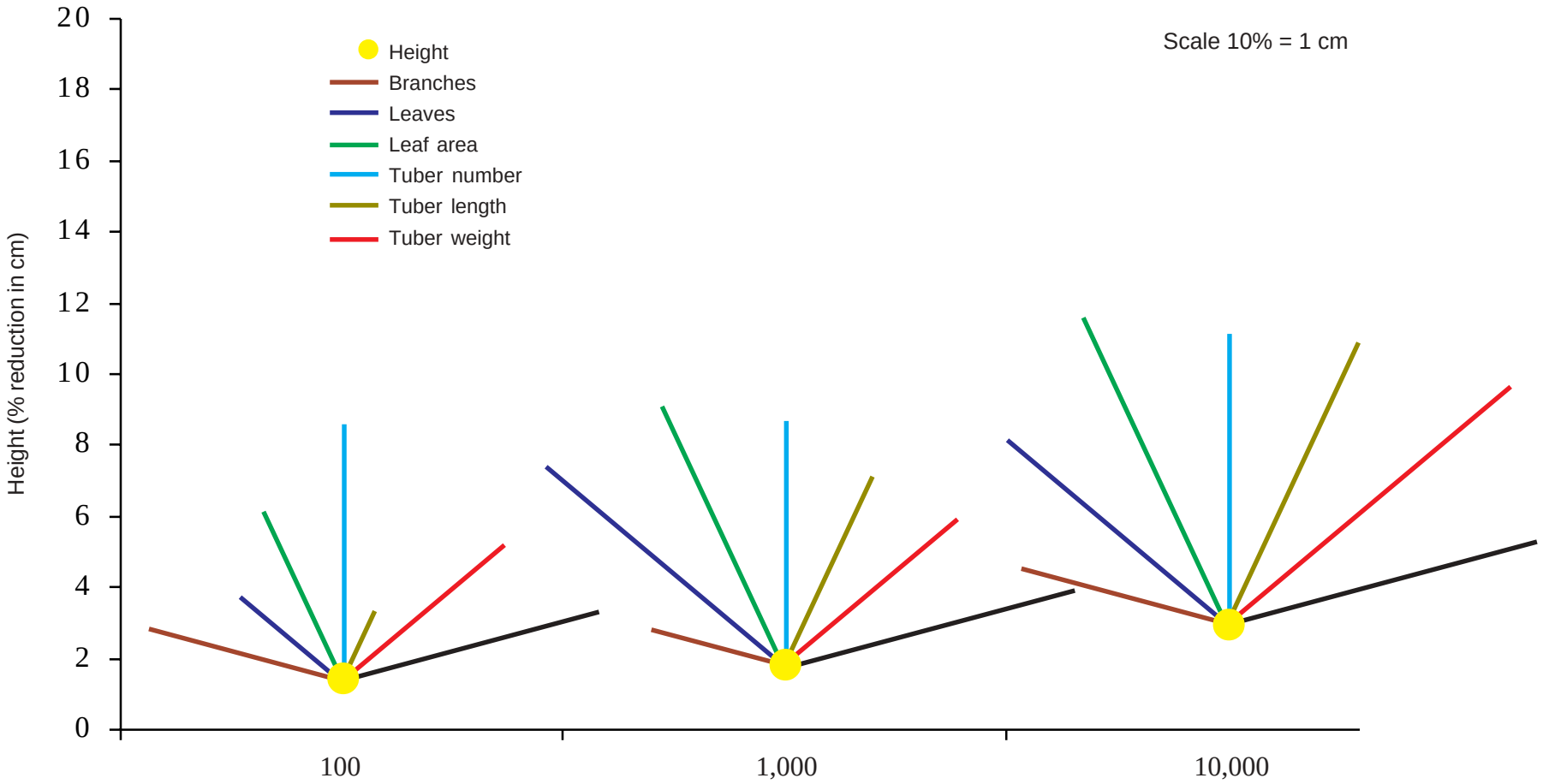


Fig. 4. Crop loss - Effect of different levels of *R. similis* on the biometric characters and yield of *P. rosea* at the time of harvest (Percentage reduction over control)

M. incognita was reported in other crops like betelvine. Nalinakumari *et al*. (1995) reported 56.9 and 67.7 per cent reduction in number of leaves in betelvine at higher level of 4000 and 5000 J_2 of *M. incognita* per plant.

Plants inoculated with all the three levels of *R. similis* (100, 1000 and 10,000) showed significant reduction in number of leaves over control and uninoculated check from three months after inoculation onwards. In 1000 level of *R. similis* inoculated plants there was 44 to 46 per cent reduction in the number of leaves over control during the period of three to 12 months. Thus 1000 *R. similis* per plant accorded an avoidable loss of 44 to 46 per cent in leaf production over a period of three to 12 months. This type of studies are scanty in *P. rosea* and other medicinal plants. But similar results were reported by Geetha *et al*. (1995) in betelvine. In their study there was 38.67 per cent reduction in the number of leaves of betelvine at 100 *R. similis* per 2500 cm^3 soil.

Comparing the loss in leaf production of these nematodes in *P. rosea*, *R. similis* caused more loss than *M. incognita*. At the lower level of 100 nematodes there was 21 per cent reduction in leaf number over the untreated plants whereas in *M. incognita* it was only 17 per cent. The highest level (10,000) of *R. similis* showed 33 per cent reduction over uninoculated whereas in *M. incognita* it was only 25 per cent.

Results presented in para 4.4.3 revealed that there was statistically significant variation in the number of branches of *P. rosea* at various inoculum level of *M. incognita* and *R. similis* (Fig. 3 & 4). In the case of *M. incognita* statistically significant variation was noticed from 7 MAI onwards whereas in *R. similis* it was from 4 MAI onwards. The level 10,000 J_2 of *M. incognita* only showed significant variation from the control from 7 to 9 MAI and after nine months 1000 J_2 level also showed significant variation from the control. There was 10 to 29 per cent reduction in the number of branches of *P. rosea* over control from 7 to

12 MAI with *M. incognita* @ 1000 J_2 per plant. This indicated that there was an avoidable reduction of 29 per cent in number of branches of *P. rosea*. The reports on reduction in number of branches due to infestation by these nematodes in medicinal plants is lacking.

All the three levels of *R. similis* showed significant variation from the control from 4 MAI onwards and at the time of harvest the levels 1000 and 10,000 only showed significant variation over control. There was 35 to 37 per cent reduction in the number of branches of *P. rosea* over control from 4 to 12 MAI when the plants were inoculated with *R. similis* @ 1000 nematodes / plant thus indicating that 1000 *R. similis* per plant produced an avoidable loss of 37 per cent at 12 MAI.

Comparing the effects of these two nematodes on the number of branches of *P. rosea*, *M. incognita* reduced more branches than *R. similis* from 100 level onwards. At 1000 level of *R. similis* there was 37 per cent reduction in the number of branches over untreated while in *M. incognita* it was only 29 per cent.

Infestation of *M. incognita* showed statistically significant variation in leaf area over the control and untreated plants (para 4.4.5). All the three levels of *R. similis* showed significant variation over control and uninoculated plants whereas in *M. incognita* the levels 1000 and 10,000 only showed significant variation from uninoculated. There was 23 per cent reduction in leaf area of *P. rosea* at 1000 J_2 level over control (1000 J_2 + Carbosulfan) revealing that there was an avoidable reduction in leaf area of 23 per cent in *P. rosea* due to *M. incognita* infection. In *R. similis* the reduction in leaf area over control was 21 per cent at 1000 level indicating that 21 per cent reduction in leaf area can be avoided by the application of carbofuran.

The data on the fresh and dry weight of shoots and root presented in para 4.4.5 showed statistically significant difference in the effect of

various levels of *M. incognita* and *R. similis*. The levels 1000 and 10,000 J_2 of *M. incognita* only showed statistically significant loss in fresh weight of shoot over control and untreated, whereas in *R. similis* all the three levels showed statistical significance. At 1000 level there was 29 per cent avoidable loss in weight of shoot due to *M. incognita* and it was 36 per cent in *R. similis*. This results are in line with the findings of Prasad and Reddy (1984). They reported 47 per cent reduction in shoot weight of Patchouli (*P. cablin*) at higher level (10,000 J_2) of *M. incognita*. But reports on reduction in shoot weight of medicinal plants due to infestation by *R. similis* is scanty. Comparing the effect of these two nematodes maximum reduction in fresh shoot weight was recorded by 10,000 J_2 of *M. incognita* (65 per cent) whereas in *R. similis* it was only 28 per cent over the untreated. Dry weight of shoot also showed the same trend.

The effect of different levels of these nematodes on the biometric characters of *P. rosea* revealed that there was a progressive increase in percentage reduction in height, number of leaves and branches, leaf area, fresh and dry weight of shoots and roots as the initial inoculum level increased from 100 to 10,000. This result is in agreement with the reports of many workers in various crops (Sundararaju *et al*., 1979 in ginger, Jasy and Koshy, 1992 in avocado and Haseeb *et al*., 1999 in *Ocimum sanctum*).

All the levels (100, 1000 and 10,000) of *M. incognita* and *R. similis* studied recorded statistically significant reduction in fresh weight of root. Plants inoculated with 1000 J_2 of *M. incognita* recorded 44 per cent loss in fresh root weight and in *R. similis* it was 35 per cent over control. Thus there was an avoidable loss of 44 and 35 per cent in fresh root weight of *P. rosea* at 1000 level of *M. incognita* and *R. similis* respectively. There was a reduction of 33 and 37 per cent at 10,000 level of *M. incognita* and *R. similis* respectively over untreated check. These results are in agreement with Prasad and Reddy (1984). They reported 27.90 per cent reduction in root weight at 10,000 J_2 level of *M. incognita* in *P. cablin*.

But Mohandas and Ramakrishnan (1997) reported significant reduction in fresh and dry fibrous root weight of *Dioscorea rotundata* at an initial inoculum level of 100 J_2 of *M. incognita* per plant.

The results on the yield and yield characters of *P. rosea* presented in para 4.4.6 indicated significant variation at different levels of *M. incognita* and *R. similis* in *P. rosea* (Fig. 3 & 4).

In the case of *M. incognita* inoculated plants, all the three levels (100, 1000 and 10,000) showed statistically significant variation in number of tubers over control and untreated. At 1000 J_2 level there was 55 per cent reduction in number of tuber over control revealing that this level of nematode produce an avoidable loss of 55 per cent in tuber number.

Length of tubers of *P. rosea* in *M. incognita* inoculated plants, showed statistically significant variation from the control and untreated at all the levels. There was 33 per cent reduction over control at 1000 J_2 level. There was 46 per cent reduction at 1000 J_2 level over uninoculated. Tuber width also showed the same trend and there was 47 per cent avoidable reduction at 1000 level. Regarding the yield in terms of weight of tubers, all the levels showed significant reduction over control. There was 44 per cent yield loss over control at 1000 J_2 level indicating that 44 per cent loss in yield can be avoided by application of carbosulfan @ 1 kg ai ha^{-1}. There are no reports about the loss in tuber yield in medicinal plants due to these nematodes. But in *D. rotundata*, Mohandas and Ramakrishnan (1997) reported significant reduction in tuber yield at a lower initial inoculum level of 100 J_2 of *M. incognita* per plant. But, Charles (1978) reported 46.4 per cent reduction in rhizome weight (yield) in ginger at higher inoculum level of 5000 J_2 of *M. incognita*.

Plants inoculated with *R. similis* showed statistically significant variation in yield and yield characters at various levels of inoculum. All the three levels showed significant variation over control and check.

There was an avoidable reduction of 48, 32, 44 and 35 per cent in tuber number, tuber length, tuber width and tuber weight (yield) respectively at 1000 level over control plants. Maximum loss were recorded at 10,000 level, the per cent reduction in number, length, width and weight of tubers being 49, 48, 44 and 37 respectively over the uninoculated. This kind of results are reported for the first time. There are no reports on the effect of *R. similis* on the tuber yield of medicinal plants especially *P. rosea*. But, Sundararaju *et al*. (1979) reported a higher loss of 73.60 per cent in the rhizome weight of ginger at same initial inoculum level of 10,000 nematodes over a period of six months. This may be due to the difference in susceptibility of crops to nematodes.

Regarding the population of nematodes in soil and root (root-knot count, root-knot index, number of lesions, lesion index), there was a progressive increase as the initial inoculum level increased from 100 to 10000. Maximum population of nematodes in soil and root was recorded in 10,000 level and this was followed by 1000 and 100 levels. The root-knot count and index recorded was maximum in 10,000 J_2 level and this was followed by 1000 J_2 level. The number of lesions and lesion index was also maximum in 10,000 level and this was followed by 1000 and 100 levels.

5.5 MANAGEMENT

Studies were conducted to evolve an eco-friendly management strategy using biocontrol agents (*G. fasciculatum, P. fluorescens*, *P. lilacinus*), organic amendments (neem cake and groundnut cake), green leaves (*G. maculata* and *C. infortunatum*) and wood ash (farmer's practice) in microplots (2 m x 2 m). The chemical, carbosulfan @ 1 kg ai ha^{-1} was also tried for comparing the efficacy of the above treatments. Nematode infested plots (NA) served as control and denematized plots (NF) as check.

The results as detailed in para 4.5 revealed that the biometric characters of *P. rosea* recorded at different intervals showed statistically significant variation. The bioagents *G. fasciculatum*, *P. fluorescens* and *P. lilacinus* were found effective as carbosulfan in reducing nematode infestation and improving plant character. *G. fasciculatum* was found superior to carbosulfan and other treatments in the case of improvement in plant height at all monthly intervals. The effect of organic amendments and green leaves were on par. The farmer's practice of applying wood ash was also found as effective as application of green leaves. A similar trend was seen in the improvement in number of leaves and number of branches. *G. fasciculatum* and *P. fluorescens* showed superiority over carbosulfan 4, 5 and 6 months after treatment (MAT) increasing the number of leaves and at 11 MAT *G. fasciculatum* established its superiority over *P. fluorescens*, *P. lilacinus* and carbosulfan. Regarding the branch formation, maximum improvement was brought about by *G. fasciculatum* at 7 MAT.

The effect of *G. fasciculatum* and *P. fluorescens* in the improvement of plant growth characters were already reported by various authors (Ray and Dalei, 1998 in greengram, Habte *et al*., 1999 in white clover, Santhi and Sivakuamr, 1995 and Verma *et al*., 1998 in tomato). But the effect of *G. fasciculatum* and *P. fluorescens* in *P. rosea* is reported for the first time. The efficacy of these two bioagents as seed treatment in kacholam (*Kaempferia galanga* L.), a popular medicinal plant of Kerala were reported earlier (Nisha and Sheela, 2003).

Among the organic amendments and green leaves the efficacy of neem cake and *Clerodendron* were also reported earlier by several workers. The effect of neem cake in managing plant parasitic nematodes and improving the growth parameters were reported earlier by many workers in several crops (Sundararaju and Sudha, 1993 in arecanut, pepper and banana, Rajani, 1998 and Nisha and Sheela, 2003 in kacholam). Groundnut cake was also reported to suppress the nematode population

and improve the growth parameters (Kamalakshiamma, 1986 in brinjal, Alam 1989 in eggplant, chilli, okra, cabbage and cauliflower). But the effect of these organic cakes on nematode suppression and improving the biometric characters and yield are reported for the first time in *P. rosea*. Several others reported that green leaves like *Glyricidia* and *Clerodendron* suppress the *R. similis* population and improve the growth parameters and yield in many crops (Jasy *et al*., 1992 in black pepper and Sudha and Sundararaju, 2001 in arecanut). These leaves also manage *M. incognita* population (Nisha and Sheela, 2003) in kacholam. But the effect of these green leaves and wood ash is reported for the first time in *P. rosea*. The potential of *P. lilacinus* also is reported for the first time in medicinal plants. But there are several reports of this biocontrol agent in many other crops (Khan and Goswamy, 2000a against *M. incognita* in tomato, Sosamma *et al*., 1998 against *R. similis* in betelvine and Sudha *et al*., 2000 against *R. similis* in arecanut).

The periodical observations on the reduction in nematode population in the soil revealed that at the initial stage (2 and 4 MAT) carbosulfan showed its superiority over other treatments. A 6 MAT *G. fasciculatum* was the best treatment but at 8 and 12 MAT the effect of *G. fasciculatum* was on par with *P. lilacinus*, carbosulfan and *P. fluorescens*. Carbosulfan was also on par with groundnut cake at 8 MAT and neem cake at 10 MAT. These findings are in agreement with the reports of Rajani (1998) and Nisha (2001) on kacholam. But the potential of *P. lilacinus* and groundnut cake in *P. rosea* are reported for the first time.

Observations recorded at the time of harvest, revealed that *G. fasciculatum* recorded maximum height (62.66 cm) followed by *P. fluorescens* (60.33 cm), carbosulfan (59.33) and *P. lilacinus* (57.66). These treatments recorded 81, 74, 71 and 66 per cent improvement in plant height respectively over the control (NA). Treatments like neem

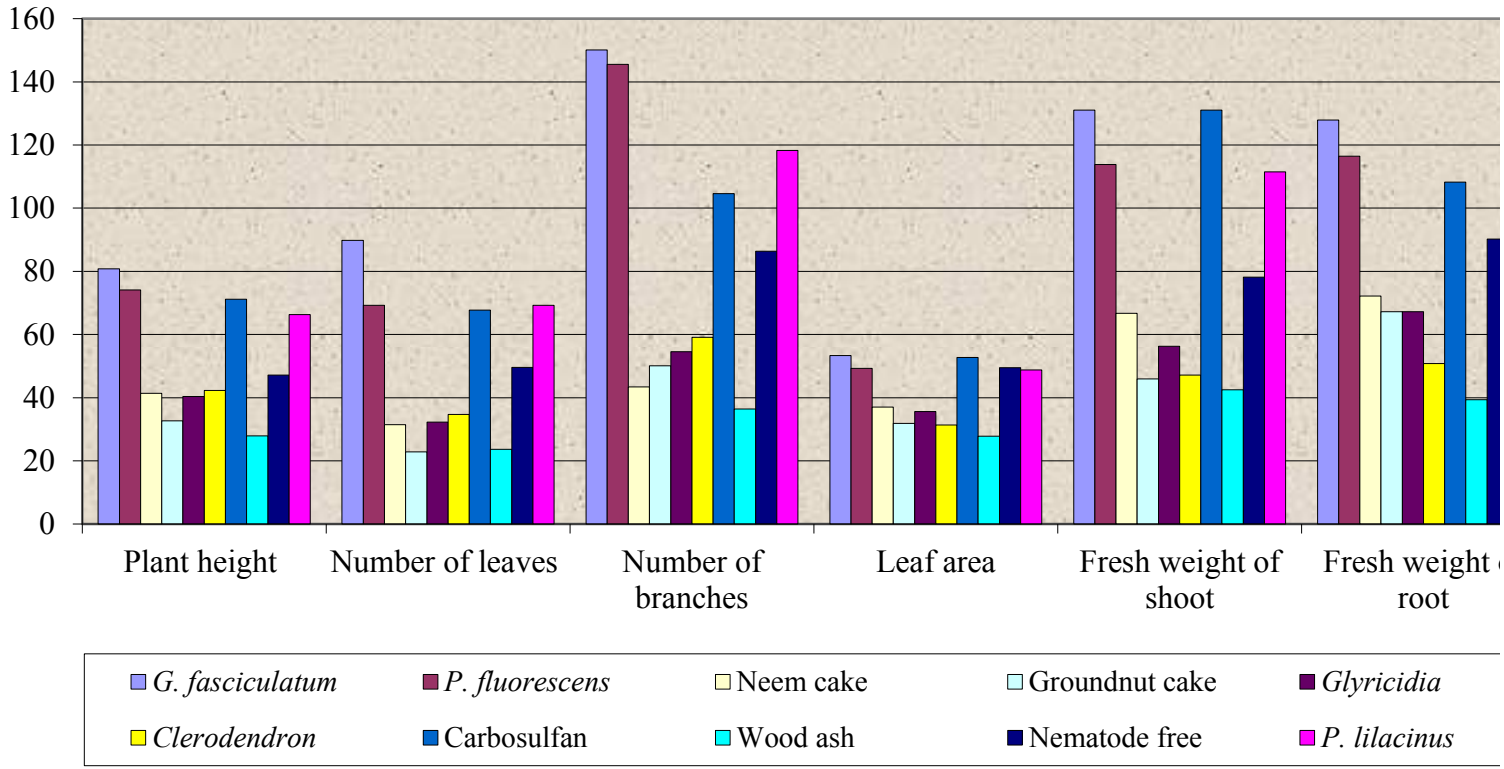


Fig. 5 Effect of different treatments on the biometric characters of *P. rosea* (percentage variation over contr

cake, *Glyricidia* and *Clerodendron* recorded 41, 40 and 42 per cent improvement respectively over control (Fig. 5). Regarding the number of leaves maximum was recorded in *G. fasciculatum* (80.33 leaves) and this showed 90 per cent increase in the number of leaves over control and 27 per cent increase over check (nematode free). *P. fluorescens*, *P. lilacinus* and carbosulfan recorded 69, 69 and 68 per cent improvement respectively over control (Fig. 5). Plants treated with organic amendments and green leaves showed 23 to 35 per cent increase in the number of leaves. Number of branches recorded maximum in plants treated with *G. fasciculatum* and was followed by *P. fluorescens*, *P. lilacinus* and carbosulfan. These treatments recorded 150, 145, 118 and 105 per cent increase in number of branches respectively over control (Fig. 5). In the case of leaf area, maximum was recorded in *G. fasciculatum* and this was followed by carbosulfan, *P. fluorescens* and *P. lilacinus*, the improvement in leaf area being 53, 52, 49 and 49 per cent respectively over control. In the case of fresh weight of shoot and root, the bioagents recorded maximum increase in weight. The percentage improvement being 131, 114 and 111 (shoot weight) and 128, 116 and 98 (root weight) respectively. Organic amendments and green leaves also recorded 46 to 67 per cent increase in shoot weight and 51 to 71 per cent increase in root weight over control.

The improvement in biometric characters by the bioagents may be due to the growth promoting activity of these beneficial fungi and bacteria in addition to the inhibition of nematode activity. The improvement in biometric characters (plant height, number of leaves, branches and fresh weight of roots and shoots) due to the reduction in the population of root-knot and burrowing nematodes by the action of AMF in the root zone of various crops was already reported by several workers (Rajani *et al*., 1998, Koshy *et al*., 1998; Sosamma *et al*., 1998). The effect of *P. fluorescens* in improving the biometric characters of kacholam was already reported by Nisha (2001).

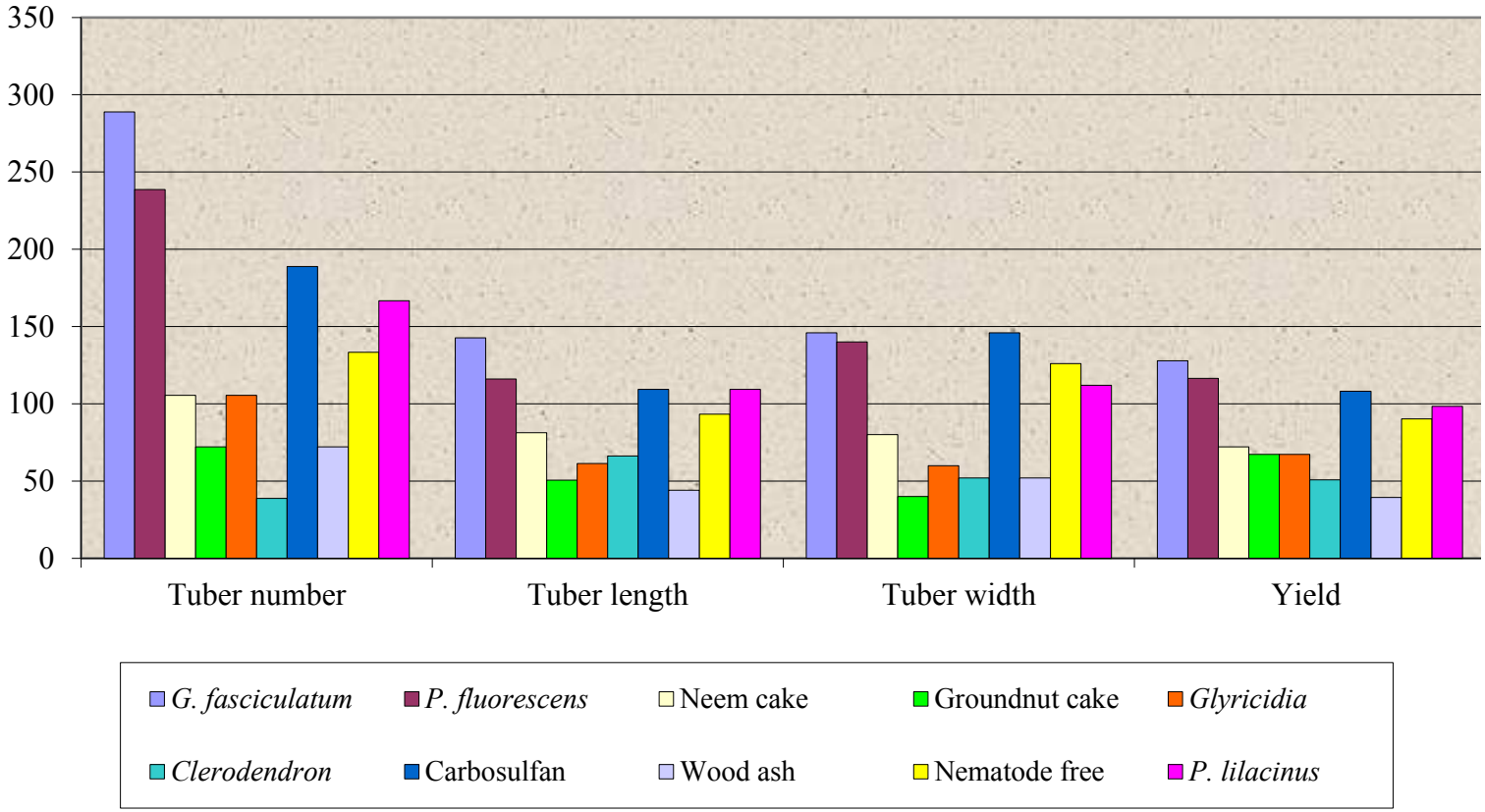


Fig. 6 Effect of different treatments on the yield characteristics of *P. rosea* (percentage variation over control)

The results presented in Para 4.5.6 revealed the effect of different treatments on the yield characters of *P. rosea* (Fig. 6). In the case of tuber number all the treatments showed significant improvement over control. Maximum tuber number was recorded by *G. fasciculatum*. This was followed by *P. fluorescens*, carbosulfan and *P. lilacinus*. The percentage improvement in tuber number was 289, 237, 189 and 167 respectively in the above treatments. Neem cake and *Glyricidia* application also increased the tuber number. There was 105 per cent increase over control in both cases. Regarding length and width of tuber same trend was noticed. Maximum yield in terms of weight of tubers per plant and per plot was recorded in *G. fasciculatum* and was statistically on par with *P. fluorescens* and were superior to all the treatments. The percentage increase in yield was 128 and 116 respectively over control. The next superior treatments were carbosulfan and *P. lilacinus* and were statistically on par. There was 108 and 98 per cent increase in yield over control. The effect of *P. lilacinus* in improving the yield may be due to the suppression of nematode activity by this fungus thus improving the biometric characters and nutrient uptake by the plant. The effect of organic amendments (neem cake and groundnut cake) and green leaf, *Glyricidia* were statistically on par. The improvement in yield and yield characters are due to the improvement in biometric characters and suppression of nematode population in soil and root due to inhibition of nematode activity by these treatments. The effect of *G. fasciculatum* in improving the yield by nematode suppression and plant growth promotion was already reported by several workers in various crops (Sivaprasad *et al*., 1990 in pepper and Sivaprasad and Sheela (1998) in pepper, ginger turmeric and cardamom). Nisha and Sheela (2003) also reported the effect of *G. fasciculatum* and *P. fluorescens* in improving the yield in kacholam.

The nematode population characters presented in para 4.5.7 revealed that the bioagents effectively reduced the population of

nematodes in soil and in roots and the effect was found to be statistically on par with that of carbosulfan, neem and groundnut cake. The effect of *G. fasciculatum* in reducing the nematode population in soil has already been reported by several workers (Sivaprasad *et al*., 1990; Asha, 1997; Sivaprasad and Sheela, 1998). Organic cakes (neem cake and ground nut cake) were found to be the next to best treatment in nematode suppression and reduction in root-knot count and this results were in accordance with reports of Kamalakshiamma (1986) in brinjal, Sundararaju and Sudha (1993) in arecanut based cropping system, Rajani (1998) and Nisha and Sheela (2003) in Kacholam.

The green leaves *Glyricidia* and *Clerodendron* also significantly suppressed nematode population in soil and roots of *P. rosea*. The effect of *Glyricidia* in reducing the population of *R. similis* was reported by Jasy *et al*. (1992) in black pepper. Nisha (2001) reported the effect of green leaf mulching using *Glyricidia* and *Clerodendron* in reducing the nematode population in kacholam. These reports also support this study. But the effect of above leaves as green mulch at the time of planting of Chethikoduveli in managing nematode population is reported for the first time.

'N' content was maximum in the leaves of plants treated with *P. lilacinus* followed by *G. fasciculatum* and *P. fluorescens* and these were statistically on par. In the 'P' content also, *P. lilacinus* ranked first followed by neem cake, carbosulfan, *G. fasciculatum* and these were on par. In the 'K' content of leaves *P. fluorescens* treated plants ranked first followed by *G. fasciculatum*, neem cake and carbosulfan. The reports on changes in NPK content of leaves of *P. rosea* and related plants due to nematode infestation is lacking and this was reported for the first time.

Summary

/6. SUMMARY

Investigations were done to evolve an ecofriendly management strategy for controlling nematodes (*M. incognita* and *R. similis*) associated with medicinal plant Chethikoduveli, *Plumbago rosea* L. using bioagents (*Glomus fasciculatum, Pseudomonas fluorescens* and *Paecilomyces lilacinus*) and organic amendments (neem cake, groundnut cake). Green leaves (*Glyricidia maculata* and *Clerodendron infortunatum*) having antihelminthic properties screened earlier and available in the farmers field were also studied as low cost method. Chethikoduveli, *P. rosea* being a newly domesticated medicinal plant, information on the pathogenic level of nematodes, their loss in yield due to various pests and diseases are lacking. Hence pathogenicity and crop loss due to the two major nematodes infesting *P. rosea* was studied in detail. The histopathological changes due to the infestation by *M. incognita* and *R. similis* were also investigated. The nematode infestation also induce biochemical changes in the feeding region, that is root of the crop which is used for pharmacological preparation. Hence these aspects were also studied.

In pathogenicity and crop loss trials, results were assessed in terms of biometric characters like height of the plant, number of leaves and number of branches at monthly intervals. The observations on characters like shoot weight, root weight and leaf area were recorded at the time of harvest of crop. The plants were uprooted 12 months after treatment and recorded the yield and yield attributing characters as well as nematode population characteristics. The build up of nematode population was also recorded at periodic intervals in crop loss estimation.

Pot culture studies were conducted on the pathogenicity of *M. incognita* and *R. similis* using different levels of inoculum *viz*., 0, 10,

100, 1000 and 10000 nematodes per pot. The result revealed that there was no significant difference in the height of the plant upto five and six months after inoculation in *R. similis* and *M. incognita* respectively. After that the levels 1000 and 10,000 showed statistically significant difference over the control. During this period plants inoculated with 10,000 and 1000 levels recorded minimum height of plant (39.6 and 43.4 cm in *M. incognita* inoculated plants and 50.00 and 54.60 cm in *R. similis* inoculated plants) and the effect of these two levels were statistically on par in both cases.

Significant reduction in number of leaves of *P. rosea* was observed six and seven months after inoculation (MAI) in the case of *R. similis* and *M. incognita* respectively. Here the effect of levels 1000 and 10000 were on par in *R. similis* while 100, 1000 and 10000 J_2 were on par in *M. incognita* infestation. In the case of number of branches significant reduction was observed in 1000 and 10000 J_2 levels at 6 MAI onwards in the case of *M. incognita* and at 11 MAI 100 J_2 also became on par with 1000 and 10,000 J_2 and pathogenic along with the above two levels. But in the case of *R. similis* significant reduction in number of branches was observed from 8 MAI and the levels 1000 and 10000 were on par.

At the time of harvest of the crop the effect of levels on the plant height due to 100, 1000 and 10000 J_2 were statistically on par and pathogenic while in *R. similis* 1000 and 10000 were statistically on par. In the case of number of branches and number of leaves the same trend was observed both in *M. incognita* and *R. similis*. But in the case of leaf area the lower levels 10 and 100 levels were also found pathogenic in *M. incognita* and *R. similis* infestation respectively. The fresh weight of shoot was reduced significantly at all the levels studied both in *M. incognita* and *R. similis* and they are statistically on par. In the case of fresh weight of root 100, 1000 and 10000 were on par in both *R. similis*

and *M. incognita* and in *R. similis* 100 level also reduced the root weight above 34 per cent.

The yield in terms of weight of tubers showed significant difference in 100, 1000 and 10000 levels in both the nematodes and these were on par but above 25 per cent reduction in yield was recorded from 1000 J_2 on wards. In the number and length of tubers also the same trend was noticed. But in the case of width of tubers all the levels were found pathogenic giving more than 25 per cent reduction. The nematode population estimated at the time of harvest showed that there was graded difference in the population of nematodes in soil and root in accordance with the initial logarithmic levels. Maximum root-knot index of 5 was recorded in 1000 and 10000 J_2 but the lesion index was maximum in 10,000 level (lesion index 3) and a same index of two was observed in 100 and 1000 levels.

The histopathological changes were studied at five days intervals after inoculation of *M. incognita* and *R. similis*. In the case of *M. incognita* the second stage juveniles penetrate the root tissue and they established feeding sites on the vascular parenchyma within five days. The head of the nematode was seen directed towards stellar region. The larvae increases in width to form sausage stage after five days of inoculation. The endodermal, pericycle and xylem and phloem cells in the vicinity of nematode head underwent hypertrophy and hyperplasia (10-15 days). The giant cell formation was noticed during 15-25 days. The adult females become sac like and the back portion of the nematode touched the surface of the root and they produced egg mass in a gelatinous matrix which protruded out from the root surface (25 – 30 days).

In the case of *R. similis*, five days after inoculation the nematodes entered the root and started feeding in the cortical region. After 10 days longitudinal burrows developed underneath the outer cortical cell layer. After 15 days necrotic regions were formed around the head of the

nematode. Burrows were formed due to the disintegration of cytoplasm and coalescence of cells were observed after 15 days. After 20 days the coalescence and breaking led to tunnel formation.

Regarding the biochemical changes caused by various levels of *M. incognita* and *R. similis*, there was considerable variation in phenol and total free amino acids. The phenol production was increased with increase in initial inoculum level (10, 100, 1000 and 10,000) in both cases. The variation in total free amino acid content expressed in terms of 0.79 µg equivalent of leucine revealed that the percentage increase in different levels of inoculation were 12, 44, 96 and 216 for *M. incognita* and 8, 24, 104,240 in *R. similis*. The rate of production of plumbagin was less in *R. similis* when compared to *M. incognita*. Maximum reduction of 7.67 per cent was recorded in 1000 J_2 level while there was a slight increase (3.62 per cent) in the case of *R. similis*. In different levels of inoculation the percentage reduction plumbagin showed only slight variation only in *R. similis*.

Microplot studies were conducted to estimate the crop loss incurred by *M. incognita* and *R. similis* in Chethikoduveli. The different levels found pathogenic in the earlier trial (100, 1000 and 10000) were compared with control (chemical carbosulfan along with 1000 level) and uninoculated plants (check).

The results revealed that there was no significant difference in the height of the plant upto two and six months in *R. similis* and *M. incognita* inoculated plots respectively. During these periods the plants exhibited minimum height in 1000 and 10000 levels inoculated (47.60 and 45.60 cm in *M. incognita* and 22.00 and 26.00 cm in *R. similis* inoculated plants). The effect of these two were statistically on par in both cases and showed significant reduction from the check. The effect of 100 level also found statistically on par with other two higher levels in the case of *R. similis* and showed significant reduction over control and check. The same trend

was continued upto to eleven months after inoculation in both the nematodes. *M. incognita* inoculated plants recorded 50 and 45.8 cm for 1000 and 10000 level of inoculation and *R. similis* recorded 35.60 and 30.00 cm for the above two levels and showed significant reduction over the control and check. But in *R. similis* inoculated plants, the 100 level was also showed significant reduction over the control and check giving 31.67 and 23.20 per cent respectively.

The loss in number of leaves of *P. rosea* was observed seven and two MAI onwards in the case of *M. incognita* and *R. similis* respectively. Here the effect of 100, 1000 and 10000 were on par and showed significant reduction over control in *R. similis* inoculated plants and 10000 J_2 only showed the effect (34.22 per cent reduction over control) in the case of *M. incognita*. In the case of number of branches significant reduction over the control and check was noticed in the case of *M. incognita* and 10000 J_2 only showed significant reduction upto 9 MAI and at 10 MAI. At 11 MAI the levels 100 and 1000 J_2 were on par with 10000 J_2 and these levels showed significant reduction over control (21.4 to 35.7) and check (19.5 to 34.14). But in *R. similis* inoculated plants significant reduction was noticed from 4 MAI on wards and all the three levels (100, 1000 and 10000) recorded statistically significant reduction over control. Levels 1000 and 10000 showed significant reduction over check at 7 MAI on wards in *R. similis* infestation.

The population of nematodes in soil at monthly intervals after inoculation recorded maximum in 10,000 level in both the nematodes and this was followed by levels 1000 and 100. There was significant variation between these three levels at all intervals except at 2 MAI.

At the time of harvest the effect of different levels of nematodes showed statistically significant variation in plant height. All the three levels (100, 1000 and 10000) showed statistically significant reduction in height over control and check in both the nematodes. Minimum height

was recorded in 10,000 levels and this was followed by 1000 and 100 levels. In the case of number of leaves, number of branches and leaf area same trend was observed both in *M. incognita* and *R. similis*. The fresh weight of shoot was reduced significantly by all the levels studied for both *M. incognita* and *R. similis* and the effect of all the levels were on par in *R. similis* whereas in *M. incognita* the effect of 10,000 J_2 showed significant reduction from all other levels and the effect of 100 and 10,000 were statistically on par. Dry shoot weight also recorded the same trend.

Fresh root weight was minimum for 10,000 level and was followed by 1000 and 100 levels in both the nematodes and the effect were statistically on par. Dry root weight also showed the same trend.

Regarding the yield and yield attributing characters all the three levels showed significant reduction over control and check. Minimum was recorded by 10,000 level and was followed by 1000 and 100 levels. Regarding the yield the effects of all the levels were statistically on par in *M. incognita* whereas in *R. similis* the effect of the levels 10,000 and 100 were on par.

Regarding the yield characters, 10,000 J_2 level significantly reduced to a tune of 71.75 and 56.73 per cent over control and check respectively while in 1000 J_2 the reduction being 55 and 31.2 per cent over control and check respectively in *M. incognita*. *R. similis* also recorded 57.14 and 49.23 per cent reduction over control and check respectively for 10,000 level and 48.05 and 38.46 per cent for 1000 level.

In the case of length of tubers there was 57.35 and 51.59 per cent reduction over control for *M. incognita* and *R. similis* respectively at 10,000 level. Tuber width also there was 66.29 and 47.92 per cent reduction over control for *M. incognita* and *R. similis*.

In the case of tuber yield in terms of weight, there was a percentage reduction of 47.82 and 22.85 over control and check respectively for *M. incognita* at 10,000 J_2 level. At 1000 J_2 also recorded 43.96 per cent reduction over control. Whereas in the case of *R. similis* there was 44 and 37 per cent reduction over control and check respectively at 10,000 level and at 1000 level also there was more than 25 per cent reduction. The loss increased with increase in inoculum level and the avoidable yield loss due to *R. similis* is 44 per cent.

Regarding nematode population in soil there was graded increase in accordance with the increase in initial inoculum level in logarithmic proportions for both the nematodes. Regarding root populations also the same trend was recorded. Maximum nematode population in soil and root were recorded in 10,000 level followed by 1000 and 100 levels respectively.

Root-knot count recorded maximum in 10,000 level with an index 5 and the level 1000 also recorded root-knot index '5' with more than 20 root-knot per gram of root. Number of lesions was maximum in 10,000 level with lesion index '3' and the level 1000 and 100 recorded and index '2' with more than 4 lesions per gram of roots.

Regarding the management of nematodes all the treatments recorded significant variation in biometric characters and yield of *P. rosea*. In the case of biometric characters at monthly intervals significant variation in plant height was noticed only after three months of application. The bioagent treated plots recorded maximum height in all the monthly observations. This was followed by the chemical carbosulfan, organic cakes and green leaves respectively.

Number of leaves recorded the same trend but significant variation between different treatments were recorded from three months after

treatment (MAT) onwards. Number of branches also recorded the same trend.

In the case of nematode population in soil assessed at different periods also showed significant variation, carbosulfan was found superior at 2 and 4 MAT. Next best treatment was *G. fasciculatum* and it was statistically on par with other two bioagents, *P. fluorescens* and *P. lilacinus* and chemical carbosulfan. The effect of neem cake was also found on par with the above treatments in later observations (10 and 12 MAT). The effect of other treatments (organic amendments and green leaves) were also found to be effective in managing the nematode population in Chethikoduveli rhizosphere.

At the time of harvest maximum plant height was recorded by *G. fasciculatum* (62.66 cm) and this was statistically on par with the effect of *P. fluorescens* and carbosulfan. *P. lilacinus* also recorded significant increase in plant height over control and other treatments and the effect was on par with *P. fluorescens* and carbosulfan. The effect of organic cakes and green leaves were statistically on par and showed significant importance in plant height over the control.

The number of leaves recorded at the time of harvest was maximum in *G. fasciculatum* which was significantly superior to all the treatments. The effect of *P. fluorescens*, *P. lilacinus* and carbosulfan were statistically on par and showed significant increase over other treatments. The effect of organic amendments and green leaves were statistically on par. In the case of number of branches maximum was recorded by *G. fasciculatum* (18.33) and was on par with *P. fluorescens*. The effect of *P. lilacinus* was on par with *P. fluorescens* and carbosulfan. The effect of organic cakes and green leaves was on par. Leaf area recorded maximum in bioagents treated plants and was statistically on par with carbosulfan. Organic cakes and green leaves also recorded significant increase in leaf area over control and the effects were statistically on par. Fresh weight of shoot

was maximum in *G. fasciculatum* and was on par with the effect of carbosulfan. The effect of other bioagents were statistically on par and recorded significant superiority to other treatments. Fresh shoot weight recorded by organic cakes and green leaves were also statistically on par showed significant increase over the control. In the case of dry weight of shoot the same trend was noticed.

Fresh weight of root was maximum in *G. fasciculatum* (231 g) and the improvement was statistically on par with *P. fluorescens* and carbosulfan. *P. lilacinus* was found to be on par with *P. fluorescens* and carbosulfan but showed significant variation from *G. fasciculatum*. All other treatments also recorded significant increase over the control. The same tend was noticed in the case of dry weight of shoots.

Regarding the yield and yield characters maximum tuber number was recorded in *G. fasciculatum* (23.33) and was significantly superior to all other treatments. The next best treatment was *P. fluorescens*. This was followed by *P. lilacinus* and carbosulfan and their effects were statistically on par. The effect of organic cakes and green leaves were statistically on par. In the case of tuber length and tuber width same trend was noticed. Maximum was recorded by *G. fasciculatum* (60 cm length and 1.23 cm width). The yield in terms of weight of tubers/plant and per plot recorded maximum in *G. fasciculatum* (231.66 g per plant and 2085 g per plot). This was followed *P. fluorescens*, carbosulfan and *P. lilacinus* and their effects were statistically on par. Treatments like organic cakes and green leaves also recorded best results and their effects also statistically on par.

Nematodes extracted from soil at the time of harvest recorded minimum in *G. fasciculatum* and the effect of this was statistically on par with other bioagents and carbosulfan. The effect of organic cakes and green leaves also gave good results but was inferior to the above treatments. Number of larvae and number of leaves in 5 g root sample

recorded minimum in carbosulfan and this was followed by *G. fasciculatum*, neem cake and other bioagents. Root-knot index was also minimum in *G. fasciculatum*, carbosulfan and neem cake. All other population characters of nematode (number of viable eggs, number of larvae/ egg mass, root-knot with egg mass etc.) the bioagents recorded minimum along with carbosulfan. Other treatments also recorded significant reduction compared to the control.

The nitrogen and phosphorus contents were maximum in the leaves of *P. lilacinus* (1.43 and 0.158 per cent respectively) treated plants followed by *G. fasciculatum* and *P. fluorescens* and these were on par. Neem cake and carbosulfan also showed higher 'P' content in the leaves as that of the above bioagents. In the 'K' content of leaves *P. fluorescens* (1.92 per cent) treated plants showed high value followed by *G. fasciculatum*, neem cake and carbosulfan.

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HOST PARASITE RELATIONSHIP AND MANAGEMENT OF IMPORTANT NEMATODES ASSOCIATED WITH CHETHIKODUVELI, *Plumbago rosea* L.

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ABSTRACT

Chethikoduveli, *Plumbago rosea* L. being a newly domesticated medicinal plant, experiments were conducted to get information on the pathogenic level of the nematodes, *Meloidogyne incognita* (Kofoid and White, 1919) Chittwood, 1949 and *Radopholus similis* (Cobb, 1893) Thorne, 1949 and their effects in loss in yield. The histopathological changes due to infestation by the nematodes were also investigated. These nematode infestation also induce biochemical changes in the feeding region *i.e.*, the root of the crop which is used for pharmacological preparations, hence these aspects were studied.

Studies were also conducted to evolve an eco-friendly management strategy for controlling nematodes using bioagents (*Glomus fasciculatum*, *Pseudomonas fluorescens* and *Paecilomyces lilacinus*) and selected organic amendments (neem cake and groundnut cake). The effect of green leaves (*Glyricidia maculata* and *Clerodendron infortunatum*) having antihelminthic properties available in the farmer's field were also studied as a low cost method.

In pathogenicity and crop loss trials the results were assessed in terms of biometric characters, (height of the plant, number of leaves and number of branches) at monthly intervals and characters like shoot weight, root weight and leaf area, yield and yield characters (length, width and number of tubers) at the time of harvest of the crop. The population of the nematodes in soil was recorded at periodic intervals and at harvest of the crop. There was significant reduction in biometric characters at two and six months after inoculation onwards in the case of *R. similis* and *M. incognita* respectively. The pathogenic level of *R. similis* was above 100 nematodes and 1000 and above in the case of *M. incognita* (above 25 per cent). Both these nematodes recorded more than 25 per cent reduction in yield.

In the case of crop loss there was an avoidable loss of 43.96 per cent at 1000 J_2 level of *M. incognita* and 35.32 per cent in the case of *R. similis*. At 100 level the losses were 36 per cent and 29 per cent in *M. incognita* and *R. similis* respectively.

The histopathological studies revealed that the entry of second stage juveniles of *M. incognita* took place within five days and penetrated the root tissue and established its feeding cite on the vascular parenchyma. Formation of hypertrophy and hyperplasia took place during 10 to 15 days at the surface of the root and they produce egg mass in a gelatinous matrix. In the case of *R. similis* nematode entered the root and started feeding in the cortical region. After 10 days longitudinal burrows developed underneath the outer cortical cell layer. Burrows were formed due to the disintegration of cytoplasm and coalescence of cells after 15 days of entry. Then the cavities were formed and they coalesce and broke down leading to tunnel formation in 25 to 30 days.

Biochemical changes caused by various levels of *M. incognita* and *R. similis* revealed that there was considerable variation in phenol and total free amino acids. The phenol production was increased with increase in initial inoculum levels (10, 100, 1000 and 10000) in both the cases. The variation in the total free amino acids revealed that the percentage increase in different levels of inoculum were 12, 44, 96 and 216 for *M. incognita* and 8, 24, 104 and 240 in *R. similis*. The rate of reduction of plumbagin was less in *R. similis* infested plants when compared to *M. incognita*. Maximum reduction of 7.67 was recorded in 1000 J_2 level while there was only 3.62 per cent increase in the case of *R. similis*.

The field experiment on management of nematodes revealed that *G. fasciculatum* @ 20 g m^{-2} having 100 chlamydospores / gram of media was found to be the best treatment followed by *P. fluorescens* @ 10 g m^{-2} @ 10^6 cells / gram and *P. lilacinus* @ 10 g m^{-2} having 10^6 spores / gram of media and these were seen even better than the chemical carbosulfan

@ 0.1 g ai m^{-2}, in managing nematode population and improving the biometric characters and yield of *P. rosea*. The yield in terms of weight of tubers revealed that *G. fasciculatum*, *P. fluorescens*, carbosulfan and *P. lilacinus* were statistically on par. *G. fasciculatum* recorded the maximum yield (231.7 g / plant). *G. fasciculatum*, *P. fluorescens* and Carbosulfan showed more than cent per cent increase while *P. lilacinus* recorded 98 per cent increase. The effect of neem cake and groundnut cake @ 100 g m^{-2} and the green leaves of *G. maculata* and *C. infortunatum* @ 5 kg m^{-2} were also found effective in managing the nematodes and improving the biometric characters and yield of the plant but these treatments were inferior to the above bioagents. The farmers practice of application of wood ash @ 5 kg m^{-2} also had some effect on nematode management and slightly increased the yield of tubers. In comparison with plants raised in denematised soil, effect of *G. fasciculatum*, *P. fluorescens* and carbosulfan were found better because of the growth promoting character of bioagents and phytotonic effect of carbosulfan. The nitrogen and phosphorus contents were maximum in the leaves of *P. lilacinus* (1.43 and 0.158 per cent respectively) treated plants followed by *G. fasciculatum* and *P. fluorescens* and these were on par. Neem cake and carbosulfan also showed higher 'P' content in the leaves as that of the above bioagents. In the 'K' content of leaves *P. fluorescens* (1.92 per cent) treated plants showed high value followed by *G. fasciculatum*, neem cake and carbosulfan.