

Development of *Meloidogyne incognita* (a root-knot nematode) in *Dolichos lablab* (country bean) and its management through root extracts of selected medicinal plants

By

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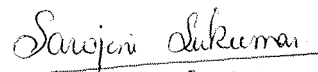
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CERTIFICATE

This is to certify that the dissertation entitled "**Development of *Meloidogyne incognita* (a root-knot nematode) in *Dolichos lablab* (country bean) and its management through root extracts of selected medicinal plants**" submitted to the Avinashilingam Institute for Home Science and Higher Education for Women, Deemed University, Coimbatore - 641 043, in partial fulfilment of the requirements for the award of the **Degree of Master of Philosophy in Life Sciences** is a record of original research work done by **Suganthi, M.**, during the period of her study in the Department of Life Sciences, Avinashilingam Institute for Home Science and Higher Education for Women, Deemed University, Coimbatore - 641 043, under my supervision and guidance and the dissertation has not formed the basis for the award of any Degree / Diploma / Associateship / Fellowship or other similar title to any candidate of any university and it represents entirely an independent work on the part of the candidate.


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DECLARATION

I hereby declare that the dissertation entitled "**Development of *Meloidogyne incognita* (a root-knot nematode) in *Dolichos lablab* (country bean) and its management through root extracts of selected medicinal plants**" submitted to the Avinashilingam Institute for Home Science and Higher Education for Women, Deemed University, Coimbatore - 641 043, in partial fulfilment of the requirements for the award of the **Degree of Master of Philosophy in Life Sciences** is a record of original research work done by me under the supervision and guidance of **Dr.(Tmt.) B.Shyamala Vijayavathi**, M.Sc., Dip. Ed. (Madras), M.Phil. (Bharathiar), Ph.D. (Avinashilingam), Reader in Zoology, Department of Life Sciences, Avinashilingam Institute for Home Science and Higher Education for Women, Deemed University, Coimbatore - 641 043, and that it has not formed the basis for the award of any Degree / Diploma / Associateship / Fellowship or other similar title to any candidate of any other university.


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Introduction

1. INTRODUCTION

Nematodes, which belong to the Phylum Aschelminthes are one of the major pests identified in the animal kingdom. Most of them are free living saprophytes but several hundred species are known to attack plant organs including roots, bulbs, leaves and flowers. Plant parasitic nematodes, otherwise known as phytonematodes are the oldest existing life forms which infect almost every crop on this earth causing heavy economic losses. They can directly cause plant disease or may act as predisposing agents to several fungal and bacterial diseases. Being ubiquitous in nature, they attack a wide range of host plants and are therefore responsible for the deterioration of health of standing crops (Swarup *et al.*, 1989). The major damage done to the plants is by the feeding activity of these nematodes which usually result in stunting and unthrifty plant growth, reduced crop yield and occasionally plant death.

In United States, the estimated annual damage caused by these plant parasitic nematodes is about 100 billion dollars (Atkinson, 1995) and in fruits and nuts it was about 12 per cent or 225 million dollars per year. It was also estimated that the losses in chick pea was 13.4 per cent, in egg plant it was 16.7 per cent, in tomato 17.7 per cent, in cowpea 15.1 per cent, in coconut 17.1 per cent and in tobacco it was 14.7 per cent.

In India, some attempts have been made to estimate the losses caused due to these plant parasitic nematodes in important commercial crops.

Annual losses reported was to the extent of Rs.40 million in wheat and Rs.30 million in barley. Around 20 million worth of loss in coffee due to root lesion nematode occurs annually. Perennial crops like citrus, coconut, arecanut, tea, coffee and banana and annual crops like vegetables, tobacco, cotton, rice and sugarcane grown under intensive cultivation in irrigated areas are particularly liable to damage by parasitic nematodes. Tomato, brinjal, okra, potato, papaya, jute, groundnut and pigeon pea suffer most.

Among the best known phytonematodes, root-knot nematodes (*Meloidogyne* spp.), stand out as the most dominant group which have an extensive host range of more than 2000 plant species (Trivedi, 2001).

Over 50 species have been reported (Sasser and Carter, 1985) in the genus *Meloidogyne* with a number of races. These nematodes are widely distributed in temperate as well as tropical areas of the world. Their occurrence in cultivated fields and green houses make them as one of the dangerous agricultural pests. Basically, root-knot nematodes parasitize roots or underground parts of various host plants. Their infection in the roots result in the formation of galls (root-knot). The above ground stem symptoms such as yellowing of leaves, wilting and premature shedding off foliage, dwarfing and decline of the plants and a subsequence reduction in crop yield (Daniel and Boothryod, 1987) are the other features which can be observed due to root-knot nematode infection. The root-knot nematode infection was first reported in cucumber in England (Berkeley, 1855).

In India, of the 12 species reported (Dasgupta, 1998), *Meloidogyne incognita*, *Meloidogyne javanica* and *Meloidogyne arenaria* are the most predominant (Jain, 1992) root-knot nematodes. Among the above species, *M. incognita* which is commonly known as “cotton root-knot nematode” is continuously receiving attention because of its wide spread occurrence and polyphagous nature. Krishnappa (1985) reported that in India, *M. incognita* has the widest host range (vegetables, pulses, fiber crops, fruits, ornamentals and other important cash and plantation crops) infecting more than 250 genera of plants.

There are six stages with four moults in the life cycle of root-knot nematode. Egg, first stage (L₁), second stage (L₂), third stage (L₃), fourth stage (L₄) and adult. The second stage juveniles (J₂) is the infective stage which infects the roots of the host plants by penetrating through their stylets. The invading larvae penetrate the meristematic tissue and migrate until the head become established in an intercellular space near the endodermis. Around the mouths of the feeding females giant cells are formed due to host parasitic interaction. These giant cells are characteristics feature of root-knot infection. Eggs are laid in the gelatinous matrix secreted by the six rectal glands which extruded from vulva. The gelatinous matrix protects and nourishes the eggs in adverse environmental conditions. Numerous females of the nematodes are present in the root galls indicating multiple infection in the host roots. The time taken for the completion of life cycle is about 20-40 days under optimum environmental conditions but takes longer in

cooler temperatures (Mahrota and Aggarwal, 2003). Several generations are completed in one crop season.

The penetration of root-knot nematode into the tissues of host tissues and further their development cause disruption in the physiological equilibrium by altering the biochemical components of the host plants and the plants in turn, react in a number of ways to offset their disturbances and the overall plant response determines, to a large extent, the success or failure of the interactions. Earlier investigations have documented that infection of plants by nematodes trigger mechanisms resulting in the alteration of metabolic activities of hosts (Owens and Specht, 1966 ; Sinha and Sukul, 1983 and Ganguly *et al.*, 1991).

On infection, it was reported that the proteins of the hosts are likely to change quantitatively resulting in the formation of new polypeptides (Bell, 1981 and Dasgupta, 1988). There are also reports on the increase in the concentration of existing proteins. Such changes in proteins, following root-knot nematode infection has been reported by Ganguly and Dasgupta (1981). Decrease in the content of carbohydrates was also reported due to the interaction of root-knot nematodes with host tissues affecting the photosynthetic activity (Sinha and Sukul, 1983). Phenols, which play a major role in the defense mechanism gets altered based on the susceptibility or resistance of the host plant to the root-knot attack (Bajaj and Mahajan, 1977).

Since root-knot nematodes cause extensive damage to the agricultural crops and their management is necessary in the fields using various methods such as chemical, physical, cultural and biological. The nematicidal chemicals though control these nematode pests more efficiently and instantaneously, provides only a short term solution. More over, their application in the soil cause several ill effects like pest resistance, pest resurgence, pesticide residues in food, pollution of water, soil and atmosphere. The nematicides are also costly, phytotoxic and they leave toxic residues in the soil. Therefore, recent emphasis on the non-chemical nematode management technologies have gained much interest amongst the scientists, agrofarmers and consumers.

Utilization of antagonistic plants offer alternative means for the nematode population management and control because generally, they do not leave their toxic residues. Recently, there has been growing interest in the suppressive effect of some plant extracts on plant parasitic nematodes (Burhan and Shaukat, 2000 ; Cayrol *et al.*, 1989 ; Akhtar *et al.*, 1990 and Ali *et al.*, 2001).

Several researchers (Goswami and Vijayalakshmi, 1981 ; Zaki and Bhatti, 1981 and Singh *et al.*, 2001) have reported the utilization of various plants which are known to possess nematicidal properties which effectively control root-knot nematodes.

Plants, antagonistic to parasitic nematodes have been recommended as organic amendment for the management of nematode populations (Alam *et al.*, 1988 and Patel *et al.*, 1991).

Using different plant extracts at various concentrations was found successful in the prevention or inhibition of egg hatch of *M. incognita* which might be due to certain toxic substances or factor present in them (Taylor and Murant, 1967).

The present investigation was therefore carried out to study the effect of aqueous root extracts of two medicinal plants, namely, *Tephrosia purpurea* and *Phyllanthus neruri* for the control of root-knot nematodes based on the following objectives.

- (1) To study the developmental stages of *M. incognita* in the host plant, *Dolichos lablab*.
- (2) To estimate the changes that have taken place in the biochemical components like proteins, carbohydrates and phenols of the host plant due to the interaction of *Meloidogyne incognita* with the host plant, *Dolichos lablab*.
- (3) To conduct a preliminary study to assess the efficacy of root extracts of two medicinal plants, *T. purpurea* and *P. neruri* on the egg hatch of *M. incognita*.

- (4) To evaluate the efficacy of root extracts of *T. purpurea* and *P. neruri* at two concentrations on the growth performance of the host plant, *Dolichos lablab* infected with *M. incognita*.
- (5) To study the efficacy of root extracts of *T. purpurea* and *P. neruri* on the root galling, egg mass production in the host plant infected with *M. incognita*

and
- (6) To find out the final nematode population in the soil.

Review of Literature

2. REVIEW OF LITERATURE

Root-knot nematodes of the genus *Meloidogyne* are one of the most important plant parasitic nematodes, which attack almost every crop, causing considerable losses of yield and are affecting the quality of produce. It is of historical as well as scientific interest since sustained nematological investigations all over the world have started with this genus. A vast number of literature is available on different aspects, such as development of these nematodes in different crops, biochemical alterations caused in crops due to their inhabitation and various eco-friendly methods adopted by several authors in the management of these nematodes from time to time.

The first root-knot nematode injury to vegetable was reported by Berkeley (1855) in cucumber. In India, Ayyar (1934) studied root-knot nematodes infecting vegetables and other crops in South India.

2.1 Life cycle of *Meloidogyne* spp. in various crops

Studies conducted on the life history of *Meloidogyne graminicola* (rice root-knot nematode) by Rao and Israel (1973) showed that the second stage juveniles entered the roots five hours after inoculation and continued to invade till 12th day. Third and fourth stage larvae appeared on sixth and ninth days respectively after inoculation. Males were observed on 24th day and females with ovisacs on 27th day. Time taken for completion of one generation varied from 26 – 51 days during different seasons.

Mathur and Varaprasad (1979) have investigated the duration of life cycle of *M. incognita* in sugarbeet and it took 39 days to complete its life cycle. Young females were found to be reported 27 days after inoculation and the first egg mass at 33 days. Second stage larvae started to hatch on 39th day.

Life cycle stages of *M. incognita* and *M. javanica* were studied on both susceptible and resistant tomato plants (Narayana and Reddy, 1980). They observed progressive increase in the rate of penetration even upto seventh day after inoculation in both plant varieties. Completion of life cycle of both *M. incognita* and *M. javanica* in Pusa Ruby was at 32 days and egg masses were observed after 32nd day following inoculation. Fully developed females were observed 28th day after inoculation. The development of *M. incognita* and *M. javanica* was found to be different in resistant variety.

Inserra *et al.* (1985) reported that the post infection development of *Meloidogyne chitwoodi* from second stage juveniles (J₂) to mature females and egg deposition on 'Nugaines', winter wheat required 105, 51, 36 and 21 days at 10, 15, 20 and 25°C.

Observations was made by Kaushik and Bhatti (1986) on the different life cycle stages and generations of *M. javanica* on tomato under different seasons. Life cycle was reported to be completed in 26 days during June / July, 31 – 42 days in March – July and 46 – 66 days in October – December. They also reported that penetration of larvae took 48 hours at a comparatively high temperature.

A comparative study on the development, reproduction and aggressiveness of *M. incognita* races three and fourth was carried out by Veech and Starr (1986) on cotton. They were able to observe the adult females 16 days after inoculation and eggs 23 days after inoculation.

Ravichandra *et al.* (1989) conducted experiments on the development and maturity of *M. javanica* and three races of *M. incognita* in susceptible and resistant brinjal cultivars. They reported that in susceptible brinjal cultivar (Erengere), the time required to complete development from second stage juveniles (J₂) to adult was 25 – 29 days and it took 51 and 44 days in a resistant cultivar (Gulla) of brinjal infected with *M. javanica* and race-3 of *M. incognita* respectively.

The life cycle of *Meloidogyne incognita* on mung var. RS-4 was reported (Datta *et al.*, 1990) to be completed within 29 days of inoculation (from egg to egg) whereas it took 44 days in gaur-var. RGC-915 at 35 ± 2°C day temperature. The second moult was observed on seventh day, third on 12th day and fourth on 16th (males) to 17th day (females) while in gaur the third and fourth moults were delayed.

The life cycle of the root-knot nematode, *M. incognita* was studied by Sharma and Trivedi (1992) in two cultivars of *Trigonella foenumgraceum*. The second stage juveniles entered the roots within 24 hours of inoculation. Egg formation was seen in the female body 36 days after inoculation in susceptible cv um – 83 and 44 days after inoculation in resistant cu um – 34.

The life cycle was completed in 42 – 44 days in um – 83 and 48 – 50 days in um – 34.

Tang *et al.* (1994) studied the penetration and development of *M. incognita* in roots of highly susceptible cotton germplasm M8. At 16 days after inoculation egg laying females were apparent. Numerous secondary infective juveniles (J₂) were found inside the younger cotton roots after 30 days of inoculation.

The life cycle of the root-knot nematode, (*M. incognita*) on tomato (cv. Ranco 823) have been investigated (Langellotti *et al.*, 1995) in the nocerino – sarnese area (Southern Italy). The results showed that the nematode had three generations. The first was completed within 60 days and took twice the time needed for the other two (about 30 days each).

Anwar *et al.* (1996) studied penetration, development and reproduction of *M. incognita* on seven cultivars of tomato. In their study, they observed that more second stage juveniles (J₂) had penetrated the roots of money maker than those of other cultivars (Peelo, USSR, Roma 365 – 8, A.H. Selection and long tipped) in 24, 48 and 72 hours after inoculation. The development was more rapid in money maker resulting in completion of second stage juveniles (J₂) to second stage juveniles (J₂) in 28 days as compared to 33 days on other cultivars.

Pedrosa *et al.* (1996) studied the penetration, post-infectious development, reproduction, and fecundity of *Meloidogyne arenaria* races

1 and 2 were studied on susceptible (CNS), partially resistant (Jackson), and highly resistant (PI 200538 and PI 230977) soybean genotypes in the green house. At 10 days after inoculation, 56 per cent and 99 per cent to 100 per cent of race 1 second stage juveniles (J_2) were vermiform or sexually undifferentiated in CNS and the resistant genotypes, respectively. By 20 days after inoculation, 88 per cent to 100 per cent of race 2 nematodes in roots of all genotypes were females, whereas only 25 per cent and one per cent of race 1 were females in CNS and the resistant genotypes, respectively. For all four genotypes, race 1 produced 85 per cent to 96 per cent fewer eggs per root system 45 days after inoculation than race 2. At 45 days after inoculation race 2 produced more eggs on CNS than the other genotypes.

Observations made on life cycle studies indicated that second stage juveniles (J_2) of *M. incognita* started invading the young mint roots 48 hours after inoculation and continued upto 16 days of inoculation. Second moult occurred on fifth day after inoculation indicating the appearance of third stage which lasted for four days when third moult occurred resulting into fourth stage male and female juveniles. Mature males and females were observed on 18th day after inoculation. Deposition of gelatinous matrix without any egg was observed on 22nd day after inoculation. Second generation J_2 were appeared on 29th day after inoculation. Thus, life cycle of *M. incognita* on Japanese mint cultivar 'Shivalik' was completed from J_2 to J_2 in 29 days at

ambient temperature 13 - 37°C during March and April (Singh and Kumar, 1998).

Studies on the life cycle of *M. incognita* on pointed gourd (*Trichosanthes dioica* Roxb.) showed that the penetration of second stage juveniles (J₂) in roots continued upto nine days with maximum numbers penetrating on sixth day and started moulting in 72 hours. Young females appeared on 18th day after inoculation. Deposition of eggs in gelatinous matrix and formation of egg masses started from 20 – 24 days followed by emergence of juveniles of second generation (Verma and Anwar, 1999).

Mahapatra and Swain (1999) observed the life cycle (J₂ – J₂) of *M. incognita* on black gram (cv T – a) which was completed within 32 days at a temperature range of 18 – 34° C. They observed the entry of juveniles 12 hours after inoculation which was continued upto six days. Minute spindle shaped galls were observed on the roots two days after inoculation. Second stage juveniles (J₂) moulted eight days after inoculation. Third moult was on 16th day and final moult at 22nd day of inoculation and young females developed. Egg sacs were secreted 26th day of inoculation and eggs were found to be laid on 30th day. Emergence of second generation juveniles started on 32nd day.

An experiment was conducted by Sanjay *et al.* (2000) to find out the number of days required by *M. incognita* to complete its one life cycle on groundnut cv GG₂. Penetration of second stage larvae took place after 48 hours of inoculation. Second moult was observed on 8th day and the spike

tail stage on 10th day. The third moult was observed on 13th day. Fourth stage larvae were observed on 16th day of inoculation. Pre-adult female was observed on 22nd day and fully mature adult female on 25th day. Fully matured females laid eggs on 29th day. Males were not observed at any stage in the life cycle. They reported that, the nematode completed its life cycle from second stage larvae (J₂) to the formation of adult female with egg mass in 29 days.

Anju Jain *et al.* (2001) studied the developmental stages of *M. incognita* in tomato. They observed the continuation of juvenile penetration till 12th day of inoculation. Third stage was observed on 12th day, immature female on 20th day and egg laying adult female on 28th day after inoculation.

Dabur *et al.* (2004) studied the life cycle of *M. graminicola* on paddy. Larval entry commenced on fifth day of sowing of sprouted rice seeds. Third (J₃) and fourth stage juveniles (J₄) were seen on eight day of sowing. On 10th day of sowing all larval stages as well as adult males and females were recorded in the roots. On 20th day egg laying females were noted on 24th day, second stage juveniles (J₂) of the second stage generation were recorded. The life cycle was completed at 24 days of inoculation.

2.2 Biochemical changes in vegetable crops infected by root-knot nematodes (*Meloidogyne* spp.)

Several investigators have documented that infection of host plants by root-knot nematodes are always accompanied by disturbances in metabolic

activities which bring about physiological and biochemical changes in the host.

Alam *et al.* (1976) reported that total soluble proteins increased in the roots of tomato seedlings infected with *M. incognita*.

Bajaj and Mahajan (1977) made investigation on the phenolic content present in the leaves and roots of two tomato cultivars (resistant and susceptible) infected with the root-knot nematode, *M. incognita*. Quantitatively both leaves and roots of resistant tomato had higher concentration of total phenols.

An increased amount of protein was observed in the roots of brinjal (egg plant) infected with *M. incognita* over that of healthy roots. A decrease was also recorded in starch and reducing sugar content. Phenol content was increased in infected roots (Singh *et al.*, 1978).

Anwar *et al.* (1979) undertaken studies to determine the effect of oil cakes amendment on the biochemical changes in the tomato plants infected with *M. incognita*. They found an increased amount of total phenols, proteins and carbohydrates in the uninoculated plants than in inoculated plants both in unamended soil and soil amended with oil cakes.

Biochemical estimation of total phenolic content of both shoots and roots of resistant (NTDR – 1) tomato showed higher amount than that of susceptible (Pusa Ruby) tomato (Narayana and Reddy, 1980).

Chatterjee and Nirmal (1981) studied the quantity of total proteins in the root galls to determine the degree of root-knot nematode infestation.

Changes in the total protein and carbohydrates in the roots of *Hibiscus esculentus* resulting from infection with root-knot nematodes were studied by Sinha and Sukul, (1983) and they found the total protein in the inoculated plants were higher and carbohydrates were lower.

Upadhyay and Banerjee (1986) noted an increase in the amount of proteins in the stem and roots of chickpea infected with root-knot nematode, *M. javanica*. Molinari (1990) reported an increase of protein content in mitochondrial and washed cell wall fraction of resistant tomato (cv Rossol) when infected with *M. incognita*.

Ganguly *et al.* (1991) observed the changes in protein both qualitatively and quantitatively in cow pea (cv Pusa Do Fash) infected with *M. incognita*. They recorded a considerable increase of total soluble protein during post infectional intervals.

Healthy and *M. incognita* infected roots of susceptible and resistant cultivars of fenugreek collected after 30, 60 and 90 days showed an increase in the amount of total proteins and total phenols, whereas total sugars and non-reducing sugars were decreased in infected roots (Sharma and Trivedi, 1992).

Studies conducted on the effects of *M. incognita*, *Paratrichodorus minor*, *Pratylenchus scribneri* on the partitioning of carbohydrates between

root and shoot showed that glucose, starch and sucrose contents in the leaves and roots of uninoculated plants of tomato were higher when compared with infected plants (Anwar, 1995).

Patel *et al.* (1996) reported that infection of *M. incognita* and *M. javanica* at 5000 and 10,000 second stage juveniles (J₂) / plants showed an increased level of total phenol contents i.e., 140.26 per cent and 92.93 per cent respectively over the control i.e., 98.23 and 73.89 per cent respectively.

Mohanty *et al.* (1997) reported that *M. incognita* infection of green gram, interfered on the root biochemistry in addition to its pathogenicity. Higher concentration of total sugar was observed in nematode, rhizobium and both rhizobium and nematode inoculated roots.

Biochemical studies were also conducted in various other plant parasitic nematodes by many workers. Biochemical changes were also reported in cowpea cv Pusa komal inoculated with reniform nematode *Rotylenchulus reniformis* (Mohanty *et al.*, 1999).

Anguina trifici infected wheat seedlings were reported (Nandini *et al.*, 1988) to exhibit an increased amount of soluble protein and carbohydrates.

A pot culture experiment with eight banana clones, comprising five hybrids and three diploid parents, was conducted to study the biochemical alterations induced by the burrowing nematode, *Radopholus similis* in banana

(*Musa* spp.). The results revealed the presence of higher quantities of phenols in the uninoculated roots of resistant clones than susceptible clone matti. The amount of total proteins, carbohydrates and sugars were more in the susceptible clone than other clones. Accumulation of phenols increased with the infestation of *R. similis*. Amount of total protein increased with the infection of nematodes. Decrease in the level of carbohydrates and increase in sugars was observed in the nematode infested roots. (Devarajan and Rajendran, 2002).

2.3 Effect of aqueous extracts of different parts of plants on the egg hatch of *Meloidogyne* spp.

Several plant parts, their products and extracts belonging to different botanical families have been reported to possess nematicidal or nemostatic properties. The toxic nature of plant extracts to various plant parasitic nematodes have been reported earlier by various researchers (Abivardi, 1971 ; Desai *et al.*, 1973). They found that different plant extracts inhibited hatching of eggs of *M. incognita*.

Yadav (1970) reported the inhibitory effect of the root exudates of *Euphorbia hitra* on the hatching of *Meloidogyne incognita* eggs.

Studies on the toxicity of extract of root and shoot of certain medicinal plants (*Aloe barbedensis*, *A. peryii*, *Gloriosa superba* and *Scillia indica*) were conducted (Rakesh and Akthar, 1988) on the larval hatching and mortality of root-knot nematode, *Meloidogyne incognita*.

An *in vitro* experiment was conducted (Renu and Trivedi, 1992) to study the effect of various root extracts on the hatching of *Meloidogyne incognita* eggs. It was observed that the root extracts of *Ocimum sanctum* and *Tagetes erecta* were found to be nematocidal whereas *Euphorbia hirta*, *Artemesia absinthium* Linn. and *Aegle marmelos* Linn. were found to be nemostatic in nature.

List of plants falling in 43 families and 128 species having nematocidal properties were identified and studied (Mehta and Sundararaj, 1992). In about 16 genera the nematocidal chemicals have been isolated.

Sasanelli and D'Addabbo (1993) investigated *in vitro* effect of leaf and root aqueous extract and root leachates of *Tagetes erecta* L., *Cineraria maritima* and *Ruta graveolens* on egg hatch of Italian populations of *M. arenaria* (Neal), *M. hapla*, *M. incognita* and *M. javanica* and found that *R. graveolens* was the most effective in having high nematocidal activity against these four *Meloidogyne* species.

Walia and Gupta (1997) studied the efficacy of *Tagetes* spp. on the egg hatchability of *Meloidogyne incognita* in aqueous extract form of the root exudates. Aqueous extracts from 30 and 60 day old marigold plants (*Tagetes* spp.) inhibited juvenile hatch of nematodes considerably at all concentrations compared to control. Root exudates without dilution irrespective of the age of the plant, inhibited maximum hatch which decreased with the increase in dilution.

Studies were conducted by Kayani *et al.* (2001) using root extracts of some plants for the control of root-knot nematode (*Meloidogyne incognita*) on tomato plants. *M. incognita* eggs were exposed to root extracts of *Melia azedarach* Linn. (dharek), *Azadirachta indica*, *A. jass* (neem), *Ricinus communis* Linn. (castor) and *Datura alba* Linn. (Datura metel). They reported standard root extracts of neem and dharek exhibited 100 per cent inhibition of egg hatching and larval mortality. Egg inhibition and larval mortality decreased with an increase in the dilution of the extracts. Similarly, with an increase in exposure time, juvenile mortality was also increased.

Extracts of two medicinal plants, *Curcuma aromatica* and *Swertia chirata*, showed a marked nematicidal and nematode hatching inhibitory activity against root-knot nematode, *Meloidogyne incognita*. The nematicidal activity was recorded in the hexane extract of *S. chirata* while the butanol extract of *C. aromatica* exhibited maximum inhibition to hatching of *M. incognita* eggs after 120 hours exposure period at 1000 ppm concentration (Pandey *et al.*, 2001).

Jayakumar *et al.* (2002) made an attempt to study the nematicidal activity of root exudates of cassava against *Meloidogyne incognita* under *in vitro* conditions. They found that the root exudates from three and four weeks old plants at all tested concentrations induced larval mortality compared to distilled water after 48 hours of exposure time. They also reported that irrespective of period of exposure, the larval mortality increased

with increase in concentration of root exudates which indicated that the root exudates of cassava contained nematicidal principles.

Singh and Singh (2002), evaluated *Adhatoda vasica*, *Aloe barbedensis*, *Calendula officinalis*, *Cannabis sativa*, *Papaver rhoeas*, *Salvia officinalis*, *Tagetes minuta* and *Vinca rosea* for their nematicidal properties. Extracts of all tested plants were toxic to larva of *M. incognita* and mortality was directly correlated with concentration of extracts. It increased with period of exposure. Highest mortality was observed after 72 hours in S concentration. Of the tested plants *T. minuta*, *C. sativa*, *P. rhoeas* and *A. barbedensis* were highly nematicidal and *S. officinalis*, the least.

Antinemic effect of aqueous extracts of weeds viz., *Parthenium hysterophorus*, *Ageratum conyzoides*, *Achyranthes aspera* and *Euphorbia hirta* were observed (Saxena and Sharma, 2003) under *in vitro* conditions against second stage juveniles of root-knot nematode, *M. incognita*. Among six different concentrations viz., S, S / 2, S / 4, S / 8, S / 16 and S / 32 of each extract tried, highest per cent mortality i.e., 100 per cent was showed by S concentration of *P. hysterophorus* and *E. hirta* after 72 hours of exposure.

Aqueous extracts of different parts of onion viz., leaves, root and bulbs were tested *in vitro* for their nematicidal effect on the larval hatch of root-knot nematode, *Meloidogyne incognita* by Goel and Gupta (2004). Their findings showed that all the aqueous plant parts extracts inhibited larval hatch at all concentrations i.e., 100, 50 and 25 per cent at all the exposure periods tested

i.e., 3, 6, 9, 12 and 15 days. Highest inhibition of larval hatch was exhibited by leaf extract followed by root extract at 100 per cent concentration at 15 days exposure period, while least inhibition was recorded in bulb extract at the same concentration and exposure time. Cumulative hatch was significantly reduced in all the extracts, i.e., leaves, roots and bulbs as compared to that in distilled water.

Investigation was carried out to see the nematicidal effect of 15 plant products viz., leaves of *Albizzia amara*, *Aristolochia bractiata*, *Tagetes erecta*, *T. patula*, *Origanum majorana*, *Azadirachta indica*, *Butea monosperma* and *Calotropis gigantea*, roots of *Acorus calamus*, bulbs of *Allium sativum*, seeds of *Citrullus lanatus*, *Areca catechu* and *Anona reticulata*, latex of *C. gigantea* and *Carica papaya* on egg hatching of root-knot nematode, *Meloidogyne incognita*. The seed extract of *Areca catechu* recorded the highest inhibition rate at 0.1 per cent concentration. Latex of *Carica papaya* caused 98.22 and cent per cent inhibition of hatching at 1.0 and 10.0 per cent concentrations respectively. Latex of *C. gigantea* also caused cent per cent inhibition at 10 per cent concentration (Saravanapriya *et al.*, 2004).

2.4 Management of root-knot nematodes (*Meloidogyne* spp.) on vegetable crops with various parts of plant extracts through soil amendments

Sundarababu *et al.* (1990) conducted pot culture experiments to manage nematodes with plant products. Chopped leaves of *Bougainvillea spectabilis*, *Ocimum sanctum*, *Onion*, *Prosopis juliflora*, *Calotropis procera*

and *Leucaena leuccephala* were applied in the soil at the rate of 5 g / kg soil, against *Meloidogyne incognita* on tomato. They reported that all plant products enhanced plant growth and suppressed final nematode populations with *P. juliflora* having the greatest effect.

In a greenhouse experiment Muller and Sturhan (1994) tested the influence of growing *Chromolaena odorata* (weed) roots on the multiplication rate of *M. incognita* and on the galling of roots of tomato cv. Moneymaker. For comparison, *Tagetes erecta* L. and *Crotalaria retusa* L. which are known to have suppressive effects on root-knot nematodes were included in the experiment. The results of the experiment showed that there was neither a toxic effect of *C. odorata* roots on *M. incognita* juveniles in soil nor an influence on the multiplication rate of the nematode in roots of a good host plant grown in close contact with *C. odorata*.

The plant extracts (leaf) of *Calotropis procera*, *Tagetes erecta*, *Catharanthus rosea* and *Bougainvillea spectabilis* were introduced in the soil to manage *Meloidogyne incognita* infecting black gram through pot culture experiments. There was significant increase in all growth parameters due to addition of leaf extracts. Leaf extracts recorded higher spore population and mycorrhizal colonization than the nematicides but the latter recorded less gall index and nematode populations (Sankaranarayanan and Sundarababu, 1997).

The nematicidal effect of fresh, exhausted and composted olive pomace and fresh grape pomace at dosages of 10, 20 and 40 t / ha was

tested on *Meloidogyne incognita* on cantaloupe in a sandy loam of Southern Italy (Daddabbo *et al.*, 2000). All treatments caused a significant reduction of nematode population on plant roots and in the soil, but only the highest dosage of grape pomace increased yield.

Mateeva and Ivanova (2000) tested the nematicidal potential of some plant extracts, *Ocimum basilicum*, *Datura stramonium*, *Tagetes patula*, *Allium sativa* and *Allium cepa* for the control of root-knot nematode (*Meloidogyne* spp.) on tomato leaf and root extracts were prepared and applied in different concentrations. One per cent and 0.5 per cent by preplanting and four times after planting. Pre-planting treatments of extracts were more effective than post planting treatments. The number of galls varied according to the concentration of plant extracts. Leaf extracts from *Ocimum basilicum* and *Datura stramonium* were more effective than root extracts of some other plants.

A pot culture experiment was carried out to study the effect of soil and foliar application of neem leaf extract at two concentrations viz., 10 and 15 per cent on the root-knot nematode, *Meloidogyne incognita*. Soil application showed higher reduction in nematode population compared to foliar application. Soil application at 15 per cent recorded minimum number of nematodes in soil and root which was 90 and 90.3 per cent decrease over control, respectively. The gall index was only 1.3 at 15 per cent concentration, whereas it was five in untreated control. The plant growth was promoted by soil application of neem leaf extract at 15 per cent followed by 10 per cent

concentration. Number of pods were 3.8 at 15 per cent while it was nil in control (Uma Maheswari and Rajeswari Sundarababu, 2001)

Trials were made for to assess the nematicidal property of the leaves of *Catharanthus roseus* under pot culture experiment against *Meloidogyne incognita*. Different aqueous concentrations of *Catharanthus roseus* leaf extracts were found to inhibit gall formation when compared to infected control. Both length and weight of root and shoot were increased with the increase in concentration in all the cases except in infected control (Saxena and Tabassum, 2001).

In a study conducted (Al-Saba *et al.*, 2001) to determine the effect of *Datura stramonium* extracts on *Meloidogyne javanica* in tomato plants, the results indicated that the aqueous extract of the plant was more effective on the second stage juveniles (larvae) than the ethanol extract. The aqueous seed extract was also more effective in reducing both gall formation and nematode population in the soil. The effect of pre-planting treatment with either extract was reported to be more considerable than post-planting treatments. The aqueous extracts of *D. stramonium* improved tomato growth compared with the infected plants.

Two mangroves namely, *Avicennia marina* and *Rhizophora mucohata* when amended in the soil showed a significant reduction in root-knot infection due to *M. javanica* in tomato seedlings under glass house conditions (Mehdi *et al.*, 2001).

Adekunle and Fawole (2002) have conducted field studies to investigate the effects of air-dried milled samples of leaves and roots of siam weed (*Chromolaena odorata*) and leaves of neem (*Azadirachta indica*) at 30 and 50 kg / ha on *Meloidogyne incognita* infecting cowpea cv. IT86D – 715. The neem leaf treated field, grain yield was recorded as 1.3 t / ha and root gall rating was 1.4. The siam weed root treatments were the least effective although these were significantly superior to untreated control.

Studies were conducted to find out the efficacy of water extract and dry powder of seven plant products viz., leaves of *Calotropis gigantea* Ait., *Tagetes erecta* L. and *Azadirachta indica*, A. juss, seeds of *Areca catechu* L. and *Citrullus lanatus* (Thunb), latex of *C. gigantea* and *Carica papaya* L. in glass house on tomato against *M. incognita*. Water extract of all the plant products resulted better performance than dry powder. Of both these forms, leaves of *C. gigantea* have resulted in increased plant growth parameters and recorded the lowest galling and soil nematode population whereas latex of *C. papaya* and *C. gigantea* recorded the poorest performance (Saravanapriya and Sivakumar, 2003)

Materials and Methods

3. MATERIALS AND METHODS

Seeds of *Dolichos lablab* (country bean) an economically important pulse crop, which is cultivated throughout India and other parts of Asia and Africa were obtained from Central Seed Depot, Coimbatore, for the present investigation entitled **“Development of *Meloidogyne incognita* (a root-knot nematode) in *Dolichos lablab* (country bean) and its management through root extracts of selected medicinal plants”**.

The unripe pods and seeds, rich in vitamins and minerals are eaten as green vegetables. The seeds are also considered febrifuge, stomachic, antispasmodic and aphrodisiac (Pandey, 2001). Because of its economic values, this pulse crop (Plate I A) is selected as a host plant to study the life cycle of a root-knot nematode (*Meloidogyne* spp.) and to assess the biochemical changes in the roots of host plants due to the interaction of root-knot nematodes (Plate I B). The root extracts of two selected medicinal plants *Tephrosia purpurea* (Kolinji) and *Phyllanthus neruri* (keelanelli) were used to evaluate their efficacy on the hatching of *M. incognita* eggs and to study their effect on the plant growth and parasitic (root-knot nematode) reproduction by amending them in the soil.

Soil mix

The soil mix (sand and red soil 1 : 1 ratio) for the present investigation was steam sterilized and stored in polythene bags. This sterilized soil was used for the maintenance of stock culture of *Meloidogyne incognita* and for

various experiments to study the life cycle of *M. incognita*, biochemical changes in the infected roots of host plants and for the management of root-knot nematodes (*M. incognita*).

Maintenance of stock culture

Pure culture of *M. incognita* was raised from a single egg mass progeny and maintained in tomato seedlings in earthen pot (20 x 36 cm) containing 7 kg of steam sterilized soil (Plate I C). Second stage juveniles hatched from egg masses were poured into holes made in the soil around tomato seedlings, raised in sterilized soil. When tomato plants mature, aerial parts were cut off at soil level, leaving the root undisturbed in the soil. New tomato seedlings were planted regularly for continuous maintenance of stock culture. The tomato plants from the soil were carefully uprooted and the roots were washed in running water. Egg masses were picked up from the root galls and placed in small petridishes with tissue paper and immersed in water for hatching. Second stage juveniles hatched out from the egg masses were collected in glass vials and stored in refrigerator for further studies.

Extraction of nematodes from the soil

Extraction of nematodes from the soil samples was carried out following Cobb's sieving and decanting technique (Cobb, 1918). *M. incognita* infected soil was soaked in 250 ml of water for five minutes in a plastic basin and mixed gently to break up the lumps of soil. The supernatant containing nematodes was passed through a series of test sieves of mesh size 20, 60,

100, 200 and 350 μ . Final suspension retained over 350 μ mesh was collected in a beaker.

From the above soil suspension, nematodes were extracted by centrifugal floatation technique (Coveness and Jensen, 1955). Nematode suspension was centrifuged for five minutes at 3000 g to settle nematodes along with residual soil. Supernatant water was then discarded and sugar solution at specific gravity of 1.18 (484 g sugar / litre of water) was added to the residue in test tube, mixed thoroughly with a glass rod and centrifuged again at 3000 g for one minute, when nematodes float in the solution and other debris settled down. The supernatant containing nematodes was poured over 350 μ mesh sieve, washed rapidly in running tap water. The clear nematode suspension in the sieve was collected, labelled and stored in refrigerator at 4°C for further use.

Estimation of Nematode Population

Final clear nematode suspension extract was poured into a rectangular plastic counting dish, with sloping sides and graduated bottom and counted under a stereoscopic zoom microscope to study the nematode population in the soil.

3.1 Development (life cycle) of *M. incognita* on the host plant, *Dolichos lablab* (country bean)

Penetration time and life cycle stages of *M. incognita* were studied individually on host plant, *Dolichos lablab*. Fifteen days old seedlings of

Dolichos lablab were planted in 10 x 7 cm plastic tumbler pots (Plate II A) containing 250 g of sterilized soil mix. After the plants were well established, 100 second stage juveniles (J₂) of *M. incognita* obtained from stock culture were poured around the roots by making three holes in the surface soil. Roots of host plants after inoculation, were carefully removed for studies at every 24 hours interval for the first five days, followed by an interval of five days till 20th day thereafter with one day interval till 40 days period. The optimum temperature maintained was 32°C – 36°C.

The infected roots removed were chopped into small pieces (approximately 2 – 4 cm) blotted dry and stained in hot lactophenol – acid fuchsin (Alexander *et al.*, 1979). Stained roots were examined and mounted on glass slides with clear lactophenol to study various stages of development of *M. incognita* in the host roots. Period of life cycle stages in host plant was recorded. Appropriate microphotographs were taken.

3.2 Biochemical studies in roots of host plant, *Dolichos lablab* infected with *M. incognita*

The roots of both inoculated and uninoculated host plants were subjected for biochemical analysis of total proteins, total carbohydrates and total phenols.

Five seeds of country beans / pot were sown in the earthen pots (22 x 16 cm) containing 2 kg of sterilized soil mix. Fifteen days after, when the seedlings were grown well, two inoculum levels, (i.e.), 500 and 1000 freshly

hatched second stage juveniles of *M. incognita* per pot were inoculated. An uninoculated check was maintained as control for comparison. The experiment was replicated thrice. 40 days after inoculation both inoculated and uninoculated plants were carefully removed from the pots and their roots were washed and processed for quantitative estimation of total proteins, total carbohydrates and total phenols.

Individual chemical components were estimated by adopting the standard methods described by Sadasivam and Manickam (1992) by using spectrophotometer spectronic 2000.

3.2.1 Total proteins

Reagents used

Reagent A

2 per cent sodium carbonate in 0.1 N sodium hydroxide.

Reagent B

0.5 per cent copper sulphate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) in 1 per cent potassium sodium tartrate.

Reagent C

Alkaline copper solution

Mixture of 50 ml of A and 1 ml of B (To be prepared prior to use).

Reagent D

Folin – Ciocalteu Reagent.

Stock solution

50 mg of bovine serum albumin dissolved in 50 ml with distilled water in a standard flask.

Working standard

10 ml of the stock solution diluted with 50 ml of distilled water in a standard flask.

Extraction of protein from sample

1 gram each of root sample from both inoculated (500 nematodes / pot and 1000 nematodes / pot) and uninoculated (control) plants and were weighed and ground separately in a mortar and pestle using 10 ml of the phosphate buffer. The extracts were centrifuged separately and the supernatants were used for protein estimation.

Estimation of protein

0.2, 0.4, 0.6, 0.8 and 1 ml of working standard solutions were pipetted out in series of test tubes. 0.1 ml of extracts from three root samples (control and two inoculated root samples) were taken separately in three other test tubes. The volume was made up to 1 ml in each test tube by adding distilled water. A tube with 1 ml of water served as the blank. 5 ml of reagent C was

added to each tube including the blank and was allowed to stand for 10 minutes. To this mixture 0.5 ml of reagent D was added and incubated at room temperature in dark for 30 minutes. The blue colour was developed. Readings were taken spectrophotometrically at 660 nm. A standard graph was drawn and the amount of protein in the samples were calculated and expressed as mg / g sample.

3.2.2 Total carbohydrates

Reagents used

- 2.5 N – HCl

Antrone reagent was prepared a fresh before use

200 mg anthrone was dissolved in 100 ml of ice cold 95 per cent H_2SO_4 .

Standard glucose

100 mg of glucose was dissolved in 100 ml of distilled water.

Working standard

This was prepared by diluting 10 ml of stock in 100 ml distilled water. To this few drops of toluene was added few drops and kept in refrigerator.

Extraction

100 mg of infected (two samples) and uninfected (control) root samples were taken in three separate boiling tubes and hydrolysed by keeping them in

water bath for 3 hours each with 5 ml of 2.5 N HCl were cooled to room temperature. The sample extracts were then neutralised with sodium carbonate until the effervescence ceased. The volume in each tube was made upto 100 ml by adding distilled water and centrifuged. The supernatant aliquots is used for analysis.

Procedure

Working standard solutions of 0.2, 0.4, 0.6, 0.8 and 1 ml were taken in series of test tubes. The volume in each tube was made upto 1 ml by adding distilled water. 1 ml of water in a test tube served as blank. 1 ml of sample from both infected and uninfected (control) roots was taken in separate test tubes. To these test tubes, 4 ml of anthrone reagent was added and were heated for 8 minutes in boiling water bath and cooled to room temperature. A dark green colour was developed. This was read at 630 nm in a spectrophotometer. A standard graph was drawn by plotting concentration of the standard on the X-axis and absorbance on the Y-axis. The amount of carbohydrate present in the sample was calculated and expressed as mg / g.

3.2.3 Total phenol

Reagents used

- 80 per cent Ethanol
- Folin – Ciocalteu Reagent
- Na_2CO_3 , 20 per cent

Stock standard

100 mg catechol dissolved in 100 ml distilled water.

Working standard

Stock was diluted to 10 times to get working standard.

Extraction

One gram each of the samples of both infected and uninfected were ground in a mortar and pestle with 10 time volume of 80 per cent ethanol. The homogenate was centrifuged at 10,000 rpm for 20 minutes. The supernatants were collected by reextracting the residues with 80 per cent ethanol and centrifuged. The pooled supernatants were evaporated to dryness. The residues were dissolved in 5 ml of distilled water. The aliquots of 0.2, 0.4, 0.6, 0.8 – 2 ml were pipetted out in test tubes and each test tube volume was made upto 3 ml with distilled water. 0.5 ml of Folin – Ciocalteu reagent was added to each test tube. After 3 minutes, 2 ml of 20 per cent Na_2CO_3 solution was added to each test tube and was mixed well. Tubes were placed in boiling water for one minute. The test tubes were cooled and absorbance was measured at 650 nm with spectrophotometer. Concentration of phenols in the samples were calculated and expressed in mg / g material.

3.3 Management of *M. incognita* using root extracts of two medicinal plants, *T. purpurea* and *P. neruri*

3.3.1 Nematotoxic effect of roots of *T. purpurea* and *P. neruri* on the egg hatch of *M. incognita*

Before amending the root extracts of *T. purpurea* and *P. neruri* in the soil, a pilot study has been conducted to find out the effect of the aqueous root extracts on the hatching of *M. incognita* eggs.

Preparation of root extracts of *T. purpurea* and *P. neruri*

Fresh roots of *T. purpurea* and *P. neruri* were collected, washed with the distilled water and cut into small pieces. 500 mg of these roots were taken separately and aqueous extracts were prepared by grinding the roots of each plant with 100 ml of distilled water in a mortar and pestle. The extracts obtained were filtered separately through a four ply muslin cloth and passed through Whatman No. 1 filter paper. The filtered extracts were then centrifuged at 4000 rpm for 10 minutes, the supernatant was separated and used as stock solutions.

From the stock solution four different concentrations viz., 0.1 per cent, 1 per cent, 10 per cent and 100 per cent of extracts were prepared by adding proportionate quantity of distilled water.

Preparation of different concentrations of root extracts from the stock solution

- 0.1 per cent - 0.1 ml of stock solution + 99.9 ml of distilled water
- 1 per cent - 1 ml of stock solution + 99 ml of distilled water
- 10 per cent - 10 ml of stock solution + 90 ml of distilled water
- 100 per cent - 100 ml of stock solution

5 ml of each concentration of the extract to be tested was poured separately in glass cavity blocks. Five healthy, equal sized and matured egg masses of *M. incognita* containing an average of 250 – 300 eggs / egg masses were placed in each cavity block. Distilled water with five egg masses in the cavity block served as control for comparison. Each treatment was replicated thrice. Two sets of arrangements were maintained to study the nematicidal action of the root extracts of these two plants on the hatchability of *M. incognita* eggs.

Number of second stage juveniles hatched were counted after 1st, 3rd, 5th and 7th days of treatment in a counting chamber under stereoscopic zoom binocular microscope. Test extracts with hatched juveniles were discarded after counting. Fresh root extracts of each dilution was added to unhatched eggs in the cavity block in order to eliminate the bacterial contamination. This method was repeated until the termination of the experiment. Data recorded was subjected to statistical analysis.

3.3.2 Management of *M. incognita* infected *Dolichos lablab* through the root extracts of *T. purpurea* and *P. neruri* amended in the soil

A pot culture experiment (Plate II B) was carried out to study the efficacy of the roots of two medicinal plants, *T. purpurea* and *P. neruri* on the management of *M. incognita* infected country bean, *Dolichos lablab*, as an alternative measure for chemical treatment.

Seedlings of *Dolichos lablab* were raised in 22 x 16 cm clay pot containing 7 kg of autoclaved soil. Fifteen days old healthy seedlings were picked out and transplanted in separate earthen pots (2 kg capacity) each with three plants. 1000 second stage juveniles were inoculated per pot. 50 g and 100 g roots of *T. purpurea* and *P.neruri* were ground well separately in 100 ml of water and the extracts were added into the soil 10 days after inoculation. Untreated, inoculated pots served as control. Treatments were replicated thrice. Extract applications was repeated once in 15 days. The experiment was terminated 40 days after treatment. Data obtained on plant growth parameters (plant height and weight), number of galls, number of egg masses, number of females / five galls and nematode population in soil were recorded. Data was subjected for statistical analysis. Photographs were taken in support of the study.

Results and Discussion

4. RESULTS AND DISCUSSION

The results of the present study entitled “**Development of *Meloidogyne incognita* (a root-knot nematode) in *Dolichos lablab* (country bean) and its management through root extracts of selected medicinal plants**” are presented in the form of Tables I to IX, Figures 1 to 11 and Plates III and IV.

Table I represents duration of developmental stages of *M. incognita* on *Dolichos lablab* and Plate III shows the life cycle stages observed under light microscope.

Results of estimation of biochemical components such as total proteins, total carbohydrates and total phenols are presented in Table II and Figures 1, 2 and 3.

Results obtained on the hatchability of *M. incognita* eggs using aqueous root extracts of *Tephrosia purpurea* and *Phyllanthus neruri* are presented in Tables III, IV and V and Figures 4, 5 and 6.

Results of the pot culture experiment on soil amendment of root extracts of *T. purpurea* and *P. neruri* to assess the growth performance, root galling and egg mass production in the host plant infected with *M. incognita* are presented in Tables VI, VII and VIII, Figures 7, 8, 9 and 10 and Plates IV A, B and C.

Table IX and Figure 11 represents the nematode population in root extracts treated and untreated soil.

4.1 Development (life cycle) of *Meloidogyne incognita* in *Dolichos lablab*

The life cycle of *M. incognita* was studied on the country bean, *Dolichos lablab* under laboratory conditions at a temperature range of 32-36°C. On examining the roots of *M. incognita* infected country bean, stained with lactophenol acid fuchsin second stage juveniles (J₂) were found lying in the cortex parallel to vascular bundle, 48 hours after the inoculation. Second stage juveniles were small and vermiform. Further, observation on the roots proved that their penetration continued till 5th day of inoculation. The appearance of different larval stages observed from the roots at regular intervals till the appearance of second stage juveniles (J₂) of next generation are presented in Table I.

The second stage juveniles (J₂) increased in body width and moulting was initiated 4 days (96 hours) after inoculation and transformed into third stage juveniles (J₃) which could be observed 5 days after nematode inoculation, near the vascular bundle region. Gall formation was also visible at this period (5th day). The emerged out third stage juveniles (J₃) further increased in width and turned to fourth stage juveniles (J₄) 10 days after inoculation. Males were visible on the 10th day. Young females were seen feeding the roots 15 days after nematode inoculation.

Table I
Duration of developmental stages of *Meloidogyne incognita* in
***Dolichos lablab* (country bean)**

Developmental stages	Hours / Days after inoculation	Time interval for each stage (in days)
Penetration of second stage juveniles (J ₂)	2 nd day (48 hours)	
Penetration continued	till 5 th day	
Appearance of third stage juveniles (J ₃) and gall formation	5	3
Appearance of fourth stage juveniles (J ₄)	10	5
Appearance of male	10	-
Young female	15	5
Appearance of adult female with gelatinous egg sac	20	5
Deposition of eggs in the gelatinous egg sac	23	3
Embryonated eggs inside the egg sacs	30	7
Appearance of first stage juveniles (J ₁)	32	2
Emergence of second stage juveniles (J ₂) second generation in the egg sac	35	3
Appearance of J ₂ (infective stage) in the soil	38 //	3

Changes in the root histology was also observed during this period along with formation of pronounced galls. Fully developed pear shaped matured females completely embedded in the host roots with empty gelatinous egg-sacs were seen 20 days after inoculation. Variable number of eggs were deposited in the gelatinous matrix at 23 days of inoculation. At 30th day of life cycle study, embryonated eggs were observed in the egg-sacs attached to the root tissues which were turned to first stage juveniles (J₁) at 32 days of inoculation. Between 32-35 days after inoculation second stage juveniles (J₂) were appeared in the egg sacs after the moulting of first stage juveniles (J₁).

Newly hatched second stage juveniles (J₂) were found in the soil at 38th day of experiment, in search of new feeding sites in the roots of host tissues which was evident from the soil examination.

The life cycle of *M. incognita* on the host plant, *Dolichos lablab* was completed within 35 days starting from the penetration of second stage juveniles (J₂) into the root tissues till the migration of second generation second stage juveniles (J₂) into the soil.

Life history of root-knot nematodes has been reported to be characterized by five developmental stages starting from first stage juveniles (J₁) in the egg to the adult stage by undergoing four moults. Similar such stages of *M. incognita* were observed in the present study in the roots of host plant, *Dolichos lablab*.

Time taken for penetration (Plate III A) of second stage juveniles (J_2) was 48 hours after inoculation. Same duration of penetration was also reported by Raj and Jain (1988) in tomato and Sanjay *et al.* (2000) in groundnut. Kaushik and Bhatti (1986) also recorded longer duration of penetration (48 and 96 hours) of *M. javanica* in tomato roots under varied seasons in Haryana. Penetration time can be varied from plant to plant. However, in certain instances minimum time (less than 24 hours) was required for penetration, i.e., 5 hours in rice by *M. graminicola* (Rao and Israel, 1973) and 12 hours in cotton by *M. javanica* (Ibrahim *et al.*, 1981) and six hours in jute by *M. incognita* (Malakar and Mian, 1994). The penetration of second stage juveniles (J_2) into the host root tissues might be possible by the mechanical action of stylet aided by the secretion of oesophageal glands as reported earlier by Bird *et al.* (1975).

Second stage juveniles (J_2) after their entry, migrated through cortical cells and settled in vascular parenchyma and started feeding on the adjacent cells and increased in body width (Hussey, 1985) and also initiated giant cell formation. These juveniles became sedentary and moulted to third stage juveniles (J_3) (Plate III B) and then to fourth stage juveniles (J_4) (Plate III C) and finally into sexually differentiated young males and females (Plate III A).

Males observed in the present studies were liberated into the soil, moved freely and finally died. Eisenback and Triantaphyllou (1991) have also stated the same during their work in root-knot species. No males were

reported at any stages in the life cycle of *M. incognita* in groundnut (Sanjay *et al.*, 2000).

Young females increased in size very rapidly by feeding on the tissues (Plate III D) continuously became pyriform or pearshaped adult females within a time interval of 15 days. These adult females secreted gelatinous egg sacs (Plate III E) of glycoproteinaceous nature by their six rectal glands which passed through anus to the external surface of female body and surrounded the vulval aperture as reported by Bird and Rogers (1965) and Orion (1995).

Egg laying females were observed on 29th day in groundnut (Sanjay *et al.*, 2000) and 28th day in tomato (Anju Jain *et al.*, 2001) whereas it was little earlier to observe adult egg laying females i.e., 20th day after inoculation in the present study. Varying number of eggs (256 to 289) were deposited in gelatinous matrix by mature females (Plate III F). In pointed gourd the number of eggs deposited was ranged between 50 and 385 eggs / egg mass (Verma and Anwar, 1999). The eggs were deposited at 3 days interval from the time of secretion of gelatinous matrix in the present study. It took 2-6 days in pointed gourd (Verma and Anwar, 1999) and 4 days interval in *M. incognita* infected black gram (Mahapatra and Swain, 1999) whereas egg laying was delayed in tomato plants grown under varied seasons in Haryana (Kaushik and Bhatti, 1986). Variation in egg deposition time of other phytonematodes also has been reported in other crops. It was one day interval in *Heterodera sorghi* on maize from the formation of gelatinous matrix (Srivastava and Chawla, 1991).

After undergoing cleavage, first stage juveniles (J_1) were observed inside the egg shells which after first moult emerged as fully developed second stage juveniles (J_2) of second generation and were liberated into the soil in search of new roots of the host plant (Plate III G).

M. incognita thus completed its life cycle in 35 days in the host plant, *Dolichos lablab*. Life cycle of *M. incognita* has been studied by various workers on various other hosts. It took 35 days in apparently healthy egg plant (Dhawan and Sethi, 1976), 39 days in sugar beet (Mathur and Varaprasad, 1979), 31 – 42 days (March – July) and 46 – 66 days (October – December) in tomato under varied seasons (Kaushik and Bhatti, 1986), 30 days in tomato (Rai and Jain, 1988), 29 days in mung var RS-4 and 44 days in gaur RGC – 915 (Datta *et al.*, 1990), 60 days on tomato (Langellotti *et al.*, 1995), 28 and 33 days in susceptible and resistant cultivars of tomato (Anwar *et al.*, 1996). 29 days in Japanese mint (Singh and Kumar, 1998). Life cycle (J_2 – J_2) of *M. incognita* on black gram (cv – I – 9) was reported to be completed with 32 days (Mahapatra and Swain, 1999) and 29 days from J_2 to adult with egg mass in groundnut (Sanjay *et al.*, 2000). 105, 51, 36 and 21 days in wheat infected by *M. chitwoodi* under varying temperatures (Inserra *et al.*, 1985). It was reported by Dabur *et al.* (2004) that *M. graminicola* took 24 days to complete its life cycle on paddy. Life cycle of *M. incognita* from egg to egg was completed in 42 – 44 days in susceptible cultivar of *Trigonella foenumgraecum* and that in resistant cultivar was 48 – 50 days at $22 \pm 3^\circ\text{C}$ day temperature.

Life cycle of various other plant parasitic nematodes hosted by different crops are studied by several authors. Life cycle of *Heterodera sorghi* (Sorghum cyst nematode) was reported to complete in 24 days on maize (Srivastava and Chawla, 1991) and 26 and 30 days on sorghum cv. CSH – 6 (Sharma and Swarup, 1984 and Sakhuja and Singh, 1985).

4.2 Biochemical analysis of total proteins, total carbohydrates and total phenols in the roots of *M. incognita* inoculated and uninoculated host plants, *Dolichos lablab*

After observing the developmental stages, it is felt necessary to estimate the amount of certain biochemical components (total proteins, total carbohydrates and total phenols) at the time of egg mass production and liberation of second stage juveniles so as to study the alterations caused by them in the physiological equilibrium of the host plant.

4.2.1 Total proteins

From the results obtained (Table II and Figure 1) on the total proteins, it is clearly evident that there is a significant increase in the roots of the host plant (*Dolichos lablab*) inoculated with 500 and 1000 nematodes / pot when compared with the healthy, uninoculated (control) plants. The values recorded were 49.67 and 64.67 mg / g respectively in the plants inoculated with 500 and 1000 nematodes / pot. The amount of total proteins present in the roots of uninoculated control plants was 35 mg / g. The total proteins recorded in the two inoculated plants were significantly different from each other.

Table II

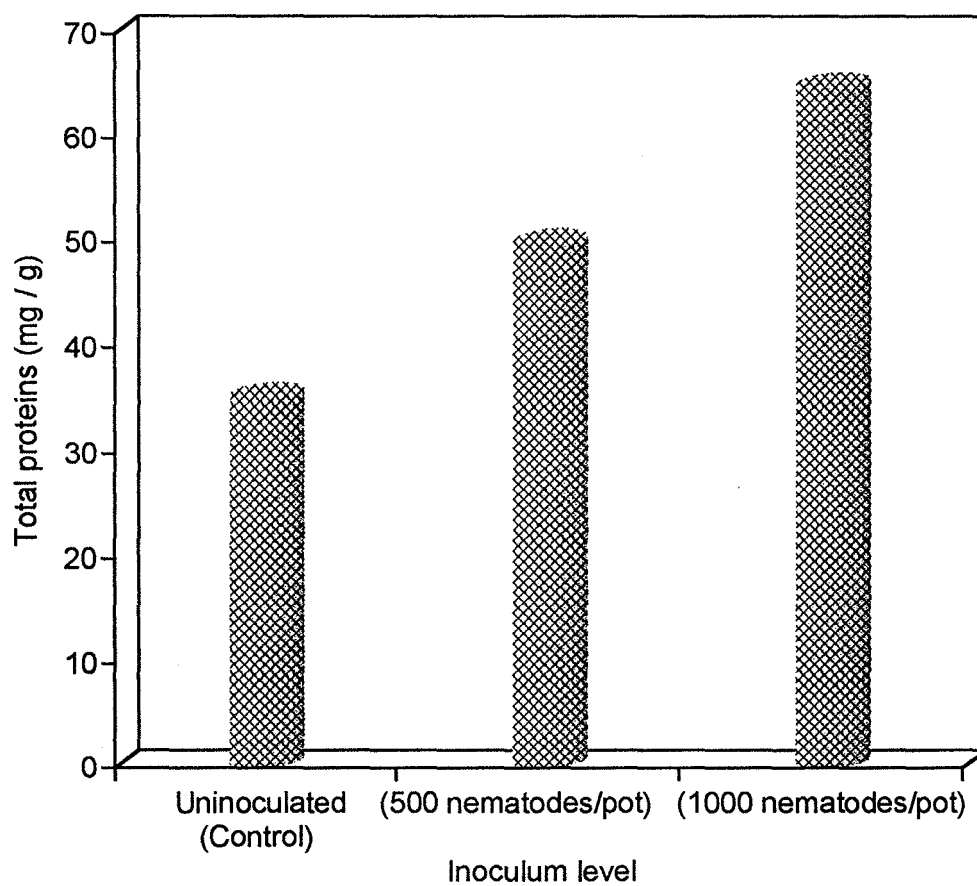
Effect of two inoculum levels of *M. incognita* on total proteins, total carbohydrates and total phenols in the roots of *Dolichos lablab*

Inoculum level	Total proteins (mg / g)	% increase over control	Total carbohydrates (mg / g)	% decrease over control	Total phenols (mg / g)	% decrease over control
Uninoculated (control)	35.00	-	58.33	-	75.00	-
500 nematodes / pot	49.67	41.9	30.00	48.5	71.50	4.6
1000 nematodes / pot	64.67	84.7	19.67	66.2	58.00	22.6
SEd	0.385		0.38		0.82	
CD (0.05)	0.94		0.94		2.00	

Mean of three replications.

FIGURE 1

Effect of *M. incognita* on total proteins in the roots of *Dolichos lablab*



Increase in the amount of total proteins was a maximum of 84.7 per cent in the host roots inoculated with 1000 nematodes / pot and 41.9 per cent in the plants inoculated with 500 nematodes / pot over the control.

Results obtained from the present investigation on total proteins revealed the fact that the roots of the host plants (*Dolichos lablab*) inoculated with two levels (500 and 1000) of nematodes / pot exceeded the amount that present in the uninoculated (control roots). Amount of total proteins recorded in the roots of plants inoculated with higher density (1000 nematodes / pot) of nematodes is of two fold increase when compared with the roots inoculated with lower density (500 nematodes / pot) over the control. Higher protein content in plants with higher inoculum level (1000 larvae / 500 g soil) of *M. incognita* on *Allium porum* was also reported by Rombati and Dhanachand (2000) which may support the present study. The increase in the amount of total proteins in inoculated roots may be due to the infestation caused by the entry of root-knot nematodes. Such increase in total proteins due to invasion of different phytoparasitic nematodes was reported earlier by several researchers (Hanks and Feldman, 1966 ; Singh *et al.*, 1978 ; Giebel, 1974 and Sinha and Sukul, 1983) in different crops. Reports on the increase of total proteins due to root-knot nematode infection in various agricultural crops such as lady's finger (Chatterjee and Sukul, 1981 and Sinha Basu and Sukul, 1983), cowpea (Ganguly *et al.*, 1991), rice (Swain and Prasad, 1991) and *Trigonella forumgracum* L. (Sharma and Trivedi, 1992) add support to the present investigation. This increase in protein content as reported by various nematologists may be due to the synthesis of new

enzyme proteins or it may be due to the contribution from the nematode itself (Ganguly and Dasgupta, 1981 and Simte and Dasgupta, 1987). Changes in the quantity of proteins in inoculated roots irrespective of the nematode density may also be due to the formation of new polypeptides (Bell, 1981 ; Dasgupta, 1988 and Simte and Dasgupta, 1987) or it may be due to synthesis of new proteins (Sharma and Trivedi, 1992) in order to substantiate the nutritive requirements of the growing nematodes in the root system of host crops (Shetty and Rudramuniappa, 1992). Increase in protein in the host roots inoculated with 1000 nematodes / pot may also be due to higher nematode population. Similar trend is reported in the findings of Singh *et al.* (1978) due to *M. incognita* infection in brinjal. Devarajan and Rajdneran (2002) reported the same in banana clones infected with burrowing nematode, *Radopholus similis* which agrees with the present investigation, whereas reports of Vijendra Singh *et al.* (2000) state that protein content showed a decrease in the roots treated with increased concentration of nematicides.

4.2.2 Total carbohydrates

Total carbohydrates estimated for uninoculated healthy plant, *Dolichos lablab* was 58.33 mg / g. The amount of total carbohydrates recorded for the plants inoculated with 500 nematodes / pot was 30 mg / g and that recorded for the plants inoculated with 1000 nematodes / pot was only 19.67 mg / g. Percentage reduction of total carbohydrates was 48.5 and 66.2 in the plants inoculated with 500 and 1000 nematodes / pot respectively over the control.

Table II and Figure 2 showed a decrease in carbohydrates in the infected roots of the host plant when compared with control plants. Reduction in total carbohydrates was recorded in the host plants at both inoculum levels of *M. incognita* when compared with uninoculated control plants. Reduction in carbohydrates due to root-knot nematode infection which alter the metabolism of several other host plants was reported by Chahal and Grover (1972), Masood *et al.* (1979) and Upadhyay and Banerjee (1986). Decrease in carbohydrates might have resulted due to the action of hydrolysing enzymes secreted by *M. incognita* or its influence in the production of these enzymes in host which might have helped in the conversion of total carbohydrates (Singh *et al.*, 1978 and Roy, 1979) or may be due to rapid consumption of these substances by nematodes (Basu and Sukul, 1983). However, reduction in carbohydrates in the host tissues is suggested to be due to pathogenesis of the nematode.

A decreasing trend in the total carbohydrates was evident in the roots of host plants at two inoculum levels was observed in the present study. Decrease in carbohydrates was also reported in different cultivars of tomato and brinjal infected with phytoparasitic nematode, *Rotylenchulus reniformis* (Mehmood, 1985) and in banana clones infected with *Radopholus similis*, a burrowing nematode (Devarajan and Rajendran, 2002).

4.2.3 Total phenols

Statistical analysis of the data presented in Table II and Figure 3 revealed that the inoculated roots of host plant *Dolichos lablab* showed a

FIGURE 2

Effect of *M. incognita* on total carbohydrates in the roots of *Dolichos lablab*

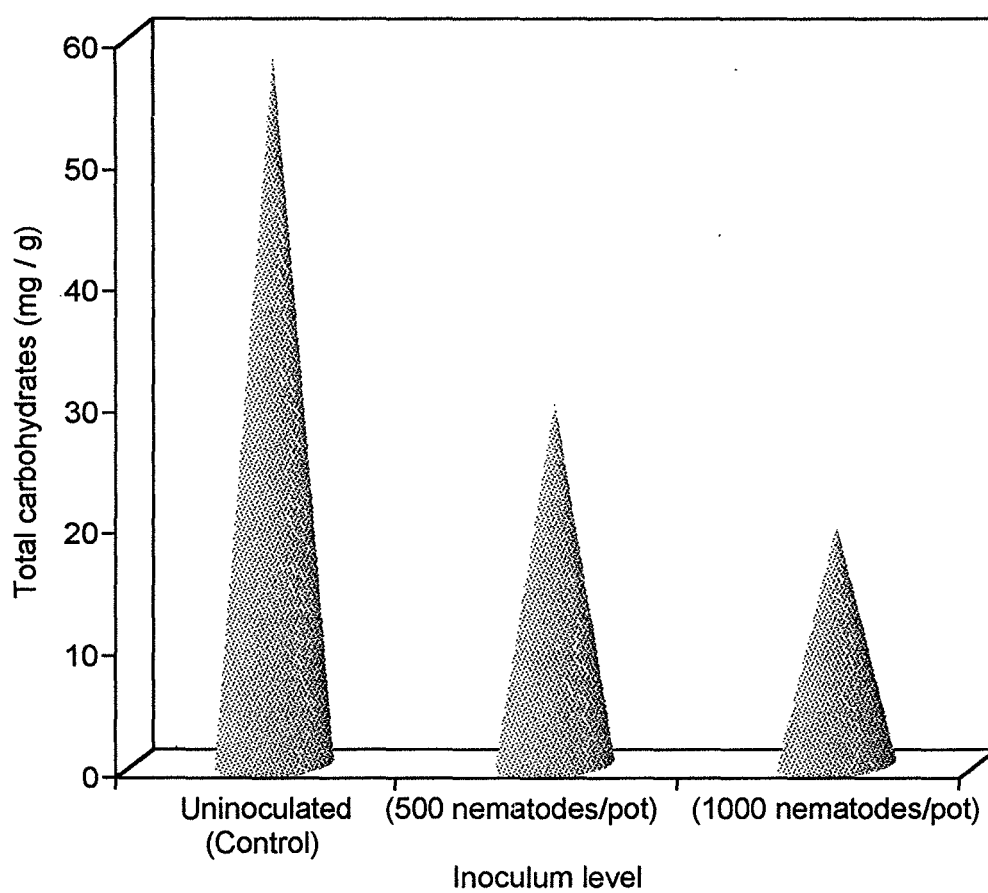
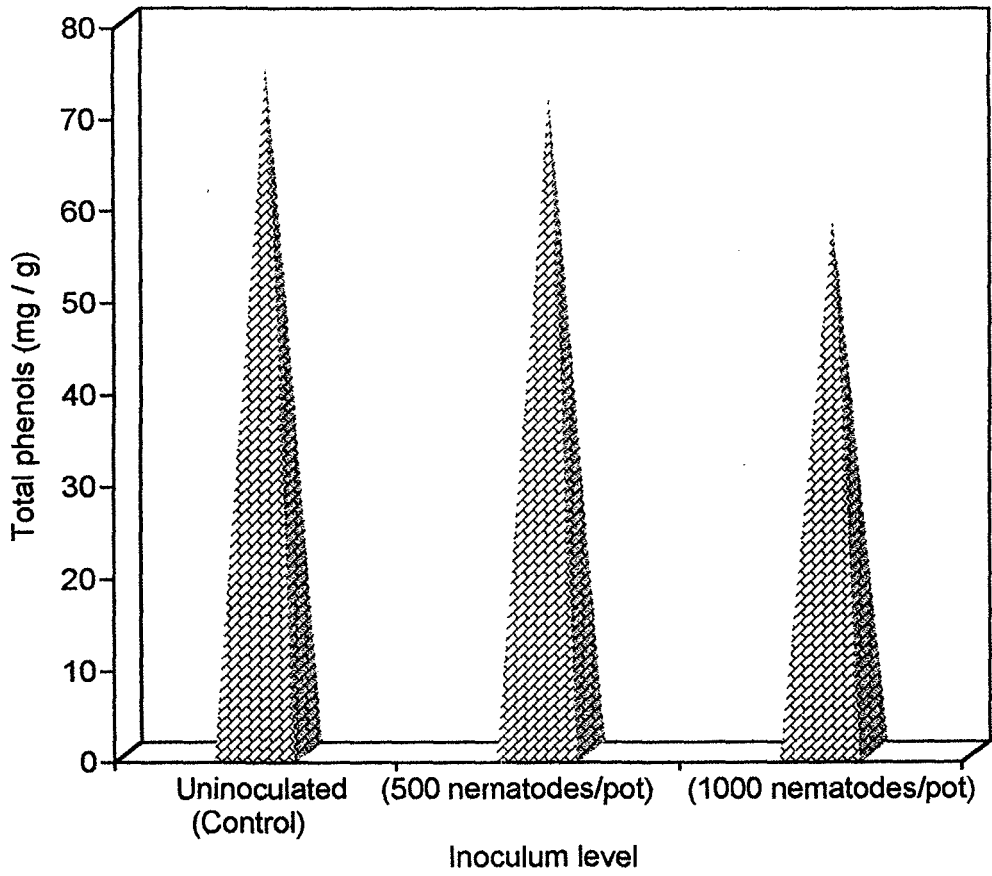


FIGURE 3

Effect of *M. incognita* on total phenols in the roots of *Dolichos lablab*



significant decrease in the amount of total phenols when compared with uninoculated control roots of the host plants. The amount of total phenols recorded for the control plant was 75 mg / g whereas that recorded for the inoculated plants were 71.5 mg / g (500 nematodes / pot) and 58 mg / g (1000 nematodes / pot) respectively. The decrease was a maximum of 22.6 per cent for the plants inoculated with higher density (1000 nematodes / pot) and a minimum of 4.6 per cent for the plants inoculated with lesser density (500 nematodes / pot) of nematodes when compared with control.

Phenols are important chemical components which play major role in defense mechanism exhibited by host plants against the invading pathogens. Lesser phenolic content in both inoculated host plants in the present study is an indication that these host plants were not able to fight against the infection caused due to root-knot nematodes. Role of phenolic compounds against nematode invasion have been studied by several authors. An increase in phenolic content was reported to be a contributory factor developed for resistance in host plants during nematode infections (Pi and Rhode, 1973 ; Giebel, 1974 and Narayana and Reddy, 1986) whereas in the present study the host plants tested recorded a decreased amount of phenols when compared with uninoculated control plants. Dasgupta and Ganguly (1986) reported that the phenols and their oxidative products are toxic to invading pathogens but in the present investigation it may be due to susceptibility of host plants, phenols released might have been reduced.

In contrast to the present study, Patel *et al.* (2001) reported in their findings a significant increase in total phenols in chickpea plants infected with *Meloidogyne* spp. at higher inoculum levels. The increase in phenol concentration which indicates the resistance of the plants against *Meloidogyne* spp. may be due to the release of free phenols and related compounds by the action of enzymes like polyphenol oxidase (Bajaj *et al.*, 1985).

4.3 Effect of aqueous root extracts of two medicinal plants (*Tephrosia purpurea* and *Phyllanthus neruri*) on egg hatch of *M. incognita*

4.3.1 Effect of aqueous root extract of *Tephrosia purpurea* on the hatchability of *M. incognita* eggs

The perusal of data presented in Table III and Figure 4 reveals that the egg hatch was maximum in the distilled water treatment when compared with the treatment of aqueous root extracts of *Tephrosia purpurea*. Number of second stage juveniles emerged from the eggs of *M. incognita* after first day of treatment with distilled water was a maximum of 276 and that from 0.1 per cent concentration was 241. Both these values are statistically not different from each other. Number of eggs hatched in root extracts of 1 and 10 per cent concentration was 212 and 109 respectively. At 100 per cent concentration the egg hatch gave rise to only 23 juveniles. Number of juveniles emerged showed a significant decrease as the concentration of root extracts increased.

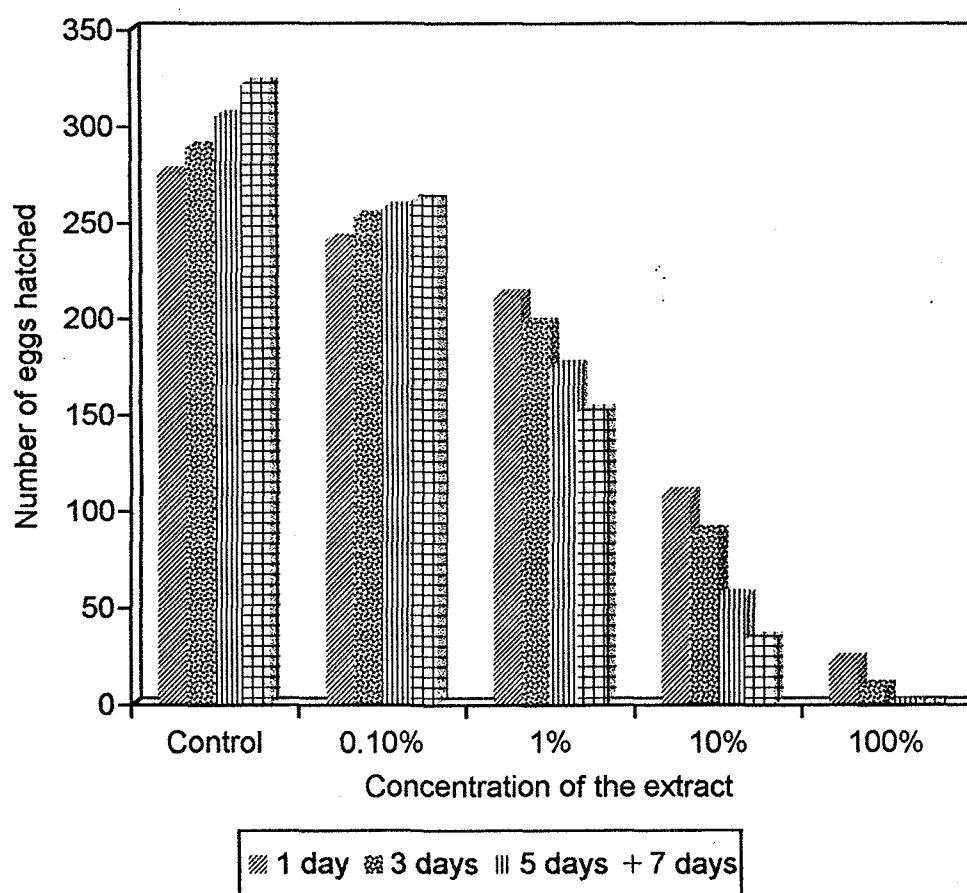
Table III
Effect of different concentrations of aqueous root extract of *Tephrosia purpurea* (Kolinji) on the egg hatch of *M. incognita*

Concentration of the extract	Number of eggs hatched after			
	1 st day	3 rd day	5 th day	7 th day
Control (Distilled water)	276.00	289.00	305.00	322.00
0.1 per cent	241.00	253.00	257.00	261.00
1 per cent	212.00	197.00	175.00	152.00
10 per cent	109.00	89.00	56.00	34.00
100 per cent	23.00	9.00	0.00	0.00
SEd	16.39	15.99	22.11	21.50
CD (0.05)	36.51	35.63	49.28	47.91

Mean of three replications.

FIGURE 4

Effect of different concentrations of aqueous root extract of *Tephrosia purpurea* on the egg hatch of *M. incognita*



The same trend in the egg hatch was observed even after third day's treatment with distilled water and the extracts of different concentration. Maximum number of 289 juveniles was emerged from distilled water treatment and a minimum of juveniles from pure extract solution. Number of juveniles emerged in 0.1, 1 and 10 per cent of root extract treatment was 253, 197 and 89 respectively. Number of eggs hatched from all concentrations of root extracts except that of 0.1 per cent are significantly varied from those eggs hatched with distilled water.

Juveniles emerged from the egg masses soaked in distilled water counted after 5th of treatment were 305 in number and that counted in 0.1 per cent of the root extract of *Tephrosia purpurea* was 257. These values are on par with each other. Number of juveniles emerged from the root extract treatment of 1 and 10 per cent concentration was 175 and 56 respectively. No egg hatch was recorded in root extract of 100 per cent concentration.

Maximum number of juveniles was recorded in distilled water treatment which was 322 and a minimum of 34 juveniles in 10 per cent root extract concentration, 7 days after treatment. Number of eggs hatched in other treatments i.e., 0.1 and 1 per cent concentration of the extracts was 261 and 152 respectively. No egg hatch was reported at pure extract concentration.

4.3.2 Effect of aqueous root extract of *Phyllanthus neruri* on the egg hatch of *M. incognita*

Results obtained on the effect of root extracts of *P. neruri* on the hatching of *M. incognita* eggs at different concentrations are presented in Table IV and Figure 5.

The trend in hatching of eggs is similar to that of *T. purpurea* root extract treatment. Maximum number of eggs were hatched in distilled water treatment with an increasing trend from the start till the termination of the experiment.

Number of juveniles emerged from the *M. incognita* eggs placed in distilled water (control) is a maximum of 226 after first day of treatment which is followed by a number of 217 juveniles at 0.1 per cent concentration of the root extract. Number of eggs hatched between control and 0.1 per cent root extract was statistically on par with each other whereas the eggs placed in 1 and 10 per cent root extracts showed a decreasing trend in their hatch with 143 and 39 juveniles respectively. A minimum number only 3 eggs were hatched at the highest concentration (pure extract of 100 per cent). However, at the end of first day of treatment, the number of eggs hatched showed a decreasing trend as the concentration of root extracts increased

At the end of 3rd day the number of eggs hatched from the distilled water showed an increasing trend when compared with first day. A maximum of 282 eggs were hatched from distilled water treatment. Number of eggs

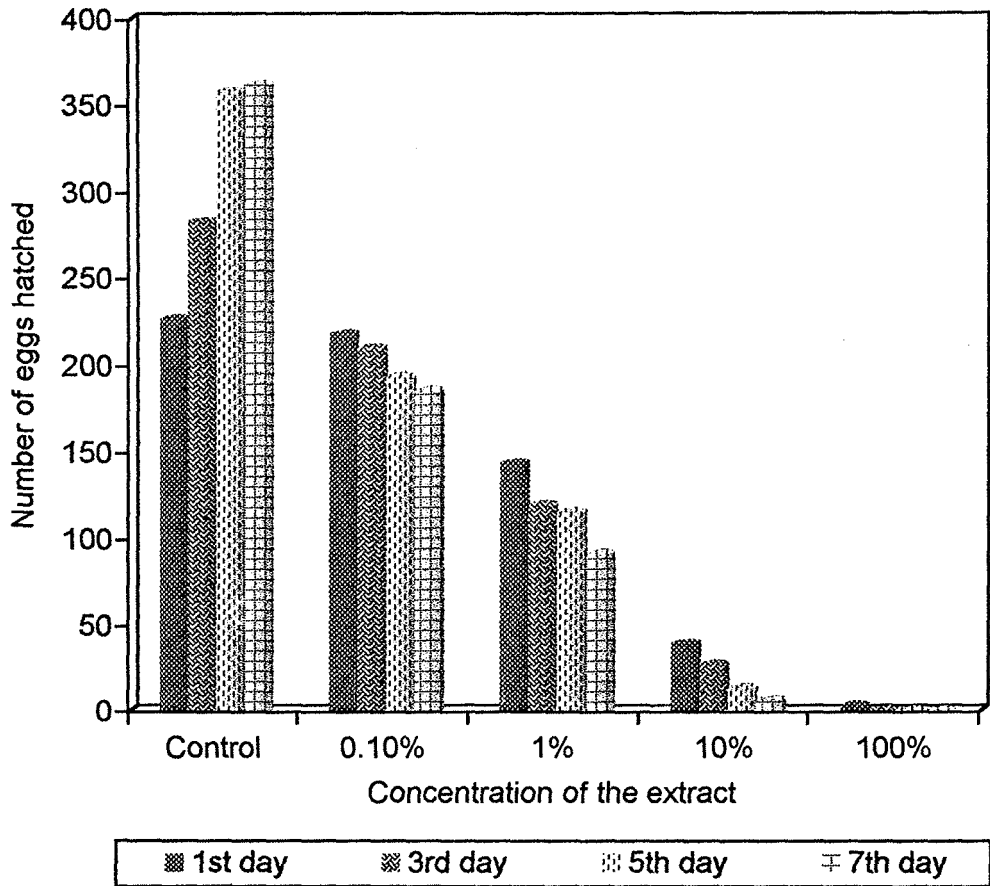
Table IV
Effect of different concentrations of aqueous root extract of
Phyllanthus neruri* (keelanelli) on the egg hatch of *M. incognita

Concentration of the extract	Number of eggs hatched after			
	1 st day	3 rd day	5 th day	7 th day
Control (Distilled water)	226.00	282.00	357.00	361.00
0.1 per cent	217.00	209.00	193.00	185.00
1 per cent	143.00	119.00	115.00	91.00
10 per cent	39.00	27.00	13.00	6.00
100 per cent	3.00	0.00	0.00	0.00
SEd	16.01	9.88	14.03	11.27
CD (0.05)	35.67	22.01	31.26	25.10

Mean of three replications.

FIGURE 5

Effect of different concentrations of aqueous root extract of *Phyllanthus neruri* on the egg hatch of *M. incognita*



hatched in different concentrations of root extract was 209 in 0.1 per cent concentration, followed by 119 in 1 per cent concentration and a minimum of 27 in 10 per cent concentration. No larval emergence was observed in 100 per cent (pure) concentration of the extract. All treatments are significantly different from each other and also with control treatment. As the concentration of the extracts increased, there was a decrease in the number of larvae emerged.

Number of larvae emerged from the distilled water treated eggs after 5th day of treatment showed an increase over the 3rd day's emergence. The number of juveniles counted in distilled water treatment was 357. Number of eggs hatched from the root extracts of 0.1, 1 and 10 per cent concentration was 193, 115 and 13 respectively. No juveniles were observed at the highest concentration (100 per cent) of the root extract of *P. neruri* even after 5th day of treatment.

Larval emergence was found to be still more increased in number with 361 from the egg soaked in distilled water, seven days after treatment whereas the number of juveniles emerged showed a decreasing trend in all the concentrations of root extract treated eggs. Number of eggs hatched was 185, 91 and 6 in the root extracts of 0.1, 1 and 10 per cent concentrations. No egg hatch was recorded at the pure extract (100 per cent) concentration.

From the Table IV and Figure 5 it is clearly evident that the hatchability of eggs was decreased in number correspondingly as the concentration of the root extracts and the number of days increased. Hence, egg hatch is inversely

proportional to the time or duration of the treatment and concentration of the root extract whereas number of eggs hatch showed an increase in distilled water along with number of days of treatment.

The total number of egg hatch after the termination (7 days) of the experiment in brief is shown in Table V and Figure 6. Total number of juveniles emerged from the eggs treated with distilled water (control) set up of *T. purpurea* was 1192 and that of *P. neruri* was 1226 are higher than the values recorded for all other concentrations of the root extract treated eggs. As the concentration (0.1, 1, 10 and 100 per cent) of the root extracts increased there was a corresponding decrease in the number of eggs hatched. Among the root extracts of two plants tested in the present study on egg hatch of *M. incognita* almost negligible percentage of egg hatch was observed in the 100 per cent root extract concentration of *P. neruri* after first day's treatment and then on no egg hatch was reported till the termination of the experiment. Egg hatch was slightly higher in 100 per cent root extract concentration of *T. purpurea* when compared with *P. neruri*, root extract.

The perusal of data given in Tables III, IV and V indicate that the root extracts of the two medicinal plants (*T. purpurea* and *P. neruri*) used in the present experiment are proved to be significantly effective in their inhibitory action on the hatching of *M. incognita* eggs at all concentrations when compared with control treatment. Maximum eggs were hatched from distilled water treatment. Hatching was very minimum at higher concentration of root extracts of both the plants tested. No hatch was reported from the eggs

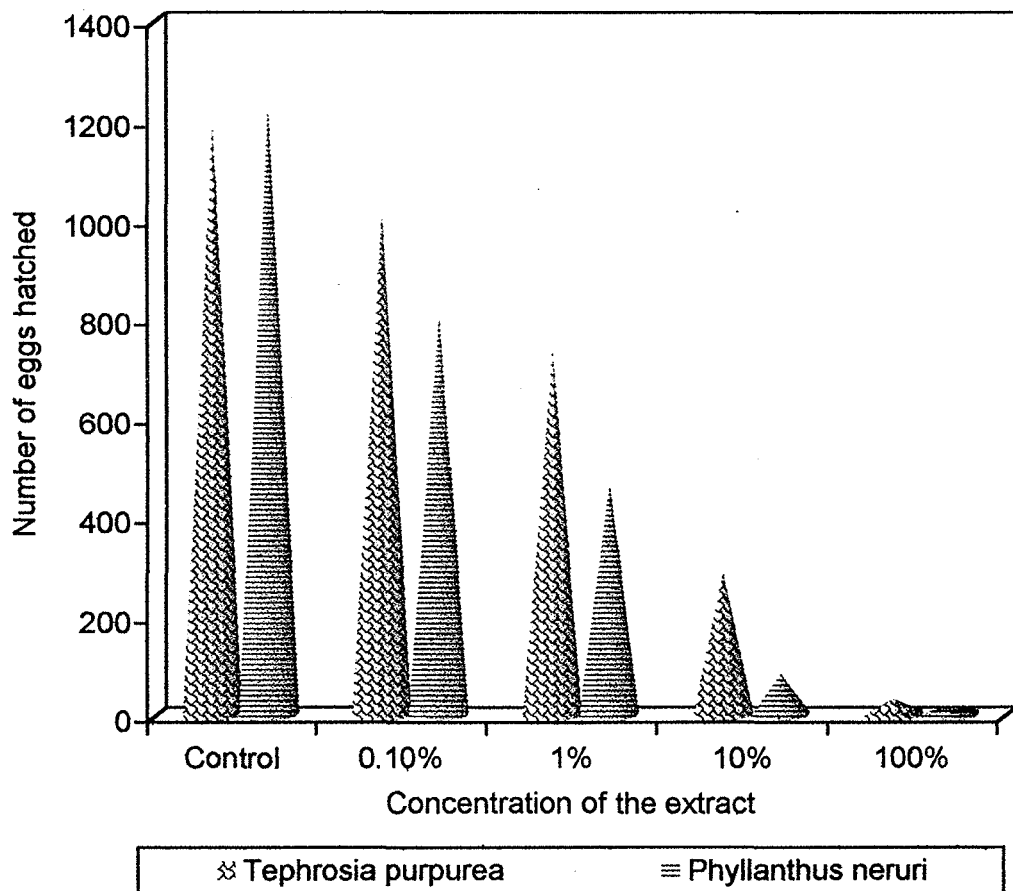
Table V

**Total number of juveniles hatched from the egg masses of
Meloidogyne incognita after seven days of treatments with
root extracts of *T. purpurea* and *P. neruri***

Concentration of the extract	Number of eggs hatched after	
	<i>Tephrosia purpurea</i>	<i>Phyllanthus neruri</i>
Control (Distilled water)	1192	1226
0.1 per cent	1012	804
1 per cent	736	468
10 per cent	288	84
100 per cent	32	3

FIGURE 6

Total number of juveniles hatched from the eggs of *Meloidogyne incognita* after seven days of treatments with root extracts of *T. purpurea* and *P. neruri* at different concentrations



treated with 100 per cent concentration of *P. neruri* root extracts from 3rd day and the same trend was continued till the last day (7th) of the experiment. Same trend was observed in the eggs treated with 100 per cent concentration of *T. purpurea* root extracts from 5th day till the termination of the experiment. It is also evident that the root extracts of 10 per cent and one per cent concentration of both plants also showed higher inhibitory effect on egg hatch which increased correspondingly as the exposure time increased.

It is therefore clear that the juvenile emergence was significantly reduced after 3rd, 5th and 7th day exposure time to the root extracts of *T. purpurea* and *P. neruri* compared with control. 10 and 100 per cent concentrations of each extract was significantly more effective than 1 and 0.1 per cent concentrations. Higher the concentrations of two extracts greater was the effect of inhibition on the egg hatch. As the concentration of roots extracts of *T. purpurea* and *P. neruri* increased the egg hatch in *M. incognita* was correspondingly decreased. Larval hatching was therefore inversely proportional to the extract concentrations and exposure time.

Similar such findings were reported by various authors using different parts (leaves, stem, roots, flowers, seeds, fruits) of several plants on the egg hatchability of *Meloidogyne* species. Much work has been done on this aspect using leaf extracts of various plants whereas very little is done with root extracts.

Inhibition of *M. incognita* juvenile hatch was reported (Yadav, 1970 ; Toida, 1972 and Alam *et al.*, 1975) when the root exudates of *Euphorbia hirta*,

Azadirachta indica and *Tagetes* were used. Cassava root exudates were reported to induce larval mortality of *M. incognita* due to its nematicidal property (Jayakumar *et al.*, 2002). Significant inhibition of egg hatch in *M. javanica* using the aqueous extract of *Argemone mexicana* was reported by Shahid and Imran (2001) with an increase in extract concentration inhibitory action was found to be more. Similar trend was observed in the present investigation. Inhibition of egg hatch in *Meloidogyne* species using various plant parts of *Tagetes* species which are known for their nematicidal and nematotoxic effect by various authors (Alam *et al.*, 1975 ; Kumari *et al.*, 1986 ; Walia and Gupta, 1997 ; Siddiqui and Alam, 1988a ; Siddiqui and Alam, 1988b and Husan – Bano *et al.*, 1999) add support to the present study. Aqueous extracts of seeds of leguminosae plants were evaluated for their nematicidal and nematostatic effects. These seed extracts were reported to have strong inhibitory effect on the egg hatch even in lower concentrations (Khurma and Kumari, 1996 ; Kumar and Singh, 1997 and Khurma and Chanday, 1999).

Further, Hussain and Massood (1975) ; Shahda *et al.* (1998) reported that the extract of *Cassia occidentalis* was known to be more toxic than their ethyl acetate extracts. Aqueous root extracts of *Gaillardia pulchella* (blanket flower) showed inhibitory effect on the egg hatch of *M. incognita* (Tsay *et al.*, 2004) hence it is considered to be an ideal antagonistic plant. Same effect is evident with the two medicinal plant parts used in the present investigation. Maximum suppression of hatching in *M. incognita* eggs was also reported by Ameer – Zareen *et al.*, (2003) while they were using the

aqueous ginger extracts at 100 per cent concentration. Root extracts of various other plants (*Ocimum sanctum*, *Tagetes erecta*, *Euphorbia hirta*, *Artemisia absinthium*, *Aegle marmelos*) were found to be more effective on hatching of *M. incognita* eggs (Renu Sharma and Trivedi, 1992). Root exudates of *Chromolaena odorata* also showed inhibitory effect on the egg hatch of *M. incognita* (Amosu, 1981). Root extract of *Crotalaria spectabilis* was found to be more effective than the leaf extract in controlling the nematodes (Subramanian and Vadivelu, 1990).

4.4 Management of *M. incognita* infestation on *Dolichos lablab* through soil amendment using root extracts of *Tephrosia purpurea* and *Phyllanthus neruri*

Results obtained on the efficacy of two-different concentrations (50 and 100 per cent) of root extracts of *T. purpurea* and *P. neruri* applied in the soil as a measure to control the root-knot nematodes (*Meloidogyne incognita*) infecting the host plant, *Dolichos lablab* (country bean) are presented in Tables VI, VII, VIII, IX ; Figures 7, 8, 9, 10, 11 and Plate IV.

The influence of root extracts of the two medicinal plants on the growth characters of *Dolichos lablab* infected with *M. incognita* is shown in Table VI ; Figure 7 and 8 and Plate IV A and B.

Number of galls and egg masses produced per host plant and nematode population in soil are presented in Tables VII and VIII ; Figures 9, 10 and Plate IV C.

Symbols T₁, T₂, T₃ and T₄ represent two different concentrations of each root extracts of *T. purpurea* and *P. neruri* used in the present study to manage the root-knot nematode (*M. incognita*) infecting the host plant, *Dolichos lablab*.

Symbols	Root extract concentration
T ₁	50 per cent (<i>T. purpurea</i>)
T ₂	100 per cent (<i>T. purpurea</i>)
T ₃	50 per cent (<i>P. neruri</i>)
T ₄	100 per cent (<i>P. neruri</i>)
C	Untreated control

4.4.1 Efficacy of root extracts of *T. purpurea* and *P. neruri* amended in the soil on the growth characters of *Dolichos lablab* infected with *M. incognita*

4.4.1.a. Shoot length

Perusal of data in Table VI reveals that host plants treated with higher concentration (100 per cent = T₄) of root extract of *P. neruri* showed a maximum shoot length of 153.2 cm when compared with other treatments. Shoot length recorded was a minimum of 30.2 cm in untreated, inoculated control plant. Increase in shoot length was slightly higher in plants treated with 50 per cent (T₁) concentration of root extract of *T. purpurea* which is 51.3 cm. The shoot length recorded in plants treated with 100 per cent (T₂) concentration of *T. purpurea* roots was 71.7 cm and plants treated with 50 per cent (T₃) concentration of *P. neruri* roots was 83.2 cm. A very negligible difference in shoot length was found between C and T₁.

Length between T₁ and T₂, T₂ and T₃ are statistically on par with each other. Variation in shoot length was highly significant in T₄ plants. However, shoot length of *Dolichos lablab* showed significant difference between control and all the root extract treated plants (Plate IV A, B).

Percentage increase was 70.1 in T₁, 137.5 in T₂, 175.8 in T₃. Four fold increase (407.8 per cent) in shoot length was recorded in T₄ plants over control.

4.4.1.b. Root length

From the Table VI and Figure 7 and Plate IV C it was clear that there was a two fold increase in root length of the host plant treated with higher concentration (100 per cent = T₄) of extracts obtained from the roots of *P. neruri* when compared with untreated control plants. All the other treatments (T₁, T₂ and T₃) showed no significant difference in root length with control plants. Root length recorded for control plant was 10.8 cm and that recorded for T₁, T₂ and T₃ were 11.6, 14.5 and 16.6 cm respectively. The root length between control and T₁, T₁ and T₂, T₂ and T₃ are statistically on par with each other.

Percentage increase was negligible in T₁ in root length when compared with control. Increase in root length was 34.3 per cent in T₂, 53.4 per cent in T₃ and that 117.9 per cent in T₄.

Table VI

Efficacy of root extracts of *T. purpurea* and *P. neruri* on the growth parameters of *Dolichos lablab* infected with *M. incognita*

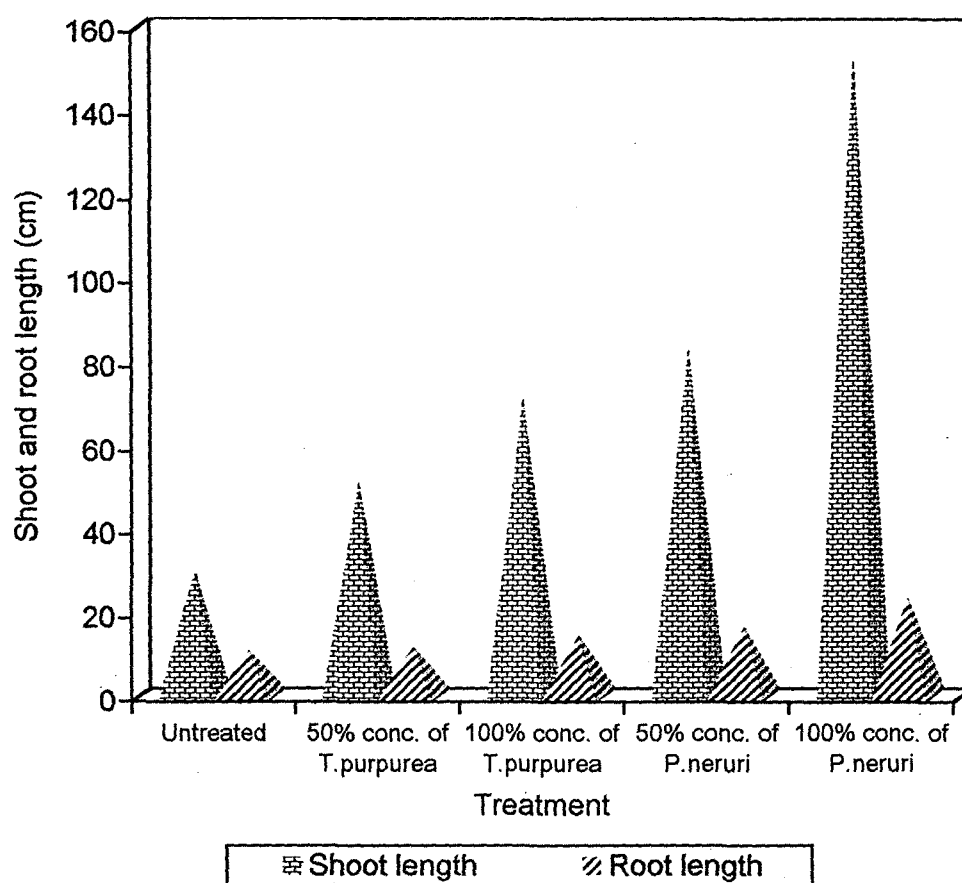
Treatment	Shoot length (cm)	Percentage increase over control	Root length (cm)	Percentage increase over control	Shoot weight (g)	Percentage increase over control	Root weight (g)	Percentage increase over control
Untreated (Control)	30.2	-	10.8	-	5.9	-	0.58	-
50% conc. of <i>T. purpurea</i> (T ₁)	51.3	70.1	11.6	7.1	10.5	78.0	0.87	50.0
100% conc. of <i>T. purpurea</i> (T ₂)	71.7	137.5	14.5	34.3	12.2	107.8	1.24	113.8
50% conc. of <i>P. neruri</i> (T ₃)	83.2	175.8	16.6	53.4	13.6	130.9	1.57	170.7
100% conc. of <i>P. neruri</i> (T ₄)	153.2	407.8	23.5	117.9	17.9	203.4	1.96	237.9
SEd	8.99		2.63		1.30		0.13	
CD (0.05)	20.04		5.87		2.89		0.23	

Mean of three replications.

SR

FIGURE 7

Efficacy of root extracts of *T. purpurea* and *P. neruri* on the shoot and root lengths of *Dolichos lablab* infected with *M. incognita*



4.4.1.c. Shoot weight

Perusal of data presented in Table VI and Figure 8 indicate that the weight of shoot is a maximum of 17.9 g in plants treated with 100 per cent (T₄) root extract concentration of *P. neruri* as against the control (untreated) plants with 5.9 g. The shoot weight of plants treated with *T. purpurea* at two concentrations i.e., 50 (T₁) and 100 per cent (T₂) was 10.5 g and 12.2 g respectively. Weight of shoot recorded for the host plants treated with root extracts of *P. neruri* at 50 per cent (T₃) concentration was 13.6 g.

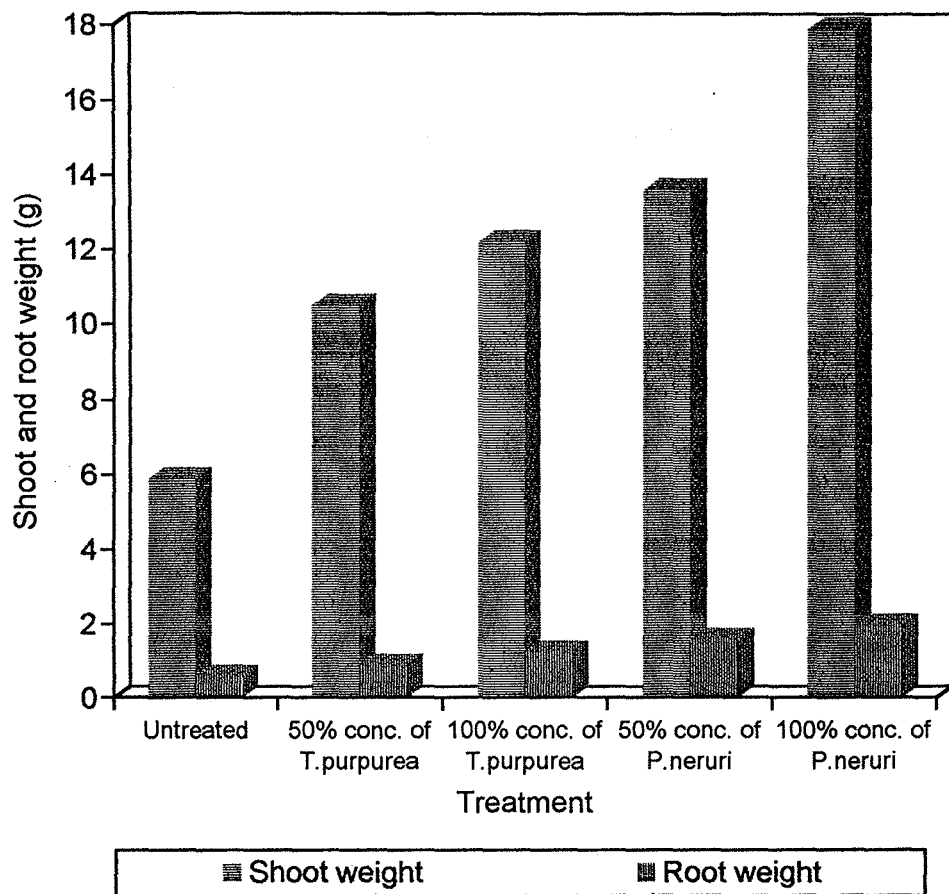
Significant variation in shoot weight was observed between untreated (control) plants and plants treated with two different concentrations of root extracts of the two medicinal plants tested. Shoot length between T₁ and T₂, T₂ and T₃ were not significantly different from each other.

Maximum of 203.4 per cent increase in shoot weight was recorded in T₄ (100% concentration of *P. neruri*) host plants followed by T₃ (50% concentration of *P. neruri*) with 130.9 per cent and T₂ with 107.8 per cent over control was noted from the table. An increase of 78 per cent shoot weight was observed in plants treated with 50 per cent (T₁) concentration of root extracts of *T. purpurea* over the control.

However, all the host plants treated with root extracts of *T. purpurea* and *P. neruri* showed significant increase in shoot weight when compared with that of untreated control plants.

FIGURE 8

Efficacy of root extracts of *T. purpurea* and *P. neruri* on the shoot and root weights of *Dolichos lablab* infected with *M. incognita*



4.4.1.d. Root weight

Statistical analysis of the data presented in Table VI and Figure 8 for the root weight of the host plant, *Dolichos lablab* infected with *M. incognita* proves that the plants treated with higher concentration (100 per cent) of the root extracts of *P. neruri* in the soil, gained more weight when compared with all the other treatments. The maximum weight recorded was 1.96 g for T₄ plants. The weight recorded was slightly lesser in T₃ plants with 1.57 g. Root weights recorded for plants treated with *T. purpurea* was comparatively lesser than *P. neruri* treated plants, i.e., in T₂ it was T₁ were 1.24 and in T₁ it was 0.87 g. Minimum root weight of 0.58 g was recorded in untreated (control) plants. Statistically there is no significant variation found between control and T₁ plants. However, significant increase was found in other treated plants (T₂, T₃ and T₄) when compared with control plants.

Increase in root weight of T₁ plants over control was 50 per cent. Root weight showed a significant increase of 113.8 per cent in T₂, 170.7 per cent in T₃ and 237.9 per cent in T₄ over control plants.

4.4.2 Efficacy of root extracts of *T. purpurea* and *P. neruri* on the number of galls and egg mass production in *Dolichos lablab* infected with *M. incognita*

Significant decrease in the intensity of galling and egg mass production was observed in all the host plants treated with two different concentrations of the root extracts of *T. purpurea* and *P. neruri* in comparison to control (Table VII and Figure 9 and Plate IV C).

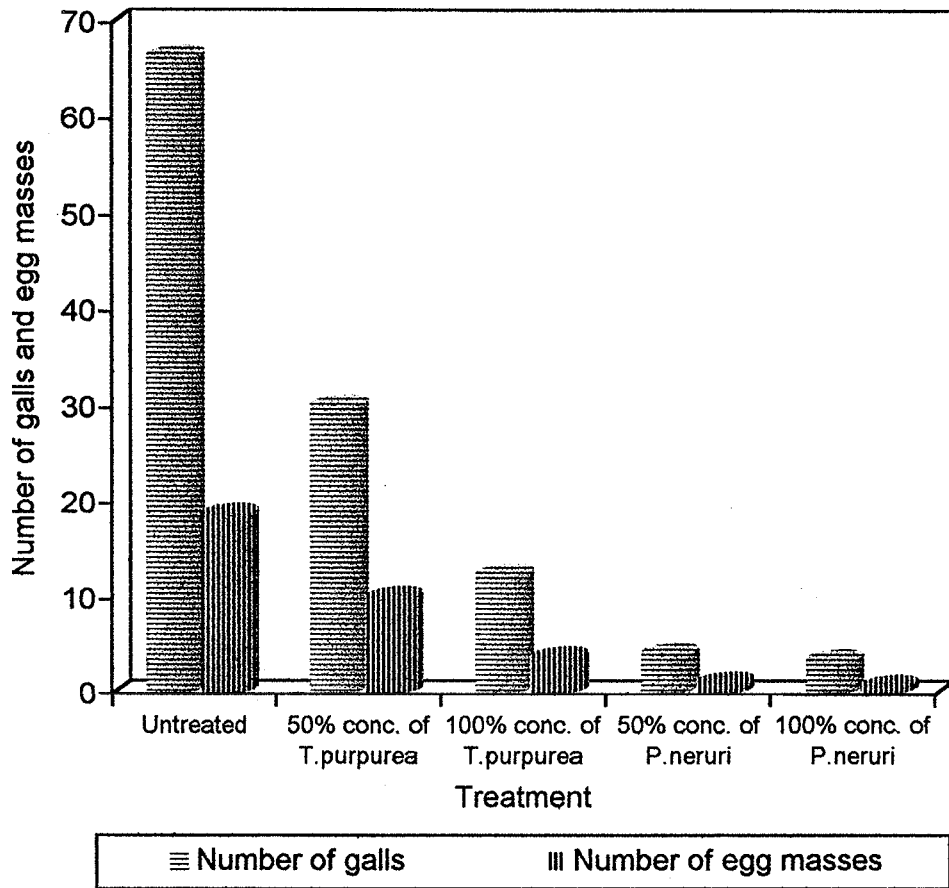
Table VII
Efficacy of root extracts of *T. purpurea* and *P. neruri* on the
number of galls and egg mass production in
Dolichos lablab* infected with *M. incognita

Treatment	Number of galls	Percentage decrease over the control	Number of egg masses	Percentage decrease over the control
Untreated (Control)	66.33	-	18.67	-
50% conc. of <i>T. purpurea</i> (T ₁)	30.00	54.8	10.00	46.4
100% conc. of <i>T. purpurea</i> (T ₂)	12.33	81.4	3.67	80.3
50% conc. of <i>P. neruri</i> (T ₃)	4.00	94.0	1.00	94.6
100% conc. of <i>P. neruri</i> (T ₄)	3.33	95.0	0.66	96.7
SEd	5.92		2.07	
CD (0.05)	13.19		4.60	

Mean of three replications.

FIGURE 9

Efficacy of root extracts of *T. purpurea* and *P. neruri* root galling and egg mass production in *Dolichos lablab* infected with *M. incognita*



4.4.2.a. Number of galls

Number of galls recorded in untreated plants was a maximum of 66.33. Galls produced in plants treated with 50 per cent (T₁) and 100 per cent (T₂) concentration of root extract of *T. purpurea* were 30 and 12.33 respectively. Minimum number of galls i.e., 4 and 3.33 was recorded in the plants treated with 50 (T₃) and 100 per cent (T₄) concentrations of the root extracts of *P. neruri* respectively.

Decrease in root galling was observed to be very high i.e., 95 per cent in T₄ plants when compared with control followed by 94 per cent in T₃ (50 per cent concentration of *P. neruri*). Root galling was also reduced to 54.8 per cent in plants treated with 50 per cent concentration of root extracts of *T. purpurea* (T₁) and 81.4 per cent in plants treated with 100 per cent concentration of *T. purpurea* roots (T₂).

However, there was significant reduction in the number of galls produced in all the root extracts treated host plants when compared with the host plants which are not treated.

4.4.2.b. Number of egg masses

It was observed from the Table VII and Figure 9 that there was significant reduction in the number of egg masses produced in the roots of host plants infected with *M. incognita* when treated with root extracts of both the medicinal plants when compared to the untreated (control) plants.

Greater number of egg masses i.e., 18.67 was observed in the root system of control plants. Egg masses recorded from the root system of plants treated with 50 (T₁) and 100 (T₂) per cent concentration of root extracts of *T. purpurea* were 10 and 3.67 respectively. Egg mass production was 1 in the case of T₃ plants (50% concentration of *P. neruri* roots). Less than 1 egg mass was found in plants treated with 100 per cent (T₄) concentration of *P. neruri* roots. Egg mass production in T₂, T₃ and T₄ plant shows no significant variations when compared with control plants.

Percentage reduction in egg mass production was maximum in T₃ and T₄ i.e., 94.6 and 96.7 per cent respectively when compared with control. Reduction in the number of egg masses produced in T₁ and T₂ was 46.4 and 80.3 per cent respectively. All the treated plants showed a decrease in number of egg mass formation when compared with untreated host plants.

4.4.2.c. Number of females / 5 galls

An average number of females present in the untreated control was 5.2 whereas the number gets reduced in the treated host roots. Average number of females present in T₁, T₂, T₃ and T₄ was 4.4, 3.6, 2.2 and 1.4 respectively. Number of females present in the roots treated with 50 and 100 per cent concentrations of root extracts of *P. neruri* was very low when compared with control and other treatments (Table VIII and Figure 10).

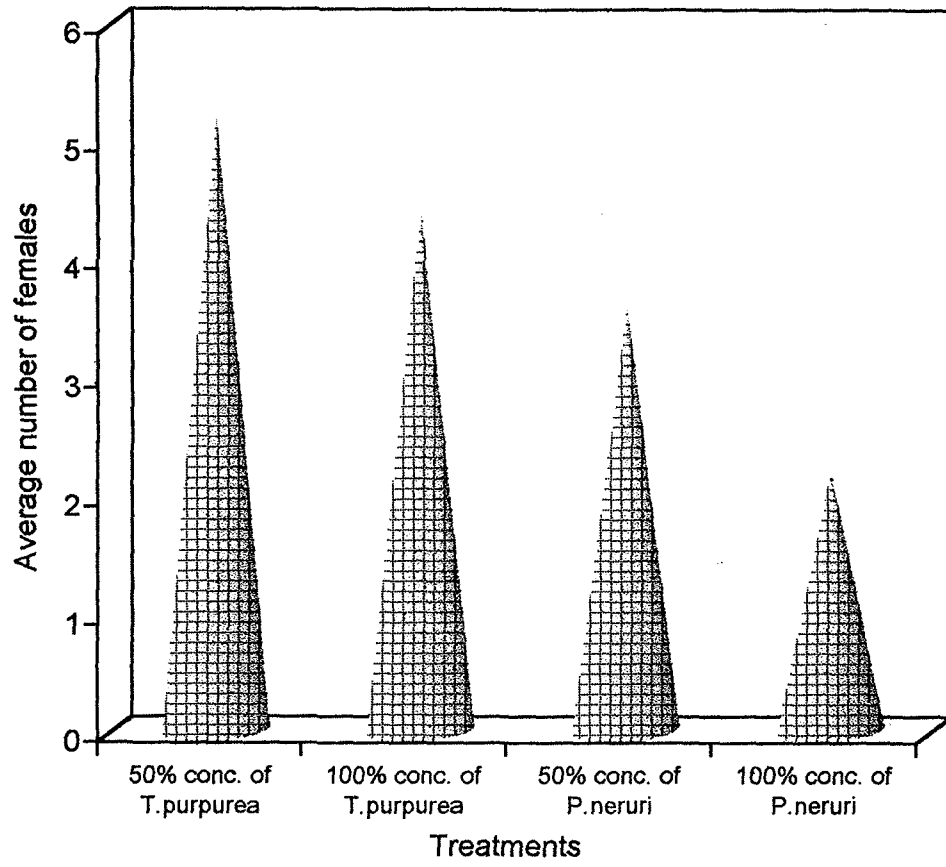
Number of females was found to be maximum in the host roots which are not treated whereas the number gets reduced in root extract treated roots.

Table VIII
Number of females of *M. incognita* present in five root galls

S.No.	Treatments	Number of galls					Average number of females / gall
		1	2	3	4	5	
1.	50% conc. of <i>T. purpuria</i> (T ₁)	5	4	6	5	6	5.2
2.	100% conc. of <i>T. purpuria</i> (T ₂)	4	5	4	4	5	4.4
3.	50% conc. of <i>P. neruri</i> (T ₃)	4	3	4	4	3	3.6
4.	100% conc. of <i>P. neruri</i> (T ₄)	1	3	3	2	2	2.2

FIGURE 10

Average number of females / gall



4.4.2.d. Nematode population in soil

Data presented in Table IX and Figure 11 revealed that the number of nematodes present in the untreated soil was a maximum of 163 in untreated soil. Number of nematodes recorded in the soil treated with 50 and 100 per cent root extracts of *T. purpurea* was 93.3 and 65.67 respectively and the number of nematodes present in the soil treated with 50 and 100 per cent concentration of root extracts of *P. neruri* was 43.6 and 16 respectively. Nematode population was significantly varied between the untreated soil and root extracts treated soils whereas no significant difference was shown in the number of nematodes present in the soil treated with 100 per cent *T. purpurea* and 50 per cent *P. neruri*. The values are statistically on par with each other.

Reduction in the nematode population was 42.7, 59.7, 73.2 and 90.2 per cent in T₁ (50 per cent *T. purpurea*), T₂ (100 per cent *T. purpurea*), T₃ (50 per cent *P. neruri*) and T₄ (100 per cent *P. neruri*) respectively in treated soils over the control.

Regarding the nematode population, maximum number of nematodes was recorded in untreated control soil whereas at 100 per cent concentration of root extracts of *P. neruri* amended soil very minimum number of nematodes was recorded.

From the results obtained on the amendment of root extracts of the two economically important medicinal plants *T. purpurea* and *P. neruri* in the soil, there was greater increase in the growth of *Dolichos lablab* plants (country bean) infected with *M. incognita* and maximum reduction in the root galling, egg mass production and final nematode population in the soil.

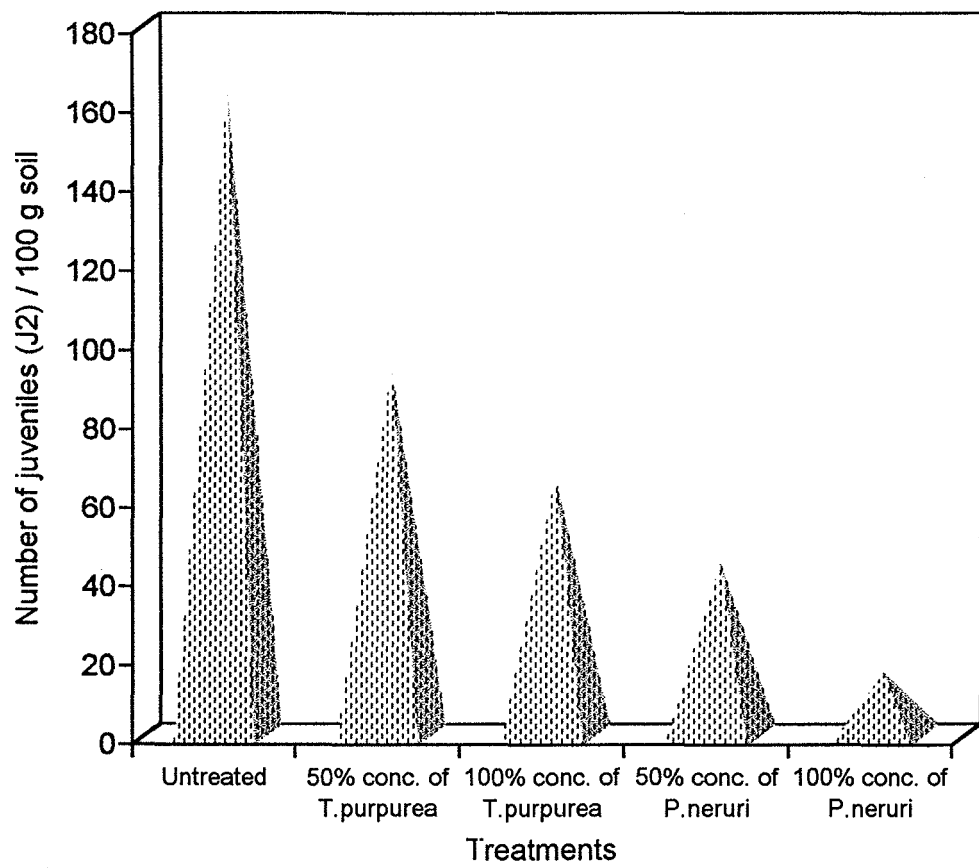
Table IX
Population of *M. incognita* in the soil treated with root extracts of
T. purpurea* and *P. neruri

S.No.	Treatments	Number of juveniles present / 100 g soil	Percentage decrease over control
1.	Untreated (Control)	163.0	-
2.	50% conc. of <i>T. purpurea</i> (T ₁)	93.3	42.8
3.	100% conc. of <i>T. purpurea</i> (T ₂)	65.7	59.7
4.	50% conc. of <i>P. neruri</i> (T ₃)	43.6	73.2
5.	100% conc. of <i>P. neruri</i> (T ₄)	16.0	90.2
	SEd	10.56	
	CD (0.05)	23.50	

Mean of three replications.

FIGURE 11

Effect of root extracts of *T. purpurea* and *P. neruri* amended in the soil on the nematode (*M. incognita*) population



Various plants and their parts have been found to be very effective in controlling nematodes infecting agricultural crops when amended in the soil (Hackney and Dickerson, 1975 ; Halbrendt, 1996 and Wang *et al.*, 2002).

Several authors have reported enhanced growth of plants infected with root-knot nematodes and reduction in the number of galls, egg masses in roots of those host plants and nematode population in soil due to the addition of various botanicals in the soil.

The present study regarding the increase in plant growth parameters due to application of botanicals as soil amendments are in confirmation with the findings of Rao and Reddy (1992) on tomato and Ramakrishnan *et al.* (1997) on okra infected with *M. incognita*.

Haider (1993) studied the effect of incorporation of plant extracts of leaf, stem, fruit and root of *Anagallis arvensis*, *Clerodendrum infortunatum* and *Cuscuta reflexa* in the soil and recorded a significant suppression in the number of galls and root-knot index of *M. incognita* and increase in the growth characters of tomatoes which may support the present study.

Drastic reduction in the number of galls, egg masses, final nematode population in soil and improved plant growth compared to checks was observed when highest dose (100 per cent) of roots of *P. neruri* was amended in the soil. Similar result was obtained in tomato infected with *M. incognita* when the parts of *Tagetes* plants are incorporated (Walia and Gutpa, 1997) in the soil and on egg plant (Stephen *et al.*, 2001) infected with *M. javanica*.

Similar such reduction in the number of galls and final nematode population in soil was reported when water extracts of *Azadirachta indica* leaves (Vats *et al.*, 1995), leaf powder of *Calotropis* (Jain and Trivedi, 1997) were applied in the soil. Chopped leaves of various plants such as Bougainvillea, *Calotropis procera*, *Leucaena leucacephala* when applied in the soil against *M. incognita*, showed enhanced plant growth in tomato plants and suppressed final nematode population (Sundarababu *et al.*, 1990).

Root extracts of the two concentrations from the medicinal plants *T. purpurea* and *P. neruri* were found to be most effective in reducing galls and enhancing growth of the host plant. Similar such findings of Kumar (1996) in okra infected with *M. incognita* is reported when root extract of *Catharanthus roseus* was applied in the soil. An increase in shoot weight and decrease in galling and final nematode levels were found to be due to the amendments of root extracts of two different concentrations of *T. purpurea* and *P. neruri*. Similar results were reported in the findings of Ploeg (2000) when he amended the soil with root or tops of tomato or marigold on the tomato infected with *M. incognita*.

Reduction in the incidence of *M. incognita* was also reported when the tissues of *Calendula officinalis* L., extracts of *Helianthus annus* L. and *Xanthium strumarium* L. amended in the soil and were found to be toxic to *M. incognita* (Prakash and Rao, 1997).

Aqueous extracts of roots, stems or leaves of *Gaillardia pulchella* was effective in controlling *M. incognita* on *Ipomoea repans* (Tsay *et al.*, 2004) which also supports the present study.

Summary and Conclusion

5. SUMMARY AND CONCLUSION

A study entitled “**Development of *Meloidogyne incognita* (a root-knot nematode) in *Dolichos lablab* (country bean) and its management through root extracts of selected medicinal plants**” was undertaken under laboratory and lath house conditions. The results of the investigation on the developmental stages and time taken for the completion of life cycle of *Meloidogyne incognita* on the host plant, *Dolichos lablab*, the resultant biochemical changes that have taken place in the amount of total proteins, total carbohydrates and total phenols in the host roots due to the interaction of this plant parasitic nematodes at the time of formation of galls and egg masses, its management through aqueous root extracts of two medicinal plants and also by assessing their efficacy by undertaking a pilot study on egg hatchability are summarised in this chapter.

A study was conducted on the life cycle of *M. incognita* on the host plant, *Dolichos lablab* as a pot (tumbler) culture experiment.

1. The time taken for the penetration of second stage juveniles (J_2) was 48 hours (2 days) after inoculation and is continued till 5th day.
2. Third stage juveniles (J_3) appeared on 5th day and the gall formation was also visible on the same day. Time interval for the formation of J_3 from J_2 was 5 days.
3. After third moult, fourth stage juveniles (J_4) were seen on 10th day of inoculation. Time interval from J_3 – J_4 was 5 days.

4. Sex differentiation was taken place ; males appeared on 10th day and young females on 15th day. Time taken for appearance of females from J₄ was 5 days.
5. Adult females (pear shaped) were seen with gelatinous matrix secreted by six rectal glands on 20th day. Number of days taken for young females to become adult females was 5 days.
6. Egg deposition inside the egg sac was observed on 23rd day (3 days interval) and embryonated eggs on 30th day after inoculation (7 days interval).
7. First stage juveniles (J₁) were noticed on 32nd day (2 days interval). After first moult, second stage juveniles (second generation) were seen on 35th day in the egg sacs (3 days interval).
8. Infective second stage juveniles (J₂) were visible in the soil on 38th day (3 days interval). Life cycle of *M. incognita* took 35 days on the host plant, *Dolichos lablab* under laboratory conditions at a temperature range of 32°C - 36°C.
9. Physiological disturbance in the host roots due to the entry of *M. incognita* was studied through the estimation of certain biochemical components of the plants inoculated with two (500 and 1000 nematodes / pot) densities of nematodes at the completion of the developmental cycle of *M. incognita*.

Following are the results obtained on the biochemical estimation of total proteins, total carbohydrates and total phenols which indicate the effect of *M. incognita* on the host plant.

10. An increase in the amount of total protein was noted in the host plants at both inoculum levels (500 and 1000 nematodes / pot) when compared with control. Percentage increase was 41.9 per cent and 84.7 per cent in plants inoculated with 500 and 1000 nematodes / pot respectively.
11. A decrease in the amount of total carbohydrates was recorded in the inoculated plants compared with control. Percentage decrease was 48.5 per cent and 66.2 per cent in plants inoculated with 500 and 1000 nematodes / pot respectively.
12. Phenol content was also shown a decrease in the amount of 22.6 per cent and 4.6 per cent in the plants inoculated with 500 and 1000 nematodes / pot respectively when compared with uninoculated control plants.

A preliminary study was conducted on the egg hatch of *M. incognita* using aqueous root extracts of two medicinal plants, *T. purpurea* and *P. neruri* to find out their inhibitory effect on them so as to amend these plant parts in the soil to evaluate their efficacy in managing these root-knot nematodes.

13. Four different concentrations (0.1, 1, 10 and 100 per cent) of extracts were prepared from the roots of two medicinal plants and distilled water

served as control. Juveniles hatched from five egg masses from each treatment was counted after 1st, 3rd, 5th and 7th days of treatment.

14. Maximum number of larvae was emerged from distilled water (1192 / 5 eggs in *T. purpurea* and 1226 / 5 eggs in *P. neruri*) after 7 days of treatment.
15. At 100 per cent concentration of the root extract minimum hatch from eggs was noticed. *P. neruri* extract was found to be most effective in inhibiting egg hatch at this concentration.
16. At 10 per cent concentration, slightly higher percentage of egg hatch was recorded from both the root extracts.
17. Even at lower concentrations (1 per cent and 0.1 per cent) of root extracts of both plants, the egg hatch was found to be lesser when compared with the control treatment.
18. Because of the high inhibitory action of the two root extracts, these plants were selected for amendment in the soil for the investigation on the management of root-knot nematodes.
19. The aqueous root extracts of medicinal plants with two concentrations (50 and 100 per cent) each, were applied in the soil to study the growth improvement of the host plant, *Dolichos lablab* infected with *M. incognita*.
20. Maximum increase in shoot and root length was recorded in the plants treated with *P. neruri* when compared with the plants treated with *T. purpurea* root extracts. Same trend was also observed in the weight

gain of shoot and root of *Dolichos lablab* treated with these two medicinal plant extracts.

Significant reduction in the intensity of galling and egg mass production was observed in the root system of *Dolichos lablab* treated with both the concentrations of the root extracts of the above mentioned medicinal plants.

Number of females was found to be more in control root galls when compared with root extract treated galls. Minimum number of females was observed in galls treated with *P. neruri* root extracts.

Final nematode population in the soil was found to be very much reduced in the treated soil when compared with untreated soil. Maximum of 90.2 and 73.2 per cent reduction was recorded at 50 and 100 per cent concentrations of *P. neruri* root extracts.

The results of the present study indicate clearly that the test plant, *Dolichos lablab* (country bean) has responded very well to the attack of the root-knot nematode, *M. incognita* and therefore can be considered as a good host. Because of the susceptibility of the host plant, this root-knot nematode could complete its life cycle in 35 days from second stage juveniles (J_2) to the second generation juveniles (J_2) within the root tissues. Numerous eggs were deposited in the egg sacs and many females were located in a single gall indicating multiple infection in the host plant. The entry of these nematodes and further their development in the root tissues have caused alterations in

their biochemical components like total proteins, total carbohydrates and total phenols.

An increase in total proteins in the inoculated plants is an useful indicator to measure the level of root-knot nematode infestation of host plants as reported by Chatterjee and Sukul (1981). Decrease in carbohydrate might have resulted either due to rapid consumption by nematodes or to the metabolic shift. The decrease in carbohydrate due to nematode infestation may result in reduced photosynthetic activity. Phenols play a role in defense mechanism. An increase in the amount of phenols have been correlated with the resistance of the host plants to various nematode diseases (Giebel, 1970 and Pi and Rohde, 1967) whereas a decrease in total phenols was observed in the present study which confirms that the host plant, *Dolichos lablab* was not able to resist the attack of these nematodes and thereby proving its susceptibility.

It is therefore, clearly evident that these nematodes can bring about biochemical changes by disrupting the physiological balance of the host plants along with changes in their morphology and root histology. Pathak *et al.* (1983) made an attempt in this aspect and concluded that there was considerable interference in the metabolism of proteins and carbohydrates in the host tissues infected with the nematodes.

Since, root-knot nematodes, *M. incognita* cause extensive damage to various agricultural crops, their control in the field is essential. Therefore, an effort has been made to study the effect of aqueous root extracts of two

medicinal plants, *T. purpurea* and *P. neruri* on hatching of *M. incognita* eggs and by applying them in the soil to find out their efficacy on growth of the host plant, *Dolichos lablab* and intensity of galling, egg mass production in roots and final nematode population in the soil.

Both the root extracts used in the present study on egg hatch of *M. incognita* were found to be highly inhibitory in action. The inhibitory effect of these extracts might be attributed to the presence of some toxic chemical compounds having the nematicidal / nemostatic activity (Singh and Sitaramaiah, 1973 and Khan, 1974) or due to a hatch inhibitor (Hamlen and Bloom, 1968) and this should have been responsible for the biotoxicity of the roots. Because of the high inhibitory effect of the root extracts of both the medicinal plants tested, they were administered in the soil with two concentrations.

The results obtained on the plant growth performance and nematode reproduction indicate that the root extracts applied in the soil have enhanced plant growth and reduced the nematode population in soil and galls and egg masses in the roots of the host plant, *Dolichos lablab*. This may be due to the presence of certain substances that were lethal to parasitic nematodes (Tsay *et al.*, 2004). Root extracts of *T. purpurea* and *P. neruri* reduced the incidence of *M. incognita* in the soil because they might have possessed toxic substances (Prakash and Rao, 1997) or precursors of substances toxic to these nematodes.

From the above study, it can be concluded that the incorporation of the root extracts of *T. purpurea* and *P. neruri* in aqueous form in the field will improve the plant growth. Their amendment in soil will reduce the intensity of galling and egg mass production thereby hindering the nematode multiplication in the soil.

Among the two medicinal plants investigated, *P. neruri* stands the most effective one in controlling root-knot nematode when compared with *T. purpurea*.

Hence, it is suggested that *P. neruri* root extracts can be administered in the soil by the agro farmers as an eco-friendly means in the place of synthetic nematicides.

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PLATE - IV

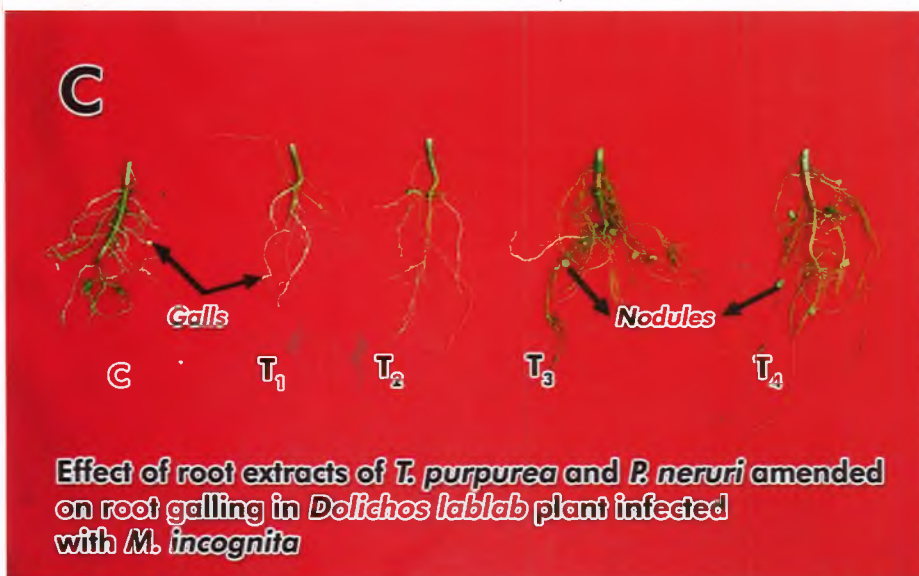


PLATE - III

Different stages of life cycle of *M. incognita* in *Dolichos lablab*

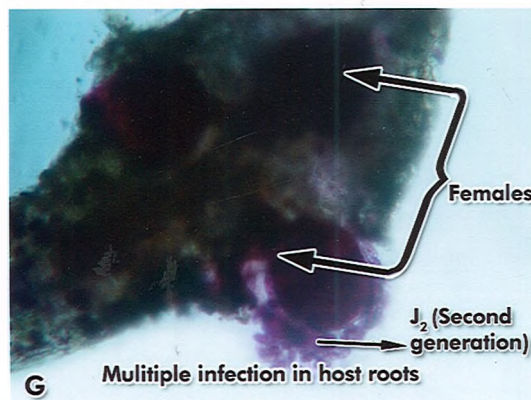
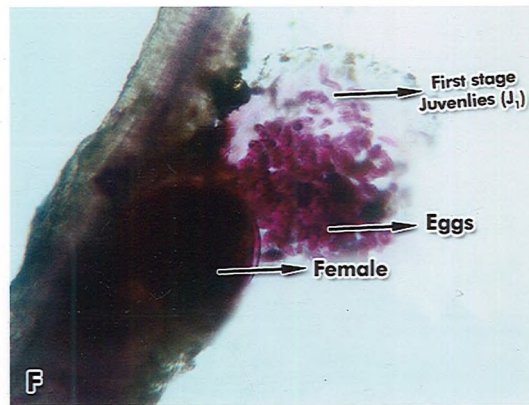
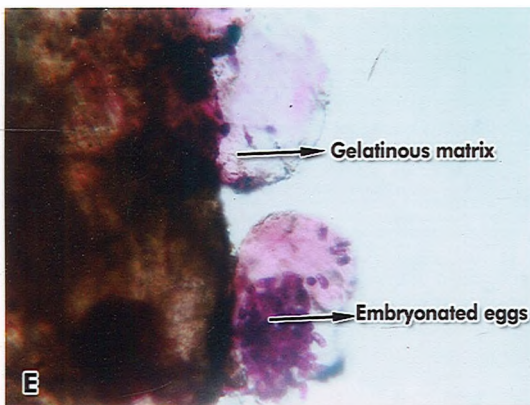
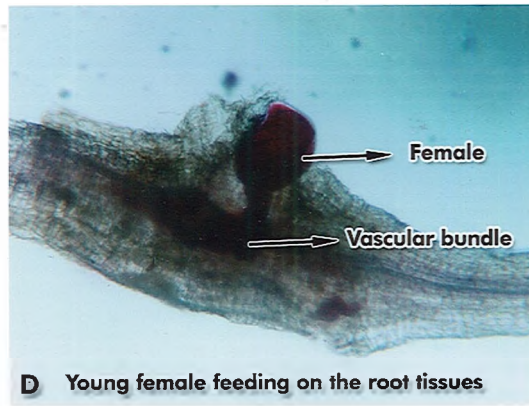
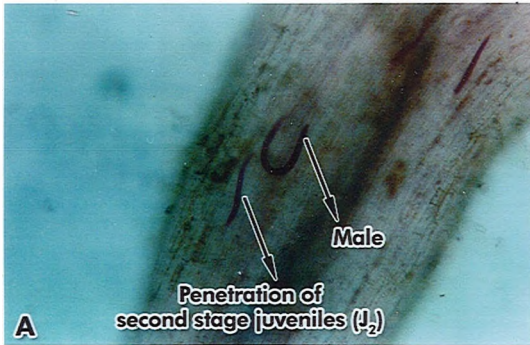
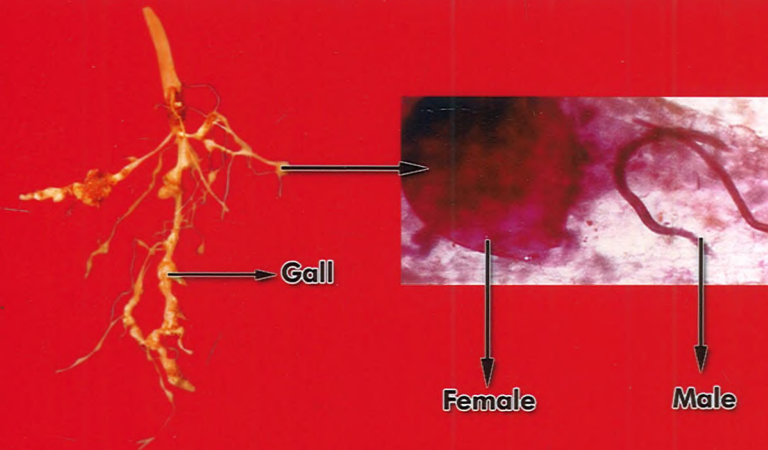


PLATE - I



A. Host plant, *Dolichos lablab* (country bean)



B. *Meloidogyne incognita* (root knot nematode) the Parasite inside root gall



C. Stock culture of *Meloidogyne incognita* maintained in tomato plants