INTERACTION OF VESICULAR-ARBUSCULAR MYCORRHIZAE AND BIOCHEMICAL VARIATION WITH *MELOIDOGYNE INCOGNITA* (Kofoid and White,1919) Chitwood,1949 ON BRINJAL (*Solanum melongena* L.)

Thesis submitted in part fulfilment of the requirement for the award of the Degree of *Doctor of Philosophy (Agriculture) in Plant Nematology* to the Tamil Nadu Agricultural University,Coimbatore-3

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CERTIFICATE

This is to certify that the thesis entitled "**INTERACTION OF VESICULAR-ARBUSCULAR MYCORRHIZAE AND BIOCHEMICAL VARIATION WITH *MELOIDOGYNE INCOGNITA* (Kofoid and White,1919) Chitwood,1949 ON BRINJAL (*Solanum melongena* L.)**" submitted in part fulfilment of the requirements for the award of the degree of **DOCTOR OF PHILOSOPHY (PLANT NEMATOLOGY)** to the Tamil Nadu Agricultural University, Coimbatore is a record of *bonafide* research work carried out by Mrs. **G. JOTHI** under my supervision and guidance and that no part of this thesis has been submitted for the award of any other degree, diploma, fellowship or other similar titles or prizes and that the work has not been published in part or full in any scientific or popular journal or magazine.

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ABSTRACT

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INTERACTION OF VESICULAR-ARBUSCULAR MYCORRHIZAE AND BIOCHEMICAL VARIATION WITH *MELOIDOGYNE INCOGNITA* (Kofoid and White.1919) Chitwood,1949 ON BRINJAL (*Solanum melongena* L.)

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Crop losses inflicted by nematode pests continue to increase and are becoming a limiting factor in stabilizing or maximising crop yield on world wide basis. Nematode management through biological agents in relation to crop protection is a subject of considerable current interest, because of a perceived urgency to develop and adopt safe, economic and efficient methods for managing nematode pests. Among the various kinds of organisms engaged in natural control of nematodes, VAM is now attracting greater attention.

Application of 10 g *Glomus mosseae* per kg soil gave the maximum growth parameters. Although root colonization by mycorrhiza was reduced a little in plants inoculated with nematodes, the spore production was not adversely affected. Since the mycorrhiza was inoculated prior to nematodes, they were able to offset the ill effect caused by the nematodes and had more vigorous roots as against the healthy plants.

It was observed that among the different species *viz*., *G.fasciculatum*, *G.mosseae*, *G.intraradices* and *G.fulvum*, *G.fasciculatum* recorded increased growth parameters and a decrease in soil nematode population with increased spore densities and VAM colonization. It was on par with *G.mosseae*. The reproduction potential and the fecundity of the root knot nematode was very much reduced due to VAM application which was observed by the presence of fewer egg masses per gram of root. The development of gelatinous matrix was delayed and only fewer eggs per egg sac were observed on plants with mycorrhiza. In addition, the plants had lower root knot indices and the yield considerably increased in *G. fasciculatum* followed by *G.mosseae* when compared to nematode alone. By cluster analysis, it was observed that *G. fasciculatum* inoculated with nematode was able to perform better than other species with nematode.

In the crop rotation, brinjal was followed by cumbu which is a non host for root knot nematode and a good host for VAM multiplication, decreased the soil nematode population and increased the spore densities. The growth parameters of brinjal was enhanced due to *G. mosseae*. A significant increase in root colonization and chlamydospore densities was observed. When greengram was followed by cumbu, the nematode population had increased a little since greengram is a host crop. This was followed by brinjal which showed a stimulated effect on plant growth with increased shoot and root weight. The soil nematode population though showed an increase after greengram, was less when compared to the first brinjal crop before rotation. *G. mosseae* fungus had the greatest effect on over all plant growth of all the crops in the rotation.

G.mosseae in different doses applied in the nursery for the management of *M.incognita* was transplanted to the main field. The seedling growth, vigour and weight was observed to be maximum in plots which received 2.0 and 2.5 kg VAM per m^2. The growth parameters, VAM colonization and yield had increased in the

mainfield, observed at harvest in all VAM treated plots, but it was maximum in 2 kg VAM per m^2. Plants treated with carbofuran yielded significantly higher than those of untreated plants. Significant increase in VAM colonization and chlamydospore densities was observed in 2.0 kg VAM per m^2 treated plots. The P content was more in 2.5 kg per m^2 treatment. The gall index was lesser in all VAM treatments. Transplanting of mycorrhizal seedlings into root knot nematode infested soil was able to perform better than non-mycorrhizal seedlings both quantitatively and qualitatively.

In nematode infested roots, many giant cells were formed with multinuclei due to hyperplasia and hypertrophy. Because of that, the xylem and phloem vessels were shifted to one side. The diameter of the cortical cells proximate to the developing females were greater than average, and when two or more resided at the same locus, the root became swollen, thus producing the characteristic gall formation symptom of infection. The females with egg masses were seen external to the epidermis. When VAM and nematode were present together, it was observed that the hyphae penetrated the epidermis, invaded the cortex, giving rise to arbuscules and vesicles and reduced the number and size of the giant cells. Arbuscules in some cortical cells were formed in close proximity to the nematodes.

A comparison of electrophoresed protein profiles obtained from VAM and VAM infected with nematodes revealed, appearance and disappearance of several protein bands. The disappearance of protein was accompanied by the appearance of new protein patterns in nematode infested VAM plants. The increase in concentration of protein in nematode infested root may be due to new enzyme protein or may be the contribution from the nematodes.

Biochemical alterations of the plants due to VAM-nematode interaction revealed that, total phenol compounds which play a role in disease resistance were found in plants inoculated with mycorrhizal fungi. There was an increase in total phenol content in nematode inoculated VAM and VAM alone plants. The protein content was more in nematode alone and comparatively it was less when VAM and nematodes were inoculated. The total sugars and reducing sugars were more in VAM and reduced a little in nematode inoculated VAM plants. There were varying numbers of amino acids in each species of VAM with nematode when the plants were analysed by paper chromatography. The amino acids like arginine, phenylalanine and glutamic acids were found in *G. fasciculatum*, *G.mosseae* and *G.intraradices* inoculated with nematode and were absent in nematode alone, which indicated that, these amino acids might have some role in suppressing the nematode. The amino acids like the cystine and tryptophan were found in nematode alone and in *G.fulvum* where maximum number of nematodes were present indicating that these amino acids favoured multiplication of nematodes.

The peroxidase activity was more in nematode inoculated VAM plants. When nematode alone was considered, it was comparatively less. It is suggested that elevated level of peroxidase activity was due to denovo synthesis of peroxidase isozymes. The chitinase activity was found to be more in all the VAM species inoculated with nematode when compared to nematode alone.

The macro nutrients like N, P and K were more in all VAM species and reduced a little when nematode was inoculated into them. It was observed that Fe, Cu and Zn were on the increase both under VAM and VAM + nematode, whereas Mn was found decreased both in VAM and VAM + nematode inoculated plants.

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(G. JOTHI)

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CHAPTER I

INTRODUCTION

Vegetables form an important constituent of diet of human beings. In India, according to the standards fixed by Nutritional Advisory Committee of Indian Council of Medical Research, we need to produce 0.23 million tonnes of vegetables per day and the present annual requirement is over 83.95 million tonnes for an estimated population of 700 million. But currently, we are producing only about 45 million tonnes with a total area around 4 million ha under vegetable crops in India (Singh *et al.*, 1989). In recent years, nematode diseases of vegetables have been gaining increased attention of both agriculturists and scientists because of direct and indirect losses caused by them.

Root-knot nematode, *Meloidogyne incognita* (Kofloid and White, 1919) Chitwood 1949, is one of the most important nematode that parasites the vegetable crops. Darekar and Mhase (1988) reported that yield losses in tomato and brinjal due to *M.incognita* under field condition in North India were 46.92 and 32.73 per cent respectively.

Vesicular-Arbuscular Mycorrhizal (VAM) fungi are obligate symbionts that colonize the roots of most cultivated plant species. This favours plant growth by increasing nutrient uptake, growth rates and hormonal activity (Abbot and Robson, 1984; Linderman, 1992). Mycorrhizae also increase plant tolerance to biotic and abiotic stress conditions, such as transplanting, soil salinity, drought, attack by soil borne pathogens, alters root attractiveness, reduce larval penetration, impending larval development, retards giant cell formation, competition for space and parasitism of eggs (Gerdemann, 1968; Smith, 1987).

Plant parasitic nematodes and VAM fungi commonly occur together in the roots or rhizosphere of the same plant, each having a characteristic but opposite effect on plant vigour. The obligately symbiotic VAM fungi may stimulate plant growth, whereas the obligate plant parasitic nematodes usually suppress plant growth. Recent research indicates that VAM fungi have potential as biocontrol agents when both groups of microorganisms occur simultaneously in roots or rhizosphere of the same plant (Hussey and Roncadori, 1982a).

Mosse (1957) demonstrated for the first time the importance of VAM fungi in phosphate uptake. It is also an established fact that zinc, copper, and sulphur uptake is also improved in the presence of VAM. The most important role of VAM is in increasing plant resistance to drought conditions, protection against soil borne pest and diseases and improving plant establishment. Mycorrhizal fungi provide great absorptive surface compared with root hair, and thus help in the absorption of relatively immobile ions in soil, such as phosphate, copper and zinc (Bagyaraj, 1992). In addition, mycorrhizal plants were shown to have greater tolerance to toxic metals, high soil temperature, adverse soil pH and to transplant shock than do non-mycorrhizal plants (Bagyaraj, 1995).

Phenolic compounds have long been thought to play a role in disease resistance (Goodman *et al.*, 1967) and they have been shown to be formed after infection by mycorrhizal fungi (Syliva and Sinclair, 1983). Mycorrhizae have also been demonstrated to contribute for the reduction in nematode population densities (Bagyaraj *et al.*, 1979; Sitaramaiah and Sikora, 1982). Biochemical alteration of the plant root has been hypothesized as one explanation of reduced nematode infection (Smith, 1987). The nematode enzyme and biochemical aspects of plant nematode interaction has been well documented by Dasgupta and Ganguly, (1986), Ganguly and Dasgupta, (1994) and Ganguly *et al.* (1994).

It is very well established now that VAM fungi improve the growth of plants that are important in agriculture, horticulture and forestry. Today, many developing countries of the world are faced with problems of providing more food, fibre and fuel to people. The challenges for the future are the utilization of mycorrhizae to

- reduce the use of phosphate fertilizers that are expensive and in short supply
- biologically control root pathogens
- establish a sustainable agriculture, less dependent on the energy-rich practices, and
- develop marginal and wastelands

One of the obstacles coming in the way of use of VAM fungi in practical agriculture is the limited information available on the ecology of these fungi and especially the effect of agricultural practices on these fungi. Therefore, it was aimed to take up the studies on the interaction of four VAM species on brinjal to control *M.incognita* using the biocontrol attributes of these symbionts. The present investigations were carried out to elucidate information on

1. The effect of four species of VAM *viz.*, *Glomus mosseae* (Nic & Gerd.), *Glomus fasciculatum* (Thaxt.) Gerd And Trappe, *Glomus intraradices* (Schenck and Smith) and *Glomus fulvum* (Bk. & Br.) Trappe and Gerd on brinjal Cv. Co. 2 to control *Meloidogyne incognita.*

2. To evaluate the optimum dose of *G.mosseae* to suppress nematode population in brinjal under pot culture conditions.

3. Management of *M.incognita* in brinjal with crop rotation under microplot condition.

4. Field evaluation of VAM for the management of *M.incognita* in nurseries and to evaluate the cost effectiveness.

5. Histopathological studies of VAM and nematode infected brinjal roots.

6. Elucidation of the biochemical changes associated with nematode management in vegetables induced by VAM infestation.

CHAPTER II

REVIEW OF LITERATURE

Plant parasitic nematodes and mycorrhizal fungi are commonly found inhabiting the same soil and colonizing roots of their host plants. These two groups of organisms exert a characteristic but opposite effect on plant health. The severity of nematode diseases are generally reduced in mycorrhizal plants. It has been shown in numerous studies that VAM fungi suppress the population of plant parasitic nematodes. Interaction of nematode and VAM result in the biochemical alteration of the plants and the scientific information accumulated is documented in this review.

2.1. EFFECT OF VAM ON VEGETABLES

Several workers have studied the interaction of *Glomus fasciculatum* with *Meloidogyne incognita* and their effect on growth and yield of tomato plants. Bagyaraj *et al.* (1979) observed that inoculation with *G. fasciculatum,* reduced the number and size of gall produced by *M. incognita* in tomato. The experiment to study the effect of integrated effect of *G. fasciculatum* and castor cake for the control of *M. incognita* on tomato (Rao *et al.*, 1994) showed that there was a significant increase in the growth of tomato seedlings with more colonization and spore density which adversely affected the development of nematodes.

When the nursery beds of tomato were inoculated with *G. fasciculatum* for the control of *M. incognita,* it was observed that VAM was able to offset the adverse effect of nematode and increased the yield by 91.3 per cent compared to untreated control (Rajeswari Sundara Babu and Sankaranarayanan, 1995). A similar positive observation was made by Rao *et al.* (1997) by inoculating *G. mosseae* for the control of *M. incognita* on tomato. The VAM inoculation

resulted in increased growth of tomato and decreased root-knot index and final nematode population.

In brinjal also *M. incognita* was found to be controlled by *G. fasciculatum* when incorporated in soil with castor cake and gave significant increase in colonization of roots of brinjal and chlamydospore densities (Rao *et al.*, 1998).

2.2. EFFECT OF DIFFERENT SPECIES OF VAM ON NEMATODES

2.2.1. Effect of *Glomus fasciculatum* on nematodes

Atilano *et al.* (1981) reported that the root zones of grape vine cuttings when infested with spores of *G. fasciculatum* showed increased plant growth. Non mycorrhizal plant growth was reduced to a greater extent due to *M. arenaria* (Neal, 1889) Chitwood,1949. The final nematode population was high in non-mycorrhizal plants.

Mac Guidwin and Bird (1982) observed that the root penetration and development of *Meloidogyne hapla* (Cooper and Grandison, 1986) Chitwood, 1949 were inhibited on *Allium cepa* L. colonized by *G. fasciculatum* under green house conditions. Final root nematode population increased two folds in non-mycorrhizal than in mycorrhizal plants. Only 60 per cent of the nematodes reached adult stages in mycorrhizal plants.

Saleh and Sikora (1984) studied the interaction between *G. fasciculatum* and *M. incognita* on cotton, and reported that the plant growth was increased by 41 per cent and nematode and eggs were reduced by 59 per cent due to the interaction.

Since the colonization of *G. fasciculatum* in the roots of *A. cepa* appeared to prevent *M. hapla* infection of roots, an increase in the leaf and bulb growth in the plants was observed (Mac Guidwin *et al.*, 1985). In 1985, Kotcan *et al.*,

experimented on *A. cepa* in organic soil inoculated with *G. fasciculatum*. In the absence of *G. fasciculatum*, *M.hapla* significantly retarded the plant growth. Final root population densities of *M. hapla* were directly proportional to the initial population densities. Root colonization of *G. fasciculatum* significantly enhanced the growth and development of all the experimented crops. The rate of increase of growth and spore density were directly proportional to the initial spore densities.

Thomas *et al.* (1989) tested six species of VAM on cardamom and concluded that the root colonization was maximum in *Gigaspora margarita* (Becker and Hall) and *G. fasciculatum*. They were able to promote maximum growth response in the absence as well as in the presence of *M. incognita*.

Lower root-knot index was recorded in tobacco by Krishna Prasad (1990) and suggested a possible use of mycorrhizal fungus, *G. fasciculatum* for bio control of root-knot nematode in transplantable crops such as tobacco, tomato, brinjal, etc. both under nurseries and field crops.

Siva Prasad *et al.* (1990) pre inoculated *G. fasciculatum* to the cuttings of *Piper nigrum* L. which reduced the gall index by 32 per cent and also reduced the nematode population both in the roots and surrounding soils. Mycorrhizal plants increased plant growth even in the presence of nematode.

Krishna Prasad (1991) incorporated *G. fasciculatum* in root-knot nematode infested sandy soil of tobacco seedlings. In non-mycorrhizal nursery plots, the percentage infestation of the nematode was 67.5 at 50 days after sowing which was increased to 95 at 75 days after sowing. In mycorrhizal seedlings it was 48-52 per cent on 50 days and 73-75 per cent on 75 days after sowing and the number of galls, endoparasites and egg masses per infested seedling were reduced by 61 to 89 per cent due to mycorrhizal inoculation.

Rao *et al.* (1994) conducted an experiment to study the effect on the integration of *G. fasciculatum* and castor cake to control *M. incognita* on tomato. Mycorrhizal seedlings showed enhanced plant growth than the non-mycorrhizal seedlings. The interrelationship of *M. incognita*, *G. fasciculatum* and three commonly used herbicides were studied by Mishra (1996). After 60 days VAM inoculated soil improved growth of tomato, when compared to other treatments.

Rao *et al.* (1997) inoculated G. *fasciculatum* at the rate of 500 mg inoculum per m^2 for the control of *M. incognita* in tomato, which gave a significant reduction in root galling and fecundity.

2.2.2. Effect of *Glomus mosseae* on *Meloidogyne* sp.

Cason *et al.* (1983) inoculated the tomato plants with *G. mosseae* for the control of *M. incognita*, which increased the plant growth, reduced the nematode penetration, egg mass production per plant and increased the colonization of VAM. On the same crop Al-Raddad (1995) inoculated *G. mosseae* for the control of *M. javanica* and observed that the gall index and average number of galls per root were reduced by 52 and 60 per cent respectively compared to *M. javanica* alone. *G. mosseae* reduced *M. incognita* population on tomato with increased growth of plants and decreased root-knot index and final population. There was an increase in root colonization and chlamydospore densities also (Rao *et al.*, 1995).

Jaizme-Vega *et al.* (1997) conducted a study to investigate the effect of *G. mosseae* on root-knot nematode, *M. incognita* on banana. The mycorrhizal banana plants responded with an increased plant growth and suppressed the nematode reproduction and galling during early stages of plant development.

G. mosseae gave an increased root colonization that helped in reducing the root-knot nematode, *M. incognita*, multiplication rate on *Crossandra undulaefolia* which was observed by Nagesh and Reddy (1997).

2.2.3. Effect of *Glomus intraradices* on *Meloidogyne* sp.

The increase in yield of cotton by 31 per cent due to inoculation of *G. intraradices* which increased the plant tolerance to *M. incognita* was reported by Smith *et al.* (1984). The influence of *G.intraradices* on the penetration and development of *M. incognita* on cotton was observed by Smith *et al.* (1986b). The rate of development of second stage juveniles to ovipositing females was unaffected by *G. intraradices* or 'P' when *M. incognita* was added at planting, but the ovipositing of the females delayed in root system when *M. incognita* was added 28 days after planting.

2.3. TIME OF INOCULATION OF VAM AND NEMATODE

Sikora and Schenck (1975) reported that due to prior inoculation of *Endogone mosseae* on tomato, oat and tobacco, the proportion of *M.incognita* larvae developing to the adult stage were reduced in the mycorrhizal plants, compared with plants inoculated with nematode alone. This suppression of the *M. incognita* population is attributed to the presence of mycelium in the host tissue and to the resultant physiological changes.

Soybeans were inoculated with *G. macrocarpus* (Tul and Tul) ten days prior to *M. incognita* or both organisms simultaneously or 10 days after planting. Among these, the plants which were infested with both nematode and VAM simultaneously had significantly fewer galls per gram of roots (Kellam and Schenck, 1980).

Interaction between *G. fasciculatum* and *M. incognita* was studied in tomato by Suresh and Bagyaraj (1984) and it was observed that mycorrhizal inoculation was found to reduce the root-knot infection when the mycorrhizal inoculation was followed by nematode than the simultaneous application of mycorrhizae plus nematodes or nematodes first followed by mycorrhizae. Cooper

and Grandison (1987) reported that on tomarillo, *M. incognita* infection and development was less in plants pre infected with mycorrhizal fungi than in plants inoculated simultaneously with both organisms. When *G. fasciculatum* was inoculated 15 days earlier than nematode it was able to enhance the growth of tomato Cv.Co.3 and suppress *M.incognita* multiplication, in pot experiment. Simultaneous inoculation followed a similar pattern but fungi were unable to suppress nematode multiplication when the nematode was inoculated 15 days prior to the fungus (Rajeswari Sundara Babu *et al.*, 1996a).

Abha Mishra and Shukla (1997) observed that when *G. fasciculatum* and *M. incognita* were applied simultaneously to tomato Cv-Pusa Ruby, there was a reduction in number of the nematode and also the root galls. Application of *G. fasciculatum* 15 days prior to the nematode significantly increased the plant growth compared with the nematode alone.

Earlier establishment of *G. fasciculatum* on penetration and development of *Heterodera cajani* (Koshy, 1967) was studied by Jain and Sethi (1988a) on cowpea and observed that over 60 per cent root penetration was adversely affected by 15 days early inoculation than simultaneous inoculation.

Jain and Sethi (1988b) reported that two weeks prior to inoculation of *G. fasciculatum* to *Vigna unguculata* L. alleviated the detrimental effect of root-knot nematode damage and reduced gall formation due to *M. incognita*.

Umesh *et al.* (1988) observed that the banana root and soil population of *R. similis* was reduced significantly when *G. fasciculatum* was applied seven days prior than simultaneous inoculation of VAM and the nematode.

Inoculation of black gram (*Vigna mungo* L) with *G. fasciculatum* 15 and 20 days earlier than inoculation with *M. incognita* reduced the nematode population

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Inoculation of black gram (*Vigna mungo* L) with *G. fasciculatum* 15 and 20 days earlier than inoculation with *M. incognita* reduced the nematode population

and also increased the biomass production (Sankaranarayanan and Rajeswari Sundara Babu 1994).

2.4. EFFECT OF NEMATICIDES ON INTERACTION

2.4.1. Effect of nematicides on nematode

M. javanica was controlled in brinjal by the application of carbofuran 3G @ 0.3 g a.i/m^2 with a lowered root-knot index than the untreated plants (Jain and Gupta, 1991).

Zahid *et al.* (1992) applied carbofuran at the rate of 30 kg /ha to brinjal infested with *M. incognita* under glass house condition. They observed the severity of disease was reduced and the number of galls, larvae and females per root was decreased.

Tomato cv. Sudan Gola and Brinjal cv. Pusa purple long were grown in field infested with *M. incognita* in Himachal Pradesh. Half of the plots were treated with 30 kg/ha carbofuran and half were untreated. Carbofuran reduced the root gall index to 34 and 34.6 per cent in brinjal and tomato and increased the yield by 16.34 and 19.8 per cent respectively in tomato and brinjal (Sharma and Khan, 1994).

2.4.2. Effect of nematicides on VAM and nematode

Thapar and Kamalauniyal (1990) studied the toxic effect of 4 pesticides *viz.*, BHC, aldrin, carbofuran and hexanema at 0.2 - 0.8 per cent, on mycorrhizal fungi in pure culture to control nematode and termites. The least toxic was BHC at all concentration and hexanema at 0.2 per cent.

A field experiment was conducted by Rao and Krishnappa (1995) to study the management of *M.incognita-Fusarium oxysporum*, disease complex in chickpeas cv.Annegiri. The results indicated that integration of soil solarization (for 6 weeks),

VAM fungi, *G. fasciculatum* inoculation (12g/hill) and seed treatment carbosulfan (3% w/w) was highly effective in reducing nematode population.

Rajeswari Sundara Babu and Sankaranarayanan (1995) applied carbofuran 3G and phorate 10G for the control of *M. incognita* in tomato along with *G. fasciculatum*. The fungus was able to off set the adverse effect of nematode and increased the yield. Both the nematicides were equally effective in decreasing nematode number and increased yield.

Sankaranarayanan and Rajeswari Sundara Babu (1997) conducted an experiment by application of phorate 10G one week later to nematode inoculation. All the treatments were effective in reduced nematode population of *M. incognita* on blackgram. VAM spore population and mycorrhizal colonization was highest in VAM alone while chemical treated plants had minimum number of spores and per cent colonization of VAM.

2.5. HISTOPATHOLOGICAL CHANGES DUE TO VAM - NEMATODE INTERACTION

2.5.1. Histopathological changes due to *Meloidogyne sp.*

Hung and Maggenti (1969) observed the larval penetration through the cortex and meristematic tissues of the root tip and the establishment of feeding sites in the central vesicular region. Syncytia induced by root-knot nematode were due to multinucleated state of giant cells from repeated mitosis without cytokinesis of a single stimulated cell in *Vicia faba* roots infested by *M. javanica*.

Dalal and Thakur (1971) observed the abnormal extension of some giant cells towards the vascular tissues resulted in the discontinuity of vascular tissue. The tracheidal elements of the infected roots formed a transverse series instead of the normal longitudinal series in the healthy plants. Disruption of epidermal cells and suberisation of outer cortical layers were observed in brinjal infected by *M.incognita*.

Giant cells were formed with well marked and varying groups of 4, 5, 7 or 8 giant cells were present around the female parasite, not only the number but also size varied. The size of the cortical cells was also affected and the cytoplasm appeared denser in the infected sections, as compared to those of the non-infected roots. Extensive hyperplasia and disorganisation of cortical layers and giant cells formation occured (Tandon and Praveen Kumar,1978).

The presence of the nematode in the cortical region of the root showed hyperplasia and derangement resulting in extensively large galls in the infected region. The parasite caused marked shift in the vascular system away from the site of infection. Both the cortical and stelar system were disorganized and the general histological appearance of the cells had unrecognizably been changed in the vicinity of the parasite which was observed by Orr and Morey (1978) in grain sorghum roots infected by *M. incognita*.

Singh *et al.* (1984) raised the resistant and susceptible lines of cowpea infected by *M. incognita*. It was observed that there were fewer giant cells around the head of *M. incognita* in the resistant plants. The cytoplasm in the giant cells of the susceptible lines appeared to be dense as compared with that in adjacent cells.

Root-knot nematode induced multinucleated giant cells. The vascular structures developed by the expansion of about half a dozen parenchyma cells with in the differentiating vascular cylinder, each become multinucleated by synchronous mitoses in the absence of cytokinesis. Mature giant cells are metabolically active and contain aneuploid nuclei with 14-16 times more DNA than unaffected roots (Jones, 1981; Hung, 1985).

Kaul and Chhabra (1997) observed that the nematode galls were usually small to very large and the nematode infected sunflower were stunted. *M. arenaria* had penetrated large number of meristematic zone of developing roots and prevented

further growth. The development of juvenile stages were observed embedded in the cortical tissues with the formation of syncytium. The syncytium varied from 3-5 in number. The xylem elements were compressed by the development of syncytium. During cell fusion, wall dissolution of the adjacent cells were evident. Cortical hypertrophy was more pronounced near the posterior region of the nematode body whereas hyperplasia reaction was observed near the anterior region.

2.5.2. Histopathological changes due to VAM and *Meloidogyne* spp.

On infection due to *Meloidogyne* spp. the nematode penetrated the roots colonized by endomycorrhizae. It migrated to the stelar region and developed into adults with their heads embedded in vascular tissues. The vesicles and the arbuscules were formed in the cortex cells. As the female developed, cortical cells and the associated fungus at and near its body were compressed and collapsed. A gelatinous matrix containing many nematode eggs protruded from the surface. Giant cells developed as a cluster of multinucleated cells in the vascular tissues immediately adjacent to the head of the nematode. Cytoplasm in the actively functioning giant cells was dense, very granular in texture and contained greatly enlarged nuclei with irregularly lobed membrane. As the giant cells become senescent, their cytoplasm become highly vacuolated, deteriorated and cavities usually appear in the vascular tissues. Xylem in the immediate vicinity of the giant cells were destroyed, crushed and even scattered in irregular isolated patches (Riffle, 1973).

O'Bannon *et al.* (1979) observed that the hyphae penetrated the epidermis and invaded the cortex giving rise to arbuscules and vesicles. Arbuscules were observed in some cortical cells in proximity to nematode induced nurse cells. *G. mosseae* infected soil have shown that the fungus rapidly invaded, which produced vesicles as well as arbuscules before nematode invasion. In some roots, vesicles and arbuscules developed in almost 50 per cent of the cortical cells.

The microtome sections of VAM and *M. incognita* infected roots revealed that the fungal hyphae penetrated the epidermis and invaded the cortex giving rise to arbuscules in some cortical cells and in the nematode induced giant cells (Sankaranarayanan, 1995). Due to *M. incognita* infection, conspicuous and well formed giant cells of spherical to slightly elongated shape was noticed near the nematode head. The cytoplasmic granules and the nuclei were condensed either at the centre of the cell or at the inner wall of cells, leaving clear space in the giant cells. The xylem and phloem vessels were pushed to one side of the root cortex and the space was occupied by the giant cells.

2.6. BIOCHEMICAL CHANGES DUE TO INTERACTION

2.6.1. Effect of nematodes on sequential changes in proteins

Simte and Dasgupta (1987a) observed the sequential changes in protein due to the inoculation of *M. incognita* on soybean. Disc-electrophoretic analysis showed that there were 3 additional bands which occurred 3-21 days after inoculation.

Quantitative and qualitative changes of soluble protein of root and shoot of cowpea cultivar Pusa Do Fasli was determined at two intervals following inoculation with root-knot nematode *M. incognita* race 1. The infection of cowpea cultivar Pusa Do Fasli with the nematode resulted in considerable increase of total soluble protein during the two post infectional intervals. A comparison of electrophoresed protein profiles obtained from healthy and infected roots and shoots revealed appearance and disappearance of several protein bands during post infection period of observation (Ganguly *et al.*, 1991).

2.6.2. Biochemical changes due to nematode interaction

Nasr *et al.* (1980) reported a significant increase in the concentration of N, K, Ca, Mn and Cu in plants inoculated with *M. incognita* and *M. javanica*. Concentration of K, P, Ca, Mn and Cu were greater in the leaves of nematode infected almond plants

than in control, whereas N and Zn were unaffected. The concentration of reducing, non-reducing and total sugars were greater in infected plants.

Singh *et al.* (1985) amended the soil with saw dust, urea, cowdung and oil cakes like castor, mustard and neem which reduced the development of *M. incognita* on tomato and observed an increase of total free phenols, O-dihydroxyphenols and amino acid contents in the infected plants.

The presence of high total phenol contents in resistant variety of pigeonpea (0.86 mg/g fresh root) inoculated with *Rotylenchulus reniformis* (Linford and Oliveira, 1940) as compared to susceptible variety (0.27 mg/g fresh roots) was the reason for its resistance to the reniform nematode (Thakar and Yadav, 1986). In 1986, Akhtar Haseeb *et al.*, observed that lignin was present in cortical and vascular bundle region of both infected and healthy roots but the infected roots showed negative reaction at the feeding site. The cortical cells become lignified in due course of time during the growth of plants. Infection with nematode might be delaying the process of lignification of cortical and pericycle tissue.

The effect of *M. incognita* race 1 and 3 and *M. javanica* on the phenolic content of barley and wheat roots were determined. The initial phenolic contents in healthy roots of barley was lower when infested with race 1 and then increased. In the case of race 3, there was an initial increase. In the case of wheat, the amount of phenols in resistant, infected roots were higher than those of susceptible healthy and infected roots (Rezk *et al.*, 1987).

Rao *et al.* (1988) reported that the amount of protein in shoots and roots of healthy rice plants were 0.8 and 0.7 mg/gm. But after infection by *Meloidogyne graminicola* Golden and Birchfield, 1965 the concentration of protein increased in shoot and roots by 1.42 times and 75 per cent respectively. The amount of phenols in shoot and roots of healthy plants were 19.5 and 28.4 mg/g and in

infected plants the phenols were reduced in shoots by 35 ` per cent and increased in roots by 31.5 per cent.

Nandini Gokte *et al.* (1988) reported that in *Anguina tritici* (Steinbuch, 1799) Filipjev, 1936 infected plants, the amount of reducing and non-reducing sugars, IAA, Soluble protein, Carbohydrate etc. were found to be increased. Cook *et al.* (1992) studied the change in minerals in different plants infested by cyst nematodes. In clover syncytia, increased P was observed and in non-syncytia, Cl was more. They also recorded less amount of Na and S in synctia associated with *Globodera rostochiensis* (Wollen weber, 1923) Mulvey and Stone, 1976 in tomato than in non-syncytial cells but other elements like Mg, P, Cl, K,Ca etc were found unchanged in both the conditions. In wheat, associated with *Heterodera avenae*, (Mortensen, Rostop and Ravn, 1908) Filipjev, 1934 the syncytia had more S and P.

The changes in total phenolic contents in the three barley cultivars (i.e.) two susceptible and one resistant were studied after 30 days of *H.avenae* inoculation. The total phenolic content in the shoots of healthy and resistant cultivars was higher as compared to the susceptible cultivars. However, in the presence of nematode, the phenol content in the two resistant cultivars, significantly increased as compared to the susceptible one (Pankaj *et al.*, 1992).

Akhtar Haseeb *et al.* (1993) studied the influence of various initial inoculum densities of *M. incognita* on the growth of *Hyoscyamus albus*, root-knot development, multiplication, physiological changes, concentration of Mn, Zn, Cu and Fe and total alkaloid 60 days after inoculation. There was a negative relationship between initial density and other parameters. The concentration of Mn and Cu in shoot decreased significantly with the increase in initial inoculum densities of nematode whereas in roots, the concentration of Mn, Zn and Fe increased with the increase in nematode population.

Mohanty *et al.* (1995) observed, increased amount of total sugars when brinjal was inoculated with root-knot nematodes. Mohanty *et al.* (1997) found that there was an increase in total sugars in the roots of greengram infected with *M. incognita*, rhizobium and both rhizobium and nematode.

2.6.3. Biochemical changes due to VAM-nematode interaction

Strobel *et al.* (1982) tested the peach leaves infected with *M. incognita*. The beneficial effects of *G. etunicatum* fungi on growth were accompanied by improved foliar P, Cu, and Zn status and were greater on nematode free plants.

Singh *et al.* (1991) inoculated *G. fasciculatum* which caused the biochemical changes such as increase in lignin and phenols in the roots which caused Pusa Ruby resistant to root-knot nematode, *M. javanica.*

Root colonization of *G. mosseae* was not affected by the presence of *Pratylenchus vulnus* (Allen and Jensen, 1951). The analysis showed that there was no nutrient deficiency in foliar analysis, although low levels of S, Mg, Mn and Zn were detected in *P. vulnus* inoculated plants, where as mycorrhizal plants had the highest levels of N, Na, P, K and Fe. (Pinochet *et al.*, 1993).

Pinochet *et al.* (1995a,b) observed that the plants inoculated with *G. intraradices* and *P. vulnus* recorded low levels of Ca, Mn and Fe whereas mycorrhizal plants achieved the highest value of N, P, S, Fe and Zn and increased absorption of Ca and Mn. Cu was the only deficient element detected by foliar analysis, although low levels of Na, Mg, Mn and Zn were detected in *P. vulnus* inoculated plants. *G. mosseae* inoculated plants had the highest values of Cu and Al.

Mishra (1996) and Abha Mishra and Shukla (1997) inoculated *M. incognita* and *G. fasciculatum* in tomato cv. Pusa Ruby and at different timings and observed that NPK content of tomato plants was significantly higher with prior or simultaneous application of VAM with the nematode.

In 1997, Jaizme Vega *et al.*, studied the interaction *of G. mosseae and M. incognita* on banana. Mycorrhizal plants fertilized at low 'P' rates exhibited higher N, P, K Ca and Mg contents compared with non-mycorrhizal plants low in 'P' with or without the nematodes.

2.6.4. Effect of nematodes on amino acids

Nasr *et al.* (1980) reported that nine combined amino acids *viz.*, histidine, arginine, serine, aspartic acid, glutamic acid, alanine, phenylalanine, leucine and an unidentified amino acid were the same in healthy as well as nematode infected plants in both the roots and the leaves of the bitter almond . The free amino acids *viz.*, cystine, ornithine, histidine, glutamine, asparagine, glutamic acid, alanine, tyrosine, methionine and phenylalanine detected were similar in both healthy and infected plants.

Changes in enzymes and free amino acids of sugarcane roots infected by lesion nematode were investigated by Sundararaj and Usha Mehta (1991) and it was found that polyphenol oxidase, peroxidase, ascorbic acid etc., were on the increase. In the control plant roots, seven amino acids were identified while the inoculated roots contained nine amino acids. Aspartic acid, cystine, histidine, hydroxy proline and proline were common for both, but glutamic acid and serine were present only in control and butyric acid, leucine, methionine and ornithine were present only in nematode inoculated plants.

A study was conducted on the healthy leaves and the *A. tritici* infested leaves of wheat Kalyan Sona plants. Ten amino acids were separated for both uninfected and infected leaves. But the concentration of the amino acids viz., lysine, histidine, proline and tyrosine increased significantly where as the concentrations of other seven decreased significantly in the infected leaves as compared to the uninfected leaves (Indra Rajvanshi, 1992).

Mohanty *et al.* (1995) investigated the biochemical changes in two brinjal varieties *viz.*, Pusa Purple long (susceptible) and Ghatikia white (resistant) inoculated with root-knot nematode. Five amino acids like L-cystine, L-serine, L-tryptophan, L-leucine and L-isoleucine were found to be common in both the varieties, but the concentration was higher in each variety in nematode inoculated plants except L-tryptophan. Mohanty *et al.* (1996) studied the biochemical alteration in Okra cultivar Pusa Sabani inoculated with root-knot nematode and observed that nine amino acid were common in both healthy and inoculated plants. Out of fourteen amino acids, where L-alanine was present only in healthy and L-histidine, L-lysine, L-proline, L-glysine were present only in inoculated. Higher concentration of all the amino acids were detected in diseased tissue except L-tryptophan.

Mohanty *et al.* (1997) inoculated *M. incognita* to greengram and found an increased concentration of all the 20 amino acids except L-tryptophan. L-tyrosine, L-proline, L-methionine which were absent in healthy, were present in nematode inoculated plants. L-aspartic acid, L-histidine, L-arginine, L-serine which were present in uninoculated plants were absent in nematode inoculated plants.

2.6.5. Effect of VAM- nematode interaction on amino acids

Suresh and Bagyaraj (1984) observed fifteen amino acids in both nematode alone and in combination of *M. incognita* and *G. fasciculatum*. There was an increase in amino acids like glutamic acid, aspartic acid, phenylalanine and serine when compared to nematode alone.

Nemec and Meredith (1981) observed that *G. etunicatum* inoculated citrus root stocks accumulated both total and free amino acids in leaves. Arginine, proline, lysine and free ammonia were found to be more due to the inoculation of VAM fungi. Twenty two free amino acid, urea and ammonia were detected.

2.7. EFFECT OF ENZYME ACTVITY ON INTERACTION

2.7.1.Effect of peroxidase activity due to nematodes

Akhtar Haseeb *et al.* (1986) inoculated *M. incognita* to the tomato plants and the peroxidase activity, oxidase and lignin in the roots were estimated. It was observed that, there was a negative peroxidase in healthy roots as against a positive in case of infected roots.

Mohanty *et al.* (1986) investigated the development of peroxidase activity at two intervals in two cowpea cultivars *viz.* susceptible and resistant inoculated with root-knot nematode *M. incognita.* Quantitative increase in peroxidase activity was observed at both the intervals. On the basis of electrophoretic analysis, it was found that new isozyme of peroxidase was synthesized during post infection period.

Studies on the peroxidase activity due to the effect of *M. incognita* on Pusa Ruby was observed by Ganguly and Dasgupta (1987) and the activity was found to be higher in galled root extract than the non-galled part of the same root.

Simte and Dasgupta (1987b) observed that there was an increase in peroxidase activity due to *M. incognita* on soybean. It is suggested that the elevated level of peroxidase activity was due to de novo synthesis of peroxidase isozymes.

Sujatha and Usha Mehta (1998) observed changes in enzyme levels of peroxidase and polyphenol oxidase in sugarcane roots infected with *P. zeae* and *M. javanica.* There was an increase in two oxidases which can be attributed as a defence mechanism to the most invading pathogens.

2.7.2. Effect on chitinase activity due to interaction

Chitin is the most abundant renewable natural resource after cellulose (Deshpande, 1986; Gooday, 1991; Nicol, 1991). It is widely distributed in nature

in marine invertebrates, insects, fungi and algae (Muzzarelli, 1977). Chitin is a component of middle layer of the tylenchoid egg shell (Bird and McClure, 1976).

Addition of chitin amendments to soil would be expected to stimulate development of microbial species capable of degrading similar compounds present in the nematode. Chitinase is expected to destroy eggs and egg masses of plant parasitic nematodes. (Culbreath *et al.*, 1985; Gooday, 1991).

Information on the influence of chitinase on plant parasitic nematodes is lacking. Chitinase could have interrupted juvenile development within the egg during embryogenesis (Mercer *et al.*, 1992). Chitinases increased hatch rates of *Meloidogyne* eggs (Mercer *et al.*, 1992). However, premature hatching led to mortality of juveniles and in some cases the juveniles die with in the eggs (Zamir Punja and Ye-Yan-Zhang, 1993).

2.8. EFFECT OF MACRONUTRIENTS ON VAM-NEMATODE INTERACTION

2.8.1. Effect of phosphorus on VAM and nematode interaction

Cason *et al.* (1983) reported that, tomato plants grown in high P level (30 µg/g) had greater root weight, increased nematode penetration and egg production per plant but decreased colonization by mycorrhizal fungi compared with plants grown in low phosphorus soil (3 µg/g). The rate of nematode development was not influenced *by Gigaspora margarita* at high soil phosphorus. Mycorrhizal tomato and clover plants were more resistant than non mycorrhizal plants to *M.hapla* at all phosphate levels.

Smith *et al.* (1984) inoculated *M. incognita* to cotton, inoculated by *G. intraradices* in glass house condition. Plants with high P was susceptible to *M. incognita* and yielded less than those in low soil P. The average yield was increased by 31 per cent and suppressed the nematode by 63.1 per cent compared to control in low P.

Cooper and Grandison (1986) observed that mycorrhizal root, increased the resistance to *M. hapla* by alteration in the physiology of root system and also as a result of better host nutrition due to improved P uptake by mycorrhizal plants. Smith *et al.* (1986) found that *M.incognita* population was least in *G. intraradices* infected cotton roots and greater in plants grown with supplemented phosphorus.

At more than 100 ppm, phosphorus was found to inhibit the development of nematode and *G. etunicatum* on bean plants (Oliverira and Zambolim, 1986). Smith and Kaplan (1988) reported that the mycorrhizal infected citrus plants and non-mycorrhizal high P plants had higher shoot weight, root weight, lower nematode population density, greater P content in leaf than non-mycorrhizal low P plants. Enhanced growth associated with root colonization by the mycorrhizal fungus appeared to result from improved P nutrition and not from antagonism between the fungus and the nematode.

Carling *et al.* (1989) reported that maximum growth and yield occurred at P fertilization rates of 50-150 µg/g in soybean plants which was inoculated with *Gigaspora margarita*, *G.etunicatum* and *M. incognita*. Egg production on VAM plants was suppressed at the lowest P rate where as the induced resistance was observed in the host at high P fertilization rate.

Both high (50 µg/g) and low (10 µg/g) P levels had little effect on mycorrhizal plants than non mycorrhizal plants. Increased P levels declined the mineral contents in plants shoots of *Cucumis melo* L. and were not significantly influenced by *G. intraradices* or *M. incognita* (Heald *et al.*, 1989).

Krishna Prasad (1990) experimented and found that by addition of *G. fasciculatum*, the roots-knot indices was reduced and required half of its dose of phosphorus fertilizer compared to non-mycorrhizal seedlings of tobacco.

Tylka *et al.* (1991) conducted an experiment both in microplot and at green house to study the effect of VAM and soil P fertility on the parasitism of soybean cyst nematode and reported that the nematode population was unaffected by VAM in microplot but was suppressed by VAM fungi in green house experiments. The effect of VAM fungi on nematode population varied with time. Soil 'P' fertility generally had no effect on population of nematode.

Santhi and Rajeswari Sundara Babu (1995) conducted a pot experiment to study the effect of different levels of phosphorus (50 and 100 µg/g) against *M. incognita*. The difference among the growth parameters of cowpea at different phosphorus levels were significant. VAM treated plants resulted in increased total phosphorus.

The individual and combined effects of two VAM fungi, *Gigaspora margarita* and *G. etunicatum* with *M. arenaria* and phosphorus fertilization (0, 25, 75 and 125 µg/g soil) on groundnut plant growth and pod yield were determined in a glass house study by Carling *et al.* (1996). Best growth and yield occurred at 75 or 125 µg/g regardless of inoculation treatment. Groundnut growth and yield were generally stimulated by *M. arenaria* at 0 to 25 µg P.

2.8.2. Effect of nitrogen and potassium on VAM-nematode interaction

Suresh and Bagyaraj (1984) studied the effect of *G. fasciculatum* and *M. incognita* on tomato. Mycorrhizal plants had increased quantities of phosphorus, potassium, calcium, total and reducing sugars, also amino acids like phenylalanine and serine. The possible role of some of these chemical constituents help in suppressing the development of root-knot nematode. Singh *et al.* (1985) amended the soil with different amendments and organic matters and found there was an increase of total free phenols, O-dihydroxyphenols and amino acid contents in infected tomato plants grown in amended soils.

The interaction of *G. fasciculatum* and *Radopholus similis* on banana Cv. Dwarf Cavendish plants inoculated with mycorrhizae alone or with the nematode had higher contents of N, P, K, Ca and Mg, reducing sugars and total sugars, phenols and total amino acids in their roots (Umesh *et al.*,1988).

MATERIALS AND METHODS

MATERIALS AND METHODS

CHAPTER III

MATERIALS AND METHODS

The details of materials and methods adopted to findout the effect of macro and micro nutrients and biochemical changes on the interaction of VAM fungi with nematode on the growth, nutrient uptake, yield, nematode population and VAM colonization of brinjal are described in this chapter.

3.1. VESICULAR - ARBUSCULAR MYCORRHIZAE INOCULUM

3.1.1. Starter inoculum

Vesicular - arbuscular mycorrhizal fungal cultures *viz.*, *G.fasciculatum*, *G.mosseae*, *G.intraradices* and *G.fulvum* maintained in the culture collection of the department of Nematology, Tamil Nadu Agricultural University, Coimbatore were used in this study. The culture used, consisted of spores and root tissues of the host along with soil.

3.1.2. Extraction of VAM fungi from soil

The VAM fungal spore in soil were estimated by recovering the spores by the wet sieving and decanting method of Gerdemann and Nicolson (1963) as described below.

A small amount of soil close to the plant was dug out with a shovel from a depth of 10-15 cm after scraping away the top one cm. Roots were carefully collected avoiding damage to the root cortex. During processing, 50 g of soil was mixed with 200 ml lukewarm water in a large beaker until all soil aggregates were broken. The supernatant was decanted through a 20 mesh sieve and the residue was resuspended in more water and decanted. This was repeated three times to give about 700 ml of suspension and leaving only grit, sand and heavy organic particles in the beaker. The roots and other organic matter on the sieve were

washed with a fine jet of water from a squeeze bottle and the washings were collected in the cylinder. The material in the cylinder was resuspended by stirring several times and decanted through a 100 mesh sieve into a second, one litre cylinder, retaining a small volume which was then resuspended in a further 300 ml of water and poured through the sieve to the second cylinder. The materials on the sieve were washed and the washings were added to the second cylinder. The materials in the second cylinder were resuspended and most of them poured through a 200 mesh sieve. The residue was resuspended in another litre of water and poured through the same sieve. The materials on the sieve were washed and added to the same cylinder. The materials in the last two cylinders were resuspended and poured through a 325 mesh sieve. The matter in sieve were washed into a small beaker and examined. The residue in large beaker and cylinders were discarded (Plate 1a and b).

3.1.3. Assessment of spore density

One ml of the extracted water was pipetted out into a nematode counting dish. Spores were calculated by counting the spores in the counting dish and by multiplying the number of spores per ml of the extract with total volume of that extract (Plate 2a).

3.1.4. Assessment of root colonization

The roots of the host plants, inoculated with VAM fungi, were examined for the colonization and presence of VAM hyphae, arbuscules and vesicles by clearing and staining the roots by a modified method of Philips and Hayman (1970) as described below.

The plants were pulled out carefully without damaging even the finer feeder roots. The roots were washed in water to remove the adhering soil particles. Then the finer roots were cut into small segments and fixed in FAA (13 ml formalin, 5 ml glacial acetic acid and 200 ml of 50 % ethanol). These fixed root segments

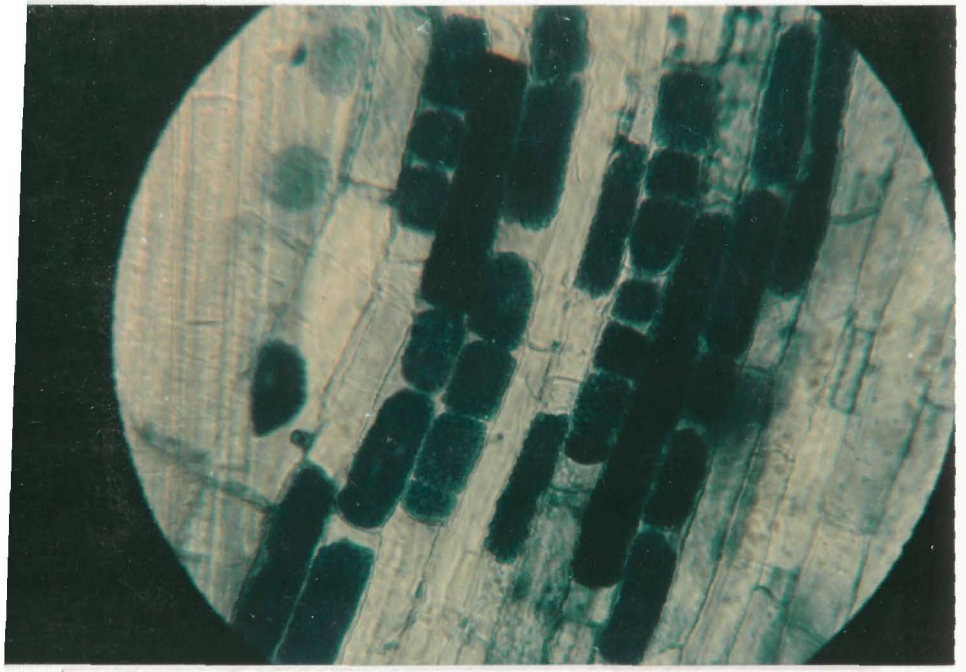


Plate 1a. L.S. of root showing arbuscules

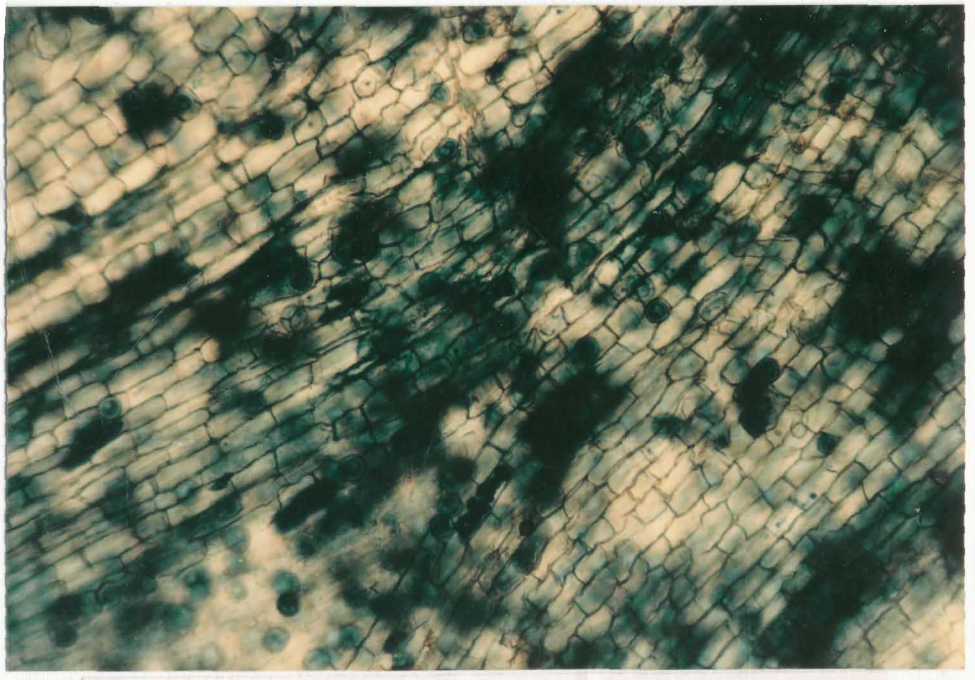


Plate 1b. L.S. of root showing vesicules

were placed in five cm specimen bottles and ten per cent KOH was added to cover the root bits. These bottles were then placed in an autoclave at 15 psi for 20 min. The purpose of the KOH solution is to clear the host cytoplasm, nuclei and also to readily allow penetration of the stain. Then these roots were rinsed in water two times and acidified by soaking in two percent HCl for three min and then the HCl solution was poured off. After that, the root segments were stained by simmering for five min in 0.05 per cent tryphan blue dissolved in lactophenol and the excess stain was removed in clear lactophenol. Hundred such segments were examined under the microscope for the presence of vesicles and arbuscules and VAM hyphae (Plate 2b). The per cent colonization of roots was calculated.

3.2. PURE CULTURES

3.2.1. Pure culture of root-knot nematode, *M. incognita*

The inoculum required for raising the pure culture was obtained from tomato plants grown in Nematology glass house, Tamil Nadu Agricultural University, Coimbatore. Roots with conspicuous galls were selected, washed gently but thoroughly in water and examined for the presence of egg masses under microscope. Galls which showed the protruding egg masses covered with gelatinous matrix were dissected and the egg masses were then kept individually in embryo cups half filled with water. Perineal pattern of the females were prepared for confirmation of the species. The egg masses collected were utilized for raising pure culture.

In two kg capacity earthen pots, steam sterilized pot mixture (Red soil: Sand : FYM - 2:2:1) was taken. Tomato Cv. Co.3 seeds were sown in the pots. In each pot three plants were maintained. The larvae that emerged from each individual egg mass were collected and added with fresh distilled water and mixed well. Then the suspension was inoculated in the soil at the base of the tomato plants. These pots were maintained at the glass house and regularly irrigated with tap water passed through 325

Plate 2a. *Glomus mosseae* spore

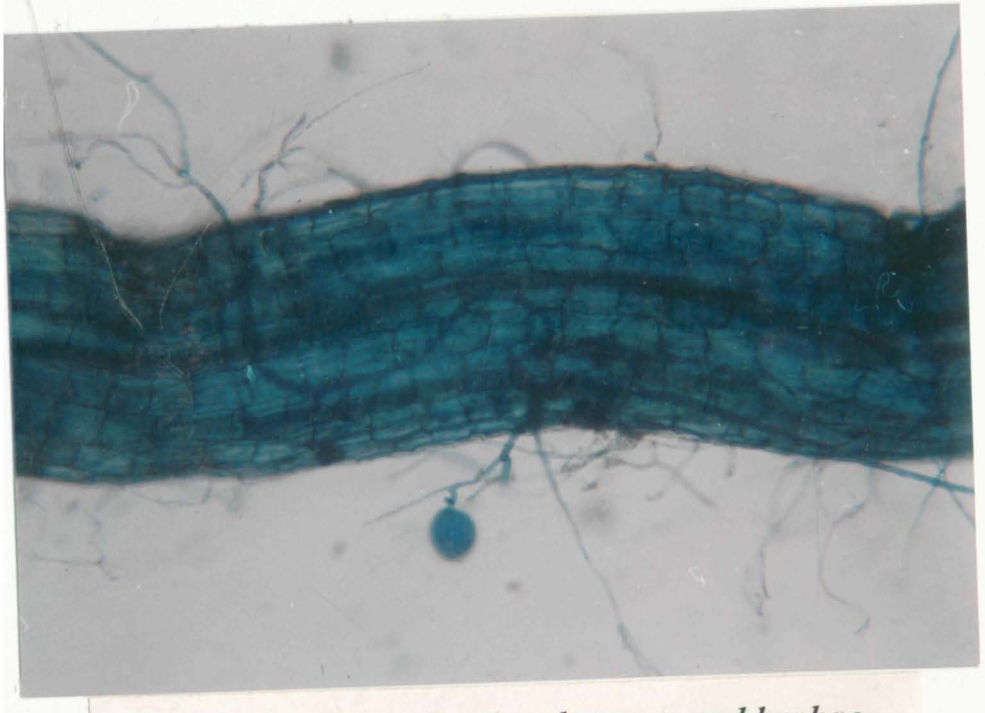


Plate 2b. *Roots showing the spore and hyphae*

mesh sieve. The plants were uprooted gently, forty five days after inoculation. The roots were carefully washed in water and examined for well developed egg masses. Such egg masses were transferred to the petridishes, containing adequate amount of distilled water and incubated in the laboratory condition. The juveniles that hatched out were used for inoculation purpose.

3.2.2. Gall Index

Gall index were graded with 1 - 5 scale rating (Heald *et al.*, 1989).

Percentage of galls / Plant	Gall Index
No galls	1
1 - 25	2
26 –50	3
51-75	4
> 75	5

3.3. EVALUATION OF DIFFERENT DOSES OF *G.mosseae* WITH *M. incognita* ON BRINJAL Cv. Co.2

A pot culture experiment was conducted to study the effect of different doses of *G.mosseae* with *M.incognita* on brinjal. The sterilized pot mixture (Red soil : Sand : FYM - 2:2:1) was filled in two kg capacity pots. One month old brinjal seedlings Cv.Co.2 were transplanted at the rate of two seedlings per pot. At the time of planting, different doses of VAM, which was commercially available from Microbiology Department, Tamil Nadu Agricultural University were mixed in the soil according to the treatments with five replications.

T_1 - 10 g VAM / kg soil

T_2 - 5 g VAM / Kg soil

T_3 - 2 g VAM / kg Soil

T_4 - 1 g VAM / kg soil

T_5 - Nematode alone

T_6 - Control (No treatment)

Fifteen days after transplanting, the second stage juveniles of *M.incognita* were inoculated @ one nematode/ml soil by making holes in the soil around the plant stem where ever necessary except control and the same were covered with sterilized soil. The plants were pulled out after 110 days and the shoot length and weight, root length and weight, gall index, soil nematode population, spore population and VAM colonization were recorded.

3.4. STUDIES ON THE INTERACTION OF FOUR DIFFERENT SPECIES OF VAM WITH *M. incognita* ON BRINJAL

A pot culture experiment was carried out to study the interaction of *G.mosseae, G.fasciculatum, G.intraradices* and *G.fulvum* with *M.incognita* on brinjal. Two kg capacity pots were filled with pot mixture and different *Glomus* species at 10 g/kg soil (100 spores/g soil) and one nematode/ml soil were mixed according to the treatments mentioned below with three replications.

T_1 - *G. fasciculatum*
T_2 - *G. fasciculatum* + *M. incognita*
T_3 - *G. mosseae*
T_4 - *G. mosseae* + *M. incognita*
T_5 - *G. intraradices*
T_6 - *G. intraradices* + *M. incognita*
T_7 - *G. fulvum*
T_8 - *G. fulvum* + *M. incognita*
T_9 - *M. incognita* alone
T_{10} - Control

Brinjal seedlings Cv. Co.2 which were one month old were transplanted at the rate of two seedlings per pot. After fifteen days, the second stage juveniles which were collected from the egg mass of the pure culture were inoculated in the rhizosphere holes and covered with sterilized soil. After 150 days, the trial was concluded. At that time, the final observation like the shoot length, weight, root

length, weight, nematode population, spore count, VAM colonization in the roots and yield were recorded.

3.5. EFFECT OF CROP ROTATION ON THE MANAGEMENT OF *M. incognita* WITH VAM

A microplot experiment was conducted to study the interaction effect of *M.incognita* with VAM using crop rotation. Brinjal was followed by cumbu then greengram and again brinjal.

3.5.1. Brinjal

The earthern thali pots of diameter 0.65 m and height 1.20 m were filled with sterilized pot mixture. The soil in the pots were mixed with *G.mosseae* culture at the rate of 10g/kg soil according to the treatment with six replications.

T_1 - Nematode alone @ 1 nematode /ml of soil

T_2 - VAM @ 10 g / kg of soil

T_3 - Nematode + VAM

T_4 - Control (Untreated)

One month old brinjal seedlings Cv. Co.2 were transplanted at the rate of three seedlings per pot. After fifteen days the second stage juveniles of *M.incognita* were inoculated at the rate of one nematode/ml soil, by making holes around the plant. The plants were pulled out after ninety days and the shoot length, weight, root length, weight, gall index, soil nematode population, spore count and VAM colonization in the roots were recorded.

3.5.2. Cumbu

After the removal of brinjal, cumbu Cv.Co.1 seeds were sown in two rows. After germination, it was thinned to ten plants per pot. The trial came to a close after three months. At that time, the shoot length, weight, root length, weight,

earhead height, soil nematode population, spore count and VAM colonization in the roots were recorded.

3.5.3. Green gram

After termination of cumbu, greengram seeds Cv. Co.2 was sown in the same pots at the rate of ten seeds per pot. After germination, it was thinned to five plants per pot. The plants were pulled out after ninety days and the shoot length, weight, root length, weight, number of pods, soil nematode population, spore count, VAM colonization and gall index in the roots were recorded.

3.5.4. Brinjal

One month old brinjal seedlings were transplanted in the same pots after greengram. This experiment was terminated after five months and all the biometric observations like soil nematode population, spore count, VAM colonization were recorded.

3.6. FIELD EXPERIMENT

3.6.1. Effect of *G.mosseae* for the control of *M.incognita* in brinjal Cv. Co.2

A field experiment was carried out during Jan-Feb (thaipattam) in field No.37 in Tamil Nadu Agricultural University, Coimbatore, on sandy loam soil to study the effect of *G.mosseae* with *M.incognita* on brinjal Cv. Co.2. A randomized block design comprising seven treatments with three replication was adopted. Each plot of the size 0.25 cents consisted of 6 rows with a spacing of 60 cm apart, containing 7 plants per row. The treatments were:

T_1 - 0.5 g VAM / m^2 - Nursery
T_2 - 1.0 g VAM / m^2 - Nursery
T_3 - 1.5 g VAM / m^2 - Nursery
T_4 - 2.0 g VAM / m^2 - Nursery
T_5 - 2.5 g VAM / m^2 - Nursery
T_6 - Carbofuran @ 1 Kg a.i / ha - Main field
T_7 - Control (No treatment)

life processes, survival mechanisms under diverse conditions, host parasite relationships, biochemical and molecular mechanism of resistance. Biochemical methods are precise and revealed exact quantitative changes in the amount of various metabolities, enzymes and mineral element. The influence of biochemical attributes like protein, phenol, total sugars, reducing sugars were carried out according to their standard procedures for micorrhizal plants along with and without nematode.

3.8.1. Estimation of proteins

3.8.1.1. Polyacrylamide-Sodium Dodecyl Sulphate Slab Gel.Electrophoresis (SDS-PAGE) of Proteins

One g of the shoot and root were used for the SDS-PAGE. It was macerated with phosphate buffer. pH 7 and the protein was extracted by centrifuging at 10,000 g for ten min at 4°C.

The separating gel of fourteen per cent was prepared and poured in between two glass plates and allowed to set for 30 to 60 min. Then the stacking gel (4%) was prepared, poured on the top and the comb was placed and allowed to set. These plates were placed in the electrophoresis apparatus and the wells were loaded with the samples (25 µl each). The standard marker protein was added to one well. The current used was 10 mA for ten min and then increased to 30 mA for four hours till the bromophenol blue from the sample reached the bottom according to the molecular weight.

Then the gel was separated from the plates and placed in the staining solution (i.e.) Coomassie brilliant blue R 250 for overnight and then destaining was done until the background of the gel was stainless (Laemmli, 1970). The R_f value was calculated.

$$R_f = \frac{\text{Distance (cm) moved by the solute from origin}}{\text{Distance (cm) moved by solvent from origin}}$$

3.8.1.2. Calorimetry

The amount of protein in the plant sample was estimated by Bradford's (1976) method. From the sample, 100 mg was taken and macerated with 3 ml of phosphate buffer and centrifuged. From the supernatant, 0.5 ml was taken and 2.5 ml of Bradford reagent was added to this and the absorbance was read at 595 nm. The concentration of protein present was calculated from the standard graph.

Standard solution: 100 mg of Bovine Serum albumin was taken and diluted in 100 ml water. 10 ml was taken and it was made up to 50 ml. From this series of solution were prepared for standard graph.

3.8.2. Estimation of phenol

The amount of phenol content in the plant sample was estimated as per the method of Malick and Singh (1980). Five ml of 80 per cent ethanol was added to100 mg samples and the extract was taken. After centrifuging, 0.5 ml of this extraction was taken and evaporated until it dried. Then 5 ml of distilled water and 0.5 ml of Folin-Ciocalteau reagent were added to this. After 3 min, 2 ml of 20 per cent $Na_2 Co_3$ solution was added. The tubes were kept in boiling water for 1 min and the absorbance was read at 650 nm.

Standard solution: 100 mg of catechol in 100 ml of water was taken and 10 per cent solution was prepared and maintained as standard solution.

3.8.3. Estimation of total sugars

The amount of total sugars in the plant sample was estimated by Anthrone method (Hedge and Horreiter, 1962). Hundred mg sample was taken and homogenised in mortar and pestle with 3 ml of 70 per cent aqueous ethyl alcohol. From this, 0.5 ml was taken and kept in boiling water bath for evaporation. One ml of water and 4 ml of anthrone reagent from burette was added to this and

heated in water bath for 8 min and cooled rapidly and read at 630 nm (green colour). From the standard graph, the concentration of total sugars was calculated.

Standard solution : From 100 per cent glucose solution, 10 ml was taken and made upto 100 ml with distilled water. This was taken as standard solution.

3.8.4. Estimation of reducing sugars

The amount of reducing sugars in the plant sample was estimated by Nelson-Somogyi method (Somogyi, 1952). The shoot and root materials collected from different treatments were taken for estimating the reducing sugars, From the sample, 100 mg was taken and homogenised with mortar and pestle. The sugars were extracted with hot 80 per cent ethanol and centrifuged. Then the supernatant was allowed to evaporate and the volume was made up to 5 ml with distilled water. From this, 0.5 ml was pipetted and the volume was made upto 2 ml with distilled water. To this, 1 ml of alkaline copper tartrate reagent was added and kept in the boiling water for 10 min. Then the tubes were cooled and 1 ml of arsenomolybdate reagent was added with a blank. After 10 min, the volume was made up to 10 ml and the absorbance was read at 620 nm. From the standard graph, the amount of reducing sugars was calculated.

Standard solution: 100 mg of glucose in 100 ml distilled water was taken and from that 10 ml was taken and made upto 100 ml as a working standard.

3.8.5. Amino Acid separation by paper chromotography

Leaf extraction was done taking 100 mg of leaf sample with 10 ml of 70 per cent ethanol. This was kept for evaporation in water bath and after that made up to 1 ml with ethanol. In Whatman No.1 filter paper sheet of the size 30x20 cm, a line was drawn two cm from the bottom. The sample was spotted with the help of a micropipette on the line three cm apart. The spots were dried with hot air blower. The standard amino acid was also applied similar to the sample. After

ɔotting, the sheet was kept in the stainless steel trough in the chromatography hamber. The spot end was kept down in the organic solvent and the chamber was losed air tight. The movement of the solvent was observed after leaving it for ver night.

The sheet was oven dried at 120°C for ten min and then sprayed with inhydrin reagent using an atomiser. Then it was again oven dried at 120°C for en min and the spots were identified and the R_f value was calculated.

$$R_f = \frac{\text{Distance (cm) moved by the solute from origin}}{\text{Distance (cm) moved by solvent from origin}}$$

The amino acids present in the samples were then identified by comparing ne R_f values with that of the authentic amino acids co-chromatographed Jayaraman, 1981).

.9. ESTIMATION OF ENZYME ACTIVITY

.9.1. Estimation of peroxidase enzyme

One g of the plant sample was taken in three ml of 0.1 M phosphate buffer pH 7). The sample was macerated with mortar and pestle and centrifuged at 8,000 g at 5 °C for fifteen min in a cuvette. From that 0.1 ml of the solution was aken and 3 ml of 0.05 M pyrogallol solution and 0.5 ml of one per cent hydrogen eroxide solution was added. The cuvette was read in a spectrophotometer at 30 nm. The change in absorbance for every thirty seconds upto three min were ecorded. The peroxidase activity was then calculated (Reddy *et al.*, 1985).

3.9.2. Estimation of chitinase enzyme

One g of plant sample was collected and was homogenised in three ml of 0.1 mM sodium citrate buffer (pH 5) with a mortar and pestle at 4°C. The omogenate was centrifuged for 15 min at 10,000 g. The supernatant was used as in enzyme source and 0.4 ml of this enzyme solution was taken into a 1.5 ml

Eppendorf tube and was added with 10 µl sodium acetate buffer (pH 5) and 0.1 ml of colloidal chitin. This was incubated in water bath at 37°C for two hours and then centrifuged at 1000 g for three min. An aliquot of 0.3 ml was taken into a glass tube containing thirty ml of phosphate buffer and twenty ml of snailgut enzyme (30 mg/ml) and incubated for one hour. To the samples, blank and standard, seventy ml of borate buffer was added. The tubes were heated in a boiling water bath for exactly three min and rapidly cooled in ice water. Into the tubes, two ml of p-dimethyl amino benzaldehyde (DMAB) was added and immediately after mixing, the tubes were incubated for twenty min at 37°C. After twenty min the tubes were cooled in tap water and read without delay at 585 nm in Hitachi model 200-20 spectrophotometer. The chitinase in leaf and root was expressed as n-mole N-acetyl glucosamine released per min per g of fresh tissue (Boller and Mauch, 1988).

3.10. ESTIMATION OF MACRO NUTRIENTS

3.10.1. Estimation of total nitrogen content in plants

The Nitrogen content was estimated by MicroKjeldhal method (Black, 1965). n a block digester tube, 0.5 g of plant sample was taken and to this, ten ml of con. H_2 SO_4 acid was added to convert organic nitrogen to inorganic nitrogen. The digestion vas done at 290-295°C. The digested material was distilled with forty per cent NaOH nd liberated ammonia was collected in two per cent boric acid which was kept in the tration vessel of autoanalyser. Then it was titrated against 0.1 N H_2SO_4 with mixed ndicator until the colour changed from red to green.

.10.2. Estimation of total phosphorus content in plants

Phosphorus in plant material was estimated by the Vanadomolybdate ethod in nitric acid system (Jackson. 1973). Five ml of the diacid extract was ken in twenty ml volumetric flask and 0.5 g of plant material was added to this. piece of red litmus paper was added to the solution and made slightly alkaline

by adding ammonia solution drop by drop till the red litmus paper changed to blue. Then five ml of Vanadomolybdate reagent was added and the solution was made up to twenty five ml with distilled water. The solution was read in a spectrophotometer at 470 nm wave length using diacid blank. The ppm was arrived by referring the chart. Total P content was calculated.

3.10.3. Estimation of potassium content in the plants

In a 250 ml conical flask, 0.5 g of the plant sample was weighed and taken. To this, fifteen ml of triple acid mixture was added and the contents were allowed to digest over a sand bath, till a clear solution was obtained. It was filtered through whatman No.1 filter paper and the volume was made up to 100 ml. This was directly read in a flame photometer after adjusting to zero with blank. From the standard chart, the concentration of K was calculated (Piper, 1966).

3.11. ESTIMATION OF MICRONUTRIENTS

To ten ml of triple acid extract, 0.5 g of the plant sample was added and digested and then the volume was made up to 100 ml. The solution was directly read in Atomic Absorption Spectrophotometer (AAS) varian Techtra 20 BQ. The concentration of different nutrients like Fe, Cu, Mn and Zn was read and the contents were calculated (Lindsay and Morvell, 1978).

3.12. STATISTICAL ANALYSIS

The experiments were conducted in a randomised block design. All the data were statistically analysed using RBD. (Murugesan *et al.*, 1974).

CHAPTER IV

EXPERIMENTAL RESULTS

Among the various kinds of organisms engaged in natural control of nematodes, VAM is gaining greater attraction because of its ability not only to control nematode but also to increase host nutrition. The effect of VAM for the control of nematode in brinjal has been studied and the results of various experiments conducted to analyse the effect of VAM-nematode interaction with biochemical variations are presented in this chapter.

4.3. EVALUATION OF OPTIMUM DOSES OF *G. mosseae* FOR THE CONTROL OF *M. incognita* ON BRINJAL CV. CO.2

There was an increase in shoot length, weight, root length and weight when *G. mosseae* was applied @ 10 g/kg soil followed by 5 g/kg soil. The above treatments were significantly different from each other. Control was on par with plants which received one g VAM per kg soil with respect to shoot length and weight with 35.6 cm and 34.2g and 37.5 cm and 32.4g respectively. The nematode alone recorded 31.8 cm (Table 1). Shoot weight was more in 10 g/kg soil with 79.2 g with an increase of 401.2 per cent over nematode alone. It was significantly different from 5 g/kg soil and 2 g/kg soil. In the case of root, the highest length and weight was recorded in 10 g/kg soil with 30.6 cm and 32.8 g respectively. It was significantly different from other treatments. In the case of root length, 1 g/kg soil and 2 g/kg soil were on par and all other treatments were significantly different. The root weight of 10 g/kg soil (32.8 g) and control (18 g) were significantly different. All other treatments were on par with each other (Fig. 1).

Colonization of VAM and spore count were greater in 10 g/kg soil followed by 5 g/kg soil with 87 and 80 per cent in case of colonization and 235 and 141

Table 1. Evaluation of optimum doses of *G. mosseae* for the control of *M. incognita* on brinjal Cv. Co. 2

Treatment	Length (cm)		Weight (g)		VAM colonization (%)	Spore count (10 g)	Soil nematode population (200 ml)	Gall Index
	Shoot	Root	Shoot	Root				
T_1 - 10 g / Kg soil	61.2 (92.4)	30.6 (82.1)	79.2 (401.2)	32.8 (25.1)	87.0	235	232	1.2
T_2 - 5 g / Kg soil	50.6 (59.1)	26.6 (58.3)	60.8 (284.8)	27.6 (5.3)	80.0	141	323	2.0
T_3 - 2 g / Kg soil	43.4 (36.4)	22.6 (34.5)	51.8 (227.8)	23.0 (- 12.2)	68.6	97	427	2.2
T_4 - 1 g / Kg soil	37.5 (17.9)	22.8 (35.7)	32.4 (105.0)	24.4 (- 6.8)	46.6	41	572	2.6
T_5 - *M. incognita* alone	31.8	16.8	15.8	26.2	-	-	752	4.6
T_6 - Control	35.6	20.0	34.2	18.0	-	-	-	-
CD (P = 0.05)	4.4	2.2	7.6	4.6	6.5	40.9	76	0.66

Figures in parenthesis indicates per cent increase over nematode alone

Fig.1. Evaluation of optimum doses of *G. mosseae* for the control of *M. incognita* on brinjal cv. Co.2

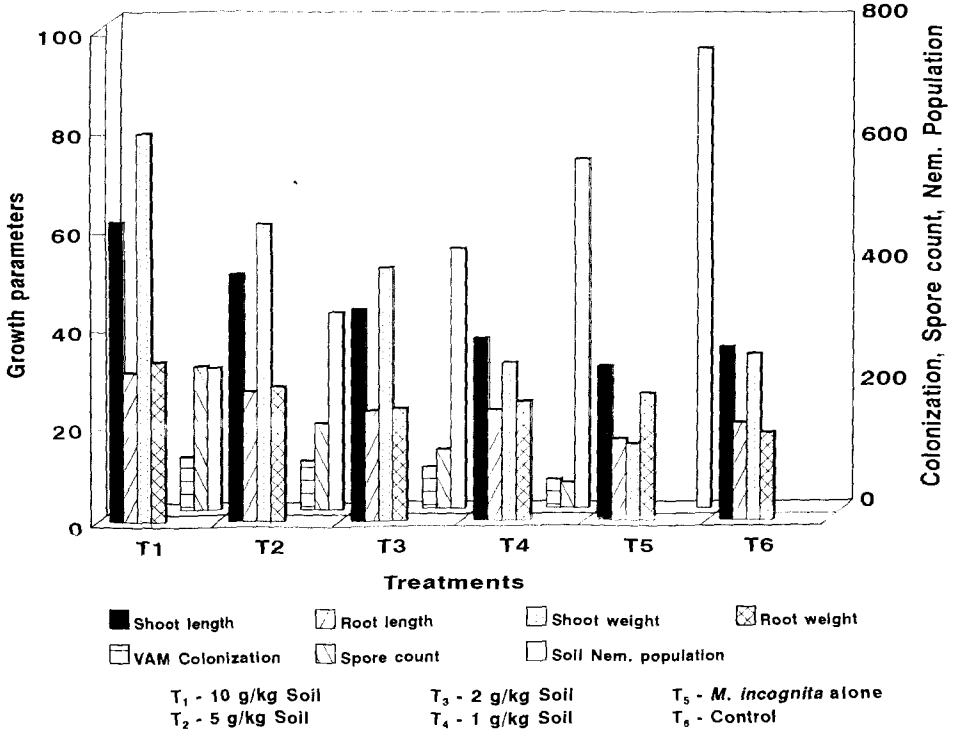


Plate 3a.

Plate 3b.

Table 2. Effect of four species of VAM, *G. fasciculatum*, *G. mosseae*, *G. intraradices* and *G. fulvum* against *M. incognita* on brinjal

Treatment	Length (cm)		Weight (g)		Soil nematode population/ 200 ml of soil	Yield (g)	per cent increase over nematode alone
	Shoot	Root	Shoot	Root			
T_1 - *G. fasciculatum*	70.3 (24.2)	46.6 (185.8)	103.6 (88.3)	96.0 (185.7)	-	198	132.9
T_2 - *G. fasciculatum* + *M. incognita*	57.3 (1.23)	31.6 (93.8)	78.3 (42.3)	61.6 (83.3)	207	143	68.2
T_3 - *G. mosseae*	77.3 (36.5)	34.3 (110.4)	93.0 (69.0)	73.3 (118.1)	-	201	136.4
T_4 - *G. mosseae* + *M. incognita*	67.0 (18.3)	28.3 (73.6)	73.0 (32.7)	58.6 (74.4)	193	111	30.5
T_5 - *G. intraradices*	72.6 (28.2)	27.0 (65.6)	90.3 (64.1)	45.6 (35.7)	-	148	74.1
T_6 - *G. intraradices* + *M. incognita*	56.3 (- 0.53)	23.0 (41.1)	78.6 (42.9)	72.3 (115.1)	276	105	23.5
T_7 - *G. fulvum*	72.6 (28.2)	31.0 (90.7)	94.6 (72.9)	70.6 (110.1)	-	165	94.1
T_8 - *G. fulvum* + *M. incognita*	60.0 (6.0)	22.0 (34.9)	88.6 (61.0)	54.6 (62.5)	286	91	7.0
T_9 - *M. incognita* alone	56.6	16.3	55.0	33.6	316	85	-
T_{10} - Control	61.0	18.6	73.3	48.0	-	135	-
CD(P=0.05)	11.1	9.7	21.6	30.1	-	24.5	-

Figures in parenthesis indicates per cent increase over nematode alone

Table 3. Effect of four species of VAM, *G. fasciculatum*, *G. mosseae*, *G. intraradices* and *G. fulvum* against *M. incognita* on brinjal

Treatment	VAM colonization	spore count	Gall index	Number of females with egg mass	Number of females without egg mass	Number of eggs/sac
T_1 - *G. fasciculatum*	72.6	148	-	-	-	-
T_2 - *G. fasciculatum* + *M. incognita*	66.6	184	2.1	17	31	43
T_3 - *G. mosseae*	76.6	208	-	-	-	-
T_4 - *G. mosseae* + *M. incognita*	61.6	177	1.8	14	30	35
T_5 - *G. intraradices*	51.6	86	-	-	-	-
T_6 - *G. intraradices* + *M. incognita*	35.0	124	2.5	22	43	66
T_7 - *G. fulvum*	35.0	85	-	-	-	-
T_8 - *G. fulvum* + *M. incognita*	31.6	69	2.8	32	41	71
T_9 - *M. incognita* alone	-	-	4.1	83	30	104
T_{10} - Control	-	-	-	-	-	-
CD (P = 0.05)	6.6	21.7	-	-	-	-

Fig.2. Effect of four species of VAM, against M. incognita on brinjal

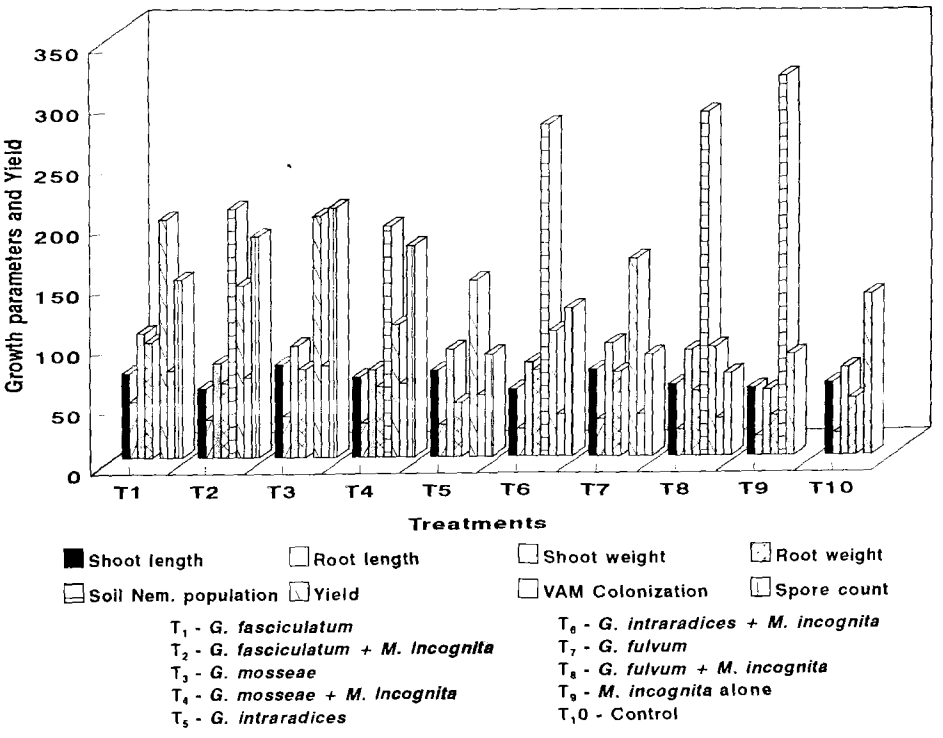


Plate 3a.

Plate 3b.

Plate 5a.

Plate 5b.

66.6 per cent and least in *G. fulvum* + *M. incognita* with 31.6 per cent. *G. intraradices* was significantly different from all other treatments, whereas *G. intraradices* + *M. incognita*, *G. fulvum* and *G. fulvum* + *M. incognito* were on par. The spore count was more in *G. mosseae* with 208 and the least in *G. fulvum* + *M. incognita* with 69. The gall index was more in *M. incognita* alone with 4.1 and least in *G. mosseae* + *M. incognita* with 1.8 (Table 3).

The penetration of juvenile in to the root and its development to adult with and without egg mass was recorded. It was more in *M. incognita* alone with 83 females with the egg mass and it was least in *G. mosseae* + *M. incognita* with only 14 females with egg mass. The females without egg mass was least in *M. incognita* alone. The number of eggs/sac was more in *M. incognita* alone with 104 and least with 35 in *G. mosseae* + *M. incognita*. The yield was maximum in *G. mosseae* with 201 g/plant followed by *G. fasciculatum* with 198 g/plant and they were on par with each other. The least was observed in *M. incognita* alone with 85 g/plant. The treatments *G. fulvum* and *G. intraradices* were on par and all the other treatments were significantly different from each other. VAM with nematode recorded the maximum in *G. fasciculatum* + nematode with 143 g/plant followed by *G. mosseae* + nematode with 111 g/plant (Plate 6 and 7).

4.4.1. Determination of best species of VAM and VAM inoculated with nematode by cluster analysis

A multivariate cluster analysis was performed to find out the suitable combination when considering the many variables used in the study. The analysis was performed with nearest neighbour with single linkage and varimax rotation involving three variables *viz.*, yield, root nitrogen and colonization. Other variables were above 0.10.

The cluster analysis was performed with K-means clustering (Table 4) to segregate these into two groups based on their performance. It was found that

Plate 6a.

Plate 6b.

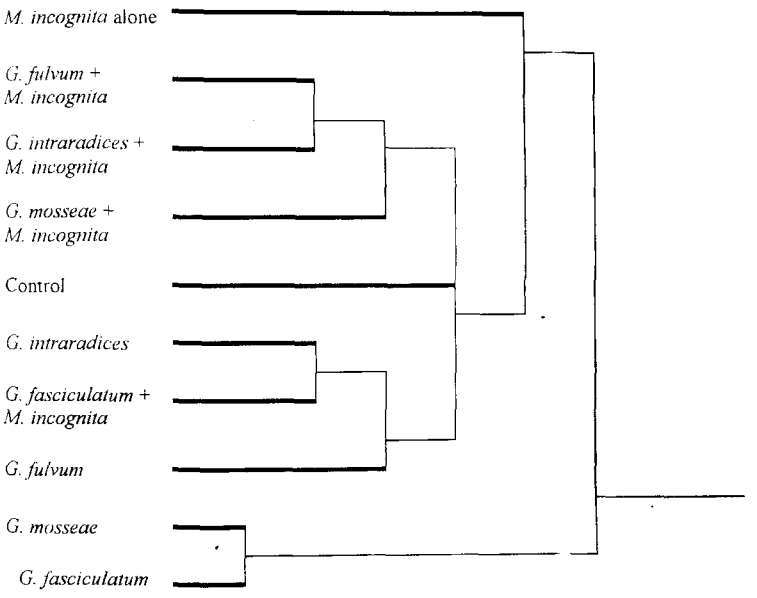
Plate 7a.

Plate 7b.

Table 4. K-means of cluster analysis for combination of different VAM species with and without nematode

Treatments	Euclidean Distance	Variable	Minimum	Mean	Maximum	Std. Dev.
Cluster I						
T_2 - *G. fasciculatum* + *M. incognita*	12.56	Yield	85.00	122.88	165.00	27.09
T_4 - *G. mosseae* + *M. incognita*	16.74	Root Nitrogen	0.47	1.02	1.27	0.23
T_5 - *G. intraradices*	17.34	Colonization	0.01	35.21	66.67	23.59
T_6 - *G. intraradices* + *M. incognita*	10.32					
T_7 - *G. fulvum*	24.32					
T_8 - *G. fulvum* + *M. incognita*	18.52					
T_9 - *M. incognita* alone	29.86					
T_{10} - Control	21.50					
Cluster II						
T_1 - *G. fasciculatum*	1.85	Yield	196.00	198.50	201.00	2.50
T_3 - *G. mosseae*	1.85	Root Nitrogen	1.33	1.59	1.84	0.25
		Colonization	72.67	74.67	76.67	2.00

Fig. 3. Cluster analysis showing combination of different VAM species with and without nematode



Cluster Tree

(Join algorithm with average linkage)

G. fasciculatum and *G. mosseae* were closely related with the shortest distance value and they were grouped in cluster number 2. Though all the other treatments were grouped in cluster number 1, it was found that nematode alone had the longest distance value.

When the dendrogram (tree diagram) was drawn using join algorithm of cluster analysis (Fig. 3), the same trend of K-means was reflected, i.e. *G. fasciculatum* and *G. mosseae* were closely linked at the shortest distance. Control was linked to *M. incognita* alone and the rest at the distance value of 21.50. It was also noticed that *G. intraradices* and *G. fulvum* inoculated with nematode were closely related which was again joined with *G. mosseae* inoculated with nematode at a farthest distance, all these treatments had *M. incognita* inoculated. But *G. fasciculatum* which had *M. incognita* inoculated joined *G. intraradices* which is not inoculated, which is again joined with *G. fulvum* which is not inoculated at a farthest distance. Thus, it could be concluded that *G. fasciculatum* could be having better check over *M. incognita* and hence the better performance even under inoculated condition.

4.5. EFFECT OF CROP ROTATION ON THE INTERACTION OF VAM WITH *M. incognita* (Brinjal - cumbu - greengram - brinjal)

4.5.1. Brinjal

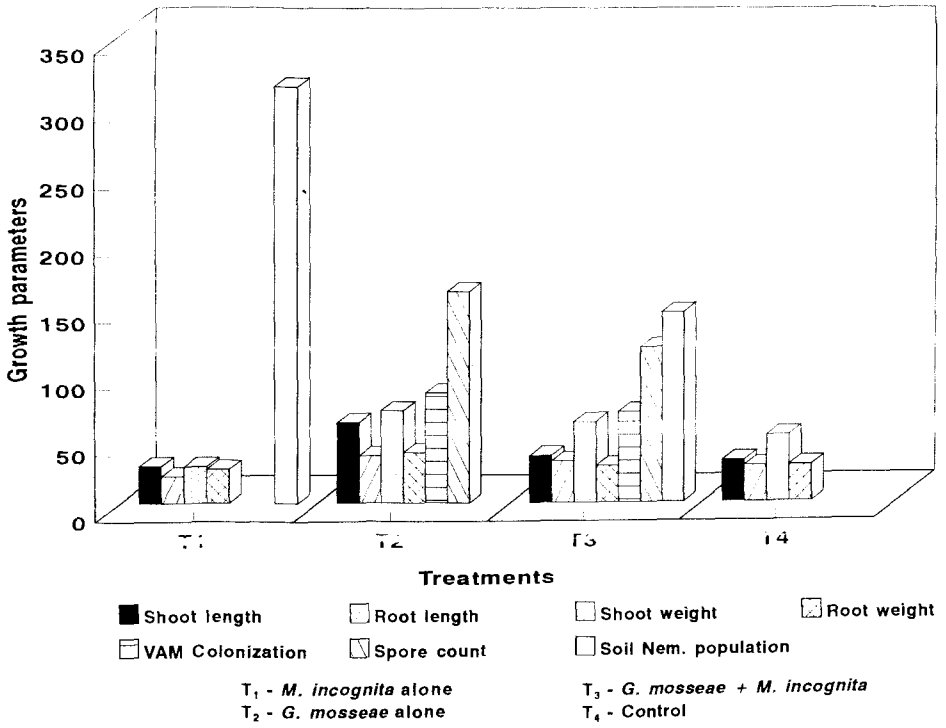
The shoot length was maximum in *G. mosseae* with 60.6 cm (Table 5). *G. mosseae* and *G. mosseae* + *M. incognita* were significantly different from each other whereas control and nematode alone were on par. The shoot weight was more in *G. mosseae* with 69.6 g followed by *G. mosseae* + *M. incognita* with 60.8 g and all the treatments were significantly different from each other. The root length and weight was maximum in *G. mosseae* with 35.6 cm and 38.1g respectively. *G. mosseae* + *M. incognita* and control were on par with each other in root weight. The VAM colonization and spore count were 83 per cent and 159

Table 5. Effect of crop rotation on the interaction of *G. mosseae* with *M. incognita* **First crop : Brinjal Cv. Co. 2**

Treatment	Length (cm)		Weight (g)		VAM colonization (%)	Spore count (10 g)	Soil nematode population (200 ml)	Gall Index
	Shoot	Root	Shoot	Root				
T_1 - *M. incognita* alone	28.0	20.3	27.5	25.6	-	-	312	4.3
T_2 - *G. mosseae* alone	60.6 (116.4)	35.6 (75.3)	69.6 (154.1)	38.1 (48.8)	83.0	159	-	-
T_3 - *G. mosseae* + *M. incognita*	35.5 (26.7)	31.3 (54.1)	60.8 (121.0)	27.6 (7.8)	68.3	117	143	3.6
T_4 - Control	31.3	27.3	50.0	27.5	-	-	-	-
CD P= 0.05	3.6	2.2	3.5	2.4	-	-	-	-

Figures in parenthesis indicates the per cent increase over nematode alone

Fig.4. Effect of crop rotation on the interaction of *G. mosseae* with *M. incognita* First Crop : Brinjal Cv. Co.2



in *G. mosseae* and 68.3 per cent and 117 in *G. mosseae* + *M. incognita* respectively. The soil nematode population was more in *M. incognita* alone with 312 nematodes per 200 ml soil followed by *G. mosseae* + *M. incognita* with 143 nematodes per 200 ml soil. The gall index was 4.3 in *M. incognita* alone and 3.6 in *G. mosseae* + *M.incognita* (Fig. 4).

4.5.2. Cumbu

The shoot length was more in *G. mosseae* with 97.1 cm followed by 94.5 cm in *G. mosseae* + *M. incognita* (Table 6). The shoot weight was 82.2 g in *G. mosseae* and it was significantly different from all other treatments. *G. mosseae* + *M. incognita* recorded 45.5 g of shoot weight and 43.4 g in *M. incognita* alone. The root length and weight was more in *G. mosseae* + *M. incognita* with 20.4 cm and 52.9 g respectively. The least in *M.incognita* alone with 11.6 cm and 38.2 g. VAM colonization was 90 per cent in *G. mosseae* and 89.1 per cent in *G. mosseae* + *M. incognita*. The spore count was more in *G. mosseae* with 191 followed by 185 spore in *G. mosseae* + *M. incognita*. The soil population was 187 nematodes per 200 ml soil in *M. incognita* and 97 in *G. mosseae* + *M. incognita*. The number of earhead was more in *G. mosseae* with 8 numbers and least in control with 3.8. The treatment *G. mosseae* and control were significantly different from each other.

4.5.3. Greengram

The shoot length and weight was more in VAM alone with 43.8 cm and 76.2 g. The least was 36.6 cm and 69.2 g in control. The root weight was more in *M. incognita* alone with 8.2 g and least in control (6.2 g). The VAM colonization and spore count was 81.6 per cent and 295 in *G. mosseae* and 72.5 per cent and 230 in *G. mosseae* + *M. incognita* respectively. The soil nematode population and gall index was 195 and 2.8 in *M. incognita* alone and 112 and 1.5 in *G. mosseae* + *M. incognita* respectively (Table 7).

Table 6. Effect of crop rotation on the interaction of *G. mosseae* with *M. incognita* Second crop : Cumbu Cv. Co. 1

Treatment	Length (cm)		Weight (g)		VAM colonization (%)	Spore count (10 g)	Soil nematode population (200 ml)	Number of ear head
	Shoot	Root	Shoot	Root				
T_1 - *M. incognita* alone	90.8 --	11.6 --	44.5 --	38.2 --	-	-	187	4.3
T_2 - *G. mosseae* alone	97.1 (6.9)	15.3 (31.8)	82.2 (84.7)	42.8 (12.0)	90.0	191	-	8.0
T_3 - *G. mosseae* + *M. incognita*	94.5 (4.0)	20.4 (75.8)	45.5 (2.2)	52.9 (38.4)	89.1	185	97	6.8
T_4 - Control	88.5	15.1	43.4	38.2	-	-	-	3.8
CD (P=0.05)	NS	5.5	15.2	11.02	-	-	-	1.4

Figures in parenthesis indicates the per cent increase over nematode alone

Table 7. Effect of crop rotation on the interaction of *G. mosseae* with *M. incognita* Third crop : Greengram Co. 5

Treatment	Length (cm)		Weight (g)		VAM colonization (%)	Spore count (10 g)	Soil nematode population (200 ml)	Gall Index
	Shoot	Root	Shoot	Root				
T_1 - *M. incognita* alone	32.5	14.3	64.3	8.2	-	-	195	2.8
	--	--	--	--				
T_2 - *G. mosseae* alone	43.8	15.0	76.2	6.6	81.6	295	-	-
	(11.3)	(4.8)	(18.5)	(-19.5)				
T_3 - *G. mosseae* + *M. incognita*	39.0	17.8	72.6	7.4	72.5	230	112	1.5
	(20.0)	(3.5)	(12.9)	(-9.7)				
T_4 - Control	36.6	19.1	69.2	6.2	-	-	-	-
	--	--	--	--				
CD (P=0.05)	7.2	NS	NS	1.0	-	-	-	-

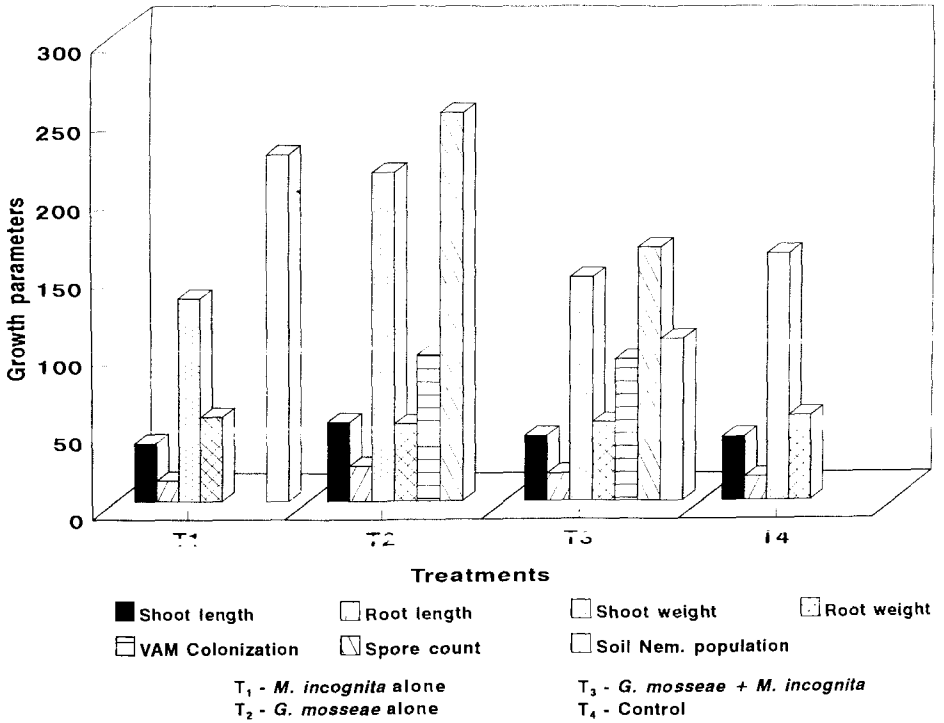
Figures in parenthesis indicates the per cent increase over nematode alone

Table 8. Effect of crop rotation on the interaction of *G. mosseae* with *M. incognita* **Fourth crop : Brinjal Cv. Co. 2**

Treatment	Length (cm)		Weight (g)		VAM colonization (%)	Spore count (10 g)	Soil nematode population (200 ml)	Gall Index
	Shoot	Root	Shoot	Root				
T_1 - *M. incognita* alone	37.6	13.3	131.1	54.3	-	-	223	4.0
	--	--	--	--				
T_2 - *G. mosseae* alone	51.0	22.8	211.8	50.1	95	250	-	-
	(35.6)	(71.4)	(61.4)	(-7.7)				
T_3 - *G. mosseae* + *M. incognita*	42.3	17.8	144.8	51.1	92	164	105	2.5
	(12.5)	(33.8)	(10.4)	(-5.8)				
T_4 - Control	41.3	15.3	160.0	54.8	-	-	-	-
	--	--	--	--				
CD (P=0.05)	4.3	2.8	29.9	NS	-	-	-	-

Figures in parenthesis indicates the per cent increase over nematode alone

Fig.5. Effect of crop rotation on the interaction of G. mosseae with M. incognita Fourth crop : Brinjal Cv. Co.2



4.5.4. Brinjal

The shoot length and weight was more in *G. mosseae* with 51 cm and 211.8 g respectively. The least was observed in *M. incognita* alone with 37.6 cm and 131.1 g respectively. The root length was more in *M. incognita* alone with 13.3 cm. The root weight was more in control with 54.8 g and lowest in *G. mosseae* with 50.1 g (Table 8). The VAM colonization and spore count was more in *G. mosseae* with 95 per cent and 250 respectively. The soil nematode population was 223 and gall index was 4 in *M. incognita* alone and least in *G. mosseae* + *M. incognita* with 105 and 2.5 respectively (Fig. 5).

4.6. FIELD EVALUATION OF *G. mosseae* FOR THE CONTROL OF *M.incognita* ON BRINJAL

The shoot weight was more in 2.5 kg VAM/m^2 with 416.6 g with an increase of 89.4 per cent over control. The least was observed in 0.5 kg VAM/m^2 with 211.1 g. The root length and weight were maximum in 2.5 kg VAM/m^2 with 123 cm and 320 g followed by 2 kg VAM/m^2 with 122 cm and 303 g respectively. The treatments 2.5 and 2 kg VAM/m^2 were on par. The initial nematode population in the field ranged from 54 to 89 per 200 ml soil. However, at harvest, the nematode population had increased, which ranged between 123 and 248. Transplanting of VAM treated seedlings recorded lower root-knot index (2.0) in 2.5 kg VAM/m^2 at harvest, than those from control with 3.6 (Table 9). The carbofuran treated plot recorded only 3.3 (Fig. 6).

The R_F value was more in Carbofuran with 4 and least in 2 kg VAM/m^2 with 1.77 (Table 10). The spore count and VAM colonization was more with 196 and 80 per cent in 2 kg VAM/m^2 followed by 194 and 75 per cent in 2.5 kg VAM/m^2 respectively. The yield was maximum in 2 kg VAM/m^2 with 80.7 kg, per plot followed by 2.5 kg VAM/m^2 with 78.8 kg and both were on par. The yield in plot receiving 1 kg/m^2 (62.2 kg) and carbofuran (64.6 kg) were on par (Fig. 7).

Table 9. Growth of brinjal plants as influenced by interaction of *G. mosseae* and *M. incognita* on brinjal

(Growth parameters)

Treatment	Length (cm)		Weight (g)		Seedling weight (g)
	Shoot	Root	Shoot	Root	
T_1 - 0.5 Kg *G.mosseae*	61.7	97	211.1	233.3	1.0
T_2 - 1.0 Kg *G.mosseae*	72.4	106	269.7	201.6	1.0
T_3 - 1.5 Kg *G.mosseae*	57.7	112	243.3	185.0	1.5
T_4 - 2.0 Kg *G.mosseae*	60.9	122	384.4	303.0	1.3
T_5 - 2.5 Kg *G.mosseae*	63.1	123	416.6	320.0	1.5
T_6 - Carbofuran 1 Kg a.i./ha	66.9	106	266.6	251.6	0.8
T_7 - Control	72.1	99	219.9	228.3	1.1
CD (P = 0.05)	NS	NS	127.4	63.8	0.33

Fig.6. Growth of brinjal plants as influenced by interaction of *G. mosseae* and *M. incognita* on Brinjal

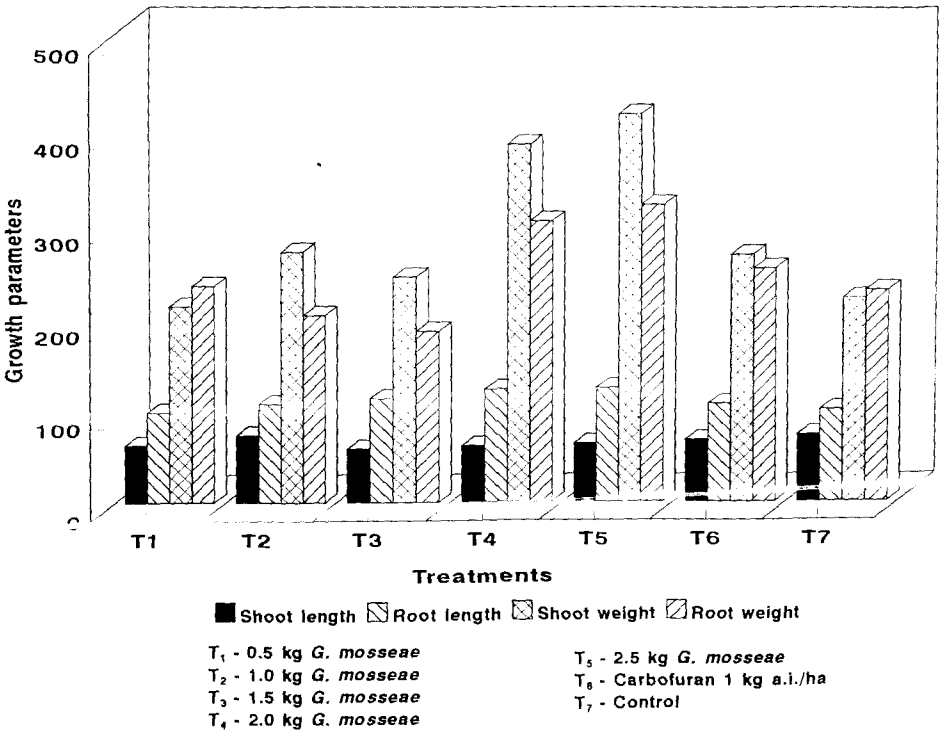
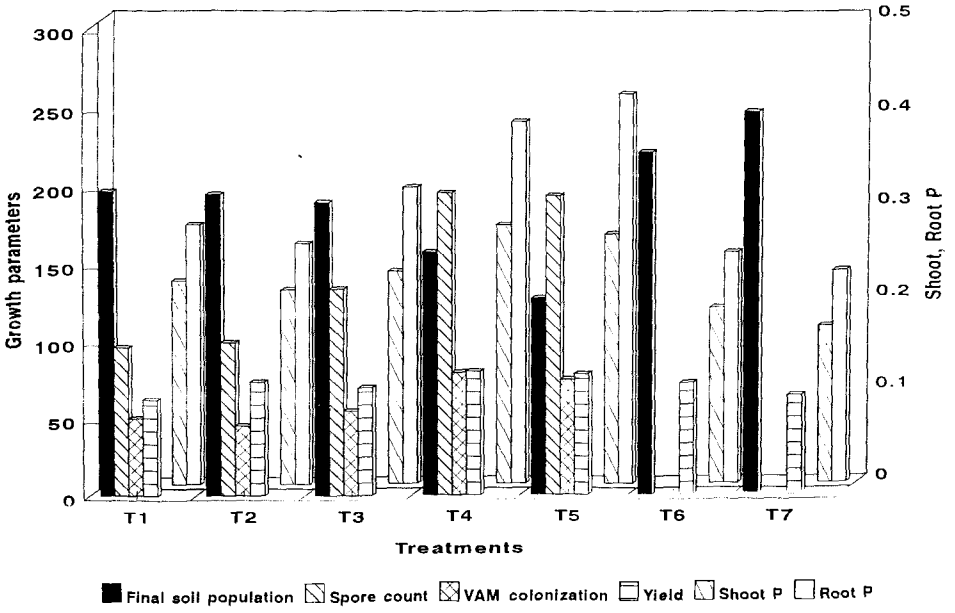


Table 10. Growth of brinjal plants as influenced by interaction of *G. mosseae* and *M. incognita* on brinjal

(Spore count, VAM colonization, Gall index and yield)

Treatment	Initial soil population	Final soil population	RF =P_f/Pi	Spore count (10 g)	VAM coloni-zation	Gall Index	Phosphorus (%)		Yield (Kg/20 m^2)	Per cent increase over control	Cost Benefit ratio
							Shoot	Root			
T_1 - 0.5 Kg *G.mosseae*	82.3	197	2.3	96	50	2.6	0.22	0.28	62.2	- 3.7	1 : -5.0
T_2 - 1.0 Kg *G.mosseae*	64.3	195	3.0	99	45	2.6	0.21	0.26	73.5	13.7	1 : 11.2
T_3 - 1.5 Kg *G.mosseae*	81.0	190	2.34	134	55	3.0	0.23	0.32	70.0	8.3	1 : 4.5
T_4 - 2.0 Kg *G.mosseae*	89.0	158	1.77	196	80	2.3	0.28	0.39	80.7	24.9	1 : 19.8
T_5 - 2.5 Kg *G.mosseae*	63.3	123	1.94	194	75	2.0	0.27	0.42	78.8	21.9	1 : 15.7
T_6-Carbofuran 1 Kg a.i /ha	54.3	222	1.00	-	-	3.3	0.19	0.25	72.7	12.5	1 : 10.5
T_7 - Control	67.6	248	3.60	-	-	3.6	0.17	0.23	64.6	-	-
CD (P=0.05)	NS	22.4	-	20.7	NS	0.95	-	-	2.3	-	-

Fig.7. Growth of brinjal plants as influenced by interaction of *G. mosseae* and *M. incognita* on Brinjal



T_1 - 0.5 kg *G. mosseae*
T_2 - 1.0 kg *G. mosseae*
T_3 - 1.5 kg *G. mosseae*
T_4 - 2.0 kg *G. mosseae*
T_5 - 2.5 kg *G. mosseae*
T_6 - Carbofuran 1 kg a.i./ha
T_7 - Control

When the cost benefit ratio was worked out, it was observed that the maximum benefit was, when 2 kg/m^2 VAM was applied in the nursery with 19.8 times increase, followed by 2.5 kg/m^2 VAM with 15.7 times increase whereas in the case of carbofuran, it was only 10.5 times increase over control.

4.7. HISTOPATHOLOGICAL STUDIES

The information about the internal structures of a plant infected by a nematode can be examined in stained serial sections of paraffin embeded material of 5-10 μ thickness. The histopathological changes brought about by nematodes which involves the formation of several atyptical tissues such as gaint cells, abnormal xylem, hyperplastic parenchyma and hypertrophy of cells.

4.7.1. Histopathology of nematode infested brinjal roots

The roots of healthy plant have a diarch radical protostele with the two protoxylem points abutting the pericycle directly (Plate 8). The primary phloem was radially arranged with respect to the protoxylem. The larvae penetrated through the cortex and meristamatic tissues of the root tip established the feeding site in the central vascular region (Plate 9). The female head of *M. incognita* was found in the cortex feeding on the cells causing hyperplasia and hypertrophy. The cells became progressively larger, multinucleated and highly vacuolated. The number of giant cells observed at each infection site varied (Plate 10a). In the giant cells there were seven to eight nuclei (Plate 10b). The diameter of the cortical cells proximate to the developing females was greater than average and the root became swollen, thus producing the characteristic external symptoms of infection. All the egg masses are external to the epidermis (Plate 11a). First stage larvae were observed inside the eggs.

The walls of the stelar tissues in contact with the nematode gave the appearance of being lignified, due to the presence of increased activity of

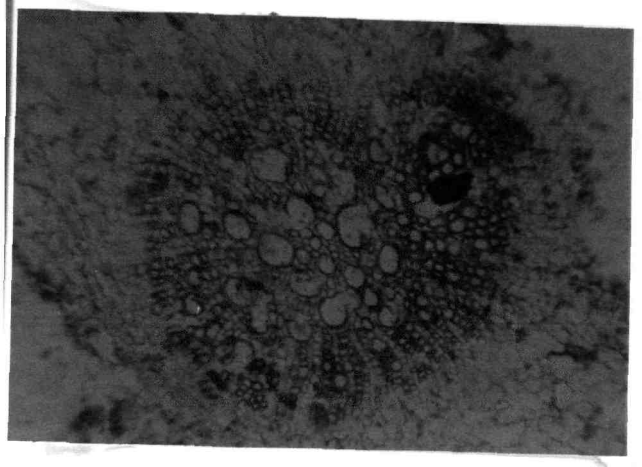


Plate 8. Healthy root

Plate 9. Feeding site in the vascular region

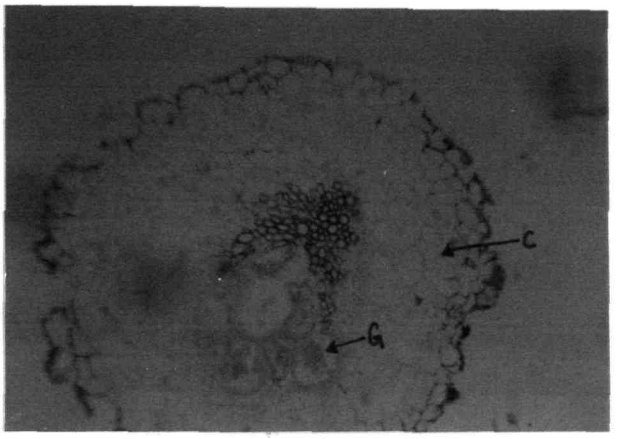


Plate 10a. Gaint cells with multinuclei

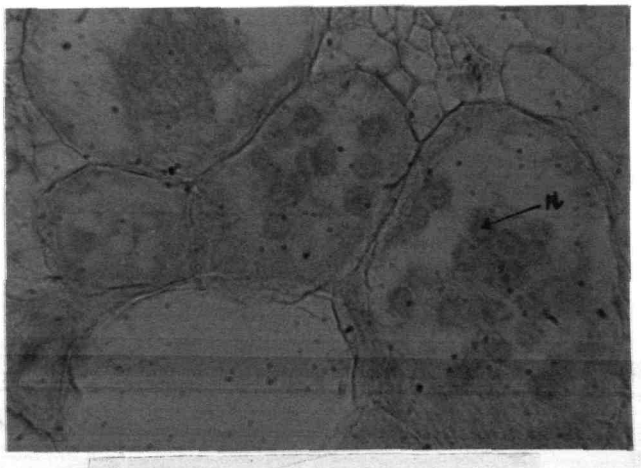


Plate 10b. Gaint cell with multinuclei

peroxidase which activated the synthesis of lignin and enhanced lignin deposition in the cell wall of endodermis (Plate 11b).

4.7.2. Histopathology of VAM and nematode infested brinjal roots

The microtome sections (10 μ thickness) of VAM infested roots revealed that the fungal hyphae penetrated the epidermis and invaded the cortex intercellularly and intracellularly (Plate 12a & b). Arbuscules were formed in the cortical cells. Vesicles were also found which was globose to irregularly shaped (Plate 13). Arbuscules were observed in some cortical cells in proximity to nematode. The xylem and phloem were pushed to one side inside the root. Due to *M. incognita* infection, the vesicular bundle was pushed towards the epidermis and few giant cells were formed.

4.8. BIOCHEMICAL ANALYSIS OF VAM-NEMATODE INTERACTION

Plant parasitic nematode are capable of altering the normal nematode processes of the host, which is manifested in the form of cellular, physiological and biochemical changes occurring in the infested host. The infection of plants by nematodes trigger certain mechanisms causing high metabolic activities on host as evident by higher levels of various metabolites in infected host than that of healthy counterpart.

4.8.1. Estimation of protein

4.8.1.1 *Qualitative changes of protein by polyacrylamide gel electrophoresis*

The qualitative changes of soluble protein of shoot and root of brinjal was observed in 1 mm thickness of slab gel. The VAM inoculated shoot stained more than the nematode + VAM shoot (Plate 14a), but the nematode + VAM root stained more than the VAM inoculated root (Plate 14b).

A comparison of electrophoresed protein profiles obtained from VAM alone and VAM + nematode inoculated shoot and root provided the following

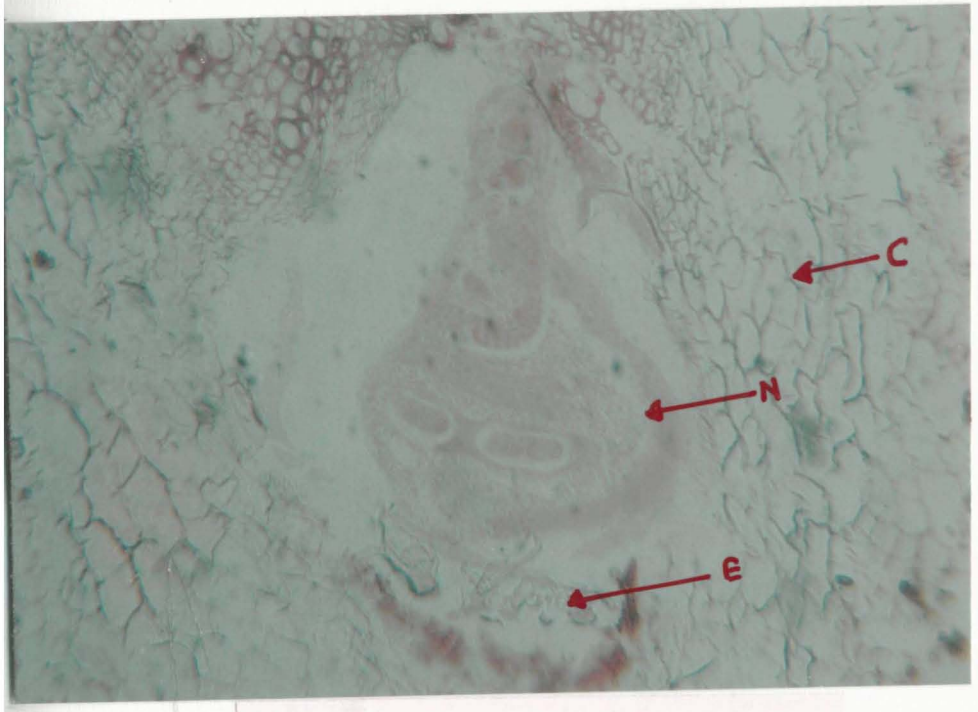


Plate 11a. Female with egg mass

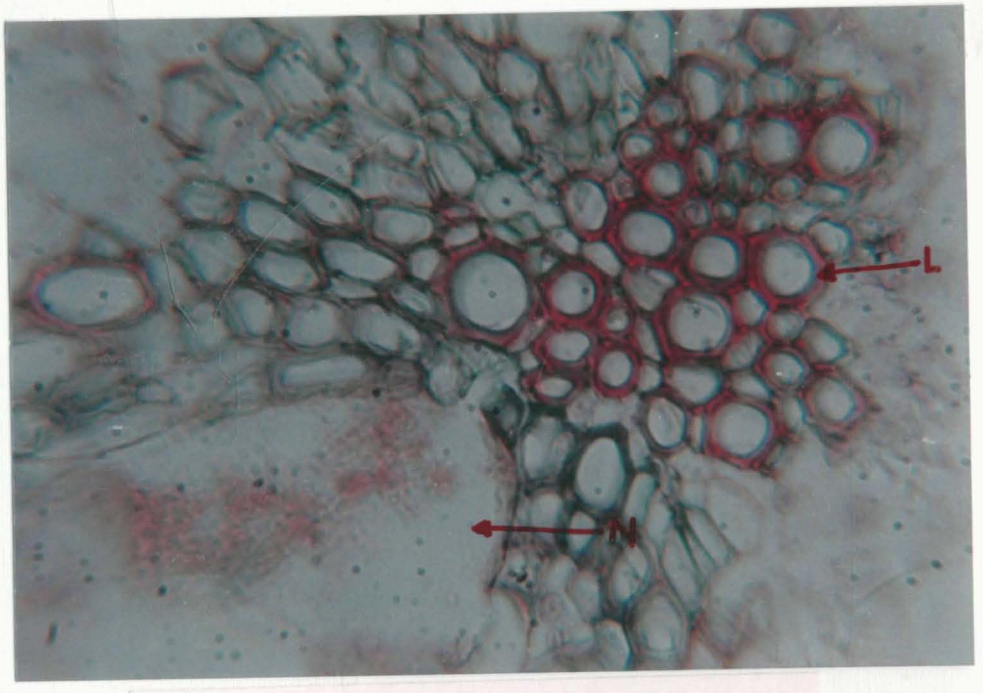


Plate 11b. Lignification of endoderms

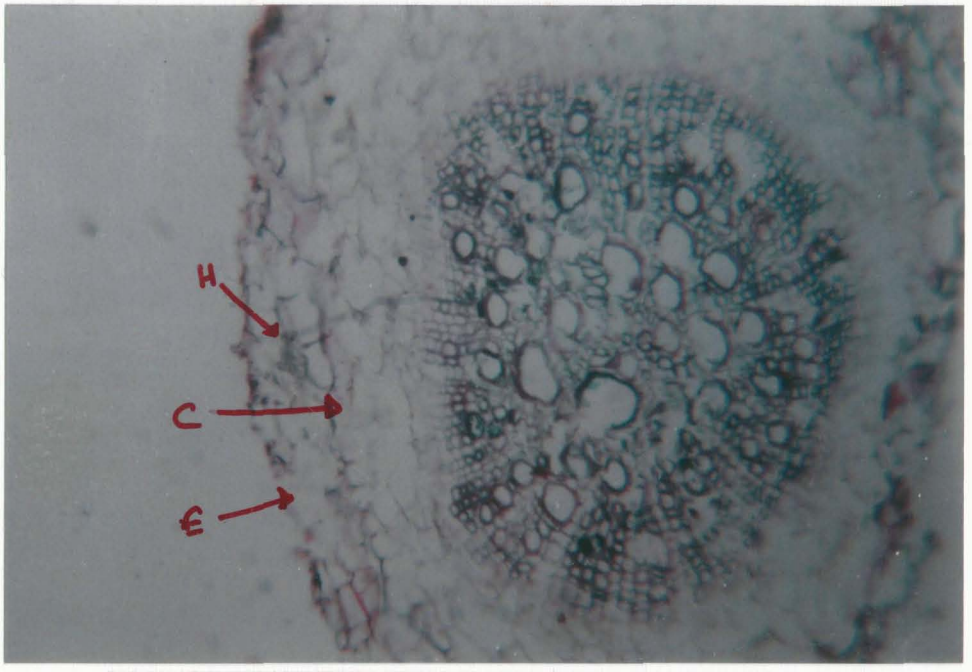


Plate 12a. Hyphae penetrating epidermis

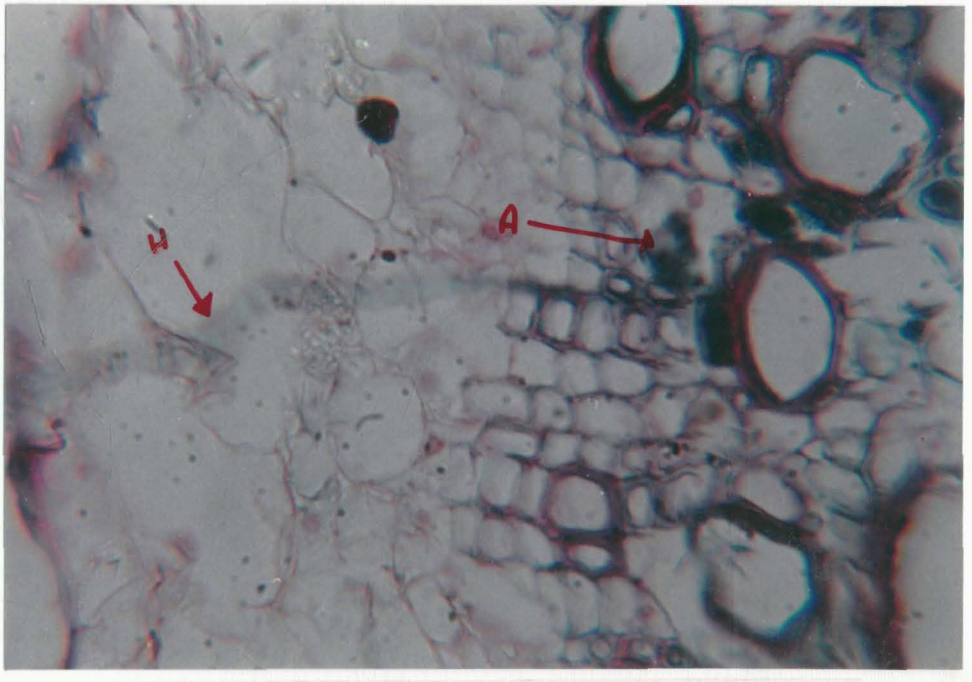


Plate 12b. Hyphae penetrating the cortex

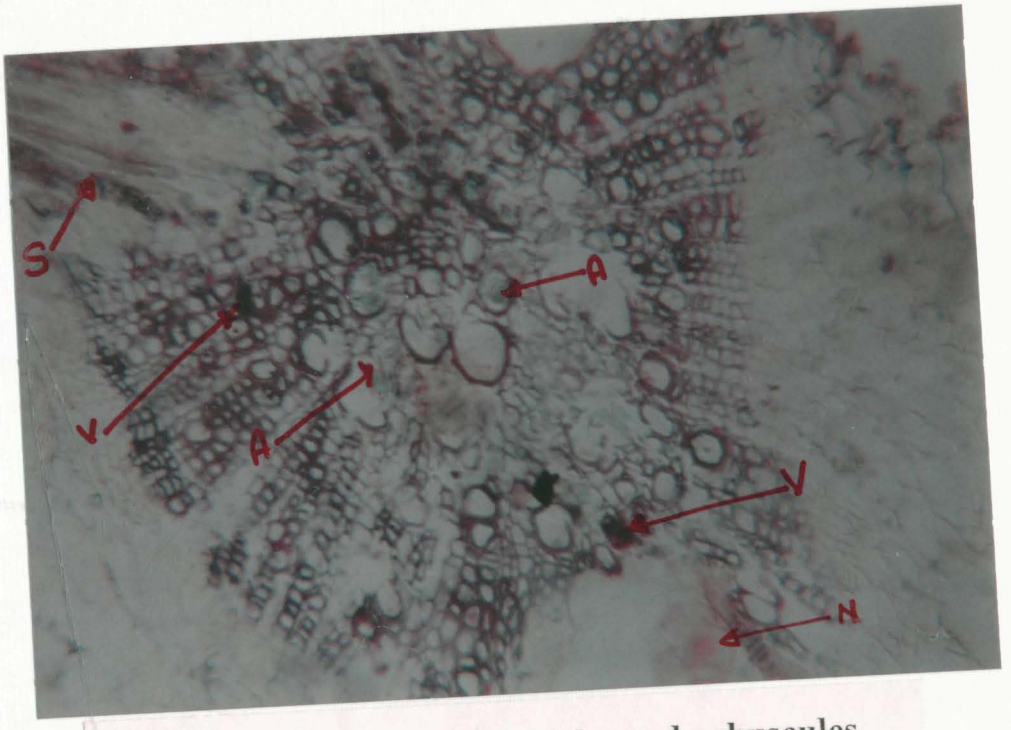


Plate 13. Presence of vesicles and arbuscules

information. There was an appearance and disappearance of some bands in VAM and VAM + nematode inoculated plants. The number of protein bands resolved for *G. fasciculatum* and nematode inoculated with *G. fasciculatum* shoot were twelve and ten respectively. Out of these, eight bands were common for both. The bands with R_f values of 0.20, 0.31, 0.81 and 0.85 were present only in *G. fasciculatum* and the bands with R_f values of 0.02 and 0.56 were present only in nematode inoculated *G. fasciculatum*. In *G. mosseae* and *G. mosseae* inoculated with nematodes the number of bands were fifteen and thirteen respectively and out of these, nine bands were common for both. In *G. mosseae* alone, the bands with R_f values of 0.35, 0.45, 0.77, 0.20, 0.81 and 0.86 were found and the bands with R_f values of 0.02, 0.18, 0.48 and 0.33 were present only in nematode + *G. mosseae* in shoot (Table 11).

In *G. intraradices* alone, the bands with R_f values of 0.05,0.10, 0.20, 0.31 and 0.35 were present and the bands with R_f values of 0.02 and 0.33 were observed when nematode inoculated. Out of thirteen and ten bands in *G. intraradices* and *G. intraradices* + nematode respectively, eight bands were common. In *G. fulvum*, five bands were present which are common for both *G. fulvum* and *G. fulvum* + nematode, whereas the bands with R_f values of 0.02 and 0.10 were present only in nematode inoculated VAM plants. In the case of nematode alone, the bands with R_f value of 0.02 was present in nematode alone and in all species of VAM inoculated with nematode. The bands with R_f values of 0.05, 0.10, 0.15 were present in nematode alone, all the VAM species and VAM + *M. incognita*, except *G. intraradices* + *M. incognita* and *G. fulvum*. The bands with R_f values of 0.26 and 0.55 were common for all the treatments. The band with R_f value of 0.35 was present in all the treatments except *G. mosseae* + *M. incognita* and *G. intraradices* + *M. incognita*. The band with R_f value of 0.62 was absent in both *G. fasciculatum*, *G. fulvum* and in combination with nematode.

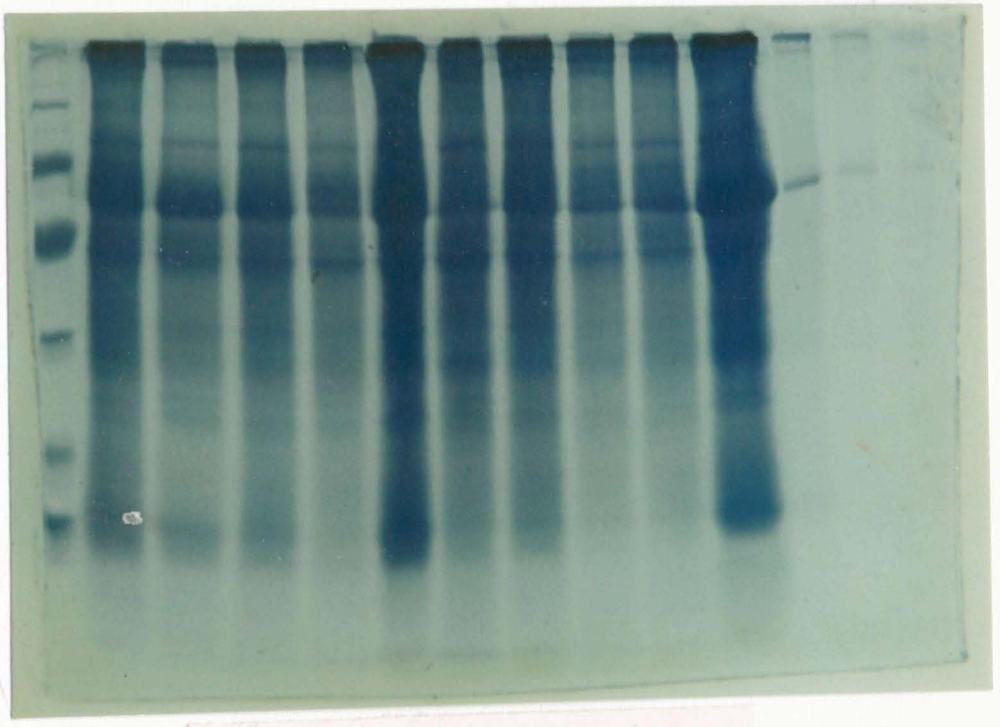


Plate 14a. SDS-PAGE – Shoot

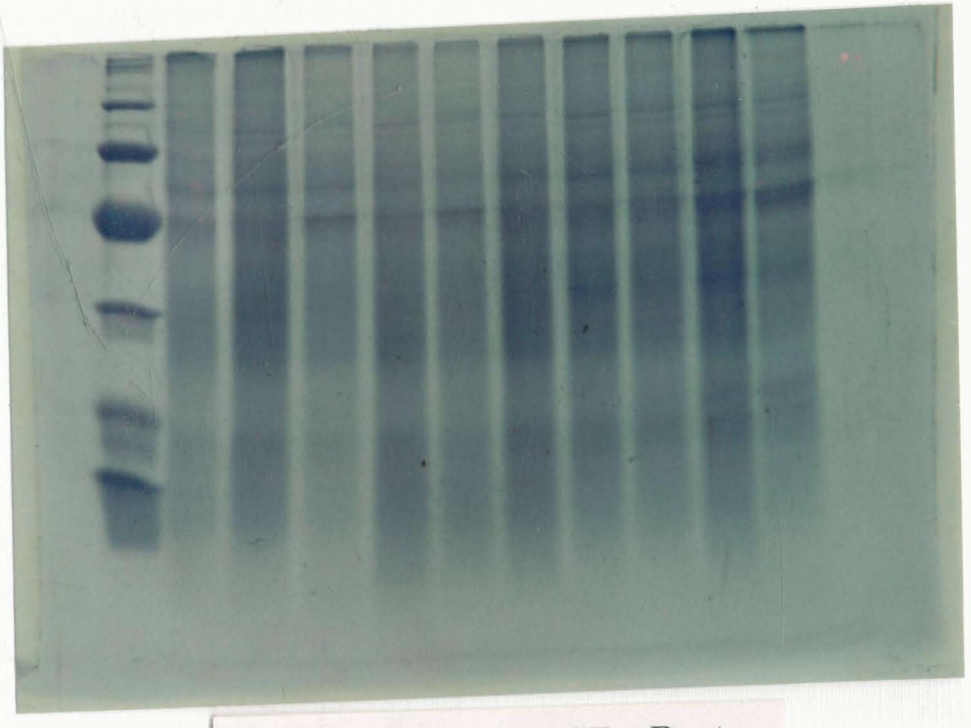


Plate 14b. SDS-PAGE – Root

Table 11. R_f values of soluble protein of shoot on SDS-PAGE

T_1 - *G. fasciculatum*	T_2 - *G. fasciculatum* + *M incognita*	T_3 - *G. mosseae*	T_4 - *G. mosseae* + *M. incognita*	T_5 - *G. intraradices*	T_6 - *G. intraradices* + *M. incognita*	T_7 - *G. fulvum*	T_8 - *G. fulvum* + *M. incognita*	T_9 - *M. incognita* alone	T_{10} - Control
-	0.02	-	0.02	-	0.02	-	0.02	0.02	-
0.05	0.05	0.05	0.05	0.05	-	0.05	0.05	0.05	-
0.10	0.10	0.10	0.10	0.10	-	-	0.10	0.10	-
0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	-
-	-	-	0.18	-	-	-	-	-	-
0.20	-	0.20	-	0.20	-	-	-	-	0.20
0.26	0.26	0.26	0.26	0.26	0.26	0.26	0.26	0.26	0.26
0.31	-	0.31	0.31	0.31	-	-	-	-	-
-	-	-	0.33	-	0.33	-	-	-	0.33
0.35	0.35	0.35	-	0.35	-	0.35	0.35	0.35	-
-	-	0.36	0.36	0.36	0.36	-	-	-	-
0.45	0.45	0.45	-	0.45	0.45	-	-	0.45	0.45
-	-	-	0.48	-	-	-	-	-	0.48
0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55
-	0.56	-	-	-	-	-	-	-	-
-	-	0.62	0.62	0.62	0.62	-	-	0.62	0.62
-	-	0.68	0.68	-	-	-	-	-	0.68
0.77	0.77	0.77	-	0.77	0.77	-	-	-	-
0.81	-	0.81	-	-	-	-	-	-	-
0.85	-	-	-	-	-	-	-	-	-
-	-	0.86	-	0.86	0.86	-	-	-	0.86

The bands are numbered according to their increasing electrophoretic mobility

In case of roots, out of nine and ten bands respectively for *G. fasciculatum* and *G. fasciculatum* + *M. incognita* with eight in common for both, the band with R_f values of 0.08 was present only in *G. fasciculatum* and the bands with R_f values of 0.041 and 0.53 were present in nematode inoculated with *G. fasciculatum*. In *G. mosseae*, the bands with R_f values of 0.18, 0.25, 0.30, 0.50 and 0.68 were present. Out of seven and eight respectively for *G. mosseae* and *G.mosseae* + *M. incognita*, only two bands were common. In *G. intraradices* and *G.intraradices* + *M. incognita*, six bands were common and the bands with R_f values of 0.21, and 0.55 were present only in *G. intraradices* and the bands with R_f values of 0.25, 0.32, and 0.56 were present when *M. incognita* was added to it. In *G. fulvum*, the bands with R_f values of 0.43 and 0.68 were present and *G. fulvum* inoculated with nematode, the bands with R_f values of 0.13, 0.37 and 0.65 were present. In *G. fulvum* and *G. fulvum* + *M. incognita* seven and eight bands were present with five bands in common for both.

The band with R_f value of 0.12 was present only in nematode alone. The band with R_f value of 0.13 was present in all the treatments except in *G. fulvum*. The band with R_f value of 0.21 was present only in *G. mosseae* + *M. incognita* and *G. intraradices*. The band with R_f value of 0.30 was present in *G. intraradices*, *G. mosseae*, *G. intraradices* with nematode and nematode alone. The band with R_f value of 0.65 was present in nematode alone and nematode inoculated *G. mosseae*, *G. intraradices*, *G. fulvum* and *G. intraradices* without nematode (Table 12).

4.8.1.2. Effect of VAM-nematode interaction on protein content

The final result of the analysis is given in table 13. Lower concentration was seen in VAM + nematode inoculated shoot and higher concentration in root when compared to uninoculated VAM. Among the different species of VAM, the protein content was found to be more in *G. mosseae* (5.70 mg/g) with an increase of 83.8 per cent over nematode alone in the shoot and 3.5 4 mg/g in the root. This

Table 12. R_f values of soluble protein of root on SDS-PAGE

T_1 - *G. fasciculatum*	T_2 - *G. fasciculatum* + *M. incognita*	T_3 - *G. mosseae*	T_4 - *G. mosseae* + *M. incognita*	T_5 - *G. intraradices*	T_6 - *G. intraradices* + *M. incognita*	T_7 - *G. fulvum*	T_8 - *G. fulvum* + *M. incognita*	T_9 - *M. incognita* alone	T_{10} - Control
0.08	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	0.12	-
0.13	0.13	0.13	0.13	0.13	0.13	-	0.13	0.13	0.13
0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
.018	0.18	0.18	-	-	-	0.18	0.18	-	-
-	-	-	0.21	0.21	-	-	-	0.21	-
0.23	0.23	-	-	-	-	-	-	-	0.23
-	-	0.25	-	-	0.25	0.25	0.25	0.25	0.25
0.27	0.27	-	0.27	0.27	0.27	0.27	0.27	0.27	-
-	-	0.30	-	0.30	0.30	-	-	0.30	-
0.31	0.31	-	-	-	-	-	-	-	0.31
-	-	-	-	-	0.32	-	-	-	-
-	-	-	-	0.37	0.37	-	0.37	-	-
-	-	-	0.38	-	-	-	-	-	-
-	0.41	-	-	-	-	-	-	-	-
-	-	-	0.43	-	-	0.43	-	0.43	0.43
-	-	0.50	-	-	-	-	-	0.50	050
-	0.53	-	-	-	-	-	-	-	-
-	-	-	0.55	0.55	-	0.55	0.55	-	-
-	-	-	-	-	0.56	-	-	-	-
-	-	-	-	-	-	-	-	-	0.61
0.62	0.62	-	-	-	-	-	-	0.62	-
-	-	-	0.65	0.65	0.65	-	0.65	0.65	-
-	-	0.68	-	-	-	0.68	-	-	0.68
0.75	0.75	-	-	-	-	-	-	-	-

The bands are numbered according to their increasing electrophoretic mobility

Table 13. Effect of VAM and *M. incognita* on protein and phenol content of brinjal Cv. Co. 2

Treatment	Protein content				Phenol content			
	Shoot (mg/g)	Per cent increase over nematode alone	Root (mg/g)	Per cent increase over nematode alone	Shoot (mg/g)	Per cent increase over nematode alone	Root (mg/g)	Per cent increase over nematode alone
T_1 - *G. fasciculatum*	4.80	54.8	4.32	-4.4	5.34	47.2	1.29	486.3
T_2 - *G. fasciculatum* + *M. incognita*	3.60	16.1	4.59	0	2.31	-36.1	0.99	350.0
T_3 - *G. mosseae*	5.70	83.8	3.54	-22.2	5.55	52.7	1.38	550.0
T_4 - *G. mosseae* + *M. incognita*	5.19	64.5	3.66	-20.0	5.31	47.2	0.81	300.0
T_5 - *G. intraradices*	4.59	45.1	3.45	-24.4	3.87	5.5	1.41	600.0
T_6 - *G. intraradices* + *M. incognita*	3.07	-0.9	4.32	-4.4	3.51	-2.7	0.60	200.00
T_7 - *G. fulvum*	4.42	41.9	3.36	-26.6	5.55	52.7	0.87	295.4
T_8 - *G. fulvum* + *M. incognita*	3.59	12.9	3.60	-20.0	2.73	-25.0	0.84	281.8
T_9 - *M. incognita* alone	3.10	--	4.59	--	3.69	--	0.22	--
T_{10} - Control	4.23	--	3.33	--	4.26	--	0.81	--
CD (P=0.05)	0.60		0.59		0.49		0.27	

was followed by *G.fasciculatum* (4.80 mg/g) with an increase of 54.8 per cent in the shoot and 4.32 mg/g in the root. The lowest was in *G. fulvum* with 4.42 mg/g with 41.9 per cent increase in shoot and 3.36 mg/g in the root. In nematode alone, the protein content was 3.1 mg/g and in root, it was 4.59 mg/g.

4.8.2. Effect of VAM - nematode interaction on total phenol content

The phenol content of the various treatments are given in table 13. The phenol content was more in all VAM incorporated shoot and root. In *G. mosseae*, the total phenol content was 5.55 and 1.38 mg/g in shoot and root respectively. This was followed by *G.fasciculatum* with 5.34 mg/g in shoot and 1.29 mg/g in root with 47.2 and 486.3 per cent increase in shoot and root respectively. When nematode was inoculated with VAM species, the total phenol content was more in *G. mosseae* + nematode inoculated shoot and root with 5.31 and 0.81 mg/g respectively followed by *G. fasciculatum* with 0.99 mg/g in roots, whereas in control, the total phenol content was 4.26 and 0.81 mg/g in shoot and root respectively. In nematode alone, the total phenol content was 3.69 and 0.22 mg/g in shoot and root (Figures 8 and 9).

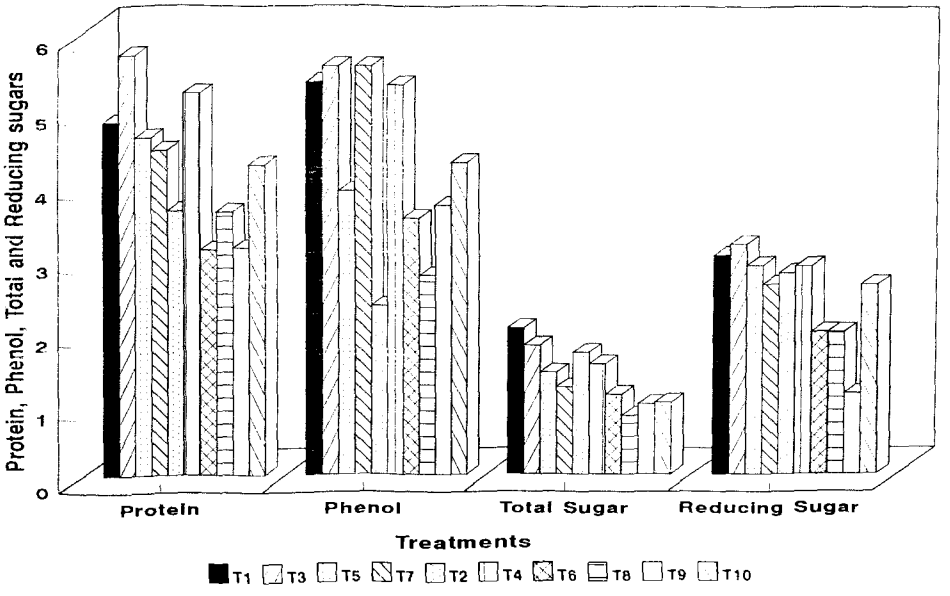
4.8.3. Effect of VAM - nematode interaction on total sugars

It was observed from the results that the total sugars was more in *G. fasciculatum* both in shoot and root (i.e.) 2.01 mg/g and 0.60 mg/g with 107.2 and 172.7 per cent increase respectively, followed by *G. mosseae* with 1.77 mg/g and 0.80 mg/g with 82.4 and 263.6 per cent increase over nematode alone in shoot and root. In nematode alone, the total sugar was 0.97 and 0.22 mg/g in shoot and root respectively, but in *G. fasciculatum* + nematode, the total sugar was 1.68 and 0.48 mg/g and in *G. mosseae* + nematode, the total sugar was 1.52 and 0.63 mg/g in shoot and root respectively. The total sugar content compared to VAM alone was less in all the treatments inoculated with nematode. However, the total sugars was less in nematode alone in shoot and more in root when compared to control (Table 14).

Table 14. Effect of VAM and nematode interaction on total and reducing sugars of brinjal Cv. Co. 2

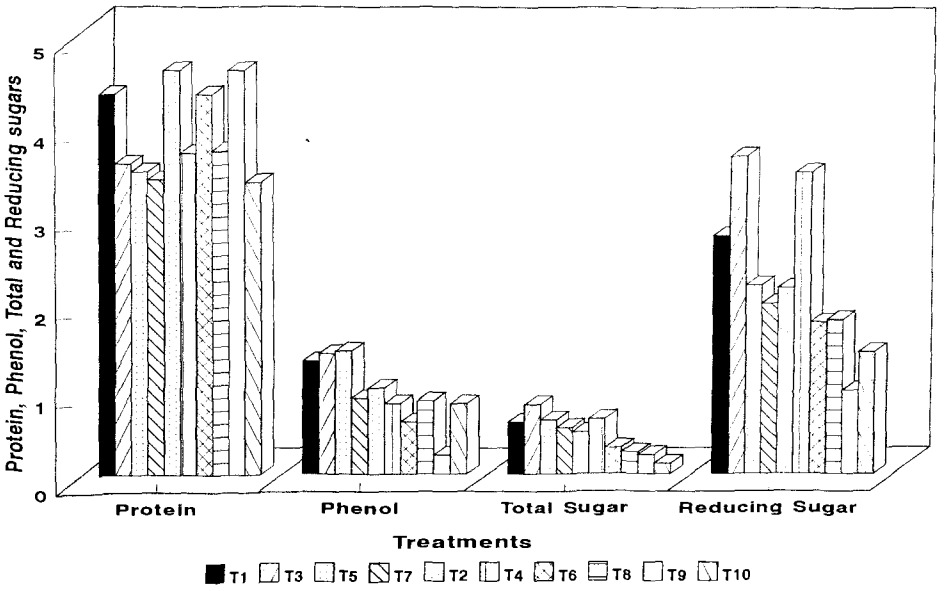
Treatment	Total sugars				Reducing sugars			
	Shoot (mg/g)	Per cent increase over nematode alone	Root (mg/g)	Per cent increase over nematode alone	Shoot (mg/g)	Per cent increase over nematode alone	Root (mg/g)	Per cent increase over nematode alone
T_1 - *G. fasciculatum*	2.01	107.2	0.60	172.7	3.00	170.2	2.73	200.0
T_2 - *G. fasciculatum* + *M. incognita*	1.68	73.1	0.48	118.1	2.76	148.6	2.13	133.3
T_3 - *G. mosseae*	1.77	82.4	0.80	263.6	3.15	185.4	3.63	300.0
T_4 - *G. mosseae* + *M. incognita*	1.52	56.7	0.63	186.3	2.85	156.7	3.45	283.3
T_5 - *G. intraradices*	1.40	44.3	0.62	181.8	2.86	157.6	2.16	133.3
T_6 - *G. intraradices* + *M. incognita*	1.10	13.4	0.30	36.3	1.95	75.6	1.74	88.8
T_7 - *G. fulvum*	1.20	23.7	0.53	140.9	2.60	134.2	1.95	111.1
T_8 - *G. fulvum* + *M. incognita*	0.81	-16.4	0.25	13.6	1.95	75.6	1.77	96.6
T_9 - *M. incognita* alone	0.97	--	0.22	--	1.11	--	0.96	--
T_{10} - Control	0.99	--	0.12	--	2.60	--	1.40	--
CD (P=0.05)	0.32	--	0.19	--	0.66	--	0.22	--

Fig.8. Effect of VAM and nematode interaction on shoot protein, phenol, total and reducing sugars of brinjal Cv. Co.2



T_1 - *G. fasciculatum*
T_2 - *G. fasciculatum + M. incognita*
T_3 - *G. mosseae*
T_4 - *G. mosseae + M. incognita*
T_5 - *G. intraradices*
T_6 - *G. intraradices + M. incognita*
T_7 - *G. fulvum*
T_8 - *G. fulvum + M. incognita*
T_9 - *M. incognita* alone
T_{10} - Control

Fig.9. Effect of VAM and nematode interaction on root protein, phenol, total and reducing sugars of brinjal Cv. Co.2



T_1 - *G. fasciculatum*
T_2 - *G. fasciculatum + M. incognita*
T_3 - *G. mosseae*
T_4 - *G. mosseae + M. incognita*
T_5 - *G. intraradices*
T_6 - *G. intraradices + M. incognita*
T_7 - *G. fulvum*
T_8 - *G. fulvum + M. incognita*
T_9 - *M. incognita* alone
T_{10} - Control

4.8.4. Effect of VAM - nematode interaction on the reducing sugars

The reducing sugar was found to be more in all the VAM species (Table 14) when compared to VAM with nematode inoculation. Among the VAM species, the highest was recorded in *G. mosseae* with 3.15 and 3.63 mg/g in shoot and root which is 185.4 and 300 per cent increase over nematode alone. This was followed by *G. fasciculatum* with an increase in shoot and root when compared with *G. intraradices* and *G. fulvum*. The reducing sugars was the least in nematode alone with 1.11 and 0.96 mg/g in shoot and root respectively, but when nematode was inoculated along with VAM, i.e. *G. mosseae* + nematode, the reducing sugar was 2.85 and 3.45 mg/g followed by *G. fasciculatum* + nematode with 2.76 and 2.13 mg/g in shoot and root respectively. A similar increase was observed in *G. intraradices* and *G. fulvum* when compared to nematode alone (Figures 8 and 9).

4.8.5. Effect of amino acids on VAM-nematode interaction

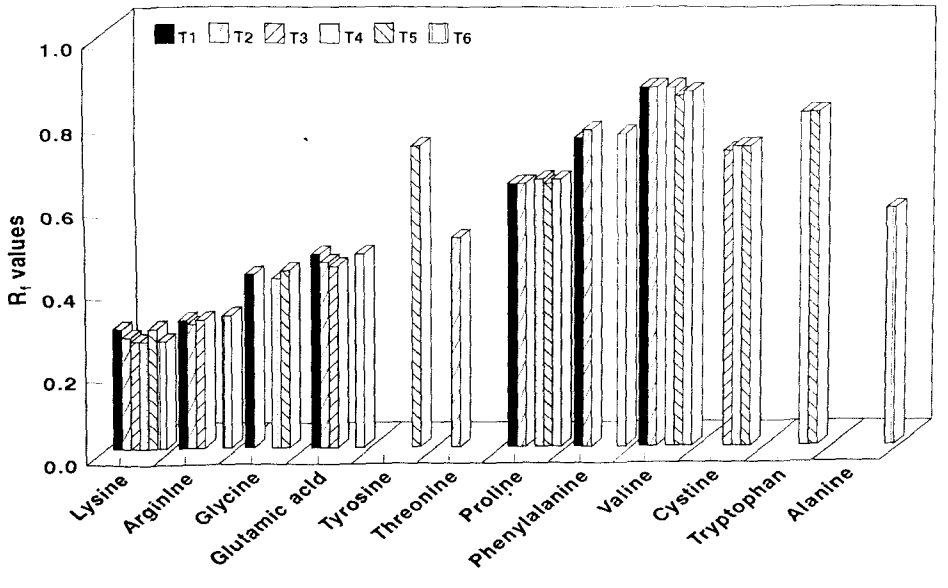
R_f value of amino acids were calculated for different treatments. It has been observed that about seven amino acids *viz.*, lysine, arginine, glycine, glutamic acid, proline, phenylalanine and valine were found in plants inoculated with *G. fasciculatum* and nematode. In *G. mosseae* + nematode inoculated plants, all the above amino acids were present except glycine instead threonine was present. In *G. intraradices* + nematode inoculated, there were only four amino acids recorded *viz.*, lysine, arginine, glutamic acid and cystine. In *G. fulvum* + nematode inoculated plants, it was observed that only six amino acids like lysine, glycine, proline, valine, cystine and tryptophan (Table 15) were present.

Lysine present in nematode alone, was present in all the species of VAM. Glysine present in nematode alone was also present in nematode inoculated *G. fasciculatum* and *G. fulvum*, whereas tyrosine was present only in nematode alone. Proline was present in *G. fasciculatum*, *G. mosseae*, *G. fulvum* along with nematode and also in nematode alone. Valine was present in nematode alone and

Table 15. R_f values of amino acids separated by paper chromatography

Sl. No.	Amino acids	T_1 - *G. fasciculatum* + *M. incognita*	T_2 - *G. mosseae* + *M. incognita*	T_3 - *G. intraradices* + *M. incognita*	T_4 - *G. fulvum* + *M. incognita*	T_5 - *M. incognita* alone	T_6 - Control
1.	*Lysine*	0.29	0.27	0.26	0.26	0.29	0.26
2.	Arginine	0.31	0.30	0.31	--	--	0.32
3.	Glycine	0.41	--	--	0.41	0.43	--
4.	Glutamic acid	0.47	0.45	0.44	--	--	0.47
5.	Tyrosine	--	--	--	--	0.73	--
6.	Threonine	--	0.51	--	--	--	--
7.	Proline	0.64	0.64	--	0.65	0.64	0.65
8.	Phenylalanine	0.75	0.77	--	--	--	0.76
9.	Valine	0.87	0.87	--	0.87	0.85	0.86
10.	Cystine	--	--	0.72	0.73	0.73	--
11.	Tryptophan	--	--	-	0.81	0.81	--
12.	Alanine	--	--	--	--	--	0.58

Fig.10. R_f values of amino acids separated by paper chromatography



T_1 - *G. fasciculatum + M. incognita*
T_2 - *G. mosseae + M. incognita*
T_3 - *G. intraradices + M. incognita*
T_4 - *G. fulvum + M. incognita*
T_5 - *M. incognita* alone
T_6 - Control

also in *G. fasciculatum*, *G. mosseae* and *G. fulvum* inoculated with nematode. Cystine was present both in nematode alone and also in *G. intraradices* but cystine and tryptophan were present in *G. fulvum* (Fig. 10).

4.9. EFFECT OF ENZYME ACTIVITY DUE TO INTERACTION

There are various enzymes which are activated due to nematode infection. The changes in enzymic activity in turn are mainly responsible for the altered metabolities and the metabolic processes. Among the various enzymes, peroxidases and chitinase are considered to play a role indirectly in inducing resistance. Peroxidase is an important enyme in the synthesis of lignins. It catalyze the oxidation of many mono and dephenols and aromatic amines to the highly toxic quinones, in the presence of hydrogen oxides.

4.9.1. Effect of peroxidase activity due to interaction

The results show that there is a remarkable increase in peroxidase activity in VAM inoculated plants when compared to control (Table 16). In plants inoculated with nematode and *G. mosseae*, the peroxidase activity was the highest with 57.5 per cent increase over nematode alone in shoot followed by *G. fasciculatum* and nematode, which was 27.5 per cent increase over nematode alone in shoot. Among the VAM species, the peroxidase activity was more in *G. mosseae* with 7.5 per cent increase over nematode alone. In nematode inoculated VAM species, there was an increase in peroxidase activity when compared to nematode alone in shoot Fig.11).

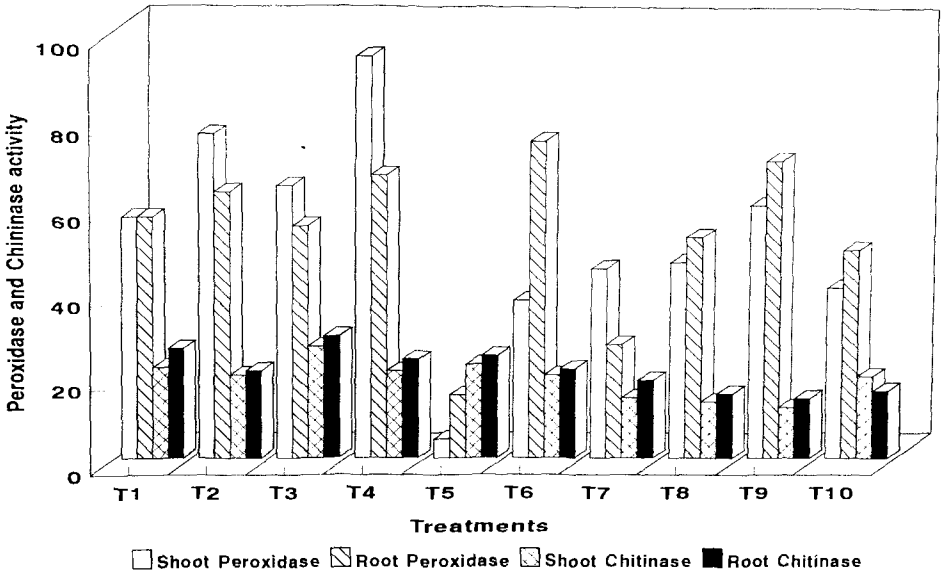
4.9.2. Effect of chitinase activity due to interaction

Among the VAM species, the highest was observed in *G. mosseae* inoculated plants with 26.4 and 28.9 n mol / mit / g in shoot and root (Table 16) respectively. In *G. fasciculatum* and *G. intraradices* inoculated plants, the chitinase activity was 21.5 and 22.3 n mol / mit / g in shoot and 25.9 and

Table 16. Effect of peroxidase and chitinase activity due to interaction

Treatment	Peroxidase activity				Chitinase activity (n mol of N-acetyl glucosamine/min/g)			
	Shoot	Per cent increase over nematode alone	Root	Per cent increase over nematode alone	Shoot	Per cent increase over nematode alone	Root	Per cent increase over nematode alone
T_1 - *G. fasciculatum*	57.0	5.0	57.0	-19.1	21.5	77.6	25.9	82.3
T_2 - *G. fasciculatum* + *M. incognita*	76.5	27.5	63.0	-10.6	19.7	62.8	20.7	45.7
T_3 - *G. mosseae*	64.5	7.5	55.0	-21.9	26.4	118.1	28.9	103.5
T_4 - *G. mosseae* + *M. incognita*	94.5	57.5	67.0	-4.9	20.7	71.0	23.5	65.4
T_5 - *G. intraradices*	4.5	-92.5	15.0	-78.7	22.3	84.2	24.4	71.8
T_6 - *G. intraradices* + *M. incognita*	37.5	-37.5	75.0	6.3	19.7	62.8	21.2	49.2
T_7 - *G. fulvum*	45.0	-25.0	27.0	-61.7	14.3	18.1	18.5	30.2
T_8 - *G. fulvum* + *M. incognita*	46.5	-22.5	52.5	-25.5	13.4	10.7	15.3	7.7
T_9 - *M. incognita* alone	60.0	--	70.5	--	12.1	--	14.2	--
T_{10} - Control	40.5	--	49.5	--	19.6	--	16.0	--

Fig.11. Effect of peroxidase and chitinase activity in shoot and root due to interaction



T_1 - *G. fasciculatum*
T_2 - *G. fasciculatum + M. incognita*
T_3 - *G. mosseae*
T_4 - *G. mosseae + M. incognita*
T_5 - *G. intraradices*
T_6 - *G. intraradices + M. incognita*
T_7 - *G. fulvum*
T_8 - *G. fulvum + M. incognita*
T_9 - *M. incognita* alone
T_{10} - Control

24.4 n mol / mit / g in root respectively. In *G. fulvum* inoculated plants, the chitinase activity was 14.3 and 18.5 in shoot and root. In all the nematode inoculated treatment, the chitinase activity was found less with 19.7 and 20.7 n mol / mit / g in *G. fasciculatum* + *M. incognita* followed by 20.7 and 23.5 n mol/ mit / g in *G. mosseae* + nematode. But least was recorded in nematode alone with 12.1 and 14.2 n mol / mit / g (Fig. 11).

4.10. EFFECT OF MACRONUTRIENTS ON NEMATODE-VAM INTERACTION

An adequate supply, uptake and a balanced distribution of nutrient elements within a plant are necessary for normal plant growth. When nematode infect plants, the nutrient status changes thus altering host physiology. The normal balance of all macro and micro nutrients are affected by nematodes.

4.10.1. Effect of *M. incognita* and VAM on total nitrogen content

From the results of various macronutrients analysed, the total nitrogen content was observed that the total nitrogen content was more in all the VAM species when compared to control (Table 17). The nitrogen content was lower in plants treated with mycorrhiza along with nematode. The least was in nematode alone with 1.0 and 0.47 per cent in shoot and root respectively. The highest was in *G. mosseae* with 1.72 and 1.33 per cent . followed by *G. fasciculatum* with 1.65 and 1.84 per cent in shoot and root respectively. In nematode inoculated with *G. mosseae*, the total nitrogen content was 1.52 and 1.22 per cent and in *G. fasciculatum* + nematode, the total nitrogen content was 1.51 and 1.04 per cent in shoot and root. A similar increase was observed in *G. intraradices* and *G. fulvum* also (Figures 12 and 13).

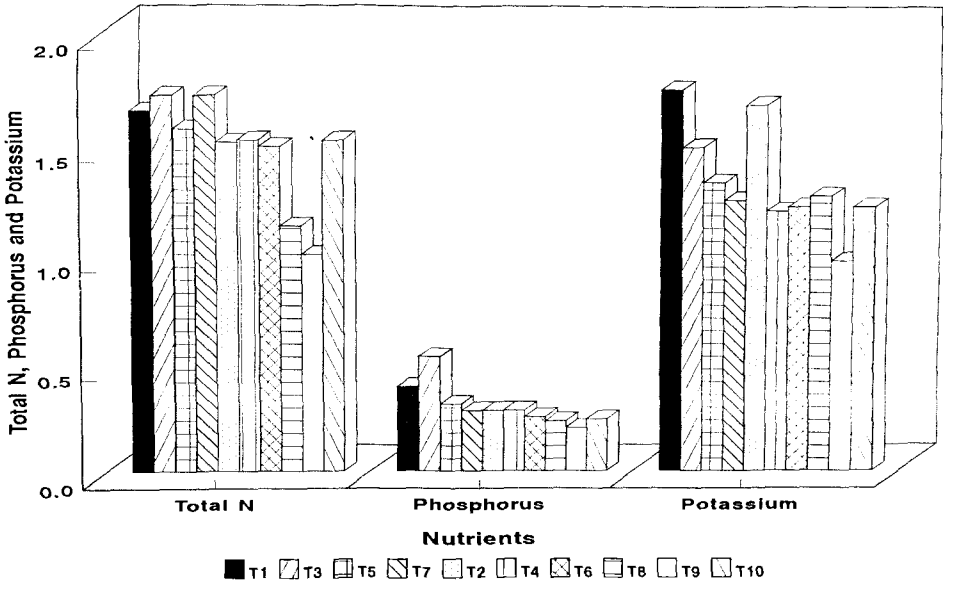
4.10.2. Effect of *M. incognita* and VAM on phosphorus content

The phosphorus content was more in mycorrhizal plants. The highest was found in *G. mosseae* with 0.53 and 0.48 per cent in shoot and root with 165 and

Table 17. Effect of VAM and *M. incognita* on total nitrogen and phosphorus of brinjal Cv. Co. 2

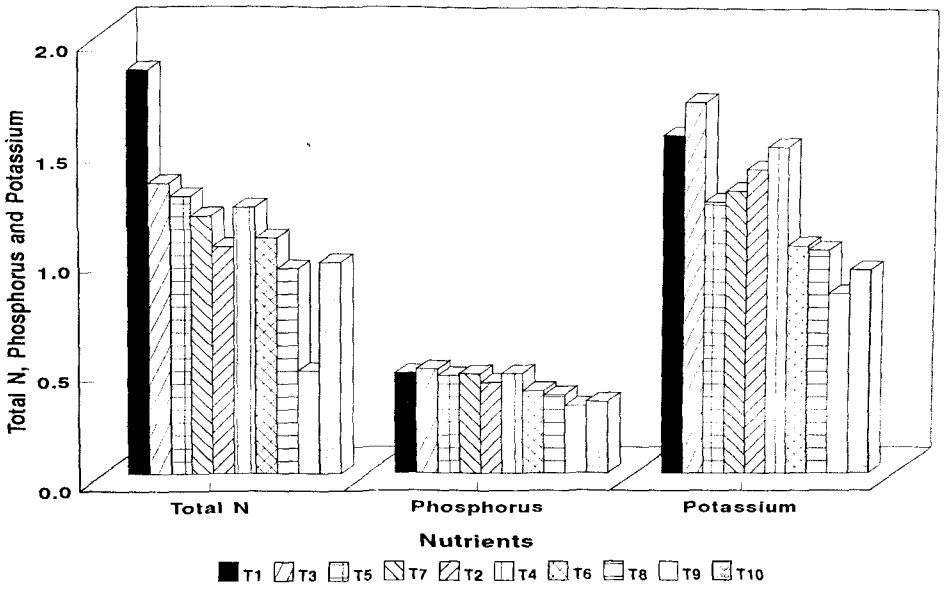
Treatment	Total nitrogen				Phosphorus			
	Shoot (%)	Per cent increase over nematode alone	Root (%)	Per cent increase over nematode alone	Shoot (%)	Per cent increase over nematode alone	Root (%)	Per cent increase over nematode alone
T_1 - *G. fasciculatum*	1.65	60.0	1.84	291.4	0.39	95	0.46	48.3
T_2 - *G. fasciculatum* + *M. incognita*	1.51	51.0	1.04	121.2	0.28	40	0.42	35.4
T_3 - *G. mosseae*	1.72	70.0	1.33	182.9	0.53	165	0.48	54.8
T_4 - *G. mosseae* + *M. incognita*	1.52	52.0	1.22	159.5	0.28	40	0.46	48.3
T_5 - *G. intraradices*	1.57	57.0	1.27	170.2	0.31	55	0.45	45.1
T_6 - *G. intraradices* + *M. incognita*	1.49	49.0	1.08	129.7	0.25	25	0.38	22.5
T_7 - *G. fulvum*	1.72	72.0	1.18	151.0	0.28	40	0.46	48.3
T_8 - *G. fulvum* + *M. incognita*	1.13	13.0	0.94	100.0	0.23	15	0.36	16.1
T_9 - *M. incognita* alone	1.00	--	0.47	--	0.20	--	0.31	--
T_{10} - Control	1.52	--	0.97	--	0.24	--	0.33	--
CD (P=0.05)	0.17	--	0.21		0.03		0.07	

Fig.12. Effect of VAM and *M. incognita* on shoot total nitrogen, phosphorus and potassium of brinjal Cv. Co.2



T_1 - *G. fasciculatum*
T_2 - *G. fasciculatum + M. incognita*
T_3 - *G. mosseae*
T_4 - *G. mosseae + M. incognita*
T_5 - *G. intraradices*
T_6 - *G. intraradices + M. incognita*
T_7 - *G. fulvum*
T_8 - *G. fulvum + M. incognita*
T_9 - *M. incognita* alone
T_{10} - Control

Fig.13. Effect of VAM and *M. incognita* on root total nitrogen, phosphorus and potassium of brinjal Cv. Co.2



T_1 - *G. fasciculatum*
T_2 - *G. fasciculatum + M. incognita*
T_3 - *G. mosseae*
T_4 - *G. mosseae + M. incognita*
T_5 - *G. intraradices*
T_6 - *G. intraradices + M. incognita*
T_7 - *G. fulvum*
T_8 - *G. fulvum + M. incognita*
T_9 - *M. incognita* alone
T_{10} - Control

54.8 per cent increase respectively over nematode alone. In *G. fasciculatum*, the phosphorus content was 0.39 and 0.46 per cent in shoot and root with 95 and 48.3 per cent increase over nematode alone. In *G. intraradices* and *G. fulvum*, there was considerable increase over nematode inoculated. with 55 and 40 per cent in shoot and 45.1 and 48.3 per cent in root respectively. In the plants that received both *G. mosseae* and *G. fasciculatum* plus nematode, the P concentration in shoot was 0.28 per cent in both and 0.46 and 0.42 per cent in roots respectively. In the case of *G. intraradices* and *G. fulvum*, the phosphorus content was 0.25 and 0.38 per cent and 0.23 and 0.36 per cent in shoot and root respectively. In nematode alone, the phosphorus content was least with 0.20 and 0.31 per cent in shoot and root (Table 17 and Figures 12 and 13).

4.10.3. Effect of *M. incognita* and VAM on potassium content

The potassium content was highest in *G. fasciculatum* with 1.75 and 1.55 per cent in shoot and root with 88.8 and 86.7 per cent increase over nematode alone (Table 18). This was followed by *G. mosseae* with 1.49 and 1.70 per cent in shoot and root. *G. intraradices* recorded 1.33 and 1.25 per cent and *G. fulvum* with 1.25 and 1.30 per cent in shoot and root respectively, whereas in *G. fasciculatum* inoculated with nematode, the potassium content was 1.68 and 1.40 per cent followed by *G. mosseae* with 1.20 and 1.50 in shoot and root respectively. A similar increase was observed in *G. intraradices* and *G. fulvum* also. In nematode alone, the potassium content was only 0.97 and 0.83 per cent (Figures 12 and 13).

4.11. EFFECT OF MICRONUTRIENTS ON NEMATODE-VAM INTERACTION

4.11.1. Effect of *M. incognita* and VAM on iron content

The iron content was more in *G.fasciculatum* with 1918 ppm in shoot. In *G.mosseae*, the iron content was 1286 and 1722 ppm in shoot and root respectively. The amount of iron content in *G. intraradices* was 2232 and 512 ppm in shoot and root respectively. In the plants inoculated with *G. fasciculatum*

Table 18. Effect of VAM and *M. incognita* on potassium of brinjal Cv. Co. 2

Treatment	Shoot (%)	Per cent increase over nematode alone	Root (%)	Per cent increase over nematode alone
T_1 - *G. fasciculatum*	1.75	88.8	1.55	86.7
T_2 - *G. fasciculatum* + *M. incognita*	1.68	77.7	1.40	68.6
T_3 - *G. mosseae*	1.49	53.6	1.70	104.8
T_4 - *G. mosseae* + *M. incognita*	1.20	23.7	1.50	80.7
T_5 - *G. intraradices*	1.33	37.1	1.25	50.6
T_6 - *G. intraradices* + *M. incognita*	1.22	25.7	1.05	26.5
T_7 - *G. fulvum*	1.25	28.8	1.30	56.6
T_8 - *G. fulvum* + *M. incognita*	1.27	30.9	1.03	24.0
T_9 - *M. incognita* alone	0.97	--	0.83	--
T_{10} - Control	1.22	--	0.94	--
CD (P=0.05)	0.38	--	0.24	--

+ nematode, the iron content was observed that 1572 and 936 ppm followed by *G. mosseae* with nematode with 440 and 3202 ppm in shoot and root respectively. In the nematodes inoculated alone, the iron content was 698 ppm and in control the iron content was 812 ppm in shoot and in roots the iron content was 1234 and 884 ppm respectively (Table 19 and Fig.14).

4.11.2. Effect of *M. incognita* and VAM on copper content

From table 19, it was observed that in *G. mosseae*, the copper content was 300 ppm with an increase of 114.2 per cent over nematode alone and 128 ppm with 326.6 per cent increase over nematode alone in shoot and root respectively. In *G. fasciculatum*, the copper content was 290 ppm in shoot and 360 ppm in root. Nematode inoculated with *G. fasciculatum* recorded 274 and 154 ppm followed by *G. mosseae* with 260 and 96 ppm in shoot and root respectively. The copper content was decreased in the other two species with and without nematode. In *M. incognita* inoculated treatment, the copper content was only 140 ppm and 30 ppm in shoot and root respectively (Fig.14).

4.11.3. Effect of *M. incognita* and VAM on zinc content

The zinc content was found (Table 20) to be more in *G. fasciculatum* with 138 and 152 ppm in shoot and root followed by in *G. mosseae* with 134 and 160 ppm in shoot and root. When nematodes were inoculated along with VAM, there was a slight decrease in concentration with 134 and 150 ppm and 132 and 156 ppm in *G. fasciculatum* + *M. incognita* and *G. mosseae* + *M. incognita* respectively. The other species of VAM recorded lesser concentration. Only nematode inoculated plants showed 41 ppm and 68 ppm in the shoot and root.

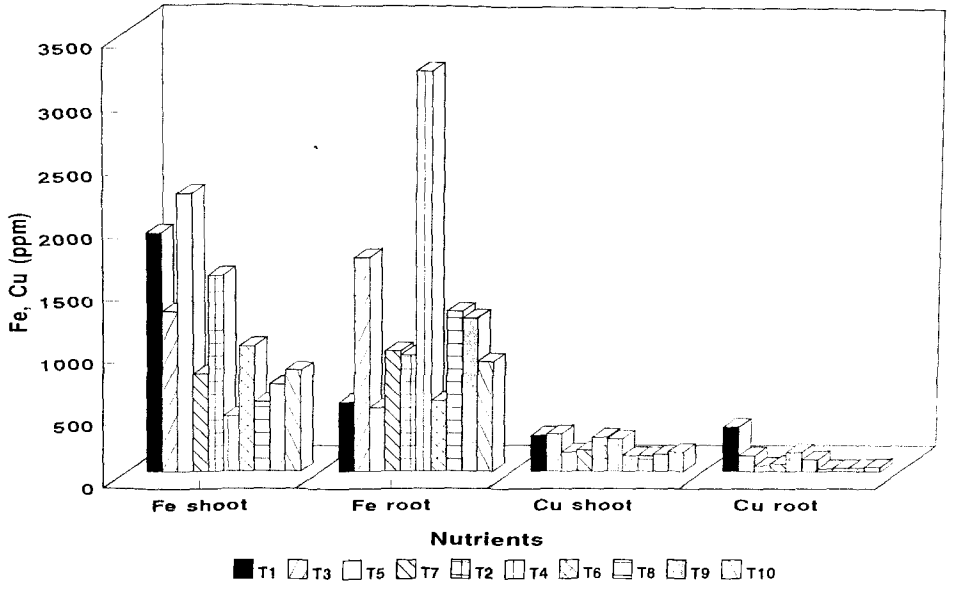
4.11.4. Effect of *M. incognita* and VAM on manganese content

The manganese content was less in all the VAM species (Table 20). *G. fasciculatum* recorded 56 ppm followed by *G. mosseae* with 20 ppm.

Table 19. Effect of *M. incognita* and VAM on copper and Iron content of brinjal Cv. Co. 2

Treatment	Iron content (ppm)				Copper content (ppm)			
	Shoot (ppm)	Per cent increase over nematode alone	Root (ppm)	Per cent increase over nematode alone	Shoot (ppm)	Per cent increase over nematode alone	Root (ppm)	Per cent increase over nematode alone
T_1 - *G. fasciculatum*	1918	174.7	558	-54.7	290	107.1	360	110.0
T_2 - *G. fasciculatum* + *M. incognita*	1572	125.2	936	-24.1	274	95.7	154	413.3
T_3 - *G. mosseae*	1286	84.2	1722	39.5	300	114.2	128	326.6
T_4 - *G. mosseae* + *M. incognita*	440	-36.9	3202	159.4	260	85.7	96	220.0
T_5 - *G. intraradices*	2232	219.7	512	-58.5	154	10.0	46	53.3
T_6 - *G. intraradices* + *M. incognita*	1004	43.8	568	-53.9	126	-10.0	22	-26.6
T_7 - *G. fulvum*	784	12.3	968	-21.5	170	21.4	62	106.6
T_8 - *G. fulvum* + *M. incognita*	554	-20.6	1290	4.5	125	-10.7	28	-6.6
T_9 - *M. incognita* alone	698	--	1234	--	140	--	30	--
T_{10} - Control	812	--	884	--	156	--	36	--
CD (P=0.05)	58.2	--	61.8	--	25.5	--	12.8	--

Fig.14. Effect of VAM and *M. incognita* on iron, copper content in shoot and root of brinjal Cv. Co.2



T_1 - *G. fasciculatum*
T_2 - *G. fasciculatum + M. incognita*
T_3 - *G. mosseae*
T_4 - *G. mosseae + M. incognita*
T_5 - *G. intraradices*
T_6 - *G. intraradices + M. incognita*
T_7 - *G. fulvum*
T_8 - *G. fulvum + M. incognita*
T_9 - *M. incognita* alone
T_{10} - Control

Table 20. Effect of *M. incognita* and VAM on zinc and manganese content of brinjal Cv. Co. 2

Treatment	Zinc content (ppm)				Manganese content (ppm)			
	Shoot (ppm)	Per cent increase over nematode alone	Root (ppm)	Per cent increase over nematode alone	Shoot (ppm)	Per cent increase over nematode alone	Root (ppm)	Per cent increase over nematode alone
T_1 - *G. fasciculatum*	138	236.5	152	123.5	56	16.6	60	-3.2
T_2 - *G. fasciculatum* + *M. incognita*	134	226.8	150	120.5	30	-37.5	52	-16.1
T_3 - *G. mosseae*	134	226.8	160	135.2	20	-58.3	68	9.6
T_4 - *G. mosseae* + *M. incognita*	132	221.9	156	129.4	17	-64.5	46	-25.8
T_5 - *G. intraradices*	50	21.9	68	0.0	17	-64.5	46	-25.8
T_6 - *G. intraradices* + *M. incognita*	42	2.4	50	-26.4	13	-72.9	33	-46.7
T_7 - *G. fulvum*	34	-17.0	88	29.4	38	-20.8	44	-29.0
T_8 - *G. fulvum* + *M. incognita*	30	-26.8	64	-5.8	30	-37.5	22	-64.5
T_9 - *M. incognita* alone	41	--	68	--	48	--	62	--
T_{10} - Control	36	--	60	--	60	--	78	--
CD (P=0.05)	5.2	--	9.1	--	6.2	--	4.2	--

G. intraradices recorded 17 ppm and *G. fulvum* recorded 38 ppm. The manganese content was more in control (60 ppm) compared to nematode inoculated (48 ppm) in the shoot. When the root was considered, the highest was noted in control (78 ppm) followed by *G. mosseae* with 68 ppm. In all the nematode inoculated VAM species, manganese content was lesser with 30 and 52 ppm in *G. fasciculatum* followed by 17 and 46 ppm in *G. mosseae* in shoot and root respectively. In *G. intraradices* and *G. fulvum* also, there was a reduction in Mn content of shoot and root.

DISCUSSION

CHAPTER V

DISCUSSION

In recent years, the possibility of using VAM fungi for biological control of parasitic nematodes is being suggested by many authors. The same aspect has been experimented and the results of various experiments carried out are discussed in this chapter.

5.3. EVALUATION OF OPTIMUM DOSES OF *G. mosseae* FOR THE CONTROL OF *M. incognita* ON BRINJAL Cv. Co. 2

The brinjal plants inoculated with nematode alone were stunted in growth with low shoot weight, while plants inoculated with different doses of VAM showed different growth parameters, but the plants inoculated with 10 g/Kg soil of VAM showed the maximum growth compared to plants inoculated with nematode alone. Mycorrhizal effects on *M. incognita* were pronounced when the fungus was allowed to develop in roots 15 days before *M. incognita* inoculation and the colonization was more in VAM inoculated plants and also they were able to enhance the plant growth and suppress nematode multiplication. Bagyaraj *et al.* (1979) recorded that inoculation of *G. fasciculatum* in tomato plants significantly reduced the number and size of the root-knot nematode galls. In fact, Saleh and Sikora (1984) reported that 55 - 60 per cent mycorrhizal colonization was required to suppress *M. incognita* reproduction on cotton. Smith *et al.* (1986a) observed that when *M. incognita* was inoculated 28 days after VAM inoculation, the effect was more pronounced, even though mycorrhizal fungi was slow to colonize roots, more than 60 per cent of the root system was colonized. Certain amount of colonization should be required for inhibition of *M. incognita*. Jain and Sethi (1988b) reported that earlier introduction of *G. fasciculatum* or *G. epigaeus* by 15 days, adversely affected root penetration of cyst nematode, *Heterodera cajani* on cowpea. The early inoculation or simultaneous

inoculation of VAM and nematode can suppress the nematode by occupying the root system (Rajeswari Sundara Babu *et al.*, 1996a). In this study, the maximum colonization (87 %) was observed in plants inoculated with 10 g/kg of VAM, followed by 80 per cent when 5 g/kg of soil was inoculated. Therefore, these root colonization percentage suggest that competition for space may account for reduced nematode infection on mycorrhizal root system.

5.4. EFFECT OF FOUR SPECIES OF VAM *G. fasciculatum*, *G. mosseae*, *G. intraradices* AND *G. fulvum* ON BRINJAL TO CONTROL *M. incognita*

There was a significant reduction in the nematode population, when VAM species were inoculated, and significant increase in plant growth parameters with a maximum in *G. mosseae*. When *G. intraradices* was inoculated, the number of nematodes reduced were minimum comparatively. This is supported by the findings of Smith *et al.* (1986b) that the rate of development of second stage juveniles to oviposting females was unaffected by *G. intraradices*. The reduction in infection suggests that *G. mosseae* altered the nematode host interaction, causing significant numbers of juveniles either to migrate out of roots or die before they could establish feeding site or that, the female, male ratio was reduced.

The *G. mosseae* inoculated plants, showed greater nematode reduction and also increased yield. *G. mosseae* reduced the adverse effect of *T. semipenetrans* in citrus lemon (O'Bannon and Nemec, 1978). The growth of the plants in VAM with nematode inoculated treatment was also considerably increased over control which is supported by Pinochet *et al.* (1995a) when *Pratylenchus vulnus* was inoculated to peach root stock, *G. mosseae* enhanced the growth of the plant even in the presence of *P. vulnus*.

When *G. fasciculatum* was inoculated, the growth parameters were good and also the nematode population was suppressed, which is supported by the

findings of Sitaramaiah and Sikora (1982) that *G. fasciculatum* increased the resistance of tomato plants *to R. reniformis*.

When the VAM colonization was considered, there was a little reduction when nematode was inoculated. In *G. fasciculatum* alone, the colonization was 72.6 per cent and in combination with nematode, the colonization was 66.6 per cent. The same has been observed by Umesh *et al.* (1988) when *G. fasciculatum* alone colonized 83 per cent and in combination with nematode it is colonized to 70 per cent in banana. In the case of *G. mosseae*, it was 76.6 per cent in VAM and 61.6 per cent in nematode VAM combination. Jaizme-Vega *et al.* (1997) has reported more than 80 per cent colonization in banana inoculated with *M. incognita*.

In *G. intraradices*, the colonization was 51.6 per cent in VAM alone and 35 per cent in VAM nematode combination. Smith *et al.* (1986b) has reported more than 50 per cent colonization in cotton inoculated with *G. intraradices*.

Schenck *et al.* (1975) using different levels of nematode population found that lower levels of *M. incognita* stimulated spore production of the mycorrhizal fungus, *Endogone herogama* in soybean. In the case of *G. fasciculatum* and *G. intraradices*, there was an increase in spore count when nematode was inoculated. Enhanced production of mycorrhizal spores in the presence of nematodes has also been observed earlier by Roncadori and Hussey (1977). At very high nematode levels, the spore production decreased. When spore count was considered, there was a decrease in spore count when nematode was added. This has been observed by Umesh *et al.* (1988) also.

The number of females with the egg mass was lesser in all the VAM species inoculated plants, but females without the egg mass was more. The inhibition in nematode development on mycorrhizal roots is responsible for the delayed gelatinous matrix formation and the reduced number of eggs per egg sac.

The reduction in penetration and development was also found by Sikora and Schenck (1975) who reported 75 per cent decrease in the number of *M. incognita* juveniles that developed into adults on tobacco plants inoculated with *G. mosseae*. Sikora (1978) also reported inhibition in growth of *M. incognita* on *G. mosseae* colonized tomato root. Egg production by *M. incognita* (eggs per root system and eggs per gram of root) on soybean inoculated with *Gigaspora margarita* or *G. etunicatum* was suppressed at low phosphorus as well as by increased phosphate fertilization (Carling *et al.*, 1989).

There was a little difference in yield increase between *G. mosseae* and *G. fasciculatum*, whereas the other two species showed a marked difference. But, when nematode was inoculated, there was difference in yield. The yield and root colonization was always enhanced by mycorrhizal inoculation, suggesting that increased yield and host tolerance to *M. incognita* may be species -specific phenomena. Other specific host-symbiont interaction have also been reported on cotton and soybean (Schenck *et al.*, 1975; Hussey and Roncadori,1982b).

Among the four species of VAM, *G. fasciculatum* was found to be more effective in controlling the nematodes and increasing the biometric observations. The VAM colonization was also found to be maximum with maximum yield. This was followed by *G. mosseae* which controlled the nematode population and also increased the yield. When *G. intraradices* and *G. fulvum* was compared, *G. intraradices* recorded more yield when inoculated with nematode. *G. fulvum* showed an increased yield when compared to nematode alone.

5.5. EFFECT OF CROP ROTATION ON THE INTERACTION OF VAM WITH *M. incognita*

Highly polyphagous nature of the root-knot nematode leaves little scope for exploitation of practicing non-chemical methods. Although chemicals are

effective, their use is highly restricted because of environmental pollution hazards. In addition, their use is out of reach of common Indian farmers due to their prohibitive cost.

In the crop rotation studies, after brinjal, cumbu was sown followed by greengram and then again brinjal. From the table, it was clear that the growth parameters were enhanced due to VAM and the population of nematode was found decreased when brinjal was followed by cumbu, since it was a non-host. There after a slow increase in population was observed, since the next crop was greengram which is a host crop. When brinjal was sown again the population was again raised a little, but not much due to the presence of fungal hyphae.

Inoculation of the plants with the fungus before nematode infestation seemed necessary to allow the fungal symbiont to become established and colonized extensively in the cortical cells of brinjal roots. This could cause changes in root exudate patterns and cell wall composition which could adversely affect nematode attraction and penetration (Sikora, 1981).

The present study indicates that the nematodes are checked if mycorrhiza are present before the nematodes are able to infect the plants. This supports a similar observation made by Hussey and Roncadori (1982b) in peach. When cumbu, which is a host for VAM fungi was grown, the VAM colonization and spore count were increased and the nematode population was drastically decreased. Graminaceous and leguminous crops are generally believed to increase VA mycorrhizal population, while non-mycotropic plants decrease the population of VAM fungi (Sieverding and Leihner, 1984). Greengram increased the population of nematodes to an appreciable level, when the legumes were inoculated with VAM, there was an increase in growth parameters. Carling and Brown (1980) observed that colonization of soybean roots by *Glomus sp* significantly increased the yield and plant dry weight. Ramraj and

Shanmugam (1986) reported that in pot culture trials, when the soil or seed is incorporated with three species of VAM, the growth of blackgram, greengram and cowpea was increased.

When the first brinjal crop was compared with the brinjal after rotation, there was a significant change in shoot length and weight and root weight. VAM colonization and spore count was also increased when compared with the first crop. There was a drastic reduction in nematode population in VAM plants inoculated with nematode. The gall index also showed a greater change.

The mechanism for reduction of nematode population in mycorrhizal plants may be due to increased amino acid content in mycorrhizal roots (Nemec and Meredith, 1981) or the presence of additional amount of phosphorus in roots (Thomas and Hussey, 1981). The possible mechanism or the beneficial effect of mycorrhiza is that it offsets the damage or the physiological stress on the plants (Suresh and Bagyaraj, 1984; Smith *et al.*, 1986b).

5.6. FIELD EVALUATION OF *G. mosseae* FOR THE CONTROL OF *M. incognita* ON BRINJAL CV. Co. 2

Through out the experiment, VAM fungi had a beneficial effect on plant growth. The nematode parasitism resulted in a decreased growth of plants. But when VAM treated plants were transplanted, there was an increase in growth and yield which may be due to nematode suppression as a result of competition for plant nutrients between VAM fungi and root-knot nematode. This has been reported previously on soybean by Carling and Brown (1980). The stimulatory effect of VAM fungi on soybean growth apparently resulted from increased P nutrition of mycorrhizal plants. VAM fungi is very much dependent on host photosynthates during the early stages of root colonization, when fungal structures are formed throughout the root cortex (Harley and Smith, 1983).

Effective utilisation of VAM was seen since the seedlings were incorporated with VAM and it was transplanted to the field only after 30 days. Further, transplanting of mycorrhizal seedlings in root-knot nematode infested, field increased the yield both quantitatively and qualitatively and reduced the nematode damage considerably.

Rajeswari Sundara Babu and Sankaranarayanan (1993) incorporated VAM into nursery beds of tomato which prevented the adverse effect of root-knot nematodes and increased the yield by 91.3 per cent over control. VAM as biofertilizer was applied in ragi nursery to offset the ill-effect of *R. reniformis* (Rajeswari Sundara Babu *et al.*, 1996b)

The yield was maximum in plots treated with higher VAM inoculum. When compared to control, the carbofuran treated plot yielded higher. The carbofuran application reduced the soil nematode population and increased the yield. It is suggested that the mycorrhizal fungus @ 2 or 2.5 Kg/m^2 could be advantageously used in the management of the root-knot nematode in brinjal nurseries and also to decrease the phosphorus requirements of the plant by 50 per cent. This biocontrol could be efficient in other transplanted crops such as tomato, tobacco and chillies where root-knot nematodes are limiting factor in crop production.

When cost benefit ratio was considered, it was effective when VAM was applied in nurseries. The benefit was more when 2 Kg VAM/m^2 was applied. So, VAM can be applied for all nursery transplantable crops. The benefit of carbofuran was comparatively less when VAM was considered, but there was an increase over untreated control.

5.7. HISTOPATHOLOGICAL STUDIES

5.7.1. Histopathology of nematode infested brinjal roots

The formation of giant cells, dislocation of vascular bundle, density of cytoplasm etc., were observed by many scientists. The presence of the nematode in the

cortical region of the root caused large galls in the infected region. The parasite caused marked shift in the vascular system away from the site of infection. Dalal and Thakur (1971) observed the abnormal extension of some giant cells towards the vascular tissues resulted in the discontinuity of vascular tissue in a transverse series instead of the normal longitudinal series in the healthy plants. Disruption of epidermal cells and suberisation of outer cortical layers were observed in brinjal infected by *M.incognita*, which is observed in this study also. Both the cortical and stelar system were disorganized and the general histological appearance of the cells had unrecognizably changed in the vicinity of the parasite which was observed by Orr and Morey (1978) in grain sorghum roots infected *by M. incognita.*

The nematode induced gall cells with varying number of nuclei were noticed in the present study. Giant cells were formed with well marked and varying groups of 4, 5, 7 or 8 giant cells were present around the female parasite, which was reported by Huang and Maggenti (1969); Tandon and Praveen kumar (1978) and Finley, (1981).

Singh *et al.* (1984) observed that the cytoplasm in the giant cells appeared dense with increased size and number of nuclei and cell wall was thickened as compared with that of the adjacent cells. Each of the giant cell became multinucleated by synchronous mitosis in the absence of cytokinensis. Matured giant cell was metabolically active and contains aneuploid nuclei with 14 -16 times more DNA than unaffected roots which was reported by Jones (1981) and Hung (1985). This is in accordance with the present study.

5.7.2. Histopathology of VAM and nematode infested brinjal roots

The histopathology of VAM and nematode infested root revealed that the hyphae penetrated the epidermis and invaded the cortex which resulted in the formation of vesicles and arbuscules. In the citrus seedling infested by *Tylenchulus*

semipenetrans, *G. mosseae* was found in 50 per cent of the cortical cells. In the cortex, vesicles, were formed along the pericycle. The fungus rapidly invaded the roots and produced vesicles as well as arbuscles before nematode invasion (O'Bannan *et al.*, 1979).

Riffle (1973) observed that *Meloidogyne sp* penetrated ectomycorrhizae colonized roots and migrated to the stelar region and developed to adult. As the nematode developed, the vascular elements were pushed to one side along with the fungus. The female with egg mass protruded from the surface of the epidermis.

Sankaranarayanan (1995) observed that VAM infected roots gave rise to vesciles and arbuscules. The cytoplasmic granules and nuclei were condensed in the centre leaving clear space in the giant cells. The xylem and phloem vessels were pushed to one side of the root cortex and the space was occupied by the giant cells, which is also observed in the present study.

In VAM inoculated plants, the wall thickening in the cortex cells of root prevented the penetration of pathogen. Infection by *G. mosseae* enhanced lignin deposition of cell walls. This phenomenon was the result of increased phenol propanes which were lignin precursors. The same has been observed by Dehne and Schonbeck (1979).

In the sections of nematode infected plant tissues, cuticle, nucleoli, chromatin and lignin stain red and the remaining plants coloured green, when (Parvatha Reddy, 1987) safranin and fast green was the basic dye combination. VAM increased wall thickening in the stelar tissues of mycorrhizal roots which deter penetration of pathogen. Increased lignins in mycorrhizal roots were associated with reduced reproduction of *M. javanica* on tomato (Singh *et al.*, 1990).

5.8. BIOCHEMICAL ANALYSIS OF VAM AND NEMATODE INTERACTION

5.8.1. Estimation of protein

5.8.1.1. Qualitative changes of protein by polyacrylamide gel electrophoresis

Electrophoretic analysis of protein profiles shows differences in the banding patterns of VAM inoculated and VAM + nematode inoculated shoot and root. Good resolution of higher, middle and lower molecular weight proteins were obtained for both the treatments. Infection of the roots by the nematodes invariably results in the disappearance of protein components and is accompanied by the appearance of new protein pattern. Such changes are seen in all the VAM species and VAM inoculated nematode plants. This has been observed by earlier workers (Ganguly and Dasgupta, 1981; Smite and Dasgupta, 1987a).

There was an increase in protein concentration in the shoot of VAM species and decrease in concentration in the shoot when nematode was inoculated. Whereas, the concentration was more in the case of roots, when nematode was inoculated with VAM species as well alone. One of the possible reasons for the increase in the protein concentration in infected plant may be due to the synthesis of new enzyme proteins or may be the contribution from the nematode (Grzelinska, 1969; Ganguly and Dasgupta, 1981; Smite and Dasgupta, 1987a).

There were newly synthesized protein bands in nematode infected plants. The increased number of protein bands in the inoculated plants could possibly reflect on the attempt on the part of the host to adjust against pathogenic invasion and also possible to some extent contribute to the increase in the soluble protein content. This may be the reason for the appearance and disappearance of protein in shoot of nematode inoculated plants. The same has also been reported by Shashi Singh *et al.* (1998).

5.8.1.2. Effect of VAM and nematode interaction on protein content

In the present investigation, an elevated level of soluble protein concentration was observed in all the species of VAM, in shoot as well as in root.

In the case of nematode alone also the concentration was higher when compared with control both in shoot and root. The increased protein synthesis in the galls was also observed by Bird (1961); Littrell (1966); Chylinska *et al.* (1972) and Singh *et al.* (1978). The increase in the protein content of infected roots agrees with the report of Singh *et al.* (1978) in brinjal. In the case of nematode inoculated VAM plants, there was a decrease in the shoot protein and increase in root protein. This increase in the concentration of protein in the root may be either due to the synthesis of new proteins or increase in the concentration of existing proteins or it may be from the adult nematode embedded in the roots. This was also observed by Ganguly *et al.* (1991).

5.8.2. Effect of VAM and nematode interaction on total phenol contents

From the results, it was observed that the phenol content was less in nematode infected plants. Among the various VAM species, more phenol content was observed in VAM alone and less in VAM + nematode. O'Bannon *et al.* (1979); Suresh and Bagyaraj (1984); Umesh *et al.* (1988) and Singh *et al.* (1990) also reported that in nematode infested plants the phenolic content was less. The phenolics are associated with disease resisting compounds responsible for hypersensitivity of plants and increased percentage is observed in resistant cultivars (Sharma *et al.*, 1990).

Some phenols are known to form complexes of amino acid, chlorogenic acid complex (Clarke *et al.*, 1959) which is highly toxic to the parasite. The phenolics stimulate IAA oxidase which favours (Giebel, 1974) auxin decomposition and formation of necrosis in plants resisting sedentary parasites. The phenols in mycorrhizal roots are also associated in the reduced reproduction of nematodes which is in agreement with Singh *et al.* (1990), who observed the reduction of *M. javanica* reproduction on tomato.

5.8.3. Effect of VAM and nematode interaction on total sugars

Batemann and Miller (1966) observed that resistant plants had higher sugar content. From the results, it was observed that the total sugars were more in mycorrhizal plants than in mycorrhizal + nematode infested plants both in shoot as well as in roots. The same has been reported by Suresh and Bagyaraj (1984) and Umesh *et al.* (1988). Therefore high sugar content in mycorrhizal plants help in the resistance of plants to nematode.

When nematode infected plants and uninoculated control were compared, it was observed that the total sugars were less in untreated control compared to nematode infested roots. It can be concluded that the localized invertase in oesophagus and intestine of nematode parasite causes the secretion in the host tissue, which causes the change in carbohydrate metabolism in host parasite relationship (Roy, 1979). Increased total sugars in *Meloidogyne* infected cucumber and tomato roots was observed by Zinovew (1969) and Farooqi *et al.* (1980) which is in accordance with the present findings.

5.8.4. Effect of VAM and nematode interaction on reducing sugars

From the analysis of reducing sugars, it was observed that, the amount of reducing sugars was more in VAM treated plants and less in mycorrhizal plants inoculated with nematode. But in *M. incognita* inoculated plants, it was the least. The decrease in reducing sugars causes the disease incidence which was also observed by Horsfall and Diamond (1957). Disease incidence was observed to be greater when the level of sugars in host plant was low. Pandey and Trivedi (1991) and Sharma (1992) have also proved the same.

5.8.5. Effect of amino acids on VAM-nematode interaction

Qualitative amino acids in brinjal leaves inoculated with VAM and *M. incognita* were analysed. The mycorrhizal plants inoculated with *G. mosseae*

and *G. fasciculatum* had more amino acids, compared to *G. intraradices and G. fulvum*. Further, the amino acid, phenylalanine was observed in *G. mosseae* and *G. fasciculatum* which is known to reduce the growth and reproduction of the root-knot nematodes (Krishna Prasad, 1971; Parvatha Reddy, 1974).

Arginine was present in all the three species of VAM, *G. mosseae*, *G. fasciculatum* and *G. intraradices* but was absent in *G. fulvum* and nematode inoculated plants which may be due to the conversion of arginine to proline through ornithine cycle (Mohanty and Pradhan, 1990). Glutamic acid was present in all the three species of VAM but was absent in *G. fulvum* and nematode inoculated plants. In sugarcane, it was found that high concentration of glutamic acid inhibit the peroxidase activity. So the absence of glutamic acid in *Pratylenchus* infected roots resulted in enhanced peroxidase levels (Glasziov *el al.*, 1967). Proline which is important for resistance to nematode was present in *G. mosseae*, *G. fasciculatum* and *G. fulvum* but absent in *G. intraradices*. Cystine was absent in *G. mosseae* and *G. fasciculatum* but present in *G. intraradices*, *G. fulvum* and nematode inoculated plants, which may be due to the presence of more nematodes in the above species. Tryptophan was present in *G. fulvum* and nematode inoculated plants only. The presence of tryptophan in nematode infected plants get metobolised to Indole Acetic acid (IAA). The excessive accumulation of IAA in the cortical region leads to hypertrophy and hyperplasia (Giebel,1982). The appearance and disappearance of amino acids indicates possible inter conversion of various amino acids (Steward and Bidwell, 1962).

5.9. EFFECT OF ENZYME ACTIVITY DUE TO INTERACTION

5.9.1. Effect of peroxidase activity due to interaction

In VAM inoculated plants, the peroxidase activity was lesser and elevated in the case of nematode inoculation. Increased peroxidase activity is associated with resistant reaction due to increased phenol concentration, where phenols were cofactors of peroxidase and hence influence the resistance (Giebel, 1974). The

elevated peroxidase activity in the diseased plants may be due to the synthesis of new isozymes as a response to the parasitic invasion of host (Mohanty *et al.*, 1986). In the present study, the peroxidase activity was observed to be more in nematode inoculated plants than in untreated control. This is in accordance with the work of Mohanty *et al.* (1986); Ganguly and Dasgupta (1987) and Sujatha and Usha Mehta (1998). The resistance is due to the oxidation of phenolic compounds to quinone which are known to be more toxic to microorganisms (Clark and Lorbear,1975; Mote and Dasgupta, 1979; Ganguly and Dasgupta, 1979).

5.9.2. Effect of chitinase activity due to interaction

The chitinase activity was more in all VAM species, but, when VAM was inoculated with nematode, there was a reduction when compared to VAM. When nematode alone was inoculated the chitinase activity was found to be less. This may be due to the chitosan. Krebs and Grumet (1991) and Masuta *et al.* (1991) reported that chitinase in plants was induced by chitosan. Chitin is known to be a structural element in the egg shell of nematode. Chitinase is a hydrolytic element which is responsible for degrading chitin, in the egg shell during embryonic development and there by damage the development of embryo (Zamir *et al.*, 1993).

5.10. EFFECT OF MACRONUTRIENTS ON NEMATODE VAM INTERACTION

5.10.1. Nitrogen

All the treatments with mycorrhiza had higher content of nitrogen than the treatments with nematode. Among the VAM species, *G. mosseae* and *G.fasciculatum* recorded the highest. The plants having mycorrhiza along with nematode were found to have higher nitrogen status than plants with nematode alone. This is in accordance with the research work of Suresh and Bagyaraj (1984) and Umesh *et al.* (1988). The mycorrhizal fungal hyphae, extract nitrogen

from soil by its absorptive surface (Bajwa and Read, 1985). The fungus contains the enzyme nitrogen reductase which break down the organic nitrogen.

5.10.2. Phosphorus

In case of phosphorus, it was more in all the species of VAM but was reduced when nematode was inoculated with mycorrhiza. However, it was more when compared with nematode alone and control. The same has been reported by Dropkin and King (1956); Hussey and Roncadori (1982a); Tang *et al.* (1984); Umesh *et al.* (1988) and Terry-Ann *et al.* (1991). The mycorrhizal hyphae explored the bulk soil by its hyphae and transport P to the host (Bolan, 1991). The phosphorus content in the roots of VAM + nematode interaction was more than in the shoot content in all the species of VAM. This indicates that the uptake and transport of elements was inpeded by the deformation of vascular elements and also *M. incognita* may have utilized them for its own growth and thereby decreased the supply of elements available and concentrated in the roots (Rowslhorne and Hague, 1986).

5.10.3. Potassium

The amount of potassium was very much reduced in nematode infested plants with and without mycorrhiza. Remarkable difference in growth response of soybean to VAM inoculation with different isolates of *G.mosseae* were attributed to improved K rather than P nutrition of the host plant (Bethlenfalvay *et al.*, 1989). The decreased concentration of K in shoots of nematode infested plants may be due to deformation of vascular tissues.

From the results it was observed that, there was an increased concentration of N, P and K in all the species of VAM compared to control and a sharp decrease in nematode alone inoculated plants. When VAM was inoculated with nematode, there was a slight decrease in the concentration with respect to VAM alone, but

there was an increase when compared to nematode alone and control. This has been supported by many authors, Umesh *et al.* (1988) recorded an higher concentration of N, P, K, Ca and Mg in banana inoculated with *R. similis* and *G.fasciculatum*. Suresh and Bagyaraj (1984) recorded increased concentration of N, P and K in the tomato plants inoculated with *G.fasciculatum*.

5.11. EFFECT OF MICRONUTRIENTS ON VAM NEMATODE INTERACTION

From the results, it is clear that, the concentration of Fe, Zn and Cu was higher in the plants inoculated with VAM species, and reduced a little when nematode was inoculated. The concentration was least in nematode inoculated alone. The effect of nematodes on the concentration of these elements varied. But, with respect to Mn, the content was lesser in VAM inoculated plants when compared to control and nematode alone.

When Fe was considered, there were fluctuation in the concentration of shoot and root, both with VAM and VAM + nematode. In the case of copper (Cu) the concentration in shoot was more than the concentration in root when nematode was inoculated in all the VAM species and also in control. It has been observed by Lambert and Weidensaul (1991) that in white clover, the delivery of Cu from hyphae ranged from 52 to 62 per cent of the total Cu uptake. In the case of nematode inoculated plants, it was lesser than control which is in accordance with, Akhtar *et al.* (1993).

In the case of Zinc, there was an increase in the concentration in plants inoculated with VAM species compared to control. But, there was an increase in Zn content in shoot when compared to root. The same result has also been reported by Umesh *et al.*(1988) when banana plants were inoculated with *R. similis* with *G.fasciculatum*, the highest Zn content was found in plants inoculated with mycorrhiza only.

The Mn content was lesser in VAM inoculated plants compared with control and plants with nematode alone. The uptake and concentration of Mn in plants are sometimes not affected by VAM and more often are lower in VAM plants (Lambert and Weidensaul, 1991). Mycorrhiza had no significant influence on the Mn content, but plants with nematodes usually contained significantly less Mn (Suresh and Bagyaraj, 1984).

M.incognita disrupt the vascular tissue of roots which hinder the translocation of elements in roots and shoots and decrease the chlorophyll content and photosynthetic rates. The role of Fe, Zn in the synthesis of chlorophyll and Fe, Cu and Mn in the photosynthetic apparatus is very important because this affect the host physiology which leads to reduction in plant dry weight (Nasr *et al.*, 1980; Viglierchio, 1987).

SUMMARY

CHAPTER VI

SUMMARY

Plant parasitic nematode, *M. incognita* is a cosmopolitan and important menace to the vegetable crops. Brinjal is a good host for the root-knot nematode which severely reduce the crop yield.

It is very well documented that vesicular arbuscular mycorrhizal fungi improve the growth of plants. The effect of VAM on nematode-host relationships deserves special attention. Mycorrhizal fungi that render nematode-susceptible plants tolerant to plant parasitic nematodes or affect nematode development may prove to be very valuable in limiting crop losses due to these pathogens. Hence, studies were conducted to know the effect of VAM in limiting the crop losses due to nematodes. Attempts were also made to observe the biochemical variations due to VAM-nematode interaction. The results of the studies are summarised below:

1. Different doses of *G.mosseae* were evaluated and the best result was at 10 g of inoculum per Kg soil. The plant growth parameters and nematode control was maximum.

2. VAM applied earlier than the nematodes, gave better colonization of VAM and also spore count. Nematode control was also maximum

3. Among the four species of VAM tried, *G.mosseae* gave the best result and it was on par with *G.fasciculatum*, by enhancing the growth parameters and also the yield.

4. *G.mosseae* decreased the number of females present in the roots and also the number of females with egg sac compared to other species. VAM

colonization and yield were maximum in *G. fasciculatum* inoculated with nematode, which was determined by cluster analysis.

5. The crop rotation studies in microplot conditions, showed better growth and nematode control in the final host crop, brinjal after rotation with cumbu and greengram.

6. In the field study, the maximum growth and yield were obtained when the VAM inoculum was given @ 2.0 and 2.5 Kg/m^2 in nursery.

7. Carbofuran treated plots showed a decrease in nematode population immediately after treatment. But, later, the nematode population increased, though the yield increased to a considerable level.

8. The cost benefit ratio was maximum in 2.0 Kg/m^2 of VAM followed by 2.5 Kg/m^2 inoculum in the nursery. The carbofuran ranked third.

9. The histopathological studies showed that, the vesicles and arbuscles were formed in the cortex cells. Due to nematode infection and formation of giant cells, the vascular bundle was shifted to one side.

10. In the nematode infected cells, the gaint cells were seen with 7 to 8 nuclei. The females were seen inside the root with the egg mass protruding out side the epidermis.

11. The stelar tissues of mycorrhizal plants were lignified, which deter penetration of pathogen.

12. The sequential changes in protein of shoot and root was observed in 1mm thickness gel, which showed the appearance and disappearance of protein bands in VAM and VAM inoculated with nematode.

13. The protein content was more in all VAM treated plants. When the nematode was inoculated, the shoot showed lesser amount of protein compared to roots.

14. VAM inoculated plants showed a higher level of phenol content. The VAM+ nematode plants also showed a marked increase in phenol content, whereas the phenol content was less in nematode infected plants.

15. Total sugars were more in mycorrhizal plants than mycorrhizal infected nematode plants. It was more in nematode inoculated plants when compared to control.

16. The reducing sugars was less in mycorrhizal plants inoculated with nematode when compared to VAM alone. The least was in *M.incognita* plants.

17. The amino acids present in *G.mosseae* and *G.fasciculatum* were more when compared to *G.intraradices* and *G.fulvum*. The amino acid phenylalanine was present in *G.mosseae* and *G.fasciculatum* which is important for the control of nematodes.

18. Peroxidase enzyme activity was elevated in nematode inoculation and lesser in VAM inoculated plants. It is associated with resistant reaction.

19. The Chitinase activity was more in VAM inoculated plants and decreased in nematode inoculated plants.

20. The nitrogen content was more in mycorrhizal plants. *G.mosseae* and *G.fasciculatum* recorded higher content of total nitrogen. VAM inoculated with nematode recorded lesser compared to VAM alone.

21. The phosphorus content was more in mycorrhizal plants where as the control and nematode inoculated plants showed a decreased level. The P content was more in VAM + nematode when compared to nematode alone.

22. The amount of potassium was very much reduced in nematode infested plants. VAM along with nematode recorded more compared to nematode alone.

23. The micronutrients like the iron, zinc and copper were higher in VAM species and it was lesser when nematode was inoculated with VAM. The concentration was lesser in nematode inoculated alone, when compared to control. But, Mn content was lesser in VAM inoculated plants as well as VAM + nematode inoculated when compared to nematode alone.

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