MANAGEMENT OF RENIFORM NEMATODE, *ROTYLENCHULUS RENIFORMIS* Linford and Oliveira, 1940 AND *FUSARIUM OXYSPORUM* F. SP. *VASINFECTUM* (Atk.) Snyder and Hansen DISEASE COMPLEX ON COTTON WITH VESICULAR - ARBUSCULAR MYCORRHIZAE

Thesis submitted in partial fulfillment of the requirements for the award of the Degree of **DOCTOR OF PHILOSOPHY in PLANT NEMATOLOGY** to the Tamil Nadu Agricultural University, Coimbatore.

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2001

CERTIFICATE

This is to certify that the thesis entitled "**Management of reniform nematode, *Rotylenchulus reniformis* Linford and Oliveira, 1940 and *Fusarium oxysporum* f.sp. *vasinfectum* (Atk.) Snyder and Hansen disease complex on cotton with vesicular arbuscular mycorrhizae**" submitted in partial fulfillment 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 **Mr. N.SEENIVASAN** 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 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

ABSTRACT

MANAGEMENT OF RENIFORM NEMATODE, *ROTYLENCHULUS RENIFORMIS* Linford and Oliveira, 1940 AND *FUSARIUM OXYSPORUM* F. SP. *VASINFECTUM* (Atk.) Snyder and Hansen DISEASE COMPLEX ON COTTON WITH VESICULAR - ARBUSCULAR MYCORRHIZAE

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Crop losses inflicted by nematode pests continue to increase and are becoming a limiting factor in stabilizing crop yield on worldwide 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, the Vesicular Arbuscular Mycorrhizal (VAM) fungi are now attracting greater attention.

Interaction of four species of VAM *viz.*, *Glomus mosseae, G. fasciculatum, G. intraradices* and *G. fulvum* with the reniform nematode, *Rotylenchulus reniformis* on cotton cultivars *viz.*, MCU 5, K10 and TCB 209 were studied. All the VAM species increased plant growth and yield of all cotton cultivars and decreased nematode population. Among them, *G. mosseae* was effective on MCU 5 and K10 and *G. fasciculatum* was more effective in TCB 209, when VAM alone

was inoculated. When VAM along with nematodes were inoculated, *G. mosseae* performed well by increasing growth parameters and reducing nematode population with increased spore density and VAM colonization in all the three cultivars tested. Nematode penetration was lower in mycorrhizal roots than in non-mycorrhizal roots. The reproduction potential and fecundity of the reniform nematode was very much reduced due to VAM application which was observed by the presence of fewer females with eggmasses and fewer number of eggs per egg sac in the mycorrhizal roots. *G. mosseae* application increased yield parameters to the maximum extent on all the three cotton cultivars even in the presence of nematodes. In addition, fibre quality of cotton was considerably improved in *G. mosseae* treatment compared to nematode alone. Macro-nutrients *viz.*, N, P and K were more in all mycorrhizal roots but was slightly reduced when nematodes were inoculated. It was observed that Fe, Cu, Zn and Mn were on the increase both under VAM and VAM + nematode plants than in the untreated in all cotton cultivars. The VAM application nullified the physiological stress induced by *R. reniformis* on cotton by reducing leaf temperature, diffusive resistance and by increasing transpiration rate and photosynthetic rate.

Evaluation of seed treatment and soil application of VAM at two dosages for the management of reniform nematode on cotton cv. MCU 5 revealed that soil application of VAM @ 10 g per kg soil was most effective in checking the reniform nematode population. The same treatment promoted plant growth and increased cotton yield. Highest level of VAM colonization with maximum spore density was observed in soil application of VAM @ 10 g/kg soil.

Studies on the effect of *G. mosseae, Pseudomonas fluorescens* and *Trichoderma viride* as soil and seed treatments for the management of reniform nematode in cotton cv. MCU 5, showed that soil application of VAM resulted in increased plant growth and reduced *R. reniformis* population. The same treatment promoted the shoot phosphorus content and increased cotton yield. However, seed treatment with *P. fluorescens* was observed to be statistically on par with the former treatment.

Management of *R. reniformis* - *Fusarium oxysporum* f. sp. *vasinfectum* wilt disease complex by different biocontrol agents also revealed that soil application of VAM was most effective in reducing nematode population and wilt index, thereby increasing growth and yield of cotton. However, its effect was on par with seed treatment with *P. fluorescens*.

Biochemical changes in cotton cultivars MCU 5 and K10 due to VAM, nematode and *Fusarium* interaction revealed that total protein was maximum in combination of pathogens (nematode + *Fusarium*) and either pathogen alone in both cotton cultivars. However, increase in protein content to a little extent in VAM alone and along with pathogens were recorded. Total sugar and reducing sugar contents were more in VAM and VAM combination treatments than in nematode, *Fusarium* and nematode + *Fusarium* combination treatments. The same trend was observed in total free amino acids content. However, in K10 the total free aminos acids, total and reducing sugar contents were slightly more in nematode alone and nematode + *Fusarium* treatments than in control and this may be attributed to the lesser susceptibility of the cultivar to the reniform nematode.

Total phenol compounds which play a key role in disease resistance were more in mycorrhizal plants than in non-mycorrhizal plants. There was a slight increase in total phenol content in plants inoculated with nematode alone than in control in the cv. K10. Peroxidase and chitinase enzyme activities were also more in all the treatments involving VAM than in treatments without VAM. The amount of macronutrients *viz.*, N, P and K was very much reduced in nematode + *Fusarium* infected plants. VAM alone and along with pathogens recorded more nutrients compared to control and pathogens alone. The micronutrients like Fe, Cu, Zn and Mn were higher in VAM alone and it was slightly reduced when pathogens were inoculated along with VAM. The concentration of the nutrients was very much reduced when both nematode and *Fusarium* were inoculated.

Investigation on the histopathology of nematode inoculated cotton roots revealed that the permanent feeding site of adult female is initiated in an endodermal cell. Syncytia induced in cotton roots consisted of a curved layer of pericycle cells which are conspicuously hypertrophied, with densely stained cytoplasm, extended 6-10 cells either side of the feeding place, both circumferentially and longitudinally involving 100-150 modified cells. But when both the nematode and VAM were present it was observed that the VAM hyphae penetrated the epidermis and invaded the cortex producing arbuscules and vesicles. It was also found that in the mycorrhizal roots, the cortical cells were more lignified which prevented the penetration of nematodes.

ACKNOWLEDGEMENT

I place on record my deep sense of gratitude and abundant measure of thanks to my chairperson **Dr. (Tmt.) Rajeswari Sundarababu**, Professor, Department of Nematology for the inspiration, motivation, direction and expert technical guidance be benevolently bestowed on me which helped in the successful completion of this project on a personal note, her broadmindedness, frankness and meticulous care definitely needs a special mention.

My utmost courteous and gracious faithfulness to the members of the advisory committee, **Dr. G. Rajendran**, Professor and Head, Department of Nematology, **Dr. R. Samiyappan,** Professor, Department of Plant Pathology, and **Dr. M. Sahulhameed** Associate Professor, Department of Environmental Science for their adroit and manually dexterous guidance in correcting and interpreting the result of the study.

I sincerely record my gratitude for all the help and guidance given by **Dr. C. Sankaranarayanan,** scientist, Sugarcane Breeding Institute, Coimbatore.

Words fail to express my heartful thanks to **Dr. M. Sivakumar** and **Dr. S. Ramakrishnan**, Associate Professors, Department of Nematology for their agile and timely help rendered during my study.

It is a great privilege for me to put forth my sincere thanks to Professors, and other staff members of Nematology, for the help rendered by them whenever approached.

My special thanks are to my parents and friends who gave moral support and guidance during my study.

I assent the expertise of **M/S. Sowmiya Communications**, Coimbatore in neat execution of this manuscript.

With immense pleasure, I express my profound thanks to one and all concerned who have directly/indirectly helped me in this endeavour. While every effort has been made to acknowledge the helps rendered, any omission therein is inadvertent. I am obliged much to express my sense of gratitude to the Lord Almighty who has been blessing me.

(N. SEENIVASAN)

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CHAPTER - I

INTRODUCTION

Cotton (*Gossypium* spp.) is the staple fibre plant belonging to the family malvaceae. It has about thirty species and subspecies of shrubs or small trees, distributed in the tropical and subtropical regions of world. Hutchinson *et al.* (1947) registered four cultivated species, *viz. Gossypium hirsutum* L., *G. barbadense* L., *G. arboreum* L. and *G. herbaceum* L. The cotton crop is ranking first among the commercial crops and is being cultivated in an area of 33.73 million ha with an annual production of 19.7 million tonnes of Kapas worldwide. India is the third largest cotton producing country which held the credibility of the original habitat of cotton. It occupies an area of 8.87 million ha with the productivity of 12.65 million bales per annum. Tamil Nadu is one of the most important cotton growing states producing six lakh bales from an area of 2.7 lakh ha with an average productivity of 378 kg/ha (Shantanu Bardhan, 1998).

Cotton is a drought resistant crop by virtue of its long tap root which may reach to a depth of more than one metre (Prentice, 1972). Damage to this tap root by plant parasitic nematodes severely restricts the uptake of water, nutrients, leading to complete cessation of tap root system. The annual yield loss in cotton due to damage by plant parasitic nematodes on a world basis is estimated to be 10.7 % (Sasser and Freckman, 1987). Cotton suffers heavily from the attack of over 22 species of plant parasitic nematodes. Among them, the loss caused due to *Rotylenchulus reniformis* Linford and Oliveira, 1940 on cotton was found to be widespread with 40-60 per cent yield loss in tropical and subtropical regions (Sikora and Bridge, 1960). In India, the yield loss caused by reniform nematode

was estimated to be 14.8 per cent (Palanisamy and Balasubramanian, 1983) and it ranged from 10-15 per cent in Tamil Nadu.

Nowadays, management of the nematode disease is difficult particularly in developing countries where nematicides are very expensive. Indiscriminate use of chemical pesticides has been cautioned on account of their deleterious effect on the ecosystem. Development of pesticides resistance in target and non-target species is reported to be a risk in using pesticides excessively.

Biological control appears to be an alternative strategy in the management of nematode diseases. Among the various kinds of organisms engaged in biological control of nematodes, vesicular arbuscular mycorrhizae (VAM) are now attracting greater attention. VAM fungi are obligate symbionts that colonize the roots of most cultivated plant species. Plant parasitic nematodes and VAM fungi commonly occur together in the roots of the rhizosphere of the same plant, each having a characteristic but opposite effect on plant vigour. The obligate symbiont VAM fungi may stimulate plant growth, whereas the obligate plant parasitic nematodes usually suppress plant growth. Baltruschat *et al.* (1973) were the first to show that plants pre inoculated with VAM, *Glomus mosseae* were less susceptible to root knot nematode infection. Since then, a number of reviews and numerous research papers have appeared on nematode-VAM interaction which indicate that VAM fungi are potential biocontrol agents against plant parasitic nematodes.

VAM fungi favour plant growth by increasing nutrient uptake, growth rates and hormonal activity (Abbott and Robson, 1984; Linderman, 1992). Mosse (1957) demonstrated for the first time the importance of VAM fungi in phosphorus

uptake. It is also an established fact that zinc, copper and sulphur uptake is also improved in the presence of VAM. Mycorrhizal fungi provide a greater absorptive surface compared to root hair, and thus help in the absorption of relatively immobile ions in soil, such as phosphorus, copper and zinc (Bagyaraj, 1992). In addition to improved plant growth and nematode control, mycorrhizae also increased plant tolerance to toxic metals, high soil temperature, adverse soil pH and to transplantation shock, than do non-mycorrhizal plants (Bagyaraj, 1995).

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 facing with the problems of providing more food, fibre and fuel to the people. The challenges for the above are the utilization of mycorrhiza as a biological control agent for root pathogens. Therefore, it was aimed to take up the studies on the interaction of four VAM species on cotton to control *R. reniformis* using the biocontrol attributes of these symbionts. The present investigation were carried out to elucidate information on:

1. The effect of four species of VAM *viz. Glomus mosseae* (Nic and Gerd.), *Glomus fasciculatum* (Thaxt.) Gerd and Trappe, *Glomus intraradices* (Schenck and Smith) and *Glomus fulvum* (Bk. and Br.) Trappe and Gerd on three leading cultivated cotton species *viz., G. hirsutum* cv. MCU 5, *G. arboreum.* cv. K10 and *G. barbadense* cv. TCB 209 to control *R. reniformis.*
2. To evaluate the effective method of application of *G. mosseae* to suppress nematode population in cotton under pot culture conditions.

3. Evaluation of different biocontrol agents, *viz. Glomus mosseae, Pseudomonas fluorescens* and *Trichoderma viride* for the management of reniform nematode on cotton under pot culture condition.
4. To evaluate the biocontrol agents, *viz. G. mosseae, P. fluorescens* and *T. viride* for managing reniform nematode and *Fusarium oxysporum* f. sp. *vasinfectum* wilt disease complex on cotton.
5. Elucidation of biochemical changes associated with interaction of reniform nematode, *Fusarium oxysporum* f. sp. *vasinfectum* and *G. mosseae* on cotton cultivars MCU 5 and K10.
6. Histopathological studies of VAM and nematode infected cotton roots.

Review of Literature

CHAPTER - II

REVIEW OF LITERATURE

The cotton crop is ranking first among the commercial crops and is being cultivated in about 33.73 million ha worldwide (FAO, 1998). In India, cotton is cultivated in 8.87 million ha occupying about 20 per cent of the world total area of cotton cultivation (Agricultural situation in India, 1997), with an annual production of 22.3 million tonnes of kapas. Among the various plant parasitic nematodes associated with cotton, the reniform nematode is considered as a serious potential threat to cotton cultivation in Tamil Nadu which is one of the chief cotton growing states of India (Balasubramanian *et al.*, 1989). The nematode causes delayed maturity, stunting, reduction in boll size that reduced the yield to the extent of 10 to 15 and 40-60 per cent respectively in India and worldwide (Palanisamy and Balasubramanian, 1983; Jones *et al.*, 1959). Also, in association with other organisms the nematode is capable of causing disease complex and thereby greater yield reduction in cotton occurred (Prasad and Padagnur, 1980).

2.1. Effect of reniform nematode on cotton

Sivakumar and Seshadri (1971) reported that *Rotylenchulus reniformis* Linford and Oliveira, 1940 was widespread in Tamil Nadu, India occurring in different types of soil varying from sandy loam to heavy clay soils. Heald and Heilman (1971) also found that reniform nematode damage to cotton increased as soil salinity increased.

Gansman *et al.* (1975) observed that reniform nematode infested cotton plants were stunted with fewer and smaller leaves than non infected plants under both field and glasshouse condition. They also found that reniform nematode

caused decrease in leaf chlorophyll concentration, mesophyll structure and water content in cotton. Oteifa *et al.* (1976) reported that soil application of Temik 10G controlled *R. reniformis* in cotton and increased yield by 65 per cent.

Muralidharan and Sivakumar (1977) observed that 32-49 per cent reduction in cotton yield and deterioration of length and strength of cotton fibre due to *R. reniformis* infection.

Sud *et al.* (1984) studied the damage potential of *R. reniformis* on cotton and found that the damage threshold was 1000 young females per 100 cc of soil. An increase in reduction in plant growth was observed beyond 100 young females per 100 cc of soil.

Robinson *et al.* (1990) observed that the distribution of the reniform nematode coincided closely with the geographical area in which cotton is produced in USA and they reported that 1.5-2.9 per cent loss of cotton worldwide has been attributed to the reniform nematode.

Gazaway *et al.* (1996) found that reniform nematodes adversely affect cotton growth by inhibiting cotton roots from extracting potassium from the soil efficiently and thereby crop maturity delayed by as much as 2 to 3 weeks. Wulfen Barger (1996) observed that significant decrease in lint quantity and quality of cotton occurred where the plants were infected with *R. reniformis*.

Cook *et al.* (1996) studied the effect of reniform nematode combined with whitefly, *Bemesia argentifolli* on cotton and found that seed index was

significantly reduced in combination. Also seeds produced under combined infection had lower germination percentage and less radical growth.

Cook *et al.* (1997) reported that *R. reniformis* caused 52 per cent reduction of lint yield and also responsible for significant reduction in fibre quality of cotton. Cook and Robinson (1998) found that reniform nematode reduced lint yield by 29.5 per cent and also reduced fibre quality and seed quality of cotton to some extent.

2.1.1. Management of reniform nematode in cotton by chemical methods

The work conducted on field management of the reniform nematode for the past one decade through chemicals revealed that systemic granular nematicides like Temik 10G, Nemacur 10 G, (Gazaway and Kabana, 1991; Gazaway and Edmisten, 1992; Lawrence and Mclean, 1995a) and EC formulations such as Fosthiazate 7.5 EC (Lawrence and Mclean, 1995b) oxamyl 2E (Garber and Oakeley, 1996) and fumigants like 1,3 dichloropropene (Baird, 1995) were effective in checking the nematode population and also increased the productivity of the crop. The chemicals were reported to suppress the nematode population upto 135 per cent (Munier *et al.*, 1996).

Lawrence and Mclean (1995c) found that the plant stand in nematicide treated cotton plants were remarkably superior to untreated plants in their appearance with greater leaf area, more squares, bolls and cotton lint. Muller *et al.* (1997) recorded an yield increase of 23.7 per cent by applying aldicarb 10 G @ 7.01 lb/acre in furrow before sowing cotton.

2.2. Interaction of fungal pathogens with cotton nematodes

Bird *et al.* (1971) reported that the nematodes *Pratylenchus brachyurus* (Godfrey) Filipjev and Schuurmans, *Trichodorus christiei* Allen and *T. porosus* Allen and the soil borne fungi, *Rhizoctonia solani* Kuhn, *Pythium debaryanum* Hesse, *P. irregulare* Buis, *P. ultimum* Trow and *Fusarium* spp. were the pathogens most frequently found in the roots and rhizosphere of field grown cotton showing "stunt" symptoms. Field application of the nematicide D-D (1,2-dichloropropane, 1-3-dichloropene) decreased stunt symptoms and significantly increased plant growth and yield.

Elgindi *et al.* (1974) found that *R. reniformis* is associated with *Fusarium oxysporum* Schlectend. f.sp. *vasinfectum* (Atk) W.C.Snyder and H.N.Hans in increasing the susceptibility of a resistant Egyptian cotton variety to the infection of wilt pathogens. Similarly, presence of *Meloidogyne* sp. increased wilt in cotton due to *Verticillium dahliae* (Ertruck *et al.*, 1975).

When cotton cv. Deltapine 16 inoculated with *Meloidogyne incognita* (Kofoid and White, 1919) Chitwood, 1949 and *Rhizoctonia solani* together or separately, the disease incidence was severe in combined infections than with either pathogen alone (Carter, 1975). Yang *et al.* (1976) reported that *Belonolaimus longicadatus* Rau along with Fusarium fungus promoted greatest wilt development in cotton.

Krishnaprasad and Padaganur (1982) observed heavy population of reniform nematode in the rhizosphere of Verticillium wilt infected cotton. Saadabi

et al. (1986) reported that the greater and faster infection of *F.oxysporum*.f.sp. *vasinfectum* occurred in the presence of *Pratylenchus sudanensis* in cotton plants.

Hillock and Bridge (1992) reported that the nematodes associated with cotton plants showing symptoms of *Fusarium* wilt were *Meloidogyne incognita, Scutellonema* spp., *R. reniformis* and *Pratylenchus* spp. The nematodes increased the incidence of wilt and variations in the population level of nematodes had a greater effect on wilt incidence than in population levels of wilt pathogens.

Ruano *et al.* (1992) reported that the most important nematode species infecting cotton crop are *M. incognita* and *R. reniformis* which aggravate symptoms of diseases such as *Fusarium* wilt and *Rhizoctonia solani*. Patel *et al.* (1992) found that reniform nematode was found associated with *Rhizoctonia bataticola* in cotton and increased disease severity.

Sankaralingam and McGawley (1994) studied the interrelationship between reniform nematode and the cotton seedling blight fungus, *Rhizoctonia solani* and found that the colonization of cotton hypocotyl tissue by *R. solani* resulted in the increase in nematode population density.

Ma *et al.* (1994) observed the symptoms of *Fusarium* wilt in cotton cultivar (*Fusarium* resistant) only when the plants were inoculated with both *Fusarium oxysporum* f.sp. *vasinfectum* and *Helicotylenchus pseudorobustus*. Johnson *et al.* (1998) reported the association of *M. incognita* and *Belonolaimus longicadatus* with *Sclerotium rolfsii* on cotton.

2.3. Vesicular Arbuscular Mycorrhiza

Plant roots provide an ecological niche for many of the soil microorganisms. Frank (1885) coined the term "Mycorrhiza" to describe the symbiotic association of plant roots and fungi. Mycorrhiza literally means "Fungus root" and by far, the most common mycorrhizal association is the vesicular arbuscular type, which produces structures (Vesicles and arbuscles) in the cortex region of the root. The Vesicular Arbuscular Mycorrhizal (VAM) association is found in most of the plant families growing in arctic, temperate and tropical regions. They are formed by non-septate zygomycetous fungi belonging to the genera *Glomus*, *Sclerocystis*, *Entrophosphora*, *Acaulospora*, *Scutellospora* and *Gigaspora* (Bagyaraj, 1995).

It is very well documented now that VAM fungi improve the growth of plants that are important in agriculture. Mycorrhizal fungi provide a great absorptive surface compared with root hair, and thus help in the absorption of relatively immobile ions in soil, such as phophorus, copper and zinc (Bagyaraj, 1992). In addition, recently mycorrhizal fungi were shown to increase host tolerance or resistance in many plants to nematode attack

2.3.1. Effect of VAM on cotton

Several workers have studied the effect of VAM on growth and yield of cotton plants. Pugh *et al.* (1980) observed that inoculation with *Gigaspora margarita* Becker and Hall and *Glomus etunicatum* (Thaxter Sensu. Gerd) Gerd Trappe stimulated the plant growth and increased absorption of phosphorus from soil. The experiment to study the effect of *G. margarita* on the growth of cotton in phosphorus deficient soil (Pugh *et al.*, 1981) revealed that *G. margarita* with

inoculum levels of 200 or 400 azygospores per plant significantly stimulated cotton growth.

Siqueira *et al.* (1986) studied the effect of six species of VAM fungi on growth and nutrition of cotton and recorded that *Glomus clarum, Gigaspora gregaria* and *Glomus macrocarpum* Tul and Tul were the most effective species. VAM species showed a close relationship between colonization rates and increase in shoot dry matter, yield and phosphorus uptake.

Smith and Roncadori (1986) observed that inoculation of VAM fungi *viz.*, *Glomus intraradices* Schenck and Smith, *Glomus ambisporum* and *Gigaspora margarita* had increased leaf tissue concentration of P, Cu, Zn and Mn and stimulated plant growth. Price *et al.* (1989) also found increased 'P' uptake and stimulated plant growth of cotton plants when inoculated with VAM fungi.

2.3.2. Effect of VAM on cotton nematodes

Roncadori and Hussey (1976) observed increased shoot weight upto 40 per cent with inoculation of *Gigaspora calospora* on root knot nemotode suceptible cotton cultivar. Hussey and Roncadori (1978) also recorded that the inoculation of *Gigaspora margarita* on cotton was able to offset the adverse effect of *Pratylenchus brachyurus* and increased shoot height, shoot weight, flower production and root weight by 96, 553, 760 and 385 per cent compared to untreated control.

Inoculation with azygospores of *Gigaspora margarita* on root knot nematode susceptible cotton cultivars nullified the stunting caused by the nematode and increased vegetative growth and square production than non

mycorrhizal plants (Roncadori and Hussey, 1977). A similar positive observation was made by Smith *et al.* (1984) by inoculating *Glomus intraradices* for the control of *M. incongnita.* The VAM inoculation increased plant tolerance to root knot nematode and yield of cotton increased by 31 per cent.

Roncadori and Hussey (1980) reported that on cotton roots *M. incognita* egg production was less on plants colonized with *G. etunicatum,* which had larger root system than uninfected plants. There were significantly fewer eggs/g root at the lower fertilization rate in mycorrhizal roots suggesting physiological or physical antagonism towards *M. incognita.*

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

Smith *et al.* (1986a) studied the influence of VAM fungus *G. intraradices* on penetration, development and reproduction of *M. incognita* in cotton, and reported that least number of nematodes penetrated in to the mycorrhizal root system. Nematode reproduction rate was lower in mycorrhizal than in non mycorrhizal root system.

Smith *et al.* (1986b) also observed that population densities of *M. incognita* juveniles were significantly lower, 60 and 90 days after planting of cotton in the plots which received *Glomus intraradices* and suggested that mycorrhizal fungi can increase host tolerance to *M. incognita* under field condition.

Sitaramaiah and Sikora (1996) studied the interaction of *G. fasciculatum* and *Rotylenchulus reniformis* in cotton. In the presence of *G. fasciculatum,* reproduction of *R. reniformis* was affected and nematodes with egg mass per g root were significantly reduced. In addition, by increasing the spore concentration of fungus, it was able to reduce the soil nematode population ranging from 40-80 per cent. They suggested that the mycorrhizae colonized roots were unfavourable sites for nematode penetration and development.

2.4. Interaction of VAM and Reniform nematode

Sitaramaiah and Sikora (1981) found that when *R. reniformis* was inoculated, the penetration of the nematode in *Phaseolus vulgaris* L. was reduced by 35 and 41 per cent respectively, whereas nematode population increased when both *G. mosseae* and *R. reniformis* were inoculated simultaneously.

Sitramaiah and Sikora (1982) observed that the endotrophic mycorrhizal fungus, *Glomus fasciculatum* increased the resistance of tomato plants to *R. reniformis* infestation. The development of the gelatinous matrix was interfered and fewer eggs/egg sac were produced. Data showed that *G. fasciculatum* adversely affected *R. reniformis* during several phases of its life cycle.

Kassab and Taha (1990) studied the effect of five initial population densities (pi of 100, 500, 1000, 2000 and 4000 nematodes / plant) of *R. reniformis* on the yield of mycorrhizal and non mycorrhizal sweet potato plants. Growth of fibrous roots and yield of storage roots of mycorrhizal plants inspite of the presence of nematodes were significantly higher than those of non mycorrhizal

plant. Coyrol (1991) reported the nematicidal effect of mycorrhizal fungi on plant parasitic nematodes like *Rotylenchulus*, *Meloidogyne* and *Heterodera.*

Lingaraju and Goswami (1993) observed the interaction of vesicular arbuscular mycorrhizal fungi, *G. fasciculatum* and the plant parasitic nematode, *R. reniformis* in cowpea in greenhouse, either singly or in combination. Mycorrhizal fungus enhanced total biomass as well as fresh root weight while the nematode reduced them. The VAM induced tolerance in cowpea to the nematode, even in the presence of damaging levels of nematodes under phosphorus deficiency condition.

Field application of *Glomus fasciculatum* individually and along with biofertilizers recorded higher yield of ragi and maize by reducing the multiplication rate of the *R. reniformis* and *Pratylenchus zeae* respectively (Babu *et al.* 1996).

Jothi and Rajeswari Sundarababu (1998) studied the effect of *G. fasciculatum* against the population of *R. reniformis* in ragi and found that yield was highest in VAM inoculated plants with a significant reduction in nematode population.

2.5. Preferential Host Plant association by VAM

Vesicular arbuscular endophytes are not host-specific. However, it is observed that VAM fungi show " Host preference" if not "Host specific". Many workers have reported interspecific and intraspecific host preference of

endomycorrhizae (Dhillon, 1992) and thus suggesting the need for selecting efficient VAM fungi for a particular host (Jeffries, 1987).

Jain and Sethi (1987) observed that the presence of *G. fasciculatum* showed a profound adverse effect on cyst production and multiplication of *H. cajani* on cowpea, while *G. epigaeus* tended to exhibit a reverse trend. Hasan and Jain (1987) surveyed the occurrence of VAM species and nematode association of Berseem (*Trifolium alexandrum* L.). It was observed that three VAM species *viz.*, *G. mosseae, G. fasciculatum* and *G. epigaeus* were associated with berseem in *Tylenchorynchus vulgaris* infected field and *G. mosseae* was most prevalent than others which offset nematode damage.

Xue and Lue (1992) reported that all the three VAM fungi *viz., Glomus epigaeum (G. versiforme), G. mosseae* and *G. macrocarpus (G. macrocarpum)* had a beneficial effect on the growth of peach seedlings and resulted in marked reduction in the soil population of harmful nematodes.

Santhi and Rajeswari Sundarababu (1995a) studied the interaction between *G. fasciculatum, G. versiforme* and *G. etunicatum* with *M. incognita* on cowpea under greenhouse condition. *G. fasciculatum* was superior at reducing the nematode population in soil.

An investigation was carried out to select an efficient vesicular arbuscular mycorrhizal fungus for capsicum varieties, Bharath and California wonder. Among 10 different VAM fungi, *Glomus monosporum* responded best for both the

varieties and inoculated plants had higher shoot and root biomass, shoot and root P content and fresh weight of fruit. (Mallesha and Bagyaraj, 1997).

Wani and Konde (1996) observed the response of ten genotypes of garlic to inoculation with a VAM fungus, *G. mosseae.* All the genotypes tested could exhibit different degrees of root colonization by the inoculated mycorrhiza leading to varied response in respect of dry matter production and mycorrhizal colonisation. They suggested that response of garlic to *G.. mossae* was genotype dependent.

Mallesha and Bagyaraj (1997) found that inoculation of tomato with *G. intraradices* had higher fruit yield, shoot P concentration, per cent mycorrhizal colonization and mycorrhizal spore number in the rhizosphere soil compared to the plants inoculated with *G. monosporum.*

Five different arbuscular mycorrhizal fungi *viz., Glomus fasciculatum , G. macrocarpum, Gigaspora margarita, Acaulospora laevis* and *Sclerocystis dussi* were screened for their ability to form efficient symbiotic association with chilli cv. Byadagi. Plants inoculated with *G. macrocarpum* had significantly highest per cent mycorrhizal root colonization, spore count, plant P concentration, growth and yield parameters. *G. fasciculatum* was found to be second best to *G. macrocarpum* (Shrihari and Sreenivasa, 1997).

Sreeramulu and Bagyaraj (1997) reported that among the nine arbuscular mycorrhizal fungi tested, plants inoculated with *G. monosporum* performed best in improving plant growth and uptake of P, Zn, Cu and Fe.

Jaizme-vega and Pinochet (1997) studied the effects of the interaction between three arbuscular mycorrhizal fungi and *pratylenchus goodeyi* on the growth of banana cv. Grand Naine. Plants inoculated with *G. mosseae* and *G. aggregatum* showed increased plant growth and nitrogen level in *P. goodeyi* infested soil.

Rivasplateron and Andrade (1998) observed that the inoculation of *Enterophospora colombiana* and *Gigaspora margarita* reduced the root knot nematode, *M. exigua* gall index by 20 per cent compared to nematode alone, but the foliar area and dry weight were greater with *G. margarita* inoculation in coffee.

Pinochet *et al.* (1998) observed that early mycorrhizal inoculation with *G. mosseae* favoured plant growth of plum rootstock, but in soils infected by *P. vulnus,* only *G. intraradices* increased the tolerance of mycorrhizal rootstock to the damaging nematode levels by stimulating plant nutrition and vegetative growth.

Harikumar and Potty (1999) surveyed natural distribution of arbuscular mycorrhizal fungi in sweet potato soils of Kerala and found that the species of *Glomus macrocarpum* was dominant among *Acaulospora, Gigaspora, Glomus, Sclerocystis* and *Scutellospora.*

Blackgram inoculated with two species of VAM fungi, *Glomus fasciculatum* and *G. mosseae* significantly increased plant growth, plant dry matter, yield attributes, nutrient uptake, spore density and per cent root

colonisation. There was no significant difference between the two mycorrhizal symbionts (Hazarika *et al.*, 1999).

2.6. Effect of different species of VAM on nematodes

2.6.1. Effect of *G. mosseae* on nematodes

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 in the root. Simultaneous inoculation of *G. mosseae* along with nematode neutralized the adverse effect of the citrus nematode and stimulated growth of citurs seedlings (Baghel *et al.*, 1990).

Al-Radded (1995) also inoculated *G. mosseae* on tomato for the control of *M. javanica* and observed that the gall index and average number of galls per root were reduced by 53 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 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). Sosamma *et al.* (1998) reported that inoculation of *G. mosseae* was found effective in reducing the nematode populations especially *M. incognita* and *Radopholus similis* infesting banana under field condition.

Rao *et al.* (1998) found that integration of *G. mosseae* with biocontrol fungus *Paecilomyces lilacinus,* for the management of *M. incognita* on egg plant did not affect each others colonization on the roots resulting in an additive effect.

Sankaranarayanan and Rajeswari Sundarababu (1999) reported that application of *G. mosseae* on blackgram recorded higher plant growth, VAM spore population, colonization, plant phosphorus content and minimum nematode population of *M. incognita.*

Rao *et al.* (1999) studied the effect of integration of *Pasteuria penetrans* and *Glomus mosseae* for the management of *M. incognita* infesting tomato revealed significant increase in plant growth parameters and reduction in root galling, nematode population in roots, eggmass production and fecundity of the nematode. The bacterial bioagent did not affect the root colonization of endomycorrhiza after transplanting.

Maximum enhancement in various growth parameters such as fresh and dry root/shoot weight and total oil yield of *Mentha arvensis* L. were obtained in soil treated with VAM fungi, *G. mosseae* by the reduction of soil and root population of *M. incognita* (Rakesh Pandey *et al.*, 1999).

2.6.2. Effect of *G. fasciculatum* on nematodes

Atilano *et al.* (1981) observed that the application of *G. fasciculatum* increased plant growth and reduced population of *M. arenaria* on grapevine. MacGuidwin and Bird (1982) observed that the root penetration and development of *M. hapla* were inhibited on *Allium cepa* L. colonized by *G. fasciculatum* under greenhouse conditions. Final nematode population in the roots increased two folds in non mycorrhizal than in mycorrhizal plants.

The gall formation by *M. incognita* and cyst production by *Heterodera cajani*, as also their multiplication was hampered by the early establishment of *G. fasciculatum* on cowpea (Jain and Sethi, 1988).

Thomas *et al.* (1989) tested six species of VAM on cardamom and concluded that the root colonization was maximum in *Gigaspora margarita* 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 he 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 in field. Sivaprasad *et al.* (1990) also found that *G. fasciculatum* on black pepper reduced root knot gall index by 32 per cent and also reduced the nematode population both in the roots and in the surrounding soils.

The germination and stand of flue cured tobacco was not affected by the root knot nematode, *M. incognita* with the incorporation of *G. fasciculatum* in infested sandy soils. The number of galls was reduced by 61-89 per cent as a result

of mycorrhizal inoculation and also the reproductive potential of the nematode was very much reduced by producing fewer eggmasses per infested seedling (Krishna prasad, 1991).

Tomato plants inoculated with *G. fasciculatum* and castor cake showed enhanced plant growth and reduction of root knot nematode population (Rao *et al.*, 1994). Rama and Reddy (1996) observed that combination of *Pasteuria penetrans* and *G. fasciculatum* significantly increased the plant growth characters, *viz.* shoot length, shoot weight, root length, root weight and yield of tomato. In addition, reduction in root knot nematode population, eggmass production, root galling and root knot index were also observed in this combination.

Rajeswari Sundarababu *et al.* (1996) found that 15 days prior inoculation of *G. fasciculatum* was able to enhance the growth of tomato and suppress *M. incognita* multiplication. Abhamisra and Shukla (1997) also observed that prior application of *G. fasciculatum* improved growth and increased tolerance to root knot nematode on tomato. Integration of *G. fasciculatum* at 500 g/m^2 inoculum with castor oil cake resulted in a significant reduction in root galling and fecundity of *M. incognita* and an increase in root colonization by endomycorrhiza.

Prior establishment of VAM fungus, *G. fasciculatum* by two weeks tended to mitigate the adverse effect of both *M. incognita* and *Tylenchorynchus vulgaris* on the plant growth of berseem. Gall formation by *M. incognita* and also multiplication of both nematodes was reduced by 28-50 per cent (Jain *et al.*, 1998). Rao *et al.* (1998) found that egg plant seedlings colonized with *G. fasciculatum* were least infected by *M. incognita* when transplanted in main field.

Nageswari and Rajeswari Sundarababu (1998) observed that the colonization of cowpea roots by *G. fasciculatum* hampered the root invation by *Heterodera cajani* leading to reduction in cyst population. VAM inoculated plants recorded good biomass production, yield and high uptake of phosphorus.

The pathogenic effect of root knot infestation on plant growth of pepper was generally reduced with VAM colonization particularly in *G. fasciculatum* inoculated plants (Sivaprasad and Sheela, 1998).

Nagesh *et al.* (1999) observed that *G. fasciculatum* @ 2 spores/g soil were required for 50 per cent root colonization under pot condition and 4 spores/g were required under nursery condition which reduced root knot nematode population by 59 and 61 per cent respectively on tomato.

2.6.3. Effect of *G. intraradices* on nematodes

Smith and Kaplan (1988) found that rough lemon seedlings, grown in *G. intraradices* infested field had enhanced growth by improved P nutrition, but mycorrhizal colonization had no effect on *Radopholus citrophilus* population in roots.

Heald *et al.* (1989) studied the interaction of *G. intraradices* and *M. incognita* on cantaloupe and revealed that growth of mycorrhizal plants inoculated with *M. incognita* was retarded by only 21 per cent, but mycorrhizal infection had no effect on the degree of root knot gall formation and did not affect the number of nematode eggs per egg mass also.

Pinochet *et al.* (1997) observed that *G. intraradices* infection on cherry root stock did not affect the number of *P. vulnus* per g of root, but increased the plant growth and nutrients inspite of the nematodes' presence which indicated that mycorrhizal colonization increased the tolerance of plants to nematodes. He also reported that *G. intraradices* colonization significantly increased growth of banana, but did not affect *M. javanica* population build up in the roots.

2.7. Effect on plant nutrients by VAM - nematode interaction

2.7.1. Effect of nematodes on plant nutrients

Oteifa (1952) found that root knot infected lima bean plants had lower total amount of N, P, K, Ca and Mg as compared to control.

Cotton plants inoculated with *R. reniformis* showed significant reduction in nitrogen, potassium and manganese even when the soil received complete nutrient level (Oteifa and Elgindi, 1974).

Nagesh and Dhawan (1988) observed that nitrogen, phosphorus and potash content in shoots of wheat plans were decreased significantly when 20 and 10 eggs and larvae of *Heterodera avenae* per cm^2 soil were inoculated. A negative correlation was recorded between inoculum level of *H. avenae* and mineral content of wheat.

Haseeb *et al.* (1990) recorded that with the increase in inoculum level of root knot nematode on *Hyoscyasmus niger*, there was corresponding decrease in iron, manganese, copper and zinc concentrations in roots and shoots except for sodium and potassium which increased in shoot. A significant decrease in N, K, Ca, Mg was reported on banana infected with *Helicotylenchus multicinctus* (Rajendran and Sivakumar, 1996).

2.7.2. Effect of VAM - nematode interaction on plant nutrients

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

Root colonization of *G. mosseae* was not affected by the presence of *Pratylenchus vulnus* on apple rootstock. 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, whereas mycorrhizal plants had the highest levels of N, Na, P, K and Fe (Pinochet *et al.*, 1993).

Pinochet *et al.* (1995a) observed that the peach plants inoculated with *G. intraradices* and *P. vulnus* recorded low levels of Ca, Mn, Fe whereas mycorrhizal plants achieved the highest value of N, P, S, Fe and Zn and increased absorption of Ca and Mn.

Pinochet *et al.* (1995b) also found that Cu was the only deficient element detected by foliar analysis of cherry plants, although low levels of Na, Mg, Mn and Zn were detected in *P. vulnus* inoculated plants. *G. mosseae* inoculated plants had the highest value of Cu and Al.

Calvet *et al.* (1995) recorded low levels of Al, Fe, Mn and Zn in non mycorrhizal nematode infected quince plants and *G. intraradices* inoculated plants recorded the highest foliar levels of N, Ca, Mg, Mn, Cu and Zn. Mycorrhizal plants infected with *P. vulnus* maintained normal to high level of Mn, Cu and Zn. Prior and simultaneous application of *G. fasciculatum* and *M. incognita* on tomato

caused significant increase in NPK content of tomato plants (Mishra, 1996; Abha Mishra and Shukla, 1997).

Lopez *et al.* (1997) observed that nitrogen and phosphorus were deficient in *P. vulnus* inoculated pear plants without mycorrhizae. *G. mosseae* inoculation with and without the nematodes resulted in increased foliar levels of N, P and Zn.

Banana plants inoculated with *G. mosseae* and *M. incognita* exhibited higher N, P, K, Ca and Mg contents compared with non-mycorrhizal plants (Jaizme-Vega *et al.*, 1997).

2.7.3. Effect of phosphorus on VAM - nematode interaction

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

Mycorrhizal roots increased the resistance to *M. hapla* on tomato by altering the physiology of root system and also as a result of better host nutrition due to improved P uptake by mycorrhizal plants (Cooper and Grandison, 1986).

Smith *et al.* (1986b) found that *M. incognita* population was least in *G. intraradices* infected cotton roots and greater in plants grown with supplemented phosphorus. Maximum growth and yield of soybean plants were reported at P

applied at 50-150 µg/g along with inoculation of *Gigaspora margarita, G. etunicatum* and *M. incognita* (Carling *et al.*, 1989).

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

Krishnaprasad (1990) recorded that by addition of *G. fasciculatum*, the root knot indices were reduced and required only half of its dose of phosphorus fertilizer compared to non mycorrhizal seedlings of tobacco.

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

2.8. Effect on plant physiology by VAM - nematode interaction

2.8.1. Effect of nematodes on physiology of plants

The spiral nematode, *Helicotylenchus multicinctus* (Cobb 1893), Golden (1956) decreased photosynthetic rate by 10.2 per cent in rice (Dutta *et al.*, 1990). Swain and Prasad (1988) recorded 25.7 per cent reduction in photosynthetic rate in plants infected with *Meloidogyne graminicola* on rice and they suggested as the nematode activity increased, the effect on photosynthesis and associated physiological process diminished productivity.

Haseeb and Shukla (1995) studied the influence of *Pratylenchus thornei* Sher and Allen on the photosynthetic rate of *Mentha citrata* Ehrn. indicated that with increase in initial population level of the nematode there was corresponding decrease in photosynthetic rate.

Kirkpatrick *et al.* (1995) observed that cotton plants infected with *M. incognita* had lower transpiration rate and higher diffusion resistance and leaf temperature than healthy plants. Patel *et al.* (1996) also found that *M. incognita* infection on cotton significantly increased leaf temperature, and diffusion resistance and decreased photosynthetic rate and transpiration rate.

Ramakrishnan and Rajendran (1998) observed that *M. incognita* on papaya increased leaf temperature and diffusion resistance and lowered the evaporation rate and photosynthetic rate.

2.8.2. Effect of VAM on physiology of plants

Anguilera-Gomaz *et al.* (1998) recorded maximum photosynthetic rate in *G. fasciculatum* treated maize plants. Makus (2000) recorded reduced leaf temperature and increased transpiration rate by application of *G. intraradices* on cotton.

Subramanian and Charest (1999) studied the impact of VAM fungus on physiological response of maize plants and observed that VAM plants exploited available soil moisture more efficiently with increased leaf evaporation rate and decreased stomatal diffusive resistance.

2.9. Biochemical changes due to nematode - VAM interaction

2.9.1. Biochemical changes due to nematode interaction

Nasr *et al.* (1980) reported a significant increase in the concentration of reducing, non-reducing and total sugars in root knot nematode affected peach rootstock. Nandini Gokte *et al.* (1988) reported that *Anguina tritici* infected wheat plants showed higher amount of reducing sugars and non reducing sugars, IAA, soluble protein, carbohydrate, etc.

Mohanty *et al.* (1995 and 1997) observed increased amount of total sugars in root knot nematode inoculated brinjal and green gram plants. Infection of banana roots by *H. multicinctus* caused increase in reducing sugars and decrease in non-reducing sugars (Rajendran and Sivakumar, 1996).

Singh *et al.* (1985) found that soil amended with oil cakes reduced the development of *M. incognita* on tomato with an increase in total free phenol, ortho-dihydroxy phenols and amino acid contents in the infected plants. The presence of high total phenol content in resistant variety of pigeonpea inoculated with *R. reniformis* as compared to susceptible variety was the reason for its resistance to the reniform nematode (Thakar and Yadav, 1986).

Pankaj *et al.* (1992) studied the changes in total phenol contents in the three barley cultivars (i.e.) two susceptible and one resistant with *H. avenae* inoculation. The total phenol content in the shoots of healthy and resistant cultivars was higher as compared to the susceptible cultivars.

Ganguly and Dasgupta (1987) studied the peroxidase activity due to the effect of *M. incognita* on tomato and found that the peroxidase activity was higher in galled root extract than the non-galled part of the same root. Simte and Dasgupta

(1987) observed that there was an increase in peroxidase activity due to *M. incognita* on soybean. Sujatha and Usha Mehta (1998) observed changes in enzyme levels of peroxidase and polyphenol oxidase in sugarcane roots affected with *P. zeae* and *M. javanica*. There was an increase in two peroxidases which can be attributed as a defense mechanism to the most invading pathogens.

Sundararaj and Usha Mehta (1991) investigated the changes in enzymes and free amino acids of sugarcane roots infected by lesion nematode and it was found that polyphenol oxidase, peroxidase, ascorbic acid were on the increase. In the control plant root, 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.

Wheat cv. Kalyansona infested with *A. tritici* was compared with control for their amino acid contents. Ten amino acids were separated for both infected and uninfected leaves. But the concentration of the amino acids *viz*., lysine, histidine, proline and tyrosine increased significantly whereas the concentrations of other seven decreased significantly in the infected leaves as compared to the uninfected leaves (Indra Rajvanshi, 1992).

Mohanty *et al.* (1996) studied the biochemical alteration in okra cultivar Pusa savani inoculated with root knot nematode and observed that nine amino acids were common in both healthy and inoculated plants. Out of fourteen amino acids, L. alanine was present only in healthy and L. histidine, L. lysine,

L. proline, L. glycine were present only in inoculated plants. Higher concentration of all the amino acids were detected in diseased tissue except L. tryptophan.

Mohanty *et al.* (1997) inoculated *M. incognita* to green gram and found an increased concentration of all the 20 amino acids except 2-tryptophan. L-tyrosine, L-proline, L-methionine which were absent in healthy were present in nematode inoculated plants.

2.9.2. Bio chemical changes due to VAM interaction

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 were detected.

Krishna and Bagyaraj (1982) observed that peanut plants inoculated with *G. fasciculatum* expressed higher concentration of OD phenol when compared to control which gave protection against pathogens.

Dighton (1983) observed that mycorrhizal fungi possess phosphatase enzyme which can hydrolyze isositol hexa phosphate and involved in the increased uptake of 'P' from the soil and drought tolerant mechanism.

Suresh and Bagyaraj (1984) reported that *G. fasciculatum* inoculation had increased total sugars, reducing sugars, amino acids, phenylalanine and serine content in tomato plants which helped in the suppression of population of root knot nematodes.

VAM colonized plants had higher concentration of amino acids in xylem sap compared to non-mycorrhizal plants (Coxwell and Johnson, 1985). Jeanmaire *et al.* (1985) observed that in VAM infected plants the neutral phosphatase activities are localized along the host membrane extending around the arbuscular hyphae.

Ocampo and Azcon (1985) recorded an increase of total and reducing sugars in roots of wheat plants inoculated with vesicular arbuscular mycorrhizae.

Panu *et al.* (1989) observed that in *Allium porum* L. the chitinase activity was high in mycorrhizal roots than in the uninfected control. Schubert *et al.* (1992) reported that trehalose was the main soluble carbohydrate in sporocarps of *G. versiforme*. It was present in mycorrhizal roots, where as in non-mycorrhizal roots it was absent or detected only in traces.

Belrhlid *et al.* (1993) isolated and identified six flavanoids from VAM inoculated root exudates and identified them as three flavanol and three flavones. Association of *G. versiforme* in the roots of *Medicago truncatula* increased the levels of phenyl ammonialyase activity and isoflavanoids accumulation (Harrison and Dixon, 1993).

Chen *et al.* (1998) observed that the concentration of total amino acids in root tissues of *Glomus caledonium* inoculated eucalyptus seedlings increased by 31 per cent than uninoculated plants.

Schwob *et al.* (2000) found that increased lignin content in rubber tree roots due to inoculation with *G. mosseae*. Ramesh *et al.* (2000) observed that cumbu

plants inoculated with *G. mosseae* induced increase in protein content and enzyme activities viz., acid phosophatase, alkaline phosphatase, superoxide dismutase and chitinase.

2.10. Effect of Fluorescent pseudomonads on nematodes

Fluorescent *Pseudomonas* spp. is emerging as the largest and potentially most promising group of plant growth promoting rhizobacteria involved in the biocontrol of plant disease (Kloepper *et al.*, 1988) and plant parasitic nematodes (Oostendorp and Sikora, 1989).

Jowarski *et al.* (1986) reported that root knot galling was reduced by *P. fluorescens* when applied around the plant root. Gokte and Swarup (1988) observed that *P. fluorescens* was effective in suppressing the population of *Heterodera avenae, H. cajani, H. zeae* and *M. incognita* under *in vitro* condition.

Seed treatment with *P. fluorescens* which was isolated from sugar beet rhizosphere reduced *H. schachtii* penetration by 75 per cent in sugar beet under field condition (Oostendorp and Sikora, 1989 and 1990). They reported that the antagonistic activity of *P. fluorescens* was probably caused by bacterial alteration of root exudates that influenced nematode hatch, attraction and penetration behaviour. In addition they suggested that the mechanism responsible for the reduction in penetration may be related to the ability of the bacteria to envelop or bind to the root surface lectins, thereby interacting with normal host recognition.

Hergarden and Sikora (1992) reported that application of sugar beet specific rhizobacterium, *P. fluorescens* (strain T58) to seed caused 42 per cent reduction in

early root penetration of *H. schachtii*. Weidenborner and Kunz (1993) showed that *P. fluorescens* cultivated in plate count broth reduced the number of *Panagrellus* sp. to 57.4 per cent.

Sujatha (1995) reported that *P. fluorescens* when applied as seed treatment, reduced *H. cajani* population in root and soil by 80 and 69 per cent respectively on cowpea.

Application of *P. fluorescens* as seedling bare root dip treatment increased plant growth significantly and reduced infestation by the root knot nematode in tomato (Santhi and Sivakumar, 1995).

Seed treatment with *P. fluorescens* which was isolated from rice rhizosphere caused 70 per cent reduction of *Hirschmanniella oryzae* population on rice roots under glasshouse condition. Similarly seed treatment with *P. fluorescens* along with soil application of chitin amendments before planting was found very effective in reducing rice root nematode population upto the level of 51 per cent under field condition (Swarnakumari, 1996).

Grapevine seedling root dip with *P. fluorescens* was found to be effective in reducing root knot nematode, *M. incognita* infestation and caused 42 per cent reduction in root gall index under glasshouse condition (Mani, 1996).

Cronin *et al.* (1997) reported that exposure of *G. rostochiensis* cysts to *P. fluorescens* under *in vitro* condition doubled the ability of eggs to hatch and the percentage of mobile juveniles was reduced three fold. They found that

P. fluorescens produced 2,4-diacetyl phloroglucinol which was responsible for increase in egg hatch ability and the reduction in juvenile mobility of potato cyst nematode.

Eapen *et al.* (1997) reported that the application of *P. fluorescens* isolates promoted the growth of black pepper seedlings though they had no effect on *M. incognita* population.

Fifty per cent of *R. similis* invasion was reduced by application of *P. fluorescens* on banana roots (Aalten and Gowen, 1998). Aalten *et al.* (1998) also reported that three strains of *P. fluorescens* were found to inhibit invasion of *R. similis* and *Meloidogyne* spp. in banana, maize and tomato roots.

2.11. Effect of *Trichoderma* sp. on nematodes

The plants grown in soil containing *T. harzianum* or *T. koningii* reduced the reproduction of *M. arenana* in maize (Windham *et al.*, 1989). Meenakshi Sharma and Saxena (1992) reported that the culture filtrate of *T. viride* affected the hatching of *M. incognita* juveniles.

Parveen *et al.* (1993) reported that seed treatment with *T. harzianum* and *T. koningii* on tomato gave 42 and 31 per cent control of *M. javanica* under field condition.

Stephen *et al.* (1996) found that combined application of either *Trichoderma*-vydate or *Trichoderma*-Nemacur significantly decreased root knot and *Fusarium* wilt disease complex on tomato and improved plant growth.

Rao *et al.* (1997) observed that significant increase in plant growth, and reduction in root galling and final population of *M. incognita* were observed in tomato seedlings transplanted in neem cake-amended soil incorporated with *T. harzianum*.

Combined application of *Paecilomyces lilacinus* and *T. harzianum* limited the damage caused by *M. incognita* and *Fusarium solani* disease complex and gave a 35 per cent increase in plant growth as compared to individual application (Khan *et al.*, 1997).

Soil application of culture filtrates of *T. viride* was highly nematicidal against *M. incognita* infecting okra (Goswami and Singh, 1998). Spiegel and Chet (1998) tested the nematicidal activity of *T. harzianum* and *T. viride* against *M. javanica* on tomato. Improved growth of nematode infected plants and decrease in the root galling index and the number of eggs per gram of root were achieved when nematode infected soil were pre-exposed to *T. harzianum*.

Latha and Sivakumar (1998) reported that culture filtrates of *T. viride* and *T. harzianum* as soil application against *Heterodera cajani* on black gram caused significant reduction in cyst and juvenile population.

Rekha Arya and Saxena (1998) observed that both *Trichothecium roseum* and *Trichoderma viride* reduced the harmful effects of *M. incognita* and improved germination of tomato in pot experiment.

Sankaranarayanan *et al.* (1998) found that culture filtrates of *T. harzianum* and *T. koningii* recorded 100 percent mortality of *M. incognita* and *M. javanica* within 24 hours of exposure under *in vitro*. Sharma (1999) also reported that 100 per cent concentration of *T. harzianum* culture filtrate caused 69.6 per cent mortality of *M. incognita*.

Xiujuan *et al.* (2000) observed that *Trichoderma* sp. isolated from nematode affected areas are found to reduce *Meloidogyne* sp. population by 82.6 per cent and promoting the growth of tomato.

Materials and Methods

CHAPTER - III

MATERIALS AND METHODS

3.1. Monoculture of reniform nematode

The reniform nematode, *Rotylenchulus reniformis* Linford and Oliveira (1940) required for the study was collected from the reniform nematode infected roots of cotton. They were collected from infected field and eggmasses were picked by seeing through the microscope (Plate 1). The collected eggmasses were placed in petridish containing sterile water and allowed to hatch. The juveniles were further used for mass culture (Sivakumar and Seshadri, 1971).

The sterilized pot mixture containing red soil, sand and farm yard manure in the proportion of 2:2:1 was filled in 5 kg earthen pots. Seeds of castor (*Ricinus communis* L.) cv. TMV 1 were sown in these pots, after surface sterilization with 0.1 per cent mercuric chloride for five minutes followed by washing with sterile water. The juveniles hatched out from the egg masses were inoculated to the castor plants at 15 days after sowing @ 2 infective females/g soil using localized incubation method. When the castor plants attained the age of 45 days, the plants were cut at the soil level and the pots re-sown with castor seeds.

The egg masses required for the various studies of this programme were obtained by gentle uprooting of the castor plants with intact root system followed by cautious washings in the tap water to avoid shedding of eggmasses. The egg masses were picked under the wide field stereo - microscope and allowed to hatch by planting the eggmasses in petridish containing sterile water.

Plate 1. Parasitization of cotton roots by *Rotylenchulus reniformis*.

The juveniles obtained in this method were maintained in tap water at room temperature (28 ± 2°C) with frequent aeration for seven days, so that all the nematodes in the suspension reached infective stage (Muralidharan and Sivakumar, 1976). These infective females were used as inoculum for various experiments of the present study.

3.2. Culturing of the wilt fungus, *Fusarium oxysporum* f.sp. *vasinfectum*

The fungus, *Fusarium oxysporum* f.sp *vasinfectum* was obtained from Dr. Lalithakumari, Centre for Advanced Studies in Botany, University of Madras, Chennai. Sand maize medium (19 : 1) was prepared and used for mass culturing of the fungus, having 20 per cent moisture at 30 ± 2°C. The sand was sieved to remove stones, plant debris etc. The sand at 19 parts was taken and mixed thoroughly with one part of the maize medium. The sand maize medium was filled three fourth in conical flask and autoclaved at 15 pounds pressure for 20 minutes. Then the bottles were allowed to cool and inoculated with the fungus under aseptic condition. After 4-7 days, The fungus grew well on the medium. This fungus was used for inoculation @ 100 g/one kg of soil (Plate 2).

3.3. Vesicular - Arbuscular Mycorrhizae inoculum

3.3.1. Starter inoculum

Vesicular - arbuscular mycorrhizal fungal cultures viz., *G. mosseae, G. fasciculatum, 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.

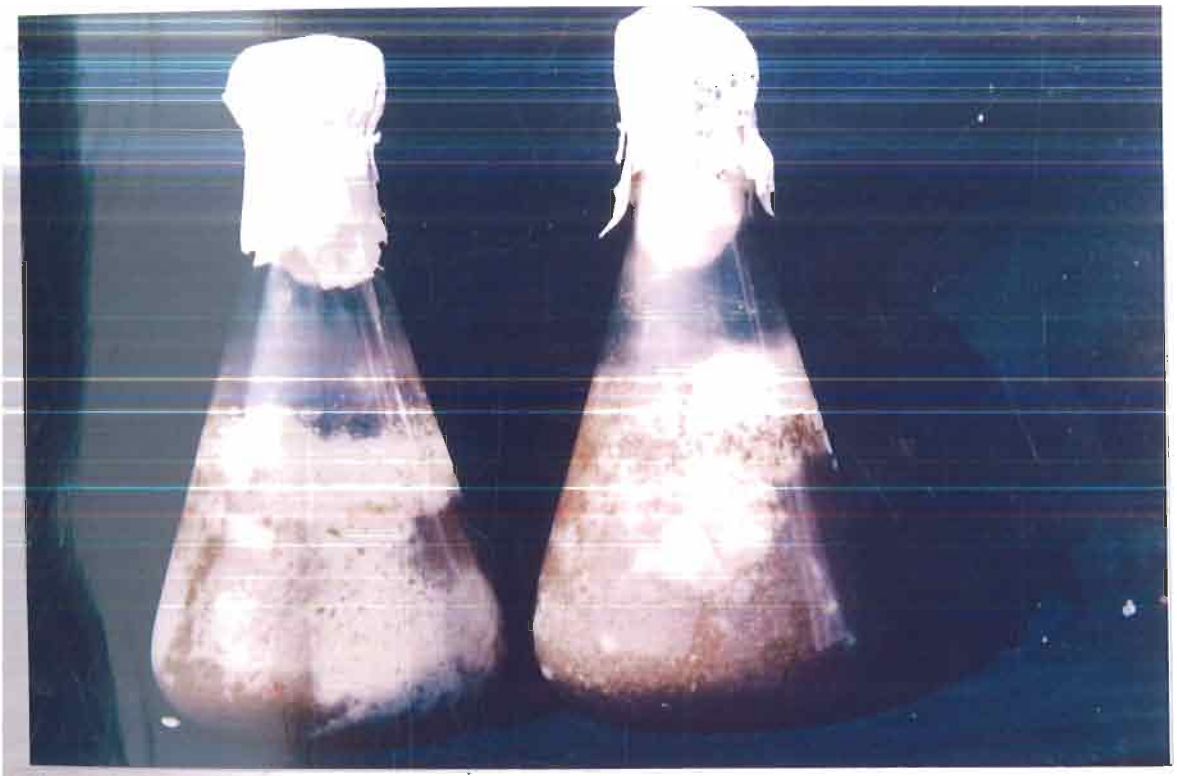


Plate 2. Mass culturing of *Fusarium oxysporum* f.sp. vasinfectum on sand maize medium

The starter cultures were transferred to mud pots containing sterile red soil, sand and FYM (2:2:1). Cumbu (Pearl millet) cv. WCC 75 seeds were sown and thinned down to eight plants/pot after germination. It was allowed to grow for 60 days. After 60 days the number of spores and per cent colonization was assessed.

3.3.2 Extraction of VAM fungal spore from soil

The VAM fungal spores in soil were estimated by recovering the spores by the net sieving and decanting method of Gerdemann and Nicholson (1963) as described below:

The 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 soil. Roots were carefully collected avoiding damage to the root cortex. During processing, 50g of soil was mixed with 200 ml luke warm water in a large beaker until all soil aggregates were broken. The supernatant were decanted through a 20 mesh sieve into a one litre measuring cylinder and the residue was re-suspended 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 re-suspended by stirring several times and decanted through a 100 mesh sieve into a second, one litre cylinder, retaining a small volume which was then re-suspended in a further 300ml 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

Plate 3. *Glomus mosseae* spore

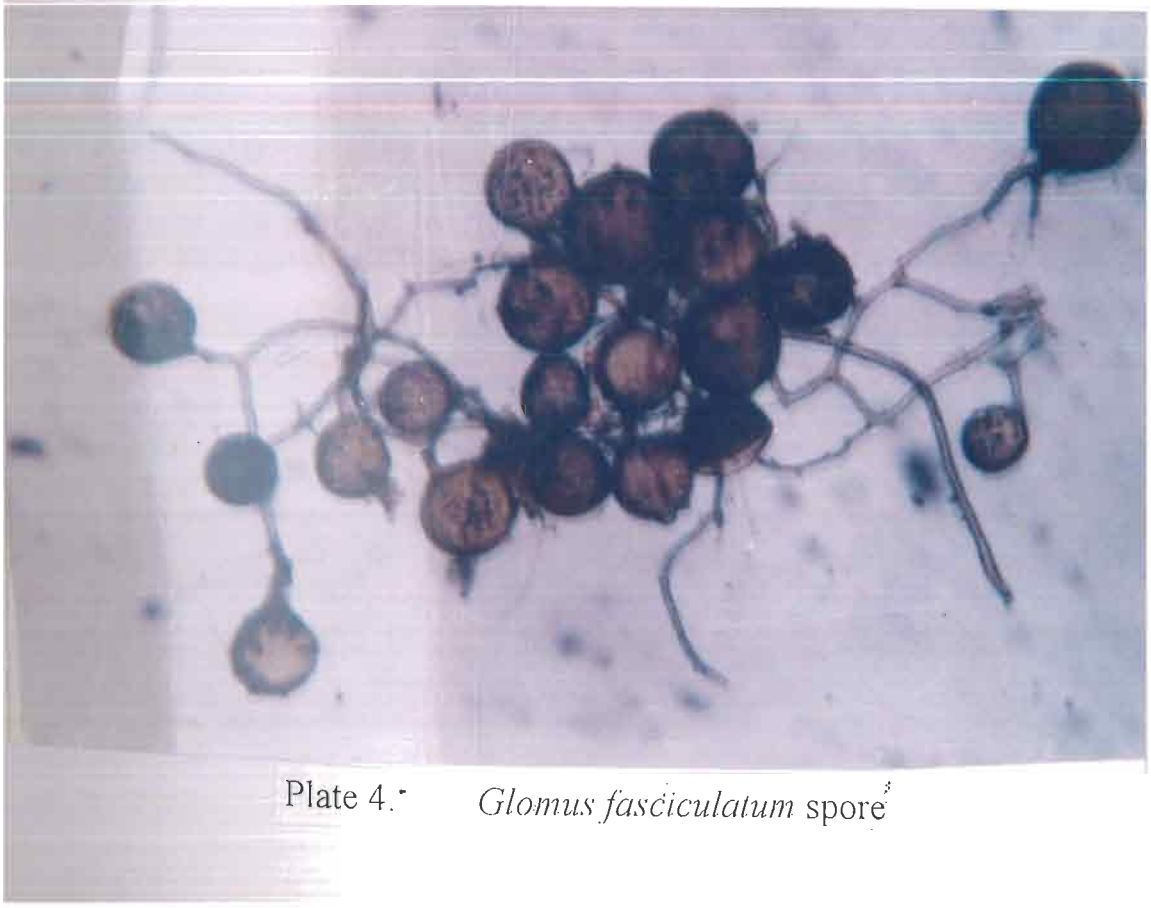


Plate 4. *Glomus fasciculatum* spore

Plate 5. *Glomus intraradices* spore

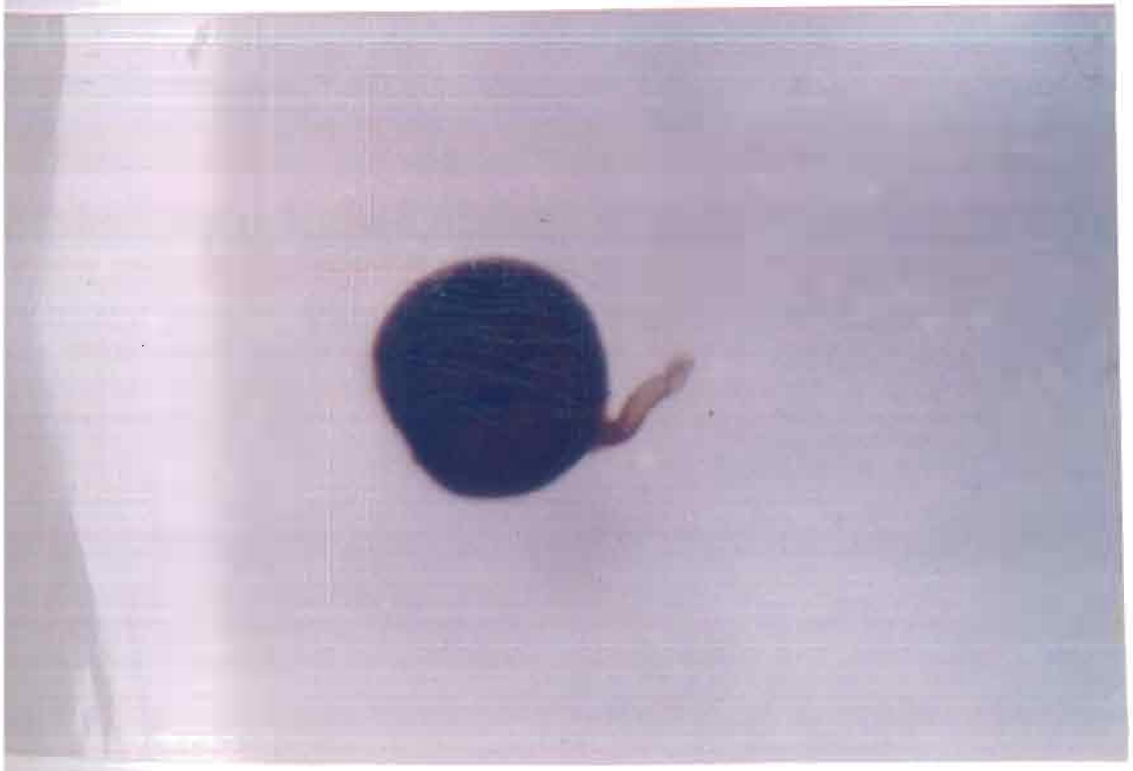


Plate 6. *Glomus fulvum* spore

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 324 mesh sieve. The matter in the sieve were washed into a small beaker and examined. The residue in large beaker and cylinders were discarded.

3.3.3. 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 (Plates 3, 4, 5 and 6).

3.3.4. Assessment of root colonization

The roots of the host plant, 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 Phillips 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 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 a 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 per cent HCl for three min and then the HCl

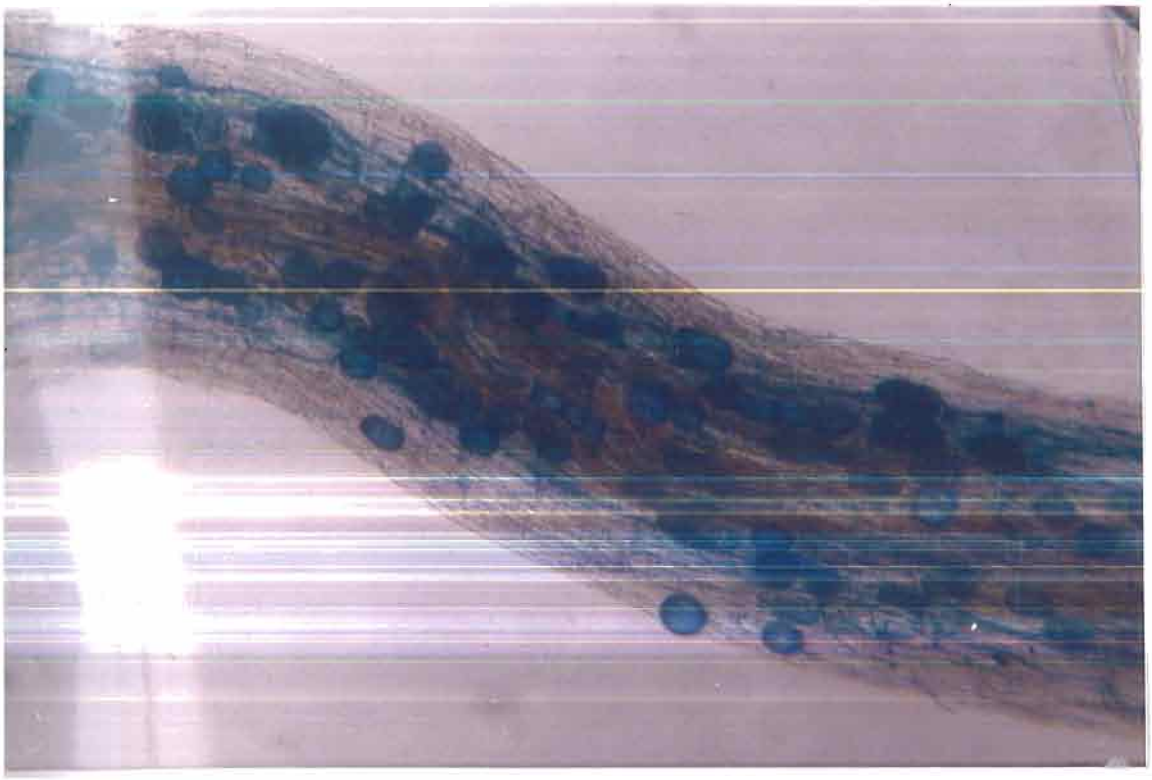
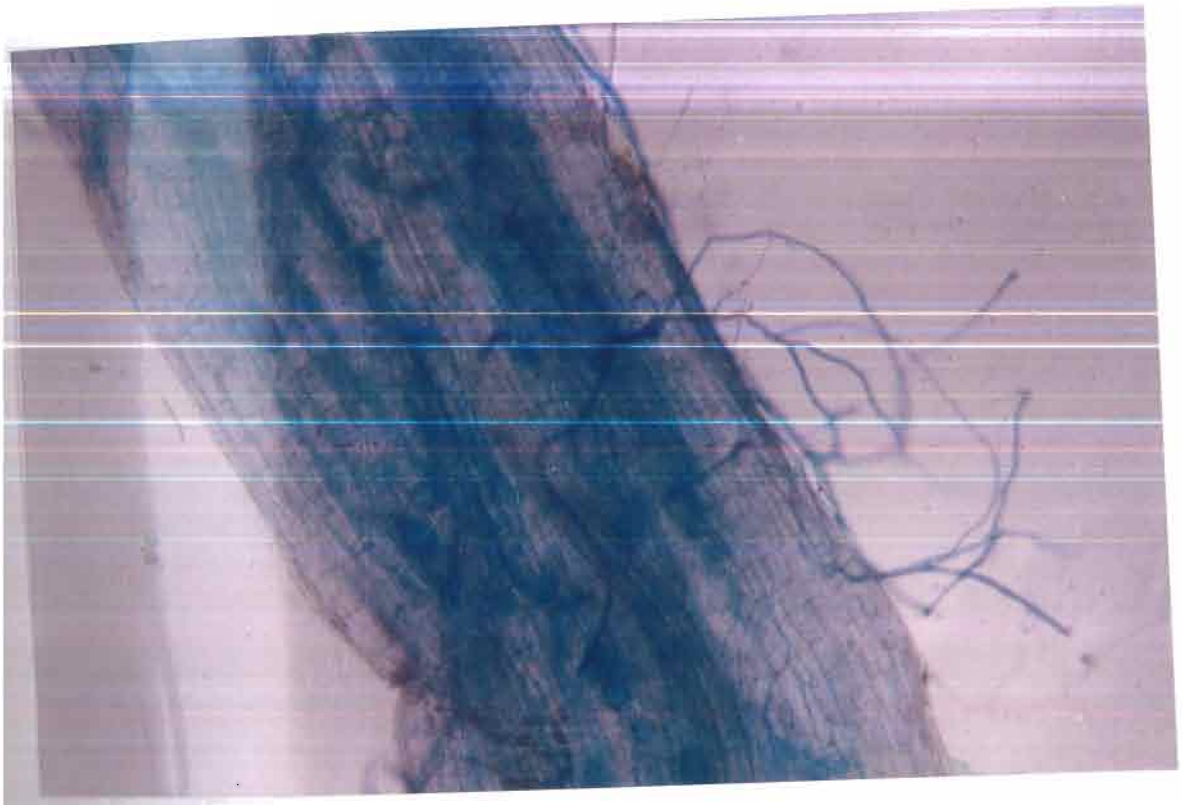


Plate 7a. Cotton roots showing vesicles

Plate 7b. Cotton roots showing vesicles and arbuscules.



.Plate 7c. Cotton roots showing vesicles and hyphae.

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. The per cent colonization of roots was then calculated (Plates 7a, 7b and 7c).

3.4. Studies on the interaction of four different species of VAM with *R. reniformis* on three cultivated cotton species

Three pot culture experiments were carried out to study the interaction of *G. mosseae, G. fasciculatum, G. intraradices* and *G. fulvum* with *R. reniformis* on cultivated *Gossypium* spp., namely *Gossypium hirsutum* cv. MCU 5, *G. arboreum* cv. K10 and *G. barbedance* cv. TCB 209. The interaction of four species of VAM with *R. reniformis* on three cotton cultivars studied as three separate experiments with the following same treatments which were replicated three times in completely randomized block design.

T_1 - *G. mosseae*

T_2 - *G. fasciculatum*

T_3 - *G. intraradices*

T_4 - *G. fulvum*

T_5 - *G. mosseae* + *R. reniformis*

T_6 - *G. fasciculatum* + *R. reniformis*

T_7 - *G. intratadices* + *R. reniformis*

T_8 - *G. fulvum* + *R. reniformis*

T_9 - *R. reniformis* alone

T_{10} - Control.

Two kg capacity pots were filled with pot mixture and different *Glomus* species were mixed with pot mixture at 10g/kg Soil (100 spores g^{-1} soil). Cotton seeds (cv. MCU5, K10 and TCB 209) surface sterilized with 0.1 per cent mercuric chloride were sown at the rate of 5 seeds per pot and the seeds germinated were thinned to 2 plant/pot at 10 Days After Sowing (DAS). The nematode, *R. reniformis* was inoculated at the rate of 2 nematodes per g of soil at 15 DAS. After 90 days, the leaf samples were collected for analysis to find out the macro and micronutrient content.

The experiment was terminated at 175 DAS, observations were made on plant growth characters, yield parameters, nematode populations in soil and root and some physiological parameters. The details of methodology adopted for study of various parameters are briefly described below.

3.4.1. Plant growth characters

3.4.1.1. Plant height

Plant height was measured from the cotyledonary node to the base of the last opened leaf.

3.4.1.2. Root length

Maximum root length of the cotton plants was recorded immediately after the termination of the experiment prior to weighing of plants and subsequent nematode estimation and expressed in cm.

3.4.1.3. Fresh weight of shoot and root

Individual plant shoot and root weight were recorded at the time of termination of the experiment and it was expressed as wet weight of shoot and root in gram.

3.4.1.4. Dry weight of shoot and root

Shoot and root samples were dried in a oven at 70°C for 24 hours and cooled in a dessicator. Dry weight was expressed in gram.

3.4.1.5. Leaf Area Index (LAI)

The leaf area was measured with LI - COR LI - 3000 area meter with conveyor belt and expressed in cm^2 per plant. The LAI was arrived by using the formula of Williams, 1946.

$$LAI = \frac{\text{Leaf area of plant}}{\text{Ground area Occupied}}$$

3.4.2. Nematode population

Population of *R. reniformis* in soil was recorded at the time of termination of the experiment by drawing a composite samples of 100 g soil from each pot. The samples were processed for nematode extraction using the method of Cobb's sieving and decanting followed by modified Baerman's funnel technique (Schindler, 1961) and the population of nematode was assessed.

For assessing nematode population from root, a representative root samples of one gram per plant collected at the time of termination of experiment. This was stained with boiled lactophenol + acid fuschsin followed by destaining in clear lactophenol (McBeth et al., 1941). From this, number of females and number of females with egg mass were assessed and expressed per plant system.

The number of eggs/eggmass was determined by dispersion of eggs with 2% sodium hypochlorite and counted under microscope (Muralidharan and Sivakumar, 1976).

3.4.3. VAM Colonization

Percentage of VAM colonization and spore count were taken as described in 3.3.

3.4.4. Yield parameters

3.4.4.1. Number of bolls per plant

The total number of bolls per plant was recorded and expressed as number per plant .

3.4.4.2. Boll weight

The cotton bolls in each plant were collected and weighed and the mean worked out and expressed in gram per boll.

3.4.4.3. Cotton yield

The cotton yield obtained from the plant in each picking made at 10 days interval commencing from 120 DAS and upto 175 DAS was recorded, and expressed in gram per plant.

3.4.4.4. Ginning percentage

Ginning percentage was calculated at the harvest stage after the last picking. It was calculated by using the following formula and expressed in percentage (Santanam, 1976).

$$\text{Ginning percentage} = \frac{\text{Weight of lint}}{\text{Weight of cotton yield}} \times 100$$

3.4.4.5. Lint index

Lint index was computed by using the following formula and expressed in g (Santanam, 1976).

$$\text{Lint index} = \frac{\text{Weight of 100 seeds x Ginning percentage}}{\text{100 - Ginning percentage}}$$

3.4.4.6. Seed index

It was calculated by taking the weight of hundred seeds and expressed in g (Santanam, 1976).

3.4.5. Fibre Quality characters

The fibre quality characters *viz.*, fibre fineness, mean fibre length and bundle strength were determined at the Cotton Technical Research Laboratory, Tamil Nadu Agricultural University, Coimbatore-3 from the lint sample.

3.4.6. Physiological parameters

The third leaf of each plant was plucked and immediately fed to leaf chamber analyser-3 of carbon dioxide Leaf chamber analysis system to read Leaf temperature and to estimate some physiological functions *viz.*, photosynthetic rate, transpiration rate and diffusion resistance (Sen et al., 1989).

3.4.7. Estimation of macronutrients

3.4.7.1. Estimation of total nitrogen content in plants

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

3.4.7.2. Estimation of total phosphorus content in plants

Phosphorus in plant material was estimated by the vanadomolybdate method in nitric acid system (Jackson, 1973). Five ml of the diacid extract was taken in twenty ml volumetric flask and 0.5 g of plant material was added to this. A 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 standard chart and total 'P' content was calculated.

3.4.7.3. Estimation of potassium content in the plants

In a 250 ml conical flask, 0.5g 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 no1. filter paper and volume was made upto 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).

3.4.8. Estimation of micro nutrients

To ten ml of triple acid extract, 0.5g of the plant sample was added and digested and then the volume was made up to 100ml. 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 Norvell, 1978).

3.5. Evaluation of seed treatment and soil application of VAM, *G. mosseae* for the management of *R. reniformis* on cotton cv. MCU 5

A pot culture experiment was conducted to study the effect of seed and soil application of *G. mosseae* at two dosages for the management of *R. reniformis* in cotton. The experiment was conducted in completely randomized block design with following six treatments replicating four times.

T_1 - *G. mosseae* as seed treatment @ 5g/kg seed.

T_2 - *G. mosseae* as seed treatment @ 10g/kg seed.

T_3 - *G. mosseae* as soil application @ 5g/kg soil.

T_4 - *G. mosseae* as soil application @ 10g/kg soil.

T_5 - Carbofuran @ 1kg a.i./ha.

T_6 - Control.

Vermiculite based commercial formulation of *G. mosseae* obtained from Microbiology Department, Tamil Nadu Agricultural University was used as inoculum. The sterilized pot mixture (Red soil; sand : FYM - 2:2:1) was filled in two kg capacity pots. At the time of planting, seed treatment and soil application of VAM at two dosages were done. For seed treatment rice gruel was mixed with the VAM formulation as a sticking agent.

Cotton (Cv. MCU5) seeds treated and untreated were sown at the rate of 5 seeds per pot according to their treatment, and the seeds germinated were thinned to 2 plant / pot at 10 DAS. The nematode, *R. reniformis* was inoculated at the rate of 2 nematodes per g of soil at 15 DAS.

The experiment was terminated 175 DAS. The plants were carefully depotted and observations on shoot height, root length, fresh weight of shoot and root, dry weight of shoot and root, LAI, number of females per plant, number of females with eggmasses per plant, number of eggs per eggmass, soil nematode population, VAM colonization in percentage, number of spores, total phosphorus content in leaf, number of bolls, boll weight, cotton yield and fibre quality parameters were made.

3.6. Evaluation of VAM and other biocontrol agents by different methods of application for the management of reniform nematode on cotton cv. MCU 5

Vermiculite based commercial formulation of *G. mosseae* and the commercial talc formulations of *P. fluorescens* and *T. viride* were evaluated as seed treatment and soil application for the management of *R. reniformis* in cotton under glasshouse condition. The following treatments were maintained with three replications and the pots were arranged in completely randomized block design.

T_1 - Seed treatment with *G. mosseae* @ 10g/kg seed.

T_2 - Soil application with *G. mosseae* @ 10g/kg soil.

T_3 - Seed treatment with *P. fluorescens* @ 10g/kg seed.

T_4 - Soil application with *P. fluorescens* @ 2.5kg/ha.

T_5 - Seed treatment with *T. viride* @ 4g/kg seed.

T_6 - Soil application with *T. viride* @ 2.5kg/ha.

T_7 - Carbofuran @ 1kg a.i./ha

T_8 - Control.

Seed treatment with VAM

VAM formulation was mixed with required quantity of rice gruel to form a slurry and cotton seeds were treated and air dried.

Seed treatment with *P. fluorescence*

Seeds were soaked in water @ 100ml/kg seed containing the talc based product of *P. fluorescens* @ 10g/kg seed for 12 hrs. Excess water was drained off and the treated seeds were incubated in dark for 24 hrs before sowing.

Seed treatment with *T. viride*

Seeds of cotton cv. MCU5 surface sterilized with mercuric chloride at 0.1 per cent for 2 min were washed with sterile distilled water and then the seeds were treated with *T. viride* @ 4g/kg seed just before sowing.

Soil application with biocontrol agents

In two kg capacity pots, sterilized pot mixture was taken and the biocontrol agents at their respective dosages were mixed thoroughly with pot mixture before sowing.

The seed of cotton (cv. MCU5) were sown @ 5 seed/pot and thinned to 2 seedlings per pot. At 15 days after germination of cotton, *R. reniformis* infective juveniles were inoculated @ 2 nematodes/g soil into the holes around the roots of the cotton plants.

The experiment was terminated 175 DAS. The plants were carefully depotted and observations on shoot height, root length, fresh weight of shoot and root, dry weight of shoot and root, LAI, number of females per plant, number of females with egg mass per plant, number of eggs per egg mass, soil nematode

population, VAM colonization in percentage, number of spores, *P. fluorescens* and *T. viride* colony forming units in roots, total phosphorus content, number of bolls, boll weight and cotton yield were made.

3.7. Evaluation of VAM and other biocontrol agents for the management of reniform nematode and Fusarium wilt disease complex on cotton cv. MUC 5

Vermiculite based commercial formulation of *G. mosseae* and the commercial talc formulations of *P. fluorescens* and *T. viride* were evaluated as seed treatment and soil application for the management of *R. reniformis* and *Fusarium oxysporum* f. sp. *vasinfectum* wilt disease complex in cotton cv. MCU5 under glass house condition. The following treatment were maintained with three replications and the pots arranged in completely randomized block design under glass house conditions.

T_1 - Seed treatment with *G. mosseae* @ 10g/kg seed.

T_2 - Soil application with *G. mosseae* @ 10g/kg soil.

T_3 - Seed treatment with *P. fluorescens* @ 10g/kg seed.

T_4 - Soil application with *P. fluorescens* @ 2.5kg/ha.

T_5 - Seed treatment with *T. viride* @ 4g/kg seed.

T_6 - Soil application with *T. viride* @ 2.5kg/ha.

T_7 - Carbofuran @ 1kg a.i./ha.

T_8 - Control.

The seeds of cotton cv. MCU5 were sown in 2kg earthern pots @ 5 seeds/pot and thinned to 2 seedlings per pot. VAM and other biocontrol agents were mixed in soil before sowing. At 15 days after germination of cotton,

R. reniformis infective juveniles @ 2 nematode / g of soil along with *Fusarium* wilt fungus @ 100 g of fungus culture/kg soil were inoculated by carefully removing the soil at the root zone of the plants.

The experiment was terminated 175 DAS. The plants were carefully depotted and observations on plant growth characters, nematode population in root and soil, wilt index, VAM colonization in percentage, number of spores, colony forming units of *P. fluorescens* and *T. viride* in roots, total phosphorus content in plants and cotton yield were made.

3.8. Biochemical changes due to interaction of reniform nematode, *Fusarium* wilt fungus and *G. mosseae* on cotton

Two pot culture experiments were conducted to investigate the effect of infection by the reniform nematode, *Fusarium* wilt fungus and VAM fungus *G. mosseae* separately and in combination on the biochemical changes of cotton cultivars *viz.*, MCU5 and K10. The experiment was conducted in CRBD with following treatments which were replicated thrice.

T_1 - Nematode alone

T_2 - VAM

T_3 - *Fusarium*

T_4 - Nematode + VAM

T_5 - Nematode + *Fusarium*

T_6 - *Fusarium* + VAM

T_7 - Nematode + VAM + *Fusarium*

T_8 - Control

Surface sterilized seeds (0.1% mercuric chloride for 2 min) of the cotton cultivars were sown in 2 kg pots containing sterilized pot mixture soil (Red soil : Sand : FYM - 2:2:1). VAM, *G. mosseae* was applied at.the time of sowing @ 10g/kg soil. Ten days after germination, seedlings were thinned to 2 plants/pot. The plants were inoculated with *Fusarium* fungus @ 100g sand maize medium per 1 kg soil and reniform nematode @ 2 females per g soil simultaneously when they were 15 day old. Inoculation of both nematode and wilt fungus were made by carefully removing the soil in the rhizosphere. Sixty days after nematode inoculation the plants were uprooted. Plants were analyzed for various biochemical contents like protein, phenol, total sugars, reducing sugars, peroxidase and chitinase enzyme action, root amino acid contents and macro and micro nutrient contents.

3.8.1. Estimation of protein

The amount of protein in the plant sample was estimated by Bradford's (1976) method. From the plant sample (shoot and root), 100 mg was taken in each 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 595nm. The calculation of protein present in the sample was calculated from the standard graph.

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

3.8.2. Estimation of phenol

The amount of phenol content in the plant sample, (shoot and root) was estimated as per the method of Malick and Singh (1980). Five ml of 80 per cent

ethanol was added to 100 mg samples and the extract was taken and centrifuged at 1000 rpm. 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, 2ml of 20 per cent Na_2CO_3 solution was added. The tubes were kept in boiling water for 1 min and the absorbance was read at 650 nm.

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

3.8.3. Estimation of total sugars

The amount of total sugars in the plant sample (shoot and root) was estimated by Anthrone method (Hedge and Horreiter, 1962). One hundred mg sample was taken and homogenized in pestle and mortar 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 up to 100ml 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, (shoot and root) 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 homogenized with pestle and mortar. The sugars were extracted with hot 80 per cent ethanol and centrifuged. Then the supernatant was allowed to evaporate and the volume was made upto 5 ml with distilled water. From this 0.5 ml was pipetted and the volume was made upto 2ml with distilled water. To this, 1ml of alkaline copper tartrate reagent was added and kept in the boiling water for 10 min, then the tubes were cooled and 1ml 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 620nm. From the standard graph, the amount of reducing sugars was calculated.

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

3.8.5. Estimation of total free amino acids

One gram of plant material (shoot and root) was taken and extraction was done in 10 ml of eight percent ethanol using a pestle and mortar. One ml of the ethanol plant extract was dissolved in 1ml of distilled water and to this, 4 ml of ninhydrin -citrate - glycerol was added and mixed well in the tube. The tubes were heated for 12 minutes in a boiling water bath. Later, the tubes were cooled to room temperature. The purple colour developed was measured at 750 nm against a reagent blank in a colorimeter. The reading was taken within one hour as the colour becomes unstable afterwards. Total free aminoacids in the sample was expressed as percentage equivalent of leucine.

3.8.6. Estimation of enzyme activity

3.8.6.1. Estimation of peroxidase enzyme

One g of the plant sample (shoot and root) was taken and three ml of 0.1 m phosphate buffer (pH7) was added. Then the sample was macerated with pestle

and mortar and centrifuged at 18,000 rpm at 5°C for fifteen min in cuvette. From that 0.1 ml of the solution was taken and 3 ml of 0.05 m pyrogallol solution and 0.5 ml of one per cent hydrogen peroxide solution was added to that. The cuvette was read in a spectrophotometer at 430 nm. The change in absorbance for every thirty seconds upto three min were recorded. The peroxidases activity was then calculated. (Reddy et al., 1985).

3.8.6.2. Estimation of chitinase enzyme

One g of plant sample (shoot and root) was collected and homogenized in three ml of 0.1 mm sodium citrate buffer (pH 5) with a pestle and mortar at 4°C. The homogenate was centrifuged for 15 min at 10,000 rpm. The supernatant was used as an enzyme source and 0.4 ml of this enzyme solution was taken into a 1.5 ml Eppendorp tube and to this 10ml sodium acetate buffer (pH 5) and 0.1 ml of colloidal chitin was added. This was incubated in water bath at 37°C for two hours and then centrifuged at 1000 rpm 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 snail gut enzyme (30mg/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 benzaldhyde (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 shoot and root was expressed as n-mole-n-acetyl-glucosamine released per min per g of fresh tissue (Boller and Mauch, 1988).

3.9. Histopathological study

Histopathological studies were done with cotton plants cv. MCU5 infected with reniform nematode and *G. mosseae*. The infected plants were collected from pot culture studies and the roots were washed gently and thoroughly in water and cut into small bits (0.5 to 1.0 cm length); then fixed and dehydrated through tertiary butyl alcohol series. After embedded in paraffin wax, it was sectioned at 10μ with the aid of the Spencer's rotary microtome. The sections were stained with safranin and counter stained with fast green and mounted in D.P.X mountant (Jensen, 1962).

Results

CHAPTER - IV

RESULTS

4.1. Effect of four species of VAM *viz.*, *G. mosseae, G. fasciculatum, G. intraradices* and *G . fulvum* on three cultivated cotton species to control *R. reniformis*

The experiment was conducted to study the interaction of four species of VAM, *G. mosseae, G. fasciculatum, G. intraradices* and *G. fulvum* with *R. reniformis* on three cultivated cotton species *viz.*, *G. hirsutum* cv. MCU 5, *G. arboreum* cv. K10 and *G. barbedance* cv. TCB 209. The results obtained are furnished below:

4.1.1. Growth parameters

4.1.1.1. Shoot length and root length (Table 1)

In *G. hirsutum* cv. MCU 5, the maximum shoot length and root length (85.3 cm and 28.3 cm respectively) was observed in *G. mosseae* inoculated plants with 59.1 and 157.2 per cent increase over nematode alone treatment. It was on par with *G. mosseae* + *R. reniformis* treatment which recorded 55.2 and 143.6 per cent increase over nematode alone treatment. The next best treatment *G. fasciculatum* recorded 81.6 cm and 25.6 cm respectively, which was on par with *G.mosseae* + *R. reniformis* treatment. *R. reniformis* alone recorded lowest values of shoot (53.6 cm) and root (11.0 cm) length and these were significantly different from all other treatments and control. Among different VAM species, *G. fulvum* recorded lowest shoot length (69.0 cm) and root length (18.6 cm) (Plates 8, 9, 10 and 11).

In *G. arboreum* cv.K10, application of *G. mosseae* produced maximum shoot length (114.7 cm) and root length (17.4 cm). The per cent increase over

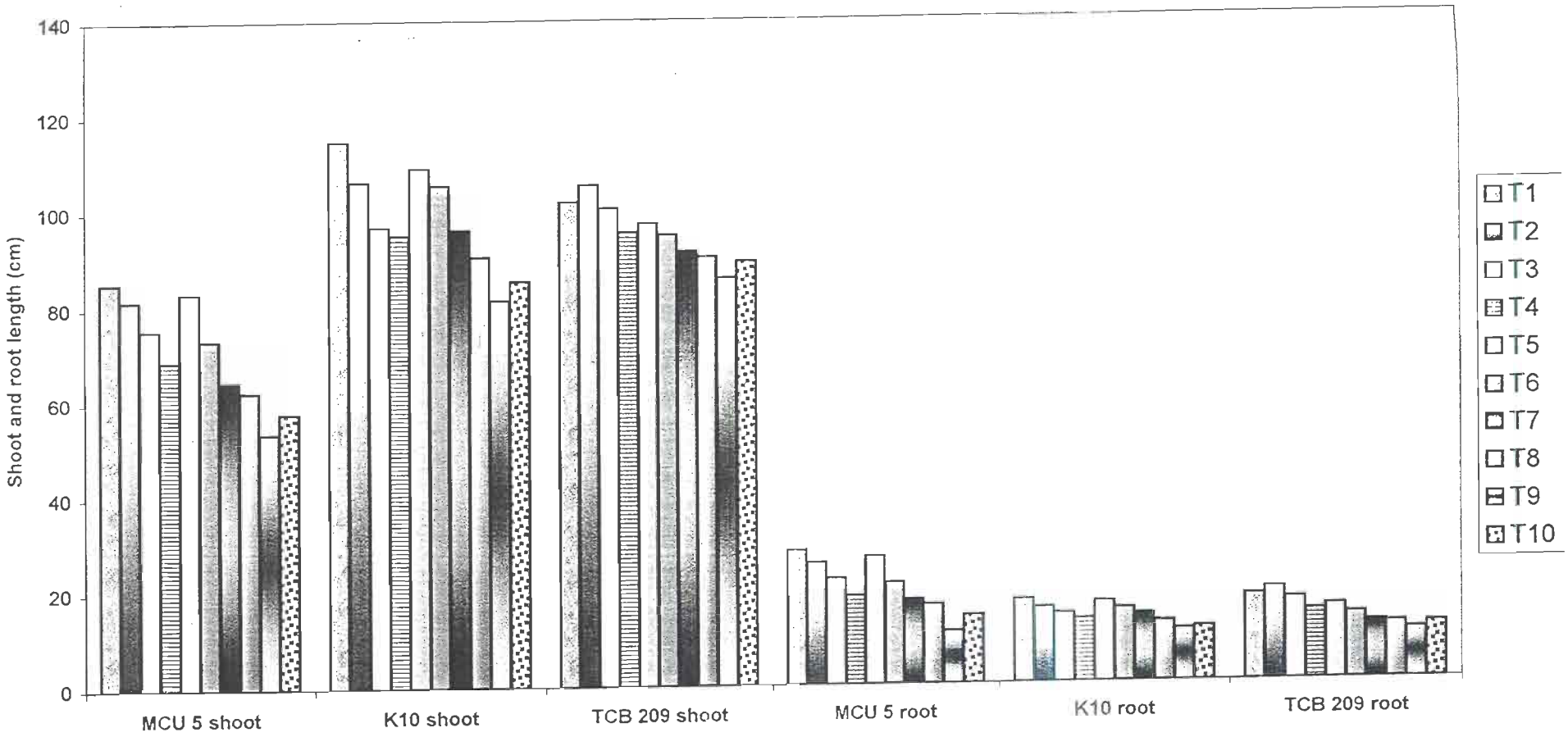
Table 1. **Effect of four species of VAM *G. mosseae, G. fasciculatum, G. intraradices* and *G. fulvum* and *R. reniformis* on shoot and root length of three cotton cultivars**

(mean of three replications)

Treatments	Cv. MCU 5		Cv. K10		Cv. TCB 209	
	Shoot length (cm)	Root length (cm)	Shoot length (cm)	Root length (cm)	Shoot length (cm)	Root length (cm)
T_1 - *G. mosseae*	85.3 (59.1)	28.3 (157.2)	114.7 (41.0)	17.4 (58.9)	101.8 (18.8)	18.0 (73.6)
T_2 - *G. fasciculatum*	81.6 (52.2)	25.6 (132.7)	106.2 (30.6)	15.7 (43.4)	105.3 (22.9)	19.4 (86.4)
T_3 - *G. intraradices*	75.3 (40.4)	22.3 (102.7)	96.8 (19.0)	14.4 (32.3)	100.4 (17.2)	17.2 (65.4)
T_4 - *G. fulvum*	69.0 (28.7)	18.6 (69)	95.1 (17.1)	13.2 (20.5)	95.4 (11.9)	14.7 (41.7)
T_5 - *G. mosseae* + *R. reniformis*	83.2 (55.2)	26.8 (143.6)	109.1 (34.1)	16.8 (53.4)	97.2 (13.4)	15.6 (50.0)
T_6 - *G. fasciculatum* + *R. reniformis*	73.3 (36.7)	21.3 (93.6)	105.5 (29.7)	15.4 (40.6)	94.8 (10.6)	13.8 (12.8)
T_7 - *G. intraradices* + *R. reniformis*	64.6 (20.5)	17.6 (60.0)	96.1 (18.3)	14.1 (28.7)	91.3 (6.5)	12.0 (15.4)
T_8 - *G. fulvum* + *R. reniformis*	62.3 (16.2)	16.6 (50.9)	90.5 (11.3)	12.5 (14.1)	90.1 (5.1)	11.8 (13.5)
T_9 - *R. reniformis*	53.6	11.0	81.3	10.9	85.7	10.4
T_{10} - Control	57.8	14.3	85.3	11.3	89.2	11.7
CD (P=0.05)	**3.5**	**2.2**	**7.8**	**0.82**	**2.35**	**1.82**

* Figures in the parentheses are per cent increase over nematode alone.

Fig.1. Effect of four species of VAM against *R. reniformis* on shoot and root length of three cotton cultivars



T1 - *G. mosseae* T2 - *G. fasciculatum* T3 - *G. intraradices* T4 - *G. fulvum* T5 - *G. mosseae* + *R. reniformis* T6 - *G. fasciculatum* + *R. reniformis*
T7 - *G. intraradices* + *R. reniformis* T8 - *G. fulvum* + *R. reniformis* T9 - *R. reniformis* T10 Control

Plate 8. Effect of *G. mosseae* against *R. reniformis* on growth of cotton cv. MCU 5

1. *G. mosseae* alone
2. *G. mosseae* + *R. reniformis*
3. *R. reniformis* alone
4. Control

Plate 8b

Plate 9. Effect of *G. fasciculatum* against *R. reniformis* on growth of cotton cv. MCU 5.

1. *G. fasciculatum* alone
2. *G. fasciculatum* + *R. reniformis*
3. *R. reniformis* alone
4. Control

Plate 9a

Plate 9b

Plate 10. Effect of *G. intraradices* against *R. reniformis* on growth of cotton cv. MCU 5.

1. *G. intraradices* alone
2. *G. intraradices* + *R. reniformis*
3. *R. reniformis* alone
4. Control

Plate 10a

Plate 10b

Plate 11. Effect of *G. fulvum* against *R. reniformis* on cotton cv. MCU 5

1. *G. fulvum* alone
2. *G. fulvum* + *R. reniformis*
3. *R. reniformis* alone
4. Control

Plate 11a

Plate 11b

nematode alone was 41.0 and 58.9 respectively. It was on par with *G. mosseae* + *R. reniformis* treatment which recorded 109.1 cm and 16.8 cm respectively. The next best treatment was *G. fasciculatum* with 106.2 cm and 15.7 cm shoot and root length which were on par with *G. fasciculatum* + *R. reniformis* treatment. In general all the VAM species increased shoot and root length of K10 cotton plants. However, VAM alone treated plants were on par with nematode treated plants. The least was recorded in nematode alone treatment with 81.3 cm and 10.9 cm which was not significantly different from control plants (Plates 12, 13, 14 and 15).

In *G. barbedance* cv. TCB 209 also application of all VAM species increased shoot and root length. *G. fasciculatum* alone recorded maximum shoot and root length with 105.3 cm and 19.4 cm respectively which were 22.9 and 86.4 per cent increase over nematode alone treatment. It was followed by *G. mosseae* application which registered 101.8 cm and 18.0 cm respectively. Among the VAM species with nematode treatments, *G. mosseae* + *R. reniformis* recorded maximum with 97.2 cm and 15.6 cm shoot and root length respectively (Plates 16 and 17).

In general application of all VAM species increased shoot and root length of cotton cultivars. Among them, *G. mosseae* performed well when applied alone and along with nematode. The next best VAM species was *G. fasciculatum* (Fig.1).

4.1.1.2. Fresh weight of shoot and root (Table 2)

In cotton cv. MCU 5, the fresh weight of shoot and root was highest in *G. mosseae* treatment with 55.3 g and 32.3 g which was on par with next best treatment *G. mosseae* + *R. reniformis* which recorded 52.8 g and 31.1 g of shoot and root weight respectively. *G. fasciculatum* registered 51.0 g and 30.39 g of shoot and root weight and it was on par with *G. mosseae* + *R. reniformis* treatment.

Plate 12. Effect of *G. mosseae* against *R. reniformis* on growth of cotton cv. K10

1. *G. mosseae* alone
2. *G. mosseae* + *R. reniformis*
3. *R. reniformis* alone
4. Control

Plate 12a

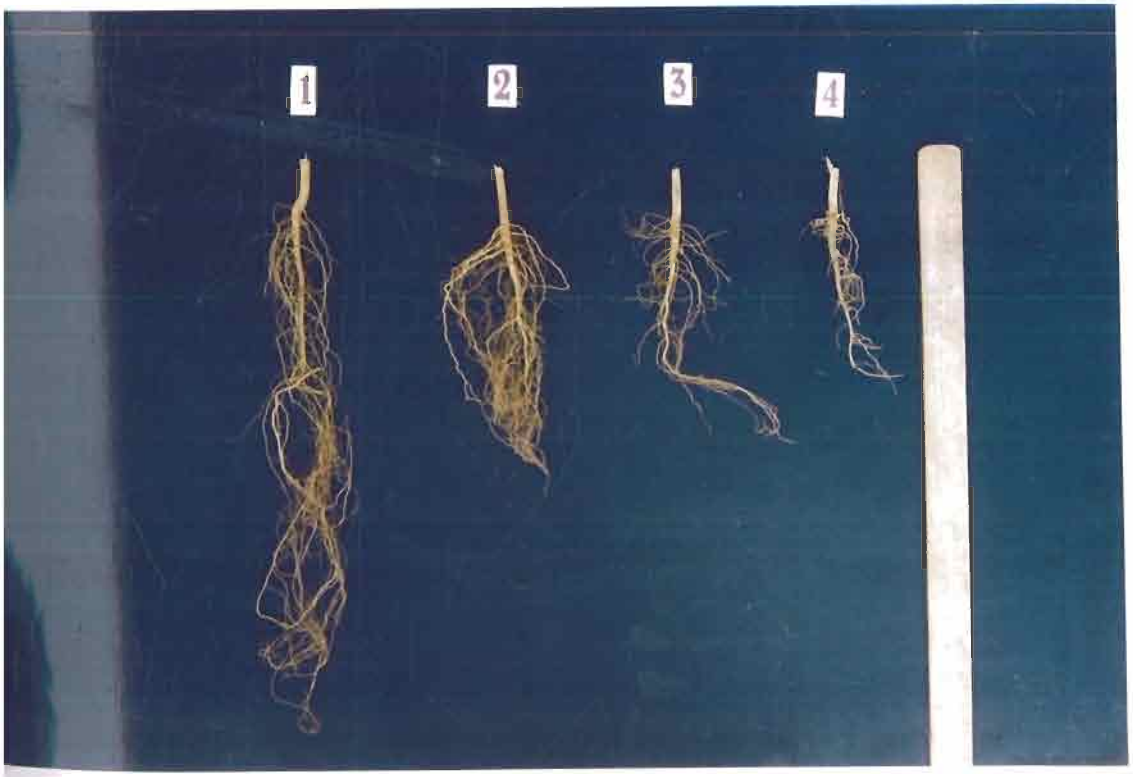


Plate 12b

Plate 13. Effect of *G. fasciculatum* against *R. reniformis* on growth of cotton cv. K10.

1. *G. fasciculatum* alone
2. *G. fasciculatum* + *R. reniformis*
3. *R. reniformis* alone
4. Control

Plate 13a

Plate 13b

late 14. Effect of *G. intraradices* against *R. reniformis* on growth of cotton cv. K10

1. *G. intraradices* alone
2. *G. intraradices* + *R. reniformis*
3. *R. reniformis* alone
4. Control

Plate 14a

Plate 14b

Plate 15. Effect of *G. fulvum* against *R. reniformis* on growth of cotton cv. K10.

1. *G. fulvum* alone
2. *G. fulvum* + *R. reniformis*
3. *R. reniformis* alone
4. Control

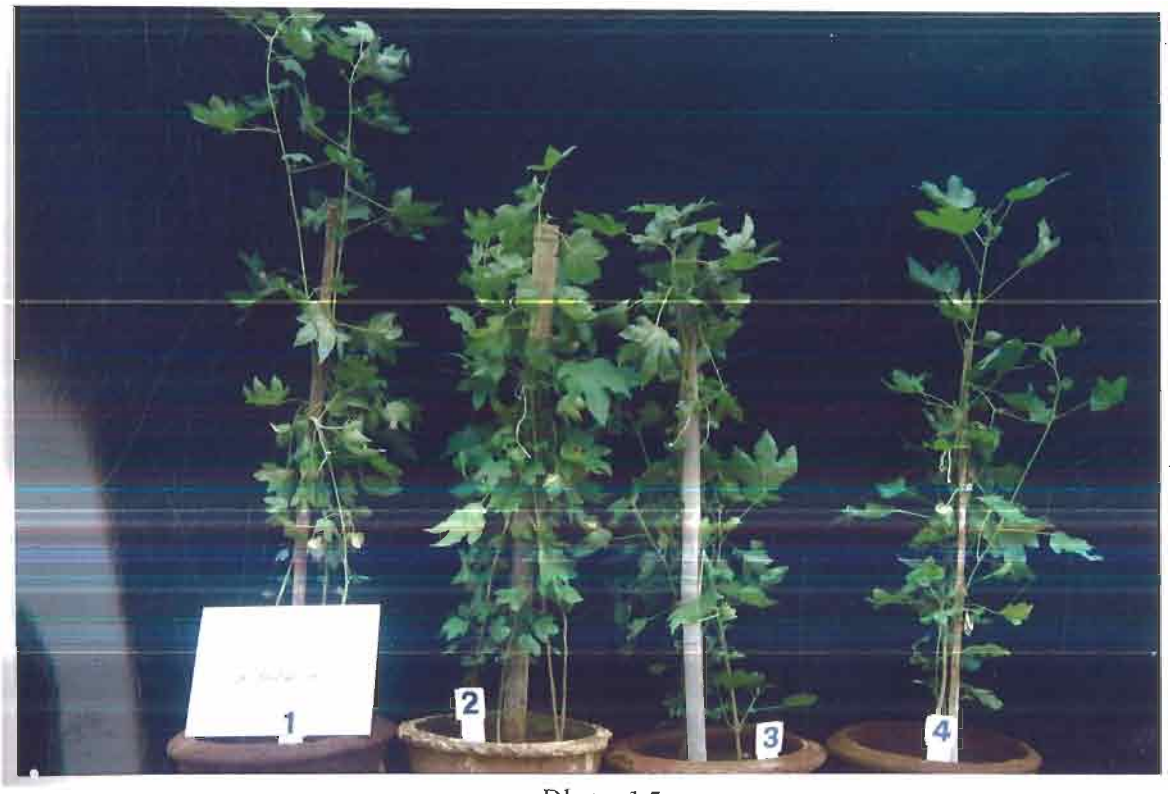


Plate 15a

Plate 15b

'late 16. Effect of four species of VAM against *R. reniformis* on cotton cv. TCB 209

Treatments:

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

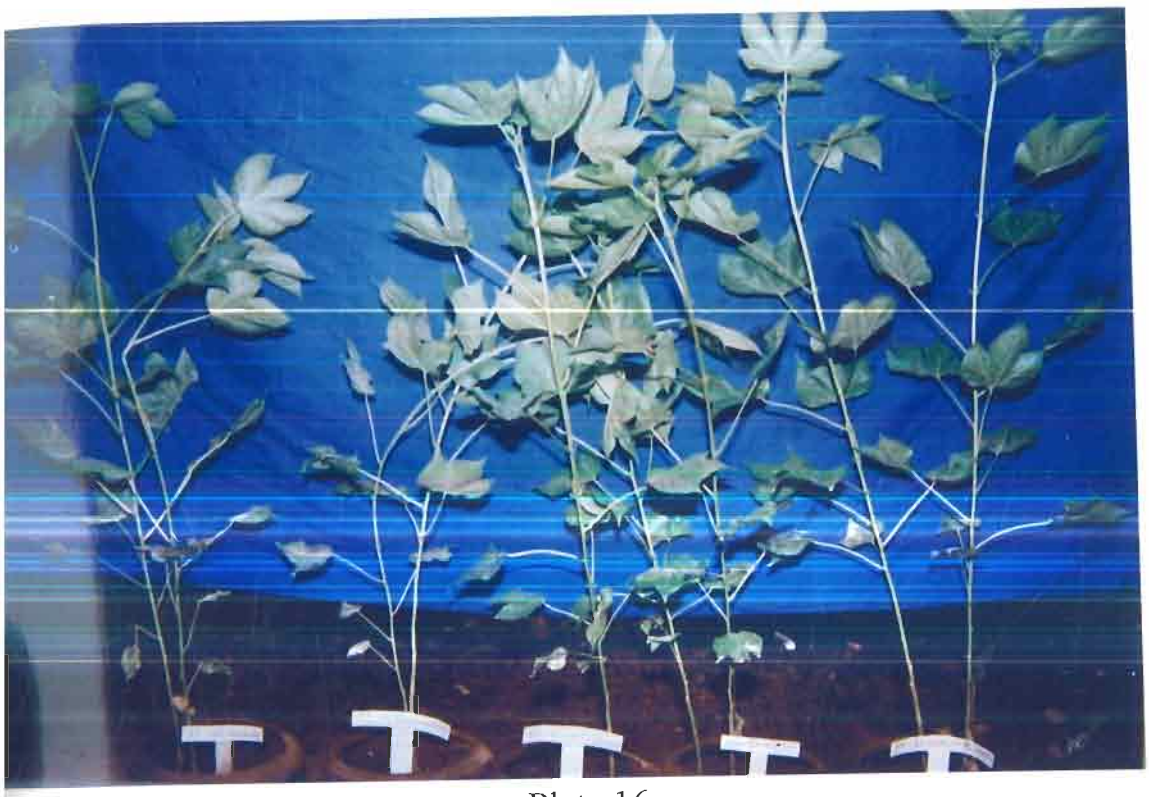


Plate 16a

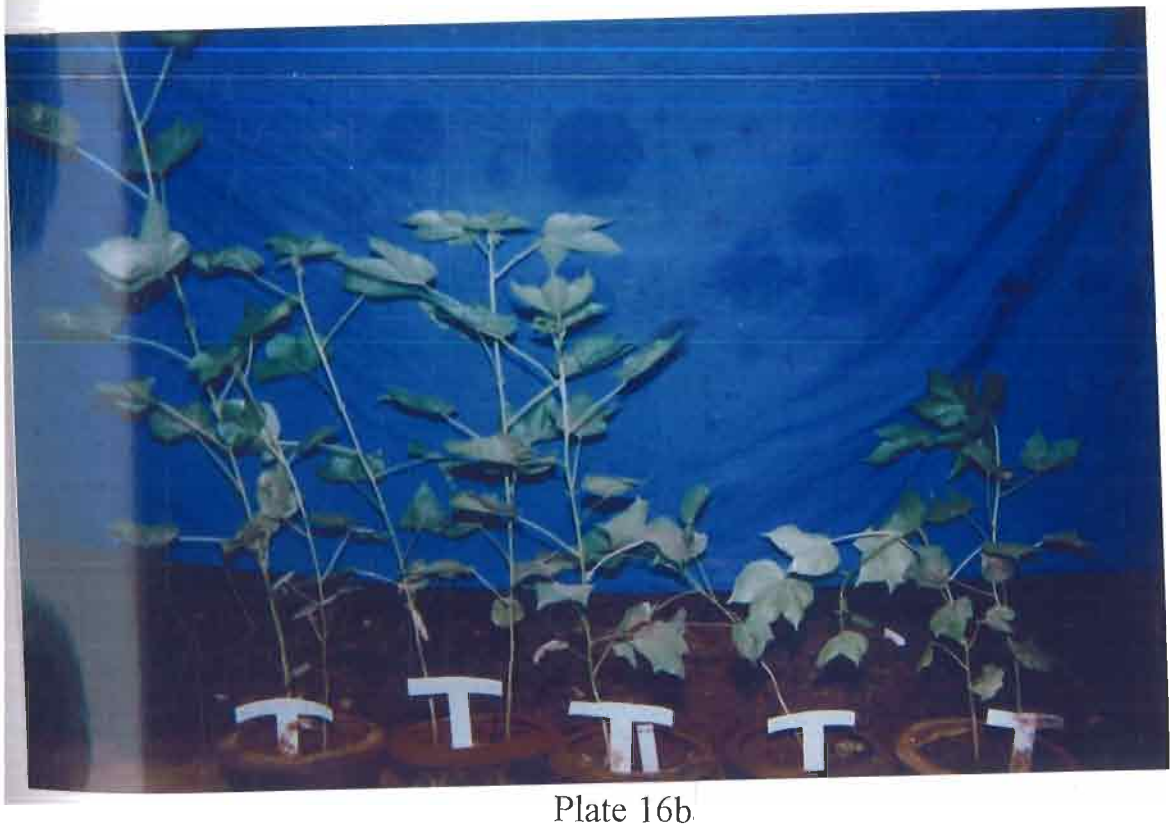


Plate 16b

e 17. Effect of four species of VAM against *R. reniformis* on root growth of cotton cv. TCB 209

Treatments:

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

Plate 17. Effect of four species of VAM against *R. reniformis* on root growth of cotton cv. TCB 209

Treatments:

T_1 - *G. mosseae*

T_2 - *G. fasciculatum*

T_3 - *G. intraradices*

T_4 - *G. fulvum*

T_5 - *G. mosseae* + *R. reniformis*

T_6 - *G. fasciculatum* + *R.reniformis*

T_7 - *G. intraradices* + *R.reniformis*

T_8 - *G. fulvum* + *R.reniformis*

T_9 - *R.reniformis*

T_{10} - Control

Plate 17

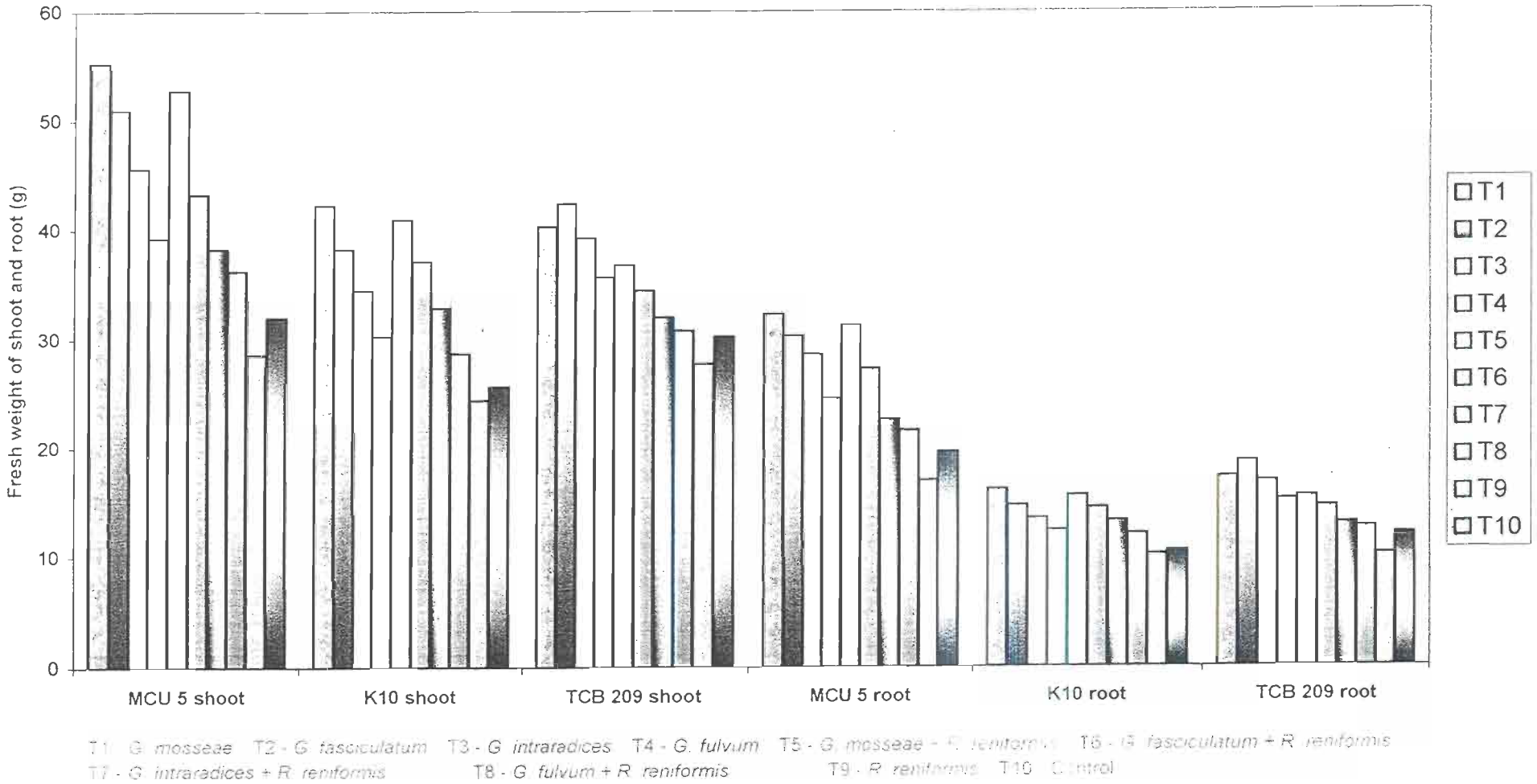
Table 2. **Effect of four species of VAM, *G. mosseae, G. fasciculatum, G. intraradices* and *G. fulvum* and *R. reniformis* on fresh weight of shoot and root in three cotton cultivars.**

(mean of three replications)

Treatments	Cv. MCU 5		Cv. K10		Cv. TCB 209	
	Shoot weight (g)	Root weight (g)	Shoot weight (g)	Root weight (g)	Shoot weight (g)	Root weight (g)
T_1 - *G. mosseae*	55.3 (93.3)	32.3 (90.0)	42.3 (73.3)	16.2 (58.8)	40.3 (45.5)	17.3 (69.3)
T_2 - *G. fasciculatum*	51.0 (78.2)	30.3 (78.2)	38.39 (57.3)	14.74 (44.5)	42.4 (53.1)	18.7 (82.9)
T_3 - *G. intraradices*	45.6 (59.4)	28.6 (68.2)	34.5 (41.3)	13.59 (33.2)	39.3 (41.9)	16.9 (65.4)
T_4 - *G. fulvum*	39.3 (37.4)	24.6 (44.7)	30.3 (24.1)	12.43 (21.8)	35.7 (28.9)	15.2 (48.7)
T_5 - *G. mosseae* + *R. reniformis*	52.8 (84.6)	31.1 (84.1)	41.0 (67.2)	15.6 (52.9)	36.8 (32.9)	15.5 (51.7)
T_6 - *G. fasciculatum* + *R. reniformis*	43.3 (51.3)	27.3 (58.8)	37.2 (52.4)	14.5 (42.1)	34.5 (24.5)	14.6 (42.9)
T_7 - *G. intraradices* + *R. reniformis*	38.3 (33.9)	22.6 (32.9)	32.9 (34.8)	13.3 (30.3)	32.0 (15.5)	13.0 (27.2)
T_8 - *G. fulvum* + *R. reniformis*	36.6 (27.9)	21.6 (27.0)	28.7 (17.6)	12.1 (18.7)	30.8 (11.2)	12.74 (24.7)
T_9 - *R. reniformis*	28.6	17.0	24.4	10.2	27.7	10.2
T_{10} - Control	32.0	19.6	25.6	10.5	30.2	12.1
CD (P=0.05)	**3.8**	**1.7**	**2.5**	**0.78**	**1.8**	**1.1**

* Figures in the parentheses are per cent increase over nematode alone.

Fig.2. Effect of four species of VAM against *R. reniformis* on fresh weight shoot and root in three cotton cultivars



All the VAM species improved shoot and root weight. *G. mosseae* alone and along with nematode was not significantly different from each other. But other VAM species when inoculated with nematodes were significantly different from VAM alone treatment. The least was observed in *R. reniformis* alone with 28.6 g and 17.0 g of shoot and root weights respectively.

In cotton cv. K10, application of VAM species increased shoot and root weight. VAM species combined with nematodes did not show any significant difference among VAM alone and with nematode treatments. The maximum shoot and root weight, 42.3 g and 16.2 g was recorded on *G. mosseae* treated plants and it was on par with next best treatment, *G. mosseae* + *R. reniformis* which registered 41.0 g and 15.6 g of shoot and root weight. The least was recorded in *R. reniformis* alone with 24.4 g and 10.2 g. It was significantly different from all other treatments, but not significantly different from control.

In cotton cv. TCB 209, *G. fasciculatum* recorded maximum of shoot and root weight of 42.4 g and 18.7 g respectively which was 53.1 and 82.9 per cent increase over nematode alone treatment. The next best treatment was *G. mosseae* which registered 40.3 g and 17.3 g of shoot and root weights respectively. Among VAM along with nematode, *G. mosseae* + *R. reniformis* registered maximum of 36.8 g and 15.5 g followed by *G. fasciculatum* + *R. reniformis* with 34.5 g and 14.6 g which were significantly different from each other. Nematode alone recorded minimum of 27.7 g and 10.2 g of shoot and root weights (Fig.2).

4.1.1.3. Dry weight of shoot and root (Table 3)

Among the different VAM species evaluated, *G. mosseae* registered the maximum dry weight of shoot and root with 12.98 g and 7.25 g which were 79.5

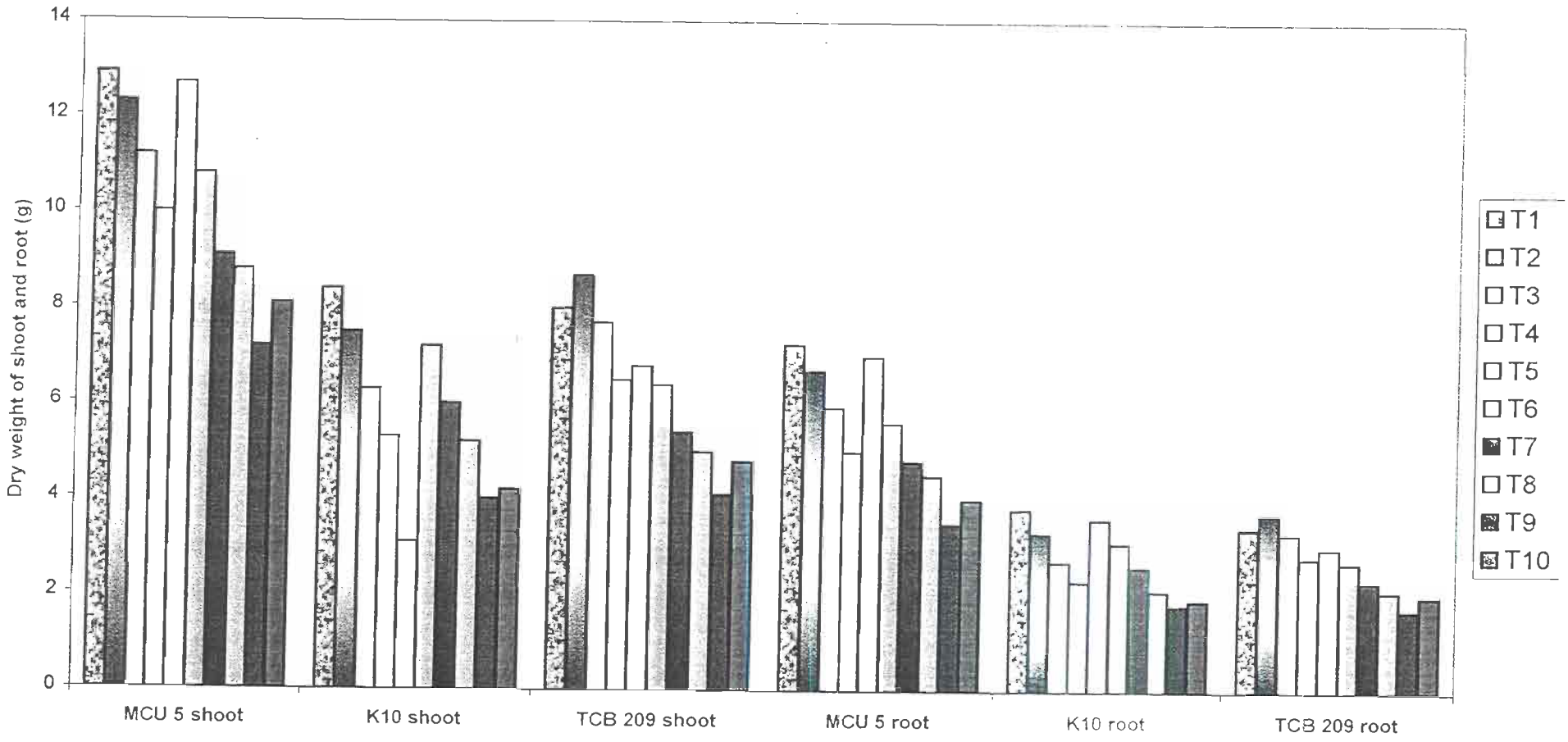
Table 3. **Effect of four species of VAM, *G. mosseae, G. fasciculatum, G. intraradices* and *G. fulvum* and *R. reniformis* on dry weight of shoot and root on three cotton cultivars.**

(mean of three replications)

Treatments	Cv. MCU 5		Cv. K10		Cv. TCB 209	
	Shoot dry weight (g)	Root dry weight (g)	Shoot dry weight (g)	Root dry weight (g)	Shoot dry weight (g)	Root dry weight (g)
T_1 - *G. mosseae*	12.98 (79.5)	7.25 (105.3)	8.4 (100.0)	3.8 (104.2)	8.0 (95.1)	3.4 (99.4)
T_2 - *G. fasciculatum*	12.36 (70.9)	6.69 (89.5)	7.5 (87.5)	3.35 (77.0)	8.7 (112.1)	3.7 (85.1)
T_3 - *G. intraradices*	11.25 (55.6)	5.92 (67.7)	6.3 (57.5)	2.77 (48.1)	7.7 (87.8)	3.3 (91.8)
T_4 - *G. fulvum*	10.07 (39.2)	5.03 (42.4)	5.3 (32.5)	2.3 (23.5)	6.5 (58.5)	2.8 (62.7)
T_5 - *G. mosseae* + *R. reniformis*	12.73 (76)	7.04 (99.4)	8.1 (102.5)	3.6 (96.2)	6.8 (65.8)	3.0 (74.4)
T_6 - *G. fasciculatum* + *R. reniformis*	10.86 (50.2)	5.66 (60.30)	7.2 (80.0)	3.1 (70.0)	6.4 (56.6)	2.7 (56.9)
T_7 - *G. intraradices* + *R. reniformis*	9.17 (26.8)	4.82 (36.5)	6.0 (50.0)	2.6 (40.1)	5.4 (33.1)	2.3 (27.5)
T_8 - *G. fulvum* + *R. reniformis*	8.85 (22.4)	4.56 (29.1)	5.2 (30.0)	2.1 (16.5)	5.0 (21.9)	2.1 (26.1)
T_9 - *R. reniformis*	7.23	3.33	4.0	1.8	4.1	1.7
T_{10} - Control	8.18	4.06	4.2	1.9	4.8	2.0
CD (P=0.05)	**0.57**	**0.49**	**0.51**	**0.24**	**0.52**	**0.21**

* Figures in the parentheses are per cent increase over nematode alone.

Fig.3. Effect of four species of VAM against *R. reniformis* on dry weight of shoot and root in three cotton cultivars



T1 - *G. mosseae* T2 - *G fasciculatum* T3 - *G intraradices* T4 - *G fulvum* T5 - *G mosseae + R*
T7 - *G intraradices + R reniformis* T8 - *G fulvum + R reniformis* T9 - *R reniformis*

and 105.3 per cent increase over nematode alone treatment in cotton cv. MCU 5. It was on per with *G. mosseae* + *R. reniformis* with 12.73 g and 7.04 g respectively. The next best treatment *G. fasciculatum* recorded 70.9 and 89.5 per cent increase over nematode alone, but it was significantly different from *G. fasciculatum* + *R. reniformis* treatment. The least was observed in *R. reniformis* alone with 7.23 g and 3.33 g of shoot and root dry weights.

In cotton cv. K10, there was increase in dry weight of shoot and root in *G. mosseae* with 110 and 104.2 per cent increase over nematode alone followed by *G. mosseae* + *R. reniformis* with 102.5 and 96.2 per cent increase over nematode alone. Nematode alone recorded 4.0 g and 1.8 g of shoot and root weight which were not significantly different from control but different from all other treatments.

In cotton cv. TCB 209, *G. fasciculatum* recorded maximum dry weight of shoot (8.7 g) and root (3.7 g) and it was followed by *G. mosseae* with 8.0 g and 3.4 g respectively. Among VAM with nematode treatments, *G. mosseae* recorded maximum with 6.8 g and 3.0 g which was 65.8 and 74.4 per cent increase of dry weight of shoot and root over nematode alone and followed by *G. fasciculatum* + *R. reniformis* with 6.4 g and 2.7 g of shoot and root weight respectively (Fig.3).

4.1.1.4. Leaf Area Index (LAI) (Table 4)

In cotton cv. MCU 5, among all the treatments *G. mosseae* was found to register more LAI of 2.53 which was 127.9 per cent increase over nematode alone treatment. All VAM species found to increase LAI significantly over control. Among VAM plus nematode treatments, *G. mosseae* performed well with 2.42 which was 118 per cent increase over nematode alone and on par with *G. mosseae*

Table 4. **Effect of four species of VAM, *G. mosseae, G. fasciculatum, G. intraradices* and *G. fulvum* and *R. reniformis* on Leaf Area Index of three cotton cultivars.**

(mean of three replications)

Treatments	Cv. MCU 5		Cv. K10		Cv. TCB 209	
	LAI	**Per cent increase over nematode alone**	**LAI**	**Per cent increase over nematode alone**	**LAI**	**Per cent increase over nematode alone**
T_1 - *G. mosseae*	2.53	127.9	2.29	60.1	2.65	45.6
T_2 - *G. fasciculatum*	2.33	109.9	2.06	44.0	2.82	54.9
T_3 - *G. intraradices*	2.01	81.0	1.85	29.3	2.59	42.3
T_4 - *G. fulvum*	1.71	54.0	1.66	16.0	2.39	31.3
T_5 - *G. mosseae* + *R. reniformis*	2.42	118.0	2.20	53.8	2.43	28.0
T_6 - *G. fasciculatum* + *R. reniformis*	1.91	72.0	2.03	55.9	2.43	28.0
T_7 - *G. intraradices* + *R. reniformis*	1.62	45.9	1.82	27.2	2.13	30.0
T_8 - *G. fulvum* + *R. reniformis*	1.54	38.7	1.61	12.5	2.02	10.9
T_9 - *R. reniformis* alone	1.11		1.43		1.82	
T_{10} - Control	1.31		1.49		1.96	
CD (P=0.05)	**0.18**		**0.13**		**0.12**	

alone. *G. fasciculatum* recorded 2.33 which was significantly different from *G. fasciculatum* + *R. reniformis* (1.91). *G. intraradices* + *R. reniformis* and *G. fulvum* + *R .reniformis* recorded low value of 1.62 of 1.54 which were on par with each other.

In K10 cotton cultivar, *G. mosseae* showed maximum value of LAI with 2.29 and it was on par with *G. mosseae* + *R. reniformis*. These two treatments were significantly different from the rest of the treatments. Nematode alone recorded least by 1.43 LAI which was not significantly different from control treatment.

In TCB 209, highest LAI was observed in *G. fasciculatum* with 2.82 and followed by *G. mosseae* (2.65). Among VAM plus nematode treatments, *G. mosseae* + *R. reniformis* and *G. fasciculatum* + *R. reniformis* recorded highest which were on par with each other.

In general *G. mosseae* performed well on all three cotton cultivars of dual inoculated plants. However, the per cent increase over nematode alone was varied for *G. mosseae* among three cotton cultivars. It was maximum with 118.0 per cent on MCU 5 followed by K10 with 53.8 per cent and 28.0 per cent on TCB 209.

4.1.2. Nematode population (Table 5)

Population of *R. reniformis* in roots as number of females and number of females with egg masses were counted. The number of females and number of females with egg masses were significantly lower in all species of VAM treated plants than nematode alone treatment (Fig.4).

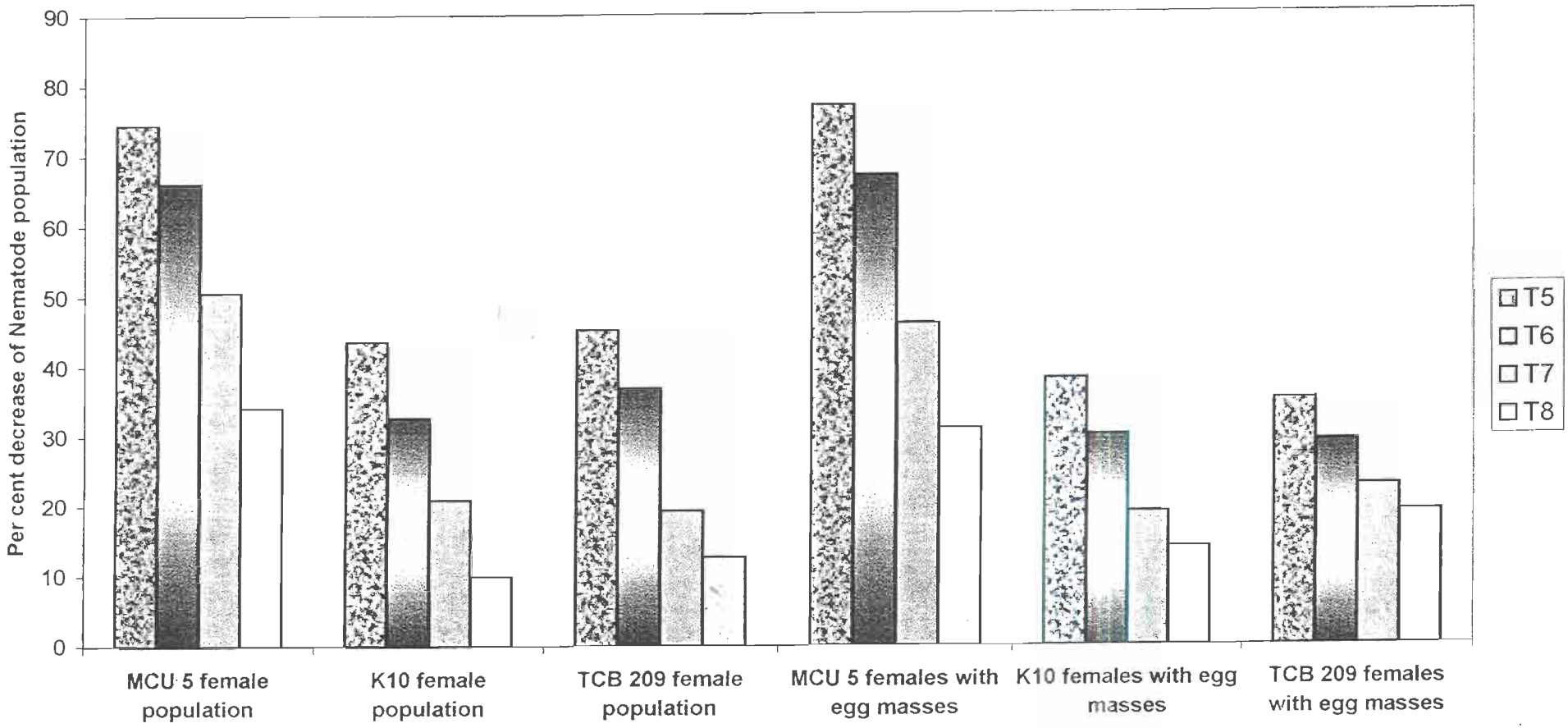
Table 5. **Effect of four species of VAM, *G. mosseae, G. fasciculatum, G. intraradices* and *G. fulvum* on *R. reniformis* (no. of females and no. of females with egg masses) in roots of three cotton cultivars.**

(mean of three replications)

Treatments	Cv. MCU 5		Cv. K10		Cv. TCB 209	
	No.of females per plant	No. of females with egg mass per plant	No. of females per plant	No. of females with egg mass per plant	No. of females per plant	No. of females with egg mass per plant
T_1 - *G. mosseae*						
T_2 - *G. fasciculatum*						
T_3 - *G. intraradices*						
T_4 - *G. fulvum*						
T_5 - *G. mosseae* + *R. reniformis*	63.6 (74.51)	27.4 (77.20)	35.2 (43.68)	25.6 (38.47)	124.6 (45.21)	84.7 (35.64)
T_6 - *G. fasciculatum* + *R. reniformis*	84.4 (66.19)	39.6 (67.05)	42.1 (32.64)	28.9 (30.02)	142.8 (36.89)	93.4 (29.03)
T_7 - *G. intraradices* + *R. reniformis*	124.6 (50.06)	64.8 (46.09)	49.4 (20.96)	33.4 (19.13)	182.5 (19.35)	101.6 (22.79)
T_8 - *G. fulvum* + *R. reniformis*	164.3 (34.15)	82.4 (31.45)	56.3 (9.92)	35.4 (14.29)	197.5 (12.73)	106.3 (19.23)
T_9 - *R. reniformis* alone	249.5	120.2	62.5	41.3	226.3	131.6
T_{10} - Control						
CD (P=0.05)	**15.2**	**11.5**	**5.2**	**4.7**	**15.3**	**7.3**

* Figures in the parentheses are per cent decrease over nematode alone.

Fig.4. Effect of four species of VAM against No. of females and No. of females with eggmasses in roots of three cotton cultivars



T5 - *G. mosseae* + *R. reniformis* T6 - *G. fasciculatum* + *R. reniformis* T7 - *G. intraradices* + *R. reniformis* T8 - *G. fulvum* + *R. reniformis*

In MCU 5, the number of females and number of females with egg masses were more in *R. reniformis* with 249.5 and 120.2 respectively and the lowest was observed in *G. mosseae* + *R. reniformis* with 63.6 and 27.4. The next best treatment in reducing number of females and number of females with egg masses was *G. fasciculatum* + *R. reniformis* which recorded 84.4 and 39.6 nematodes respectively.

In K10 cotton cultivar, the number of females and number of females with egg masses were reduced due to VAM application. It was 62.5 and 41.3 respectively on nematode alone treatment. The *G. mosseae* treatment recorded 35.2 and 25.6 respectively. In general when compared to MCU 5 cultivar the number of females and number of females with egg masses were very less even in nematode alone treatment.

In TCB 209, the maximum reduction in number of females of number of females with egg masses were recorded in *G. mosseae* + *R. reniformis* treatment with 124.6 and 84.7 respectively. The next best treatment in reducing number of females and number of females with egg masses was *G. fasciculatum* + *R. reniformis* which recorded 142.8 and 93.4. The highest was observed in *R. reniformis* alone with 226.3 and 131.6 respectively.

In cotton cv. MCU 5, the number of eggs/egg mass was highest in *R. reniformis* alone with 52.7 eggs/egg mass (Table 6). The least was observed in *G. mosseae* + *R. reniformis* treatment with 32.2 eggs/egg mass. The next best VAM species in reducing egg population was *G. fasciculatum* which registered 34.7 eggs/egg mass. The same trend was maintained in cotton cultivars K10 and

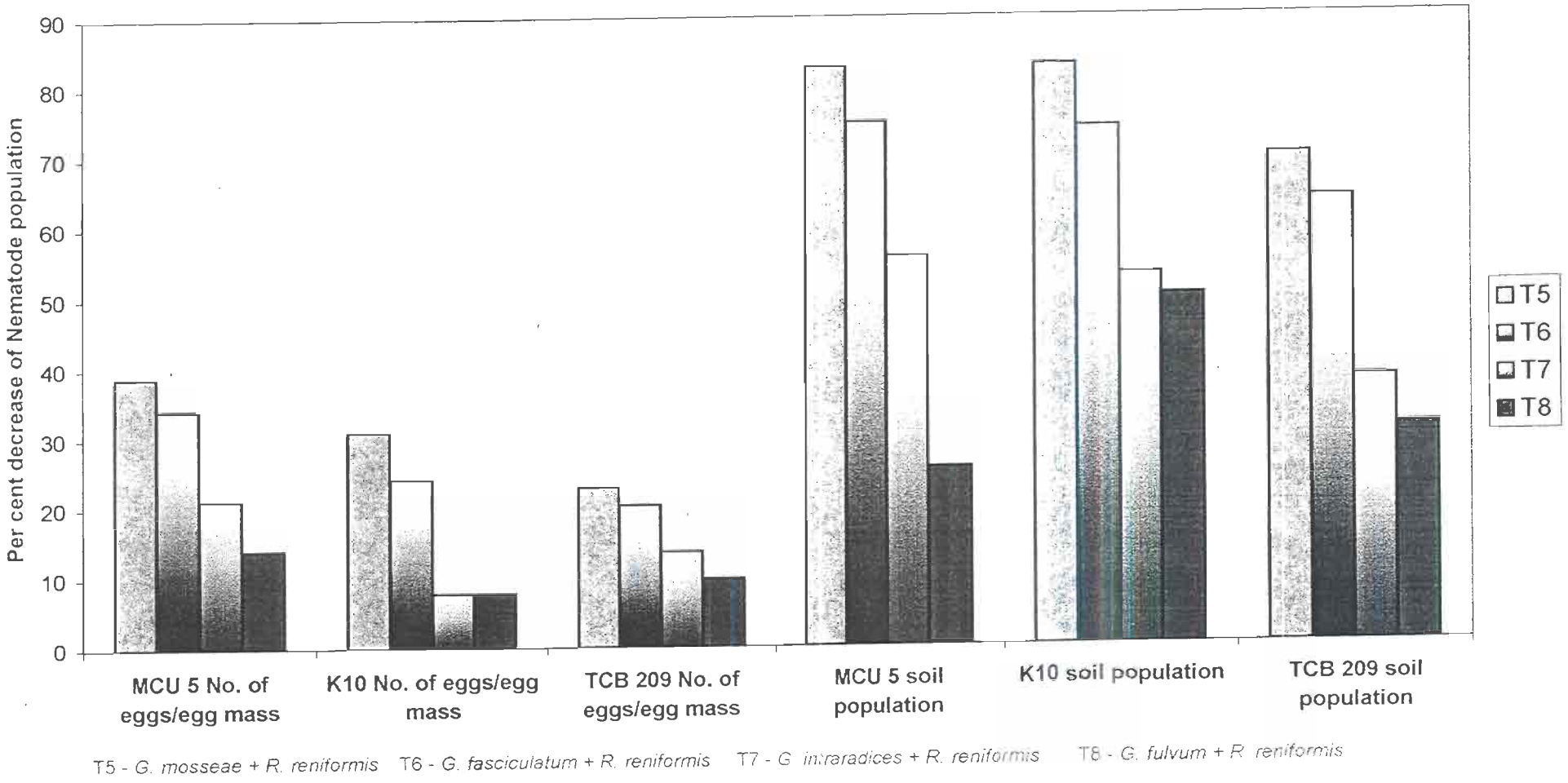
Table 6. **Effect of four species of VAM, *G. mosseae, G. fasciculatum, G. intraradices* and *G. fulvum* on *R. reniformis* (no. of eggs/egg masses and soil population) in three cotton cultivars**

(mean of three replications)

Treatments	Cv. MCU 5		Cv. K10		Cv. TCB 209	
	No .of eggs per egg mass	Soil nematode population/ 100g soil	No. of eggs per egg mass	Soil nematode population/ 100 g soil	No. of eggs per egg mass	Soil nematode population/ 100 g soil
T_1 - *G. mosseae*						
T_2 - *G. fasciculatum*						
T_3 - *G. intraradices*						
T_4 - *G. fulvum*						
T_5 - *G. mosseae* + *R. reniformis*	32.2 (38.89)	124.3 (82.85)	18.5 (30.97)	82.4 (83.02)	41.3 (22.95)	135.6 (69.99)
T_6 - *G. fasciculatum* + *R. reniformis*	34.7 (34.15)	182.6 (74.80)	20.3 (24.25)	124.8 (74.28)	42.7 (20.34)	284.7 (63.75)
T_7 - *G. intraradices* + *R. reniformis*	41.5 (21.25)	320.4 (55.79)	24.7 (7.84)	226.3 (53.37)	46.3 (13.62)	487.4 (37.93)
T_8 - *G. fulvum* + *R. reniformis*	45.3 (14.00)	540.5 (25.42)	24.7 (7.84)	242.4 (49.95)	48.4 (9.70)	536.3 (31.71)
T_9 - *R. reniformis* alone	52.7	724.7	26.8	485.3	53.6	785.3
T_{10} - Control						
CD (P=0.05)	**6.2**	**51.4**	**3.5**	**34.5**	**4.5**	**58.4**

* Figures in the parentheses are per cent decrease over nematode alone.

Fig.5. Effect of four species of VAM against No. of eggs per eggmass and soil nematode population in three cotton cultivars



TCB 209. However, number of eggs/egg mass were very low in K10 cultivars even in nematode alone treatments when compared to other cotton cultivars (Fig.5).

When the soil nematode was considered in all three cotton cultivars nematode alone recorded highest soil nematode population with 724.7, 485.3 and 785.3 in MCU 5, K10 and TCB 209 cotton cultivars respectively. In all the cotton cultivars *G. mosseae* registered lowest nematode population with 124.3, 82.4, and 135.6 respectively followed by *G. fasciculatum* with 182.6, 124.8 and 284.7 in MCU 5, K10 and TCB 209 cultivars respectively.

4.1.3. VAM colonization and spore count (Table 7)

In cotton cv. MCU 5 the VAM colonization was maximum in *G. mosseae* with 72.3 per cent (Table 7). It was followed by *G. fasciculatum* which registered 65.3 per cent and it was on par with *G. mosseae* + *R. reniformis* treatment which recorded 62.4 per cent colonization. *G. fulvum* along with *R. reniformis* recorded least of 31.7 per cent colonization. In K10 also *G. mosseae* observed to have maximum colonization percentage of 57.3 which was comparatively less than MCU 5. In TCB 209, *G. fasciculatum* recorded maximum of 46.1 followed by *G. mosseae* with 44.5 per cent. In K10 and TCB 209 *G. mosseae* + *R. reniformis* recorded 55.2 and 35.8 per cent colonization which were maximum among treatments when VAM species plus nematodes applied (Fig.6).

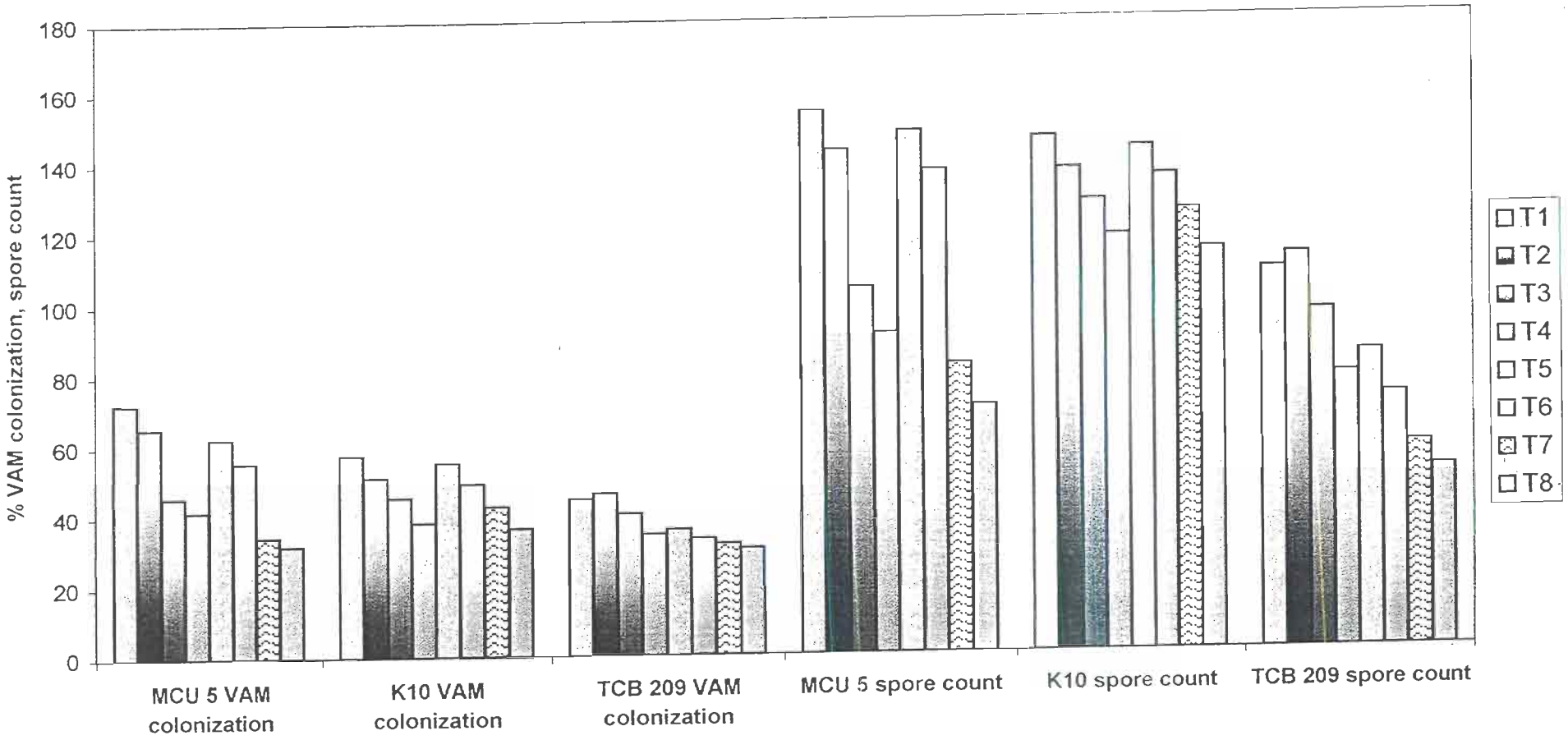
The spore count was more in *G. mosseae* with 154.3 and 146.2 in cotton cv. MCU 5 and K10 respectively (Table 7). In TCB 209 the maximum spore count was observed in *G. fasciculatum* with 112.4 and it was on par with *G. mosseae*

Table 7. **Effect of four species of VAM, *G. mosseae*, *G. fasciculatum*, *G. intraradices* and *G. fulvum* and *R. reniformis* on per cent colonization and spore count of three cotton cultivars.**

(mean of three replications)

Treatments	MCU 5		K10		TCB 209	
	VAM colonization	Spore count/ 50 g soil	VAM colonization	Spore count/ 50 g soil	VAM colonization	Spore count/ 50 g soil
T_1 - *G. mosseae*	72.3	154.3	57.3	146.2	44.5	108.2
T_2 - *G. fasciculatum*	65.3	143.7	51.1	137.4	46.1	112.4
T_3 - *G. intraradices*	45.7	104.5	45.3	128.3	40.3	96.8
T_4 - *G. fulvum*	41.7	91.6	38.1	118.7	34.4	78.4
T_5 - *G. mosseae* + *R. reniformis*	62.4	148.3	55.2	143.5	35.8	84.5
T_6 - *G. fasciculatum* + *R. reniformis*	55.4	137.4	49.2	135.1	33.1	72.6
T_7 - *G. intraradices* + *R. reniformis*	34.2	82.5	42.7	125.5	31.6	58.3
T_8 - *G. fulvum* + *R. reniformis*	31.7	70.4	36.4	114.2	30.2	51.8
T_9 - *R. reniformis* alone	-	-	-	-	-	-
T_{10} - Control	-	-	-	-	-	-
CD (P=0.05)	**5.6**	**7.4**	**3.7**	**5.2**	**3.6**	**10.2**

Fig.6. Effect of four species of VAM against *R. reniformis* on VAM colonization and spore count in three cotton cultivars



T1 - *G. mosseae* T2 - *G. fasciculatum* T3 - *G. intraradices* T4 - *G. fulvum* T5 - *G. mosseae* + *R. reniformis* T6 - *G. fasciculatum* + *R. reniformis*
T7 - *G. intraradices* + *R. reniformis* T8 - *G. fulvum* + *R. reniformis*

with 108.2. In MCU 5 the second best treatment was *G. mosseae* + *R. reniformis* with 148.3 which was on par with *G. mosseae* alone. The same trend was maintained in K10. The least spore count was observed in *G. fulvum* and *R. reniformis* with 70.4, 114.2 and 51.8 in MCU 5, K10 and TCB 209 cultivars respectively (Fig.6).

4.1.4. Yield parameters

4.1.4.1. Number of bolls per plant (Table 8)

In MCU 5, *G. mosseae* and *G. mosseae* + *R. reniformis* had shown maximum number of bolls per plant and they were on par with each other and significantly different from the rest. The per cent increase over nematode alone was 37.9, 35.6 respectively. The next best was *G. fasciculatum* which showed 31 per cent increase over nematode alone.

In K10, all VAM species recorded increase in the number of bolls per plant. Among VAM alone treatments, *G. mosseae* observed to have maximum of 17.6 followed by *G. mosseae* + *R. reniformis* and *G. fasciculatum* with 17.0 and 16.3 respectively. In K10 cultivar VAM species along with nematode treatments were on par with respective VAM alone treatments. The nematode alone recorded least (12.6) and it was not significantly different from control but significantly different from rest of the treatments.

In TCB 209, *G. fasciculatum* observed to have maximum number of bolls per plant with 14.6 and followed by *G. mosseae* which recorded 14.0 bolls per plant. Among VAM with nematode treatments *G. mosseae* + *R. reniformis* showed

Table 8. **Effect of four species of VAM, *G. mosseae, G. fasciculatum, G. intraradices* and *G. fulvum* with *R. reniformis* on number of bolls in three cotton cultivars.**

(mean of three replications)

Treatments	MCU 5		K10		TCB 209	
	No. of bolls per plant	Per cent increase over nematode alone	No. of bolls per plant	Per cent increase over nematode alone	No. of bolls per plant	Per cent increase over nematode alone
T_1 - *G. mosseae*	17.8	37.9	17.6	39.6	14.0	27.3
T_2 - *G. fasciculatum*	16.9	31.0	16.3	29.3	14.6	32.7
T_3 - *G. intraradices*	16.2	25.5	15.3	21.4	13.6	23.6
T_4 - *G. fulvum*	14.7	13.9	14.0	11.1	12.6	14.5
T_5 - *G. mosseae* + *R. reniformis*	17.5	35.6	17.0	34.9	13.0	18.2
T_6 - *G. fasciculatum* + *R. reniformis*	15.6	20.9	16.0	26.9	12.3	11.8
T_7 - *G. intraradices* + *R. reniformis*	14.1	11.6	15.0	19.0	12.3	11.8
T_8 - *G. fulvum* + *R. reniformis*	13.7	6.2	13.6	17.9	12.0	9.1
T_9 - *R. reniformis* alone	12.9	-	12.6	-	11.0	-
T_{10} - Control	13.5	-	13.0	-	11.6	-
CD (P=0.05)	**0.53**	-	**0.51**	-	**0.53**	-

higher value of 13.0 bolls per plant followed by *G. fasciculatum* + *R. reniformis* treatment with 12.3 bolls per plant.

4.1.4.2. Boll weight (Table 9)

In cotton cultivar MCU 5, maximum boll weight was recorded in *G. mosseae* (4.21 g) followed by *G. mosseae* + *R. reniformis* (4.10g) and *G. fasciculatum* (4.08 g) which were on par with each other and exhibited significant difference over the remaining treatments. *G. intraradices* was significantly different from all other treatments, where as *G. fulvum, G. intraradices* + *R. reniformis, G. fulvum* + *R. reniformis* and control were on par. The least was recorded in *R. reniformis* alone with 2.81 g which was significantly different from all other treatments.

In K10, boll weight was maximum in *G. mosseae* (3.86 g) followed by *G. fasciculatum* (3.41 g) which were significantly different from each other. Among VAM plus nematode treatments, *G. mosseae* was maximum with 3.75 g followed by *G. fasciculatum* (3.25 g). Nematode alone was least with 2.22 g which was not significantly different from control.

In TCB 209, maximum boll weight observed in *G. fasciculatum* with 4.12 g and followed by *G. mosseae* (3.86 g). Among VAM along with nematode, *G. mosseae* + *R. reniformis* recorded maximum with 3.56 g and which was on par with *G. fasciculatum* + *R. reniformis* (3.50 g). The nematode alone recorded least with 2.86 which was significantly different from all other treatments (Fig.7).

Table 9. **Effect of four species of VAM, *G. mosseae, G. fasciculatum, G. intraradices* and *G. fulvum* with *R. reniformis* on boll weight of three cotton cultivars.**

(mean of three replications)

Treatments	MCU 5		K10		TCB 209	
	Boll weight (g/boll)	Per cent increase over nematode alone	Boll weight (g/boll)	Per cent increase over nematode alone	Boll weight (g/boll)	Per cent increase over nematode alone
T_1 - *G. mosseae*	4.21	49.8	3.86	36.8	3.86	34.5
T_2 - *G. fasciculatum*	4.08	45.1	3.41	20.9	4.12	44.1
T_3 - *G. intraradices*	3.62	28.8	3.35	18.7	3.82	33.6
T_4 - *G. fulvum*	3.42	21.7	3.24	14.8	3.52	23.1
T_5 - *G. mosseae* + *R. reniformis*	4.10	45.9	3.75	32.9	3.56	24.5
T_6 - *G. fasciculatum* + *R. reniformis*	3.69	31.3	3.25	15.2	3.50	22.4
T_7 - *G. intraradices* + *R. reniformis*	3.37	19.9	3.23	14.5	3.20	11.9
T_8 - *G. fulvum* + *R. reniformis*	3.25	15.6	3.20	13.4	3.16	10.4
T_9 - *R. reniformis* alone	2.81	-	2.82	-	2.86	-
T_{10} - Control	3.22	-	2.94	-	3.12	-
CD (P=0.05)	**0.26**	-	**0.18**	-	**0.23**	-

4.1.4.3. Cotton yield (Table 10)

In MCU 5, the cotton yield was maximum in *G. mosseae* with 75.3 g/plant followed by *G. mosseae* + *R. reniformis* (70.8 g/plant) and *G. fasciculatum* (69.3 g/plant) which were on par with each other and significantly different from all other treatments. The least was observed in *R. reniformis* alone with 36.3 g/plant.

In K10, among the VAM species *G. mosseae* and *G. fasciculatum* were able to produce more cotton yield with 67.9 g and 55.5 g respectively and were significantly different from each other. When combined with *R. reniformis*, *G. mosseae* and *G. fasciculatum* recorded 63.7 g and 52.0 g which were not significantly different with respective of VAM alone treatments. The nematode alone recorded 38.2 g which was not significantly different from control but significantly different from rest of the treatments.

In TCB 209, *G. fasciculatum* produced more cotton yield (60.2 g) and it was followed by *G. mosseae* application (54.0 g). Among VAM plus nematode treatments, *G. mosseae* and *G. fasciculatum* recorded maximum of 46.2 g and 43.0 g which were on par with each other and were significantly different from the rest. The least was recorded in *R. reniformis* alone with 31.4 g which was significantly different from all other treatments (Fig.7).

4.1.5. Yield attributes (Table 11)

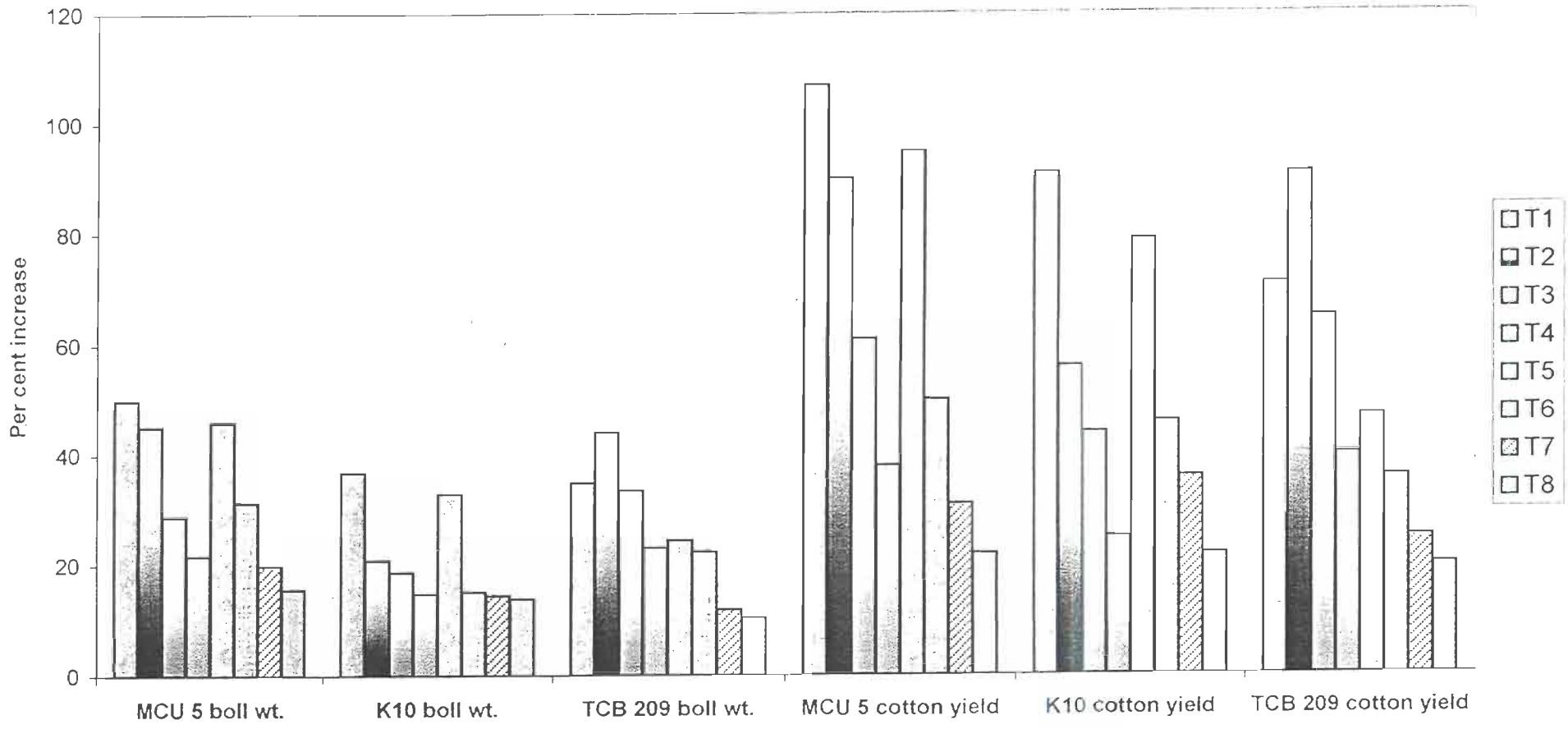
With respect to ginning percentage, in all three cotton cultivars the treatments exhibited no significant difference with each other. However, *G. mosseae* had shown a high values of ginning percentage with 36.20 and 32.61 in MCU 5 and K10 cultivars respectively. In TCB 209 *G. fasciculatum* observed to

Table 10. **Effect of four species of VAM, *G. mosseae, G. fasciculatum, G. intraradices* and *G. fulvum* and *R. reniformis* on cotton yield of three cotton cultivars.**

(mean of three replications)

Treatments	MCU 5		K10		TCB 209	
	Cotton yield/plant (g)	Per cent increase over nematode alone	Cotton yield/plant (g)	Per cent increase over nematode alone	Cotton yield/plant (g)	Per cent increase over nematode alone
T_1 - *G. mosseae*	75.3	107.4	67.9	91.2	54.0	71.7
T_2 - *G. fasciculatum*	69.3	90.0	55.5	56.3	60.1	91.1
T_3 - *G. intraradices*	58.6	61.4	51.2	44.2	51.9	65.0
T_4 - *G. fulvum*	50.4	38.8	45.3	23.3	44.3	40.8
T_5 - *G. mosseae* + *R. reniformis*	70.8	95.0	63.7	79.4	46.2	47.1
T_6 - *G. fasciculatum* + *R. reniformis*	54.6	50.5	52.0	46.4	43.0	36.8
T_7 - *G. intraradices* + *R. reniformis*	47.6	31.1	48.4	36.3	39.3	25.1
T_8 - *G. fulvum* + *R. reniformis*	44.6	22.8	43.5	22.5	37.9	20.5
T_9 - *R. reniformis* alone	36.3	-	35.5	-	31.4	-
T_{10} - Control	43.6	-	38.2	-	36.1	-
CD (P=0.05)	**7.5**	-	**5.6**	-	**4.5**	-

Fig.7. Effect of four species of VAM against *R. reniformis* on yield of three cotton cultivars



T1 - *G mosseae* T2 - *G fasciculatum* T3 - *G intraradices* T4 - *G* [illegible] *mosseae*
T7 - *G intraradices + R reniformis* T8 - *G fulvum + R reniformis*

Table 11. **Effect of four species of VAM, *G. mosseae, G. fasciculatum, G. intraradices* and *G. fulvum* and *R. reniformis* on yield parameters of three cotton cultivars.**

(mean of three replications)

Treatments	MCU 5			K10			TCB 209		
	Ginning percentage	Seed index (g)	Lint index (g)	Ginning percentage	Seed index (g)	Lint index (g)	Ginning percentage	Seed index (g)	Lint index (g)
T_1 - *G. mosseae*	36.20	11.27	5.84	32.61	9.91	4.92	35.12	11.91	6.80
T_2 - *G. fasciculatum*	35.90	11.10	5.79	32.24	9.82	4.89	35.25	11.92	6.81
T_3 - *G. intraradices*	35.40	10.89	5.69	31.90	9.71	4.87	34.92	11.83	6.74
T_4 - *G. fulvum*	35.38	10.62	5.62	31.20	9.69	4.61	34.69	11.64	6.72
T_5 - *G. mosseae* + *R. reniformis*	36.09	11.18	5.82	32.10	9.80	4.91	33.82	11.62	6.42
T_6 - *G. fasciculatum* + *R. reniformis*	35.21	10.77	5.68	31.80	9.72	4.87	33.62	11.41	6.32
T_7 - *G. intraradices* + *R. reniformis*	34.85	10.61	5.58	31.40	9.68	4.82	33.61	11.23	6.31
T_8 - *G. fulvum* + *R. reniformis*	34.72	10.60	5.57	30.00	9.62	4.21	33.48	11.04	6.21
T_9 - *R. reniformis* alone	34.40	9.95	5.53	30.30	9.60	4.18	33.21	10.92	6.18
T_{10} - Control	34.71	10.58	5.58	30.10	9.62	4.21	34.61	11.24	6.32
CD (P=0.05)	**0.283**	**0.24**	**0.08**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**

have maximum value of ginning percentage with 35.25. *R. reniformis* alone recorded least value of 34.40, 30.30 and 33.21 in MCU 5, K10 and TCB 209 cultivars respectively.

Seed index was significant character in MCU 5 with maximum value recorded in *G. mosseae* with 11.27 followed by *G. mosseae* + *R. reniformis* (11.18) and *G. fasciculatum* (11.10) which were on par with each other. The treatments *G. fasciculatum* + *R. reniformis, G. intraradices* + *R. reniformis, G. fulvum* + *R. reniformis* and control were on par with each other. The least was recorded in *R. reniformis* alone with 9.95 which was significantly different from all others treatments. In K10 and TCB 209 the seed index was found to be a non--significant character. However, *G. mosseae* recorded maximum value of 9.91 in K10 and *G. fasciculatum* with maximum value (11.92) in TCB 209 (Table 11).

In cotton cv. MCU 5 lint index was observed to be maximum in *G. mosseae* (5.84), *G. mosseae* + *R. reniformis* (5.82) and *G. fasciculatum* (5.79). They remained on par with each other and significantly different from the remaining treatments. In K10 and TCB 209 the lint index was not significant with each treatments. However, *G. mosseae* and *G. fasciculatum* recorded maximum values of 4.92 and 6.81 on K10 and TCB 209 cultivars respectively.

4.1.6. Fibre quality characters (Table 12)

Among fibre quality characters, fibre fineness exhibited no significant difference among the treatments in all the three cotton cultivars. However, it was observed to be maximum in *G. mosseae* with 3.85, 2.72 and 3.54 on MCU 5, K10 and TCB 209 cultivars respectively. Among the VAM plus nematode treatments,

Table 12. **Effect of four species of VAM, *G. mosseae, G. fasciculatum, G. intraradices* and *G. fulvum* on *R. reniformis* on fibre quality of three cotton cultivars.**

(mean of three replications)

Treatments	MCU 5			K10			TCB 209		
	Fibre fineness*	Mean fibre length (mm)	Bundle strength**	Fibre fineness*	Mean fibre length (mm)	Bundle strength**	Fibre fineness*	Mean fibre length (mm)	Bundle strength**
T_1 - *G. mosseae*	3.85	28.8	21.34	2.72	27.6	20.68	3.54	28.8	20.68
T_2 - *G. fasciculatum*	3.81	28.6	21.16	2.68	27.4	20.52	3.53	28.2	20.69
T_3 - *G. intraradices*	3.74	28.2	20.62	2.62	26.9	20.42	3.51	27.9	20.41
T_4 - *G. fulvum*	3.72	27.8	20.58	2.61	26.8	20.42	3.52	27.6	20.48
T_5 - *G. mosseae* + *R. reniformis*	3.82	28.6	21.23	2.69	27.5	20.62	3.49	27.6	20.17
T_6 - *G. fasciculatum* + *R. reniformis*	3.74	28.1	20.65	2.64	27.4	20.51	3.49	21.3	20.19
T_7 - *G. intraradices* + *R. reniformis*	3.71	27.5	20.57	2.64	27.1	20.41	3.48	26.8	20.14
T_8 - *G. fulvum* + *R. reniformis*	3.70	27.3	20.52	2.63	25.9	20.41	3.48	26.2	20.13
T_9 - *R. reniformis* alone	3.69	26.8	20.31	2.60	25.9	20.34	3.48	26.2	20.12
T_{10} - Control	3.71	27.2	20.55	2.61	25.9	20.21	3.50	27.9	20.19
CD (P=0.05)	**NS**	**0.12**	**0.21**	**NS**	**0.08**	**NS**	**NS**	**NS**	**NS**

* Micronaire 10^{-6} g $inch^{-1}$ ** (g tex^{-1})1/8" gauge

G. mosseae + *R. reniformis* recorded maximum with 3.82, 2.69 and 3.49 in three cotton cultivars respectively.

Mean fibre length was observed to be significant in MCU 5 and K10 cultivars. It was not significant in TCB 209. In MCU 5 mean fibre length was maximum in *G. mosseae* with 28.8 mm and it was followed by *G. fasciculatum* (28.6 mm) and *G. mosseae* + *R. reniformis* (28.6 mm) treatments which were on par with each other. The least was observed in nematode alone with 26.8 mm. In K10, *G. mosseae* registered highest value of mean fibre length (27.6 mm) followed by *G. mosseae* + *R. reniformis* (27.5 mm). The least was observed in *R. reniformis* alone (25.9 mm) and control (25.9 mm) treatments. In TCB 209, *G. mosseae* (28.8 mm) recorded maximum followed by *G. fasciculatum* (28.2 mm).

In MCU 5, *G. mosseae* recorded maximum bundle strength with 21.34 and it was on par with the next best treatments *G. mosseae* + *R. reniformis* (21.23) and *G. fasciculatum* (21.16) and they were significantly different from others. *R. reniformis* alone recorded lowest (20.31) which was significantly different from other treatments. The remaining treatments exhibited no significant difference with each other. In K10, and TCB 209, bundle strength was not significantly different among treatments. However, *G. mosseae* (20.68) and *G. fasciculatum* (20.69) recorded maximum in K10 and TCB 209 cultivars respectively.

4.1.7. Macro nutrient content

4.1.7.1. Total nitrogen content (Table 13)

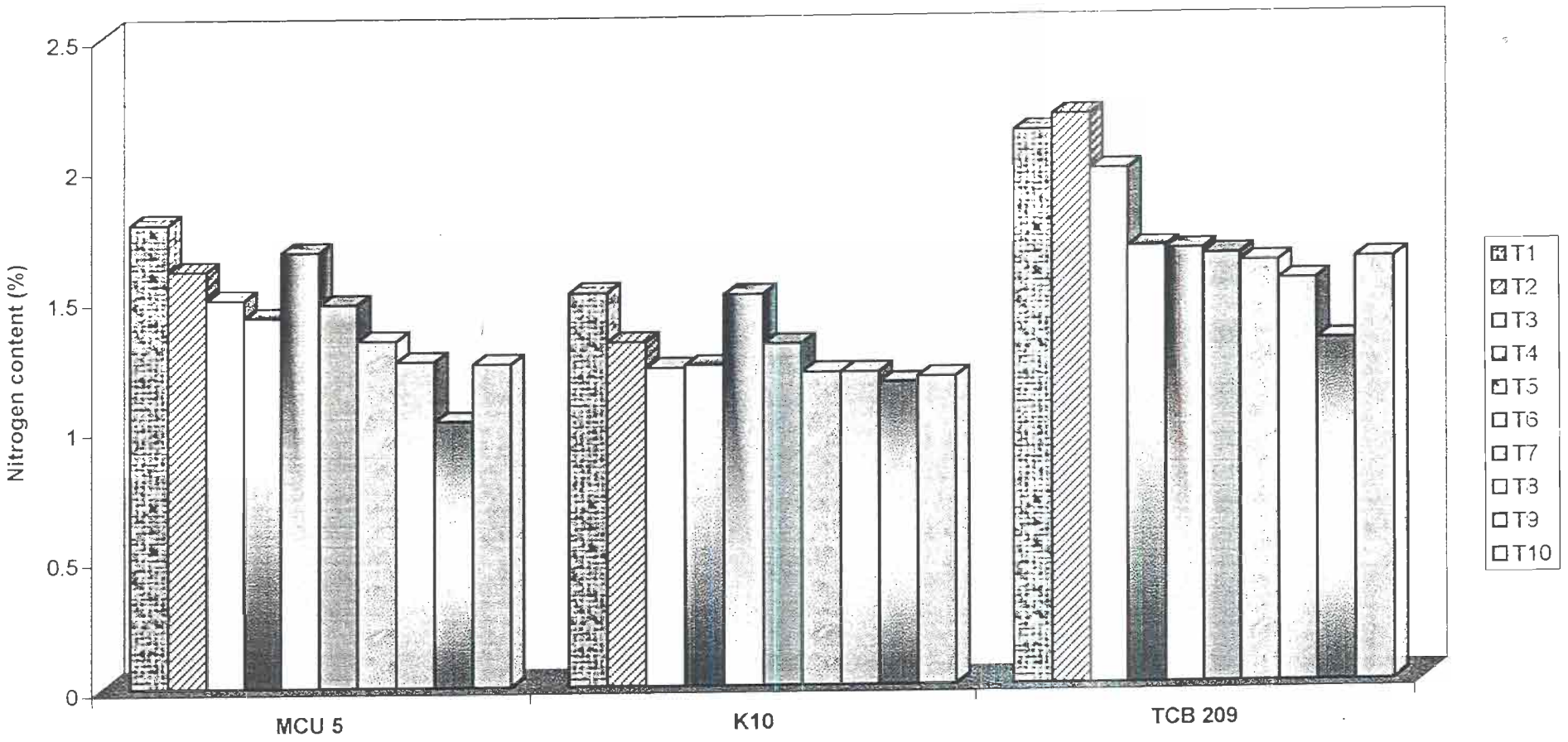
From the results of various macronutrients analyzed the total nitrogen content in plant shoot was more in all the VAM species inoculated plants when

Table 13. Effect of four species of VAM, *G. mosseae*, *G. fasciculatum*, *G. intraradices* and *G. fulvum* with *R. reniformis* on total nitrogen content in shoots of three cotton cultivars.

(mean of three replications)

Treatments	MCU 5		K10		TCB 209	
	Total nitrogen (%)	Per cent increase over nematode alone	Total nitrogen (%)	Per cent increase over nematode alone	Total nitrogen	% increase over nematode alone
T_1 - *G. mosseae*	1.78	74.5	1.51	30.1	2.12	61.8
T_2 - *G. fasciculatum*	1.60	56.8	1.32	13.7	2.18	66.4
T_3 - *G. intraradices*	1.49	46.0	1.22	5.1	1.97	50.3
T_4 - *G. fulvum*	1.42	39.2	1.23	6.0	1.67	27.4
T_5 - *G. mosseae* + *R. reniformis*	1.67	63.7	1.50	29.3	1.66	26.7
T_6 - *G. fasciculatum* + *R. reniformis*	1.47	44.1	1.31	12.9	1.64	25.1
T_7 - *G. intraradices* + *R. reniformis*	1.33	30.3	1.20	3.4	1.61	22.9
T_8 - *G. fulvum* + *R. reniformis*	1.25	23.0	1.21	4.3	1.54	17.5
T_9 - *R. reniformis* alone	1.02	-	1.16	-	1.31	-
T_{10} - Control	1.24	-	1.18	-	1.62	-
CD (P=0.05)	**0.16**	-	**0.15**	-	**0.29**	-

Fig.8. Effect of four species of VAM against *R. reniformis* on nitrogen content of three cotton cultivars



T1 - *G. mosseae* T2 - *G. fasciculatum* T3 - *G intraradices* T4 - *G. fulvum* T5 - *G. mosseae + R reniformis* T6 - *G. fasciculatum + R. reniformis*
T7 - *G intraradices + R reniformis* T8 - *G. fulvum + R. reniformis* T9 - *R. reniformis* T10 - Control

compared to control. In MCU 5, the nitrogen content was least in nematode alone (1.02%). The highest was recorded in *G. mosseae* with 1.78 per cent followed by *G. mosseae* + *R. reniformis* (1.67%) and *G. fasciculatum* (1.60%) which were on par with each other. *G. mosseae* and *G. fasciculatum* treatments were on par with inoculated alone and in combination with nematode.

In K10, all VAM species alone and in combination with nematodes observed increased total nitrogen content. *G. mosseae* recorded maximum of 1.51 per cent. The reniform alone registered least value of 1.16 per cent and it was significantly different from the rest of the treatments.

In TCB 209, among VAM species *G. fasciculatum* and *G. mosseae* recorded maximum total nitrogen content with 2.18 and 2.12 per cent respectively. Among VAM with nematode treatments *G. mosseae* recorded maximum of 1.66 per cent followed by *G. fasciculatum* (1.64%). The nematode alone registered least value of 1.31 per cent which was significantly different from rest of the treatments (Fig.8).

4.1.7.2. Phosphorus content (Table 14)

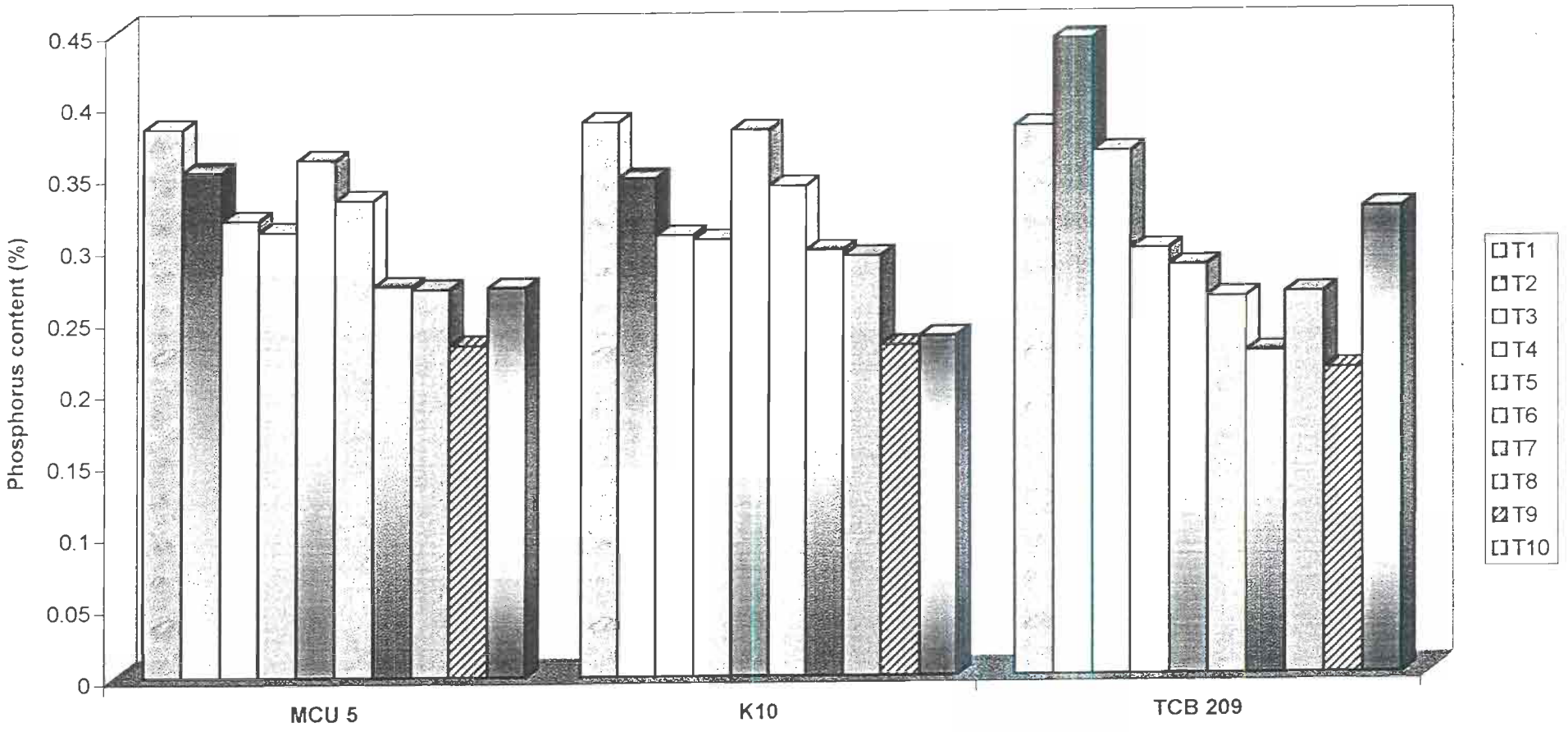
The phosphorus content was more in mycorrhizal plants. In MCU 5, the highest value was observed in *G. mosseae* with 0.382 per cent in shoot and it was 65.3 per cent increase over nematode alone. It was followed by *G. mosseae* + *R. reniformis* (0.360%) and *G. fasciculatum* (0.352%) treatments which were on par with each other and recorded 55.8 and 52.3 per cent increase over nematode alone. In nematode alone, the phosphorus content was least with 0.231 per cent in shoot.

Table 14. **Effect of four species of VAM, *G. mosseae*, *G. fasciculatum*, *G. intraradices* and *G. fulvum* with *R. reniformis* on phosphorus content in shoots of three cotton cultivars.**

(mean of three replications)

Treatments	MCU 5		K10		TCB 209	
	Phosphorus content (%)	Per cent increase over nematode alone	Phosphorus content (%)	Per cent increase over nematode alone	Phosphorus content (%)	Per cent increase over nematode alone
T_1 - *G. mosseae*	0.382	65.3	0.386	66.0	0.382	80.9
T_2 - *G. fasciculatum*	0.352	52.3	0.347	50.9	0.443	109.5
T_3 - *G. intraradices*	0.312	37.6	0.307	33.5	0.364	71.4
T_4 - *G. fulvum*	0.310	34.1	0.304	32.2	0.296	38.1
T_5 - *G. mosseae* + *R. reniformis*	0.360	55.8	0.380	65.2	0.284	33.3
T_6 - *G. fasciculatum* + *R. reniformis*	0.332	43.7	0.341	48.3	0.262	23.8
T_7 - *G. intraradices* + *R. reniformis*	0.272	17.7	0.296	28.7	0.224	24.8
T_8 - *G. fulvum* + *R. reniformis*	0.270	16.8	0.292	26.9	0.265	23.8
T_9 - *R. reniformis* alone	0.231	-	0.230	-	0.212	-
T_{10} - Control	0.271	-	0.236	-	0.324	-
CD (P=0.05)	**0.034**	-	**0.042**	-	**0.061**	-

Fig.9. Effect of four species of VAM against *R. reniformis* on phosphorus content of three cotton cultivars



T1 - *G. mosseae* T2 - *G. fasciculatum* T3 - *G. intraradices* T4 - *G. fulvum* T5 - *G. mosseae + R. reniformis* T6 - *G. fasciculatum + R. reniformis*
T7 - *G. intraradices + R. reniformis* T8 - *G. fulvum + R. reniformis* T9 - *R. reniformis* T10 - Control

In K10, *G. mosseae* recorded maximum 'P' content (0.386%) and nematode alone recorded the least (0.230%). In TCB 209 *G. fasciculatum* (0.443%) recorded maximum 'P' content followed by *G. mosseae* (0.382%). In combination with nematodes, *G. mosseae* + *R. reniformis* recorded maximum of 0.284 per cent. In TCB 209 'P' content was increased in all VAM species which was significantly reduced when *R. reniformis* was inoculated along with VAM species. The *R. reniformis* alone registered least value of 0.21 per cent (Fig.9).

4.1.7.3. Potassium content (Table 15)

In MCU 5, among VAM species, *G. mosseae* (1.63%) and *G. fasciculatum* (1.58%) recorded maximum potassium content which were on par with each other. When VAM combined with nematode, *G. mosseae* + *R. reniformis* recorded highest 'P' content with 1.56 per cent which was on par with *G. fasciculatum* + *R. reniformis* (1.44%). But these two treatments significantly different from *G. intraradices* and *G. fulvum* alone and in combination with nematodes. In nematode alone, the potassium content was only 0.94 per cent.

In K10, all VAM species significantly increased 'K' content when inoculated alone or in combination with nematodes. The nematode alone and control recorded least with 0.77 and 0.79 per cent 'K' which were on par and significantly different from all other treatments (Fig.10).

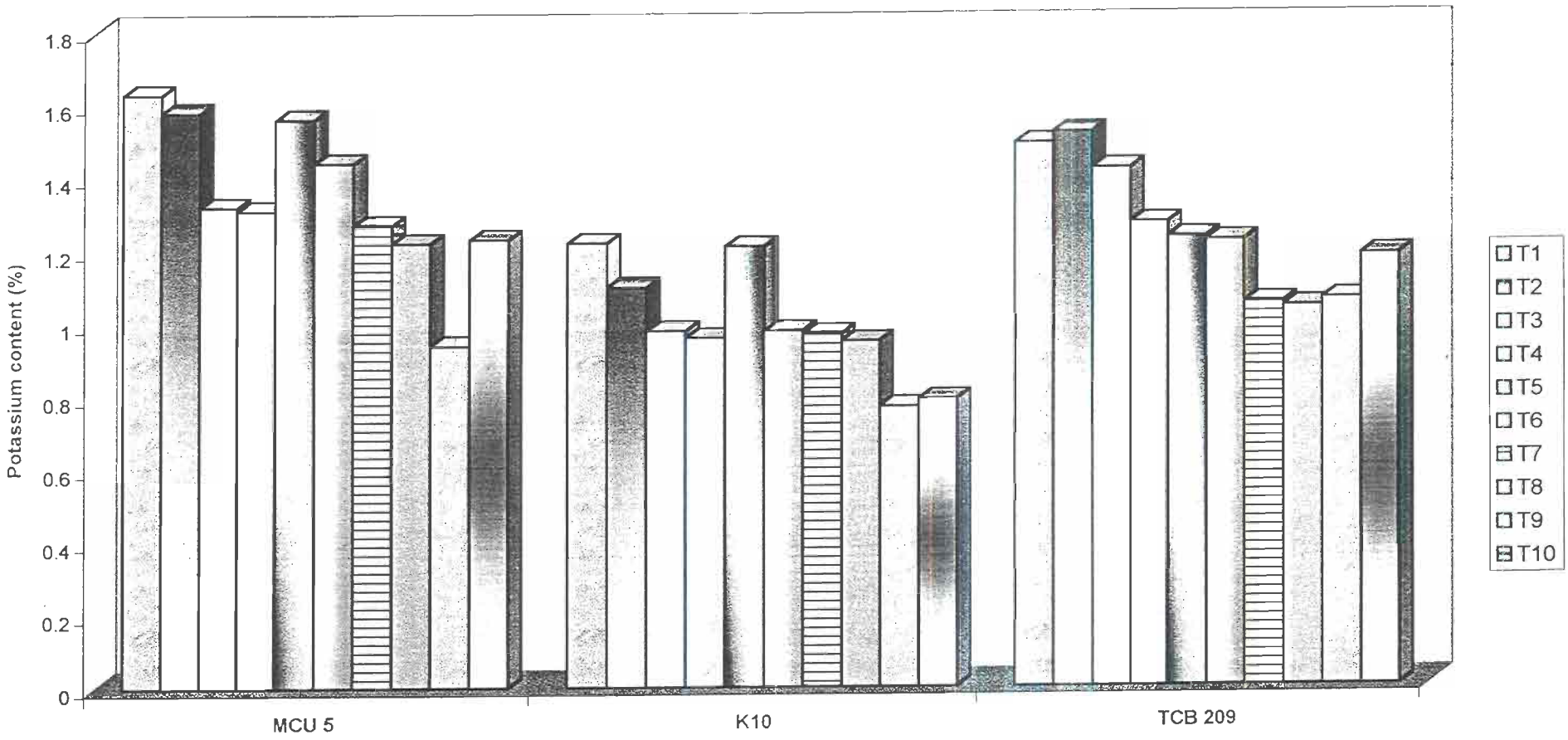
In TCB 209, maximum 'K' content recorded in *G. fasciculatum* (1.52%) which was on par with *G. mosseae* (1.49%). Among VAM with nematode treatments *G. mosseae* and *G. fasciculatum* performed well with 'K' content of 1.23 and 1.22 per cent respectively which were on par and significantly different from

Table 15. **Effect of four species of VAM, *G. mosseae, G. fasciculatum, G. intraradices* and *G. fulvum* with *R. reniformis* on potassium content in shoots of three cotton cultivars.**

(mean of three replications)

Treatments	MCU 5		K10		TCB 209	
	Potassium content (%)	Per cent increase over nematode alone	Potassium content (%)	Per cent increase over nematode alone	Potassium content (%)	Per cent increase over nematode alone
T_1 - *G. mosseae*	1.63	73.4	1.22	58.4	1.49	40.6
T_2 - *G. fasciculatum*	1.58	68.0	1.10	42.8	1.52	43.3
T_3 - *G. intraradices*	1.32	40.4	0.98	27.2	1.42	33.9
T_4 - *G. fulvum*	1.31	39.3	0.96	24.7	1.27	19.8
T_5 - *G. mosseae* + *R. reniformis*	1.56	65.9	1.21	57.1	1.23	16.0
T_6 - *G. fasciculatum* + *R. reniformis*	1.44	53.1	0.98	27.2	1.22	15.1
T_7 - *G. intraradices* + *R. reniformis*	1.27	35.1	0.97	25.9	1.05	-0.9
T_8 - *G. fulvum* + *R. reniformis*	1.22	30.5	0.95	23.4	1.04	-0.9
T_9 - *R. reniformis* alone	0.94	-	0.77	-	1.06	-
T_{10} - Control	1.23	-	0.79	-	1.18	-
CD (P=0.05)	**0.28**	-	**0.11**	-	**0.16**	-

Fig.10. Effect of four species of VAM against *R. reniformis* on potassium content of three cotton cultivars



T1 - *G. mosseae* T2 - *G. fasciculatum* T3 - *G. intraradices* T4 - *G. fulvum* T5 - *G. mosseae* + *R. reniformis* T6 - *G. fasciculatum* + *R. reniformis*
T7 - *G. intraradices* + *R. reniformis* T8 - *G. fulvum* + *R. reniformis* T9 - *R. reniformis* T10 - Control

VAM alone treatment *G. intraradices* + *R. reniformis*, *G. fulvum* + *R. reniformis* and *R. reniformis* alone were on par with each other and significantly different from control.

4.1.8. Micro nutrient content

4.1.8.1. Iron content (Table 16)

In cotton cv. MCU 5, the iron content was more in *G. mosseae* with 1275 ppm in shoot and it was on par with next best test treatments *G. mosseae* + *R. reniformis* (1258 ppm) and *G. fasciculatum* (1252 ppm). The amount of iron content in *G. intraradices* and *G. fulvum* were 1176 ppm and 1124 ppm which were significantly different from nematode alone treatments. In nematode alone the iron content was only 987 ppm and it was significantly different from control.

In K10 and TCB 209, VAM inoculation increased the iron content in shoot. *G. mosseae* recorded maximum in K10 with 985 ppm and *G. fasciculatum* recorded maximum in TCB 209 with 1286 ppm. In VAM along with nematode treatments *G. mosseae* registered maximum with 1110 ppm in TCB 209 which was significantly lower than *G. mosseae* alone treatment (Fig.11).

4.1.8.2. Copper content (Table 17)

There was a significant increase in copper content in all the VAM inoculated cotton cultivars. In MCU 5 and K10, *G. mosseae* registered highest copper content with 168 ppm and 180 ppm which were on par with *G. mosseae* + *R. reniformis* treatments in both cultivars. In TCB 209, *G. fasciculatum* recorded maximum concentration of 135 ppm. In K10 there was no significant difference exists between *R. reniformis* and control treatments (Fig.11).

Table 16. **Effect of four species of VAM, *G. mosseae, G. fasciculatum, G. intraradices* and *G. fulvum* with *R. reniformis* on iron content in shoots of three cotton cultivars.**

(mean of three replications)

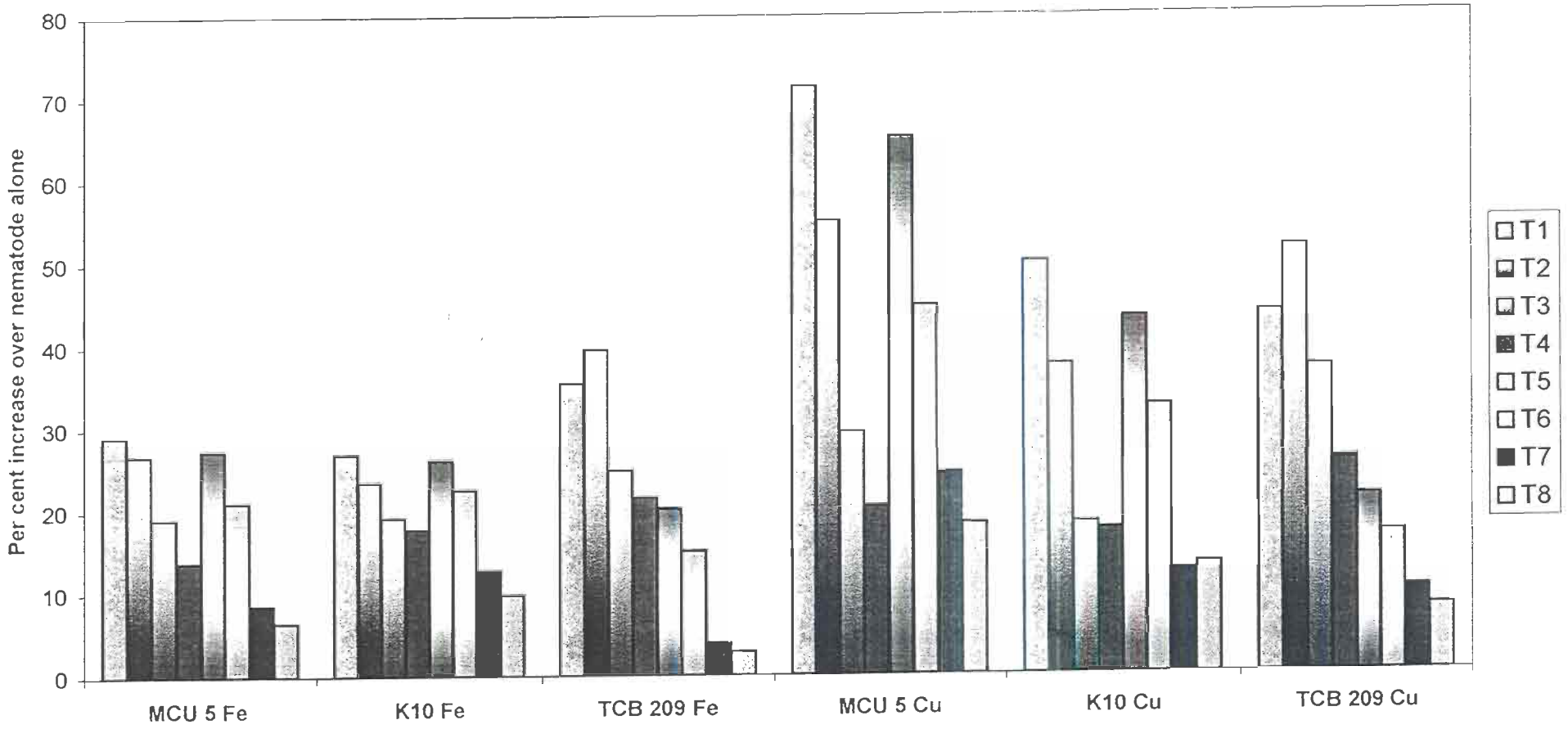
Treatments	MCU 5		K10		TCB 209	
	Iron content (ppm)	Per cent increase over nematode alone	Iron content (ppm)	Per cent increase over nematode alone	Iron content (ppm)	Per cent increase over nematode alone
T_1 - *G. mosseae*	1275	29.1	985	26.9	1248	35.5
T_2 - *G. fasciculatum*	1252	27.1	959	23.5	1286	39.6
T_3 - *G. intraradices*	1176	19.1	925	19.2	1150	24.9
T_4 - *G. fulvum*	1124	13.8	915	17.9	1120	21.6
T_5 - *G. mosseae* + *R. reniformis*	1258	28.4	979	26.2	1110	20.5
T_6 - *G. fasciculatum* + *R. reniformis*	1204	21.9	951	22.6	1060	15.1
T_7 - *G. intraradices* + *R. reniformis*	1069	8.3	875	12.8	957	3.9
T_8 - *G. fulvum* + *R. reniformis*	1052	6.5	852	9.8	948	2.9
T_9 - *R. reniformis* alone	987	-	776	-	921	-
T_{10} - Control	1045	-	785	-	961	-
CD (P=0.05)	**45.2**	-	**15.8**	-	**35.2**	-

Table 17. **Effect of four species of VAM, *G. mosseae*, *G. fasciculatum*, *G. intraradices* and *G. fulvum* with *R. reniformis* on copper content in shoots of three cotton cultivars.**

(mean of three replications)

Treatments	MCU 5		K10		TCB 209	
	Copper content (ppm)	Per cent increase over nematode alone	Copper content (ppm)	Per cent increase over nematode alone	Copper content (ppm)	Per cent increase over nematode alone
T_1 - *G. mosseae*	168	71.4	180	50.0	128	43.8
T_2 - *G. fasciculatum*	152	55.1	165	37.5	135	51.7
T_3 - *G. intraradices*	127	29.5	142	18.3	122	37.1
T_4 - *G. fulvum*	118	20.4	141	17.5	112	25.8
T_5 - *G. mosseae* + *R. reniformis*	162	65.3	172	43.3	108	21.3
T_6 - *G. fasciculatum* + *R. reniformis*	142	44.8	159	32.5	104	16.9
T_7 - *G. intraradices* + *R. reniformis*	122	24.4	135	12.5	98	10.1
T_8 - *G. fulvum* + *R. reniformis*	116	18.3	136	13.3	96	7.9
T_9 - *R. reniformis* alone	98	-	120	-	89	-
T_{10} - Control	114	16.3	121	-	97	-
CD (P=0.05)	**12.5**	-	**10.5**	-	**6.5**	-

Fig.11. Effect of four species of VAM against *R. reniformis* on Iron and Copper content in three cotton cultivars



T1 - *G. mosseae* T2 - *G fasciculatum* T3 - *G. intraradices* T4 - *G fulvum* T5 - *G mosseae + R reniformis* T6 - *G. fasciculatum + R. reniformis*
T7 - *G. intraradices + R. reniformis* T8 - *G. fulvum + R. reniformis*

4.1.8.3. Manganese content (Table 18)

In cv. MCU 5, manganese content was high in *G. mosseae* which recorded 67 ppm and it was followed by *G. mosseae* + *R. reniformis* treatment (64 ppm) and *G. fasciculatum* (63 ppm). Nematode alone recorded lowest Mn content with 48 ppm. In cv. K10 all VAM species improved Mn content. *G. mosseae* performed well with 48 ppm which was on par with *G. mosseae* + *R. reniformis* treatment with 47 ppm. The nematode alone registered only 34 ppm which was on par with control treatment (36 ppm). In TCB 209, *G. fasciculatum* recorded maximum Mn content of 46 ppm which was on par with *G. mosseae* (44 ppm). In TCB 209 all VAM species along with nematodes recorded significantly lower Mn content than they were applied alone. Among VAM with nematode treatments *G. mosseae* registered maximum value of 39 ppm. The nematode alone recorded least value of 31 ppm (Fig.12)

Zinc content exhibited a similar trend as manganese content in all cotton cultivars (Table 19).

4.1.9. Physiological parameters (Table 20)

4.1.9.1. Leaf temperature

In MCU 5, leaf temperature was found to be maximum in nematode alone (31.34°C) treatment which was on par with *G. intraradices* + *R. reniformis* (31.27°C), *G. fulvum* + *R. reniformis* (31.24°C) treatments. These treatments were significantly different from control and rest of the treatments. *G. mosseae* recorded lowest leaf temperature (29.07°C) followed by *G. mosseae* + *R. reniformis* (29.17°C) and both were on par with each other.

Table 18. **Effect of four species of VAM, *G. mosseae, G. fasciculatum, G. intraradices* and *G. fulvum* with *R. reniformis* on manganese content in shoots of three cotton cultivars.**

(mean of three replications)

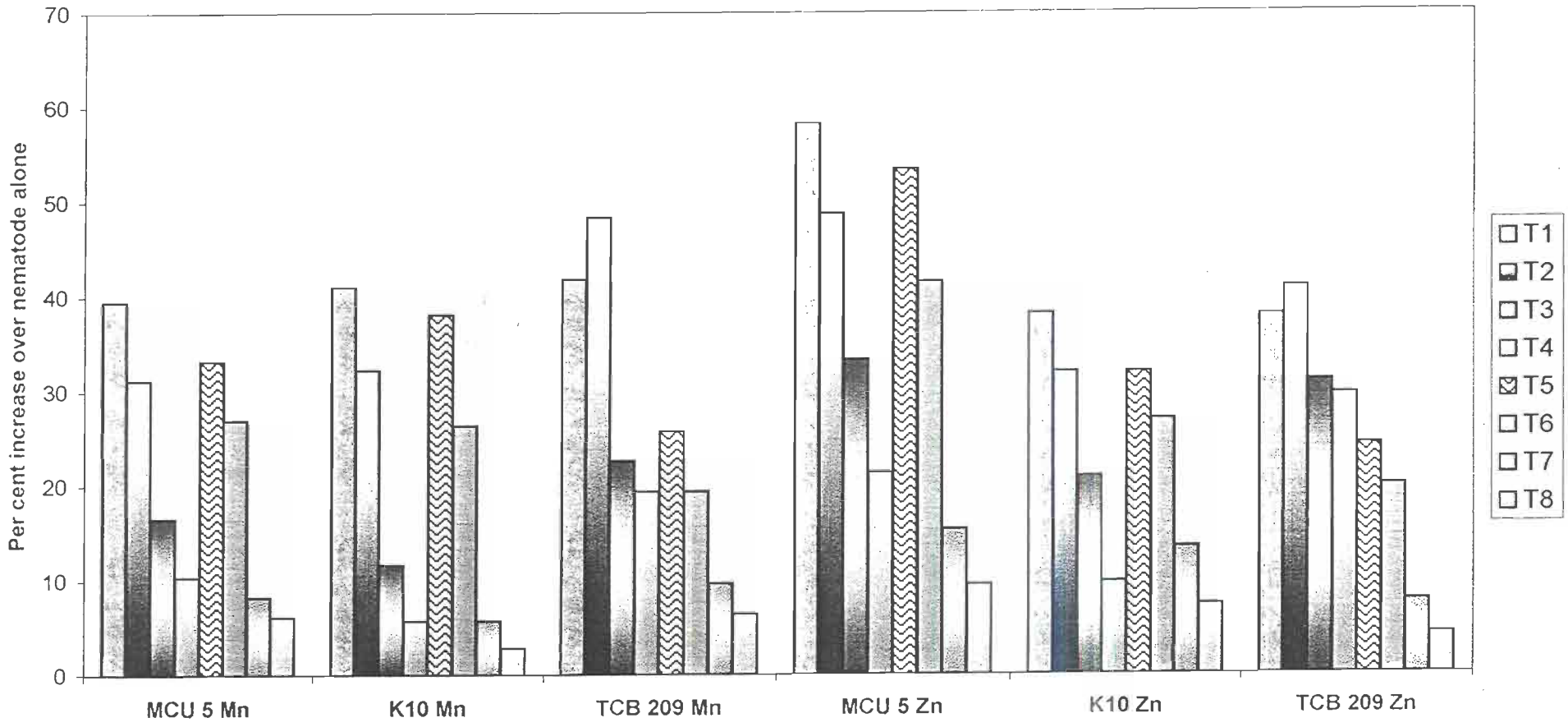
Treatments	MCU 5		K10		TCB 209	
	Shoot manganese content (ppm)	Per cent increase over nematode alone	Shoot manganese content (ppm)	Per cent increase over nematode alone	Shoot manganese content (ppm)	Per cent increase over nematode alone
T_1 - *G. mosseae*	67	39.5	48	41.1	44	41.9
T_2 - *G. fasciculatum*	63	31.2	45	32.3	46	48.4
T_3 - *G. intraradices*	56	16.6	38	11.7	38	22.6
T_4 - *G. fulvum*	53	10.4	36	5.8	37	19.4
T_5 - *G. mosseae* + *R. reniformis*	64	33.3	47	38.2	39	25.8
T_6 - *G. fasciculatum* + *R. reniformis*	61	27.0	43	26.4	37	19.4
T_7 - *G. intraradices* + *R. reniformis*	52	8.3	36	5.8	34	9.7
T_8 - *G. fulvum* + *R. reniformis*	51	6.2	35	2.9	33	6.5
T_9 - *R. reniformis* alone	48	-	34	-	31	-
T_{10} - Control	54	-	36	-	34	-
CD (P=0.05)	**3.5**	-	**2.8**	-	**2.5**	-

Table 19. **Effect of four species of VAM, *G. mosseae*, *G. fasciculatum*, *G. intraradices* and *G. fulvum* with *R. reniformis* on zinc content in shoots of three cotton cultivars.**

(mean of three replications)

Treatments	MCU 5		K10		TCB 209	
	Shoot content (ppm)	Per cent increase over nematode alone	Shoot content (ppm)	Per cent increase over nematode alone	Shoot content (ppm)	Per cent increase over nematode alone
T_1 - *G. mosseae*	133	58.3	112	38.2	159	38.3
T_2 - *G. fasciculatum*	125	48.8	107	32.0	163	41.7
T_3 - *G. intraradices*	112	33.3	98	20.9	151	31.3
T_4 - *G. fulvum*	102	21.4	89	9.8	149	29.6
T_5 - *G. mosseae* + *R. reniformis*	129	53.5	107	32.0	143	24.3
T_6 - *G. fasciculatum* + *R. reniformis*	119	41.6	103	27.1	138	20.0
T_7 - *G. intraradices* + *R. reniformis*	97	15.4	92	13.5	124	7.8
T_8 - *G. fulvum* + *R. reniformis*	92	9.5	87	7.4	120	4.3
T_9 - *R. reniformis* alone	84	-	81	-	115	-
T_{10} - Control	92	-	82	-	134	-
CD (P=0.05)	**6.5**	-	**5.6**	-	**7.5**	-

Fig.12. Effect of four species of VAM against *R. reniformis* on Manganese and Zinc content in three cotton cultivars



T1 - *G. mosseae* T2 - *G. fasciculatum* T3 - *G. intraradices* T4 - *G. fulvum* T5 - *G. mosseae* + *R. reniformis* T6 - *G. fasciculatum* + *R. reniformis*
T7 - *G. intraradices* + *R. reniformis* T8 - *G. fulvum* + *R. reniformis*

Table 20. **Effect of four species of VAM, *G. mosseae, G. fasciculatum, G. intraradices* and *G. fulvum* and *R. reniformis* on physiological parameters (leaf temperature and transpiration rate) of three cotton cultivars.**

(mean of three replications)

Treatments	MCU 5		K10		TCB 209	
	Leaf temperature (°C)	Transpiration rate (µg of H_2O_2 cm^{-2} s^{-1})	Leaf temperature (°C)	Transpiration rate (µg of H_2O_2 cm^{-2} s^{-1})	Leaf temperature (°C)	Transpiration rate (µg of H_2O_2 cm^{-2} s^{-1})
T_1 - *G. mosseae*	29.07	8.62	28.91	8.27	28.19	8.75
T_2 - *G. fasciculatum*	29.27	8.52	28.91	8.21	28.17	8.82
T_3 - *G. intraradices*	29.89	8.50	29.12	8.20	28.24	8.72
T_4 - *G. fulvum*	30.07	8.14	29.12	8.16	28.20	8.68
T_5 - *G. mosseae* + *R. reniformis*	29.17	8.24	28.94	8.26	29.04	8.62
T_6 - *G. fasciculatum* + *R. reniformis*	29.38	8.12	28.96	8.21	29.12	8.26
T_7 - *G. intraradices* + *R. reniformis*	31.27	8.13	29.16	8.19	29.21	8.15
T_8 - *G. fulvum* + *R. reniformis*	31.24	7.97	29.18	8.15	29.24	8.24
T_9 - *R. reniformis* alone	31.34	7.42	29.20	8.11	29.45	8.11
T_{10} - Control	30.12	8.18	29.19	8.11	28.45	8.45
CD (P=0.05)	**0.13**	**0.21**	**NS**	**NS**	**0.14**	**0.31**

In K10, leaf temperature was found to be a non significant character. However, the nematode alone recorded highest value of 29.20°C whereas *G. mosseae* recorded lowest value of 28.91°C.

In TCB 209, all the VAM species recorded lowest leaf temperature. But in combination with nematodes the leaf temperature increased which were significantly different from VAM alone treatments. The nematode alone recorded highest leaf temperature (29.45°C).

4.1.9.2. Transpiration rate (Table 20)

Transpiration rate was lowest in *R. reniformis* alone with, 7.42 and 8.11 in cotton cultivars MCU 5, TCB 209 respectively, *G. mosseae* and *G. fasciculatum* treatments recorded maximum with 8.62 and 8.75 in MCU 5 and TCB 209 cotton cultivars respectively. When VAM along with nematode, *G. mosseae* recorded highest with 8.24 and 8.62 in MCU 5 and TCB 209 cultivars respectively. The transpiration rate was non-significant in cotton cv. K10, though the trend was same.

4.1.9.3. Stomatal diffusive resistance (Table 21)

In MCU 5, the stomatal diffusive resistance of nematode alone treatment recorded highest value (10.41) which was on par with *G. fulvum* + *R. reniformis* (9.78) and *G. intraradices* + *R. reniformis* (9.56) treatments. *G. mosseae* recorded lowest value (7.52) followed by *G. fasciculatum* 7.82. *G. mosseae* + *R. reniformis* (8.43) which was on par with control, but significantly different than nematode alone, *G. intraradices* + *R. reniformis* and *G. fulvum* + *R. reniformis*. In K10, stomatal diffusive resistance was not significant with all treatments. However, *G.*

Table 21. **Effect of four species of VAM, *G. mosseae, G. fasciculatum, G. intraradices* and *G. fulvum* and *R. reniformis* on physiological parameters (stomatal diffusive resistance and photosynthetic rate) of three cotton cultivars.**

(mean of three replications)

Treatments	MCU 5		K10		TCB 209	
	Stomatal diffusive resistance ($s.cm^{-1}$)	Photo-synthetic rate (mg CO_2 dm^{-2} hr^{-1})	Stomatal diffusive resistance ($s.cm^{-1}$)	Photo-synthetic rate (mg CO_2 dm^{-2} hr^{-1})	Stomatal diffusive resistance ($s.cm^{-1}$)	Photo-synthetic rate (mg CO_2 dm^{-2} hr^{-1})
T_1 - *G. mosseae*	7.52	45.06	8.91	45.01	8.97	45.68
T_2 - *G. fasciculatum*	7.82	45.00	8.97	44.82	8.99	45.24
T_3 - *G. intraradices*	8.43	44.56	9.05	43.12	9.12	44.24
T_4 - *G. fulvum*	8.44	44.26	9.18	42.98	9.24	43.64
T_5 - *G. mosseae* + *R. reniformis*	8.43	44.81	8.99	44.92	9.21	43.21
T_6 - *G. fasciculatum* + *R. reniformis*	8.42	44.88	8.90	44.71	9.42	43.06
T_7 - *G. intraradices* + *R. reniformis*	9.56	42.44	9.17	43.11	9.91	42.90
T_8 - *G. fulvum* + *R. reniformis*	9.78	42.18	9.19	42.92	9.92	42.86
T_9 - *R. reniformis* alone	10.41	42.02	9.10	42.90	9.97	42.84
T_{10} - Control	8.12	43.18	9.18	42.91	9.21	43.2
CD (P=0.05)	**0.24**	**0.29**	**NS**	**0.16**	**0.21**	**0.42**

mosseae recorded lowest value of 8.91. In TCB 209, *G. mosseae* registered lowest value (8.97) which was on par with *G. fasciculatum* (8.99). In VAM plus nematode treated plants there was significant increase in stomatal diffusive resistance. The nematode alone recorded highest value (9.97) which was significantly different from all other treatments.

4.1.9.4. Phytosynthetic rate (Table 21)

In MCU 5, the photosynthetic rate was increased in VAM treated plants than nematode alone. *G. mosseae* recorded highest photosynthetic rate (45.06) which was on par with *G. fasciculatum* (45.00). The next best treatment was *G. mosseae* + *R. reniformis* which recorded 44.81. The nematode alone registered least (42.02) which was on par with *G. intraradices* + *R. reniformis* (42.44) and *G. fulvum* + *R. reniformis* (42.18) treatments. In K10, nematode alone treatment recorded least value with 42.90 but it was on par with control (42.91). All VAM species observed to increase photosynthetic rate significantly when inoculated alone and along with nematode. In TCB 209, *G. mosseae* recorded maximum of 45.68 which was on par with *G. fasciculatum* (45.24). VAM with nematode treatments significantly increased the photosynthetic rate than nematode alone *G. mosseae* + *R. reniformis* recorded 3.06 which was maximum among VAM with nematode treatments.

4.2. Effect of seed treatment and soil application of *G. mosseae* for the management of *R. reniformis* on cotton cv. MCU 5

The effect of seed treatment and soil application methods of *G. mosseae* at two dosages on *R. reniformis* was studied and the results are presented in table 22 to 27.

Maximum shoot length and root length (86.7 cm and 27.4 cm respectively) was produced in soil application of VAM @ 10 g/kg soil and it was significantly superior to control and carbofuran treatments (Table 22 and Plate 18). This was followed by soil application of VAM @ 5 g/kg soil (81.1 cm and 22.6 cm shoot and root length respectively) (Plate 19). When comparing methods of application, seed treatment with *G. mosseae* at two dosages recorded lowest shoot length and root length. Seed treatment @ 5 g/kg seed recorded minimum of 65.1 cm and 14.1 cm, however, it was significantly superior to control (Fig.13).

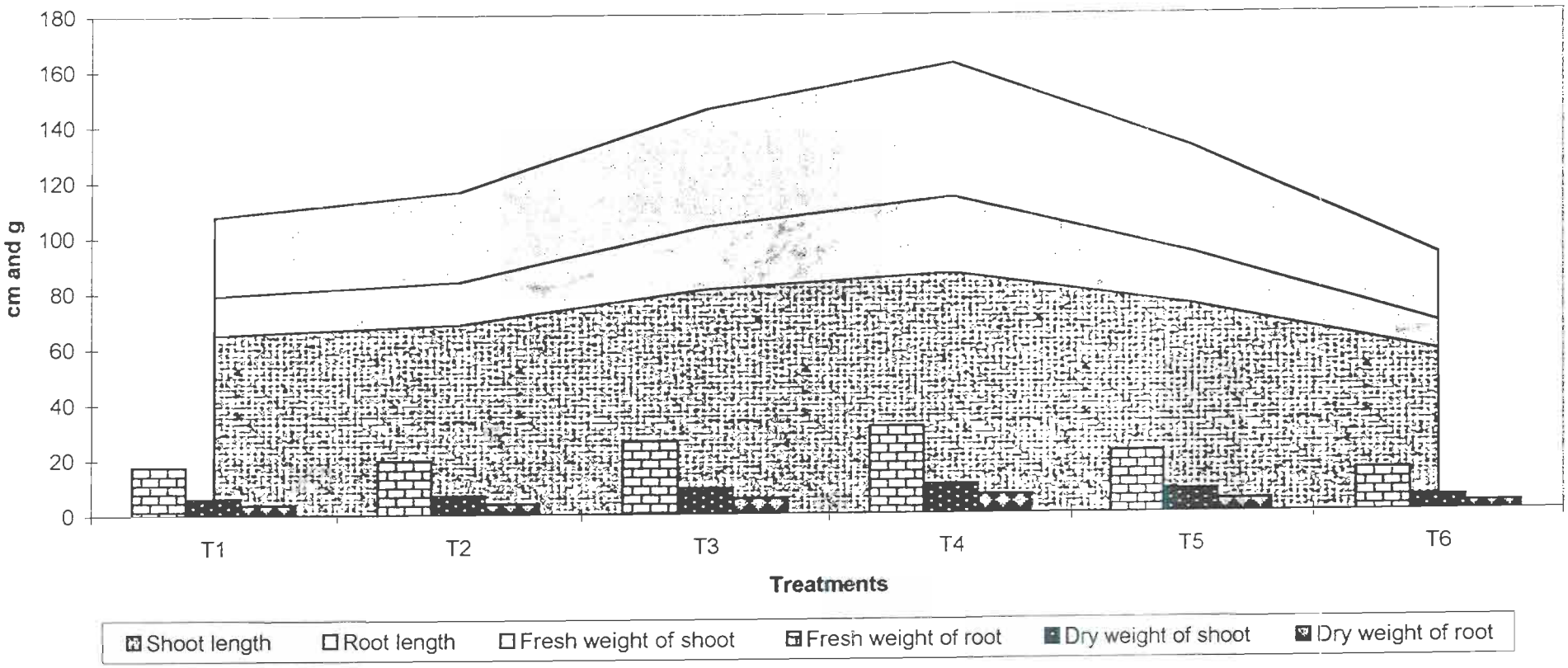
Soil application of VAM @ 10 g/kg soil recorded maximum fresh weight of shoot and root (48.4 g and 31.4 g respectively) followed by VAM applied in the soil @ 5 g/kg soil (42.4 g and 26.3 g respectively) and were significantly different from each other. VAM applied as seed treatment @ 5 g/kg seed recorded lowest fresh weight of shoot and root (28.4 g and 17.8 g respectively). With regard to dry weight of shoot and root, the highest was recorded on soil application of VAM @ 10 g/kg soil (10.7g and 6.9g) which were 105.7 and 122.5 per cent increase over control. Next to soil application, carbofuran recorded maximum of 8.2 g and 4.7 g shoot and root weight respectively. The minimum was observed in seed treatment @ 5 g/kg seed (6.3 g and 3.8 g respectively). The same trend was noticed with leaf area index also (Table 23).

VAM as soil application @ 10 g/kg soil recorded the highest per cent of shoot phosphorus content (0.389 per cent) and was significantly different from all other treatments. It increased the shoot 'P' content by 177.8 per cent over control. Among VAM application methods, seed treatment with VAM @ 5 g/kg seed

Table 22. **Effect of seed treatments and soil application of *G. mosseae* with *R. reniformis* on growth parameters of cotton cv. MCU 5 (mean of four replications)**

Treatments	Length (cm)		Fresh weight (g)		Dry weight (g)	
	Shoot	Root	Shoot	Root	Shoot	Root
T_1 - Seed treatment @ 5 g/kg seed	65.1	14.1	28.4	17.8	6.3	3.8
T_2 - Seed Treatment @ 10 g/kg seed	68.6	15.4	32.4	19.7	7.0	4.0
T_3 - Soil application @ 5 g/kg soil	81.1	22.6	42.4	26.3	9.4	5.8
T_4 - Soil application @ 10 g/kg soil	86.7	27.4	48.4	31.4	10.7	6.9
T_5 - Carbofuran @ 1 kg a.i./ha	75.1	18.7	38.2	22.4	8.2	4.7
T_6 - Control	57.8	10.2	24.6	15.4	5.2	3.1
CD (P=0.05)	**4.5**	**3.4**	**3.2**	**2.4**	**1.2**	**0.62**

Fig.13. Effect of seed treatment and soil application of VAM with *R. reniformis* on growth parameters of cotton



T1 - VAM as seed treatment @ 5g/g seed T2 - VAM as seed treatment @ 10 g/kg seed T3 - VAM as soil application @ 5g/g soil
T4 - VAM as soil application @ 10g/g soil T5 - Carbofuran @ 1 kg a.i /ha T6 - Control

Plate 18. Effect of different method of application of *G. mosseae* against *R. reniformis* on growth of cotton cv. MCU 5

Treatments:

1 - VAM as seed treatment @ 5 g/kg seed

2 - VAM as seed treatment @ 10 g/kg seed

3 - VAM as soil application @ 5 g/kg soil

4 - VAM as soil application @ 10 g/kg soil

Treatments:

1 - VAM as soil application @ 10 g/kg soil

2 - VAM as soil application @ 5 g/kg soil

3 - Carbofuran @ 1 kg a.i./ha

4 - Control

Plate 18a

Plate 18b

Plate 19. Effect of different method of application of *G. mosseae* against *R. reniformis* on root growth of cotton cv. MCU 5.

Treatments:

1 - VAM as seed treatment @ 5 g/kg seed

2 - VAM as seed treatment @ 10 g/kg seed

3 - VAM as soil application @ 5 g/kg soil

4 - VAM as soil application @ 10 g/kg soil

5 - Carbofuran @ 1 kg a.i./ha

6 - Control

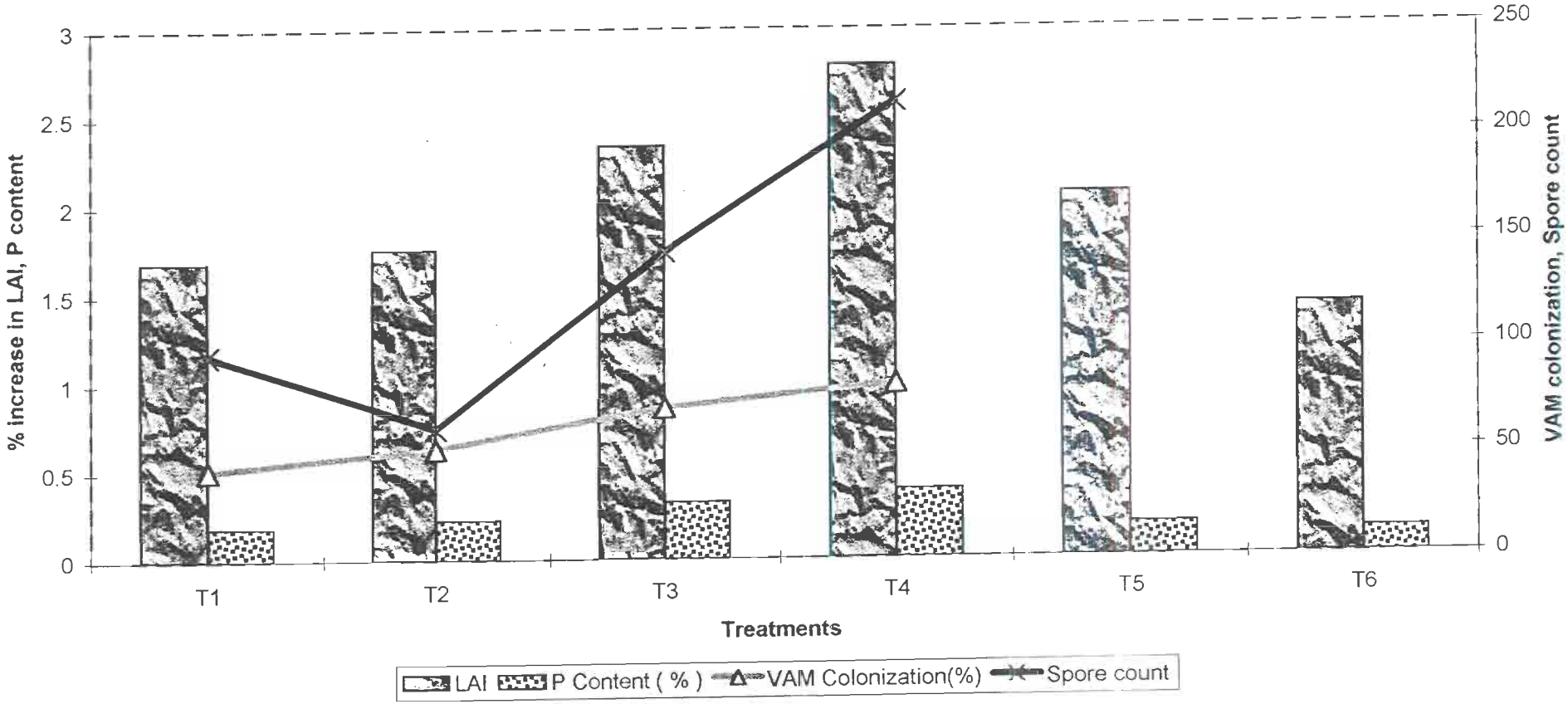
Plate 19

Table 23. **Effect of seed treatment and soil application of application of *G. mosseae* with *R. reniformis* on LAI, phosphorus content and VAM colonization in cotton cv. MCU 5**

(mean of four replications)

Treatments	Leaf Area Index	'P' content in shoot (%)	VAM colonization percentage	Spore count/ 50 g soil
T_1 - Seed treatment @ 5 g/kg seed	1.69	0.187	42.4	97
T_2 - Seed treatment @ 10 g/kg seed	1.76	0.226	52.6	62
T_3 - Soil application @ 5 g/kg soil	2.34	0.324	71.6	145
T_4 - Soil application @ 10 g/kg soil	2.79	0.389	82.3	215
T_5 - Carbofuran @ 1 kg a.i./ha	2.06	0.185	-	-
T_6 - Control	1.42	0.140	-	-
CD (P=0.05)	**0.26**	**0.04**	**5.5**	**42.6**

Fig.14. Effect of seed treatment and soil application of VAM with *R.reniformis* on LAI, P content, VAM colonization and spore count



T1 - VAM as seed treatment @ 5g/g seed
T2 - VAM as seed treatment @ 10 g/kg seed
T4 - VAM as soil application @ 10g/g soil
T5 - Carbofuran @ 1 kga.i /ha
T6 - Control

recorded lowest phosphorus content (0.187%) and it was on par with carbofuran application (Table 23 and Fig.14).

Maximum spore production and mycorrhizal colonization was recorded (215 and 82.3 per cent respectively) by plants inoculated with VAM as soil application @ 10 g/kg soil, followed by soil application @ 5 g/kg soil (145 and 71.6 per cent respectively) (Table 23). The VAM inoculated as seed treatment @ 5 g/kg seed registered lowest spore count and mycorrhizal colonization (97 and 42.4 per cent respectively).

Lowest number of females (65.3) and females with egg masses (29.6) on cotton roots was recorded in soil application of VAM @ 10 g/kg soil treatment and it was 74.7 and 75.8 per cent decrease over control (Table 24). VAM as seed treatment @ 5 g/kg seed recorded highest number of females (185.6) and females with egg masses (89.8). Nematode alone recorded maximum number of females (258.4) and females with eggmasses (122.4) (Fig.15).

The least number of eggs/eggmass (32.2) was noticed in plants when VAM applied in the soil @ 10 g/kg soil followed by VAM applied in soil @ 5 g/kg soil. The nematode alone recorded highest number of eggs/eggmass (51.8). VAM as seed treatment @ 5 g/kg seed and 10 g/kg seed and carbofuran treatments were on par with each other (Table 24).

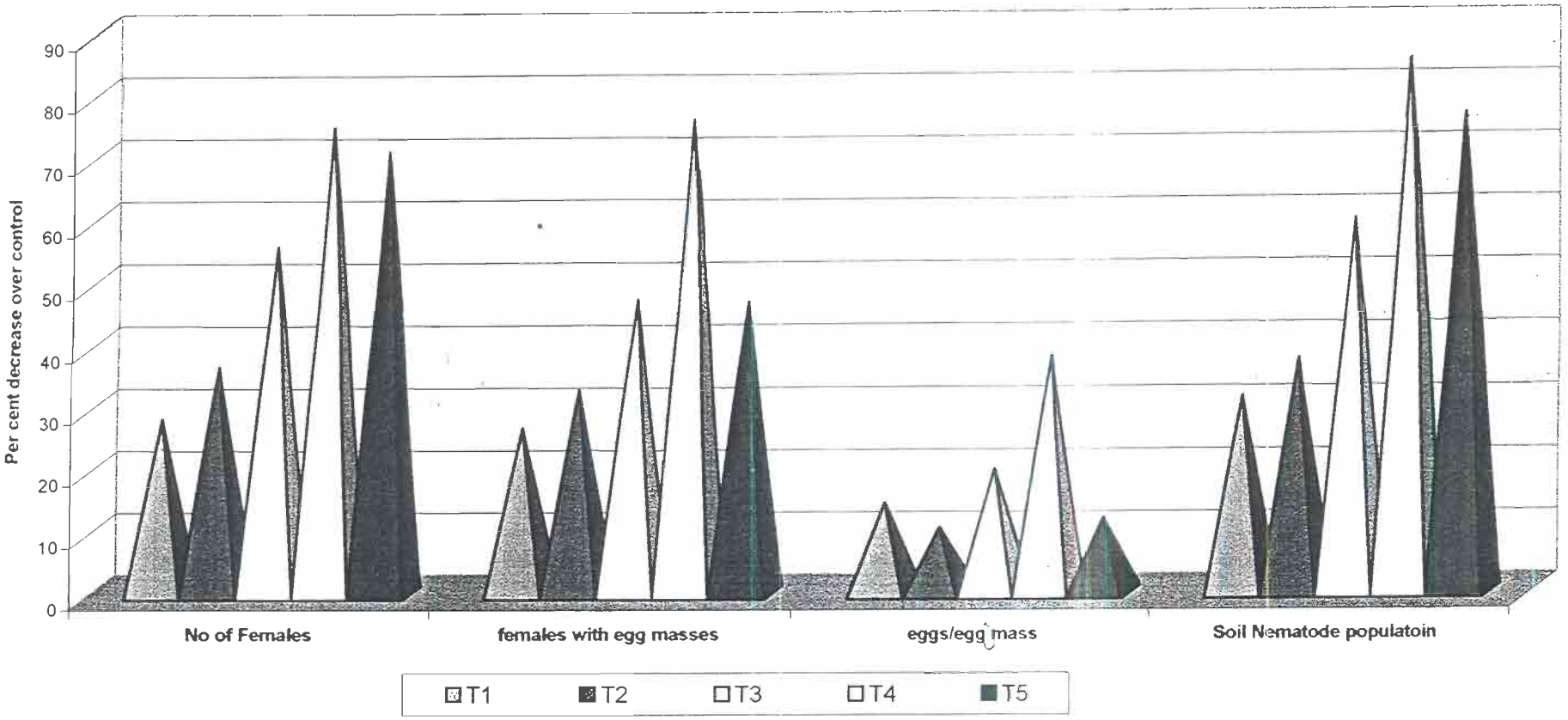
Highest nematode population in soil (764.2) was recorded in nematode alone control. VAM applied in the soil @ 10 g/kg soil recorded lowest nematode

Table 24. Effect of seed treatment and soil application of *G. mosseae* on *R. reniformis* population in cotton cv. MCU 5

(mean of four replications)

Treatments	Number of females per plant	Number of females with egg masses per plant	Number of eggs per egg mass	Soil nematode population/100 g soil
T_1 - Seed treatment @ 5 g/kg seed	185.6	89.8	44.3	524.4
T_2 - Seed treatment @ 10 g/kg seed	164.3	82.4	46.4	480.4
T_3 - Soil application @ 5 g/kg soil	114.6	64.8	41.5	310.4
T_4 - Soil application @ 10 g/kg soil	65.3	29.6	32.2	114.3
T_5 - Carbofuran @ 1 kg a.i./ha	75.3	65.3	45.6	184.2
T_6 - Control	258.4	122.4	51.8	764.2
CD (P=0.05)	**16.8**	**13.4**	3.6	**45.6**

Fig.15. Effect of seed treatment and soil application of VAM on *R. reniformis* population in cotton



T1 - VAM as seed treatment @ 5g/g seed T2 - VAM as seed treatment @ 10 g/kg see T3 - VAM as soil application @ 5g/g soil
T4 - VAM as soil application @ 10g/g soil T5 - Carbofuran @ 1 kg/ha T6 - Control

(114.3) population and significantly superior over other treatments and the decrease was 85.0 per cent over nematode alone (Fig.15).

Highest number of bolls and boll weight was recorded (16.3 and 3.86 respectively) in VAM applied as soil application @ 10 g/kg soil, followed by soil application of VAM @ 5 g/kg soil (14.6 and 3.34 g respectively). VAM as seed treatment @ 5 g/kg seed recorded lowest number of bolls (12.3) and boll weight (2.71 g) and it was significantly lower than carbofuran application. Nematode alone recorded lowest number of bolls (11.3) and boll weight (2.34 g) (Table 25).

Soil application of VAM @ 10 g/kg soil to the cotton plants gave higher cotton yield (62.9 g) followed by soil application of VAM @ 5 g/kg soil with 48.7 g and carbofuran with 41.4 g which were on par with each other. Inoculation of VAM as seed treatment @ 5 g/kg seed gave lowest yield (33.3 g) (Table 25 and Fig.16).

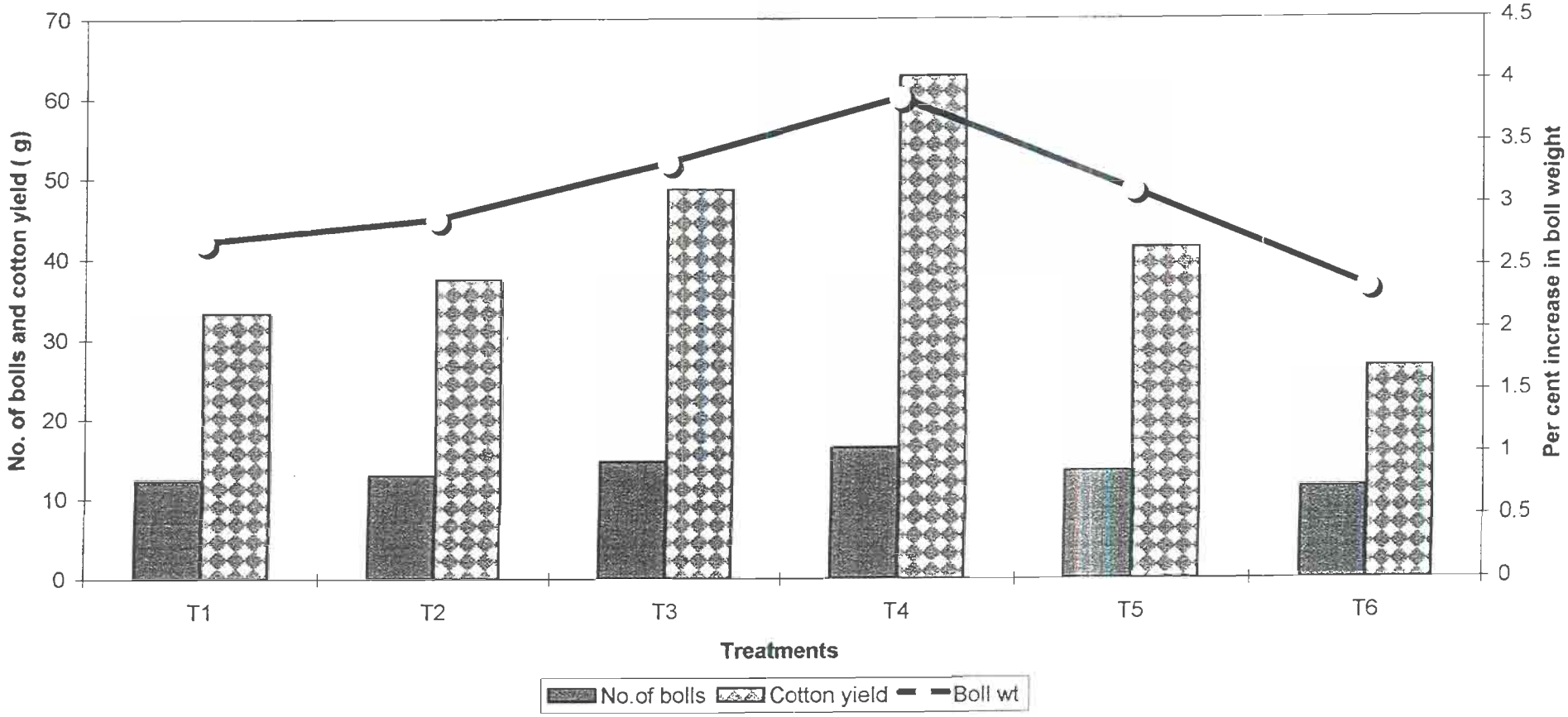
The yield attributes *viz.*, ginning percentage, seed index and lint index were observed to be maximum in soil application of VAM @ 10 g/kg soil with 36.02, 11.18 and 4.92 respectively which were significantly superior over other treatments (Table 26). It was followed by soil application of VAM @ 5 g/kg soil as 35.87, 10.94 and 4.81 which were on par with carbofuran application which recorded 35.81, 10.91 and 4.79 respectively. Nematode alone treatment recorded least value of ginning percentage, seed index and lint index with 34.71, 9.96 and 4.24 and it was on par with seed treatment of both doses.

Soil application of VAM @ 10 g/kg soil recorded maximum of 3.81, 28.4 and 21.26 fibre fineness, mean fibre length and bundle strength respectively which was on par with soil application of VAM @ 5 g/kg soil and carbofuran application.

Table 25. **Effect of seed treatment and soil application of *G. mosseae* on *R. reniformis* on yield parameters of cotton cv. MCU 5**
(mean of four replications)

Treatments	Number of bolls per plant	Boll weight (g/boll)	Cotton yield per plant (g)
T_1 - Seed treatment @ 5 g/kg seed	12.3	2.71	33.3
T_2 - Seed treatment @ 10 g/kg seed	13.0	2.81	37.5
T_3 - Soil application @ 5 g/kg soil	14.6	3.34	48.7
T_4 - Soil application @ 10 g/kg soil	16.3	3.86	62.9
T_5 - Carbofuran @ 1 kg a.i./ha	13.3	3.12	41.4
T_6 - Control	11.3	2.34	26.4
CD (P=0.05)	**0.37**	**0.24**	**9.2**

Fig.16. Effect of seed treatment and soil application of VAM with *R. reniformis* on yield parameters of cotton



T1 - VAM as seed treatment @ 5g/g seed T2 - VAM as seed treatment @ 10 g/kg seed T3 - VAM as soil application @ 5g/g soil
T4 - VAM as soil application @ 10g/g soil T5 - Carbofuran @ 1 kga.i./ha T6 - Control

Table 26. **Effect of seed treatment and soil application of *G. mosseae* on *R. reniformis* on some yield attributes of cotton cv. MCU 5**

(mean of four replications)

Treatments	Ginning percentage	Seed index (g)	Lint index (g)
T_1 - Seed treatment @ 5 g/kg seed	34.82	10.12	4.30
T_2 - Seed treatment @ 10 g/kg seed	34.85	10.14	4.31
T_3 - Soil application @ 5 g/kg soil	35.87	10.94	4.81
T_4 - Soil application @ 10 g/kg soil	36.02	11.18	4.92
T_5 - Carbofuran @ 1 kg a.i./ha	35.81	10.91	4.79
T_6 - Control	34.71	9.96	4.24
CD (P=0.05)	**0.28**	**0.25**	**0.21**

Table 27. **Effect of seed treatment and soil application of *G. mosseae* with *R. reniformis* on fibre quality characters of cotton cv. MCU 5**

(mean of four replications)

Treatments	Fibre fineness Micronaire 10^{-6}g inch^{-1}	Mean fibre length (mm)	Bundle strength (g tex^{-1})1/8" gauge
T_1 - Seed treatment @ 5 g/kg seed	3.70	27.3	20.42
T_2 - Seed treatment @ 10 g/kg seed	3.71	27.4	20.43
T_3 - Soil application @ 5 g/kg soil	3.18	27.6	20.65
T_4 - Soil application @ 10 g/kg soil	3.81	28.4	21.26
T_5 - Carbofuran @ 1 kg a.i./ha	3.78	27.6	20.62
T_6 - Control	3.70	27.2	20.31
CD (P=0.05)	**NS**	**0.13**	**0.21**

Nematode alone recorded least value of fibre fineness, mean fibre length and bundle strength with 3.70, 27.2 and 20.31 respectively which were on par with seed treatment with VAM at both dosages (Table 27).

4.3. Effect of VAM and different biocontrol agents for the management of *R. reniformis* on cotton cv. MCU 5

All the biocontrol agents *viz.*, *G. mosseae*, *P. fluorescens* and *T. viride* as both seed and soil application increased the plant growth parameters compared to control. Among them, soil application of *G. mosseae* recorded maximum growth followed by seed treatment with *P. fluorescens*. These two treatments were on par with each other in improving growth characters *viz.*, shoot and root length, fresh weight of shoot and root, dry weight of shoot and root and leaf area index. Soil application of *G. mosseae* recorded 92.6 cm and 28.2 cm of shoot and root length respectively which was 42.9 and 151.7 per cent increase over control (Table 28). Seed treatment of *P. fluorescens* registered 89.4 cm and 26.6 cm shoot and root length respectively which were 37.9 and 137.5 per cent increase over control (Plates 20 and 21).

Similarly fresh weight of shoot (44.3 g) and root (25.6 g) were higher in soil application with *G. mosseae* which were 119.3 and 148.5 per cent increase over control. It was on par with *P. fluorescens* seed treatment with 42.1 g and 24 .5 g of shoot and root weight. Further, dry weight of shoot and root were also maximum in soil application with *G. mosseae* (11.0 and 6.4 g) and it was on par with *P. fluorescens* as seed treatment (10.5 and 6.1 g) (Fig.17).

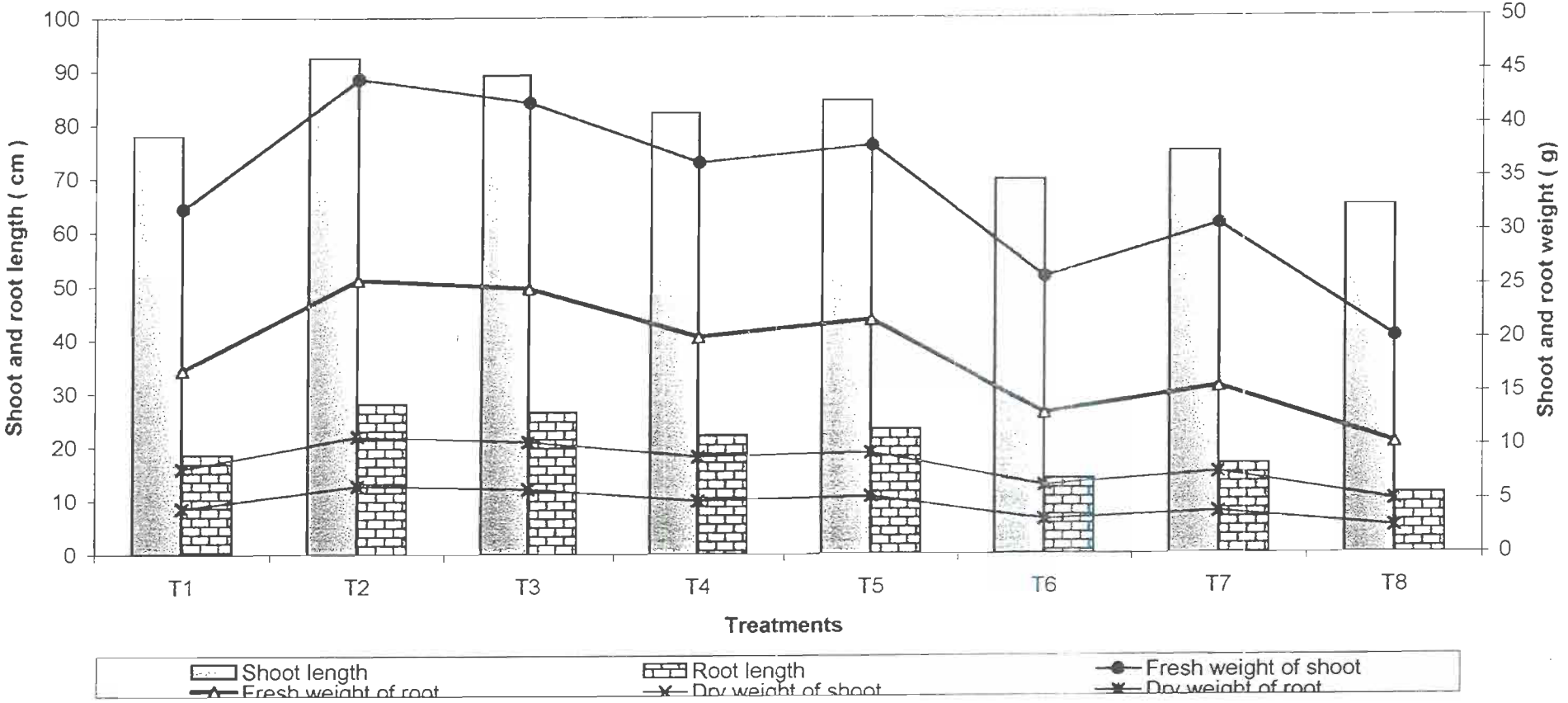
Among the different biocontrol agents tried, *G. mosseae* and *P. fluorescens* performed well in improving plant growth parameters and they were significantly

Table 28. Effect of VAM and different biocontrol agents on growth parameters of cotton cv. MCU 5 inoculated with *R. reniformis*

(mean of three replications)

Treatments	Length (cm)		Fresh weight (g)		Dry weight (g)	
	Shoot	Root	Shoot	Root	Shoot	Root
T_1 - Seed treatment with *G. mosseae* @ 10 g/kg seed	78.1	18.7	32.2	17.2	8.0	4.3
T_2 - Soil application with *G. mosseae* @ 10 g/kg soil	92.6	28.2	44.3	25.6	11.0	6.4
T_3 - Seed treatment with *P. fluorescens* @ 10 g/kg seed	89.4	26.6	42.1	24.5	10.5	6.1
T_4 - Soil application with *P. fluorescens* @ 2.5 kg/ha	82.3	22.3	36.5	20.3	9.1	5.0
T_5 - Seed treatment with *T. viride* @ 4 g/kg seed	84.6	23.4	38.1	21.9	9.5	5.4
T_6 - Soil application with *T. viride* @ 2.5 kg/ha	69.7	14.1	25.8	13.1	6.4	3.2
T_7 - Carbofuran @ 1 kg a.i./ha	74.9	16.9	30.7	15.6	7.6	3.9
T_8 - Control	64.8	11.2	20.2	10.3	5.0	2.5
CD (P=0.05)	**3.7**	**2.4**	3.7	2.2	0.93	0.56

Fig.17. Effect of VAM and different biocontrol agents against *R.reniformis* on growth parameters of cotton



T1 - VAM as seed treatment T2 - VAM as soil application T3 - *P. fluorescens* as seed treatment T4 - *P. fluorescens* in soil application
T5 - *T. viride* as seed treatment T6 - *T. viride* as soil application T7 - Carbofuran T8 - Control

Plate 20. Effect of different biocontrol agents against *R. reniformis* on growth of cotton cv. MCU 5.

Treatments:

1 - *G. mosseae* as seed treatment

2 - *G. mosseae* as soil application

3 - *P. fluorescens* as seed treatment

4 - *P. fluorescens* as soil application

5 - *T. viride* as seed treatment

6 - *T. viride* as soil application

7 - Carbofuran

8 - Control

Plate 20a

Plate 20b

Plate 21. Effect of different biocontrol agents against *R. reniformis* on root growth of cotton cv. MCU 5.

Treatments:

1 - *G. mosseae* as seed treatment

2 - *G. mosseae* as soil application

3 - *P. fluorescens* as seed treatment

4 - *P. fluorescens* as soil application

5 - *T. viride* as seed treatment

6 - *T. viride* as soil application

7 - Carbofuran

8 - Control

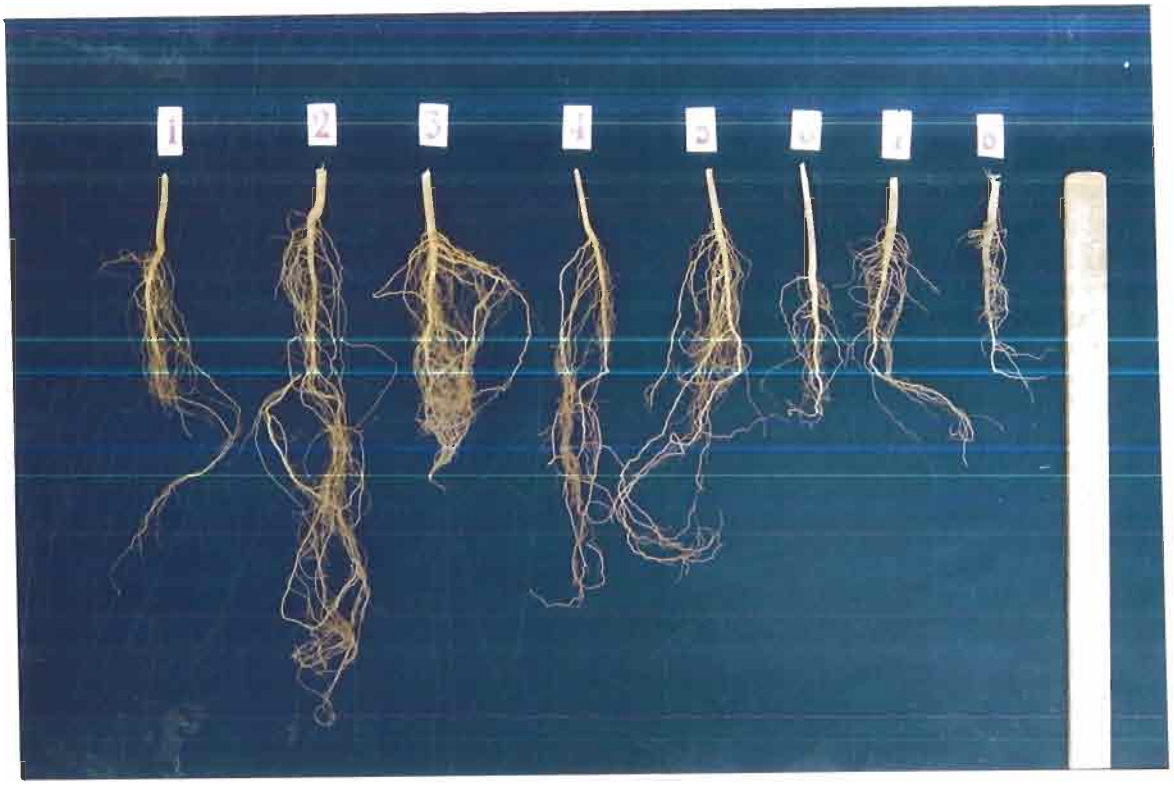


Plate 21

different from all other treatments. Soil application with *T.viride* recorded least value with 69.7 cm, 14.1 cm, 25.8 g, 13.1 g, 6.4 g, 3.2 g and 1.84 of shoot and root length, fresh weight of shoot and root, dry weight of shoot and root and leaf area index respectively.

The maximum 'P' content was observed in soil application of *G. mosseae* with 0.413% which was 220.1 per cent increase over control. It was followed by seed treatment with *P. fluorescens* (0.312%) and seed treatment with *G. mosseae* (0.287%) which were on par with each other. The least recorded in nematode alone treatment with 0.129% (Table 29) (Fig.18).

Colonization of VAM was maximum in soil application with 82.5 per cent than seed treatment by VAM. *P. fluorescens* as seed treatment recorded maximum (126 x 10^8 cfu) colony forming units than as soil treatment. *T. viride* as seed treatment recorded maximum colony forming units than as soil application (Table 29).

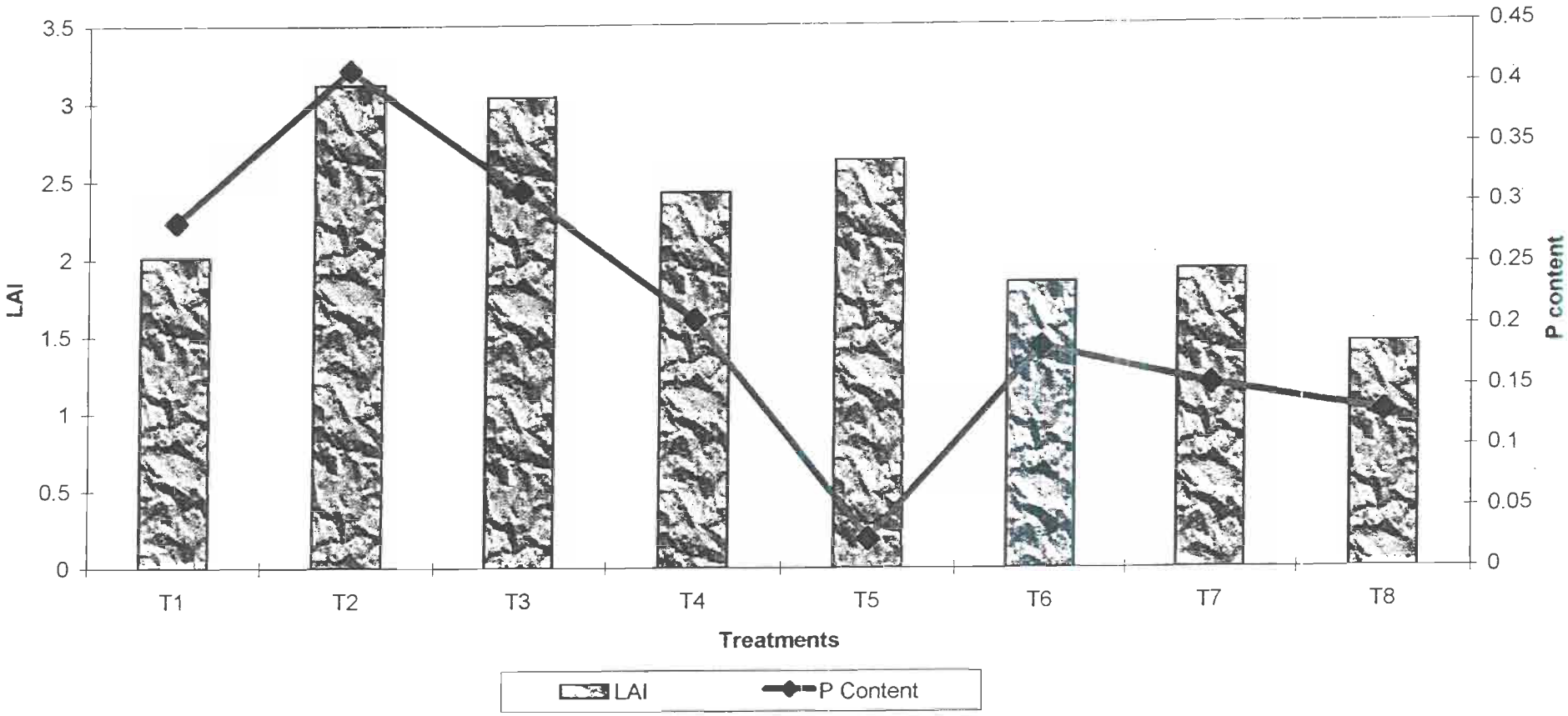
The maximum number of females and females with egg mass were recorded on nematode alone treatment. All the biocontrol agents found to reduce nematode population in roots (Table 30). *G. mosseae* recorded maximum reduction of number of females and females with egg masses with 72.1 and 76.8 per cent decrease over nematode alone. It was on par with the next best treatment, seed treatment with *P. fluorescens* with 68.5 and 72.2 per cent decrease over nematode alone. Soil application of *T. viride* recorded least reduction which was 14.4 and 12.9 per cent decrease of number of females and number of females with egg masses respectively (Fig.19).

Table 29. **Effect of VAM and different biocontrol agents on LAI, 'P' content and colonization of biocontrol agents in cotton cv. MCU 5 inoculated with *R. reniformis***

(mean of three replications)

Treatments	Leaf Area Index	'P' content in shoot (%)	Colonization by biocontrol agents
T_1 - Seed treatment with *G. mosseae* @ 10 g/kg seed	2.01	0.287	54.6%
T_2 - Soil application with *G. mosseae* @ 10 g/kg soil	3.12	0.413	82.5%
T_3 - Seed treatment with *P. fluorescens* @ 10 g/kg seed	3.04	0.312	126×10^8 cfu
T_4 - Soil application with *P. fluorescens* @ 2.5 kg/ha	2.42	0.206	87×10^8 cfu
T_5 - Seed treatment with *T. viride* @ 4 g/kg seed	2.63	0.240	11×10^3 cfu
T_6 - Soil application with *T. viride* @ 2.5 kg/ha	1.84	0.183	12×10^3 cfu
T_7 - Carbofuran @ 1 kg a.i./ha	1.92	0.152	-
T_8 - Control	1.45	0.129	-
CD (P=0.05)	0.38	0.06	-

Fig.18. Effect of VAM and different bio control agents against *R. reniformis* on LAI and P content of cotton



T1 - VAM as seed treatment T2 - VAM as soil application T3 - *P. fluorescens* as seed treatment T4 - *P. fluorescens* as soil application
T5 - *T. viride* as seed treatment T6 - *T. viride* as soil application T7 - Carbofuran T8 - Control

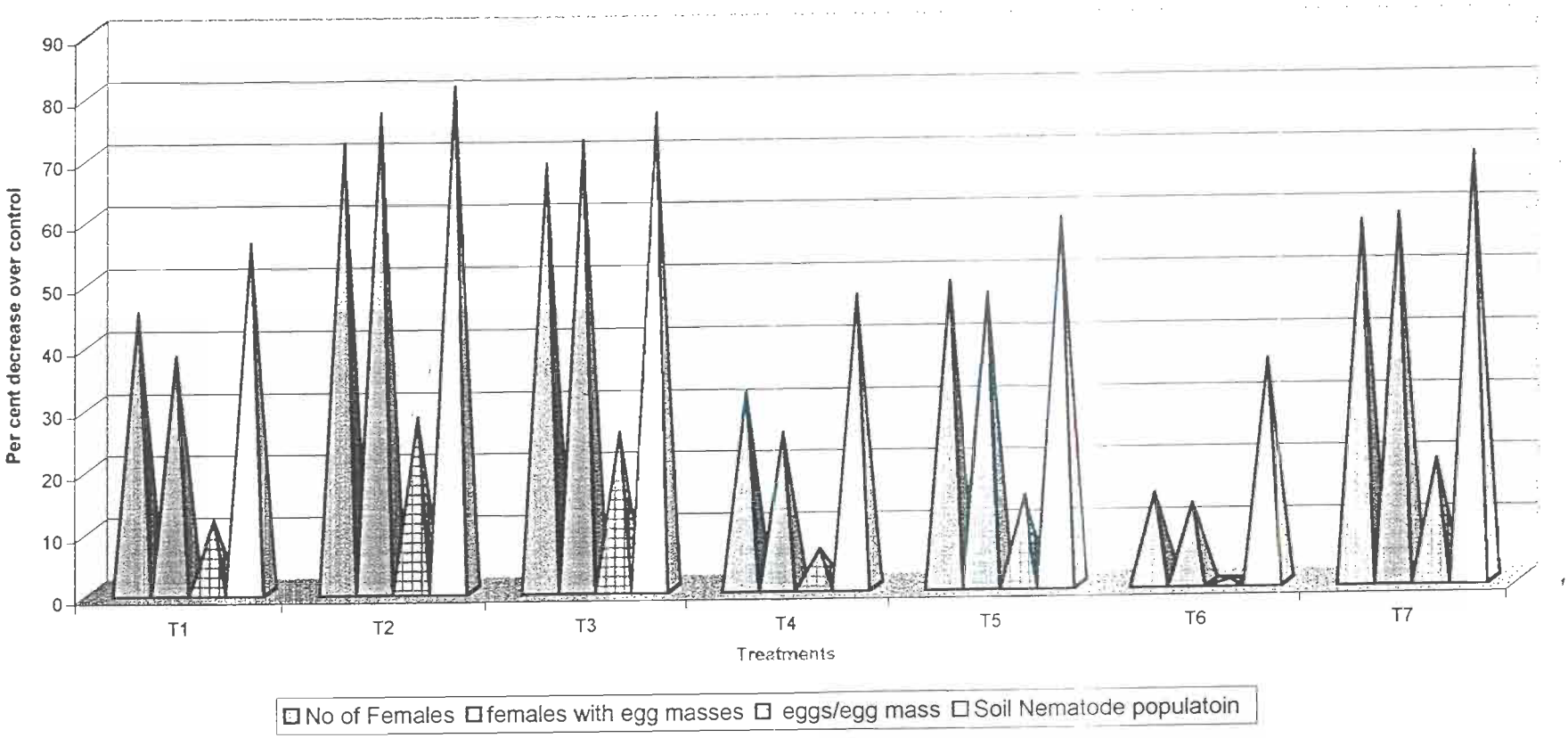
Table 30. Effect of VAM and different biocontrol agents on population of *R. reniformis* in cotton cv. MCU 5

(mean of three replications)

Treatments	Number of females per plant	Number of females with egg masses/ plant	Number of eggs per egg mass	Soil nematode population/ 100 g soil
T_1 - Seed treatment with *G. mosseae* @ 10 g/kg seed	135.9 (45.0)	87.2 (38.1)	50.9 (11.6)	295.0 (56.7)
T_2 - Soil application with *G. mosseae* @ 10 g/kg soil	68.6 (72.1)	32.6 (76.8)	41.5 (27.9)	128.6 (81.1)
T_3 - Seed treatment with *P. fluorescens* @ 10 g/kg seed	77.3 (68.5)	39.1 (72.2)	43.0 (25.3)	160.3 (76.4)
T_4 - Soil application with *P. fluorescens* @ 2.5 kg/ha	167.9 (31.7)	105.8 (25.0)	54.0 (6.2)	360.6 (47.1)
T_5 - Seed treatment with *T. viride* @ 4 g/kg seed	125.3 (49.0)	74.6 (47.1)	49.3 (14.4)	274.6 (59.7)
T_6 - Soil application with *T. viride* @ 2.5 kg/ha	209 (14.0)	122.8 (12.9)	57.3 (0.5)	434.6 (36.2)
T_7 - Carbofuran @ 1 kg a.i./ha	102 (58.2)	57.7 (59.1)	46.3 (19.6)	212.3 (68.8)
T_8 - Control	245.9	141.1	57.6	682.0
CD (P=0.05)	**15.4**	**14.5**	**2.3**	**37.5**

* Figures in the parenthesis per cent decrease over control .

Fig.19. Effect of VAM and different biocontrol agents on population of *R.reniformis* in cotton



T1 - VAM as seed treatment T2 - VAM as soil application T3 - *P. fluorescens* as seed tre

fluorescens in soil application

T5 - *T. viride* as seed treatment T6 - *T. viride* as soil application T7 - Carbofuran T8 -

The number of eggs/eggmass was least in soil application of *G. mosseae* which was 27.9 per cent decrease over control. It was on par with seed treatment with *P. fluorescens* which recorded 25.3 per cent decrease over control. The maximum number of eggs/eggmass recorded in nematode alone control with 57.6 (Table 30).

Reduction in soil nematode population was maximum in plants treated with VAM applied in soil with 81.1 per cent reduction over control. It was on par with *P. fluorescens* as seed treatment with 160.3 which was 76.4 per cent decrease over control. Nematode alone recorded maximum of 682 nematodes/100 g soil (Table 30).

Among different biocontrol agents tested, soil application with *G. mosseae* recorded maximum number of bolls per plant (19.3) with 101.4 per cent increase over control. The effect of this treatment was on par with seed treatment with *P. fluorescens* with 18.6 which was 93.7 per cent increase over control. Nematode alone recorded least value (9.6). Similarly maximum increase in boll weight was noticed in soil application with *G. mosseae* which recorded 3.92 g which was on par with seed treatment with *P. fluorescens* (3.83). Among all biocontrol agents *T. viride* as soil application recorded low value of number of bolls (11.3) and boll weight (2.58 g) than other biocontrol agents (Table 31).

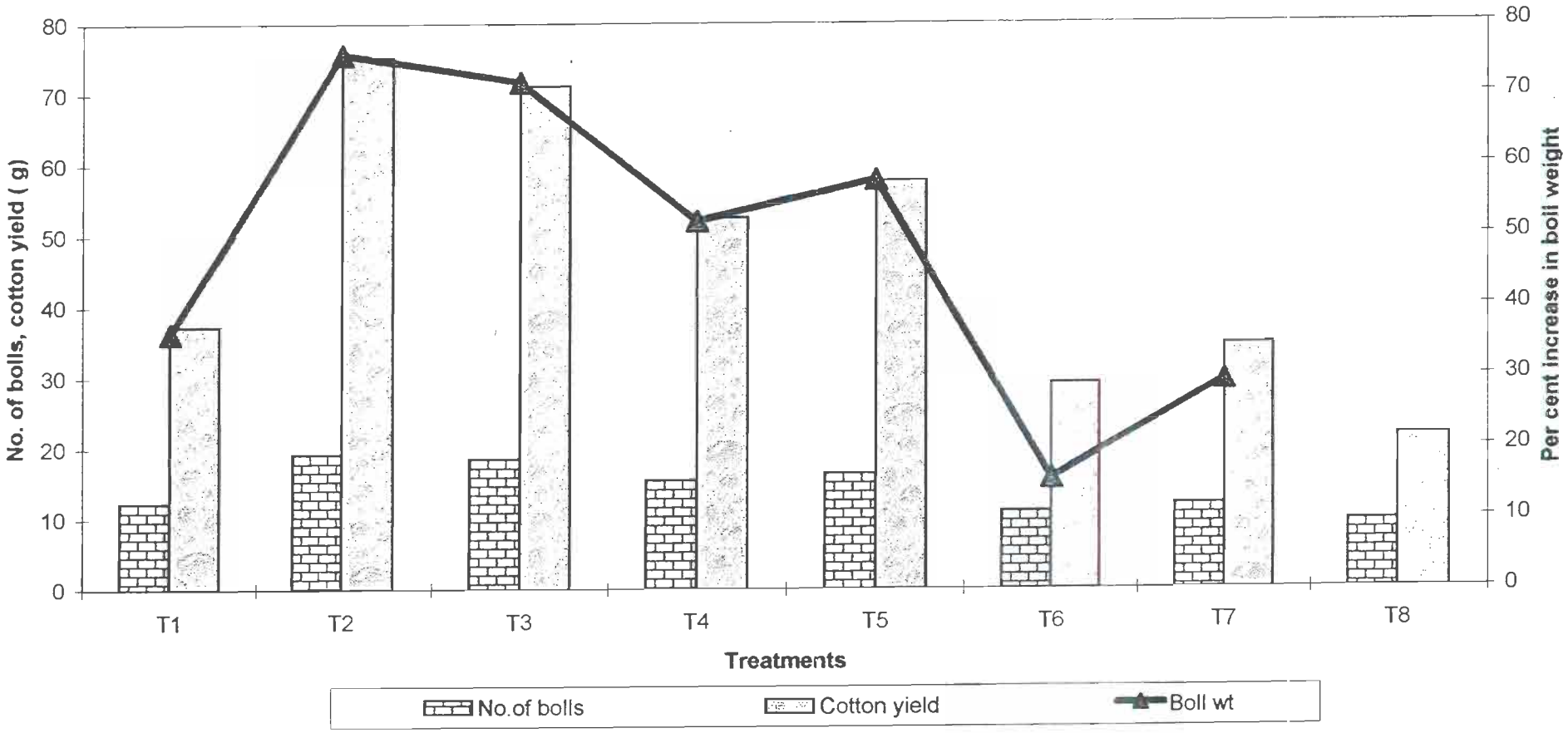
Further, application of *G. mosseae* recorded maximum cotton yield per plant with 75.6 g which was 250.0 per cent increase over control. However, it was on par with seed treatment with *P. fluorescens* which recorded 71.2 g. Nematode

Table 31. Effect of VAM and different biocontrol agents on yield parameters of cotton cv. MCU 5 inoculated with *R. reniformis*

(mean of three replications)

Treatments	Number of bolls per plant	Mean boll weight (g)	Cotton yield per plant (g)
T_1 - Seed treatment with *G. mosseae* @ 10 g/kg seed	12.3	3.04	37.3
T_2 - Soil application with *G. mosseae* @ 10 g/kg soil	19.3	3.92	75.6
T_3 - Seed treatment with *P. fluorescens* @ 10 g/kg seed	18.6	3.83	71.2
T_4 - Soil application with *P. fluorescens* @ 2.5 kg/ha	15.5	3.39	52.5
T_5 - Seed treatment with *T. viride* @ 4 g/kg seed	16.4	3.52	57.7
T_6 - Soil application with *T. viride* @ 2.5 kg/ha	11.3	2.58	29.1
T_7 - Carbofuran @ 1 kg a.i./ha	12.0	2.89	34.6
T_8 - Control	9.6	2.23	21.6
CD (P=0.05)	**1.2**	**0.26**	**5.5**

Fig.20. Effects of VAM and different biocontrol agents against *R.reniformis* on yield parameters of cotton



T1 - VAM as seed treatment T2 - VAM as soil application T3 - *P. fluorescens* as seed treatment T4 - *P. fluorescens* in soil application
T5 - *T. viride* as seed treatment T6 - *T. viride* as soil application T7 - Carbofuran T8 - Control

alone recorded least cotton yield with 21.6 g which was significantly different from all other treatments (Table 31 and Fig.20).

4.4. Effect of VAM and different biocontrol agents for the management of *R. reniformis* - *Fusarium oxysporum* f. sp. *vasinfectum* wilt disease complex on cotton cv. MCU 5

Generally there was an improvement in all plant growth parameters in all the biocontrol agents treated plants receiving either as seed treatment or as soil application in comparison to control (inoculated with *R. reniformis* + *F. oxysporum* .sp. f. *vasinfectum* - untreated). The maximum shoot and root length was recorded in soil application with *G. mosseae* with 82.3 cm and 24.3 cm which was 42.8 and 138.2 per cent increase over control. Both the treatments were on par with each other and significantly different from other treatments. The minimum shoot and root length was recorded in control with 57.6 cm and 10.2 cm. Among biocontrol agents, *T. viride* as soil application recorded lowest value of 68.2 cm and 15.7 cm of shoot and root length respectively (Table 32; Plates 22 and 23).

Fresh weight of shoot and root were 38.6g and 26.1g and dry weight of shoot and root were 9.65g and 6.52g when *G. mosseae* was applied as soil treatment. It was on par with next best treatment, *P. fluorescens* as seed treatment with 37.2g, 25.2g, 9.3g and 6.3g of fresh weight and dry weight of shoot and roots respectively over control. The control recorded minimum value of fresh weight as 17.6 g and 9.6 g and dry weight as 4.40 g and 2.40 g (Table 32 and Fig.21).

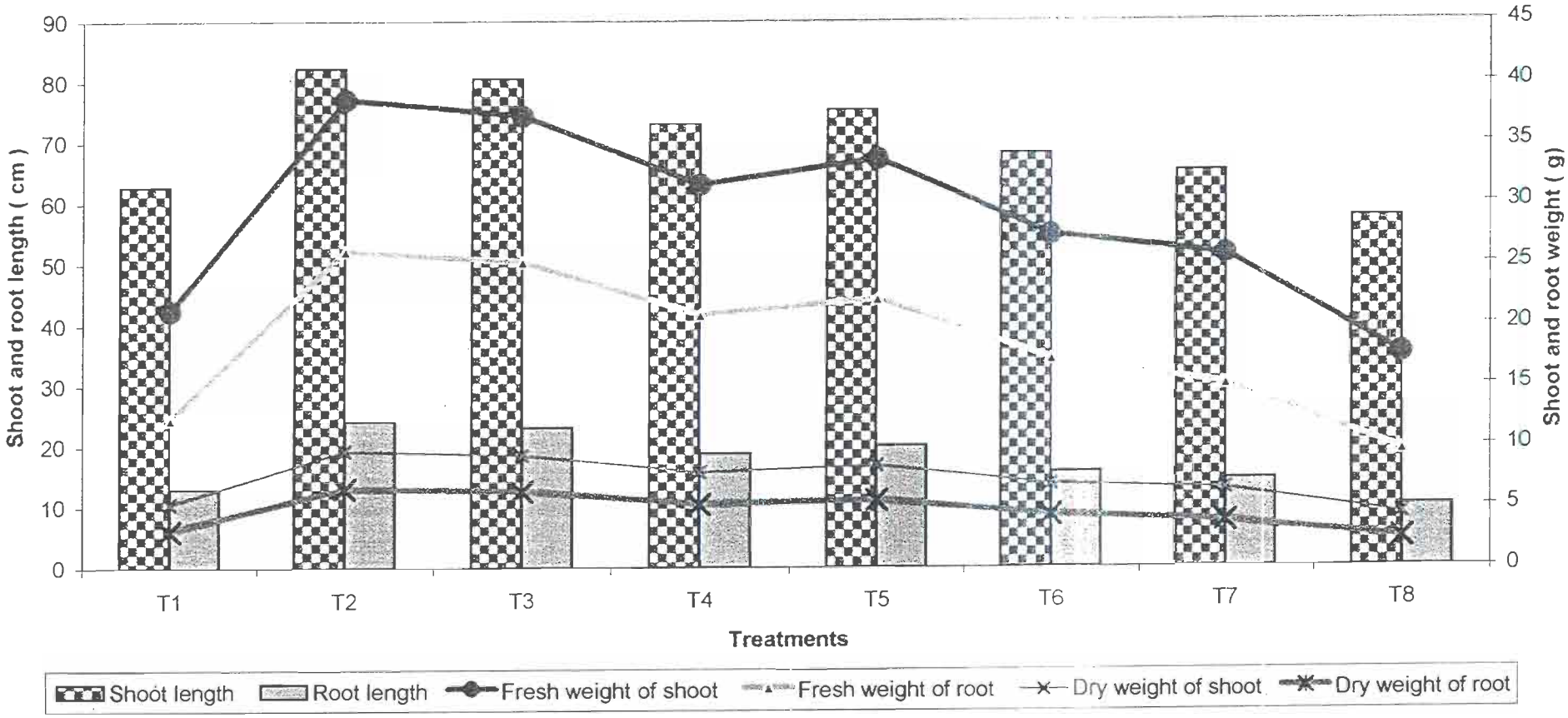
All the biocontrol agents were effective in increasing the leaf area index and shoot 'P' content compared to control (Table 33). But soil application with

Table 32. **Effect of VAM and different biocontrol agents on growth of cotton cv. MCU 5 infected with *R. reniformis* and *F. oxysporum* f. sp. *vasinfectum***

(mean of three replications)

Treatments	Length (cm)		Fresh weight (g)		Dry weight (g)	
	Shoot	Root	Shoot	Root	Shoot	Root
T_1 - Seed treatment with *G. mosseae* @ 10 g/kg seed	62.8	13.1	21.2	12.3	5.30	3.07
T_2 - Soil application with *G. mosseae* @ 10 g/kg soil	82.3	24.3	38.6	26.1	9.65	6.52
T_3 - Seed treatment with *P. fluorescens* @ 10 g/kg seed	80.6	23.2	37.2	25.2	9.30	6.30
T_4 - Soil application with *P. fluorescens* @ 2.5 kg/ha	73.1	18.8	31.5	20.8	7.87	5.20
T_5 - Seed treatment with *T. viride* @ 4 g/kg seed	75.4	20.1	33.6	22.1	8.40	5.52
T_6 - Soil application with *T. viride* @ 2.5 kg/ha	68.2	15.7	27.4	17.2	6.85	4.30
T_7 - Carbofuran @ 1 kg a.i./ha	65.2	14.5	25.8	15.1	6.45	3.77
T_8 - Control	57.6	10.2	17.6	9.6	4.40	2.40
CD (P=0.05)	**4.76**	**2.84**	**3.24**	**2.62**	**0.81**	**0.65**

Fig.21. Effect of VAM and other biocontrol agent on growth of cotton infected with *R.reniformis* -F.o.f.sp.vasinfectum



T1 - VAM as seed treatment T2 - VAM as soil application T3 - *P. fluorescens* as seed
T5 - *T. viride* as seed treatment T6 - *T. viride* as soil application T7 - Carbofuran T8

Plate 22. Effect of different biocontrol agents against *R. reniformis* and Fusarium wilt disease complex on growth of cotton cv. MCU 5.

Treatments:

1 - *G. mosseae* as seed treatment

2 - *G. mosseae* as soil application

3 - *P. fluorescens* as seed treatment

4 - *P. fluorescens* as soil application

5 - *T. viride* as seed treatment

6 - *T. viride* as soil application

7 - Carbofuran

8 - Control

Plate 22a

Plate 22b

Plate 23. Effect of different biocontrol agents against *R. reniformis* and Fusarium wilt disease complex on root growth of cotton cv. MCU 5.

Treatments:

1 - *G. mosseae* as seed treatment

2 - *G. mosseae* as soil application

3 - *P. fluorescens* as seed treatment

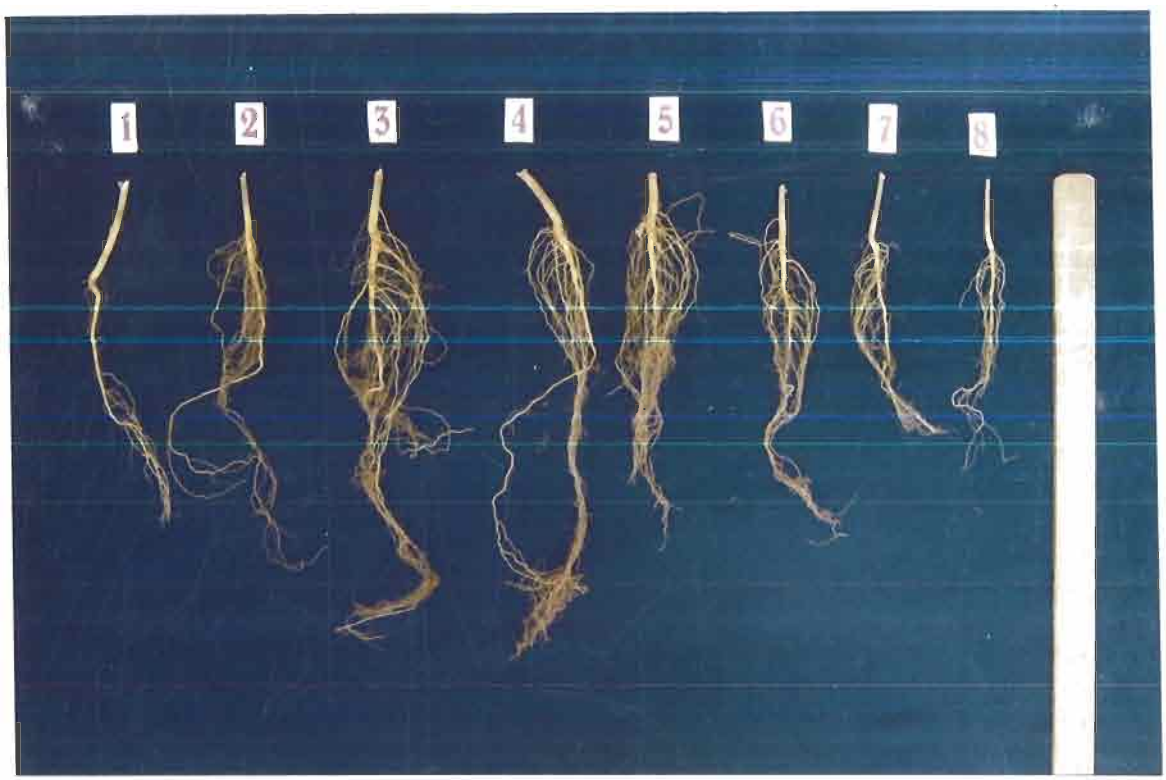
4 - *P. fluorescens* as soil application

5 - *T. viride* as seed treatment

6 - *T. viride* as soil application

7 - Carbofuran

8 - Control


1
2
3
4
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6
7
8

G. mosseae gave the maximum increase in LAI (2.86) and 'P' content (0.382%) and it was on par with seed treatment with *P. fluorescens* but significantly different from all other treatments (Fig.22).

Colonization of VAM was maximum in soil application with VAM with 71.5 per cent than seed treatment by VAM. *P. fluorescens* as seed treatment recorded maximum (110 x 108 cfu) colony forming units then as soil treatment. *T. viride* as seed treatment recorded maximum colony forming units then as soil application (Table 33).

The data on number of females and number of females with egg masses in plant roots showed significant reduction in the treatment of soil application of *G. mosseae* (58.3 and 28.3) and seed treatment with *P. fluorescens* (73.6 and 34.8) which were on par with each other in reducing nematode population (Table 34). Control recorded maximum number of females (222.30) and females with egg masses (116.6).

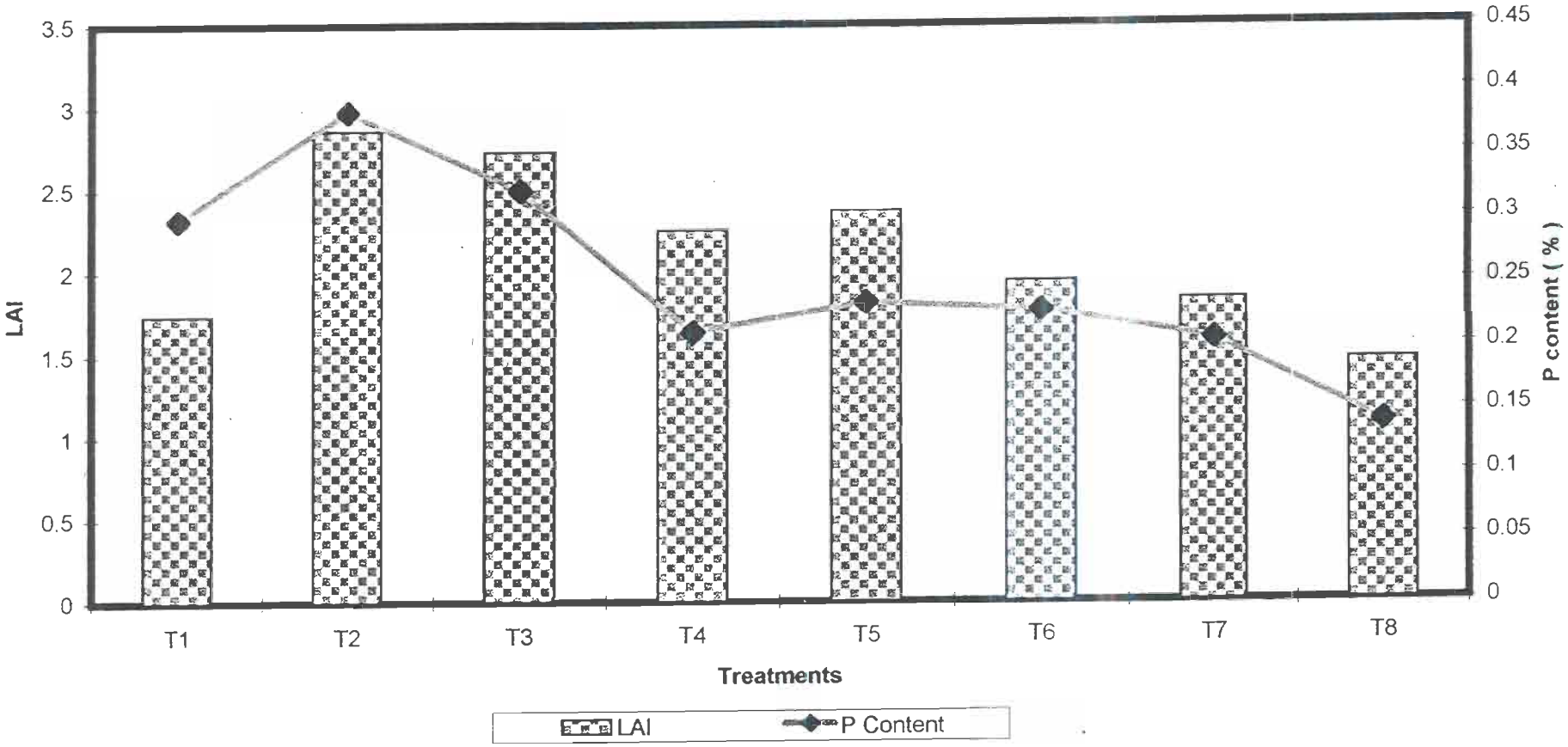
G. mosseae, *P. fluorescens* and *T. viride* either seed treatment or as soil application reduced number of eggs/egg mass and soil nematode population. Soil application of *G. mosseae* and seed treatment of *P. fluorescens* performed better than *T. viride* in reducing number of eggs/egg mass and soil nematode population which were on par with each other. Per cent decrease of number of eggs/egg mass and soil nematode population was 25.4 and 81.2 in soil application of *G. mosseae* and it was 22.6 and 77.1 per cent in *P. fluorescens* as seed treatment in comparison to control (Table 34 and Fig.23).

Table 33. **Effect of VAM and different biocontrol agents on LAI, 'P' content and colonization of biocontol agents in cotton cv. MCU 5 infected with *R. reniformis* and *F. oxysporum* f.sp. *vesinfectum***

(mean of three replications)

Treatments	Leaf Area Index	'P' content in shoot (%)	Colonization by biocontrol agents
T_1 - Seed treatment with *G. mosseae* @ 10 g/kg seed	1.74	0.298	45.5%
T_2 - Soil application with *G. mosseae* @ 10 g/kg soil	2.86	0.382	71.5%
T_3 - Seed treatment with *P. fluorescens* @ 10 g/kg seed	2.73	0.321	$110x10^8$ cfu
T_4 - Soil application with *P. fluorescens* @ 2.5 kg/ha	2.25	0.210	45×10^2 cfu
T_5 - Seed treatment with *T. viride* @ 4 g/kg seed	2.37	0.233	11×10^3 cfu
T_6 - Soil application with *T. viride* @ 2.5 kg/ha	1.94	0.227	13×10^2 cfu
T_7 - Carbofuran @ 1 kg a.i./ha	1.83	0.204	-
T_8 - Control	1.46	0.139	-
CD (P=0.05)	**0.24**	**0.054**	-

Fig.22. Efect of VAM and different biocontrol agents on LAI and P content of cotton infected with *R.reniformis* -Fusarium



T1 - VAM as seed treatment T2 - VAM as soil application T3 - *P. fluorescens* as seed treatment T4 - *P. fluorescens* n soil application
T5 - *T. viride* as seed treatment T6 - *T. viride* as soil application T7 - Carbofuran T8 - Contro

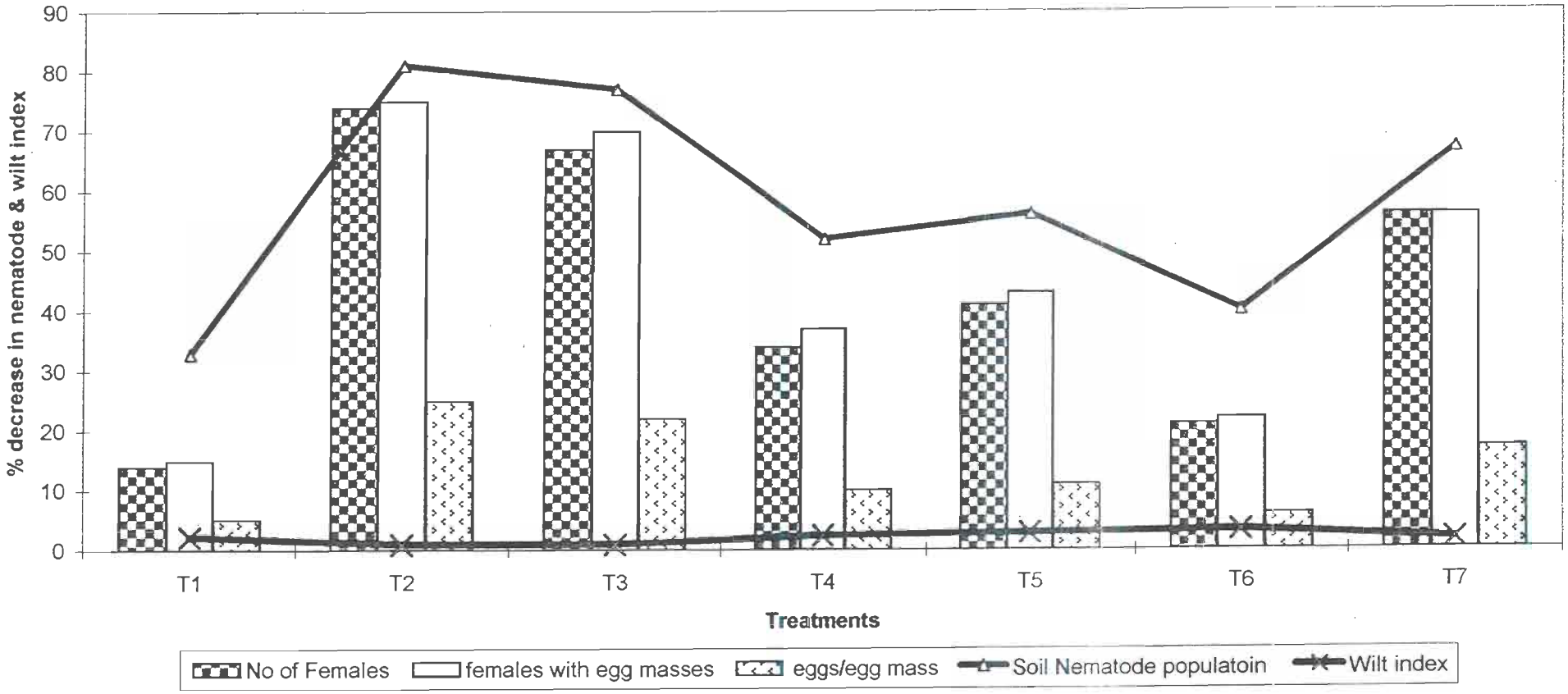
Table 34. **Effect of VAM and different biocontrol agents on nematode population and wilt index of cotton cv. MCU 5 infected with *R. reniformis* and *F. oxysporum* f. sp. *vesinfectum***

(mean of three replications)

Treatments	Number of females per plant	Number of females with eggmasses/plant	Number of eggs per egg mass	Soil nematode population/100 g/soil	Wilt index
T_1 - Seed treatment with *G. mosseae* @ 10 g/kg seed	193.3 (14.2)	98.0 (15.1)	53.9 (5.2)	481.3 (33.2)	2.2
T_2 - Soil application with *G. mosseae* @ 10 g/kg soil	58.3 (74.1)	28.3 (75.2)	42.6 (25.4)	116.0 (81.2)	0.9
T_3 - Seed treatment with *P. fluorescens* @ 10 g/kg seed	73.6 (67.4)	34.8 (70.4)	43.9 (22.6)	139.6 (77.1)	0.9
T_4 - Soil application with *P. fluorescens* @ 2.5 kg/ha	14.9 (34.1)	72.6 (37.0)	50.9 (10.4)	299.3 (52.1)	2.3
T_5 - Seed treatment with *T. viride* @ 4 g/kg seed	132.9 (41.5)	65.4 (43.1)	50.3 (11.2)	276.6 (56.4)	2.8
T_6 - Soil application with *T. viride* @ 2.5 kg/ha	177.6 (21.2)	90.9 (22.3)	53.4 (6.2)	375.4 (40.2)	3.2
T_7 - Carbofuran @ 1 kg a.i./ha	98.9 (56.2)	50.1 (56.2)	47.0 (17.1)	204.3 (67.3)	1.7
T_8 - Control	225.3	116.6	56.9	628.4	4.2
CD (P=0.05)	**22.5**	**11.5**	**2.84**	**55.4**	-

* Figures in the parentheses are per cent decrease over control.

Fig.23. Effect of VAM and different bio control agents on nematode population and wilt index



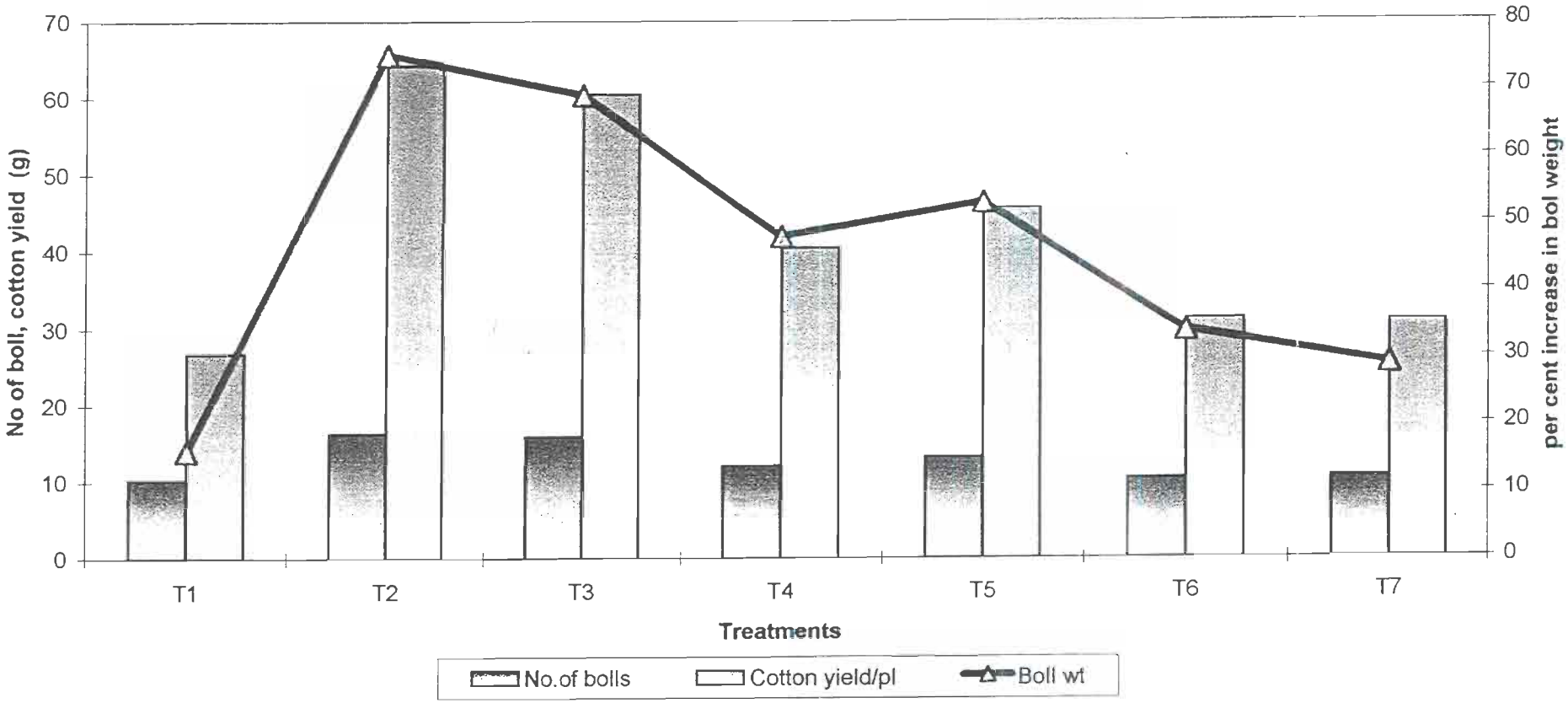
T1 - VAM as seed treatment T2 - VAM as soil application T3 - *P. fluorescens* as seed treatment T4 - *fluorescens* in soil application
T5 - *T. viride* as seed treatment T6 - *T viride* as soil application T7 - Carbofuran T8 - Control

Table 35. **Effect of VAM and different biocontrol agents on yield attributes of cotton cv. MCU 5 infected with *R. reniformis* and *F. oxysporum* f. sp. *vesinfectum***

(mean of three replications)

Treatments	Number of bolls per plant	Mean boll weight (g)	Cotton yield per plant (g)
T_1 - Seed treatment with *G. mosseae* @ 10 g/kg seed	10.3	2.61	26.8
T_2 - Soil application with *G. mosseae* @ 10 g/kg soil	16.3	3.94	64.2
T_3 - Seed treatment with *P. fluorescens* @ 10 g/kg seed	15.9	3.81	60.6
T_4 - Soil application with *P. fluorescens* @ 2.5 kg/ha	12.1	3.35	40.5
T_5 - Seed treatment with *T. viride* @ 4 g/kg seed	13.2	3.46	45.6
T_6 - Soil application with *T. viride* @ 2.5 kg/ha	10.3	3.03	31.2
T_7 - Carbofuran @ 1 kg a.i./ha	10.6	2.92	30.9
T_8 - Control	9.5	2.25	21.3
CD (P=0.05)	**O.74**	**0.282**	**4.52**

Fig.24. Effect of VAM and different biocontrol agent on yield parameters of cotton infected with *R.reniformis* - Fusarium



T1 - VAM as seed treatment T2 - VAM as soil application T3 - *P. fluorescens* as seed treatment T4 - *P. fluorescens* in soil application
T5 - *T. viride* as seed treatment T6 - *T. viride* as soil application T7 - Carbofuran T8 - Control

The wilt index in all the treatments with bioagents were significantly reduced. The highest reduction was observed in the treatment of soil application with *G. mosseae* (0.9) and seed treatment with *P. fluorescens* (0.9) which were on par with each other and significantly different from the rest. It was further observed that carbofuran treatment also significantly reduced wilt index (1.7) than control.

The number of bolls and mean boll weight in all the treatments with biocontrol agents were significantly increased. The highest was observed in soil application with *G. mosseae* (16.3 and 3.94 g) and seed treatment with *P. fluorescens* (15.9 and 3.81 g) which were on par with each other (Table 35). The number of bolls and boll weight were least in control with 9.5 and 2.25 g respectively.

The cotton yield was maximum in *G. mosseae* as soil application (64.2 g) and *P. fluorescens* as seed treatment (60.6 g) which were on par with each other and significantly different from rest of the treatments. It was 201.4 and 184.3 per cent increase over control (Fig.24).

4.5. Biochemical changes due to interaction of reniform nematode, Fusarium wilt fungus and *G. mosseae* on cotton cultivars MCU 5 and K 10

4.5.1. Total protein content (Table 36)

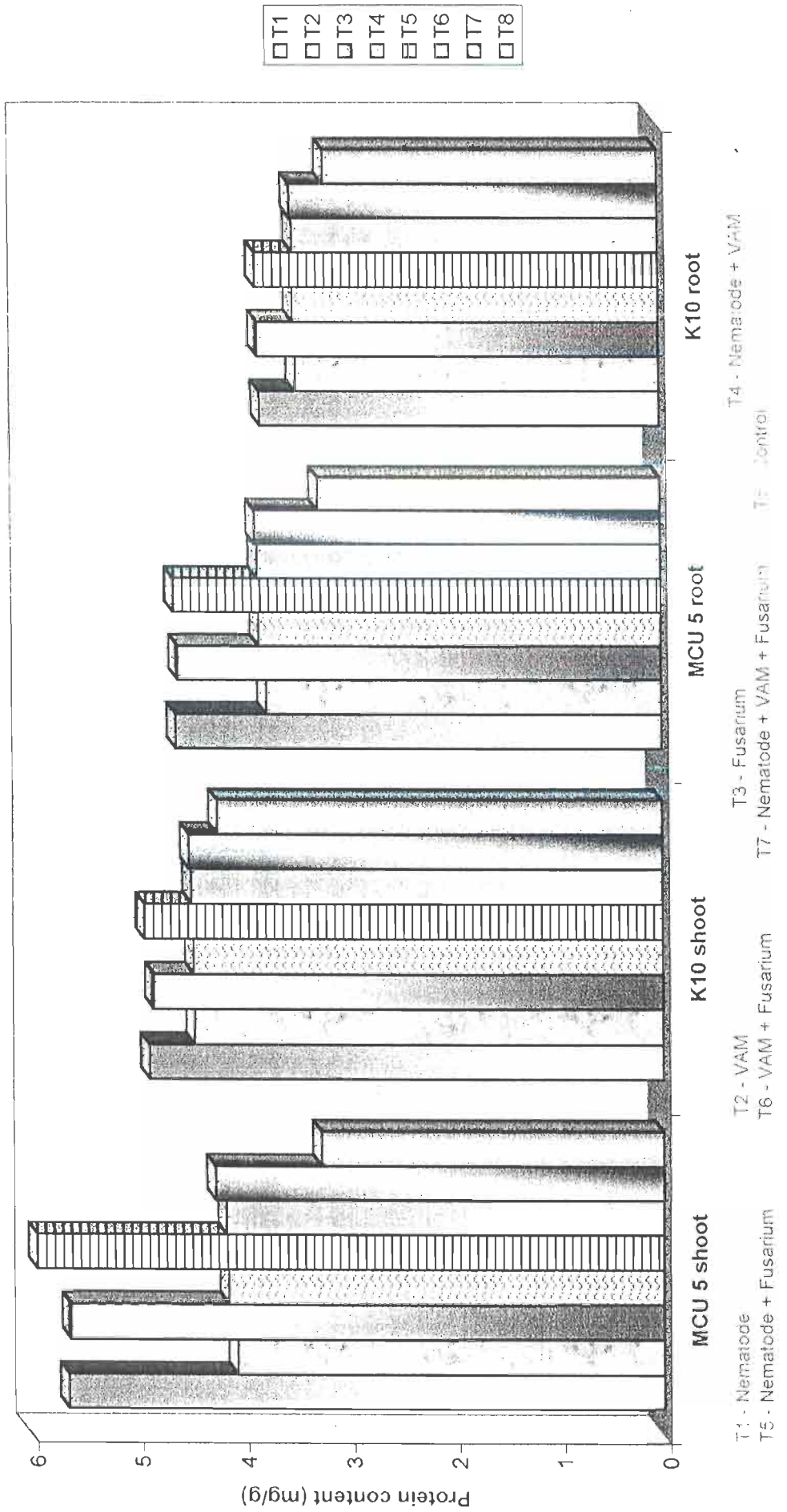
Shoot protein content was maximum in nematode + Fusarium treated plant with 5.94 mg/g and 4.91 mg/g in MCU 5 and K10 respectively, it was on par with nematode alone (5.64 mg/g and 4.86 mg/g respectively) and Fusarium alone (5.62

Table 36. Effect of VAM, *Fusarium* and *R. reniformis* on protein content of cotton cultivars, MCU 5 and K10

(mean of three replications)

Treatments	MCU 5				K10			
	Shoot (mg/g)	Per cent increase over control	Root (mg/g)	Per cent increase over control	Shoot (mg/g)	Per cent increase over control	Root (mg/g)	Per cent increase over control
T_1 - Nematode alone	5.64	74.0	4.60	43.3	4.86	15.4	3.78	19.6
T_2 - VAM	4.04	24.6	3.74	16.5	4.43	5.2	3.44	8.8
T_3 - *Fusarium*	5.62	73.4	4.58	42.6	4.82	14.4	3.80	20.2
T_4 - Nematode + VAM	4.12	27.1	3.81	18.6	4.44	5.4	3.46	9.4
T_5 - Nematode + *Fusarium*	5.94	83.3	4.62	43.9	4.91	16.6	3.82	20.8
T_6 - VAM + *Fusarium*	4.14	27.7	3.82	19.0	4.46	5.9	3.46	9.4
T_7 - Nematode+VAM+*Fusarium*	4.24	30.8	3.84	19.6	4.48	6.4	3.48	10.1
T_8 - Control	3.24	-	3.21	-	4.21	-	3.16	-
CD (P = 0.05)	**0.70**	-	**0.49**	-	**0.64**	-	**0.24**	-

Fig.25. Effect of VAM, Fusarium and *R. reniformis* on protein content of cotton cultivars (MCU 5 and K10)



mg/g and 4.82 mg/g on MCU 5 and K10 respectively) treatments. Least shoot protein was observed in control with 3.24 mg/g and 4.21 mg/g on MCU 5 and K10 respectively followed by VAM alone (4.04 mg/g and 4.43 mg/g respectively) and nematode + VAM (4.12 mg/g and 4.44 mg/g respectively). The same trend was maintained in root protein content on MCU 5 and K10 cultivars respectively.

In general, lower concentration of shoot and root protein was observed in control followed by VAM alone with pathogens in both cultivars. The higher concentration of protein was observed in nematode + Fusarium and it was on par with nematode and Fusarium alone treatments. However, extent of increase in protein content was varied among both cultivars. In MCU 5, nematode + Fusarium recorded 43.9 per cent increase of root protein over control, where as in K10 it was 20.8 per cent only (Fig.25).

4.5.2. Total phenol contents (Table 37)

In MCU 5, the total phenol content was maximum in VAM alone with 5.62 mg/g and 1.48 mg/g in shoot and root respectively, which was 36.7 and 46.5 per cent increase over control. This was on par with nematode + VAM (5.42 mg/g and 1.46 mg/g in shoot and root) and VAM + Fusarium (5.41 mg/g and 1.32 mg/g in shoot and root) and nematode + VAM + Fusarium (5.32 mg/g and 1.31 mg/g in shoot and root). The phenol content was least in control with 4.11 mg/g and 1.01 mg/g in shoot and root respectively. Nematode + Fusarium observed a slight increase in phenol content but there was no significant difference with control and it was on par with Fusarium alone and nematode alone treatments.

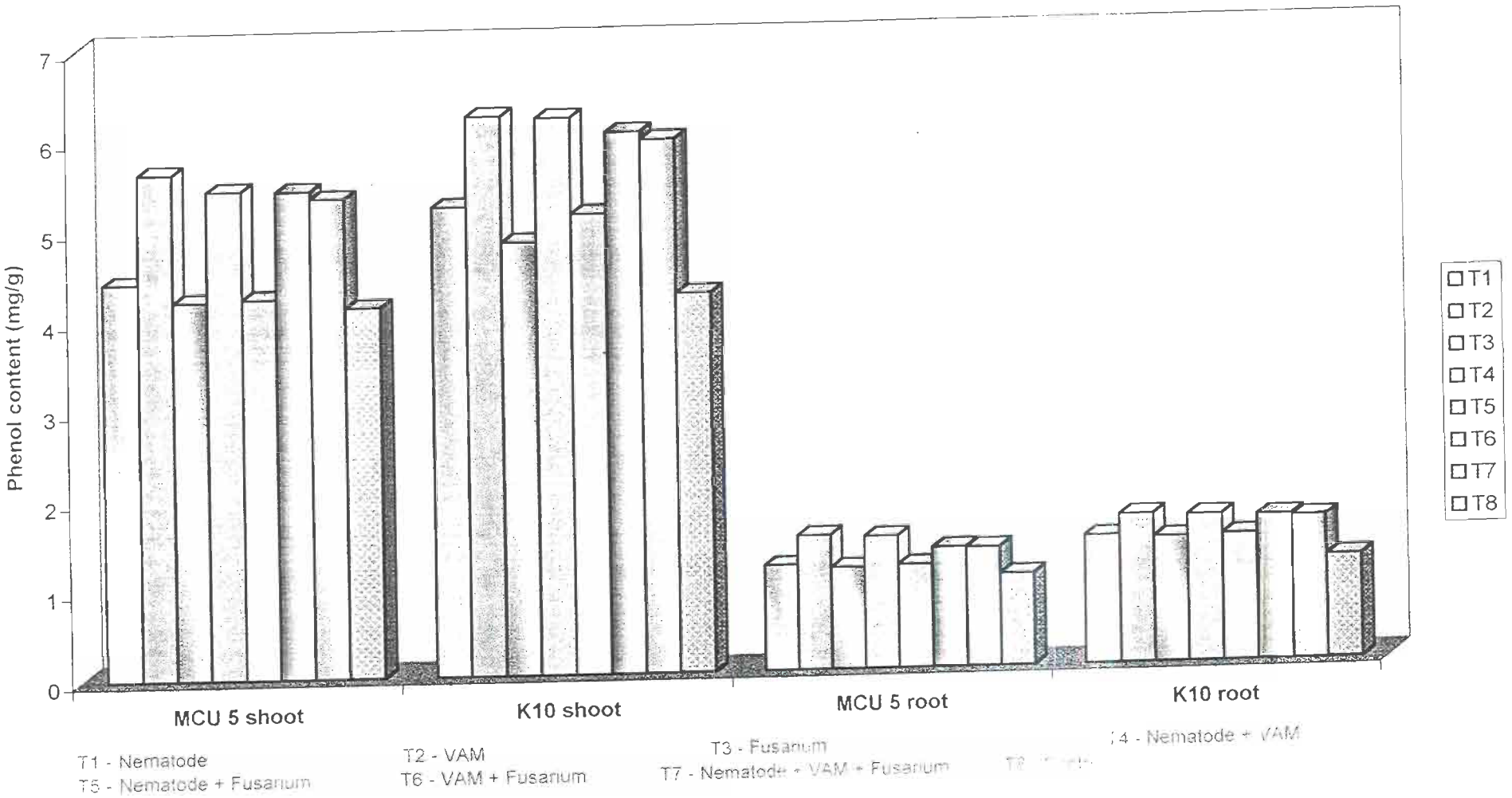
In K10, total phenol content was maximum in VAM alone with 47.5 and 43.8 per cent increase over control in shoot and root respectively. VAM along with

Table 37. Effect of VAM, *Fusarium* and *R. reniformis* on total phenol content of cotton cv. MCU 5 and K10

(mean of three replications)

Treatments	MCU 5				K10			
	Shoot (mg/g)	Per cent increase over control	Root (mg/g)	Per cent increase over control	Shoot (mg/g)	Per cent increase over control	Root (mg/g)	Per cent increase over control
T_1 - Nematode alone	4.41	7.2	1.16	14.8	5.21	23.7	1.41	23.6
T_2 - VAM	5.62	36.7	1.48	46.5	6.21	47.5	1.64	43.8
T_3 - *Fusarium*	4.20	2.1	1.12	10.8	4.81	14.2	1.38	21.0
T_4 - Nematode + VAM	5.42	31.8	1.46	44.5	6.18	46.7	1.62	42.1
T_5 - Nematode + *Fusarium*	4.22	2.6	1.14	12.8	5.11	21.3	1.40	22.8
T_6 - VAM + *Fusarium*	5.41	31.6	1.32	30.6	6.01	33.9	1.60	40.3
T_7 - Nematode+VAM+*Fusarium*	5.32	29.4	1.31	29.7	5.92	40.3	1.58	38.5
T_8 - Control	4.11	-	1.01	-	4.21	-	1.14	-
CD (P = 0.05)	**0.42**	-	**0.25**	-	**0.52**	-	**0.24**	-

Fig.26. Effect of VAM, Fusarium and *R. reniformis* on total phenol content of cotton cultivars (MCU 5 and K10)



nematode + Fusarium was on par with VAM alone treatment. Among pathogens (without VAM), nematode alone recorded maximum with 5.21 mg/g and 1.41 mg/g in shoot and root which was 23.7 and 23.6 per cent increase over control and it was on par with Fusarium alone and nematode + Fusarium treatments. Control plants recorded least with 4.21 mg/g and 1.14 mg/g (Fig.26).

4.5.3. Total sugar content (Table 38)

It was observed from the results that in MCU 5, the total sugars was more in VAM alone with 2.32 mg/g and 0.68 mg/g in shoot and root which was 85.6 and 151.8 per cent increase over control. The total sugar content of VAM alone treatment was on par with VAM + nematode (2.24 mg/g and 0.64 mg/g in shoot and root) and nematode + VAM + Fusarium (2.18 mg/g and 0.63 mg/g in shoot and root). Nematode + Fusarium recorded least amount with 0.86 mg/g and 0.16 mg/g which was on par with Fusarium alone and control. Nematode alone recorded slight increase of total sugar with 34.4 and 66.6 per cent increase over control in shoot and root respectively.

In K10 cultivars, total sugar was maximum in VAM alone (2.86 mg/g and 0.72 mg/g in shoot and root) followed by VAM along with nematode (2.82 mg/g and 0.68 mg/g), VAM + Fusarium (2.80 mg/g and 0.64 mg/g) and in all combination together (2.79 mg/g and 0.62 mg/g). The least was recorded in control with 1.62 mg/g and 0.31 mg/g in shoot and root (Fig.27).

4.5.4. Reducing sugar (Table 39)

The reducing sugar was found to be more in VAM alone and along with pathogens treatments in both the cotton cultivars. In MCU 5, VAM alone recorded maximum of 3.25 mg/g and 2.76 mg/g in shoot and root which was 43.8 and 27.1

Table 38. Effect of VAM, *Fusarium* and *R. reniformis* on total sugar contents of cotton cv. MCU 5 and K10

(mean of three replications)

Treatments	MCU 5				K10			
	Shoot (mg/g)	Per cent increase over control	Root (mg/g)	Per cent increase over control	Shoot (mg/g)	Per cent increase over control	Root (mg/g)	Per cent increase over control
T_1 - Nematode alone	1.68	34.4	0.45	66.6	2.32	43.2	0.53	41.5
T_2 - VAM	2.32	85.6	0.68	151.8	2.86	76.5	0.72	132.9
T_3 - *Fusarium*	1.11	-11.2	0.42	55.5	2.21	36.4	0.51	40.1
T_4 - Nematode + VAM	2.24	79.2	0.64	137.0	2.82	74.0	0.68	64.5
T_5 - Nematode + *Fusarium*	0.86	-31.2	0.16	-40.7	2.18	34.5	0.50	39.8
T_6 - VAM + *Fusarium*	2.22	77.6	0.62	129.6	2.80	72.8	0.64	106.4
T_7 - Nematode+VAM+*Fusarium*	2.18	74.4	0.63	133.3	2.79	72.2	0.62	100.0
T_8 - Control	1.25	-	0.27	-	1.62	-	0.31	-
CD (P = 0.05)	**0.34**	-	**0.14**	-	**0.28**	-	**0.18**	-

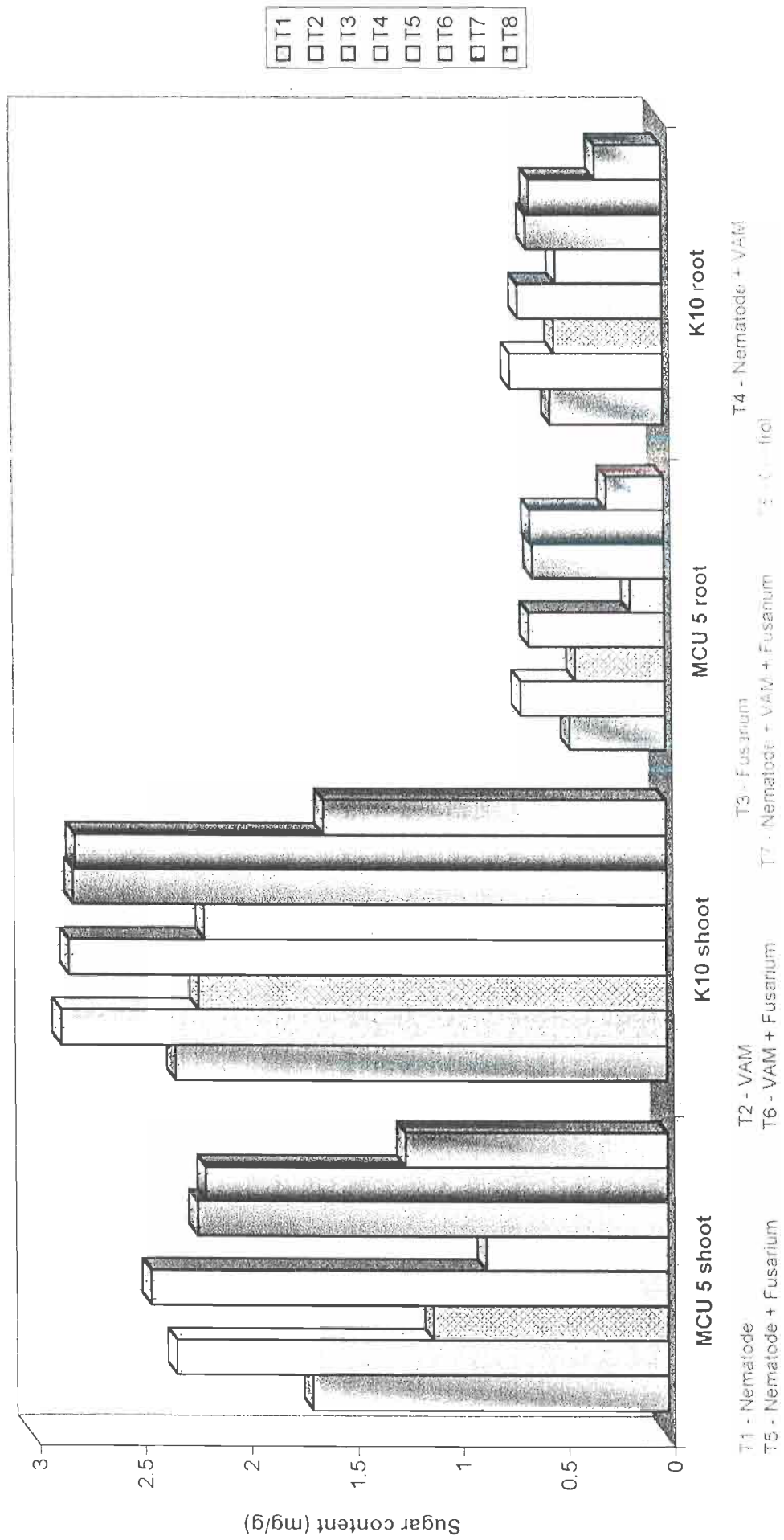


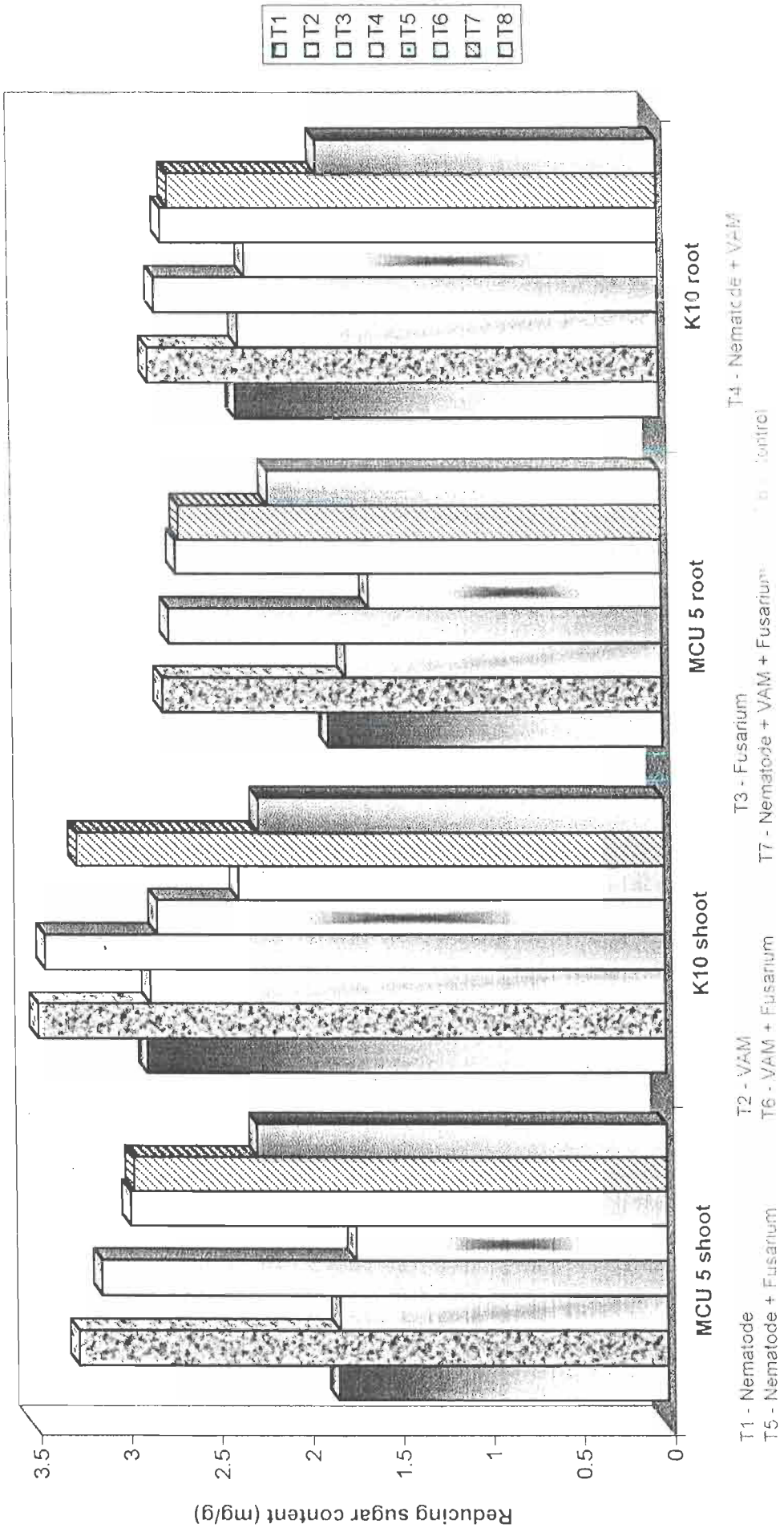
Fig.27. Effect of VAM, Fusarium and *R. reniformis* on total sugar content of cotton cultivars (MCU 5 and K10)

Table 39. Effect of VAM, *Fusarium* and *R. reniformis* on reducing sugar content of cotton cv. MCU 5 and K10

(mean of three replications)

Treatments	MCU 5				K10			
	Shoot (mg/g)	Per cent increase over control	Root (mg/g)	Per cent increase over control	Shoot (mg/g)	Per cent increase over control	Root (mg/g)	Per cent increase over control
T_1 - Nematode alone	1.82	-19.4	1.85	-14.7	2.86	27.6	2.34	24.4
T_2 - VAM	3.25	43.8	2.76	27.1	3.46	54.4	2.82	50.0
T_3 - *Fusarium*	1.81	-19.9	1.78	-17.9	2.84	26.7	2.32	23.4
T_4 - Nematode + VAM	3.12	38.0	2.72	25.3	3.42	52.6	2.78	47.8
T_5 - Nematode + *Fusarium*	1.72	-23.8	1.62	-25.3	2.80	25.0	2.28	21.2
T_6 - VAM + *Fusarium*	2.96	30.9	2.68	23.5	3.35	49.5	2.74	45.7
T_7 - Nematode+VAM+*Fusarium*	2.94	30.0	2.66	22.5	3.24	44.6	2.70	43.6
T_8 - Control	2.26	-	2.17	-	2.24	-	1.88	-
CD (P = 0.05)	0.36	-	0.22	-	0.54	-	0.32	-

Fig.28. Effect of VAM, Fusarium and *R. reniformis* on reducing sugar content of cotton cultivars (MCU 5 and K10)



per cent increase over control. This was on par with VAM + Fusarium with 30.9 and 23.5 per cent increase over control. Nematode + Fusarium recorded least with 1.72 mg/g and 1.62 mg/g in shoot and root which was 23.8 and 25.3 per cent decrease over control.

In K10, nematode + Fusarium recorded 25.0 and 21.2 per cent increase which was the least increase VAM alone recorded maximum with 3.46 mg/g and 2.82 mg/g in shoot and root which was on par with VAM along with either pathogens alone or in combination treatment (Fig.28).

4.5.5. Total free amino acid (Table 40)

In cotton cultivar MCU 5, nematode + Fusarium recorded least value of total free amino acids with 0.40 mg/g and 0.30 mg/g in shoot and root followed by nematode alone (0.44 mg/g and 0.34 mg/g) and Fusarium (0.52 mg/g and 0.36 mg/g) treatment. VAM alone recorded maximum total free amino acid with 1.82 mg/g and 1.64 mg/g in shoot and root which was 116.6 and 121.6 per cent increase over control. It was on par with VAM along with pathogens treatments.

In K10 cultivar though in nematode alone and nematode + Fusarium recorded least, there was significant increase over control. Maximum amount of total free amino acid was observed in VAM alone with 2.82 mg/g and 2.64 mg/g in shoot and root which was 200 and 221.9 per cent increase over control. The VAM along with pathogens were on par with VAM alone treatments (Fig.29).

4.5.6. Peroxidase enzyme activity (Table 41)

The results showed that there was a remarkable increase in peroxidase activity in VAM alone and along with pathogens treatments. In cotton cv. MCU 5

Table 40. **Effect of VAM, *Fusarium* and *R. reniformis* on total free amino acid content of cotton cv. MCU 5 and K10**

(mean of three replications)

Treatments	MCU 5				K10			
	Shoot (mg/g)	Per cent increase over control	Root (mg/g)	Per cent increase over control	Shoot (mg/g)	Per cent increase over control	Root (mg/g)	Per cent increase over control
T_1 - Nematode alone	0.44	-47.6	0.34	-54.0	1.44	53.1	1.34	63.4
T_2 - VAM	1.82	116.6	1.64	121.6	2.82	200.0	2.64	221.9
T_3 - *Fusarium*	0.52	-38.0	0.36	-51.3	1.52	59.5	1.36	65.8
T_4 - Nematode + VAM	1.61	91.6	1.20	62.1	2.62	178.7	2.20	168.2
T_5 - Nematode + *Fusarium*	0.40	52.3	0.30	-59.4	1.40	48.9	1.30	58.5
T_6 - VAM + *Fusarium*	1.62	92.8	2.21	198.6	2.62	178.7	2.21	169.5
T_7 - Nematode+VAM+*Fusarium*	1.54	83.3	1.14	162.1	2.54	170.2	2.14	160.9
T_8 - Control	0.84	-	0.74	-	0.94	-	0.82	-
CD (P = 0.05)	**0.24**	-	**0.54**	-	**0.54**	-	**0.64**	-

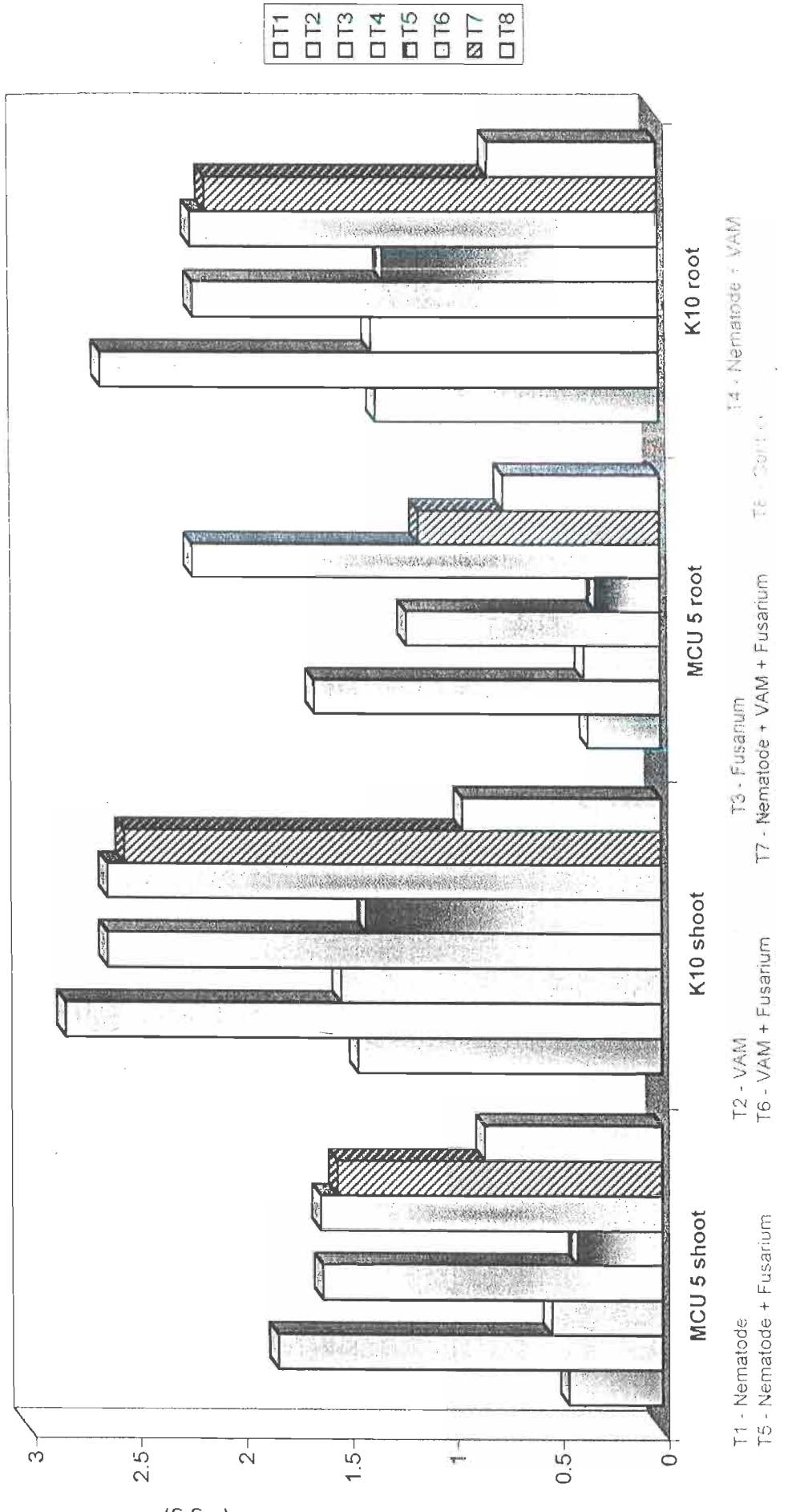


Fig.29. Effect of VAM, Fusarium and *R. reniformis* on total free aminoacid content of cotton cultivars (MCU 5 and K10)

Table 41. Effect of VAM, *Fusarium* and *R. reniformis* on peroxidase content of cotton cultivars, MCU 5 and K10

(mean of three replications)

Treatments	Peroxidase content (change in absorbance at 420 nm m^{-1} mg^{-1})							
	MCU 5				K10			
	Shoot	Per cent increase over control	Root	Per cent increase over control	Shoot	Per cent increase over control	Root	Per cent increase over control
T_1 - Nematode alone	49.0	20.9	52.0	16.8	57.0	35.7	60.5	56.0
T_2 - VAM	55.0	35.8	57.5	29.2	64.0	52.3	65.5	45.0
T_3 - *Fusarium*	47.0	16.0	51.5	15.7	56.5	34.5	59.5	32.2
T_4 - Nematode + VAM	66.5	64.1	65.0	46.0	78.5	86.9	82.0	82.2
T_5 - Nematode + *Fusarium*	45.0	11.1	50.4	13.2	54.5	82.1	58.0	28.8
T_6 - VAM + *Fusarium*	62.0	53.0	61.0	37.0	74.5	77.3	78.4	74.2
T_7 - Nematode+VAM+*Fusarium*	61.5	51.8	60.5	35.9	72.0	71.4	75.0	66.6
T_8 - Control	40.5	-	44.5	-	42.0	-	45.0	-
CD (P = 0.05)	**5.5**	-	**4.0**	-	**6.5**	-	**7.5**	-

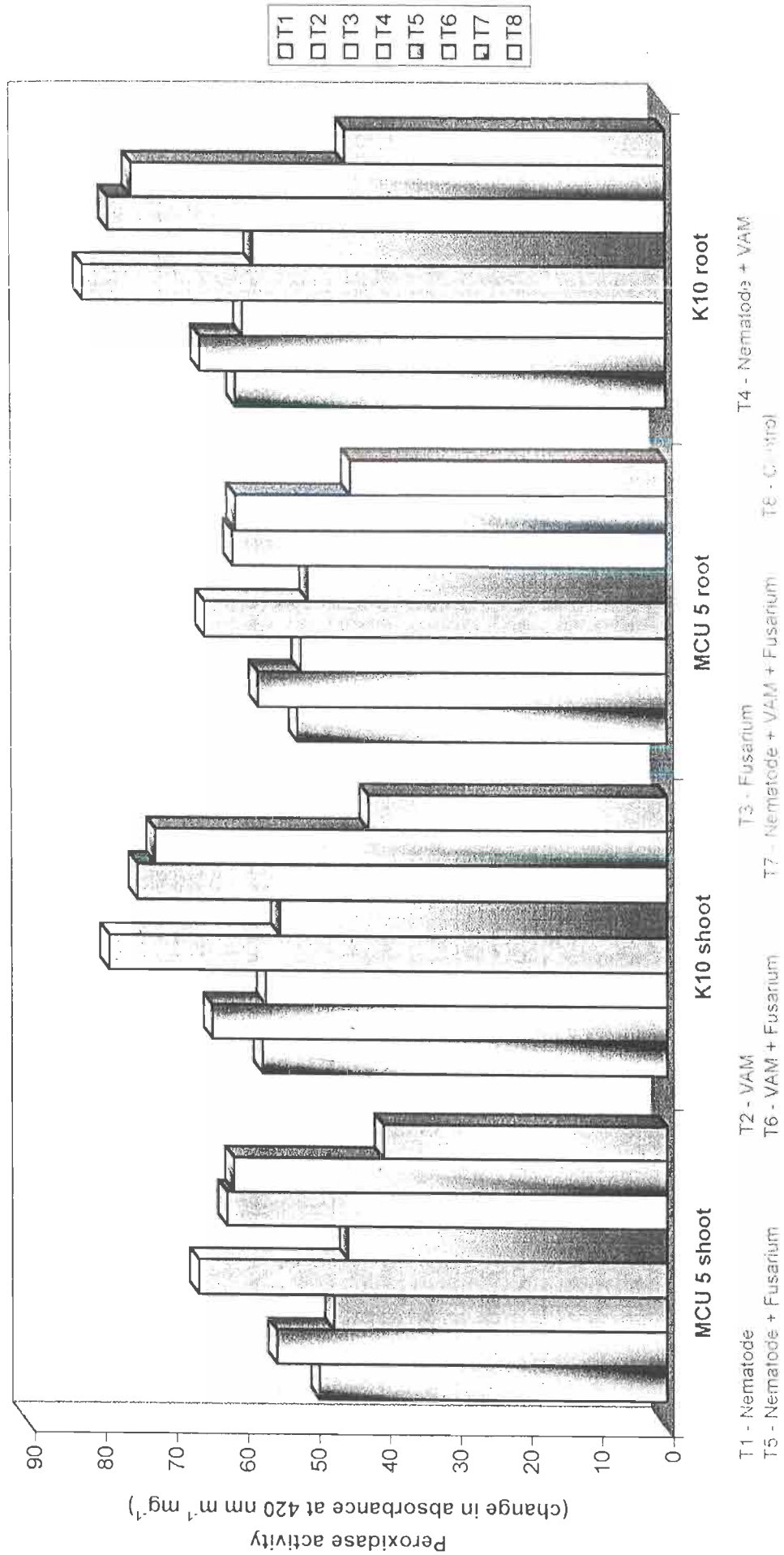


Fig.30. Effect of VAM, Fusarium and *R. reniformis* on peroxidase activity of cotton cultivars (MCU 5 and K10)

Table 42. Effect of VAM, *Fusarium* and *R. reniformis* on chitinase content of cotton cv. MCU 5 and K10

(mean of three replications)

Treatments	Chitinase content (n mol of N-acetylglucosamine/min/g)							
	MCU 5				K10			
	Shoot	Per cent increase over control	Root	Per cent increase over control	Shoot	Per cent increase over control	Root	Per cent increase over control
T_1 - Nematode alone	19.2	3.2	18.4	12.1	23.8	36.7	23.4	56.0
T_2 - VAM	26.4	41.9	28.6	74.3	29.4	68.9	29.8	98.6
T_3 - *Fusarium*	19.4	4.3	18.2	10.9	22.1	27.0	21.2	41.3
T_4 - Nematode + VAM	25.8	38.7	26.2	59.7	27.8	59.7	27.6	84.0
T_5 - Nematode + *Fusarium*	19.1	2.9	17.6	7.3	23.4	34.4	22.6	50.6
T_6 - VAM + *Fusarium*	25.2	35.4	26.0	58.5	27.4	57.4	27.4	82.6
T_7 - Nematode+VAM+*Fusarium*	24.8	33.3	25.8	57.3	27.0	55.1	27.0	80.0
T_8 - Control	18.6	-	16.4	-	17.4	-	15.0	-
CD (P = 0.05)	**2.5**	-	**3.5**	-	**2.5**	-	**3.0**	-

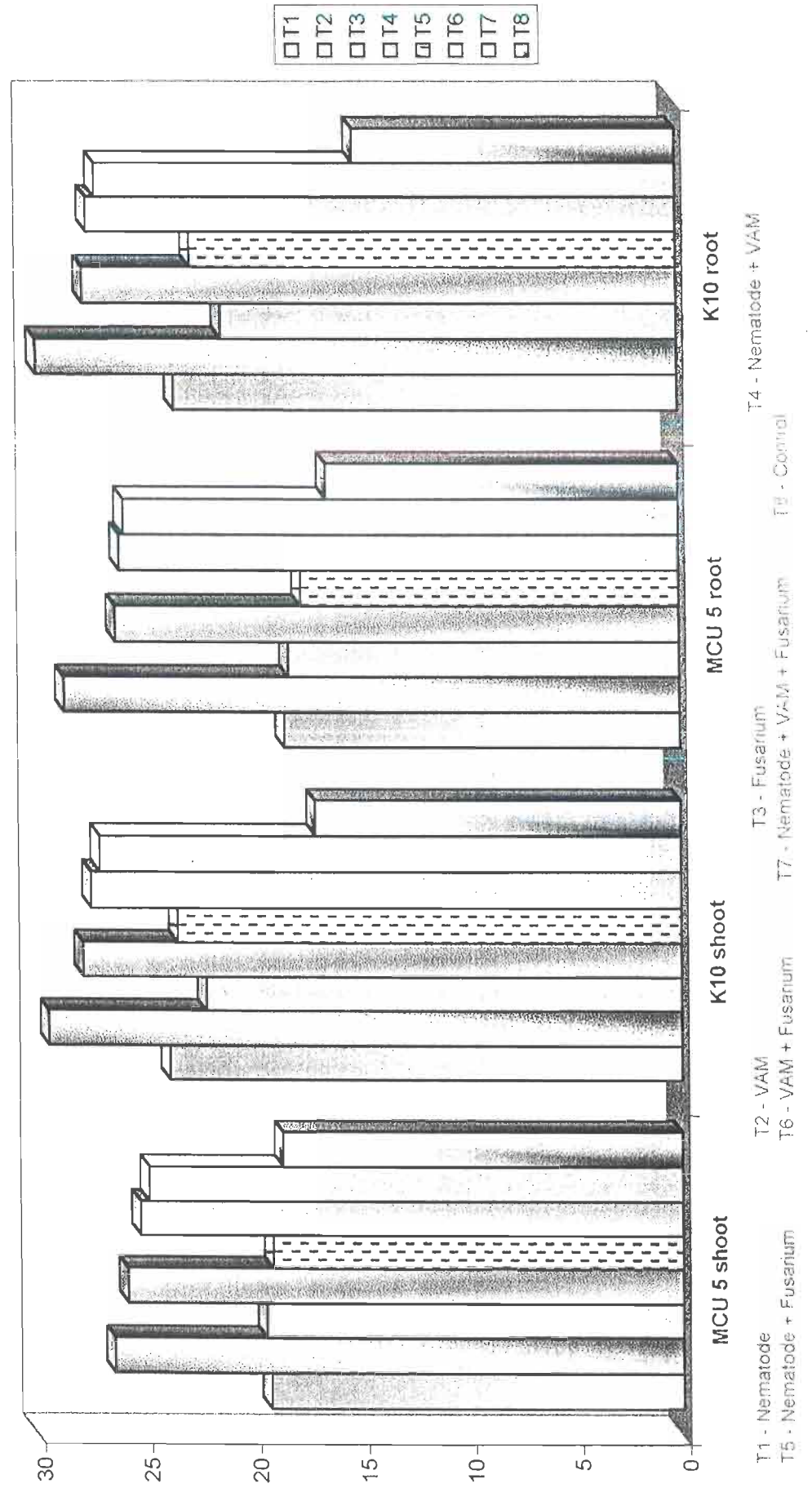


Fig.31. Effect of VAM, Fusarium and *R. reniformis* on chitinase activity of cotton cultivars (MCU 5 and K10)

the peroxidase activity was maximum in nematode + VAM treatment with 66.5 and 65.0 in shoot and root which was 64.1 and 46.0 per cent increase over control. This was on par with VAM + Fusarium and VAM + nematode + Fusarium treatment. VAM alone recorded 55.0 and 57.5 which was significantly different from all other treatments. The least was observed in control with 40.5 and 44.5 in shoot and root respectively. Nematode alone, Fusarium alone and in combination were on par with each other which were observed to increase peroxidase activity to a little extent (Fig.30).

In K10 cultivar also the trend maintained was the same. However, extent of peroxidase increase was more in K10 than MCU 5.

4.5.7. Chitinase enzyme activity (Table 42)

In cotton cv. MCU 5, the maximum chitinase activity observed in VAM alone treatment with 26.4 and 28.6 in shoot and root which was on par with VAM along with either pathogens alone and in combination treatments. Control recorded least with 18.6 and 16.4 in shoot and root respectively. Similar trend was recorded in K10 cultivar also (Fig.31).

4.5.8. Macronutrient content

4.5.8.1. Nitrogen (Table 43)

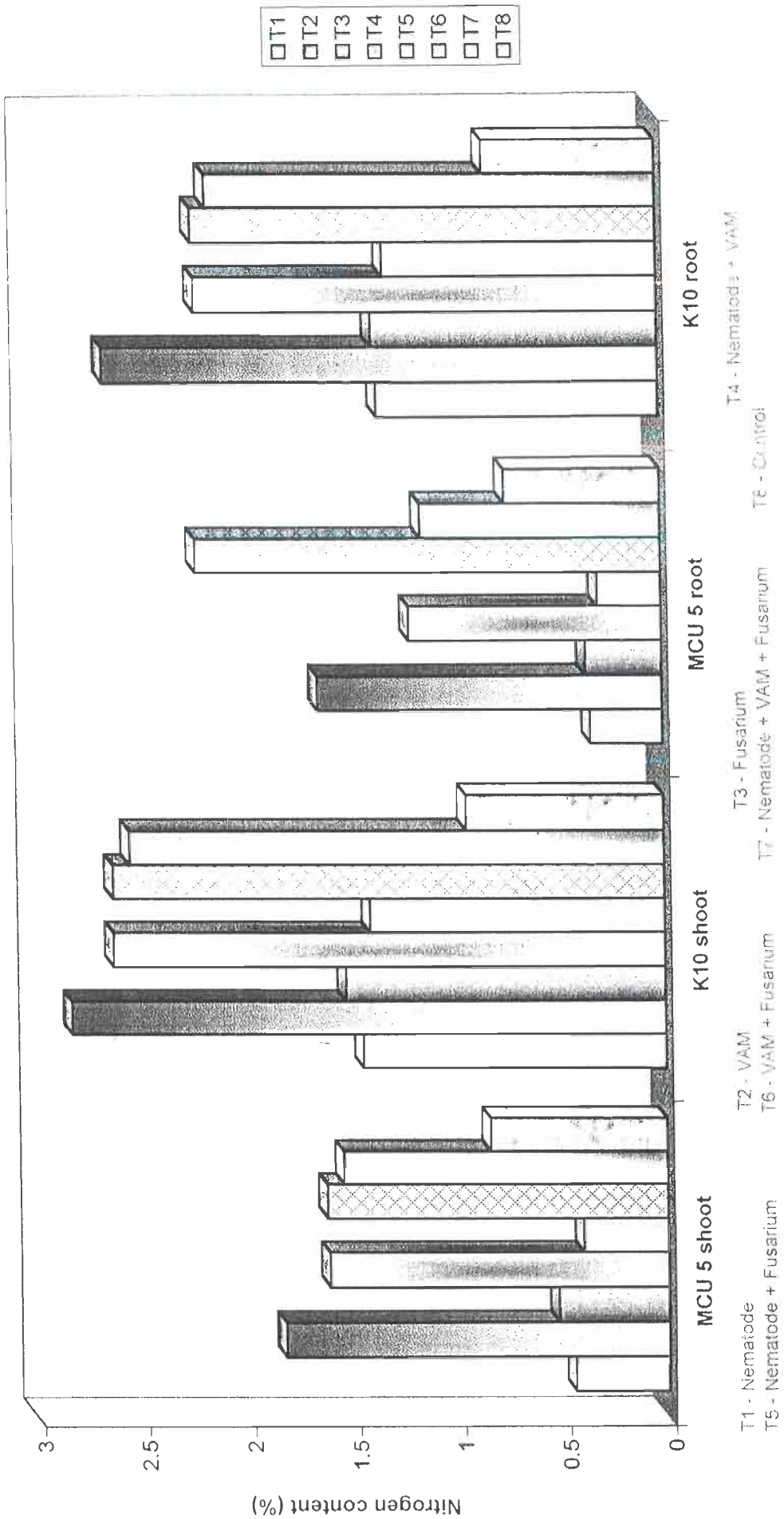
Nitrogen content in shoots of cotton cv. MCU 5 was reduced by nematode and Fusarium infestation. Maximum reduction could be seen where both nematode and Fusarium were present. Inoculation of VAM increased the shoot nitrogen content. VAM alone recorded maximum of 1.82% followed by VAM + Fusarium (1.62%) VAM + nematode (1.61%) and VAM + Fusarium + nematode (1.54%) treatments.

Table 43. Effect of VAM, Fusarium and *R. reniformis* on total nitrogen content of cotton cv. MCU 5 and K10

(mean of three replications)

Treatments	MCU 5				K10			
	Shoot content (%)	Per cent increase over control	Root content (%)	Per cent increase over control	Shoot content (%)	Per cent increase over control	Root content (%)	Per cent increase over control
T_1 - Nematode alone	0.44	-47.6	0.34	-57.0	1.44	53.1	1.34	63.4
T_2 - VAM	1.82	116.6	1.64	121.6	2.82	200.00	2.64	221.9
T_3 - Fusarium	0.52	-38.6	0.36	-51.3	1.52	27.6	1.36	65.8
T_4 - Nematode + VAM	1.61	91.6	1.20	62.1	2.62	178.7	2.20	168.2
T_5 - Nematode + Fusarium	0.40	-52.3	0.30	-59.4	1.40	48.9	1.30	58.5
T_6 - VAM + Fusarium	1.62	92.8	2.21	198.6	2.62	178.7	2.21	168.2
T_7 - Nematode+VAM+Fusarium	1.54	83.3	1.14	54.0	2.54	170.2	2.14	160.9
T_8 - Control	0.84	-	0.74	-	0.94	-	0.82	-
CD (P = 0.05)	**0.24**	-	**0.54**	-	**0.54**	-	**0.64**	-

Fig.32. Effect of VAM, Fusarium and *R. reniformis* on nitrogen content of cotton cultivars (MCU 5 and K10)



In K10 cultivar also the same trend was maintained. The extent of reduction of nitrogen due to nematode was less in K10. In K10, maximum 'N' content in shoot recorded on VAM alone treatment with 2.82 and it was on par with VAM along with nematode and Fusarium treatments.

Root nitrogen content was maximum with 2.21 recorded in VAM + Fusarium treatment in MCU 5. In K10 the VAM alone recorded maximum nitrogen content with 2.64. In addition, it was noticed that root nitrogen content increased due to nematode inoculation in both cultivars. However, the increase in the nitrogen content in the presence of nematode was less in K10 compared to MCU 5 (Fig.32).

4.5.8.2. Phosphorus content (Table 44)

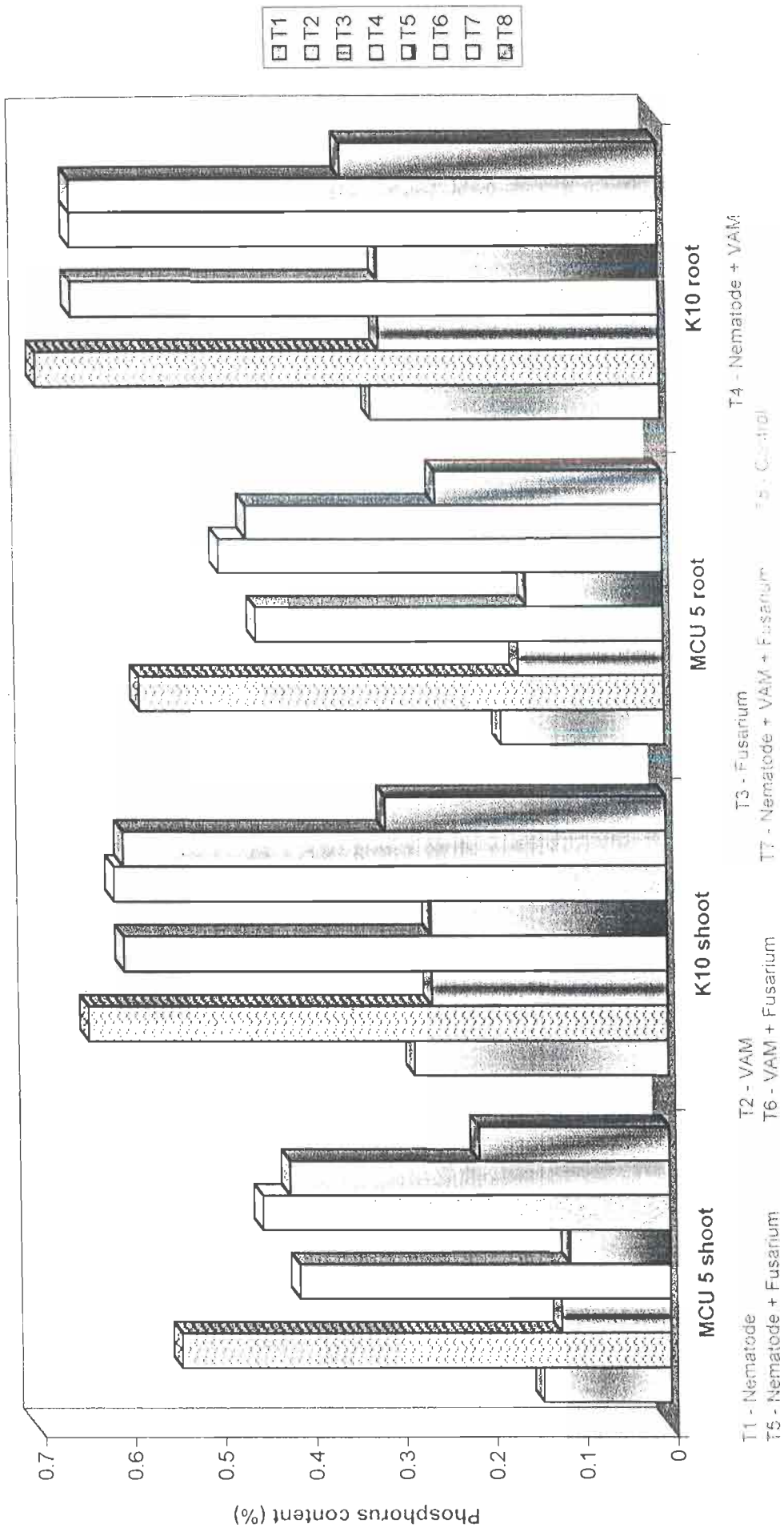
The phosphorus content in shoot and root was found to be reduced by the infestation of the pathogens. The extent of reduction was less in K10 cultivar. The reduction due to nematode alone and Fusarium alone was 33.3, 42.8 per cent in MCU 5 and it was 9.6 and 16.0 per cent in K10. Presence of both pathogens caused 47.6 and 16.1 per cent depletion of phosphorus content in shoots of MCU 5 and K10. It was 40.1 and 11.4 per cent reduction in roots of MCU 5 and K10. VAM alone recorded maximum of 'P' content in both cultivars with 0.54 per cent and 0.58 per cent in shoot and roots of MCU 5 and 0.64 per cent and 0.69 per cent in K10 cultivar. VAM along with nematode, Fusarium and in combination also recorded increased phosphorus content than control. VAM + nematode, VAM + Fusarium and VAM + nematode + Fusarium recorded 95.2, 114.2 and 100.0 per cent increase of shoot 'P' content in MCU 5 and it was 93.5, 96.7 and 93.5 per cent in K10 (Fig.33).

Table 44. Effect of VAM, Fusarium and *R. reniformis* on phosphorus content of cotton cv. MCU 5 and K10

(mean of three replications)

Treatments	MCU 5				K10			
	Shoot content (%)	Per cent increase over control	Root content (%)	Per cent increase over control	Shoot content (%)	Per cent increase over control	Root content (%)	Per cent increase over control
T_1 - Nematode alone	0.14	-33.3	0.18	-28.0	0.28	-9.6	0.32	-8.5
T_2 - VAM	0.54	157.1	0.58	132.0	0.64	106.4	0.69	97.1
T_3 - Fusarium	0.12	-42.8	0.16	-36.0	0.26	-16.0	0.31	-11.4
T_4 - Nematode + VAM	0.41	95.2	0.45	80.0	0.60	93.5	0.65	85.7
T_5 - Nematode + Fusarium	0.11	-47.6	0.15	-40.0	0.26	-16.0	0.31	-11.4
T_6 - VAM + Fusarium	0.45	114.2	0.49	96.0	0.61	96.7	0.65	85.7
T_7 - Nematode+VAM+Fusarium	0.42	100.0	0.46	84.0	0.60	93.5	0.65	85.7
T_8 - Control	0.21	-	0.25	-	0.31	-	0.35	-
CD (P = 0.05)	**0.06**	-	**0.07**	-	**0.08**	-	**0.09**	-

Fig.33. Effect of VAM, Fusarium and *R. reniformis* on phosphorus content of cotton cultivars (MCU 5 and K10)



4.5.8.3. Potassium content (Table 45)

The potassium content in shoot and root of cotton cv. MCU 5 and K10 were significantly higher in plants receiving mycorrhiza alone. The plants having mycorrhizae along with both the pathogens were found to have higher potassium content than plants with pathogen alone. In combination nematode + Fusarium treatment recorded least potassium content with 0.82% and 0.77% in shoot and root of MCU 5 and 1.16% and 1.15% in shoot and root of K10 (Fig.34).

4.5.9. Micro nutrient contents

4.5.9.1. Iron (Table 46)

The iron content in shoots and roots of cotton cultivar decreased due to the presence of the pathogens. Presence of nematode + Fusarium resulted in maximum reduction of iron content in the shoot and root with 32.0, 33.0 per cent in MCU 5 and 4.1, 4.3 per cent in K10 respectively. In general, VAM treated plants increased iron content in both shoot and root by 127.9, 131.3 per cent in MCU 5 ad 119.4, 123.6 per cent in K10. In addition VAM along with pathogens also increased iron content. Nematode + Fusarium + VAM recorded 115.7, 118.2 per cent increase of iron content in MCU 5 and 114.1, 118.1 per cent increase in K10 cultivar (Fig.35).

4.5.9.2. Copper (Table 47)

The copper content was reduced when both nematode and Fusarium present either alone or combination. The depletion was maximum when both the pathogens combined, with 26.0 and 28.1 per cent decrease over control. In MCU 5 and 11.4 and 18.0 per cent decrease in K10. VAM and VAM along with nematode and Fusarium recorded increase in copper content. VAM alone recorded a maximum increase over control with 69.6 and 75.1 per cent in MCU 5 and 116.5

Table 45. Effect of VAM, Fusarium and *R. reniformis* on potassium content of cotton cv. MCU 5 and K10

(mean of three replications)

Treatments	MCU 5				K10			
	Shoot content (%)	Per cent increase over control	Root content (%)	Per cent increase over control	Shoot content (%)	Per cent increase over control	Root content (%)	Per cent increase over control
T_1 - Nematode alone	0.91	-25.4	0.86	-26.4	1.24	5.0	1.19	5.3
T_2 - VAM	1.65	35.2	1.60	36.7	1.76	49.1	1.71	51.3
T_3 - Fusarium	0.97	-20.9	0.92	-21.3	1.26	6.7	1.21	7.0
T_4 - Nematode + VAM	1.54	26.2	1.49	27.3	1.70	44.0	1.65	46.0
T_5 - Nematode + Fusarium	0.82	-32.7	0.77	-34.1	1.16	-1.6	1.15	1.7
T_6 - VAM + Fusarium	1.58	25.5	1.55	32.4	1.74	47.4	1.69	49.5
T_7 - Nematode+VAM+Fusarium	1.51	23.7	1.46	24.7	1.69	43.2	1.65	48.6
T_8 - Control	1.22	-	1.17	-	1.18	-	1.13	-
CD (P = 0.05)	**0.25**	-	**0.24**	-	**0.22**	-	**0.21**	-

Fig.34. Effect of VAM, Fusarium and *R. reniformis* on potassium content of cotton cultivars (MCU 5 and K10)

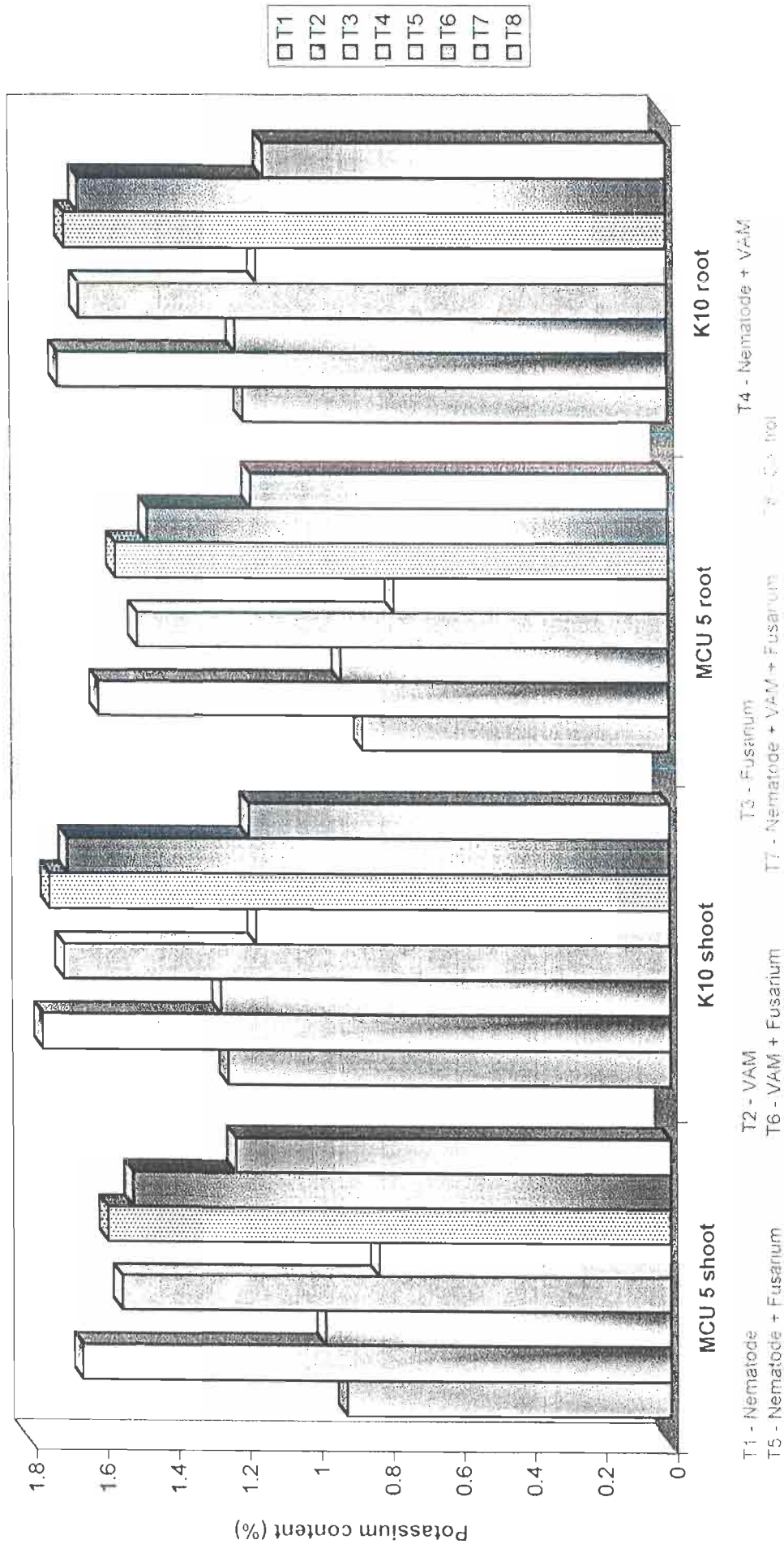


Table 46. Effect of VAM, Fusarium and *R. reniformis* on iron content of cotton cv. MCU 5 and K10

(mean of three replications)

Treatments	MCU 5				K10			
	Shoot content (ppm)	Per cent increase over control	Root content (ppm)	Per cent increase over control	Shoot content (ppm)	Per cent increase over control	Root content (ppm)	Per cent increase over control
T_1 - Nematode alone	675	-24.5	650	-17.7	855	-34.0	825	-3.5
T_2 - VAM	1858	127.9	1828	131.3	1942	119.4	1912	123.6
T_3 - Fusarium	648	-20.4	622	-21.2	862	-2.5	832	-2.6
T_4 - Nematode + VAM	1788	119.3	1757	122.4	1945	119.7	1885	120.4
T_5 - Nematode + Fusarium	554	-32.0	529	-33.0	848	4.1	818	-4.3
T_6 - VAM + Fusarium	1795	120.2	1775	124.6	1925	117.5	1890	121.0
T_7 - Nematode+VAM+Fusarium	1758	115.7	1724	118.2	1895	114.1	1865	118.1
T_8 - Control	815	-	790	-	885	-	855	-
CD (P = 0.05)	**52.5**	-	**54.6**	-	**68.4**	-	**70.4**	-

Fig.35. Effect of VAM, Fusarium and *R. reniformis* on iron content of cotton cultivars (MCU 5 and K10)

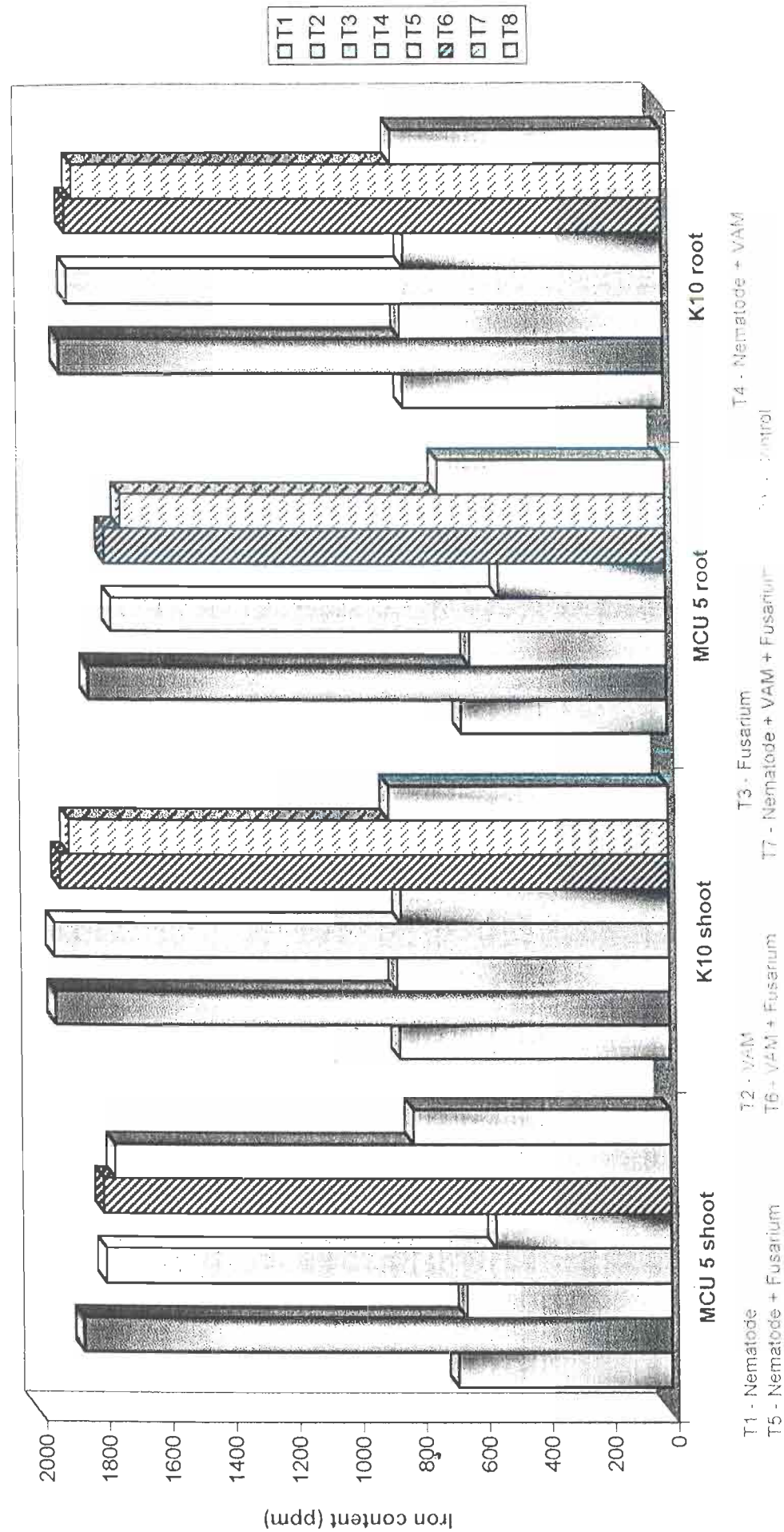
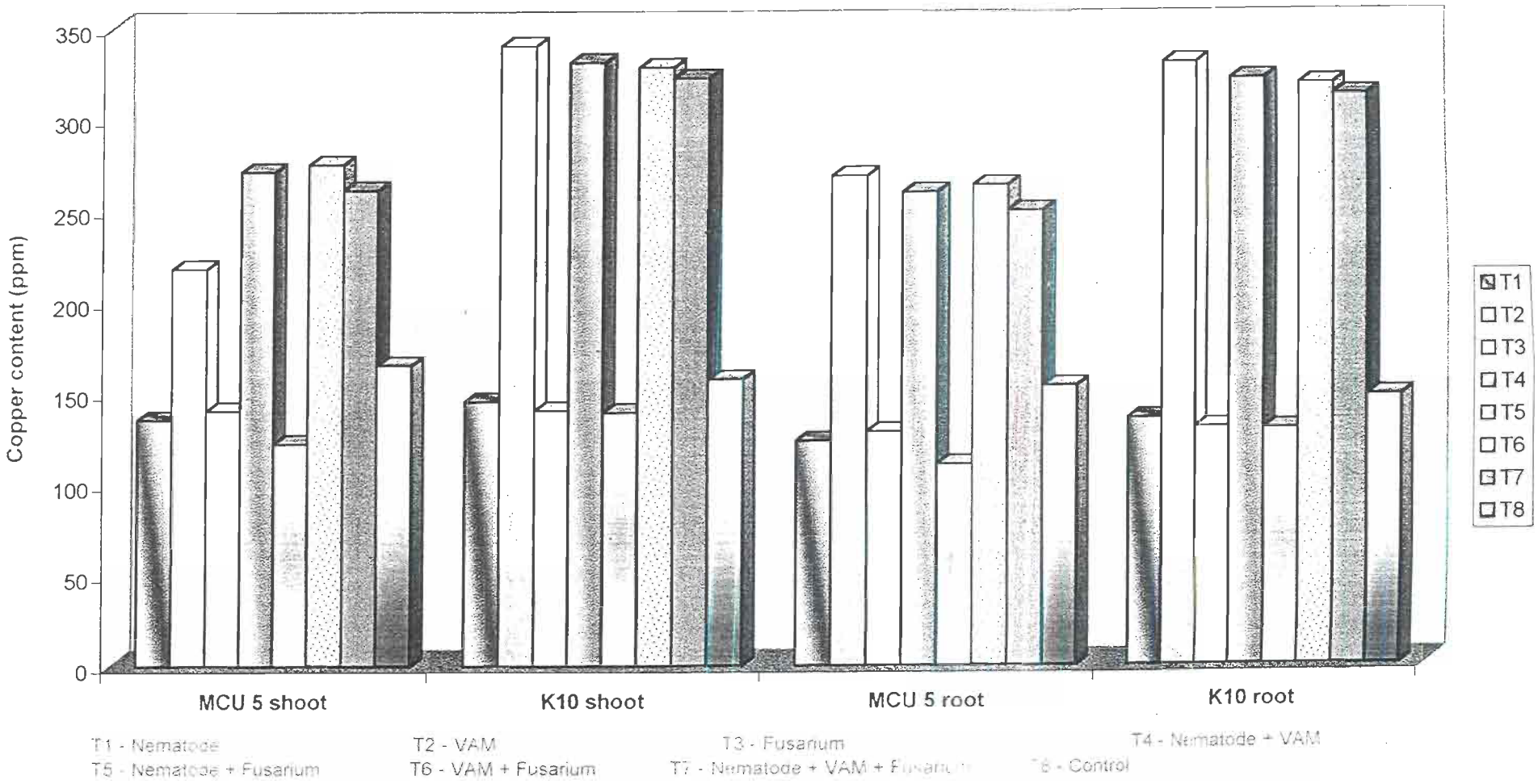


Table 47. Effect of VAM, Fusarium and *R. reniformis* on copper content of cotton cv. MCU 5 and K10

(mean of three replications)

Treatments	MCU 5				K10			
	Shoot content (ppm)	Per cent increase over control	Root content (ppm)	Per cent increase over control	Shoot content (ppm)	Per cent increase over control	Root content (ppm)	Per cent increase over control
T_1 - Nematode alone	135	-18.1	123	-13.7	145	-7.6	135	-8.1
T_2 - VAM	280	69.6	268	75.1	340	116.5	330	124.4
T_3 - Fusarium	140	-15.1	128	-16.3	140	-10.8	130	-11.5
T_4 - Nematode + VAM	271	64.2	259	69.2	331.	10.8	321	118.3
T_5 - Nematode + Fusarium	121	-26.0	110	-28.1	139	-11.4	129	-18.0
T_6 - VAM + Fusarium	275	66.6	263	71.8	328	108.9	318	116.3
T_7 - Nematode+VAM+Fusarium	261	58.1	249	62.7	322	105.0	312	112.2
T_8 - Control	165	-	153	-	157	-	147	-
CD (P = 0.05)	**22.5**	-	**21.7**	-	**25.4**	-	**26.8**	-

Fig.36. Effect of VAM, Fusarium and *R. reniformis* on copper content of cotton cultivars (MCU 5 and K10)



and 124.4 per cent in K10. But, when VAM inoculated with two pathogens there was a slight reduction in copper content, but it was superior to control (Fig.36).

4.5.9.3. Manganese (Table 48)

With inoculation of nematode and Fusarium there was reduction in the manganese content in the shoot and root of both cotton cultivars. But it was to a lesser extent in K10 cultivar. Inoculation of nematode + Fusarium resulted in 52.7, 59.3 per cent reduction in MCU 5 and 15.7 and 17.6 per cent reduction in K10 respectively compared to control. The manganese content was maximum in VAM treated plants. When VAM inoculated along with pathogens also there was an increase in manganese content compared to control. Nematode + VAM + Fusarium recorded 30.5 and 34.3 per cent increase in MCU 5 and 42.1 and 47.0 per cent increase in K10 respectively (Fig.37).

4.5.9.4. Zinc (Table 49)

The zinc content was reduced by the presence of the pathogens alone and in combination. A peak reduction of 33.3 and 36.7 per cent in MCU 5 and 14.2 and 15.6 per cent in K10 cultivars in shoot and root respectively was recorded in nematode + Fusarium. Maximum increase of Zn content was observed when VAM alone inoculated with 125.9 and 138.7 per cent increase in MCU 5 and 135.7 and 150.9 per cent in K10. VAM along with pathogens observed to reduce Zn content slightly. But it was significantly different from control. Nematode + VAM + Fusarium recorded increased Zn content by 114.8 and 126.5 per cent in MCU 5 and 128.5 and 114.1 in K10 cultivar (Fig.38).

Table 48. Effect of VAM, Fusarium and *R. reniformis* on manganese content of cotton cv. MCU 5 and K10

(mean of three replications)

Treatments	MCU 5				K10			
	Shoot content (ppm)	Per cent increase over control	Root content (ppm)	Per cent increase over control	Shoot content (ppm)	Per cent increase over control	Root content (ppm)	Per cent increase over control
T_1 - Nematode alone	25	-30.5	21	-34.3	36	-5.2	32	-5.8
T_2 - VAM	52	44.4	48	50.0	56	47.3	52	52.9
T_3 - Fusarium	27	-25.0	23	-28.1	32	-15.7	26	-23.5
T_4 - Nematode + VAM	50	38.8	46	43.7	55	44.4	51	50.0
T_5 - Nematode + Fusarium	17	-52.7	13	-59.3	32	-15.7	28	-17.6
T_6 - VAM + Fusarium	48	33.3	44	37.5	54	42.1	50	47.0
T_7 - Nematode+VAM+Fusarium	47	30.5	43	34.3	54	42.1	50	47.0
T_8 - Control	36	-	32	-	38	-	34	-
CD (P = 0.05)	**5.2**	-	**6.4**	-	**4.8**	-	**5.2**	-

Fig.37. Effect of VAM, Fusarium and *R. reniformis* on manganese content of cotton cultivars (MCU 5 and K10)

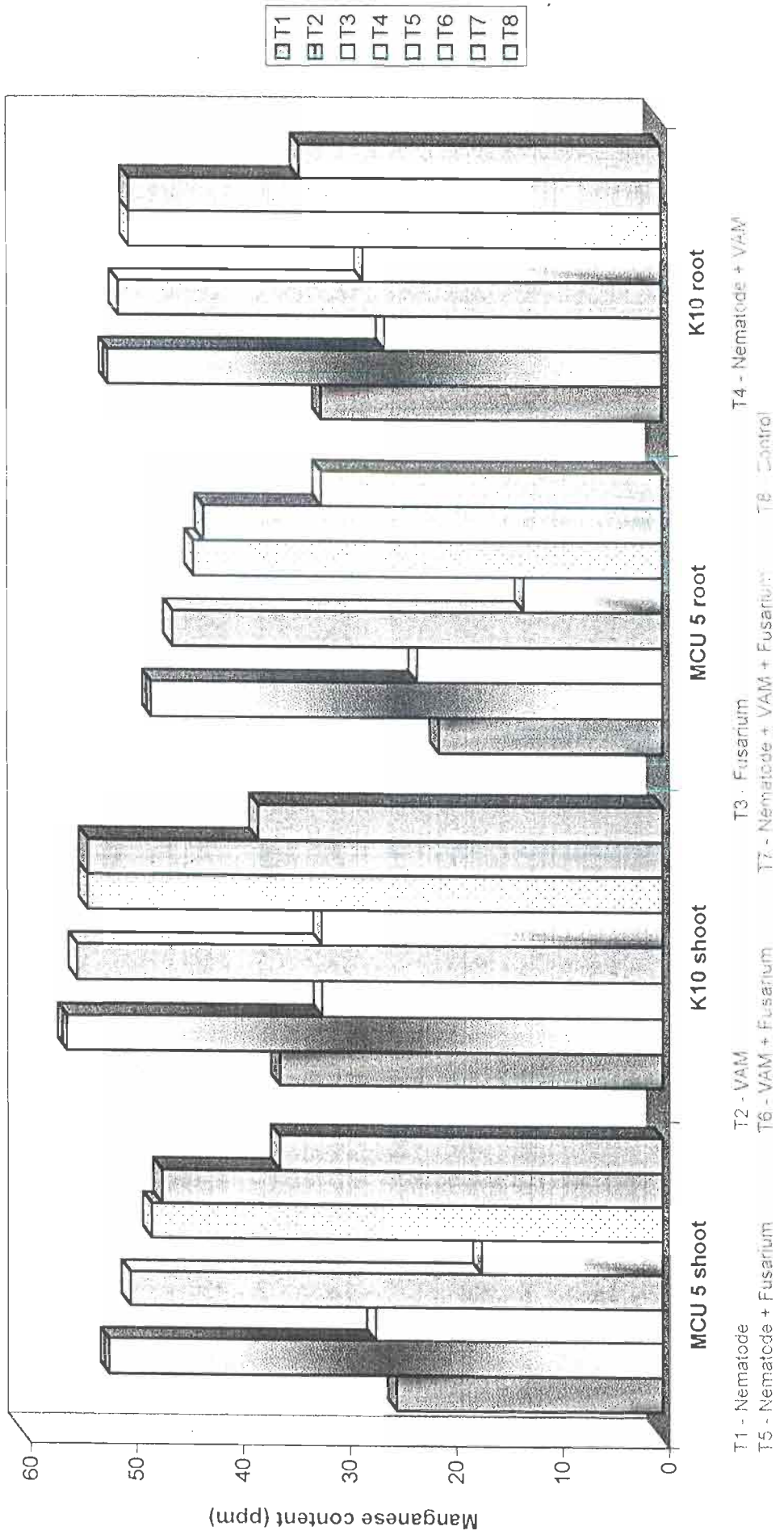


Table 49. Effect of VAM, Fusarium and *R. reniformis* on zinc content of cotton cv. MCU 5 and K10

(mean of three replications)

Treatments	MCU 5				K10			
	Shoot (mg/g)	Per cent increase over control	Root (mg/g)	Per cent increase over control	Shoot (mg/g)	Per cent increase over control	Root (mg/g)	Per cent increase over control
T_1 - Nematode alone	44	-18.5	39	-20.4	55	-1.7	50	-1.9
T_2 - VAM	122	125.9	177	138.7	132	135.7	128	150.9
T_3 - Fusarium	41	-24.0	36	-26.5	51	-8.9	46	-9.8
T_4 - Nematode + VAM	118	118.5	113	130.6	130	132.4	125	145.0
T_5 - Nematode + Fusarium	36	-33.3	31	-36.7	48	-14.2	43	-15.6
T_6 - VAM + Fusarium	120	122.2	115	134.6	129	130.3	124	143.1
T_7 - Nematode+VAM+Fusarium	116	114.8	111	126.5	128	128.5	123	114.1
T_8 - Control	54	-	49	-	56	-	51	-
CD (P = 0.05)	**6.5**	-	**6.0**	-	**5.5**	-	**6.5**	-

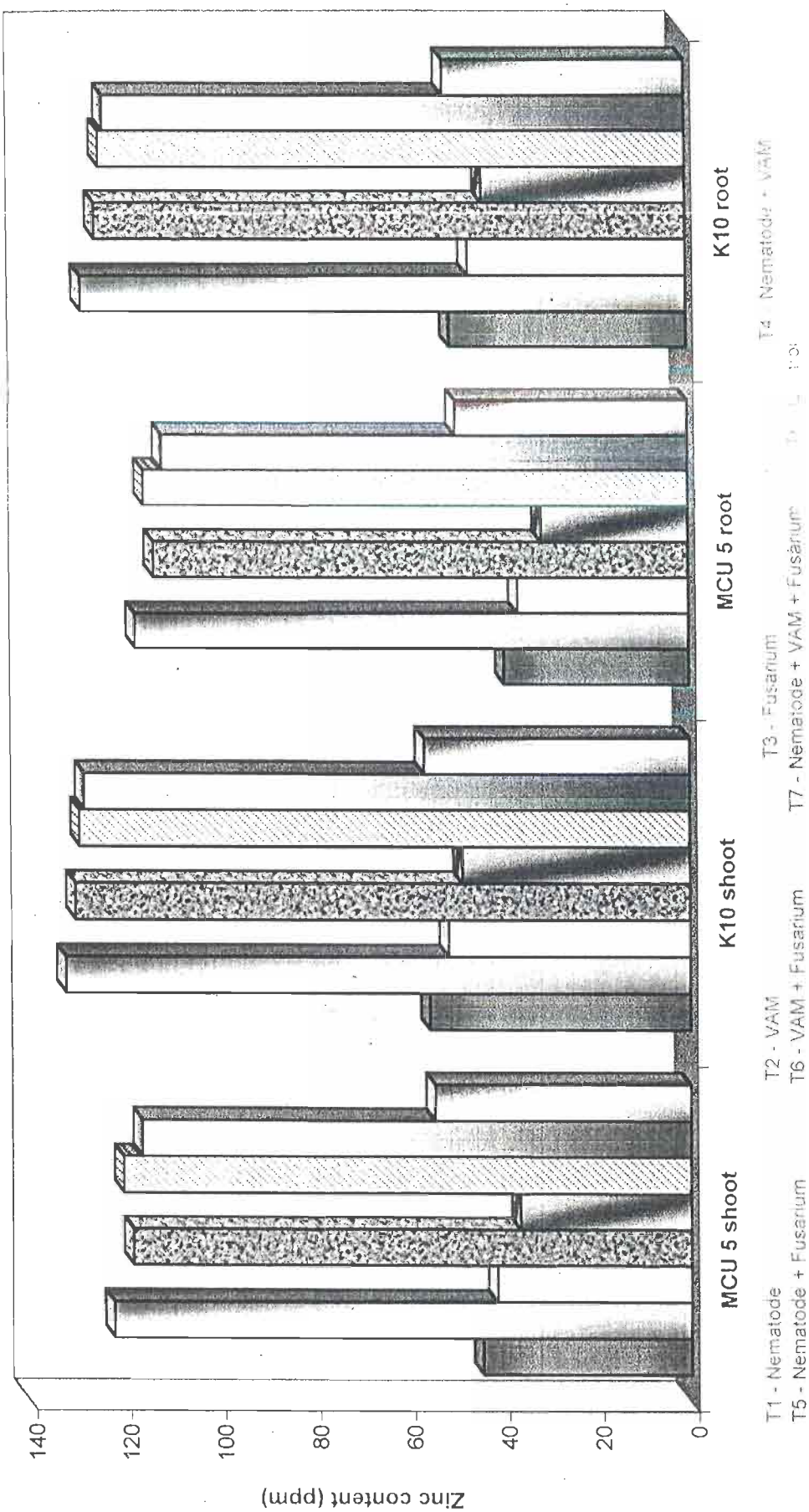


Fig.38. Effect of VAM, Fusarium and *R. reniformis* on zinc content of cotton cultivars (MCU 5 and K10)

4.6. Histopathological studies

The information about the internal structure of a cotton root infected by *R. reniformis* and VAM was examined in stained serial sections of paraffin embedded material of 10μ thickness. The results are furnished below:

4.6.1. Histopathology of *R. reniformis* infected cotton roots

The reniform nematode adult females appear with posterior end of the body protruding the root surface of cotton roots (Plate 26). They enter through the epidermis, penetrate intercellularly as well as intracellularly through the cortex, endodermis and pericycle and reach the phloem where they feed. A passage slightly wider than nematode body and with thickened wall is formed by the destruction of cortical cells (Plate 24). Syncytia induced by *R. reniformis* in cotton roots consisted of curved layer of pericycle cells which are conspicuously hypertropied with dense cytoplasm, extending 6-10 cells on either side of the feeding cell, both circumferentially and longitudinally (Plate 25). Pericycle cells near to the nematode head are usually 3-5 times the size of normal pericycle cells. Expansion, infact, occurs mostly in the initial cells of the syncytia and the degree of expansion of modified cell is reduced as the distance from these cells increases until there is no expansion is possible. The extent of cell modification caused by one nematode forming one 'syncytial unit' has been estimated to be 100-150 modified cells (Plate 27). The cytoplasm of the feeding cell and other syncytial cells was very dense and granular. The modified cells push the pericycle and endodermis towards the periphery of the root as a result of which these layers lose their identity and break down. No changes were observed in xylem.

E - Endodermis
P - Pericycle
S - Syncytium
C - Cortex
N - Nematode

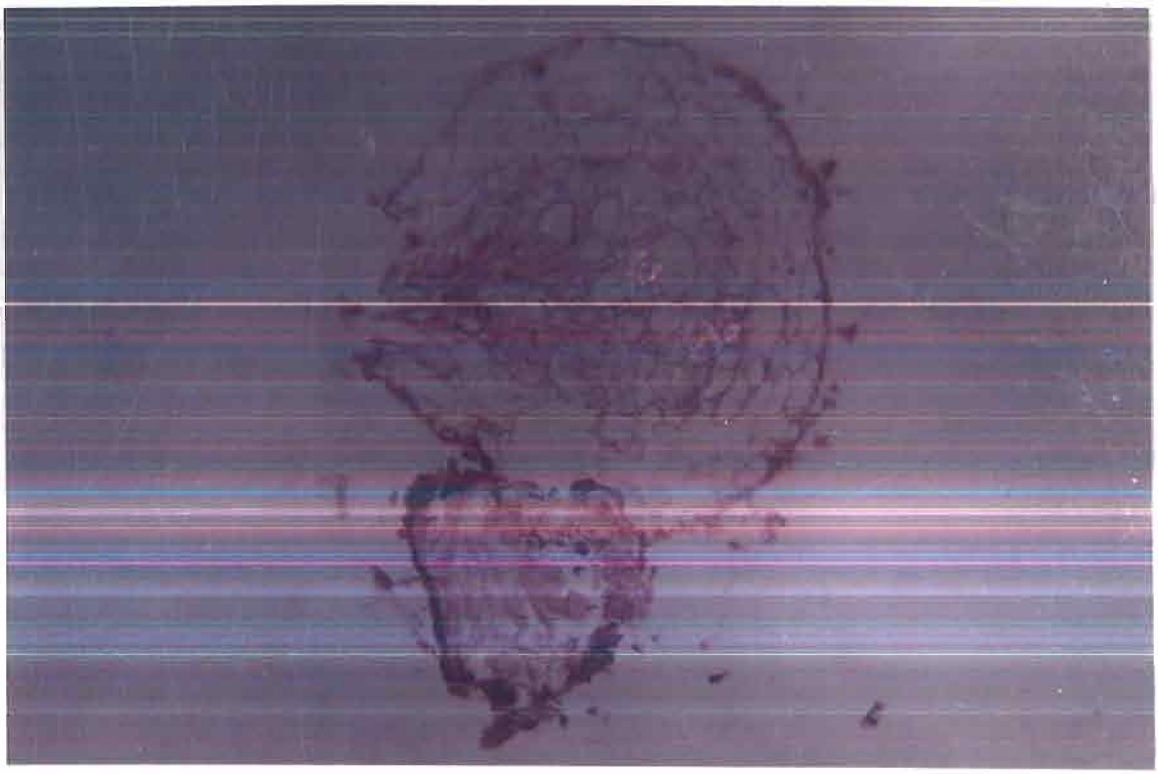


Plate 24. Cross section showing the stelar area with modified pericycle cells (Syncytia)

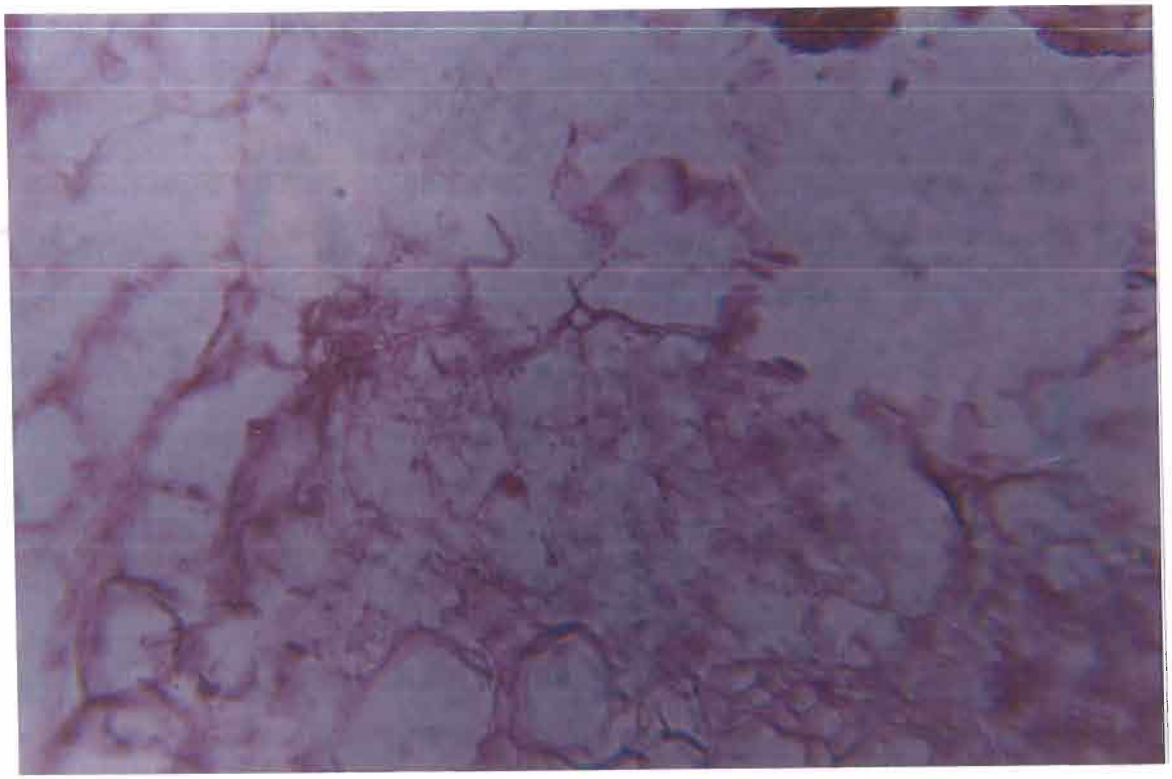


Plate 25. Enlargement of feeding zone of syncytium

A - Arbuscules
V - Vesicles
C - Cortex

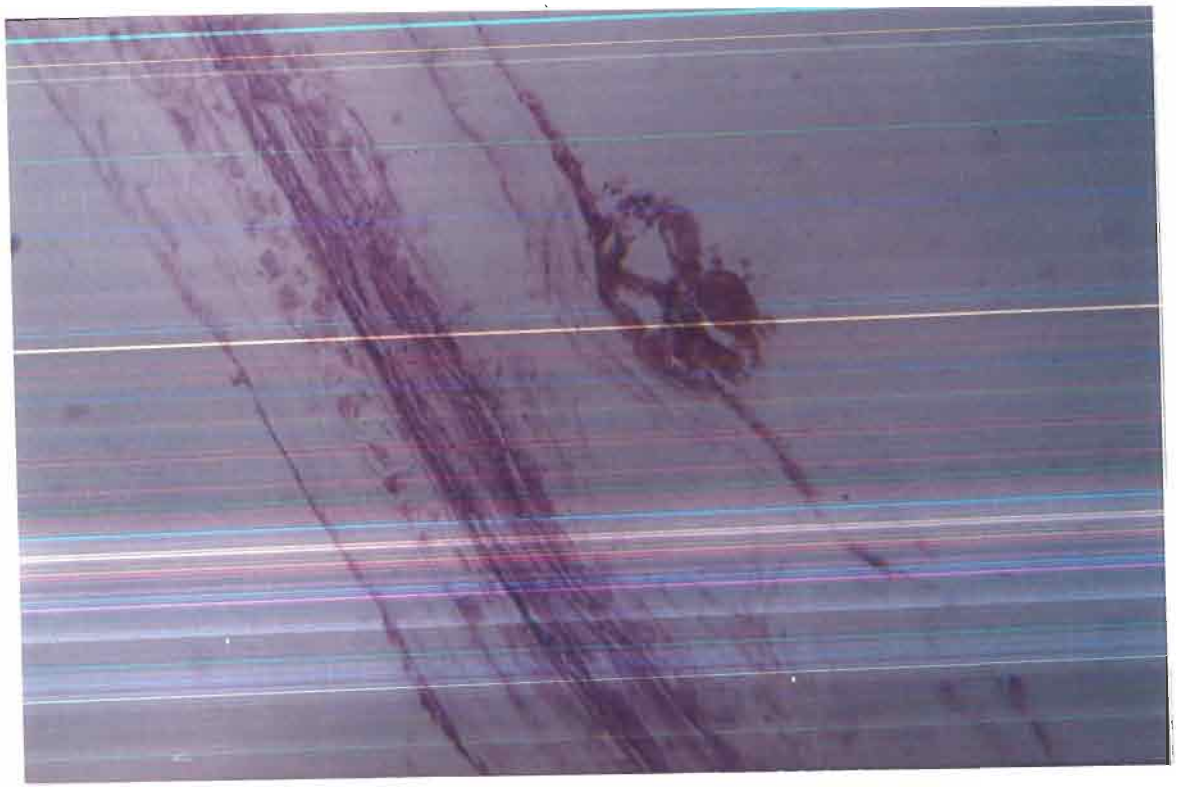


Plate 26. Longitudinal section of nematode infected root

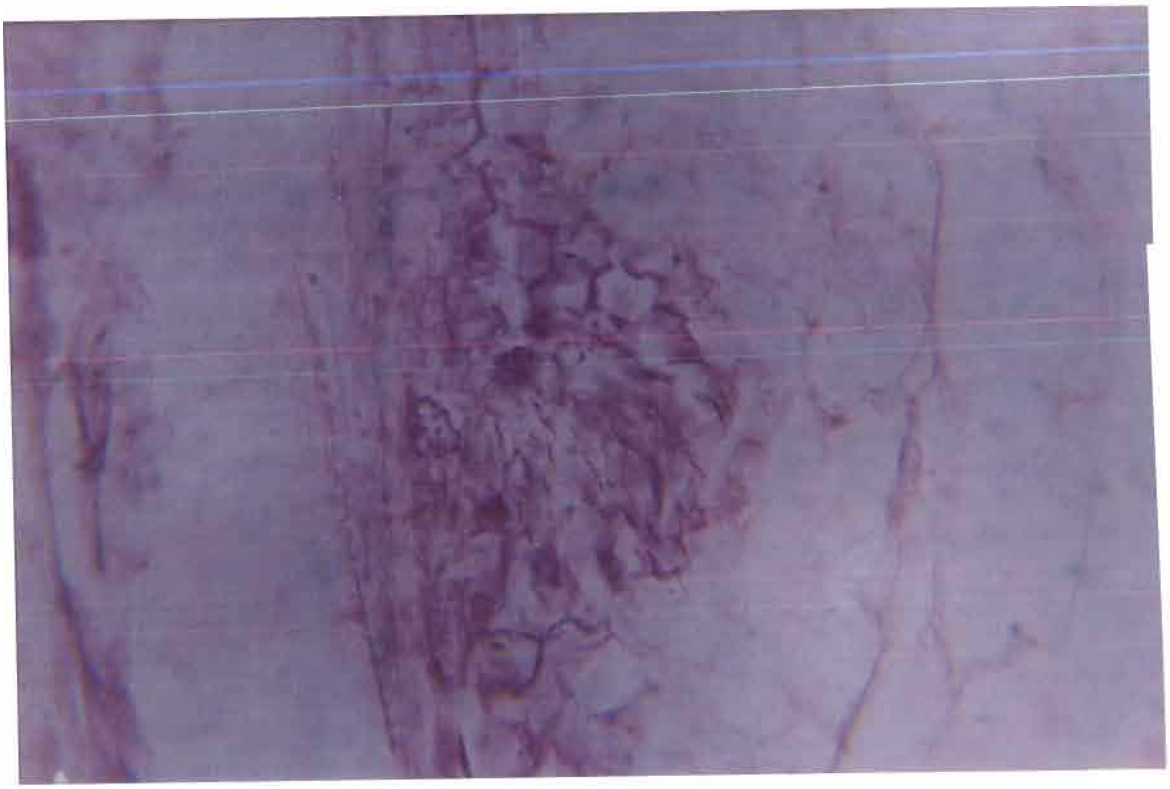


Plate 27. Enlargement of the feeding point of the longitudinal section.

C	-	Cortex
L	-	Lignification of cortical cells
N	-	Nematode
V	-	Vesicles
A	-	Arbuscules

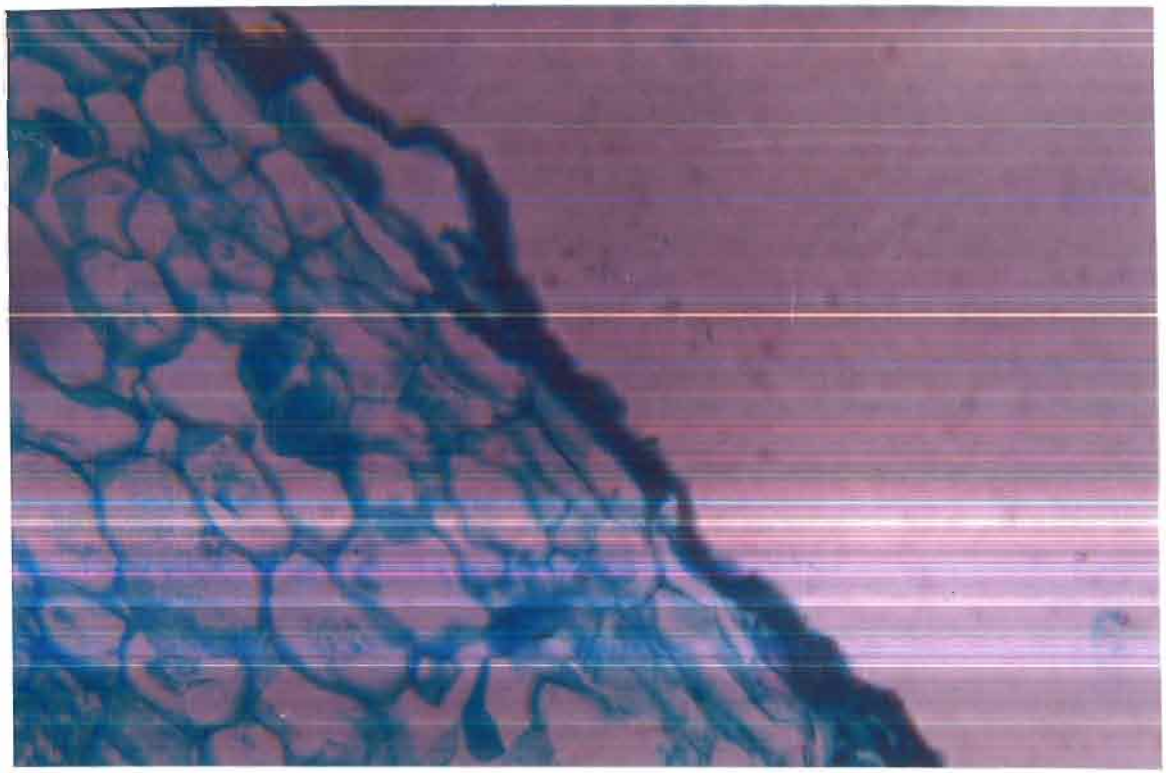


Plate 28. Presence of vesicles and arbuscles

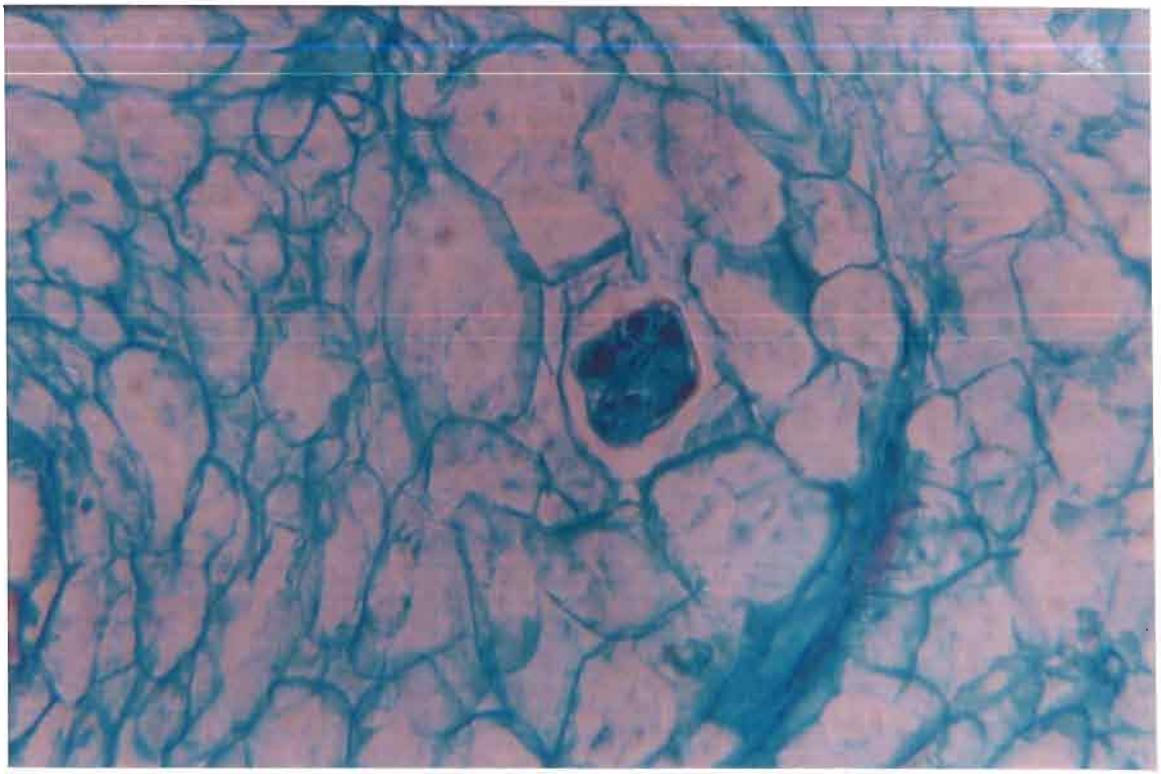


Plate 29. Presence of vesicle

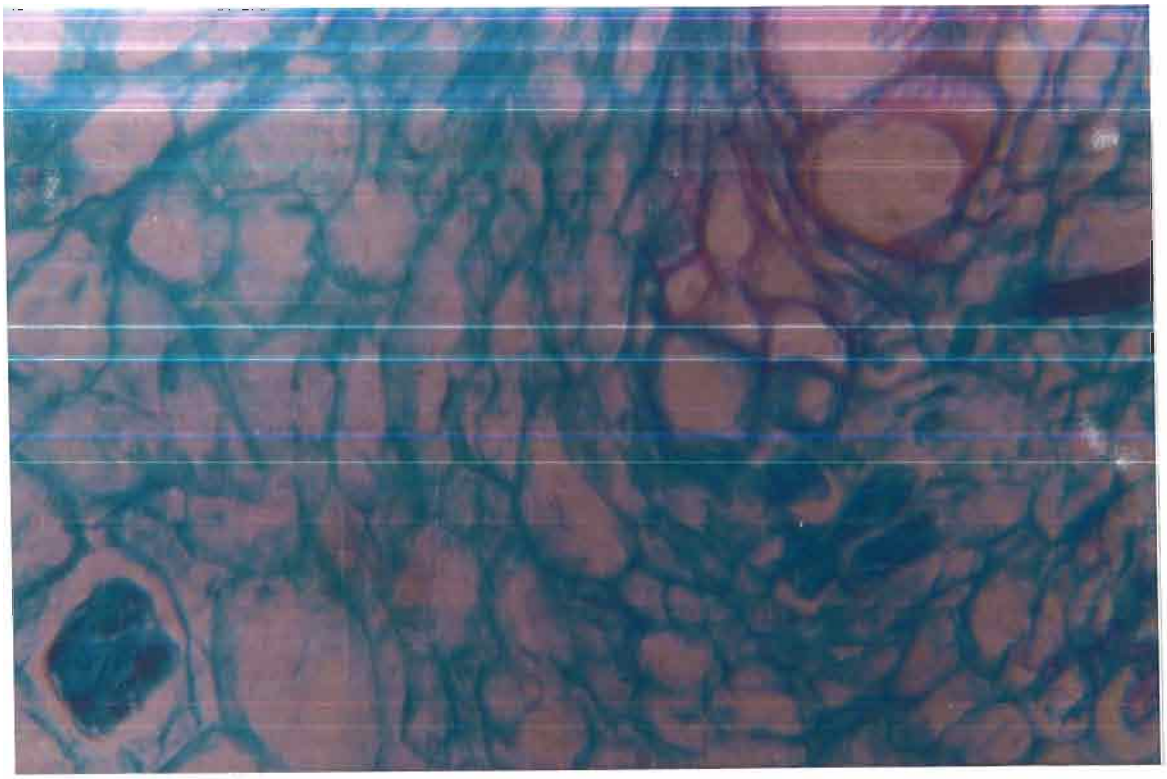


Plate 30. Lignification of cortical cells around nematode

4.6.2. Histopathology of VAM and nematode infested cotton 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. Arbuscles were found in cortical cells. Vesicles were also found which were globuse to irregular shaped (Plates 28 and 29). Arbuscles were observed in some cartical cells in proximity to entry point of nematodes. It was observed that lignification of cortical cells in VAM infected roots at the entry point of nematode may have affected further penetration of nematodes (Plate 30).

Discussion

CHAPTER - V

DISCUSSION

5.1. Effect of four species of VAM *viz.*, *G. mosseae, G. fasciculatum, G. intraradices* and *G . fulvum* on three cultivated cotton species to control *R. reniformis*

Investigations were made to study the interaction of four species of VAM with *R. reniformis* in three cotton cultivars *viz.*, MCU 5, K10 and TCB 209. It is evident from the study that all the VAM species *viz.*, *G. mosseae, G. fasciculatum, G. intraradices* and *G. fulvum* were found to have potential to increase plant growth characters *viz.*, shoot length, root length, fresh weigh of shoot and root, dry weight of shoot and root and leaf area index in all the three cotton cultivars *viz.*, MCU 5, K10 and TCB 209. The increased growth of VAM treated plants was reported by several investigators on cotton (Pugh *et al.*, 1980; Siqueira *et al.*, 1986). Potentiality of all VAM species found in our study was in accordance with the findings of Xue and Lue (1992) who reported that all the VAM fungi tried *viz.*, *G. epigaem, G. mosseae* and *G. macrocarpum* had beneficial effect on growth of peach seedlings. Shrihari and Sreenivasa (1997) also reported that five different arbuscular mycorrhizal fungi *viz.*, *G. fasciculatum, G. macrocarpum, Gigaspora margarita, Acaulospora laevis* and *Sclerocystis dussi* were found to increase growth and yield of chilli. Plant growth promotion may be related to enhanced nutrient uptake and increased tolerance to pathogens (Hayman, 1983). In addition, the plant growth regulators including indole-3-acetic acid, gibberellins and cytokinins released by VAM fungi may also contribute enhanced plant growth (Allen *et al.*, 1982; Ho, 1987).

However, VAM species examined varied in their ability for improving plant growth among and within cotton cultivars. *G. mosseae* performed well in MCU 5 and K10 but *G. fasciculatum* performed better on TCB 209 when VAM alone inoculated. Similar results were observed by Xue and Lue (1992) on peach seedlings when inoculated with three *Glomus* species. Shailaja *et al.* (1998) also reported that differential growth response by *G.mosseae* and *G. fasciculatum* on two cultivars of sesamum. Although the mechanism underlying these differentials are not well understood, it is suggested that much of the effect may be due to differences in their ability to infect host roots quickly and extensively (Abott and Robson, 1982).

In general, plants inoculated with nematode alone were stunted in growth with low shoot and root length, shoot weight, root weight and leaf area index. However, all the dually inoculated plants (VAM + nematode) were superior to nematode alone treatments in improving the biomass. The increased biomass due to VAM fungi when along with nematode has already been established by Elliott *et al.* (1984) and Suresh and Bagyaraj (1984). On combined inoculation of mycorrhiza and *R. reniformis*, the maximum biomass production was observed in *G. mosseae* + *R. reniformis* inoculated plants in all the cotton cultivars. Similarly increased plant growth on *G. mosseae* treated cotton, citrus and tomato plants inoculated along with *M. incognita, Tylenchulus semipenetrans* and *Meloidogyne incognita* were reported by O'Bannon *et al.* (1979); Saleh and Sikora (1994); and AL-radded (1995).

All the VAM species tried were found to reduce the number of infective females penetrated into the roots of all cotton cultivars tested when compared to

nematode alone treatment. Sikora and Schonbeck (1975) also reported a decrease in number of *M. incognita* larvae that penetrated in mycorrhizal plants, suggesting that some alteration in root attractiveness may play a role in the penetration of *R. reniformis* in mycorrhizal inoculated cotton plants. Similar observations were earlier reported by Hussey and Roncadori (1978) in cotton inoculated with *Pratylenchus brachyurus* and VAM fungi, *Gigaspora margarita*. Graham *et al.* (1981) reported that the presence of amino acids and reducing sugars, which are more in the root exudation of mycorrhizal plants than from nonmycorrhizal plants, may affect nematode attraction in mycorrhizal plants. Cason *et al.* (1983) suggested that the presence of VAM in plant roots must have altered the root exudates that could affect the attractiveness of roots to plant parasitic nematodes.

The number of females with eggmasses and number of eggs/egg mass in all three cotton cultivars was reduced by inoculation of all the VAM species. It indicated that VAM fungi might have caused changes in the host which affected nematode reproduction and development. The inhibition in nematode development on mycorrhizal roots is responsible for lower adult females with eggmasses and reduced number of eggs/eggmass. The reduction in penetration and development of nematode was also observed by Sikora and Schonbeck (1975) who reported 70 per cent decrease in the number of *M. incognita* juveniles that developed into adults on tobacco plants inoculated with *G. mosseae*. Sikora (1979) also reported inhibition in growth of *M. incognita* on *G. mosseae* colonized tomato root. Suppression in egg production of *M. incognita* on soybean inoculated with *Gigaspora margarita* was reported by Carling *et al.* (1989).

When comparing nematode alone treatments among the three cultivars tested, the cultivar K10 recorded less nematode population. The data on number of

females, number of females with eggmasses and number of eggs/egg mass clearly shows that the variety K10 of *G. arboreum* is the least susceptible to *R. reniformis*. *G. arboreum* has been reported to be a poor host for the reniform nematode in USA (Minton, 1964) and in India (Muralidharan and Sivakumar, 1971).

Among four VAM species tested, *G. mosseae* inoculated plants showed greater nematode reduction and maximum increase in plant growth. The next best VAM species in reducing nematode population and increasing plant growth was *G. fasciculatum*. When *G. intraradices* and *G. fulvum* were inoculated, though there was reduction in nematode population, reduction was minimum comparatively. Many studies proved that *G. mosseae* and *G. fasciculatum* were effective in reducing nematode population and improved plant growth (Rao *et al.*, 1995; Rao *et al.*, 1996). Ineffectiveness of *G. intraradices* was supported by Smith *et al.* (1986b) who observed that the rate of development of *M. incognita* was unaffected by *G. intraradices* on cotton.

In addition, the results of the present study showed that efficacy of *G. mosseae* among cotton cultivars varied in reducing nematode population and increasing plant growth. Lambert *et al.* (1980) suggested that the mycorrhizal fungi probably vary in their efficacy between different varieties of the same host so that some are more efficient than others and mycorrhizal dependency may be one of the major factors in the efficient control of nematodes. Jayaraman and Kumar (1993) also reported that the efficiency of some VAM fungus can vary markedly between different varieties of the host plant. Hence, certain host fungus combinations were more effective than others. In the present study, per cent reduction of nematode and per cent increase of plant growth was maximum in

MCU 5 than K10 and TCB 209. This suggests that mycorrhizal dependency and nematode control by VAM fungi are possibly inter-related.

When VAM colonization was considered, there was significant variation among VAM species and among cotton varieties. *G. mosseae* was observed to have maximum colonization in cotton cultivars of MCU 5 and K10. In TCB 209, the VAM colonization was maximum for *G. fasciculatum*. Colonization percentage of *G. mosseae* among the three cotton cultivars varied and it was maximum in MCU 5. Many research studies have shown that host preference exists among mycorrhizal fungi (Cruz, 1989, Silva and Siqueira, 1991). Lindeman (1988) reported that the response of the host to VAM fungi is highly influenced by host physiology, genotype, edaphic factors, environmental condition and root secretions. Ezawa *et al.* (1995) suggested that competitive ability of VAM to colonize and proliferate in the plant root varies with fungal species and the host plant. The same trend was observed in the present study also. Role of specific root exudates in selective stimulation of spore germination and hyphal growth was reported by Gianinazzi-Pearson *et al.* (1989) in clover and lupin. Further, the efficient VAM fungi are known to colonize the roots early and there by occupy as many site as possible (Afek *et al.*, 1990). In the present study also the better colonization and development of *G. mosseae* in cotton might be because of its higher competitive ability and selective stimulation by the host plant.

In VAM + Nematode treatment there was little reduction in the colonization of VAM was noticed. *G. mosseae* recorded 72.3 57.3 and 44.5 per cent colonization when inoculated alone whereas it was 62.4, 55.2 and 35.8 per cent when inoculated along with nematode on cotton cultivars *viz.*, MCU 5, K10 and

TCB 209 respectively. Similar trend of colonization was observed by Jaizme-vega *et al.* (1997) as 87 per cent colonization when *G. mosseae* alone was inoculated whereas it was 80 per cent when inoculated with *M. incognita* on banana.

In *G.fasciculatum* alone the colonization was 65.3, 51.1 and 46.1 per cent and in combination with nematode the colonization was 55.4, 49.2 and 33.1 per cent in cotton cultivars MCU 5, K10 and TCB 209 respectively. The same trend has been observed by Umesh *et al.* (1988) where *G. fasciculatum* alone colonized 83 per cent and in combination with *M. incognita* colonization was 70 per cent in banana.

Many studies indicated that mycorrhizal spore production was influenced by plant parasitic nematode. However, the reported results are varied. Shenck *et al.* (1975) using different levels of nematode population found that lower levels of *M. incognita* stimulated spore production of the mycorrhizal fungus, *Endogone heterogama* in soybean. Enhanced production of mycorrhizal spores in the presence of nematodes has also been observed by Roncadori and Hussey (1977). However, Umesh *et al.* (1988) observed that at higher nematode infestation level, there was decrease in spore production. In the present study also, *G. mosseae* treated cotton plants observed to have lower nematode penetration and development, subsequently resulting in higher spore production. *G. intraradices* and *G. fulvum* recorded higher nematode population resulting in decreased spore production.

The observations made in the present study VAM alone and in combination with nematode increased the cotton yield and yield attributes *viz.*, number of bolls,

boll weight, ginning percentage, seed index and lint index. Inoculation with *G. mosseae* alone and in combination with nematode increased cotton yield to a maximum extent in all the three cotton cultivars tested. Significant increase in the yield of several crops *viz.*, tomato (Al-Radded, 1995), banana (Jaizme-Vega *et al.*, 1997), blackgram (Sankaranarayanan and Rajeswari Sundarababu, 1999) due to *G. mosseae* application was reported earlier. Also VAM application in various crops reported to have increased yield and yield attributes (Mohamed *et al.*, 1999; Pandey *et al.*, 1999).

There was an increase in fibre quality character when VAM species applied alone and along with nematode, though some of the characters were non significant on the cotton cultivars. The findings in the present study showed decrease in the fibre quality characters when nematode alone was inoculated. Jones *et al.* (1959) reported that the quality of lint and fibre of cotton deteriorated due to *R. reniformis* infestation and by controlling nematodes using soil fumigation they improved the quality of the produce. The reduction in quality of castor oil (Sivakumar and Seshadri, 1971) and cotton lint and fibre (Murlidharan and Sivakumar, 1977) due to *R. reniformis* were reported earlier. In present study also it was found that VAM application significantly reduced nematode population which improved fibre quality simultaneously. In addition, improved quality of groundnut (Elsheikh and Mahamadzein, 1998) and rice (Lang *et al.*, 1998) due to VAM application was observed earlier which indicated that VAM itself has the potential to improve fibre quality in cotton.

In general, mycorrhizae have improved the nitrogen content in shoots of cotton plants. Among VAM species, *G. mosseae* and *G. fasciculatum* recorded

maximum nitrogen content. Even when mycorrhizae were inoculated along with nematodes it showed improved nitrogen content when compared to nematode alone treatment. The mycorrhizal fungal hyphae extract nitrogen from soil by its absorptive surface. Also the fungus contains an enzyme nitrogen reductase which breakdown the organic nitrogen (Bajwa and Read, 1985) which resulted in the increased nitrogen content in shoots of VAM inoculated cotton plants. This is in accordance with the research work of Suresh and Bagyaraj (1984) and Umesh *et al.* (1988).

Phosphorus content of all VAM species inoculated plants was more in all the three cotton cultivars, but it was reduced when nematode was inoculated along with mycorrhizae. However, VAM with nematode treatment had more P content when compared to nematode alone and control treatments. 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).

Improved potash content in cotton plants inoculated with VAM alone and along with nematode was noticed in the present study. The nematode alone treatment recorded lowest concentration of K content. The increased K content in VAM fungi alone and with nematode treated plant was earlier reported by Smith *et al.* (1984) on cotton and Smith and Kaplan (1988) on citrus.

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 in each cotton cultivars. When VAM was inoculated with nematode, there was a slight decrease in N, P, K concentration than VAM alone treatments. Significant decrease in the

nutrient in nematode alone treated plants was observed. This has been supported by many authors. Jaizme-vega *et al.* (1997) reported an higher concentration of N, P, K in banana inoculated with *M. incognita* and *G. mosseae*. Umesh *et al.* (1988) also recorded increased concentration of N, P, K in banana plants inoculated with *R. similis* and *G. fasciculatum*.

From the results, it is clear that the concentration of micronutrients *viz.*, Fe, Mn, Cu and Zn was higher in the plants inoculated with VAM species and it was least in nematode alone treatment. The concentration of these nutrients slightly reduced when nematodes were inoculated with VAM. Similar results with improved micronutrient status observed by Pinochet *et al.* (1995) on peach root stock; Calvet *et al.* (1995) on quince root stock; and Lopez *et al.* (1997) on pear root stock. The increased mineral content in mycorrhizal plants may be attributed extensively to the ability of VAM fungi to expand the volume of the absorption surface of the roots in soil by which mineral nutrients are made available to plants (Clark and Zeto, 2000).

In the present study, it was observed that *R. reniformis* affected the physiology of cotton plants. *R.. reniformis* alone inoculated plants showed to increase leaf temperature, diffusive resistance, decrease transpiration rate and photosynthetic rate. Nematode infection decreased diffusive resistance, which resulted in decreased leaf transpiration rates. Because of the lower leaf transpiration rates, evaporative cooling of leaves decreased with a concomitant increase in leaf temperature of the infected plants. Because of increased leaf temperature, infected plants may have experienced greater stress than control. Similarly root knot nematode induced stress on cotton (Patel *et al.*, 1990) and

papaya (Ramakrishna and Rajendran, 1995) was reported earlier. In addition VAM treated plants alone and along with nematode recorded reduced leaf temperature, diffusive resistance, increased transpiration rate and photosynthetic rate which indicated that VAM treatment offset the stress caused by nematodes. Similarly decreased leaf temperature, diffusive resistance, increased transpiration rate and photosynthetic rate due to VAM treatment was reported by Subramanian and Charest (1999) on maize and Syvertsen and Graham (1990) on citrus.

5.2. Effect of seed treatment and soil application of VAM, *G. mosseae* for the management of *R. reniformis* on cotton cv. MCU 5

Plants inoculated with mycorrhiza irrespective of inoculation method at both dosages increased plant growth. The increased plant growth due to VAM fungi has already been established by Elliot *et al.* (1984); Suresh and Bagyaraj (1984).

In the present investigation, a lot of variations among and within different inoculation methods of VAM were observed in their ability for stimulating plant growth. Inoculation of VAM as soil treatment gave better results in improving plant growth than seed treatment with VAM. The simple reason is VAM inoculum in the rhizosphere has easy access to the roots and it proliferates covering the entire root system. Similar findings were reported by Ross and Harper (1970) in soybean; Timmer and Leydon (1978) in citrus. Abbott and Robinson (1984) also reported that more mycorrhizal root was formed by *Glomus* sp. when inoculum was dispersed through out the soil than when it was treated with seed material.

Inoculation of VAM by both seed and soil application reduced the nematode population *viz.*, number of females, number of females with eggmasses,

number of eggs per eggmass, and soil nematode population. Similar observations have been earlier reported by Hussey and Roncadori (1978) on cotton; Bagyaraj *et al.* (1979b) on tomato, Kellam and Schenk (1980) on soybean, Sitaramaiah and Sikora (1982) on tomato and Jain and Sethi (1988) on cowpea. Such a reduction of nematode population reflecting in enhancing the plant growth characters was observed in the present study also. Further, the maximum reduction of nematode population occurred in soil application @ 10 g/kg soil. Nagesh *et al.* (1999) also reported that the nematode population was negatively correlated with initial dosage of VAM spores which was in accordance with present findings.

In the present study, VAM application reduced the severity of nematode incidence in mycorrhizal plants which may be due to altered biochemical constituents in host plant (Sikora and Schonbeck, 1975) or improved plant nutrition, especially phosphorus (Hussey and Roncadori, 1992) or alteration of the compounds in root exudates (Sikora, 1981) or alteration of physiological compounds in the root tissue (Shenck *et al.*, 1975) or hardening of root tissue due to increased lignin levels in the endo and exodermis of mycorrhizal plants (Dehne and Schonbeck, 1975).

Nemec and Meredith (1981) reported higher aminoacid content in mycorrhizal plant compared to control. Further the mycorrhizal plants had higher concentration of phenylalanine and serine which are known to reduce the growth and reproduction of root knot nematodes (Krishna Prasad, 1975). Thus, the presence of increased quantities of sugars, aminoacids, like phenylalanine and serine and phosphorus may each or collectively, play a role in suppressing the development of nematodes in the mycorrhizal plants. It is speculated that, the same

kind of mechanism might be the reason in the present study for the reduction in nematode population.

In the present investigation, the maximum spore numbers and mycorrhizal colonization was recorded in plants where VAM was applied in soil than in seed treated plants. This may be due to the uniform and complete distribution of VAM inoculum in the root proliferation zone, covering entire root system with inoculum resulting in more infection point, creating a complete colonization of the root (Jackson *et al.* 1972; Timmer and Leydon, 1978). Saleh and Sikora (1984) reported that 55-60 per cent mycorrhizal colonization was required to suppress *M. incognita* reproduction on cotton. Similarly in present study, soil application of VAM @ 10 g and 5 g/kg soil recorded 82.3 and 71.6 per cent colonization which was found to reduce nematode population effectively. But seed treatment with VAM @ 10 g and 5 g recorded only 52.6 and 42.4 per cent colonization which recorded least reduction in nematode population.

Phosphorus content of shoots of cotton plants also significantly increased by VAM fungi than control, which is in confirmity with the findings of Gerdeman (1968), Hayman (1982), where VAM fungi formed a beneficial symbiotic association with roots which increased the plants ability to absorb phosphorus.

The yield and fibre quality were reduced in the presence of *R. reniformis* which was also observed earlier by Muralidharan and Sivakumar (1977). Lawrence *et al.* (1990) also found the association of high population of *R. reniformis* resulted in stunted cotton plants with reduced yield. The yield and yield attributes were high in the plants treated with VAM as soil application at higher

dosage. Similar high yields were observed by Sitraramaiah and Sikora (1996) in cotton when VAM inoculated as soil treatment with high initial spore inoculum.

5.3. Effect of VAM and different biocontrol agents for the management of *R. reniformis* on cotton cv. MCU 5

Different biocontrol agents *viz.*, *G. mosseae, P. fluorescens* and *T. viride* both as seed and soil application were evaluated for their effectiveness against reniform nematode in cotton. Results of the experiment showed that soil application of *G. mosseae* was found to be most effective against the reniform nematode. However, its effect was on par with *P. fluorescens* as seed treatment.

The present finding is in accordance with Klyuchnikor and Kozhervin (1990) who observed that endomycorrhiza, *G. Mosseae* and *P. flourescens* had the most favourable effect in improving plant growth and decreasing pathogenic infection in the rhizosphere of potato similarly and equally. Among all biocontrol agents, *T. viride* both as seed and soil application was less effective than VAM and *P. fluorescens*. It is in accordance with findings of Priyarani *et al.*, (1998) and Sramek *et al.*, (2000). They observed that *G. mosseae* was most effective in reducing population of *M. incognita* than *T. viride* on tomato.

In general, all the biocontrol agents were found to increase the plant growth characters *viz.*, shoot and root length, fresh weight of shoot and root, dry weight of shoot and root and leaf area index. The increased growth parameters in *G. mosseae* treated plants were earlier reported by Afek *et al.* (1990) on cotton, Al- Radded (1995) on tomato and Jaizme-Vega *et al.* (1997) on banana. The increase in growth parameters in the mycorrhizal plants attributed to the increased uptake of nutrients

and better physiological status of the plants (Alexander *et al.*, 1989 and Sreenivasa, 1992).

The increased growth of *P. fluorescens* treated plants was also reported by several investigators in crops such as potato (Burr *et al.*, 1978 ; Kloepper *et al.* 1980a) citrus (Gardner *et al.*, 1978), cotton (Howel and Stipanovic, 1980) and tomato (Gamliel and Katan, 1991). Plant growth promotion may be related to the suppression of population of various parasitic and non parasitic pathogens in the rhizosphere (Burr *et al.*, 1978). In addition, growth regulators including gibberellins, cytokinins and indole 3-Acetic acid produced by the plant growth promoting bacterium also reported to constitute a mechanism for the plant growth (Brown, 1974, Lifshits *et al.*, 1987). Improvement in plant growth, parameters by *T. viride* application also reported by Stephan *et al.* (1996) on tomato and Sankaranarayanan *et al.*, (1998) on sunflower.

Among different bio control agents tested, *G. mosseae* and *P. fluorescens* were found to be the best in controlling nematode population both in soil and root in addition to improving plant growth parameters. Through *T. viride* was found to reduce nematode population, the efficacy was not comparable with rest of the biocontrol agents. The reduction in the severity of nematode infection in mycorrhizal plants may be due to altered biochemical consitituents in the host plant (Sikora and Shonbeck, 1975) or alteration of compounds in the root exudates (Sikora, 1981) or improved plant nutrition, especially phosphorus (Hussey and Roncadori, 1982). The effectiveness of *G. Mosseae* was supported by Rao *et al.* (1999) who observed reduction in penetration, development and reproduction of *M. incognita* on tomato treated with *G. mosseae*. The effectiveness of *P.*

fluorescens was supported by Oostendorp and Sikora (1989) who observed that there is a potential nature in *P. fluorescens* as a biocontrol agent for the management of plant parasitic nematodes. They suggested that the mechanism responsible for the reduction of nemsatode penetration was attributed to the ability of bacterium to envelop or bind to root surface lectins, there by interacting with normal host recognition by the nematode.

Maximum colonization of VAM was observed in soil application which was supported by Timmer and Leydon (1978). Seed treatment with *P. fluorescens* had maximum colonization than soil application. It was in accordance with the findings of Anurhadha and Gnanamanickam (1987). Colonizing ability of VAM and *P. fluorescens* helped in improving plant growth and reducing nematode population. Similarly improved plant growth and reduction in nematode population with maximum colony of VAM was recorded by Reddy *et al.* (1998) and maximum colony of *P. fluorescens* by Santhi and Sivakumar (1995).

Application of VAM in various crops was reported to improve yield and yield attributes (Makus, 2000). The observations made in the present study also increased that soil application of VAM increased yield parameters *viz.*, number of bolls and boll weight. However it was on par with seed treatment with *P. fluorescens*. Improved yield attributes by *P. fluorescens* application on cotton was also reported by Howel and Stipanovic (1986).

The effective treatment of soil application of VAM and seed treatment with *P. fluorescens* was found to increase the seed cotton yield 250.0 and 229.6 percent

respectively. Significant increase in the yield of cotton due to *G. mosseae* (Makus, 2000) and *P. flourescens* (Misaghi, 1990) was reported earlier.

5.4. Effect of VAM and different biocontrol agents for the management of *R. reniformis* and *Fusarium oxysporum* f. sp. *vasinfectum* wilt disease complex on cotton cv. MCU 5

Nematode infestation (number of females and females with eggmasses in roots) and its multiplication (number of eggs/eggmass and soil nematode population) decreased in all biocontrol agents treated cotton plants compared to control (*R. reniformis* + *Fusarium* alone). Reduction of nematode population by VAM (Rao *et al.*, 1999), *P. fluorescens* (Santhi and Siyakumar, 1995) and *T. viride* (Sharma, 1999) have been reported earlier. The nematode reduction by VAM may be due to altered biochemical constituents in the host plant (Sikora and Schonbeck, 1975) or improved plant nutrition, especially phosphorus (Hussey and Roncadori, 1982) or alteration of compounds in the root exudates (Sikora, 1981) or hardening of VAM root tissue due to increased lignin levels in the endo and exodermis of mycorrhizal plants (Dehne and Schonbeck, 1975). The nematode reduction by *P. fluorescens* may be due to alteration of root exudates (Oostendorp and Sikora, 1989) or alteration of biochemical changes in roots (Nayar, 1996), or production of antibiotics (Cronin *et al.*, 1997). *T. viride* may reduce nematode population by chitinase enzyme which hydrolyze the nematode egg shell (Jatala, 1986). It is speculated that the same kind of mechanism by the above biocontrol agents might also operate in the present study to reduce nematode population.

The wilt index was also reduced by treatment with all biocontrol agents. The reduction of disease severity by VAM (Schonbeck and Dehne, 1977),

P. fluorescens was supported by Oostendorp and Sikora (1989) who observed that there is a potential nature in *P. fluorescens* as a biocontrol agent for the management of plant parasitic nematodes. They suggested that the mechanism responsible for the reduction of nematode penetration was attributed to the ability of bacterium to envelop or bind to root surface lectins, there by interacting with normal host recognition by the nematode.

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The effective treatment of soil application of VAM and seed treatment with *P. fluorescens* was found to increase the seed cotton yield 250.0 and 229.6 percent

inoculated with *M. incognita* and *Macrophomina phaseolina* was observed on cowpea by Devi and Goswami (1992). Gianinazzi (1988) also reported that VAM improved the nutrient uptake and water absorption by increasing the absorbing surface area thereby increased the plant growth. Similar findings of improved plant growth and 'P' uptake was observed in this experiment also by *G. mosseae* treatment. Improvement on plant growth by *G. mosseae* also reported by Schenck *et al.* (1975) and Hussey and Roncadori (1982b).

Hussey and Roncadori (1982b) and Bhagawathi *et al.* (2000) postulated that VAM fungi offset the yield loss normally caused by nematodes and fungal pathogens by enhanced uptake of phosphorus and other nutrients, therefore improving plant growth and yield. This statement can be justified by the present study with *G. mosseae* inoculated plants with nematode and *Fusarium* showed reduced nematode population and wilt incidence thereby improved seed cotton yield as compared to control. However, the increase in yield of cotton due to *G. mosseae* was on par with *P. fluorescens* application. There are records of significant increase in the yield of several crops *viz.*, potato (Burr *et al.*, 1978) and wheat (Becker and Cook, 1988) due to *P. fluorescens* application.

5.5. Biochemical changes due to interaction of reniform nematode, *Fusarium* wilt fungus and *G. mosseae* on cotton cultivars MCU 5 and K10

5.5.1. Protein content

In the present investigation an elevated level of protein concentration was observed in shoots and roots of both the cotton cultivars. In both cotton cultivars protein content was maximum in combination of both pathogens and either pathogens alone. Present findings of increased protein synthesis during nematode

infestation and Fusarium pathogenesis are in line with those of Bird (1961) Litterell (1966), Singh *et al.* (1978) and Sharma and Kaul (1999). The increase might be due to synthesis of new proteins by invaded pathogens (Uritani, 1976). In addition, both cultivars observed to increase protein content to a little extent in VAM alone and VAM along with pathogens. Similar increase in protein content in mycorrhizal plants was observed earlier by Senthil Kumar (1999) on maize and Abdel-Ali (2000) on cowpea. Stahmann and Demores (1998) suggested that inoculation with either pathogens did not show any increase in protein content on resistant cultivars. The same trend was observed in K10 cultivar where protein content increase was only a little and that may be the reason for it to be less susceptible to *R. reniformis*.

5.5.2. Total phenol content

Phenols have been claimed to play a significant role in the host plant against infection of pathogens. Phenols occur in plant tissue as inactive phenolic glycosides, phenolic ester, flavonoids, coumarin derivatives, phenolic acids and terpenoid compounds. Following infection by pathogen, the bound phenols are released by the active enzyme, glucosidase. The active phenols thus released participate in the defense reaction of the host. Distinct correlation between the degree of plant resistance and the phenolics present in the plant tissue had been indicated by Pitcher *et al.* (1960); Brueske and Dropkin (1973). Results of the present study showed that VAM alone and along with pathogens induced more accumulation of total phenolics in shoot and roots of cotton plants. Accumulation of phenols in the hyphae and arbuscles of the mycorrhizal plants which helped in the reduction in the reproduction of nematodes was reported by Krishna and Bagyaraj, 1984 and Singh *et al.*, 1990.

5.5.3. Total sugar content

Increased total sugar content was observed in the host resistant to nematodes (Batemann and Miller, 1999) and pathogens (Sindhan *et al.*, 1999). From the results it was observed that the total sugar was more in mycorrhizal plants when inoculated alone and with pathogens which indicated that VAM increased resistance in the plants to the infection by nematodes and pathogens. The same has been reported by Suresh and Bagyaraj (1984) and Umesh *et al.* (1988). Therefore high sugar content in mycorrhizal plants indicated the development of resistance in cotton to nematode disease complex.

When nematode infected plants and control were compared, it was observed that total sugars were slightly increased in nematode alone plants. It can be concluded that localized invertase in esophagus and intestine of nematode parasite cause the secretion in the host tissue, which caused the changes in carbohydrate metabolism in the host (Roy, 1979). Increased total sugar in nematode infected cucumber and tomato was observed by Zinovew (1969) and Farooqi *et al.* (1986) which is in accordance with the present findings.

5.5.4. Reducing sugar content

In general, reducing sugars in nematode and Fusarium treated plants were decreased in MCU 5. Sindhan and Prashar (1996) reported plants resistant to pathogens recorded increased in reducing sugar and susceptible plants recorded decrease in reducing sugar content when pathogens were inoculated. Similar trend was observed in our study as decreased reducing sugar was observed in MCU 5 when pathogens were inoculated. And there was a slight increase in reducing sugar in K10 which may be the reason for its less susceptibleness found in our study. In

general, amount of reducing sugars was more in VAM treated plants. Increased reducing sugar content of VAM treated plants observed earlier by Rao and Rao (1998) also.

5.5.5. Total free amino acid content

VAM alone and along with pathogens inoculated plants showed high amount of total free amino acid compared to either pathogen alone or in combination. Coxwell and Johnson (1985) reported that VAM colonized plants had higher concentration of amino acids in xylem sap compared to nonmycorrhizal plants. There are indications that amino acid induce host resistance to fungal pathogens (Kuc *et al.*, 1957; Decker, 1969) and nematodes (Gonclaves *et al.*, 1995). The decrease in amino acid content in nematode and Fusarium inoculated plants was in line with the findings of Kaul and Munjal (1980) in apple tree infested with rotting fungi and Zaki and Bhatti (1987) in pigeonpea infected with cyst nematodes. The extent of increase was more in K10 which was less susceptible to *R. reniformis* and more in MCU 5 which was more susceptible to *R. reniformis*. Similar results were observed to tomato cultivars resistant and susceptible to *M.incognita* by Horeya *et al.* (1994).

5.5.6. Peroxidase activity

The plant peroxidases are important in the reinforcement of the cell wall at the border of infection in resistant plants and they are considered as important component of an active defense response of pathogen invaded tissue (Zacheo *et al.*, 1995). In the present study the peroxidase activity was observed to increase in nematode and Fusarium inoculated plants both in MCU 5 and K10 cultivars. This is in accordance with the work of Mohanty *et al.* (1986) and Sujatha and Usha

Mehta (1998). However the extent of peroxidase activity in pathogens inoculated plants was high in K10 cultivars (less susceptible to *R. reniformis*). Similarly increased peroxidase activity in resistant cultivars of tomato was reported by Shukla and Chakraborthy (1988). The present results showed that there was increase in peroxidase content in plants treated with VAM in both cultivars. Similarly enhanced peroxidase activity was documented by Salzer *et al.* (1999) and Kong *et al.* (2000) on mycorrhizal inoculated plants.

5.5.7. Chitinase activity

Application of VAM was found to increase chitinase enzyme accumulation in cotton plants in the present investigation. The same response of increased in chitinase activity due to VAM was reported by Ramesh *et al.* (2000). Chitin, a β-1-4, linked polymer of N-acetylglucosamine, is a structural component of cell wall of fungi and egg shell of nematodes. The enzyme chitinase hydrolyzes the chitin layer and subsequently disruption of hyphae of pathogenic fungi and nematode egg shell takes place (Punja and Zhang, 1993).

5.5.8. Macronutrient content

5.5.8.1. Nitrogen content

In the present studies, nitrogen content in shoots of cotton plants was reduced by pathogen infection. Earlier work by Oteifa and Elgindi (1962) and Heffes *et al.* (1991) confirmed the report of reduction of nitrogen content by nematodes and fungal pathogens. However, nematodes and in combination with *Fusarium* causes a slight increase in the nitrogen content in the roots. In general, VAM inoculation increased both shoot and root nitrogen content in both cultivars and offset the depletion of nutrients by pathogens infection. The increase in

nitrogen content due to VAM infection was earlier documented by Pinochet *et al.* (1995) and Abha Mishra (1997) also.

5.5.8.2. Phosphorus content

The phosphorus content in shoot and root was found to be reduced by nematode wilt complex in the present study. Decreased 'P' content was also observed by Dropkin and King (1956) and Heffes *et al.* (1991). It was observed that VAM inoculation increased 'P' content even in the presence of nematode or/ and Fusarium treatments. The increase in 'P' content due to VAM in nematode and pathogen inoculated plants was established earlier by Winkler *et al.* (1994) on soybean infected with *Heterodera glycines* and *Macrophomina phaseolina.*

5.5.8.3. Potassium content

From the present studies, it is evident that the potassium content in shoot and root of cotton was reduced by nematode/Fusarium infection. Fatemy and Evans (1986) found that potassium content in plants infected by *M. incognita* tend to decrease. However, VAM inoculation alone and along with pathogens improved 'K' content significantly. Similar observations was made by Jaizme-Vega *et al.* (1997) on banana.

5.6. Micronutrient content

The concentration of iron, manganese, copper and zinc in shoots and roots decreased with nematode and Fusarium alone and in combination in both cotton cultivars. But the extent of reduction was comparatively less in K10 than MCU 5. The maximum reduction in all these elements was obtained when both nematode and Fusarium were combined. It was in accordance with the findings of Kumar

(1995) who reported maximum decrease in iron, manganese, copper and zinc when *R. reniformis* and *Rhizoctonia solani* combined than separately applied. However, concentrations of these elements was maximum in VAM treated plants. The VAM along with pathogens alone and in combination also increased the concentration of these elements when compared to control. Similar increase in micro nutrients by VAM application was reported earlier by Clark and Zeto (2000).

5.7. Histopathological study

The present study has shown that the reniform nematode is mainly a phloem feeder. The damage to cortex is caused mostly due to mechanical destruction of cells. The infected tissues at the site of feeding show hypertrophy, hyperplasia, thickening of cell walls, granular cytoplasm and enlarged nuclei. These changes were brought about by *R. reniformis* on castor and papaya (Sivakumar and Seshadri, 1972). Head (1975) reported that syncytia extended 6-10 cells on either side of the feeding cells and consisted of a curved sheet of hypertrophied pericylcle cells which is observed in this study also.

In the present study it was observed that the nematode induced 'syncytia unit' formed with 100-150 number of modified cells. The extent of cell modification caused by *R. reniformis* forming one 'syncytial unit' has been estimated to be 100-200 modified cells in coffee by Vovlas and Lamberti (1990).

Rebois (1980) observed that closer examination of the feeding zone showed that the nematode stylet was inserted into one endodermal cell of the feeding unit. Where the stylet penetrated the wall of feeding cell, a small peg like structure called 'feeding peg' is formed. This is in line with the present study.

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 arbuscles. O' Bannon *et al.* (1979) observed that in citrus seedling inoculated with *Tylenchulus semipenetrans* and *G. mosseae*, it was found that *G. mosseae* colonized upto 50 per cent in the root. The fungus rapidly invaded the roots and produced vesicles as well as arbuscles before nematode invasion.

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

CHAPTER - VI

SUMMARY

The reniform nematode, *Rotylenchulus reniformis* is the potential pest of cotton and it is established to be one of the major causes for limiting cotton production. Hence, there is a need to evolve suitable strategy for the management of this nematode and to increase the production of cotton. The biocontrol means of controlling this nematode using vesicular arbuscular mycorrhizae was attempted. So far only chemical nematicides have been suggested for the control of this nematode. But it is not easily affordable by the farming community. In addition, pesticide usage in cotton ecosystem will lead to several pollution and residual toxicity problems. With the above facts in view, studies were undertaken to select efficient VAM fungi among *G. mosseae*, *G. fasciculatum*, *G. intraradices* and *G. fulvum*. Attempts were also made to evaluate two methods of application *viz.*, seed treatment and soil application at two dosages under glasshouse condition. Besides, effect of different commercial formulations of biocontrol agents viz., *G. mosseae*, *P. fluorescens* and *T. viride* as seed and soil application were evaluated for the management of reniform nematode and reniform nematode - *Fusarium* wilt complex in *G. hirsutum* cv. MCU 5, *G. arboreum* cv. K10 and *G. barbedanse* cv. TCB 209. In addition, the possible biochemical changes during VAM, nematode and *Fusarium* interactions were studied. Histological studies were made on the nematode VAM interaction. The results obtained in the present study are enumerated below:

1. Among the four species of VAM tried, *G. mosseae* performed well in improving growth and yield of cotton cultivars MCU 5 and K10 and *G. fasciculatum* was best on TCB 209. When considering VAM along with

nematode treatments, *G. mosseae* was found most effective against *R. reniformis* and increased growth and yield of all three cotton cultivars.

2. *G. mosseae* decreased the number of females, number of females with egg masses, number of eggs per egg sac and soil nematode population in all three cotton cultivars effectively.
3. *G. mosseae* not only reduced the nematode population but also increased the plant growth characters *viz.*, shoot and root length, fresh weight of shoot and root, dry weight of shoot and root and leaf area index.
4. Maximum VAM colonization and spore count occurred in *G. mosseae* on cotton cultivars MCU 5 and K10. In TCB 209, it was maximum in *G. fasciculatum*. However, *G. mosseae* was best in all three cotton cultivars when combined with nematode.
5. There was increase in yield parameters *viz.*, number of bolls, boll weight, ginning percentage, seed index, lint index and cotton yield in the treatment of *G. mosseae* along with nematode.
6. *G. mosseae* was also found to improve fibre quality characters *viz.*, fibre fineness, mean fibre length, bundle strength in all cotton cultivars tested even though these characters were non significant in K10 and TCB 209.
7. The nitrogen content was more in mycorrhizal plants. *G. mosseae* and *G. fasciculatum* recorded higher content of total nitrogen. VAM combined with nematode recorded lesser amount of 'N' compared to VAM alone in all the cotton cultivars.
8. The phosphorus content was more in mycorrhizal plants whereas the control and nematode inoculated plants showed a decreased level. The 'P' content was more in VAM + nematode in all cotton cultivars when compared to nematode alone.

9. The amount of potassium was very much reduced in nematode infected plants but the percentage of reduction was lower in K10. VAM along with nematode recorded more 'K' content compared to nematode alone in all cotton cultivars.

10. The micronutrients like iron, zinc, copper and manganese were higher in VAM treatment but reduced slightly when nematode was inoculated along with VAM. The concentration was very much reduced in nematode alone.

11. *G. mosseae* nullified the physiological stress caused by *R. reniformis* by decreasing leaf temperature and diffusive resistance and by increasing transpiration rate. *G. mosseae* also improved photosynthetic rate in all cotton cultivars.

12. Studies on the method of application to increase effectiveness of VAM (*G. mosseae*) revealed that the soil application of VAM @ 10 g/kg soil was the best method to obtain highest control of nematode population and improved cotton yield.

13. Soil application of *G. mosseae* @ 10 g/kg soil gave maximum VAM colonization and cotton yield, than seed treatment with VAM.

14. Studies on different biocontrol agents to manage reniform nematode revealed that *G. mosseae* as soil application was best to obtain highest control of nematode population. The next best treatment was seed treatment with *P. fluorescens* which was on par with the former.

15. There was an increase in plant growth parameters and yield when VAM was applied as soil treatment. However, the effect of this treatment was not significantly different from the effect of seed treatment with *P. fluorescens*.

16. Among the different biocontrol agents tried for the management of reniform nematode - *Fusarium* wilt complex, soil application with VAM was found to

be effective in reducing both nematode population and wilt fungus. It was on par with seed treatment of *P. fluorescens* treatment.

17. The lowest wilt index was observed in soil application of *G. mosseae* and seed treatment with *P. fluorescens*.

18. Improved plant growth and subsequent increase in cotton yield were noticed in soil application of VAM and seed treatment of *P. fluorescens* which were on par with each other.

19. In general, *G. mosseae* and *P. fluorescens* were more effective than *T. viride* for checking reniform nematode - *Fusarium* wilt complex.

20. The protein content was more in nematode/*Fusarium* alone and in combination. When pathogens (Nematode + *Fusarium*) were inoculated, the shoot showed lesser amount of protein compared to roots.

21. VAM inoculated cotton plants (both MCU 5 and K10) showed a higher level of phenol content. VAM along with pathogens also showed a marked increase in phenolic content.

22. The phenol content was less in nematode + *Fusarium* treatment in MCU 5. However, it increased to some extent in K10 cultivar.

23. Total sugars were more in mycorrhizal plants inoculated alone and along with pathogens. It was slightly more in nematode alone plants on MCU 5. In general, VAM + *Fusarium* caused highest reduction of total sugar content both in shoot and root in MCU 5 and K10.

24. The reducing sugars were also maximum in VAM treated plants than nonmycorrhizal plant. In MCU 5, *Fusarium* + nematode recorded minimum content of reducing sugars, whereas in K10 the reducing sugar content increased slightly over control.

25. The total free amino acid content was least in nematode + *Fusarium* treatment in MCU 5, but it slightly increased a little over control in K10

cultivar. The maximum amount of total free amino acid was recorded on VAM alone treatment in both the cotton cultivars followed by VAM along with pathogens.

26. Peroxidase activity was more in VAM along with pathogens treatment. Nematode and *Fusarium* alone and in combination recorded a lesser peroxidase activity but was slightly more than untreated.

27. Maximum chitinase activity was observed in VAM alone and along with pathogens but it was minimum in control.

28. The nitrogen content was more in mycorrhizal plants when inoculated alone and along with pathogens. However, root nitrogen content in nematode + *Fusarium* treatment was also increased to some extent compared to control and a little more in MCU 5 and less in K10.

29. The phosphorus content was more in mycorrhizal plants whereas the control and pathogens inoculated plants showed a decreased level. The 'P' content was also more in VAM along with pathogens when compared to pathogens alone.

30. The amount of potassium was very much reduced in nematode + *Fusarium* infected plants. VAM alone and along with pathogens recorded more potassium compared to control and pathogens alone.

31. The micronutrients like iron, zinc, copper and manganese were higher in VAM alone and it was lesser when pathogens were inoculated along with VAM. The concentration of the nutrients was very much reduced to the minimum extent when both nematode and *Fusarium* were inoculated.

32. The histopathological studies showed that the vesicles and arbuscules were formed in the cortex cells.

33. The cortical tissues of mycorrhizal plants were lignified, which deter penetration of nematodes.

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