STUDIES ON THE EFFECT OF ROOT – KNOT NEMATODE MELOIDOGYNE INCOGNITA (KOFOID & WHITE) CHITWOOD, ON PUERARIA PHASEOLOIDES BENTH.

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Dedicated to my father

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CERTIFICATE

This is to certify that this thesis entitled, "Studies on the effect of root knot nematode, *Meloidogyne incognita* (Kofoid & White) Chitwood on *Pueraria phaseoloides* Benth" is an authentic record of the research work carried out by Smt. Thankamony, S under our scientific supervision and guidance at the Rubber Research Institute of India, Kottayam in partial fulfillment of the requirements for the degree of Doctor of Philosophy of the Mahatma Gandhi University, under the faculty of Science and no part there of has been presented for the award of any other degree, diploma or associateship in any University.

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DECLARATION

I hereby declare that this thesis entitled , studies on the "effect of root knot nematode, *Meloidogyne incognita* (Kofoid & White) Chitwood on *Pueraria phaseoloides* Benth", has not previously formed the basis of the award of any degree, diploma, associateship, fellowship or other similar titles for recognition.

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Chapter I

INTRODUCTION

INTRODUCTION

Natural rubber (*Hevea brasiliensis* Muell. Arg.) is an important plantation crop in India. A number of agricultural practices have been introduced to improve the production of natural rubber. Establishment of leguminous cover crops in the initial stages of cultivation is an important agronomic practice adopted in rubber plantations. Leguminous cover crops enrich soil with nitrogen, prevent soil erosion, reduce soil temperature, augment organic carbon in soil and support soil microbial activity.All these beneficial effects of cover crop help in increasing the growth rate and yield of *Hevea*. Among the various cover crops used in rubber plantations, *Pueraria phaseoloides* is the most popular one in India and elsewhere (Potty *et al.*, 1980). A number of biotic and abiotic factors are reported to influence the growth, nodulation and nitrogen fixation of *Pueraria phaseoloides*. One of the important biotic factors that adversely affects *Pueraria phaseoloides* is the root-knot nematode, *Meloidogyne incognita* (Mammen, 1973).

The root-knot nematodes are the predominant group of plant parasitic nematodes. They are also the parasites on major food crops, vegetables, plantation crops, fruits and ornamental plants (Sasser, 1980). The infected plants show stunted growth, become chlorotic and loose vitality and vigour. Various mineral deficiency symptoms are also reported to occur due to nematode infection. Flowering and fruiting are suppressed

and consequently the yield is greatly reduced. Severe infection at seedling stage may cause death of the plants. The severity of damage caused by the nematodes to the plants depends upon population density of nematodes, plant age and several other biotic and abiotic factors. The optimum damaging threshold level of *Meloidogyne incognita* varies in different crops. Each crop species has a minimum threshold level below which it is not significantly affected by a given species of root knot nematode. The potential damaging threshold level of root-knot nematode is of importance in gaining insight for designing effective control measures.

The frequency and density of root knot nematodes show variations from location to location due to variations in climatic conditions, soil type and agronomic practices adopted for cultivation in each location. The damage caused by nematodes to plant root in clayey soil with little organic matter was initially lesser than that in such soils with more organic matter. Among the soil types, the rate of penetration occurs in descending order in clay, sand, clay loam respectively. Soil type and cropping systems are probably the major components that play a vital role in root knot incidence.

Root-knot nematode is highly pathogenic to different pulse crops (Gupta *et al.*, 1986; Kalita and Phukan, 1993) and affects the growth, nodulation, nitrogen fixation and total biomass of the plants. Due to the mechanical injury caused by the nematodes to root tissues, the water

absorption efficiency, mineral uptake and total root biomass are found reduced. The hydrolytic and oxidative enzymes and growth regulators secreted by the nematodes play a determinative role in the development of nodules, alteration of host nutrition and interference in the nitrogen fixing capacity of nodules. The early destruction of nodules by nematodes results in the reduction of nitrogen fixation (Chahal and Chahal, 1989).

The population of plant parasitic nematodes fluctuated with seasonal changes. Rainfall pattern, air and soil temperature, soil moisture and host root growth are the predominant factors influencing the population of nematodes in soil (Mukherjee and Dasgupta, 1993). But the population of *Meloidogyne incognita* is correlated more with the soil temperature than with soil moisture. Temperature between 26 and 28^0C induces more penetration of nematodes in plant roots irrespective of soil types. The influence of seasons on the nematode population was reported to be more evident at the upper layer of the soil than in the deeper layers. Similarly, nematode population was more in summer and low in winter months. Due to availability of fresh and actively growing roots, post monsoon period is reported to be congenial for the survival and multiplication of root-knot nematodes (Eapen,1993). Root-knot nematodes are bound to interact with soil microorganisms. The interactions of *Meloidogyne incognita* with different saprophytic fungi, vesicular arbuscular mycorrhizae(VAM) and *Rhizobium* sp. have been reported to reduce the pathogenicity of root-knot

nematodes. The symbiotic association of VAM improves the plant growth by increasing nutrition, phosphorous uptake and provides more resistance to the host plant. The mycorrhizal association is reported to cause changes in root physiology by increasing amino acid and sugar contents in the root and the wall thickness of the root cortical cells. These conditions are not favourable for the penetration and multiplication of root-knot nematodes. Thus the root infection of plants by nematodes can be reduced by the establishment of VA mycorrhizae. It is logical to state that the penetration rate of parasitic nematodes can be decreased, their development inside the root may be retarded or the degree of damage caused by the nematode may be lowered by the early establishment of VA mycorrhizae. VAM has thus been reported as a biocontrol agent against root-knot nematode, *Meloidogyne incognita* (Jain and Hasan, 1994).

Root knot infection in plant in general causes a series of changes in the plant metabolism. Accumulation of phenols has been reported as a resistance factor against many infection agents. The increased content of phenolic compounds helps in the formation of hypersensitive reaction towards nematode infestation (Shukla and Chakraborty, 1988). Phenolic compounds play a major role in the control of nematodes through larval mortality and by making the plant roots less attractive to nematodes. Polymerization and transformation of phenolic compounds are reported to act against the parasites (Anwar and Israr, 1976). Root-knot infested plants grown in soil containing

high organic matter showed increase in total phenol, *ortho* dihydroxy phenols and amino acid contents(Singh *et al.*, 1985).

Plants infested with root knot nematodes accumulate carbohydrates in the tissue. Increase in soluble and structural proteins was also observed due to the infestation of root-knot nematodes (Simte and Dasgupta, 1987). The interaction of nematodes and plants leads to the depression of novel genes, which results the de novo synthesis of RNA and protein molecules. *De novo* synthesis of the isozymes of peroxidase and ribonuclease and proteins and isoflavonoids in response to *Meloidogyne incognita* has been reported by Kaplan (1977) and Ganguly and Dasgupta (1981).

The root-knot nematode, *Meloidogyne incognita* has caused significant changes in growth, nodulation, nitrogen fixation and biochemical constituents in a variety of leguminous crops. In rubber plantation, even though there are reports on the incidence and intensity of root-knot nematode in the cover crop, *Pueraria phaseoloides*, its adverse effect on the growth of the plant and biochemical activities are lacking. A study on the effect of root-knot nematode *Meloidogyne incognita* infestation on *Pueraria phaseoloides* will help in understanding the host pathogen interaction and to device suitable management practices for their control. Therefore, this study was carried out with the following objectives.

1. Isolation of root-knot nematodes from different rubber growing soils and testing their infectivity.

2. Studying the effect of nematode infestation in *Pueraria phaseoloides* on growth, biomass, nodulation and nitrogen content.

3. Effect of seasonal variations in nematode population and gall formation on *Pueraria phaseoloides.*

4. Studies on the effect of root-knot nematode and VAM interaction on plant growth.

5. Investigations on the biochemical changes in *Pueraria phaseoloides* due to root knot infestation .

Chapter II

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Leguminous cover crops are established and maintained in rubber plantations to improve physico-chemical properties of soil, conserve soil moisture, prevent soil erosion, reduce soil temperature and prevent weed growth (Soong and Yap, 1976). All these beneficial effects of cover crops help in increasing the growth and yield of *Hevea brasiliensis* (Prathapan *et al.*, 1994; Prathapan *et al.,* 1995; Jessy *et al.,*1997 and Abraham *et al.,* 2001). Different leguminous cover crops grown in rubber plantations include *Pueraria phaseoloides, Mucuna bracteata, Calapagonium mucunoides, Centrosema pubescens,* and *Mimosa invisa* var *inermis.* Among these, *Pueraria phaseoloides* is very popular in the rubber plantations in Kerala as it is easy to establish and it fixes more atmospheric nitrogen than other crops. It is a vigorous twiner and creeper and can be propagated by seeds. The plant can withstand strong sun and smothers weeds. The seed rate is about 3-4.5 kg/ha. This cover crop is found to be attacked by number of pests including root-knot nematode, *Meloidogyne incognita.*

*Meloidogyne incognita (*Kofoid & White)Chitwood, *Meloidogyne javanica* (Treub)Chitwood, *Meloidogyne arenaria* (Neal) Chitwood and *Meloidogyne hapla* Chitwood are the four major species of root-knot nematodes (Chitwood,1949). Among these *Meloidogyne incognita* and

Meloidogyne javanica are more common than other species and are therefore, agriculturally more important (Sassser, 1980). The pathogenic effect of root-knot nematode on growth characterestics, nutrient uptake and yield of leguminous cover crops have been well documented by several workers and it is reported as a potential threat to different leguminous plants (Chahal and Chahal, 1989; Gupta *et al.*, 1986; Bhagawati and Phukan, 1991; Kalita and Phukan, 1993). The incidence of root-knot nematode, *Meloidogyne incognita* on leguminous cover crops grown in association with rubber was first reported from India by Mammen (1973). Rajendran and Jayarathnam (1977) observed the occurrence and infestation of *Meloidogyne incognita* in *Hevea* and *Pueraria phaseoloides*. Studies conducted on the relative tolerance of leguminous covers to root-knot nematode, *Meloidogyne incognita* showed that *Pueraria phaseoloides* is highly susceptible to root-knot nematodes (Thankamony *et al.*, 1989).

2.1 Geographical Distribution

The root-knot nematode was first discovered by Berkley in 1855 on cucumber in glasshouses of U.K. Being parasitic on many plant species, root-knot nematodes are recognized as one of the major group of plant pathogens adversely affecting world's food production (Sasser, 1980). They are most prevalent in tropical and sub-tropical regions of the world, where summer is longer than winter. According to Sasser (1979) crop losses due to root-knot nematodes in major geographical regions of the

tropics range from 5 to 43 per cent. This however, it depends upon the crop, root-knot nematode species and geographical region (Sasser, 1980; Sasser and Carter, 1982). Franklin (1979) has eloquently discussed the economic importance of root-knot nematodes in temperate, 'sub-tropical, mediterranean' and tropical regions of the world.

2.2 Isolation of root-knot nematodes from different rubber growing soils and testing the infectivity

Extensive surveys of root knot nematodes associated with a variety of crops have been carried out by several workers (Prasad and Dasgupta, 1964; Sethi and Swarup, 1968; Sitaramaiah *et al.*, 1971; Velasquez, 2001). Root knot nematodes associated with vegetables, fruit crops, cereals and other crops in thirty districts of Uttar Pradesh have been reported by Rashid *et al.* (1973). In soybean, Raut *et al.* (1986) reported the prevalence of root-knot nematodes in most of the areas along with other plant parasitic nematodes. The occurrence of root knot nematodes in association with black pepper was reported by Ramana and Mohandas (1987).Root-knot nematode was reported as a predominant nematode species attacking different horticultural crops in Karnataka (Singh *et al.*, 1979). Sundararaju *et al.* (1979) observed severe incidence of root-knot nematode, *Meloidogyne incognita* in spices such as pepper, cardamom, ginger, turmeric, clove, cinnamon and nutmeg. The infection of root-knot nematodes in citrus caused small elongated galls at the terminal and sub-

terminal ends and caused severe rotting of roots (Siddiqui *et al.*, 1987). Among the different plant parasitic nematodes recovered from jute, the frequency and incidence of *Meloidogyne incognita* was reported to be very high (Bora *et al.*, 1989). A multistratified random survey of nematodes with reference to the cropping patterns in the homesteads of Thiruvananthapuram district revealed that out of 100 homesteads, *Meloidogyne incognita* was present in 76 and the population reached the economic threshold level (Sheela *et al.*, 1990). In sunflower (*Helianthus annus*), Amaranatha and Krishnappa (1990) reported the occurrence of six important genera of plant parasitic nematodes including *Meloidogyne incognita.* About 50 per cent of vegetable fields in Uttar Pradesh were found severely infested with root-knot nematodes (Wajid Khan *et al.*, 1994). Severe incidence and infestation of root-knot nematodes in sugarcane was observed by Sundararaju and Mehta (1991). Community analysis of plant parasitic nematodes in the rhizosphere soils of banana in Puri district of Orissa showed highest population density of root-knot nematodes (Ray *et al.*, 1987). The groundnut growing districts of Punjab state revealed extensive distribution of root-knot nematodes,*Meloidogyne incognita* followed by *Aphelenchus avenae* and *Tylenchorhynchus vulgaris* (Sakhuja and Sethi, 1985). Rhizosphere soil analysis of cork wood tree, *Duboscia myoporoides* showed *Meloidogyne incognita* as the most dominant species compared to other parasitic nematodes and seedlings

infested with root-knot nematodes showed stunted growth also. In *Hevea*, studies on the frequency and distribution of plant parasitic nematodes revealed that *Meloidogyne incognita* was the predominant species compared to other nematode species. (Thankamony *et al.*, 1996).

2.2.1 Bioassay on nematode populations and infectivity

Bioassay is the technique in which appropriate hosts are used to determine the relative population levels of nematodes. It was used primarily for endoparasitic nematodes such as root-knot and cyst nematodes but can be used for any nematode under suitable propagation conditions. They are usually done in green house.

Godfrey (1934) described a method of estimating the root-knot nematode population of soil on the basis of gall counts on indicator plants. Rau (1944) applied indicator plant technique in testing soil from tea nurseries. Dropkin (1954) did quantitative work on infectivity and gall size in tomato and cucumber seedlings with *Meloidogyne incognita* which could be successfully applied in use of these plants as indicators. Simon (1980) reported the plant assay of soil to evaluate the potential damage caused by *Heterodera avenae* to wheat plants. Mc Sorley (1983) discussed a bioassay sampling method for *Meloidogyne incognita* under field conditions also.

2.3 Effects of root - knot nematode on growth, biomass, nodulation and nitrogen fixation in plants

Leguminous plants are reported to be the highly susceptible to root-knot nematodes. Several workers have reported the pathogenic effect of root-knot nematodes on leguminous plants e.g. *Meloidogyne* spp. on *Arachis hypogea* (Masefield, 1958), *Meloidogyne javanica* and *Meloidogyne hapla* on *Vicia villosa* (Malek and Jenkins, 1964) and on *Medicago sativa* (Nigh, 1966), *Meloidogyne javanica* on *Trifolium ripens* (Taha and Raski, 1969), *Meloidogyne hapla, Meloidogyne javanica* and *Meloidogyne incognita* on soybean (Balasubramanian, 1970), *Meloidogyne* spp. on cowpea (Bhagawat and Thomas, 1982), *Meloidogyne arenaria* on *Arachis hypogea* (Vaishnav *et al.*, 1985), *Meloidogyne incognita* on *Vigna mungo* (Verdejo *et al.*, 1989) and *Meloidogyne incognita* on *Pueraria phaseoloides* (Mammen, 1973).

The attack of root-knot nematodes causes mechanical injury to the root tissues thereby reducing the water absorption efficiency of the host roots (Alam *et al.*, 1974). Reduction in root biomass due to nematode infection or root surface area leads to the reduction of mineral uptake. The chlorophyll content of the leaf was also reduced by *Meloidogyne incognita*, thus adversely affecting photosynthesis (Wallace, 1987). The reduction in root nodulation is also reported due to the attack of *Meloidogyne incognita* (Chahal and Chahal, 1989).

Significant decrease in plant growth characters along with heavy losses in yield was observed in blackgram due to *Meloidogyne incognita* (Chahal *et al.*, 1988; Kumar *et al.*, 1993; Kalita and Phukan, 1993). Rai *et al.* (1987) studied the pathogenic effect of root-knot nematode on cotton and concluded that cotton yield was significantly reduced at and above 100 juveniles of *Meloidogyne incognita* in Desi cotton (*Gossypium arboreum*) and at above 500 juveniles per plant per 500g of soil in American cotton (*Gossypium hirsutum*). Routaray *et al.* (1987) observed the pathogenicity of *Meloidogyne incognita* in ginger and horsegram. According to Singh and Misra (1974) and Bhagawati and Phukan (1991) significant plant growth reduction was found with an initial inoculum of 1000 larvae of *Meloidogyne incognita* per plant per 500 g of soil on pea variety Boneville and the plants showed chlorosis and shedding of basal leaves at highest inoculum. Similar symptoms were observed on Chillies by Rajagopalan *et al.* (1969). Azmi (1986) recorded significant reduction in shoot weight at 1000 and above level of inoculation in subabool (*Leucaena leucocephala).* Raut and Sethi (1981); Jagdale *et al.* (1985); Mishra and Singh (1985); Ali (1986) and Chahal and Chahal (1987) also reported the adverse effect of *Meloidogyne incognita* on the growth characteristics of soybean, betelvine, jute , cardamom and on mung bean respectively. Sharma *et al.* (1978) recorded field symptoms of groundnut plants infected with root-knot nematode (*Meloidogyne arenaria*) as

yellowing and dwarfing of leaves, reduced vigour and stunted growth in patches.

McClure *et al.* (1974) reported that, age of plant at the time of inoculation affected the number of *Meloidogyne incognita* larvae penetrating roots, the penetration being inversely proportional to the age of plant. Jain and Bhatti (1986) also recorded the influence of plant age on the incidence of root-knot nematode, *Meloidogyne javanica* in tomato. They found that the advanced age seedlings suffer comparatively less than young seedlings. Similar types of observations were made by Masood and Hussain (1975). They found more severe infection when tomato seedlings were inoculated at the age of ten days than at the age of 20 or 30 days. Eapen (1994) confirmed the destructive effects of *Meloidogyne incognita* infestation in cardamom and pointed out that an initial population of 4 juveniles per 1000 cm^3 soil can inflict serious losses in cardamom yields.

Many biotic factors affect the fixation of atmospheric nitrogen and among these, nematodes occupy an important place. Earlier reports have shown that *Meloidogyne* spp. cause reduced nodulation in leguminous crops (Hussaini and Seshadri, 1975 and Singh *et al.*, 1977). Different theories like nutrient deficiency caused by nematodes in host plants, competition between nematode larvae and root nodule bacteria and on antagonistic effect of root-rot pathogens upon root nodule bacteria (Epps

and Chambers, 1962) have been reported as the cause for reduced nodulation (Masefield, 1958; Inchinohe, 1961;Epps and Chambers 1962; Malek and Jenkins,1964). Singh *et al.*, (1977) reported reduction in growth characters, nodulation and symbiotic nitrogen fixation in mungbean (*Vigna radiata*) due to the action of *Meloidogyne incognita.* *Meloidogyne incognita* was also reported to reduce the number of nodules per plant, total biomass and total nitrogen uptake of mungbean (*Vigna radiata*) cultivar ML- 131 irrespective of the initial inoculum level of nematodes (Chahal and Chahal, 1989). Hardy *et al.* (1973) and Chahal and Chahal (1988) reported the adverse effects of root-knot nematodes on the functioning of nodules to fix nitrogen as reflected by reduced leghaemoglobin content, bacteroid contents and nitrogenase activity of nodules on chick pea (*Cicer arietinum*). Verdejo *et al.* (1988) reported that nodulation in black gram was significantly decreased by root-knot nematode, *Meloidogyne incognita.* The reduction in the nitrogen content of nematode inoculated plant was also reported to be due to the early destruction of rhizobial nodules (Robinson, 1961).On cowpea inoculation of nematode, *Meloidogyne incognita* adversely affected the root nodulation and nitrogen content of the plants (Sharma *et al.*,1976). With the increase in the population level of *Meloidogyne incognita* there was a corresponding decrease in the chlorophyll content, number of nodules, nitrogen content

of shoot and protein content of grain,*Phaseolus aureus*(Singh *et al.*, 1977) and papaya,*Carica papaya* L.(Ramakrishnan *et al.*,1997).

2.4 Seasonal fluctuations

For effective nematode control and advisory service, understanding of established nematode populations and their population dynamics are the pre-requisites, which in turn are influenced by the seasonal fluctuations, host status, availability and quality of food and ability of the parasites to utilize the food materials (Nusbaum and Barker, 1971).

The seasonal behaviour of nematode population is one of the important tools in the study of plant nematode relationship (Pinochet *et al.*, 1990). There are several reports on the seasonal variations of nematode populations and their correlation with environmental factors (Jagdale *et al.*, 1981; Joshi *et al.*, 1989; Huang *et al.*,1994). General observations drawn from the earlier studies indicate that the fluctuation of nematode population depends on environmental factors and host plants. Khan *et al.* (1971) and Van Guindy (1985) reported that a temperature of 25^0C was optimum for the build up of nematode population in soil. Rao and Swarup (1975) and Krishnarao and Krishnappa(1996) observed increase in nematode population during summer months and decreased during winter. In banana field, a peak nematode population was recorded in the month of March and decline during December (Choudhary *et al.*, 1990). Two peak periods of *Hoplolaimus indicus* population around the root zones of *Psidium*

guajava, one in July and the other in October-November have been observed. The rate of reproduction was favoured at soil temperature of 15-30^{0}C (Dwivedi *et al.*, 1987). They also observed that the influence of season on nematode population was more evident at soil depth of 0 to 10 cm than in the deeper layers.

Eapen (1993) reported maximum second stage juveniles of root-knot nematode in cardamom plantations during the months of March and April. He observed rapid increase of nematode population in roots during the post monsoon period which gradually declined during summer and was the lowest in monsoon months. According to him crop phenology appears to be the major factor influencing the fluctuations in nematode populations when compared to the other ecological factors like rainfall and soil temperature. The optimum conditions for survival and multiplication of root-knot nematodes were the post monsoon period (Khan *et al.*, 1971).

Similar post monsoon build up of nematode population was observed in other perennial crops like coconut (Koshy and Sosamma, 1978), coffee (Kumar, 1991) and black pepper (Mohandas and Ramana, 1988). Rainfall pattern, air and soil temperatures, soil moisture and host root-growth appeared to be the more predominant factors in influencing the population of pathologically significant nematode species (Mukherjee and Dasgupta, 1993). Mittal *et al.* (1989) studied the effect of soil texture on

sorghum cyst nematode, *Heterodera sorghi* infesting sorghum and reported maximum plant growth and cyst multiplication in a mixture of clay loam and sand (3:1). But the juvenile population was greater in sandy loam and sand (3:1) than clay loam soil.

Soil temperature and soil type are reported to be the primary factors which influence the penetration, development and reproduction of nematode in the plant roots (Ferris *et al.*, 1970 and Elmiligy, 1971). The effect of soil type on the penetration of *Pratylenchus vulnus* to roses was also carried out by Ouden and Beuzenberg (1971). Multiplication of root knot nematode *Meloidogyne incognita* on blackgram (*Vigna mungo* L.) in different soil types was reported by Baheti and Yadev (1991). Similar study was carried out by Mehta *et al.* (1993) in sugarcane varieties cultivated in 3 soil types. Temperatures between 26 and 28^0C induced greatest penetration of *Pratylenchus zeae* irrespective of soil type. Among the soil types, the rate of penetration occurred in descending order in clay, sand and clay loam respectively. The seasonal variations in population densities of plant parasitic nematodes showed that fluctuation in the population densities of *Helicotylenchus dihystera* and *Meloidogyne incognita* correlated more with the temperature than with the soil moisture (Khan and Sharma, 1990).

2.5 Interactions with other microorganisms

Vesicular arbuscular mycorrhizae (VAM) and rhizobia are beneficial soil micro-organisms having symbiotic associations with leguminous plants (Vyas and Srivastava, 1995; Gupta *et al.,* 1999). VAM and *rhizobium* interaction improves the plant growth, nodulation, nitrogen fixation and suppresses the root-knot nematode infestation in most of the legumes (Grandison and Cooper, 1986; Ramaraj and Shanmugam, 1990; Rao *et al.*, 2003). The beneficial effect of VAM fungi on plant growth has been reported by Mosse (1973) and Sanjeev and Dohroo (1996). Symbiotic association of VAM is known to improve plant growth and general health through better host nutrition and improves phosphate uptake and provides more resistance to the plant (Sundarababu *et al.*,1996; Choudhary *et* al.,2002). The interactions between these fungi and plant parasitie nematodes have been studied by several workers (Suresh and Bagyaraj, 1984; Saleh and Sikora, 1988; Abha and Shukla, 1997; Sadasivan *et al.*,1998).Under various environmental conditions, the detrimental influences of plant parasitic nematodes were reported to be partially alleviated by the presence of an endomycorrhizal association (Sikora, 1979; Hussey and Roncadori, 1982). Mycorrhizal association is also reported to alter the root physiology by increasing aminoacid and reducing sugar content in the root and the wall thickness of cortical root cells (Dehne and Schonbeck, 1978; Schenck, 1981; Haymann, 1982) and this altered

root physiology may not be favourable for the penetration and multiplication of root-knot nematodes. The prior establishment of VAM reduced the deleterious effect of nematodes and provided better plant growth of tomato (Sundarababu *et al.*, 2000) and Peach (Hussey and Roncadori, 1982).

Sitaramaiah and Sikora (1981) and Sikora *et al.*(1995) have reported that the establishment of VAM fungi on roots protects the plants against infection by nematodes. Tobacco, tomato, oats, carrot and soybean plants pre-inoculated with VAM fungi were reported as less susceptible to *Meloidogyne incognita* (Schenck *et al.,* 1975; Sundarababu and Suguna,1998). Sitaramaiah and Sikora (1980) also reported significant reduction in the number of larvae of *Rotylenchulus reniformis* on *Glomus mosseae* inoculated cotton roots. The effect of *Glomus fasciculatum* and *Glomus epigaeus* on penetration of larvae of *Heterodera cajani* and their further development in cowpea was investigated by Jain and Sethi (1988). Number of galls of *Meloidogyne incognita* and number of cysts of *Heterodera cajani* were reported to be reduced in cowpea roots due to the prior colonisation of *Glomus fasciculatum* (Jain and Sethi, 1988). The beneficial effect of VAM fungi on the nematode susceptible cotton cultivar reduced damage caused by *Meloidogyne incognita* (Roncadori and Hussey, 1977). Soybean inoculated with *Glomus macrocarpum* and *Meloidogyne incognita* had fewer galls per gram of root, increased root weight compared

with plants inoculated with nematode alone (Kellam and Schenck, 1980). Inoculation of *Glomus fasciculatum* reduced *Meloidogyne incognita* infestation in tomato compared to simultaneous as well as prior inoculation of nematode (Suresh and Bagyaraj, 1984). The extensive colonization by VAM fungi could cause changes in root exudate pattern and nematode penetration (Sikora *et al.*, 1975; Rakesh *et al.*,1997). Suresh and Bagyaraj (1984) reported that nematode infestation was checked if mycorrhizal colonization was present before the nematodes were able to infect the plants. Significant decrease in the infectivity potential of *Meloidogyne incognita* was observed in plants which received *Glomus fasciculatum* inoculation. Several interaction studies involving VAM and plant pathogenic nematode in a plant system point to the fact that VAM generally induced resistance to nematode susceptible plants (Heald *et al.*, 1986; Jain and Sethi, 1987; Jothi and Sundarababu,2000).Lingaraju *et al.* (1993) studied the interactive relationship of a VAM fungi and *Rotylenchus reniformis* on cowpea and recorded that mycorrhizal fungi enhanced total biomass and root weight while the nematode reduced them. Saleh and Sikora (1984) observed that in the presence of *Glomus fasciculatum*, counts on *Meloidogyne* larvae and eggs were reduced in cotton. Bagyaraj *et al.* (1979) reported reduction in size of galls produced by *Meloidogyne incognita* and *Meloidogyne javanica* on tomato in the presence of *Glomus*

fasciculatum. Sundarababu *et al.* (1993) also observed better plant growth in nematode inoculated tomato in the presence of *Glomus fasciculatum.*

Thomas *et al.*(1989) reported that the growth of cardamom plants infected with root knot nematode *Meloidogyne incognita* improved significantly by inoculation with mycorrhizal fungi. Santhi *et al.* (1993) reported the effect of moisture level on the interaction of *Glomus fasciculatum* with *Meloidogyne incognita* on cowpea and observed that the nematode population was positively correlated with the moisture levels whereas VAM spore count and colonization was negatively correlated. Schwob *et al.* (1999) studied the effect of climatic factors on native VAM and *Meloidogyne exigua* in *Hevea* plantation and reported maximum spore count in rainy season. Seventy per cent moisture content and a soil pH of 7.0 was reported to be suitable for the establishment of VAM to control root-knot nematodes (Sankaranarayanan *et al.*, 2001). Anilkumar *et al.*(2003) reported the effect of vesicular-arbuscular mycorrhizal fungi on growth and nutrient uptake of leguminous plants under stress conditions. They have observed that the VAM associated plants showed more growth rate and nutrient levels than the ones without VAM association. VAM has been reported as bio control agent against root-knot nematodes, *Meloidogyne incognita* (Jain and Hasan, 1994).

2.6 Biochemical changes

Plant parasitic nematodes, like many other plant pathogens are capable of altering the host metabolism. This disturbed metabolism includes physiological and biochemical changes in the host (Ganguly *et al.*, 1991; Zinov'eva *et al.*,2004). The manifestation of these changes in the infected host decides whether the host becomes susceptible or resistant to nematode attack. Premachandran and Dasgupta (1983) and Dasgupta (1988) have reported that it was the biochemical changes induced by the nematodes that decided the fate of the host. Pathak *et al.* (1983) made an initial attempt in this direction and concluded that there was considerable interference in the metabolism of proteins, nitrogen and carbohydrates in the nematode infected plants. Ganguly and Dasgupta (1984) and Bajaj *et al.*(1988) have also reported measurable biochemical changes induced by root-knot nematode, *Meloidogyne incognita* in the roots of susceptible as well as resistant varieties of tomatoes.

2.6.1 Changes in Phenolic compounds

The role of phenolic compounds in the defense mechanism of the plants and consequently their accumulation in the cells damaged by nematode feeding have been reported by various workers (Acedo and Rohde, 1971; Valette *et al.*, 1998). Accumulation of phenols in plants has been reported as a possible resistance factor to nematode infestation (Balasubramanian and Purushothaman, 1972; Sitaramaiah and Singh, 1978; Kerry, 2000). The

importance of production, rate of production and liberation of phenolics from plant tissues after nematode infection was emphasized by Acedo and Rohde (1971) and Khaberman (1972). Increase in the concentration of free phenols following infection by *Meloidogyne incognita* was reported by Ganguly and Dasgupta (1984), Bajaj *et al.* (1985) and Gapasin *et al.* (1988). The increase in phenols help in the formation of hypersensitive reaction towards the nematode infection (Shukla and Chakraborty, 1988; Mazzafera *et al.,* 1989). The early accumulation of phenolic compound at the infection site is reported as a result of the rapid hypersensitive death of cells (Fernandez and Heath, 1989). Bell (1980); Mansfield (1982) and Matern and Knuesel (1988) have proposed that the defensive strategy of plants exists in two stages. The first is assumed to involve the rapid accumulation of phenols at the infection site which function to slow or even halt the growth of the pathogen and second, to allow for the activation of secondary strategies that would more thoroughly restrict the pathogen.

Phenolic compounds play an important role in the control of root-knot nematode through larval mortality and by rendering host roots less attractive to nematodes (Sitaramaiah and Singh, 1978; Nicholson and Hammerschmidt, 1992). Coupled with the increase in phenolic concentration, increase in the activity of polyphenol oxidase in the diseased tissues was reported by Ahuja and Ahuja (1980), Bajaj *et al.* (1983, 1985) and David (2002). The synergistic effect of higher auxin and phenolic

compounds in the infected roots showed a regulatory role in cell division and gall formation (Stonier and Yang, 1973; Shekawat and Arya, 1979). Gieble (1974) reported a distinct correlation between the degree of plant resistance and phenolics present in the plant tissues. Ganguly and Dasgupta (1982) and Bajaj *et al.* (1988) reported the cellular changes and phenolic contents in two tomato cultivars in relation to nematode infection. Razk *et al.* (1987) observed higher concentration of phenolics in healthy roots of resistant barley cultivar than the susceptible one and emphasized the possible role of phenolic compounds which may lead to resistant response towards the invading nematodes. The resistant variety of barley cultivar invaded by *Heterodera avenae* also showed higher phenolic contents than the susceptible ones (Pankaj *et al.*, 1992). Anwar and Israr (1976) observed phenolic and *ortho*-dihydroxy phenolic changes and their role in resistant and susceptible varieties of tomato to *Meloidogyne incognita.* The role of phenols in pigeon pea resistance to reniform nematode has been reported by Thakar *et al.* (1986). The nematode infested leaves bore a large number of brown spots and the increase in total phenols seemed to have been the result of host-parasite interaction, reported as a defense reaction to nematode attack (Jenkins and Taylor, 1967).

Accumulations of phenolic and *ortho*-dihydroxy phenolic compounds are reported to be rapid and more in resistant varieties than moderately resistant and susceptible varieties (Anwar and Israr, 1976). Gieble (1970)

and Hung and Rohde (1973) also reported the rapid and high accumulation of phenolic compounds in resistant varieties of nematode inoculated plants. Higher concentration of phenolic compounds in Zinnia leaves infested with *Aphelenchoides ritzemabosi* was reported by Gill and Uppal (1977). Root-knot nematode infested tomato plants grown in soil containing organic amendment showed increase in total free phenols, *ortho*-dihydroxy phenols and amino acid content (Singh *et al.*, 1985).

2.6.2 Changes in Carbohydrates

Several investigators have documented that infection of plants by plant parasitic nematodes initiates a rise in metabolic activities (Ganguly and Dasgupta, 1981; Patel and Patel, 1991). Jatala and Jensen (1963) reported quantitative changes in total soluble/reducing carbohydrates in sugar beet as a result of infestation by *Meloidogyne hapla* and *Heterodera schachtii.* Sharma and Sethi (1976) observed increased quantity of total carbohydrates in susceptible and mutant varieties of cowpea due to the infestation of *Meloidogyne incognita* and *Heterodera cajani.* They have also reported that the degree of increase was greater for *Heterodera cajani* than *Meloidogyne incognita.* Gill and Uppal (1977) reported higher concentration of total sugars and reducing sugars in the leaves of *Zinnia elegans* infested with *Aphelenchoides ritzemabosi.* Increased concentration of carbohydrates in wheat plants due to *Anguina tritici* infection was reported by Pathak *et al.* (1983). The infestation of plant parasitic nematode caused increase in sugar

content and it was also observed that increased sugars is helpful for the survival of nematodes (Owens and Specht, 1966; Dropkin, 1969). They have also suggested the reason for the increase in content of sugars in the infested leaves and it was presumed that it may be due to the degradation of starch or inhibition of starch synthesis from sugar. Farooqi *et al.* (1980) reported an increase in total as well as reducing sugar content in root-knot nematode infected tomato plants. Effect of root knot nematode *Meloidogyne incognita* on the total protein, carbohydrate and lipid in roots of *Hibiscus esculentus* was reported by Basu and Sukul(1983). In chick pea the incidence of *Meloidogyne javanica* resulted in the disturbance of metabolism of protein and carbohydrates as well as chloroplast pigments (Upadhyay *et al.*, 1986). Significant variation in carbohydrate metabolism was reported in tomato plants by Wallace (1974). He also explained that variation in carbohydrate metabolism could be due to the reduced photosynthetic capacity or increased rate of respiration. They have also observed that changes in carbohydrate were recognizable even away from the site of infection indicating that nematode's secretion products were able to diffuse across the cells, and these changes takes place either as a damage compensatory factor or the nematode triggers these changes for its own survival. In both the situations, there is an increase in certain metabolites even to the level of accumulation in galled tissues or metabolic sinks as suggested by Ishibashi and Shimizu (1970).

2.6.3 Changes in Amino Nitrogen

Many reports are available on the mechanisms involved in the incompatibility of plant with phytophagous nematodes (Premachandran and Dasgupta, 1983; Dasgupta and Ganguly, 1986; Giebel *et al.*,1994). Incompatibility and plant defense mechanisms in several plant species against fungal pathogens and nematodes are characterized by hypersensitive reaction, production and accumulation of host synthesized phytoalexin like antibiotics, hydroxyproline rich glycoproteins, deposition of lignin like polymers and increase in activity of certain hydrolases and several other enzymes (Ganguly and Dasgupta, 1982; Schmelzer *et al.*, 1984; Naresh *et al.*, 1995; David, 2002). The molecular basis of plant – nematode interaction was studied by Mote and Dasgupta (1979), Raja and Dasgupta (1986) and Guozhong *et al.*(2005). They have reported that nematode induced synthesis of polypeptides and enzyme mediated biosynthesis of antibiotic molecules that restrict or limit the activities of the parasites or pathogens during the early stages of post infectional period. Phenylanaline ammonia lyase (PAL), the first and one of the key enzymes of phenyl propanoid pathway leading to biosynthesis of certain benzoic compounds such as cinnamic acid, hydroxy cinnamic acid, flavonoids, coumarin and lignin like polymers in a metabolic sequence was investigated extensively in *Lycopersicon esculentum* - *Meloidogyne incognita* combination (Mote and Dasgupta, 1979). Sirohi and Dasgupta (1993) reported the early induction

and enhanced activity of phenylalanine ammonia-lyase by root-knot nematode, *Meloidogyne incognita* in cowpea cultivar during the early stages of infection. They studied the interaction between root-knot nematode and cowpea plant and reported the derepression of noval genes, which subsequently lead to *de novo* synthesis of RNA and protein molecules under the influence of elicitors released by the parasite. Rapid post infectional increase of enzyme activity resulting from *de novo* synthesis of enzyme or activation of pre-existing inactive forms of enzyme has been reported by Shannon *et al.* (1971). Mote and Dasgupta (1979) provided positive evidence for *de novo* synthesis of isozymes of phenylalanine anmonia-lyase associated with resistant expression in tomato against *Meloidogyne incognita.* Ganguly and Dasgupta (1981) and Goswami *et al.* (1986) reported the *de novo* synthesis of isozymes of peroxidase, ribonuclease and protein components in root-knot disease of tomato by *Meloidogyne incognita. Denovo* synthesis of an isoflavonoid compound by soybean in response to *Meloidogyne incognita* has been reported by Kaplan and Keen(1977). Simte and Dasgupta (1987) reported the *de novo* synthesis of peroxidase isozymes in soybean infected with root-knot nematode, *Meloidogyne incognita.*

The defence response in mungbean to *Meloidogyne incognita* was investigated by Hussaini and Seshadri (1976) and they reported that aminoacids like arginine, leucine, norleucine and threonine were found to be

lacking in resistant varieties. Sampath *et al.* (1970) indicated the presence of a biochemical defense mechanism which inhibited the multiplication of *Meloidogyne graminicola* on rice. Later, Jena and Rao (1977) estimated the aminoacids qualitatively and reported that the resistance in rice to *Meloidogyne graminicola* was associated with high aspartic acid and alanine contents and low valine, tryptophan and methionine contents in roots. They inferred that the aminoacids were normally present but the amount had increased in resistant rice due to nematode infestation. The antimetabolic effects of aspartic acid, alanine and glutamic acid on *Meloidogyne graminicola* was confirmed by Swain and Prasad (1991). An increased protein metabolism was observed in soybean infected with root-knot nematode, *Meloidogyne incognita* (Simte and Dasgupta, 1987). Effect of *Meloidogyne incognita* on the growth,protein and lipid contents of *Allium porrum* was reported by Romabati and Dhanachand (2000).In cotton, Noel and McClure (1978) reported increased activity of peroxidase and 6-phosphogluconate dehydrogenase due to infection of *Meloidogyne incognita*. Induction of isoperoxidase in resistant and susceptible tomato cultivars by *Meloidogyne incognita* was reported by Molianaris (1991). Comparison of aminoacids and amides in relation to plant nematode interaction has been done by many workers (Farooqi *et al.*, 1980; Sahu and Mohanty, 1986; Mohanty and Pradhan, 1989; Pokharel, 1991).

Chapter III

MATERIALS AND METHODS

MATERIALS AND METHODS

3. 1 Isolation of *Meloidogyne incognita* from rubber growing soils, assay of population and testing their infectivity

3.1.1 Collection of soil samples

A total of 349 soil samples were collected at random from twenty-one locations of rubber growing area to study the natural distribution of root knot nematodes. Soil was dug out with a trowel to a depth of 15 cm after scrapping away the top 1cm layer of soil. Sampling was confined to feeder root zone. The survey was conducted during November to May when nematode populations are found to be maximum. Composite samples were drawn by mixing ten sub samples from each site, labelled and brought to the laboratory. Each soil sample was thoroughly mixed and 250 g was drawn from the homogenous lot for processing.

3.1.2 Extraction of nematodes

Extraction of nematodes was done by Cobb's wet sieving and petridish extraction method (Chawla and Prasad, 1974). Washings (sievings) from 400-mesh sieve were collected in a beaker and transferred to two layers of tissue paper contained in a moulded aluminium wire guaze with their edges rolled down so as to rest on the upper edge of the petri dish when placed on it. A wire qauze was placed on a petridish (10 cm) containing sufficient water to maintain the paper wet for 48 hours so that all active nematodes migrated to the water inside the petridish. The nematode suspension in petridish was collected in a beaker and kept

undisturbed for two hours. The supernatant was decanted and the suspension was poured in to a counting dish. The nematodes were identified up to the generic level in the living conditions by observing the morphological characters and their population counts were made under a stereoscopic binocular microscope. From these data, the absolute density, relative density, absolute frequency and relative frequency of nematodes were calculated using the method of Norton (1978).

For specific identification, the nematodes were killed by heat, fixed in Formalin-Aceticacid (4:1v/v) processed through lacto phenol into dehydrated glycerin (Siddique, 1964) and mounted in the same medium on a glass slide. Nematodes were identified by using pictorial key to nematode genera by Mai and Lyon(1960).

3.1.3 Absolute and relative frequencies

From assay of nematode population, absolute frequency and relative frequency of *Meloidogyne* spp. in samples were calculated. Frequency denotes how often a species occurs among the samples examined.

$$\text{Thus absolute frequency} = \frac{\text{No. of samples that contained } \textit{Meloidogyne} \text{ spp.}}{\text{Total No. of samples collected}} \times 100$$

Relative frequency was calculated from absolute frequency as the per cent of frequency of a species to sum of frequencies of all species.

$$\text{Relative frequency} = \frac{\text{Frequency of } \textit{Meloidogyne} \text{ spp.}}{\text{Sum of frequencies of all species}} \times 100$$

3.1.4 Absolute and relative densities

Absolute density represents the total population of plant parasitic nematodes identified and estimated in each sample analysed. From this data, the relative density of *Meloidogyne* spp. was calculated as a per cent of *Meloidogyne* spp. to total population of plant parasitic nematodes (Sundararaju *et al.*, 1991).

3.1.5 Infectivity of soil samples

The indicator plant technique (Mcsorley *et al.*, 1983) was used to test the infectivity of soil samples. Tomato (*Lycopersicon esculentum* var Pusa Ruby) seedlings were raised in the soil (500 g) to be tested and grown for six weeks in polythene bags (15 cm diameter) by providing nutrients as per normal recommendation (N P K @ 75: 40: 25 kg/ha.).

Soil samples (500 g) were mixed with 4.5 g urea, 11.0g rock phosphate and 1.1g potash before planting. Twenty days after first application urea (3.5 g) and potash (1.0 g) were also applied. The seedlings were watered regularly.

The plants were uprooted after six weeks and the nematode infestation was recorded using a gall index on 0-5 scale based on the per cent of root system infested *viz* .0 = no gall ; 1 = < 10 per cent ; 2 = 11 – 25 per cent ; 3 = 26 – 50 per cent ; 4 = 51 – 75 per cent and 5 = 76 – 100 per cent . The incidence of *Meloidogyne* spp. as indicated by the relative extent to which the roots get infested (Dropkin, 1954), was assayed .

3.2 Effect of *M. incognita* infestation on the growth, biomass, nodulation and nitrogen fixation of *Pueraria phaseoloides* (Rao *et al.*, 2003)

Sterilized potting mixture (1kg) containing fine soil , sand and well decomposed and powdered cowdung in the ratio 2:1:1(w/w) was filled in 15 cm diameter mud pots. The pots were kept in another sterilized pot to prevent the entry of soil organisms. Acid treated seeds of *P. phaseoloides* were sown .One week after germination, the seedlings were thinned to five per pot. Axenic culture of *M.incognita* for inoculation was raised from a suitable egg mass on tomato plants raised in sterilized soil and further multiplied on the same host. Single egg mass derived one female nematode was removed from an infected root surface by observing the infected root bits under water in syracuse watch glass using a stereo binocular microscope and carefully picking it to another watch glass containing sterile distilled water .For raising pure culture of nematode, a single egg was placed close to the root system of tomato seedling raised in sterile soil, covered with soil and watered regularly. The larva which hatched out from

the egg would infect the tomato roots, grow on it and produce several egg masses within 30 days. These were collected and the process repeated to develop sufficient inoculum of the nematode. When sufficient egg masses were available these were picked, placed on extraction dish and larvae were allowed to hatch out and migrate in to the sterile water .The population of infective larvae was counted .The inoculum was adjusted to the desired level by dilution /centrifugation. An isolate of the root nodule bacterium, *Bradyrhizobium* sp.nodulating *P. phseoloides* obtained from the culture collections of Plant Pathology Division, RRII was used for the experiment. The isolate was multiplied in yeast extract mannitol broth .

The broth culture of *Bradyrhizobium* sp. after suitable dilutions in sterile distilled water was used in the experiment.

Various treatments included were

1.	R	6. R-N_1	11.N_2-R	16. N_3
2.	R+N_1	7. R-N_2	12. N_3-R	17. N_4
3.	R+N_2	8. R-N_3	13. N_4-R	18. control
4.	R+N_3	9. R-N_4	14. N_1	
5.	R+N_4	10.N_1-R	15. N_2	

Where R = *Bradyrhizobium* (10 ml, having 10^8 cfu/ml); N = infective larvae of *M. incognita*; R + N series = *Bradyrhizobium* and

nematode inoculated simultaneously. R – N series = *Bradyrhizobium* followed by nematode at 10 days interval. N – R series = Nematode followed by *Bradyrhizobium* at 10 days interval. N_1 = 1000 nematodes, N_2 = 2000 nematodes, N_3 = 3000 nematodes and N_4 = 4000 nematodes.

Suitable control without nematode and *Bradyrhizobium* were also maintained. All the treatments were replicated three times. Nematode inoculation was done by pipetting the required number of larvae held in 5 ml suspension through four holes punched close to the root zone. In the case of *Bradyrhizobium* 10 ml aliquot of broth (108 cfu/ml) was pipetted out and inoculated near the root zone. Observations were recorded on various growth characters such as shoot length , shoot weight (fresh and dry), root length, root weight (fresh and dry), number of galls, nodules and nitrogen content of shoot and root after 60 days of inoculation.

3.2.1 Shoot length

Height of the main shoot was measured from the ground level to the tip of the terminal bud and expressed in cm.

3.2.2 Shoot weight (fresh)

The shoot portions of the plants were separated and gently pressed between folds of filter paper to remove the excess moisture and fresh weight was determined

3.2.3 Shoot weight (dry)

The shoot portions after removing excess moisture was wrapped in paper and dried in a hot air oven at 80^0C to constant weight and the weight was recorded .

3.2.4 Root length

The length of the main root from the collar to the tip was measured.

3.2.5 Root weight

The root portions were separated and washed gently in running tap water to remove all the adhering soil particles. Pressed gently in folds of filter paper to remove excess moisture and fresh and dry weight was determined .

3.2.6 Root nodulation

Number of rhizobial nodules in primary and lateral roots were counted and recorded.

3.2.7 Determination of total nitrogen

Total nitrogen in the leaf sample was determined by microkjeldahl method (Jackson, 1962). The samples were dried at 70^0C for 48 h and powdered. Fifty mg of powdered sample was transferred in to a digestion flask and digested with 2 g of potassium sulphate and 40± mg of mercuric oxide and added 2.4 ml of concentrated sulphuric acid. The flask was gently heated until frothing ceased and the heating was continued more strongly until the solution was cleared. After cooling, 10 ml of distilled

water was added and the mixture was warmed to dissolve the solute material. Blanks were run similarly using the reagents alone.

3.2.7.1 Estimation of nitrogen

The digested sample was transferred into the microkjeldahl distillation unit. 2 ml sodium hydroxide – sodium thiosulphate mixture prepared by dissolving 50 g of sodium hydroxide and 5 g of hydrated sodium thiosulphate in 100ml of water was added and steam distilled. The liberated ammonia was collected in to a 5 ml of 4 per cent boric acid solution (in water), containing 2-3 drops of methyl red – bromocresol green indicator (prepared by mixing five parts of 0.2 per cent alcoholic bromocresol green solution with one part of 0.2 per cent alcoholic methyl red solution). The distillate was titrated against 0.02 N hydrochloric acid. The end point was the appearance of green colour. The blank digest was also titrated in the same way. Nitrogen in the sample was calculated by employing the factor, 1 ml of 1 N acid is equivalent to 14 mg of nitrogen.

3.3 Effect of seasonal variation on nematode population and gall formation in *P. phaseoloides*

Three different locations were selected within the experiment station of RRII and *Pueraria phaseoloides* seedlings were raised at these sites as per the recommendations. Eight replications were maintained for each location. Soil samples and root samples were collected on 16th of every month from September 2000 to August 2001 from the rhizosphere of these plants. Soil samples were collected at a depth of 15 cm from the surface

using a screw type soil augur .The soil of five samples were mixed together to form a composite sample .From the sample 250 g was taken and analysed by Cobb's sieving and petridish extraction method and population of root-knot nematode per 250 g soil was assessed. Root samples were washed thoroughly and number of nematode galls per plant was recorded from which root-knot indices were worked out. The monthly rainfall and mean monthly atmospheric temperature were recorded in the Agromaterological observatory situated in the experimental farm. The data were statistically analysed to study the correlation of nematode population in the soil with temperature, rainfall and with host infection. The seasonal variation in nematode population and in the formation of galls in *P. phaseoloides* was also studied.

3.4 Effect of *M.incognita* and VAM interaction on the formation of galls

Earthen pots of 1kg soil capacity were filled with steam sterilized potting mixture and *P. phaseoloides* seeds pre-treated with concentrated sulphuric acid were dibbled. One plant in each pot was maintained after five days of germination. Each plant was inoculated at three to four leaf stage with 1000 freshly hatched second stage juveniles of *M.incognita* obtained as described earlier from infested tomato roots grown under sterile conditions.

The treatments imposed were as follows.

1. Inoculation with VAM alone(50g/kg soil.ie 300 spores of *G.fasciculatum*/kg soil).
2. Inoculation of nematode alone (1000 nematodes /kg soil).
3. Simultaneous inoculation of VAM and nematode (VAM + M).
4. Nematode inoculation one week after VAM inoculation (VAM–M_7).
5. Nematode inoculation two weeks after VAM inoculation (VAM–M_{14}).
6. VAM inoculation one week after nematode inoculation (M–VAM_7).
7. VAM inoculation two week after nematode inoculation (M–VAM_{14}).
8. Untreated control (without VAM or nematode inoculation).

The experiment was conducted with three replications in completely randomized design. In the treatment requiring VAM inoculation, soil mixture containing 300 spores (50 g/kg soil) was placed five cm below the soil surface and mixed with the soil at the central portion of the pot and covered with a layer of soil. The experiment was concluded after 120 days and observations were recorded on shoot length, shoot weight (fresh and dry), root length root weight, VAM root infection (per cent), VAM spore count, final nematode population and gall formation as per the standard procedures described elsewhere.

3.4.1 Estimation of mycorrhizal infection (Philips and Hayman, 1970)

The roots were cut into one cm bits, washed gently in tap water without disturbing the external mycelium. The samples were heated to about 90^0C for one hour in 10 per cent potassium hydroxide solution on a water bath. It was rinsed four times in tap water, acidified by immersing for five minutes in 2 per cent hydrochloric acid.

The acid was poured off and 0.05 per cent cotton blue in lacto phenol was added. The roots were boiled in this stain for three minutes. The stain was poured off and lacto phenol was added and kept overnight to destain the host tissue. The samples were subsequently examined under a stereoscopic microscope for mycorrhizal infection. VAM colonization was expressed using the following formula.

$$\text{Per cent colonization} = \frac{\text{No. of root segments showing VAM colonization}}{\text{Total no. of root segments examined}} \times 100$$

The root segment was considered mycorrhizal even if one of the three fungal structures *i.e.,* hyphae, arbuscules or vesicles were present.

3.4.2 Enumeration of VAM spore population

The spores were collected by wet sieving and decanting method and spore counts were taken as detailed by Gerdemann and Nicolson (1963). Soil sample (50 g) was suspended in 200 ml of luke-warm water. Heavier particles were allowed to settle for a few seconds and the suspension was decanted through a 710 μm sieve to remove the larger particles of organic

matter. The residue was suspended in more water and sieving was repeated. The suspension that passed through this sieve was saved and stirred to resuspend all particles. The heavier particles were allowed to settle for a few seconds and the liquid decanted through 250 μm sieve. The suspension that passed through this sieve was again collected and the sieving was repeated using *105 μm sieve and 45 μm sieve.* The larger particles of organic matter were caught in the top sieves of higher pore size. The soil particles and spores collected in 105 μm and 45 μm sieves were taken in 100 ml conical flasks separately. The suspension in each flask was shaken thoroughly and allowed to settle for 30 seconds. The spores present in these suspensions were trapped on nylon mesh, with 45 μm pore size placed on a marked petridish and the number of spores were counted by observing under a stereoscopic microscope.

3.4.3 Recording of root-knot indices

Roots were separated, washed under running tap water to remove soil, blotted dry and root systems were examined for the presence of galls. Number of galls per root system was counted. Gall index (GI) ratings were done on a 0 to 5 scale (Taylor and Sasser, 1978).

3.5 Effect of *M.incognita* infestation on the biochemical changes in *P. phaseoloides*

P.phaseoloides seeds pre-treated with concentrated sulphuric acid were sown in 15 x 10 cm earthen pots containing 500g of sterilized potting mixture (containing soil, finely dried cowdung and river sand in the ratio

2:1:1v/v/v). Thinning of the seedlings were done after one week of germination keeping five healthy seedlings per pot. Ten days old seedlings were inoculated by freshly hatched second stage juveniles of *Meloidogyne incognita* in a series of 0, 10, 100, 1,000 and 10,000 larvae per pot. All the treatments were replicated five times and maintained in randomized block design in the glass house. The plants were watered daily with sterile water. Sixty days after inoculation, each plant was uprooted, washed and observations were recorded on growth characteristics, number of galls, number of egg masses and final nematode population. The samples were also analysed for biochemical constituents.

3.5.1 Biochemical analysis (Chandramohan *et al.*, 1967)

3.5.1.1 Ethanol extraction of plant materials

Leaves of the plants were collected chopped and used for ethyl alcohol extraction after removing excess moisture by blotting them between folds of filter paper. Exactly 1 g of chopped material was plunged in to 20 ml of 80 per cent boiling ethyl alcohol, extracted for 5 minutes on a water bath and cooled in running tap water. The material was homogenized by grinding in a porcelain mortar and pestle and the slurry was squeezed through two layers of cheese cloth. The residue was re-extracted with ethyl alcohol and the extracts were pooled. The final volume was adjusted to 20 ml with 80 percent ethyl alcohol. The residue after drying was used for the estimation of starch.

3.5.1.2 Quantitative estimation of total phenols

Total phenols were estimated by employing Folin–Ciocalteu reagent (Bray and Thorpe, 1954)(Annexure1).The reagent was diluted with equal volume of water. One ml of this reagent was added to 1ml of the alcohol extract in a 25ml marked boiling tube followed by 2ml of 20 percent sodium carbonate and the mixture was heated in a boiling water bath for one minute. The blue coloured mixture was diluted to 25ml with glass distilled water. Reagent blank was maintained with 1ml of distilled water instead of ethyl alcohol extract. The per cent of light transmittance was determined in a Spectronic – 20' colorimeter at 725nm. Total phenols were calculated from a standard curve plotted using catechol.

3.5.1.3 Quantitative estimation of *Ortho* dihydroxy phenols

Ortho-dihydroxy phenols were estimated by the method described by Johnson and Schaal (1952) employing Arnow's reagent(Annexure1). To 1 ml of the alcoholic extract in a 25 ml marked boiling tube, 1 ml of 0.5 N hydrochloric acid, 1 ml of Arnow's reagent prepared by dissolving 10 g of sodium nitrate and 10 g of sodium molybdate in 100 ml of glass distilled water and 2 ml of I N sodium hydroxide were added. The volume was made up to 25ml with distilled water and the intensity of light pink colour was read in a Spectronic – 20' colorimeter at 522 nm. Reagent blank contained 1 ml of distilled water in the place of ethyl alcohol extract.

Ortho dihydroxy phenols were calculated from a standard curve prepared using catechol.

3.5.1.4 Determination of reducing sugars

Reducing sugar in the alcohol extract was determined by the Nelson's (1944) method . To 1 ml of alcohol extract in a 25 ml marked boiling tube, 1 ml of mixture of reagent 'A' and 'B'(Annexure1) prepared by mixing 25 parts of reagent 'A' with 1 part of reagent 'B' was added. The mixture was heated for 20 minutes in a boiling water bath, cooled in tap water and 1 ml of the arseno molybdate reagent(Annexure1)was added. The solution was thoroughly mixed and diluted to 25ml with glass-distilled water. Reagent blank contained 1ml of distilled water in the place of ethyl alcohol extract. The intensity of the resulting blue colour was read in a Spectronic – 20' colorimeter at 497 nm. Glucose was used as standard to prepare a standard curve and results were expressed in glucose equivalents.

3.5.1.5 Determination of non-reducing sugars

Non – reducing sugars present in the alcohol extract were first hydrolysed to reducing sugars (Inman, 1962) and then estimated. Exactly 1 ml of the alcohol extract was taken in a boiling tube and evaporated to dryness on a water bath. One ml of glass distilled water and 1 ml of I N, sulphuric acids were added to the residue. The mixture was hydrolysed by heating at 49^0C for 30 minutes over a water bath. The solution was neutralized with I N sodium hydroxide using methyl red indicator.

Total sugar content of the hydrolysed sample was estimated by Nelson's method. Non – reducing sugars were calculated by subtracting the reducing sugar value from that of total sugars and were expressed as glucose equivalent.

3.5.1.6 Determination of amino nitrogen

Amino nitrogen was determined by the ninhydrin method of Moore and Stein (1954).To 1ml of alcohol extract in a boiling tube, 1 drop of methyl red indicator was added and the extract was neutralized with 0. 1 N sodium hydroxide. To this solution 1 ml of ninhydrin reagent(Annexure1) was added, mixed thoroughly by shaking and aluminium caps were placed on the tubes. The mixture was heated for 20 minutes in a water bath. The tubes were removed, cooled under running tap water, 5ml of diluent solution (n-Propanol + water 1:1v/v) was added and the contents thoroughly mixed.

The intensity of the purple colour of the solution was read in a Spectronic – 20' colorimeter at 475nm . Blank consisted of 1 ml of distilled water in the place of alcohol extract. Amino nitrogen was calculated from a standard graph prepared using glutamic acid.

3.5.1.7 Quantitative estimation of starch

Starch in the samples was estimated by the method of Sumner and Somers (1949).Two hundred mg of finely powdered 80 per cent alcohol insoluble residue dried in an oven at 60^0C was placed in a glass stoppered

100ml Erlenmeyer flask. Three ml of 6 N hydrochloric acid were added to the flask and steamed in an autoclave at 100^0C for 1 h. The flasks were cooled and the solution was neutralized by using I N sodium hydroxide. The volume was raised to 25ml with distilled water. An aliquot of 1ml was withdrawn and glucose was estimated by Nelson's (1944) method. The amount of starch was determined by multiplying the amount of estimated glucose by the factor 0.9.

Chapter IV

EXPERIMENTAL RESULTS

RESULTS

4.1 Isolation of root-knot nematode (*Meloidogyne* spp.) from rubber growing soils and testing their infectivity

4.1.1 Frequency and density of plant parasitic nematodes

Twelve important genera of plant parasitic nematodes were recorded from the rhizosphere of *Hevea brasiliensis*. Nematodes belonging to the genera *Meloidogyne* and *Helicotylenchus* were more predominant and were encountered in more than sixty per cent of the soil samples studied. Genera *Aphelenchoides, Hemicriconemoides* and *Longidorus* were recorded at forty per cent of frequency of occurrence followed by genera *Radopholus, Tylenchus, Hoplolaimus, Criconemoides, Trichodorus* and *Xiphinema*. Lowest frequency of 13.46 was recorded for genus *Hemicycliophora*. The relative frequency followed the same trend as the absolute frequency. The relative density was found to vary from region to region. Maximum relative density of 37.13 was noticed for *Meloidogyne* and a minimum of 1.87 for *Criconemoides* and *Hoplolaimus* ((fig.1-4).

4.1.2 Distribution of *Meloidogyne* spp.

Meloidogyne sp.was found distributed in all the rubber growing areas studied (Table1). Hundred per cent frequency of occurrence was noticed in Kulasekharam, Punalur, Perumpulickal, Vaniampara, Calicut, Kanhikulam, Padiyoor, and Alakode. The absolute density of *Meloidogyne* spp. was found to vary from region to region considerably. The highest mean nematode population of 2225 per 250g soil was observed in soil samples

collected from Punalur followed by Kanhikulam, Calicut, Vaniampara, Padiyoor, Muvattupuzha and Kottayam where in a mean population of more than 1000 nematodes per 250 g soil was recorded.

The soil samples collected from Dapchari (Maharashtra), Nettana (Dakshin Kannada), Alakode (Kerala) and Paraliar (TamilNadu) showed low levels of root-knot infestation. The lowest nematode population of 36 per 250 g soil was recorded in soil samples collected from Dapchari (Maharashtra).

4.1.3 Infectivity of soil samples(Mcsorley *et al* ., 1983)

The infectivity of soil samples collected from different location was evaluated using the indicator plant (*Lycopersicon esculentum*) (PLATE 1). Two larvae per g soil was recorded as the threshold damaging level of *M.incognita* as it could cause root galls in more than 25 per cent of the root system of the indicator plant (Table 2). The highest gall index of 4.8 was recorded from the soil sample collected from Punalur region followed by Kanhikulam,Padiyoor,Vaniampara and Muvattupuzha. The lowest gall index of 0.2 was noticed in soil samples collected from Dapchari (fig 5).

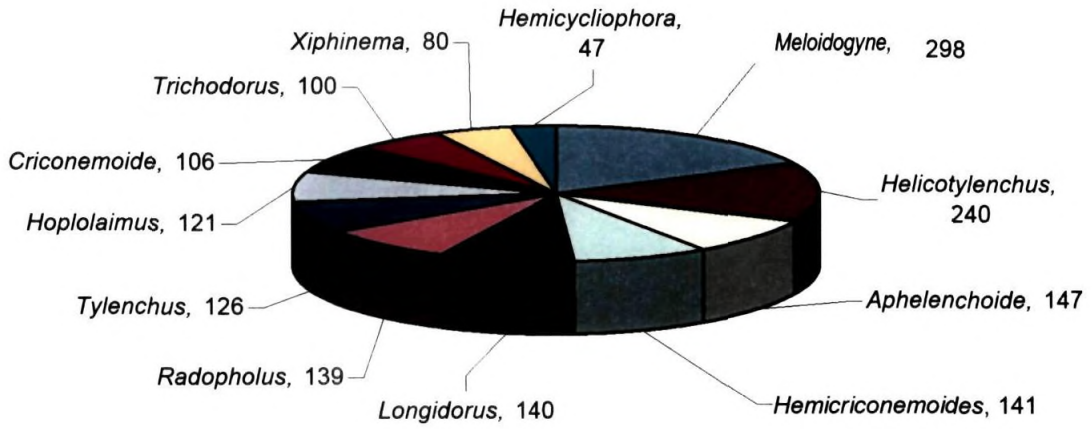

Fig.1. Number of nematode infested samples of rubber growing soils along with the genus infested

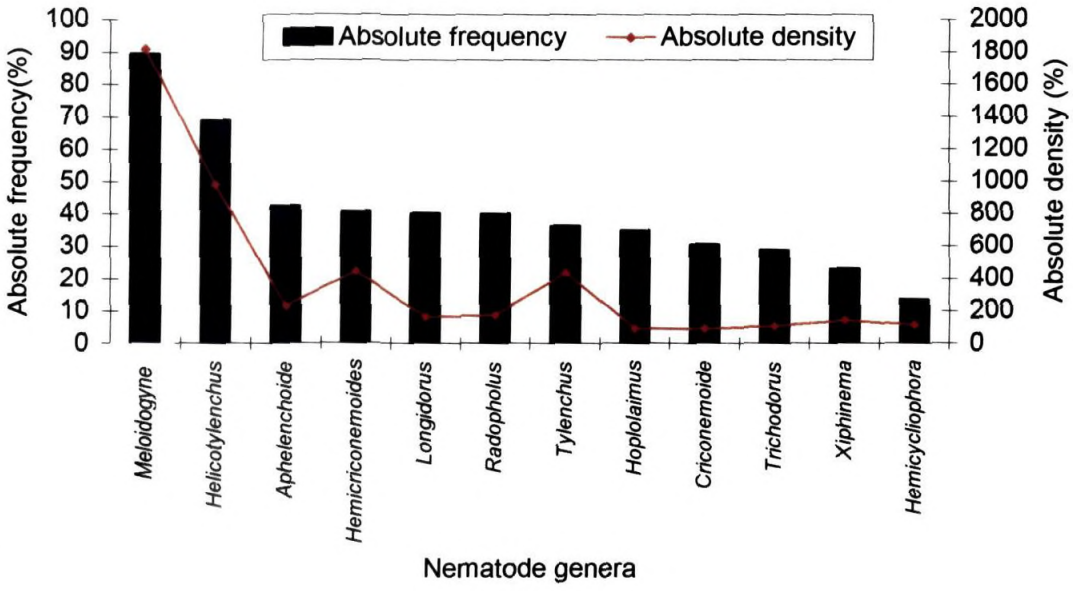

Fig.2. Absolute frequency and density of parasitic nematodes in rubber growing soils

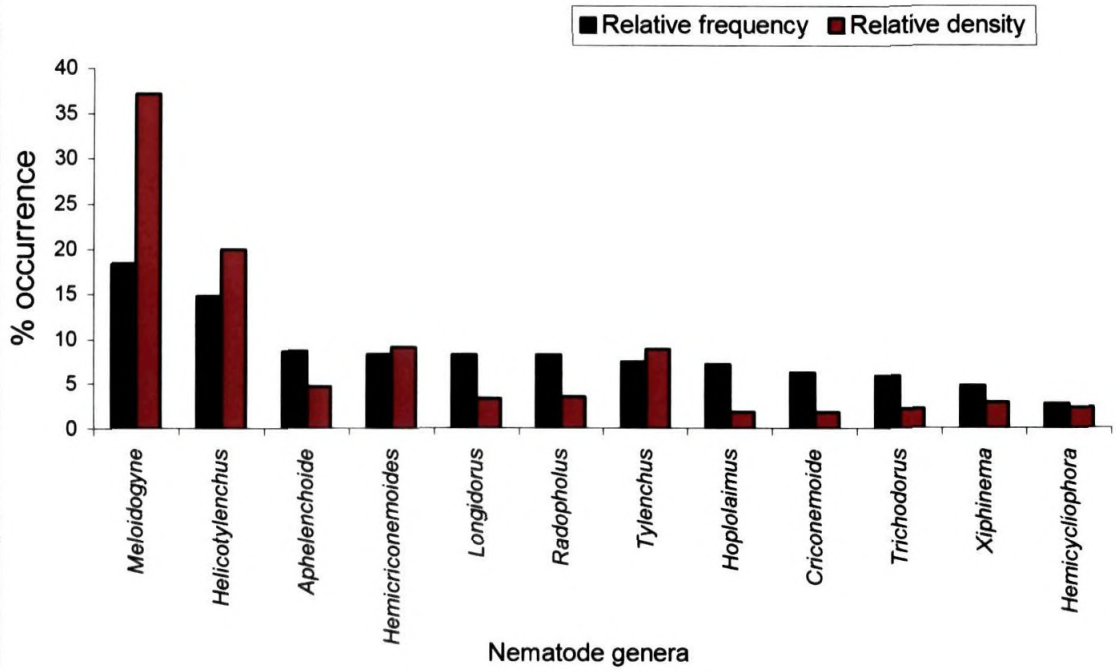

Fig.3. Relative frequency and relative density of occurrence of parasitic nematode in rubber growing soils

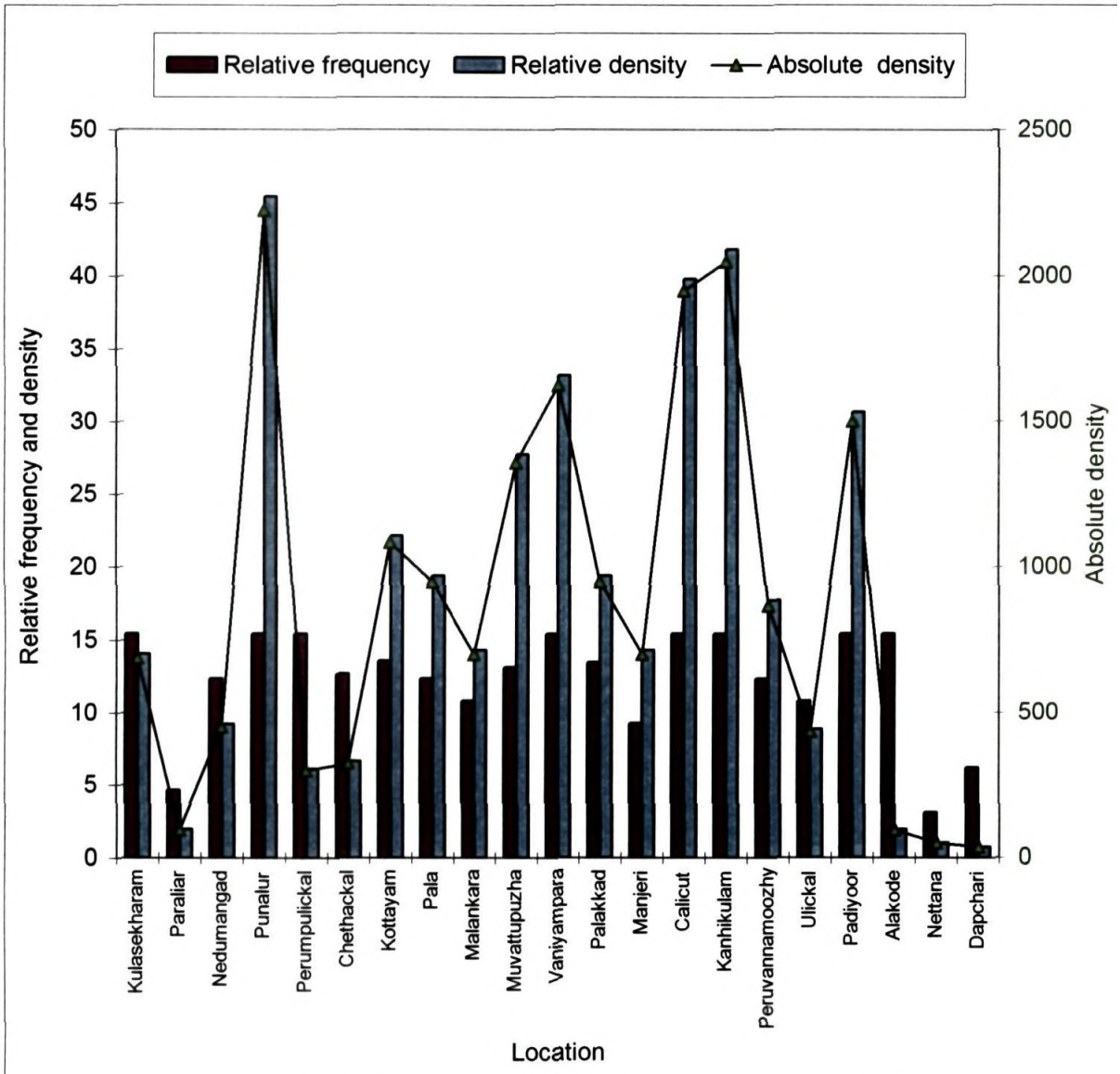

Fig. 4 Density and frequency of occurrence of *Meloidogyne* spp. in rubber plantations

Table 1. Density and frequency of occurrence of *Meloidogyne* spp.in rubber plantations.

Location	No. of samples collected	No. of samples infested	Absolute frequency (%)	Relative frequency (%)	Absolute density (nematode population per 250g.soil)	Relative density (%)
Kulasekharam	16	16	100	15.44	688	14.05
Paraliar	10	3	30	4.63	95	1.94
Nedumangad	15	12	80	12.35	450	9.19
Punalur	30	30	100	15.44	2225	45.43
Perumpulickal	10	10	100	15.44	300	6.13
Chethackal	22	18	82	12.66	325	6.63
Kottayam	27	24	88	13.59	1083	22.11
Palai	15	12	80	12.35	950	19.40
Malankara	18	14	70	10.81	700	14.29
Muvattupuzha	27	23	85	13.13	1357	27.70
Vaniampara	24	24	100	15.44	1625	33.18
Palakkad	15	13	87	13.43	950	19.40
Manjeri	10	6	60	9.27	700	14.29
Calicut	20	20	100	15.44	1950	39.82
Kanhikulam	15	15	100	15.44	2050	41.86
Peruvannamoozhy	15	12	80	12.35	868	17.72
Ulickal	10	7	70	10.81	434	8.86
Padiyoor	20	20	100	15.44	1500	30.63
Alakode	15	15	100	15.44	96	1.96
Nettana	10	2	20	3.08	50	1.02
Dapchari	5	2	40	6.18	36	0.74

Plate 1. Indicator Plant (*Lycopersicon esculentum*)

Table 2. Infectivity of *Meloidogyne* spp. in soil samples (Mean of five observations).

Location	Population/ g of soil	Gall index (GI)
Kulasekharam	2.8	2.4
Paraliar	0.38	0.0
Nedumangad	1.8	2.0
Punalur	8.9	4.8
Perumpulickal	1.2	1.0
Chethackal	1.3	1.0
Kottayam	4.33	3.4
Palai	3.8	3.2
Malankara	2.8	2.4
Muvattupuzha	5.4	3.6
Vaniampara	6.5	3.6
Palakkad	3.8	2.6
Manjeri	2.8	3.0
Calicut	7.8	3.8
Kanhikulam	8.2	4.0
Peruvannamoozhy	3.5	3.2
Ulickal	1.7	1.8
Padiyoor	6.0	3.8
Alakode	0.4	0.0
Nettana	0.2	0.0
Dapchari	0.14	0.2

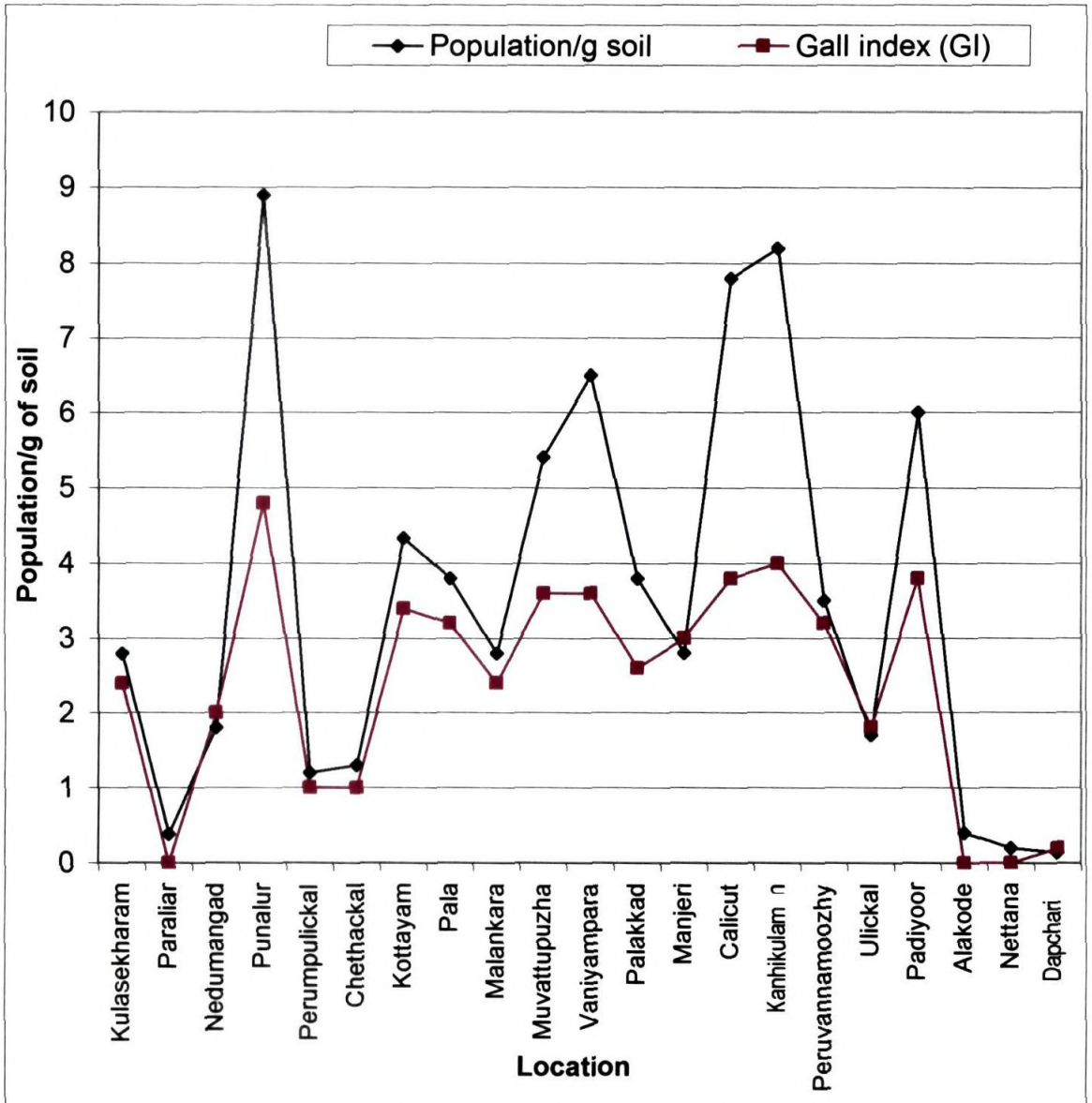

Fig. 5 Infectivity of *Meloidogyne* spp. in soil samples

4.2 Effect of inoculation with *M.incognita* on the growth, biomass, nodulation and nitrogen fixation in *P.phaseoloides*

The results presented in Table 3 show that there were significant differences in the growth characteristics of the plants between nematode inoculated and uninoculated series. However, with *Bradyrhizobium* inoculation done simultaneously or 10 days after nematode inoculation, the adverse effect of nematode was found to be overcome to some extent and a growth increase of 5 to 13 perc ent over control was recorded. But nematode inoculation at higher levels such as 3000 or 4000 larvae per pot along with *Bradyrhizobium* or 10 days prior or later to *Bradyrhizobium* caused growth reduction ranging from 8-25 per cent over control and also led to yellowing or shedding of leaves indicating that nematode infestation was a limiting factor. Even though no significant reduction in shoot length was obtained, appreciable difference could be seen between nematode inoculated and uninoculated control plants (plate2-7). An increase in shoot length ranging from 12 to 30 per cent over control was recorded in nematode inoculated series compared to control plants. Maximum percentage increase in shoot length (15.86) was observed in *Bradyrhizobium* alone inoculated plants. Significant differences in the development of root system were also recorded in inoculated and uninoculated plants. *Bradyrhizobium* inoculated plants showed 33 percent increase of root length over control. But addition of nematodes simultaneously or 10 days prior or after *Bradyrhizobium* significantly

reduced the root growth. In the nematode alone inoculated plants the percentage of reduction was more. Similar was the trend regarding the fresh and dry weight of shoot and root of plants inoculated with both the organisms simultaneously or one after the other compared to uninoculated plants. Increase in root weight was noticed in combined and pre-inoculation of *Bradyrhizobium* with nematodes at 10 level of inoculum.

Nematode infestation significantly reduced nodulation in all the treatments. In treatments where nematode inoculation preceded *Bradyrhizobium* inoculation, the reduction in nodulation was more than in other treatments. Maximum reduction of nodules (82.32 per cent) was noticed in plants inoculated with root knot nematodes at 4000 level of inoculum followed by *Bradyrhizobium* Maximum number of nodules were observed in *Bradyrhizobium* alone inoculated plants. Similarly, significant reduction in the number of galls was observed in plants inoculated with *Bradyrhizobium* and nematode either simultaneously or one after the other compared to nematode inoculation alone (Table 4). Maximum number of galls (120/plant) was recorded in nematode alone inoculated plants at 4000 level of inoculum (Plate 8).

Plate 2. **Control (*Pueraria phaseoloides* without *Meloidogyne incognita* and *Bradyrhizobium* inoculation)**

Plate 3. ***P. phaseoloides*** **inoculated with 4000 level of nematode inoculum**

Plate 4. ***P. phaseoloides*** **inoculated with** ***Bradyrhizobium*** **alone**

Plate 5. ***P. phaseoloides*** **with simultaneous inoculation of** ***Bradyrhizobium*** **and** ***M. incognita***

Plate 6. ***P. phaseoloides*** **inoculated with** ***Bradyrhizobium*** **followed by** ***M. incognita*** **at 10 days interval**

Plate 7. ***P. phaseoloides*** **inoculated with** ***M. incognita*** **followed by** ***Bradyrhizobium*** **at 10 days interval**

Table 3. Effect of *Meloidogyne incognita* and *Bradyrhizobium* on the growth of *Pueraria phaseoloides* (Average of three replications).

Treatment	Shoot length (cm)	Shoot weight (g)		Root length (cm)	Root weight (g)	
		Wet	Dry		Wet	Dry
R	80.33 (+15.86)	5.83 (+24.30)	0.73 (+23.72)	32.22 (+32.88)	2.33 (+14.22)	0.53 (+82.76)
R+N_1	72.66 (+4.80)	4.05 (-13.65)	0.50 (-15.25)	23.66 (-2.75)	2.06 (+0.98)	0.37 (+27.59)
R+N_2	65.33 (-5.70)	4.12 (-12.15)	0.48 (-18.64)	22.33 (-8.22)	1.96 (-3.92)	0.27 (-6.90)
R+N_3	63.66 (-8.18)	3.67 (-21.75)	0.42 (-28.81)	19.33 (-20.55)	1.95 (-4.41)	0.24 (-17.24)
R+N_4	52.00 (-24.94)	2.77 (-40.94)	0.34 (-42.37)	14.00 (-42.45)	1.78 (-12.75)	0.22 (-24.14)
R-N_1	74.00 (+6.74)	4.14 (-11.73)	0.53 (-10.17)	29.33 (-20.55)	2.16 (+5.88)	0.46 (+58.62)
R-N_2	62.33 (-10.09)	3.50 (-25.37)	0.47 (-20.34)	23.66 (-2.75)	2.00 (-1.96)	0.25 (-13.79)
R-N_3	59.00 (-14.89)	3.48 (-25.80	0.45 (-23.73)	21.00 (-13.69)	1.83 (-10.29)	0.22 (-24.14)
R-N_4	51.33 (-25.96)	3.63 (-22.60)	0.46 (-22.03)	18.66 (-31.52)	1.57 (-23.03)	0.18 (-37.93)
N_1-R	78.00 (+12.50)	3.68 (-23.03)	0.41 (-30.51)	16.66 (-31.52)	1.45 (-28.92)	0.28 (-3.45)
N_2-R	72.3 (+4.32)	2.64 (-43.71)	0.37 (-37.29)	15.66 (-36.99)	0.93 (-54.41)	0.26 (-10.34)
N_3-R	56.33 (-18.75)	2.18 (-53.52)	0.35 (-40.68)	15.33 (-36. 99)	0.95 (-53.43)	0.24 (-17.24)
N_4-R	54.33 (-21.15)	2.01 (-57.14)	0.28 (-52.54)	14.66 (-39.75)	1.35 (-33.82)	0.15 (-48.27)
N_1	59.00 (-14.89)	3.61 (-23.03)	0.38 (-35.29)	20.00 (-17.79)	1.96 (-3.92)	0.20 (-31.03)
N_2	61.00 (-12.01)	3.00 (-36.03)	0.31 (-47.46)	15.00 (-38.35)	1.74 (-14.70)	0.16 (-44.83)
N_3	59.66 (-13.95)	2.07 (-55.86)	0.29 (-50.85)	14.33 (-41.10)	0.98 (-51.96)	0.11 (-62.09)
N_4	48.33 (-30.29)	1.63 (-65.25)	0.26 (-55.93)	10.66 (-56.18)	1.02 (-50.00)	0.10 (-65.52)
Control	69.33	4.69	0.59	24.33	2.04	0.29
P= 0.05	N.S	2.01	0.25	7.00	0.92	0.16

R = *Bradyrhizobium* alone, R + N series = *Bradyrhizobium* and nematode inoculated simultaneously, R - N series = *Bradyrhizobium* followed by nematode at 10 days

N-R series = Nematode followed by *Bradyrhizobium* at 10 days interval.

N_1 = 1000 larvae/pot; N_2 = 2000 larvae/pot; N_3 = 3000 larvae/pot and

N_4 = 4000 larvae/pot; N.S. Not significant

Figures in parentheses represent percent increase (+)/decrease(-) over control.

Table 4. Effect of *M.incognita* and *Bradyrhizobium* on nodulation and nematode galls on *P.phaseoloides* (Average of three replications).

Treatment	No. of nodules per plant	No. of galls per plant
R	164	-
$R+N_1$	140 (14.63)	48.00 (5.25)
$R+N_2$	121 (26.22)	56.33 (3.43)
$R+N_3$	105 (35.98)	79.33 (4.02)
$R+N_4$	61 (62.80)	110.66 (7.78)
$R-N_1$	80 (51.22)	32.00 (36.83)
$R-N_2$	66 (59.76)	43.66 (25.15)
$R-N_3$	54 (67.07)	57.00 (31.04)
$R-N_4$	34 (79.27)	78.88 (34.45)
N_1-R	77 (53.05)	44.33 (12.49)
N_2-R	64 (60.98)	62.66 (8.55)
N_3-R	45 (72.56)	80.33 (2.82)
N_4-R	29 (82.32)	81.66 (31.95)
N_1	Nil	50.66
N_2	Nil	58.33
N_3	Nil	82.66
N_4	Nil	120.00
Control	Nil	Nil
CD at 5%	28.33	21.64

Figures in parentheses represents percent decrease of nodules and galls over *Bradyrhizobium* alone and nematode alone inoculated plants respectively.

Plate 8. **Root-knot nematode, *M. incognita* infestation in *P. phaseoloides* at 4000 level of inoculum**

Table 5. Effect of *M.incognita* and *Bradyrhizobium* on nitrogen content of *P.phaseoloides* 60 days after inoculation (mean of 3 replications)

Treatment	Nitrogen content (%)	
	Shoot	Root
R	2.60 (+28.07)	2.77 (+37.81)
N_1	1.91 (-5.91)	1.96 (-2.48)
N_2	1.80 (-11.33)	1.84 (-8.45)
N_3	1.57 (-22.66)	1.58 (-21.39)
N_4	1.35 (-33.50)	1.47 (-26.86)
$R + N_1$	1.98(-2.46)	1.93(-3.98)
$R + N_2$	1.91(-5.91)	1.86(-7.46)
$R + N_3$	1.84(-9.36)	1.80(-10.45)
$R + N_4$	1.78(-12.32)	1.74(-13.43)
$R\text{-}N_1$	2.49(+22.66)	2.47(+22.89)
$R\text{-}N_2$	2.45(+20.69)	2.39(+18.91)
$R\text{-}N_3$	2.30(+13.30)	2.20(+9.45)
$R\text{-}N_4$	2.19(+7.88)	2.16(+7.46)
$N_1\text{-}R$	2.14(+5.42)	2.11(+4.98)
$N_2\text{-}R$	1.77(-12.81)	1.58(-21.39)
$N_3\text{-}R$	1.70(-16.26)	1.54(-23.38)
$N_4\text{-}R$	1.57(-22.66)	1.39(-30.85)
Control	2.03	2.01
CD at 5%	0.18	0.20

Figures in parentheses denote per cent increase (+)/decrease (-) over control

Nematode inoculation adversely affected the total nitrogen content of shoot and root of *P.phaseoloides* (Table 5). Nitrogen content (per cent) of both shoot and root was significantly higher when *Bradyrhizobium* inoculation preceded nematode inoculation. Further, the nitrogen content of shoot of *P.phaseoloides* inoculated with 4000 larvae/plant was on par to that of the plants at 3000 and 2000 levels of inoculum. However, the nitrogen content of root at all the levels of nematode were on par when inoculation with *Bradyrhizobium* preceded nematode inoculation. Maximum nitrogen content was recorded in *Bradyrhizobium* alone inoculated plants with the per cent increase of 28.07 and 37.81 over control in the shoot and root respectively. In nematode alone inoculated plants a per cent decrease of 5.91, 11.33, 22.66, 33.50 and 2.48, 8.45, 21.39 and 26.86 nitrogen content in the shoot and root of *P.phaseoloides* at 1000, 2000, 3000 and 4000 level of nematode inoculum respectively was observed.

4.3 Effect of seasonal variation in nematode population and gall formation on *Pueraria phaseoloides*

Seasonal fluctuations of root-knot nematode (*M.incognita*) population and intensity of infection on *P.phaseoloides* with respect to atmospheric temperature and rainfall are furnished in Table 6. It is seen that the nematode population depends on these two environmental factors and host plant. During the study period, the maximum temperature ranged

from 29.1^{0}C to 34.5^{0}C and the temperature recorded were almost the same during September and October months (30.7^{0}C and 30.8^{0}C). Increase in the temperature was noticed from November onwards with a maximum of 34.5^{0}C during March. From April onwards, the maximum temperature declined from 33.6^{0}C to 29.1^{0}C. Highest temperature and population were recorded during March and April. The trend of population showed that at the maximum temperature of 31.9^{0}C to 34.5^{0}C the nematode population was high while decrease in temperature recorded from May onwards caused a perceptible decrease in population. Unlike temperature, significant differences were observed in the month wise distribution of rainfall and root infection. Maximum rainfall was observed during June but the nematode population and host infection were comparatively low. Highest nematode population (1400/250g. soil) and host infection (17.17 galls/plant) were noticed during the month of March when the temperature was very high (34.5^{0}C). The rainfall recorded during March was only 27.6 mm. Nematode population of soil showed an increasing trend during the summer months (Fig 6). Correlation coefficient analysis among variables showed that environmental temperature had significant influence on the nematode population and host infection. similarly the intensity of infection was highly correlated with the nematode population (Table 7)

Table 6. Mean monthly atmospheric temperature, rainfall, nematode population and infestation (RRII Experiment Station- September 2000 –August 2001).

Months	Temperature ^{0}C (Maximum)	Rainfall mm	*Nematode Population per 250 g soil	*Galls per plant
September	30.7	246.0	533	6.25
October	30.8	155.1	488	5.67
November	32.0	90.7	366	3.56
December	31.9	30.2	710	7.54
January	32.9	23.0	976	9.34
February	33.1	47.6	1000	10.04
March	34.5	27.6	1400	17.17
April	33.6	367.0	1200	11.88
May	32.1	240.0	976	8.71
June	29.5	657.2	350	3.0
July	29.1	600.07	283	3.0
August	29.7	284.9	462	3.25

* Mean of eight replications.

Table 7. Correlation of nematode population and infection at different months with temperature and rainfall

Variable	Covariance	Correlation
Temperature Vs Population	531.3109	0.9097
Temperature Vs Infection	6.1203	0.9038
Rainfall Vs Population	-36811.493	-0.4964NS
Rainfall Vs Infection	-445.99	-0.5188 NS
Population Vs Infection	1412.98	0.96815

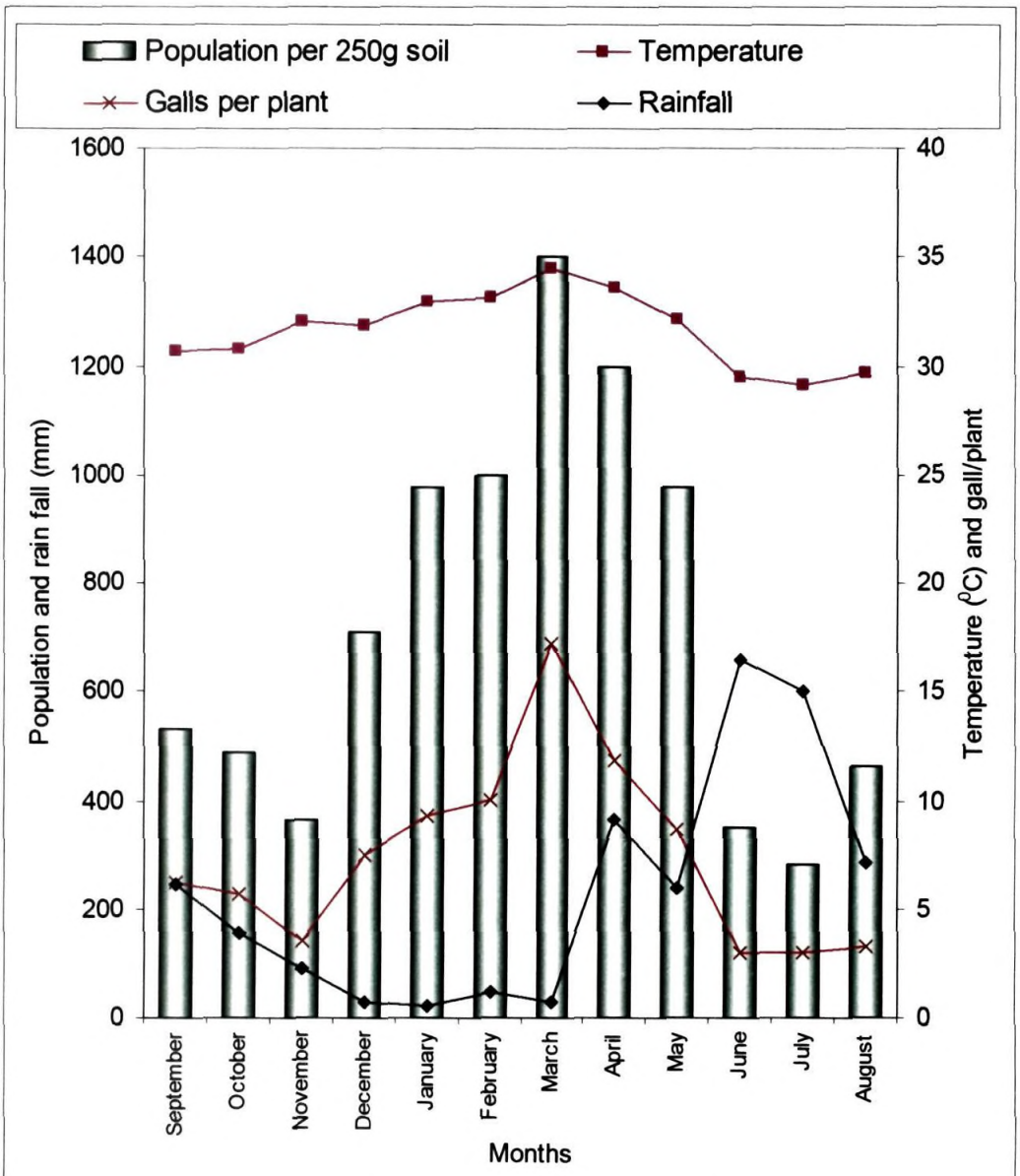

Fig 6 Seasonal variation in the population of *M. incognita* in soil and host infestation

4.4 Interaction of VAM and *Meloidogyne incognita* on *Pueraria phaseoloides*

The effect of interaction of VAM and root-knot nematode, *M.incognita* on the growth parameters of *P.phaseoloides* presented in Table 8 indicated that the root knot nematode *M.incognita* singly or in combination with mycorrhizal fungi irrespective of the time of inoculation reduced plant growth considerably. VAM alone inoculated plants had shown significantly better growth and weight of shoot and root than the other treatments. Inoculation of plants with root-knot nematode alone caused significant reduction of all growth parameters. Establishment of VAM one or two weeks prior to nematode inoculation showed better plant growth characters than simultaneous inoculation of VAM and nematode. Plants inoculated with nematode first and one or two weeks later with VAM showed significantly lower growth compared to plants inoculated first with VAM although superior to nematode alone inoculated plants. When nematode and mycorrhizal fungi were inoculated simultaneously, the growth response induced by VA mycorrhizal fungi was lesser than that in plants inoculated with VAM alone though they were superior to uninoculated control and nematode alone inoculated plants. Microscopic observations on the cleared and stained roots showed that mycorrhizal fungi colonized roots of *P.phaseoloides* at varying levels (plate 9-12).

Maximum mycorrhizal colonization and spore count were observed for VAM alone inoculated plants (Table 9).

Table 8. Effect of inoculation of *M.incognita* and VAM on growth*of *P.phaseoloides.*

Treatment	Shoot			Root		
	Length (cms)	Fresh weight (g)	Dry weight (g)	Length (cm)	Fresh weight(g)	Dry weight(g)
Control	59.67	5.82	0.77	18.00	1.33	0.15
VAM	115.00	8.59	1.34	49.33	2.78	0.32
M	19.00	2.92	0.22	6.33	0.05	0.006
VAM+M	75.00	5.78	0.48	23.00	1.69	0.18
VAM-M7	86.00	7.98	0.98	33.33	1.37	0.15
M-VAM7	45.00	5.28	0.75	18.00	0.70	0.08
VAM-M14	92.33	8.22	0.58	38.33	1.14	0.12
M-VAM14	63.33	3.92	1.17	18.33	1.77	0.19
(CDat5%)	16.23	2.34	0.66	10.04	1.12	0.13

Control = No VAM or nematode

VAM = VAM alone

M = Nematode alone

VAM+M = Simultaneous application of VAM and nematodes

VAM-M_7 } = Inoculation of VAM and nematode one week prior or later to each other

VAM_7 }

VAM-M_{14} } = Inoculation of VAM and M two weeks prior or later to each other

M-VAM_{14} }

*Values mean of 3 replications

The results also showed that inoculation of VAM and *M.incognita* one or two weeks prior or later to each other caused significant reduction in the formation of mycorrhizal spores. Maximum root galls formation was observed in nematode alone inoculated plants. Plants inoculated with VAM first and two weeks later with nematodes had significantly lower number of galls and nematode population. The multiplication rate of root knot nematode *M.incognita* was found to decrease from 41.90 to 71.43 per cent due to the inoculation of VAM. While maximum multiplication rate (2.1) was observed in the treatment inoculation with nematode alone. Lowest rate of multiplication (0.60) was recorded from plants inoculated with VAM first and two weeks later with nematode (Table 9). Plants inoculated with nematode resulted in stunted growth while those inoculated with VAM recorded improvement of plant growth. Pre-inoculation of VAM ensured higher plant growth, less number of galls/plant and reduced nematode population in soil when the nematode infection succeeded it by 14 days.

Table 9. Effect of inoculation of *M.incognita* and VAM on mycorrhizal root infection, spore count, gall formation and nematode multiplication on *P.phaseoloides*. (mean of three replications).

Treatment*	Mycorrhizzal colonization (%)	VAM spore count / 50 ml soil	Number of galls per plant	Final nematode population per 250 g. soil	Multiplication rate of nematode per/250g soil
Control	0.00	0.00	0.00	0.00	0.00
VAM	72.67	2855.33	0.00	0.00	0.00
M	0.00	0.00	91.67	2111.00	2.1
VAM + M	63.00	1751.67	23.33	1066.67	1.06
VAM- M_7	61.33	791.67	24.00	677.67	0.67
M– VAM_7	66.67	1410.00	13.33	811.00	0.81
VAM–M_{14}	67.67	1985.00	4.33	600.67	0.60
M–VAM_{14}	47.33	1293.33	39.00	1221.67	1.22
(P = 0.05)	19.55	1098.53	20.62	530.17	

* same as in table 8.

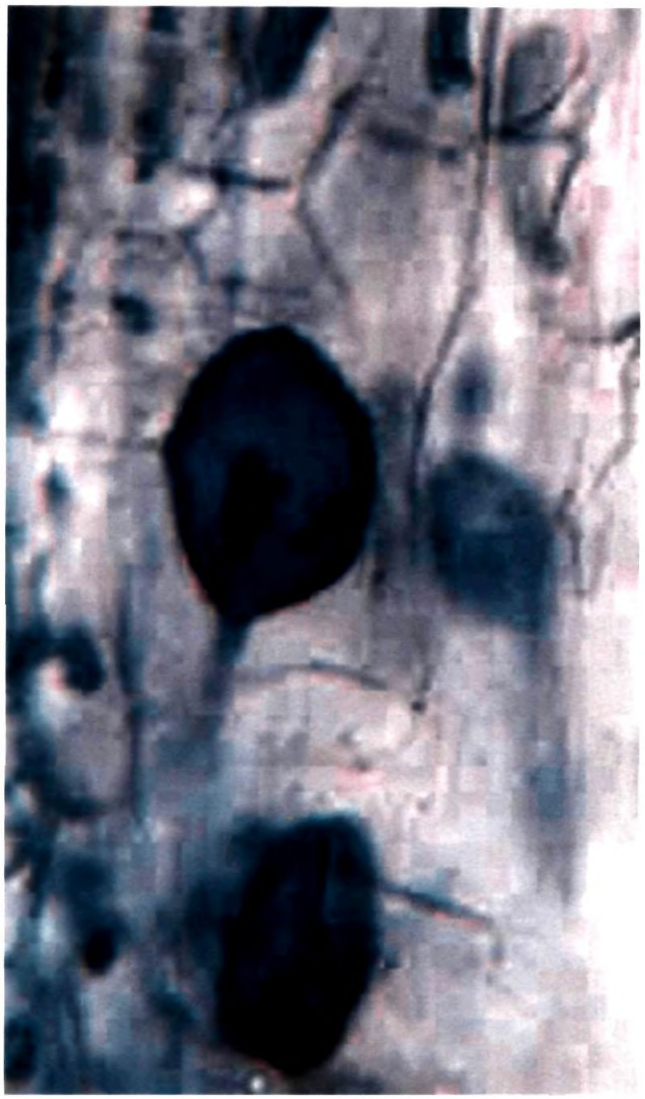

Plate 9. **Vesicles of *Glomus fasciculatum* in *P. phaseoloides***

Plate 10. **Arbuscules of *G. fasciculatum* in *P. phaseoloides***

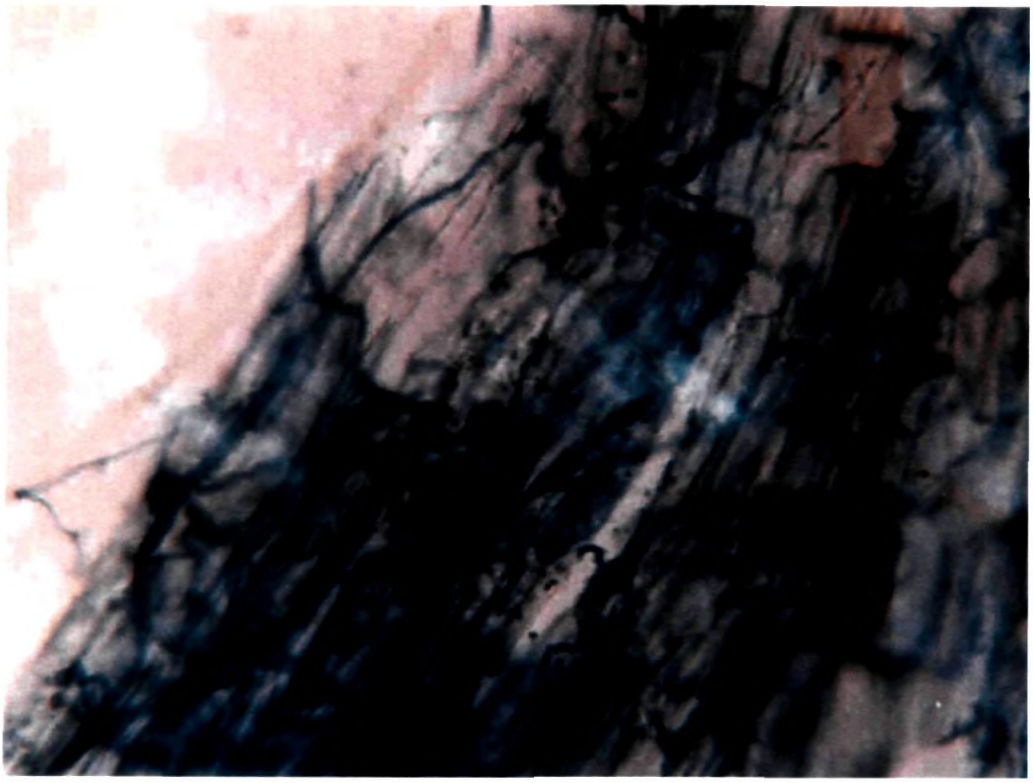

Plate 11. Hyphae of *G. fasciculatum*

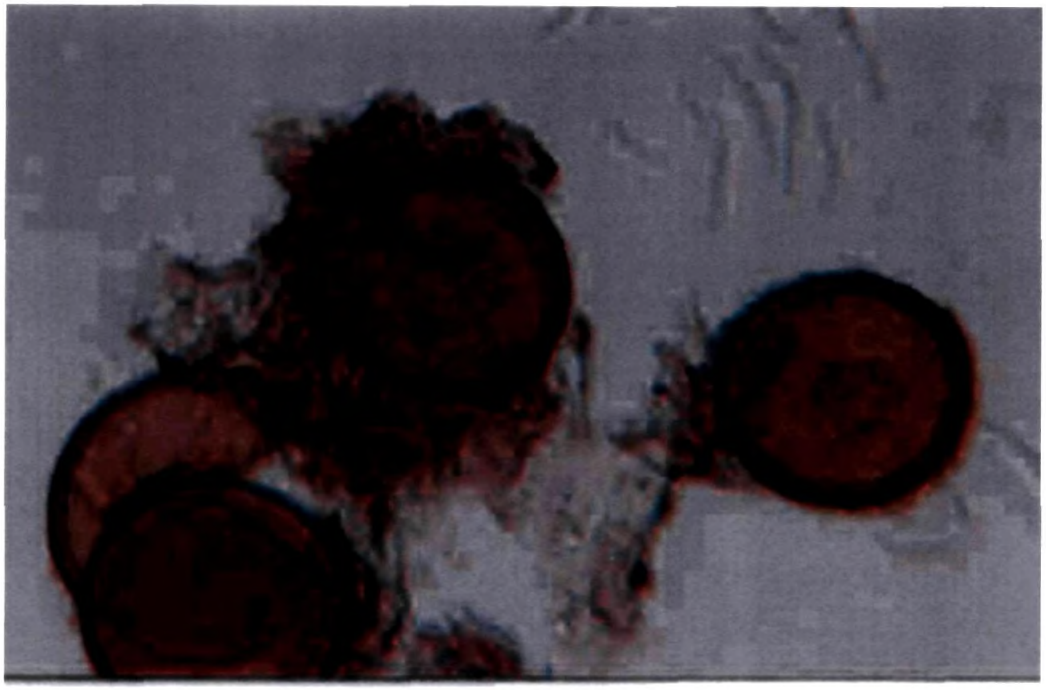

Plate 12. Spores of *G. fasciculatum*

4.5 Effect of different inoculum levels of *Meloidogyne incognita* on the growth and changes in biochemical constituents of *Pueraria phaseoloides*

The inoculated plants exhibited stunted growth compared to control plants(plate 13-16). The parasitic nature of root-knot nematode, *M.incognita* was evident as indicated by the reduction in shoot length, shoot weight, root length and root weight. The active parasitism of nematode was reflected in the increase in number of galls, number of egg masses and final population of nematodes with incremental inoculum levels (Table 10). In general, the data revealed that with increase in nematode inoculum, there was a corresponding decrease in plant growth. An initial inoculum level of 10,000 larvae caused 79.79, 86.11 and 62.84 per cent reduction in shoot length, shoot weight and root length over control where as with 10 larvae it was 20.38, 37.60, 17.57 per cent. The root weight of the plants showed a reduction of 7.02 and 26.32 per cent over control in 1000 and 10,000 levels of inoculum respectively. Total number of egg masses and galls also showed an increasing trend with the increase of inoculum levels. Maximum number of egg masses (25.80/plant) and galls (37.60/plant) were recorded from 10,000 level of inoculation. But maximum nematode population of 2504 per 250g. soil was observed in 1000 level of inoculum . Multiplication rate of nematode was found decrease with increase in inoculum levels. Highest multiplication rate of 50.8 was observed in the lowest inoculum level.

4.5.1 Changes in biochemical constituents

There was considerable interference in the metabolism of plant due to the inoculation of root-knot nematode, *M. incognita*. The results presented in Fig.7-10 show that accumulation of total phenols, *ortho*-dihydroxy phenols, reducing sugars, total sugars, amino nitrogen and reduction in starch levels were observed in the inoculated plants compared to un inoculated control.

4.5.2 Changes in total phenols and *ortho*-dihydroxy phenols

Inoculation of different levels of *M.incognita* on *P.phaseoloides* considerably altered the content of both total phenol and *ortho*-dihydroxy phenol in the leaves (fig. 7). An increase in phenol content with increase of inoculum levels of nematode was recorded.

4.5.3 Changes in reducing and non-reducing sugars

Reducing and non-reducing sugars increased with increase in nematode inoculum. Infested plants at all levels of inoculum had more reducing and non-reducing sugars compared to uninoculated plants (fig.8). The plants at 10,000 level of inoculation exhibited higher reducing / non-reducing sugars over control followed by 1,000 100, and 10 level of inoculation.

4.5.4 Changes in amino nitrogen

In general, an increase in the level of nematode inoculum from 10 to 10,000 markedly increased the content of amino nitrogen in the leaves

(fig.9). At the lowest level of inoculum, the increase of amino nitrogen over control was only marginal but it gradually increased with the increasing level of nematode inoculum.

4.5.5 Changes in starch

Unlike other biochemical constituents, starch content of *Pueraria phaseoloides* leaves was found to decrease with increase in inoculum levels. As the nematode inoculum increased, a corresponding decrease in starch content was recorded (fig.10).

Table 10. Effect of different inoculum levels of *M.incognita* on the growth of *P.phaseoloides* and nematode multiplication

Inoculum levels	Shoot		Root		No. of egg masses per plant	No. of galls per plant	Nematode population per 250 g. soil	Multiplication rate of nematode Per 250g soil
	Shoot length (cm)	Shoot weight (g)	Root length (cm)	Root weight (g)				
Control 0	114.80	8.35	29.60	1.14	Nil	Nil	Nil	Nil
10	91.40 (20.38)	5.21 (37.60)	24.40 (17.57)	1.34	2.80	5.8	508.4	50.8
100	56.40 (50.87)	4.75 (43.11)	18.00 (39.19)	1.78	7.00	17.60	794.00	7.94
1,000	44.80 (60.98)	2.85 (65.87)	13.40 (54.73)	1.06 (7.02)	15.20	31.80	2504.00	2.50
10,000	23.20 (79.79)	1.16 (86.11)	11.00 (62.84)	0.84 (26.32)	25.80	37.60	1965.20	0.20
CDat5%	55.39	3.05	13.13	0.57	6.76	10.80	395.75	

Figures in parentheses represent percent increase over control.(Average of 5 replications).

Plate 13. Effect of inoculation of *M. incognita* at 10 level of inoculum on the growth of *P. phaseoloides* (60 days after inoculation)

Plate 14. Effect of inoculation of *M. incognita* at 100 level of inoculum on the growth of *P. phaseoloides* (60 days after inoculation)

Plate 15. Effect of inoculation of *M. incognita* at 1000 level of inoculum on the growth of *P. phaseoloides* (60 days after inoculation)

Plate 16. Effect of inoculation of *M. incognita* at 10000 level of inoculum on the growth of *P. phaseoloides* (60 days after inoculation)

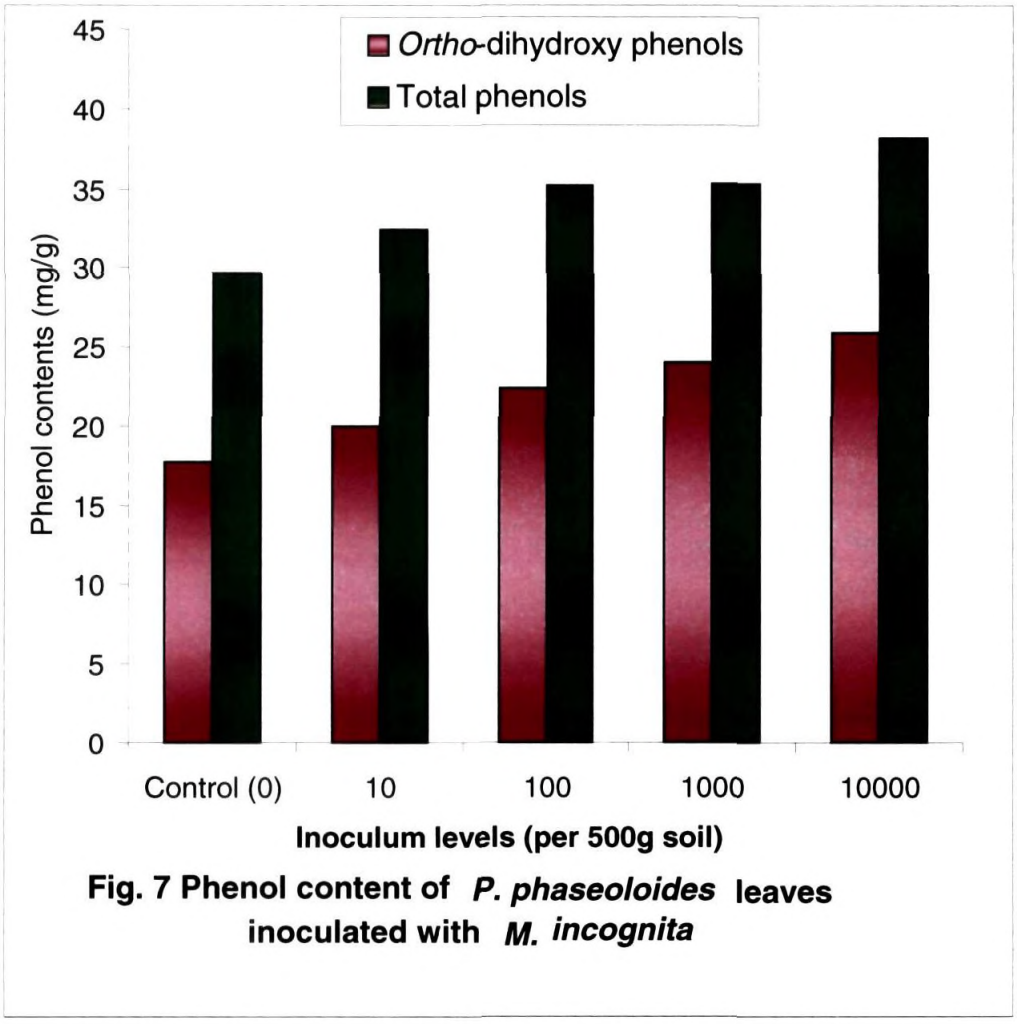

Fig. 7 Phenol content of *P. phaseoloides* leaves inoculated with *M. incognita*

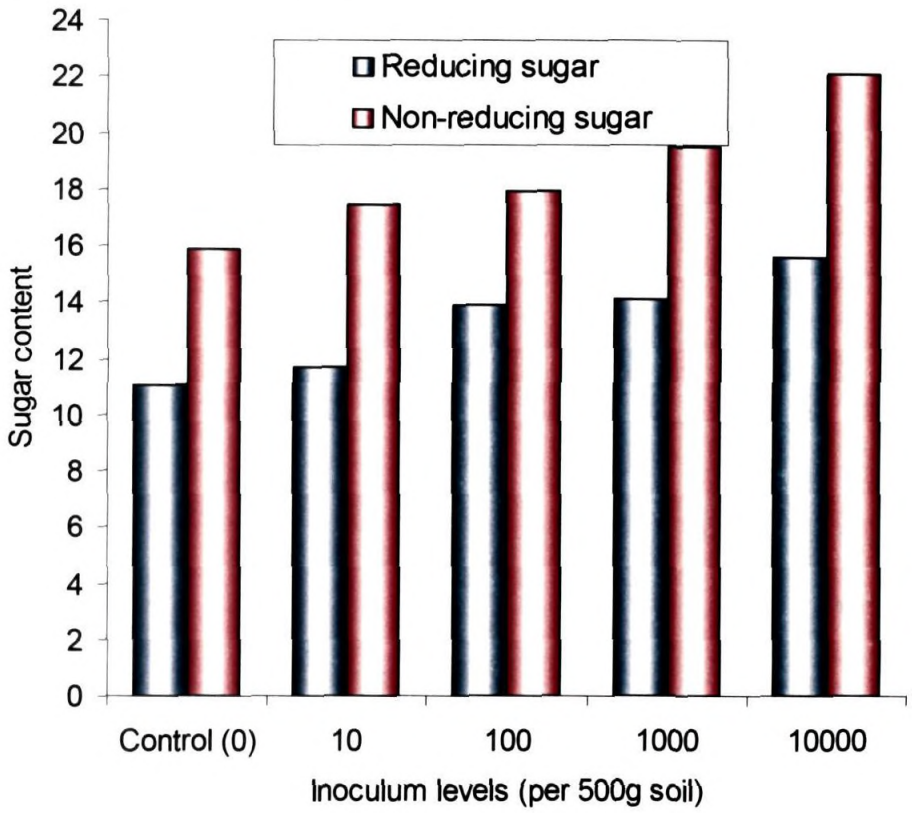

Fig.8 Sugar content of *P. phaseoloides* leaves inoculated with *M. incognita*

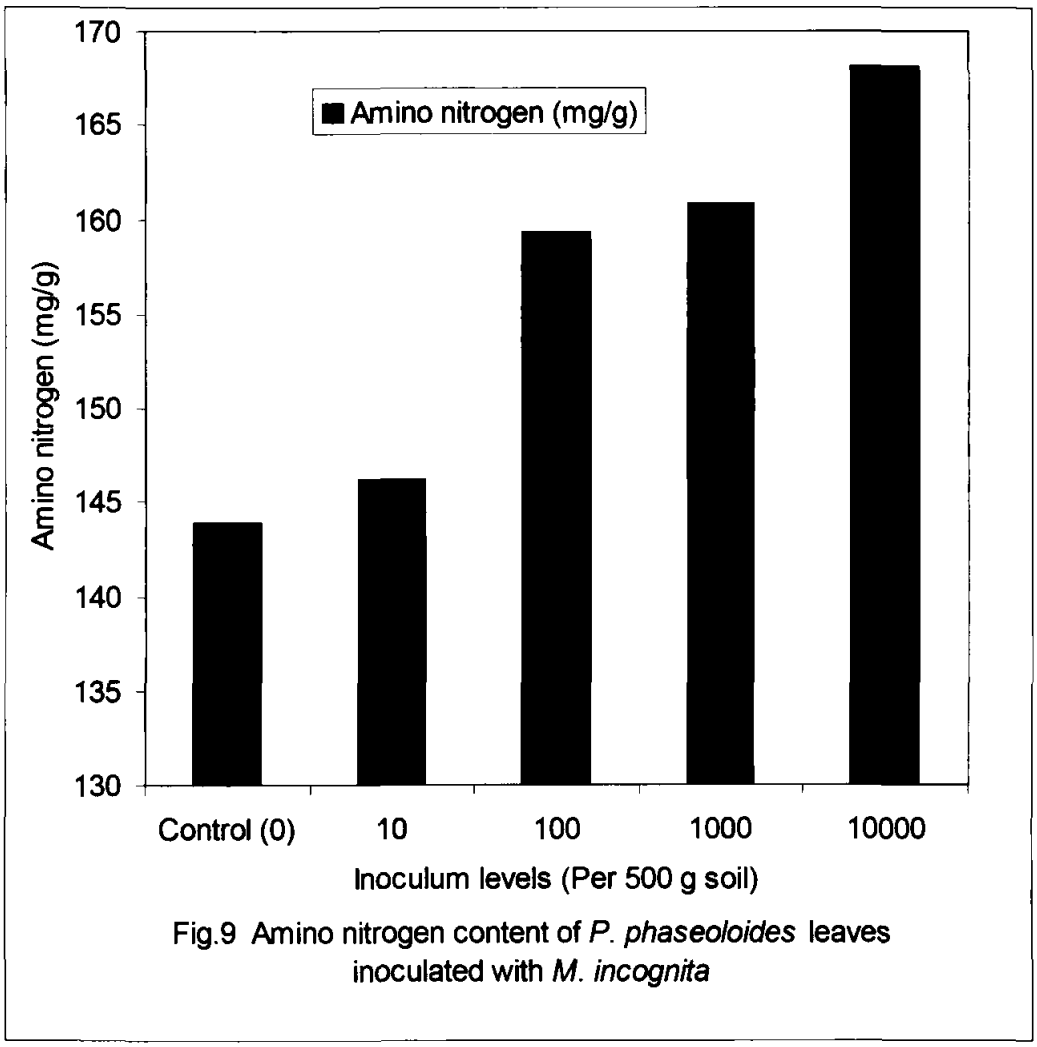

Fig.9 Amino nitrogen content of *P. phaseoloides* leaves inoculated with *M. incognita*

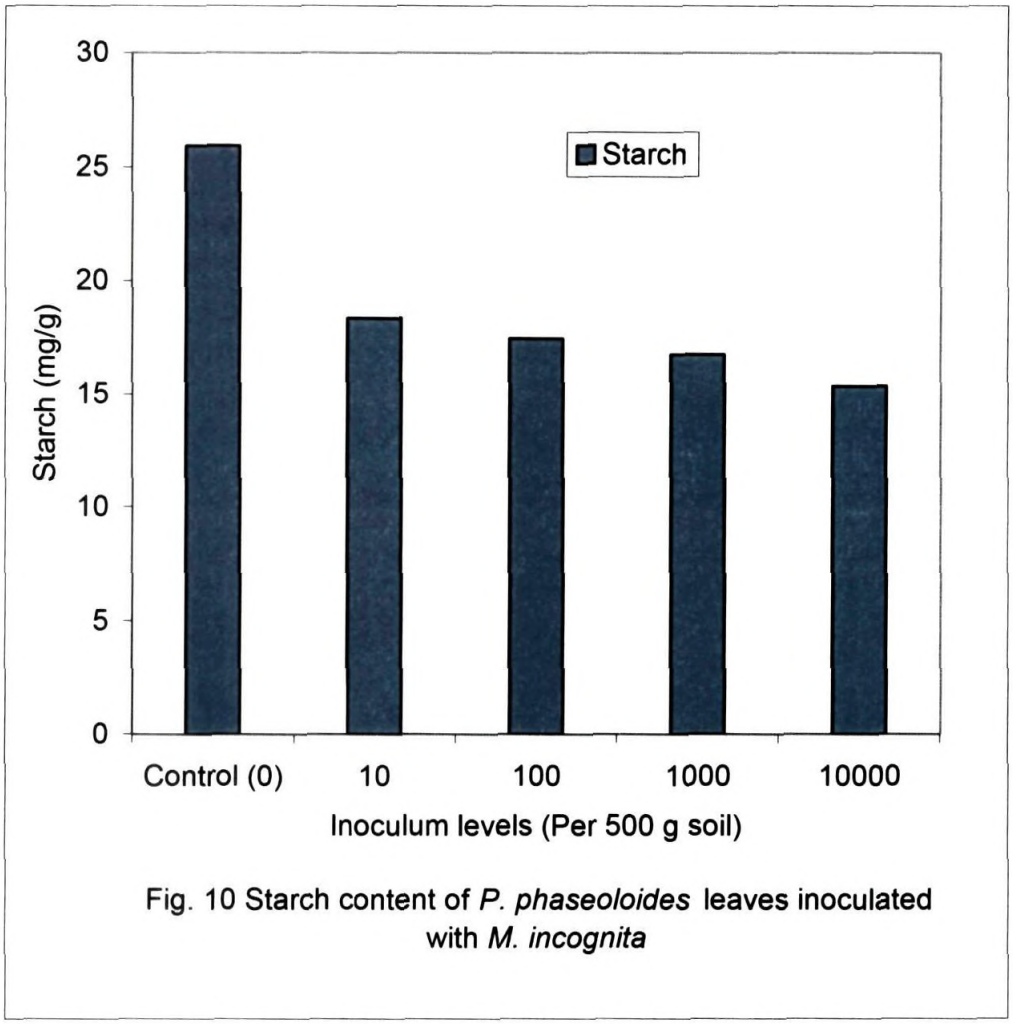

Fig. 10 Starch content of *P. phaseoloides* leaves inoculated with *M. incognita*

Chapter V

DISCUSSION

DISCUSSION

5.1 Frequency and distribution of plant parasitic nematodes in rubber plantations

Root knot caused by *Meloidogyne* spp. is one of the most economically important nematode diseases on crop plants in India. Edaphic and environmental factors greatly influence the local as well as regional distribution and population dynamics of nematodes.

A survey covering twenty one rubber growing locations of Kerala, Tamilnadu and Maharashtra for the frequency and distribution of nematodes has revealed the occurrence of twelve important genera of plant parasitic nematodes. It also exposed, the possible parasitic association of *Longidorus, Xiphinema, Trichodorus, Criconemoides, Radopholus, Hoplolaimus* and *Hemicycliophora s*pp. with rubber for the first time in India . *Meloidogyne* spp. has the wide range of occurrence (above 85%) and ubiquitous presence throughout the area. *Helicotylenchus* spp. represented more than 65% frequency of occurrence. Next in order in the range of occurrence is *Aphelenchoides, Hemicriconemoide, Longidorus, Tylenchus, Criconemoides, Radopholus* and *Hoplolaimus* spp. The rest of the nematode genera show only a limited range of distribution. The frequency and density of nematodes as explained by absolute and relative density and frequency factors in the present investigation show variations. This can also be attributed to the fact that climatic conditions, soil types

and agronomic practices vary in each location studied, thus resulting in a varied population. The occurrence of plant parasitic nematodes associated with variety of crops have been reported by several workers (Rama and Dasgupta, 1987; and Sundararaju and Mehta, 1994). However wide variations were recorded in the frequency of occurrence and population density of nematodes. Climatic and soil conditions, varieties of plants, cropping sequences and various agricultural practices are reported to determine the spread of different genera and species of plant parasitic nematodes in a particular region (Mukhopadhyaya and Prasad, 1986). Mehta *et al.* (1993) concluded that soil types of different location are probably the most important factor controlling the spread of nematode species in different areas The result of the present study showing wide variations in nematode population is in confirmity with the above findings.

The soil samples collected from different locations also showed variations in infectivity on the indicator plant, *Lycopersicon esculentum.* The samples having a population of two nematodes and above per gram soil showed high gall indices (>2) on rootlets of indicator plants. Thus two larvae per gram soil can be regarded as an optimum damaging threshold level of *Meloidogyne incognita* as reported by Nath *et al.* (1979) and Mani (1983). Rao and Krishnappa (1994) also reported an inoculum of 2 larvae per g. soil as an optimum damaging threshold level in cultivars of chickpea under glass house conditions. A range of gall index from 0.2 to

4.8 was recorded in the present study. Higher gall index indicates high population build up which may endanger the crop. Sharma (1988) and Sharma *et al.* (1991) conducted similar infectivity studies with the soil samples collected from the rhizosphere of groundnut and recorded variations in the gall forming behaviour of indicator plant.They have noticed mild to severe gall formation in indicator plant.

Out of 349 soil samples collected from rubber growing areas, 298 samples showed the presence of root-knot nematodes with considerable variations in distribution pattern. The predominance of root-knot nematode among the plant parasitic nematode is established and has been reported by many workers. In citrus Mani (1986) and in cardamom Ramana and Mohandas (1987) reported the occurence and prevalence root knot nematode, *Meloidogyne incognita*. A survey of tea nurseries in south India revealed that about 87 percent of the soil samples tested are infested with root knot nematodes (Rao *et a.*,1979). In vegetable crops about 50 percent of the fields in Meerut division in Uttar Pradesh were reported to be infested with *Meloidogyne* spp.(Abrar and Khan, 1990).

5.2 Effect of *M.incognita* on the growth, biomass, nodulation and nitrogen fixation in *P. phaseoloides*

The present study indicate that inclusion of nematode in any of the treatments at any of the levels of population cause significant decrease in the height of the plant, root length, fresh and dry weight of shoot and roots,

number of nodules and nitrogen content of shoot and root as compared to control. However with *Bradyrhizobium* inoculation made simultaneously or 10 days after nematode inoculation, the adverse effect of nematode could be reduced. It is also noticed that the number of nodules were found reduced in all treatments wherever nematode was included. The nematode inoculation prior to *Bradyrhizobium* resulted in maximum reduction of nodules. On the other hand,number of galls per root system increased with an increase in nematode inoculum.A significant decrease in the nitrogen content was recorded in nematode inoculated plants as compared to plants treated with *Bradyrhizobium* alone. The effect of interaction of *M.javanica* on the growth, nodulation and nitrogen content in *Vigna radiata* has also been reported by Bopaih *et al.*(1976).According to them, the inoculation of root-knot nematode simultaneously or 2 to 7 days preceding *Bradyrhizobium* inoculation had deleterious effect on plant growth compared to plants inoculated with *Bradyrhizobium* alone. Kumar and Vadivelu (1993) studied the inter relationship between *M. incognita* and *Rhizobium* sp. on black gram, *Vigna mungo* and reported significant decrease in the growth parameters, nodulation and nitrogen fixation by the nematodes.

Wardojo *et al.* (1963) and Taha and Raski (1969) also reported growth reduction in peas, lucerne and clover by the infestation of *Heterodera trifoli, Pratylenchus globulicola* and *M. javanica* respectively.

They have reported that the significant reduction noticed in growth and fresh and dry weight of shoot and root in the treatments with nematode alone and nematode followed by rhizobia compared to the treatment rhizobia followed by nematode.

Significant reduction in nodulation due to root-knot nematode, *M.incognita* observed in the present study is found in accordance with earlier reports such that on cowpea (Sharma *et al.*, 1976); mung bean (Chahal and Chahal, 1989); chilli(Yadav and Mathur,1993). Nutman (1958) reported that reduced nodulation in the nematode infested plants might be due to interruption in the translocation of certain materials particularly carbohydrates from the shoot and/or consumption of host plant materials during gall formation or directly by nematodes. Different theories like nutrient deficiency caused by nematodes on host plants (Masefield, 1958; Malek and Jenkins, 1964), competition between nematode and root nodule bacteria (Ichinohe, 1961; Epps and Chambers, 1962; Malek and Jenkins, 1964), antagonistic effect of root-rot organism upon root nodule bacteria (Epps and Chambers, 1962) and overall reduction of root system (Taha and Raski, 1969) has been put forth as a possible cause for reduced nodulations. Daney *et al.* (1970) reported that nematodes can secrete hydrolytic and oxidative enzymes and growth regulators which cause reduction in nodulation, host nutrition and nitrogen fixation. The reduction in nitrogen content is reported to be due to reduced root weight, overall

reduction in number of nodules, early degeneration of nodules, destruction of nodular tissue by development of nematode inside nodules and reduced leghaemoglobin content of nodules (Sharma *et al.*, 1975). It may therefore be concluded from the present study that root-knot nematode, *M.incognita* adversely affects the growth and symbiotic process of nitrogen fixation by *Bradyrhizobium* sp. on *P. phaseoloides.*

5.3 Seasonal fluctuations of *M.incognita* associated with *P.phaseoloides*

The seasonal fluctuations of root-knot nematode, *M.incognita* in the rhizosphere of *P.phaseoloides* showed variation according to temperature and rainfall. The population of *M. incognita* attained an annual peak during March-April.The lowest population was observed during June-July. The highest population of 1400 nematodes per 250 ml soil was recorded when the maximum temperature was comparatively high (34.5^0C) and the rainfall was low (27.6 mm). Temperature was observed to be directly correlated with nematode infestation. The higher temperature range of 31.9^0C - 34.5^0C induced maximum penetration of *M. incognita* into the roots of *P.phaseoloides.* These observations of the present study are substantiated by number of earlier reports. According to Elmiligy (1971), temperature is considered as a primary factor in the nematode activity, reproduction and in the host parasite relationship. Jones (1980) reported that nematode population generally increased during summer months and

decreased in winter. The rapid increase in population densities in soil during summer months suggests increased hatching of eggs (Eapen, 1993). Mohanty *et al.* (1993) reported a peak population of *M. incognita* under high temperature (30°C) and low rainfall (39mm) compared to other months in banana plantation. Azmi (1990) observed that nematode population synchronises with plant growth, rainfall and temperature.

Though no significant effect of rainfall on population was noticed, it was seen that during heavy rainfall periods of June and July the nematode population was found to be low. Vrain (1978) reported that continuous rain leads to anaerobic conditions in soil which are quite unfavourable for nematode survival.During this period the nematodes may survive as unhatched larvae in eggs which are more resistant to temperature. Similar observations were made by Jones (1980) and Choudhary and Phukan (1990). Mukherjee and Dasgupta (1993) concluded that both rainfall and continued desication depressed population near the soil surface. A significant negative correlation was observed between nematode population and monthly rainfall. Maximum root-knot nematode infestation in *P.phaseoloides* was noticed during March-April and minimum in June-July. Population peaks of *M.incognita* was found coincided with maximum root growth and infestation. Mukharjee and Dasgupta (1993) also reported the peak levels of nematodes *Helicotylenchus abunaamai* and *Helicotylenchus cocophilus* at periods of maximum root growth. Eapen

(1993) reported a rapid infestation of root-knot nematodes in plant roots during the post monsoon period and lowest during monsoon months and suggested that the optimum conditions for survival and multiplication of root-knot nematodes prevailed during the post-monsoon period. The post monsoon build up of nematode was also observed in coconut (Koshy and Sosamma, 1978)Coffee (Kumar,1991) and black pepper (Mohandas and Ramana,1988). The nematode build up in this season was reported to be mainly due to the increased availability of young and active roots (O`Bannon and Stokes, 1978). The increased root growth provides more feeding site for the nematodes (Mukherjee and Dasgupta,1993). Padhi and Das (1986) observed a co-evolutionary standpoint in host-parasite relationship and reported that the parasite may have adapted itself to the root growth of the host by timing its reproductive cycles. In the present study higher root-knots or galls per plant (17.17) was observed under maximum population density (1400/250ml soil). This observation is in confirmity with the findings of Seinhorst (1965) who reported that an association exists between soil population density of root knot nematodes and crop damage. Seinhorst (1967) reported that each crop plants have a nematode tolerance limit below which they are not affected by a given nematode species. Huijsman *et al.* (1969) reported a tolerance limit of 15-eggs per ml soils to *Globodera rostochiensis* for The Netherlands soil.

These studies are indicative of the fluctuation in population of root-knot nematodes, *M.incognita* associated with various plants and suggest that the observation as in the present study on *P.phaseoloides* population fluctuation behaviour during the various seasons of the year is expected. In general, the population is affected by temperature, rainfall and plant growth.

5.4 Influence of vesicular-arbuscular mycorrhizae on the effects and reproduction of *M. incognita* on *P. phaseoloides*

The observations in this study clearly indicated that prior establishment of mycorrhizae mitigated the deleterious effect of nematode on plant growth characters. Inoculation of *Glomus fasciculatum* one or two weeks prior to *M.incognita* caused significant enhancement in the shoot and root length, fresh and dry weight of shoot and fresh weight of root system compared to simultaneous inoculation or prior inoculation of nematode species. Similar observations were made by Hussey and Roncadori (1982) in peach. They have reported that prior application of mycorrhiza (*G.fasciculatum*) reduced the adverse effect of nematode infestation to a considerable extent than the simultaneous application or nematodes preceding mycorrhiza.

Thomas *et al.* (1989) observed that *G. fasciculatum* and *Gigaspora margarita* improved the growth of cardamom infected with *M. incognita*. *G. fasciculatum* was observed to enhance plant growth, suppress nematode population and increase yield of tomato (Sundarababu *et al.*, 1993).The

results of the present study clearly indicated that with increasing inoculum levels of *M. incognita* there was a definite decline in the plant growth, while increasing myrorrhizal levels resulted in improved the growth. Similar findings have been reported earlier by several workers (Islam *et al.*, 1980; Sundarababu *et al.*, 1996). Mycorrhizal roots are reported as more lignified than non-mycorrhizal roots (Dehne, 1982). When AM fungi penetrate into roots, they increase cell wall lignification and protect the roots from penetration by nematodes (Morandi, 1996). He also reported the occurrence of phytoalexin and phenolic compounds in endomycorrhizal interactions and their potential role in biological control. Dehne (1978) observed high Chitinase activity of mycorrhizal roots and reported that these enzymes are effective against plant pathogens like nematodes.

The effect of earlier establishment of *Glomus fasciculatum* and *Glomus epigaeus* on penetration and development of *Heterodera cajani* in cowpea was studied by Jain and Sethi (1988) who reported that pre-inoculation of mycorrhizal fungi adversely affected root penetration to a greater extent than simultaneous inoculations. Direct parasitization of galls by mycorrhizal fungi is not apparent. Upon root infection *Meloidogyne* species induce the formation of hypertrophic tissues, the galls. Inside the galls, the host reacts to nematode establishment with the formation of giant cells. The giant cells act as transfer cells for the nutrient flow towards the parasite. VAM fungi which influence the nematodes often parasitise these

cells, destroy them and thus inhibit nematode development (Dehne, 1982; Kellam and Schenck, 1980; Rakesh *et al.*, 2000).

The reduction in number of nematodes penetrating mycorrhizal roots indicate that mycorrhizal roots are undesirable sites for larval penetration and establishment of root-knot nematodes.The present findings were comparable to that of Abha *et al.*(1997) who reported reduced penetration of *M. incognita* in presence of *Glomus fasciculatum* in tomato. In addition to the reduced penetration rate of nematode into the roots of mycorrhizal host plant, the development of nematodes inside the roots is also affected adversely. Priestel (1980) found a specific decrease in the development of *Meloidogyne* larvae into females and egg production in females in the mycorrhizal roots. These observations may be of importance for sedentary nematodes in the field and may explain the present findings. Sitaramaiah and Sikora (1982) also reported that the population dynamics of nematodes can be reduced in mycorrhizal root system.

In the present study, it is found that mycorrhizal colonization percentage and spore count were significantly affected due to the presence of root-knot nematodes. Maximum root colonization percentage and spore count were obtained from VAM alone inoculated plants and minimum in VAM inoculated plants two weeks after nematode (M-VAM_{14}) and nematode inoculated plants one week after VAM inoculation (VAM-M_7)

respectively. Interference of root knot nematode, *M. incognita* with mycorrhizal infection of the root system and fungal sporulations observed in this study was similar to that reported by Schenck *et al.* (1975) in soybean . Priestel (1980) found that under the influence of *M. incognita,* the colonization of cucumber roots by *G. mosseae* was adversely affected by the nematode. Interaction between vesicular-arbuscular mycorrhiza, *M. incognita* and *Heterodera cajani* on cowpea as influenced by time of inoculation was reported by Jain and Sethi (1988). They have reported that prior establishment of VAM fungi by one or two week reduced the adverse effect of *M. incognita* and *H.cajani* on the plant growth of cowpea. They have noticed reduced number of galls in the case of *M. incognita* and number of cysts in the case of *H.cajani* by early establishment of *G.fasciculatum* in roots. Umesh *et al.* (1988) also observed significant decrease in nematode number both in roots and soil inoculated with mycorrhiza. Sitaramaiah and Sikora (1981) and Rakesh *et al.* (2000) reported the effect of vesicular mycorrhizal fungi in protecting roots against infection of nematodes. Jothi and Sundarababu (2000) and Sadasivan *et al.* (1998) showed that brinjal and cowpea plants pre-inoculated with VAM fungi were less susceptible to *M. incognita.* Sharma *et al.* (1994) also reported increased resistance in tomato plants to nematodes in the presence of *G.fasciculatum.* They have observed stunted growth in nematode inoculated plants while plants inoculated with VAM

showed the opposite trend. The pre-inoculation of VAM fungi by two weeks allowed sufficient time to the slow growing fungal symbiont to establish itself in the roots extensively prior to exposure to nematodes. The reduced number of nematodes in the presence of mycorrhiza recorded in the present study are in conformity with the findings of Suresh and Bagyaraj (1984) on tomato. They have observed that pre-establishment of mycorrhizae *(G. fasciculatum)* to nematode *(M. incognita)* reduced nematode infestation when compared to simultaneous application or nematodes preceding mycorrhiza. Bagyaraj *et al.* (1979) observed the reduction of galls produced by *M. incognita* and *M. javanica* on tomato in the presence of *G.fasciculatum.* Sharma *et al.*(1993) recorded reduction in root-knot infestation in tomato when there was interaction between *M. incognita* and *G.fasciculatum.*

The present investigation indicates that *G. fasciculatum* is beneficial to *P.phaseoloides* as it helped in increasing plant growth and overcoming the harmful effects of root-knot nematode, *M. incognita.* Final nematode populations were also found to be influenced by *G.fasciculatum.* Similar observations were recorded on cowpea where increased biomass and reduced nematode infestation was obtained by inoculating *G. fasciculatum* in varied initial inoculum densities. Wallace (1983) reported that interactions between VAM and plant parasitic nematodes are best detected when varied inoculum densities of the interacting organisms are used. The decrease in galls per plant

and nematode population in presence of VAM has been demonstrated by Kellam and Schenck (1980) in soybean and in tomato by Suresh and Bagyaraj (1984). They have also reported that nematode infestation is checked if mycorrhizal colonization is present before they are able to infect the plants.The results of this study indicate the strong potentiality of using *Glomus fasciculatum* as one of the biocontrol agent for root-knot disease in susceptible crop plants. The study also re-inforces the view that VAM can induce tolerance in plants against the attack of parasitic nematodes (Naresh *et al.*, 1995; Choudhary *et al.*, 2002).

5.5 Pathogenecity of *M. incognita* on *P. phaseoloides* at different inoculum levels

Pathogenecity studies indicate that there was a progressive decrease in growth of plant as the inoculum density of *M. incognita* increased. Significant reduction in shoot length, shoot weight and root weight were recorded with the increase of inoculum levels from 10 to 10,000 nematodes. Similar studies on plant characters at varying inoculum levels of root-knot nematodes have been earlier reported by Trivedi and Tiagi (1981), Acharya *et al.* (1987) and Raman *et al.* (1988). They have recorded a positive correlation between increase in population levels and reduction in plant growth characters. The minimum threshold level of root-knot nematodes causing pathogenecity varies in different crops. On watermelon, Dhankar *et al.* (1986) recorded 1000 larvae per kg soil as the

threshold level where the plant growth was reduced significantly over control. Similar observations have also been made by Prasad and Guar (1974) in brinjal infected with root-knot nematodes. In pigeon pea, Pathak *et al.* (1985) noticed significant decrease in plant growth with an initial inoculum of 100 juveniles per plant in 500 g soil. Eapen(1994) recorded stunting and narrowing of leaves and reduced biomass in cardamom plant which received varying levels of initial inoculums.

The results of the present study further revealed that the root weight when inoculated with 10 and 100 larvae were found to be higher than that in control and decreased gradually at higher levels of inoculum density. Such an increase of root weight at low levels of inoculum density over control was recorded by Bora and Phukan (1982) on jute plant. The increased root weight obtained at 10 and100 levels of inoculation may be partly due to galling and partly due to root proliferation by nematode infestation. Increased root weight due to root galling and root proliferation was also reported by Sakhuja and Sethi (1985). Sharma *et al.* (1975) and Castillo *et al.* (2001) reported that the host infestation and nematode multiplication were found to be density dependent.

Number of galls increased gradually with increasing level of nematode inoculums from 10 to 10,000 larvae but nematode population showed a decline at inoculum density of 10,000 larvae. Similar

observations on the increase of galls from 10 to 10,000 levels of inoculum and a decline in population density at 10,000 levels has been reported by Dhawan and Sethi (1976) on brinjal and Kalita and Phukan (1993)on black gram. The number of egg masses were also found to increase with the inoculum densities of 10, 100, 1000 and 10,000. Similar report have also been made by Sivakumar and Meerazainuddin (1974) and Pant *et al.* (1983) which confirms the present findings.

The maximum and minimum reproductive rate recorded at lowest (10) and highest (10,000) level of inoculum respectively in the present investigation is similar to the results obtained by Bora and Phukan (1982) on jute and Walia and Seshadri (1985) on chick pea. Peacock (1959) reported a negative correlation of nematode number with the reproduction factor. He noticed mass invasion of root-knot larvae at a single point of attack. The root area being the same, the crowded nematode has to struggle for establishment and thereby the reproduction factor is reduced . Lesser the nematodes, lesser the competition and therefore greater is the reproduction factor. Sudhasukumaran *et al.* (1986) and Divito *et al.*(2004) also reported maximum multiplication of nematodes at the lowest level of nematode inoculum.

5.6 Changes in biochemical constituents

Enhancement of biochemical constituents is reported as a defensive mechanism of plant against nematodes (Basu and Sukul, 1983; Romabati

and Dhanachand,2000). This was substantiated by the result of the present study which indicated that there was considerable increase in the quantity of total phenols, *ortho*-dihydroxy phenols, reducing and non-reducing sugar and amino nitrogen in *P. phaseoloides* infested with root-knot nematode, *M. incognita.* Similar biochemical changes in the roots of susceptible as well as resistant tomato plants were reported due to the infection of *M.incognita* (Ganguly and Dasgupta, 1979; Ganguly and Dasgupta, 1984; Bajaj *et al.*, 1988). Gill and Uppal (1977) reported highest concentration of total phenols, *ortho*-dihydroxy phenols, total sugars and reducing sugars of *Zinnia elegan* leaves infested with nematodes, *Aphelenchoides ritzemabosi.* Increase in total phenol is reported as a result of host parasite interaction (Taysir and Dawood , 1979) and it helps in the formation of hypersensitive reaction towards nematode infection (Shukla and Chakraborty, 1988 and Mazzafera *et al.*, 1989).The increase in phenol may be partially due to absorption of phenolic compounds released by the decomposition of organic matter through roots and partially due to increase in polyphenol oxidase activity resulting from nematode infection (Alam *et al.*, 1977). There was also significant variation in carbohydrate metabolism as indicated by increased sugar content and decreased level of starch. Sharma and Sethi (1976) also reported increased sugar content in susceptible and mutant forms of cowpea due to *M. incognita* and *Heterodera cajani.* The increased sugar is

reported to be due to the degradation of starch or inhibition of starch synthesis and this increased sugar content is observed to be favourable for the survival of nematodes (Dropkin, 1969; Vidyasekharan and Kandasami, 1972). Decrease in starch content recorded in the present investigation is similar to the findings of Agarwal *et al.*(1985). They have reported increased enzyme activities, reduction in free sugars and disappearance of starch in the root-knots infested host plants like okra (*Abelmoschus esculentus*). The variation in carbohydrate metabolism could be due to the reduced photosynthetic capacity or increased rate of respiration as reported by Wallace (1974).

An increased protein metabolism observed in the present study is found in close conformity with the findings of Simte and Dasgupta (1987) who reported increased protein content in soybean infected with *M. incognita*. On infection, the proteins of the host are likely to change quantitatively resulting in the formation of new polypeptides (Giebel, 1982; Dasgupta, 1988). An increase in protein, DNA and RNA content following infection with root-knot nematode has been reported (Sinha *et al.*, 1983; Simte and Dasgupta, 1987). Ganguly *et al.*(1991) reported increased soluble protein content in cowpea cultivars infected with root-knot nematodes. The interactions of cowpea cultivars with root-knot nematodes results in enhanced synthesis of *m* RNA which ultimately directs synthesis of polypeptides required for the production of low

molecular weight metabolites having antibiotic properties. Upadhyay *et al.* (1986) reported that synthesis of protein was stimulated by the infection of root knot nematodes. The increase in total protein and aminoacids due to nematode infection was also reported (Stewart *et al.,* 1996 and Patel *et al.*, 1995) on different crops. These authors concluded that increase in proteins and aminoacids in nematode infested plants are disease related. From these findings it may be concluded that infection by *M. incognita* resulted in the disturbance in metabolism of proteins, carbohydrates and phenolic compounds in infected plants of *P. phaseoloides*.

The findings of the present study clearly showed differences in the population density of root knot nematode, *Meloidogyne incognita* in rubber growing soils. The nematode infested *Pueraria phaseoloides* showed stunted growth and changes in biochemical constituents like sugars, aminoacids and phenols. VAM and bradyrhizobial interaction with the nematode alluviated the adverse effect of root knot nematodes in *P.phaseoloides.* The study also indicated seasonal variations of root knot nematode population with environmental factors.

Chapter VI

SUMMARY

SUMMARY

A survey was conducted in twenty one different rubber growing regions of Kerala, Tamil Nadu, Dakshin Kannada and Maharashtra to determine the frequency of occurrence and distribution of plant parasitic nematodes in rubber plantations. The common genera of plant parasitic nematodes encountered in rubber growing soil included *Meloidogyne*, *Helicotylenchus*, *Aphelenchoides*, *Hemicycliophora*, *Longidorus*, *Radopholus*, *Tylenchus*, *Hoplolaimus*, *Criconemoides*, *Trichodorus* and *Xiphinema*. The density and frequency of *Meloidogyne* was found more in all the locations compared to other nematode genera. Maximum population density of 2225 nematodes per 250 ml soil was recorded with hundred percent frequency of occurrence in Punalur region followed by Kanhikulam, Calicut, Vaniampara, Padiyoor, Muvattupuzha and Kottayam. Lowest population and frequency of *Meloidogyne* was observed in Nettana and Dapchari region.

Infectivity test of soil samples conducted with the indicator plant, tomato (*Lycopersicon esculentum*) showed variations in the formation of galls. Two larvae of *Meloidogyne incognita* per g. soil was recorded as the minimum inoculum potential.

Effect of the root-knot nematode, *M.incognita* on the growth, biomass, nodulation and nitrogen fixation on the cover crop grown in

young rubber plantations, *Pueraria phaseoloides* was studied by applying different inoculum levels of root-knot nematodes. The results showed significant differences in the growth characteristics between inoculated and uninoculated plants. Inoculation of *Bradyrhizobium* simultaneously or 10 days after nematode inoculation could reduce the adverse effect of nematode and increase growth by 5 to 13 percent over control. Significant reduction in nodulation was also observed due to nematode infestation. Reduction percentage of nodules was more where nematode inoculation preceded bradyrhizobial inoculation. Maximum gall formation was recorded in plants inoculated with nematode alone. Significant decrease in number of galls was observed on simultaneous application of *M. incognita* and *Bradyrhizobium* or by the application of the two organisms 10 days prior or later to each other.

Nitrogen content in the shoot and root of *P.phaseoloides* was considerably reduced by the infestation of root-knot nematode, *M.incognita* at different inoculum levels. A per cent decrease of 5.91, 11.33, 22.66 and 33.50 of nitrogen in shoot and 2.48, 8.45, 21.39 and 26.86 in root over control was observed at 1000, 2000, 3000 and 4000 levels of nematode inoculum respectively. By bradyrhizobial inoculation a per cent increase of 28.07 and 37.81 nitrogen content of shoot and root over control was recorded. Nitrogen content of both shoot and root of *P. phaseoloides* was

significantly higher when bradyrhizobial inoculation preceded nematode inoculation.

Root-knot nematode, *M. incognita* showed seasonal fluctuations in soil population and in host infestation. An increase in the population density was recorded in summer months and decrease in winter. Peak populations of *M.incognita* were recorded during March and April months (1400 and 1200 per 250 ml soil) when the mean temperature was 34.5^0 C and 33.6^0 C respectively. Soil population of nematodes and infestation was very low during June-July. The results of the present study showed a post-monsoon build up of nematode population in soil. Correlation coefficient analysis showed that environmental temperature is significantly correlated with nematode population and host infestation. A high correlation was also observed between the intensity of infection and soil population of nematodes. Even though no significant effect of quantity of rainfall on nematode population and host infestation was observed, monthly distribution of rainfall showed reducing effect in the population and infestation in succeeding months.

A pot culture study on the effect of interaction of root-knot nematode, *M.incognita* and VAM (*Glomus fasciculatum*) in *P. phaseoloides* indicated that the pre-inoculation of VAM or inoculation of VAM simultaneously with nematode or one or two weeks later to

nematode can reduce the adverse effect of root-knot nematode considerably and favour plant growth. Significant reduction in mycorrhizal colonization percentage and spore count were recorded due to the infestation of *M .incognita* in *P.phaseoloides*. The multiplication rate of *M.incognita* was found decreased by the inoculation of VAM. Plants inoculated with mycorrhiza first and two weeks later with nematode showed minimum multiplication rate(0.60) as against the maximum value (2.1) observed in plants inoculated with nematode alone.

Enhancement of some biochemical constituents was recorded due to the infestation of root-knot nematode, *M.incognita* on *P.phaseoloides*. Significant increase in the content of total phenols, *ortho*-dihydroxy phenols, reducing sugars, non-reducing sugars and amino nitrogen was observed at 1000, 2000, 3000 and 4000 levels of nematode inoculum. Low starch content was recorded in nematode inoculated plants. Increase in the level of larval inoculum resulted in proportional decrease in plant growth and increase in root-knot disease on *P.phaseoloides*. The possibility of using VAM as biocontrol agent for the management of *Pueraria phaseoloides* in rubber plantation is discussed.

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Annexure

Preparation of Reagents

1. Folin-Ciocalteu reagent

Dissolved 100 g sodium tungstate (Na_2WO_4 $2H_2O$) and 25 g sodium molybdate ($NaMO_4$-$2H_2O$) in 700 ml water in 1 litre flask. Added 50 ml 85 per cent *ortho* phosphoric acid (H3 PO4) and 100 ml Con. HCl and boiled under reflux gently for 10 h. Cooled and added 150 g lithium sulphate (Li_2SO_4) dissolved in 50 ml water and 4-5 drops of liquid bromine. Boiled the mixture without condenser for 15 minutes to remove the excess bromine. Cooled, diluted the volume with water and filter. The reagent, golden yellow in colour was stored in brown bottles. It is stable for many months. Just before use, dilute one volume of this stock solution with 2 volumes of distilled water.

2. Copper reagent 'A'

In 800 ml of the glass distilled water 25 g of anhydrous sodium carbonate, 25 g of Sodium Potassium tartarate (Rochelle Salt), 20 g of Sodium bicarbonate and 200 g of anhydrous sodium sulphate were dissolved and diluted to 1 litre with glass distilled water.

3. Copper reagent 'B'

To 100 ml of glass distilled water, 15 g of copper sulphate and 1-2 drops of concentrated sulphuric acid were added.

4. Arsenomolybdate colour reagent

To 450 ml of glass distilled water, 25 g of ammonium molybdate, 21 ml of concentrated sulphuric acid and 3 g of sodium arsenate dissolved in 25 ml of glass distilled water were added and the mixture was kept in an incubator at 37^0C for 48 h. The reagent was stored in a glass stoppered brown bottle.

5. Citrate buffer 0.2M (pH 5.0)

Exactly 21 g of AR grade citric acid was dissolved in 200 ml of 1 N sodium hydroxide in a standard flask and the volume was raised to 500 ml with glass distilled water.

6. Ninhydrin reagent

To 500 ml of 0.1 N citrate buffer at pH 5.0, 800 mg of hydrated stannous chloride was added. The solution was mixed with 20 g of recrystalised ninhydrin, dissolved in 500 ml methyl cellosolve and the solutions were mixed together. Fresh reagent was prepared on the day of use.

7. Diluent solution

Equal volume of glass distilled water and n-propanol were mixed and used.