INTERACTION BETWEEN VA MYCORRHIZA AND *BRADYRHIZOBIUM* IN COWPEA

By

S. BEENA

THESIS

Submitted in partial fulfilment of the requirement for the degree of

DOCTOR OF PHILOSOPHY

(PLANT PATHOLOGY)

Faculty of Agriculture
Kerala Agricultural University

DEPARTMENT OF PLANT PATHOLOGY
COLLEGE OF HORTICULTURE
Vellanikkara, Thrissur-680 656
KERALA, INDIA
1999

DECLARATION

I hereby declare that this thesis entitled **'Interaction between VA mycorrhiza and *Bradyrhizobium* in cowpea'** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, fellowship or other similar title, of any other University or Society.

Vellanikkara
4-6-99

S. BEENA

CERTIFICATE

Certified that this thesis entitled **`Interaction between VA mycorrhiza and *Bradyrhizobium* in cowpea'** is a record of research work done independently by **Smt. S. Beena,** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

Dr.M.V. Rajendran Pillai
Chairman, Advisory Committee
Associate Professor
Department of Plant Pathology
College of Horticulture

Vellanikkara
9-6-99

CERTIFICATE

We, the undersigned members of the Advisory Committee of **Smt.S.Beena,** a candidate for the degree of **Doctor of Philosophy in Agriculture,** agree that the thesis entitled **'Interaction between VA mycorrhiza and *Bradyrhizobium* in cowpea'** may be submitted by Smt.S.Beena, in partial fulfilment of the requirement for the degree.

20/7/99

Dr.M.V. Rajendran Pillai
Associate Professor
Department of Plant Pathology
College of Horticulture
Vellanikkara
(Chairman)

Dr.A. Sukumara Varma
Associate Professor & Head
Dept. of Plant Pathology
College of Horticulture
Vellanikkara
(Member)

Dr.Koshy Abraham
Associate Professor (Plant Pathology)
Cadbury Cocoa Research Project
College of Horticulture
Vellanikkara
(Member)

Dr.S.Rajan
Associate Professor & Head
Dept. of Olericulture
College of Horticulture
Vellanikkara
(Member)

Sri.S. Krishnan
Assistant Professor
Dept. of Agricultural Statistics
College of Horticulture
Vellanikkara
(Member)

EXTERNAL EXAMINER

ACKNOWLEDGEMENT

With immense pleasure, I express my deep sense of gratitude and indebtedness to **Dr.M.V.Rajendran Pillai**, *Associate Professor, Department of Plant Pathology, College of Horticulture, Vellanikkara and Chairman of the Advisory Committee for his personal attention, constant encouragement, guidance and constructive criticism throughout the course of this study and in the preparation of the manuscript.*

I am very much obliged to **Dr.A.Sukumara Varma**, *Associate Professor and Head, Department of Plant Pathology, College of Horticulture, Vellanikkara and member of the advisory committee for his valuable help and expert suggestions during this investigation and preparation of the thesis.*

I extent my great sense of gratitude to **Dr.Koshy Abraham**, *Associate Professor of Plant Pathology, College of Horticulture, Vellanikkara and member of the Advisory Committee for the encouragement, timely help, critical suggestions and for the valuable corrections suggested in the preparation of the thesis.*

My sincere gratitude is also due to **Dr.S.Rajan**, *Associate Professor and Head, Department of Olericulture, College. of Horticulture, Vellanikkara and member of the Advisory Committee for the guidance and suggestions in the conduct of the investigation and preparation of the thesis.*

I express my sincere thanks to **Sri.S.Krishnan**, *Assistant Professor, Department of Agricultural Statistics, College of Horticulture, Vellanikkara and member of the Advisory Committee for the valuable guidance and help rendered during the statistical analysis of the data and preparation of the thesis.*

I am extremely grateful to Dr.James Mathew, Retd. Professor and Head, Department of Plant Pathology, College of Horticulture, Vellanikkara for the *guidance,encouragement and help as the member of my Advisory Committee in the early stages of the investigation.*

I am very much indebted to each and every member of the Department of Plant Pathology for their help at many stages. My sincere thanks are due to Dr.Sally K. Mathew, Dr.T.N.Vilasini and Sri.T.C.Radhakrishnan, Associate Professors and Dr.Sheela Paul and Dr.D.Girija, Assistant Professors for their help in conducting the research work successfully.

I thankfully acknowledge the help rendered by Dr.K.Surendra Gopal, Assistant Professor, Department of Plant Pathology for the identification of VAM cultures.

I respectfully acknowledge Dr.A.I.Jose, Associate Dean, College of Horticulture, Vellanikkara for his encouragement and help rendered during this investigation.

My sincere thanks are due to Dr.M.Mini, Assistant Professor, Department of Microbiology, Dr.T.Sreekumaran, Professor and Sri.Sanjayan, Research Assistant, Centre of Excellance in Pathology, College of Veterinary and Animal Science, Mannuthy for their help in conducting the electron microscopic studies.

I am thankful to Dr.D.J.Bagyaraj, Professor and Head, Department of Microbiology, U.A.S., Banglore for the supply of VAM cultures.

I am also thankful to Dr.Sasikumar Nair, Professor, Department of Plant Pathology, College of Agriculture, Vellayani for the supply of Bradyrhizobium culture.

I express my thanks to Sri.C.S.Gopi, Associate Professor and Dr.Sam.T.Kurumthotical, Assistant Professor, Department of Agricultural Chemistry for their help during the chemical analysis of plant and soil samples.

My sincere thanks are due to Dr.K.Anita Cherian, Assistant Professor, Banana Research Station, Kannara for her valuable suggestions and help rendered during this investigation.

I am thankful to Kum. Raji,P., Mrs.Vimi Louis, Assistant Professors, R.A.R.S., Pattambi, Mrs.Bindu Menon, Mrs.Mini Abraham, Kum. Nimmi Jose, Kum. T.P.Aysha, Kum.Binimole, Kum.Bini Philip and Sri.K.V.Shaju for their enourmous help in conducting the research work successfully.

Thanks are due to Sri.K.A.Joy and his family for the prompt and neat typing of the manuscript. I also express my thanks to Smt.Joyce for rendering help in statistical analysis.

I am grateful to Kerala Agricultural University for the grant of study leave.

My sincere thanks are due to Dr.C.T.Abraham, Dr.V.K.Mallika, Dr.K.T.Prasannakumari and Dr.V.S.Sujatha for taking the photographs.

I am deeply indebted to my husband, children, parents and in-laws for their constant encouragement and warm blessings for completing this endevour.

Above all, I bow my head before God, the ALMIGHTY for the success of this venture.

S. BEENA

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

Introduction

1. INTRODUCTION

Various associations of microbes with the plants are often studied and their beneficial effects are being explored in recent times. Among them, the association of Vesicular-arbuscular mycorrhizal fungi (VAM) with the roots of cultivated crops have much practical significance. VA mycorrhizal association with roots of higher plants is a rule rather than an exception. Its importance is multifold.

VAM is proved to enhance the absorption of nutrients like phosphorus and many micronutrients. Moreover, it has a role to play in checking many root diseases, in absorbing water in water deficient areas and even in the establishment of orchids and crop plants. Further, the beneficial effects of VAM are being commercially utilized by the farmers with the use of commercial inoculants. The symbiotic activity of *Bradyrhizobium* is well established and its efficiency in fixing the elemental nitrogen is exploited on a commercial scale, with the limitation that this nitrogen fixing process work with a legume *Bradyrhizobium* system only.

Cowpea is an important legume vegetable cultivated in Kerala, which enrich the soil by fixing atmospheric nitrogen and cater the protein requirements of Keralites.

In this era of preference to ecofriendly cultivation, any attempt to reduce the use of chemical fertilizers by microorganisms will be of great significance. The higher productivity with lower cost input will be an attractive proposition in the present scenario. In this background, the present study on "Interaction between VA mycorrhiza and *Bradyrhizobium* in cowpea" was taken up and the following aspects were studied.

1. Estimation of the natural occurrence of VAM in cowpea rhizosphere in five locations of Trichur district.

2. Characterisation and identification of predominantly occurring VAM in the survey locations.
3. Preparation of mass inoculum of native VAM and screening them for efficiency in cowpea.
4. Purification and characterisation of the cowpea *Bradyrhizobium* recommended for Kerala.
5. Infection pattern of VAM and *Bradyrhizobium* in cowpea.
6. Anatomical studies of nodulation and infection pattern of VAM.
7. Efficiency of the microsymbionts on the uptake of nutrients under different levels of nitrogen and phosphorus in pot culture.
8. Efficiency of the microsymbionts on the uptake of nutrients under different levels of nitrogen and phosphorus in the field condition.

Review of Literature

2. REVIEW OF LITERATURE

In the struggle for existence, different forms of inter-relationships have developed among living beings over millions of years. Among various such relationships symbiotic associations are common. The well known example for symbiotic association is that between *Rhizobium* and legumes. Inoculation of legume seeds with the appropriate culture of *Rhizobium* is practiced world wide to ensure better establishment and nitrogen fixation by legumes. Mycorrhiza, another symbiotic association between fungus and roots of higher plants have been known to mankind for over 100 years. Frank in 1885 recognized the fungus root structure and coined the term `Mykorrhizen' to indicate fungus root. Some fungi are found to form vesicles and arbuscules in the host roots and they are called VA mycorrhiza.

A typical VA mycorrhizal association was first reported by Treub (1885) in sugarcane. Since then a number of reports were published on the occurrence of VA mycorrhiza in plants. Janse (1896) reported the presence of VA mycorrhiza in about 69 plant species belonging to 56 families. Presence of VA mycorrhiza was reported by Dangeard (1900) in poplar, Butler (1939) in *Phaseolous*, *Stylosanthes* and *Leucaena* and Gerdmann (1968) in grapes, citrus and tomato. The presence and beneficial association of VA mycorrhiza in various cultivated crops like rubber, cocoa, cassava and vegetables in Kerala has been reported by various workers (Potty, 1978, Sivaprasad *et al.*, 1982, Sivaprasad *et al.*, 1984, Girija and Nair, 1985, Sulochana and Nair, 1985 and Nair and Girija, 1986).

2.1 Screening for the efficiency of VA mycorrhiza on growth improvement of crops

Effect of different VAM fungi under varying levels of phosphorus on growth and nutrient uptake in pigeon pea was studied by Champawat (1990b).He reported significant increase in phosphorus and nitrogen content of shoot and root at all levels of phosphorus applied with all the three VAM fungi screened. He also found that *Glomus constrictum* was better than the other two VAM fungi at all levels of phosphorus except at 75 kg ha^{-1}, when *Gigaspora calospora* was superior.

Costa and Paulino (1990) also investigated the effect of four VAM fungi in *Leucaena* with or without added phosphorus at the rate of 22 kg ha^{-1}. They recorded increased dry matter, yield and also phosphorus and nitrogen content and their uptake. VAM infection and nodulation in both the treatments was also found to increase. They stated that the most efficient VAM fungi varied with the parameters measured.

In another study conducted by Paula *et al.* (1990) to evaluate the efficiency of eight VA mycorrhizal fungi in soybean, observed that *Glomus etunicatum* significantly increased the root colonisation, phosphorus concentration, dry weight and grain yield of plants.

Devi and Sitaramaiah (1991) reported increased mycorrhizal root colonisation, greater root volume and greater dry weight of shoot and root in blackgram inoculated with four species of endomycorrhizal fungi. The maximum mycorrhizal root colonisation of 84.1 per cent and root volume of 251 per cent were recorded in *Glomus constrictum* among the four VAM fungi tested. But the root dry weight was significantly increased by *Glomus fasciculatum* (222 per cent) and the shoot dry weight by *Glomus epigaeum* (271 per cent). They observed significant differences among the four species of fungi in their ability to stimulate the vegetative growth of black gram.

In a pot culture experiment with soil containing several mycorrhizal fungi, Lee and Ryu (1992) observed that the infection of roots with VAM was independent of phosphate level in the soil for sorghum, but was inversely related to phosphate and to plant growth for soybean. The results of the experiment conducted by

Ho (1993) showed that among the different VAM fungi tested, *Scutellospora calospora* was the most efficient inoculant in increasing the nutrient uptake by maize plants.

Pattanaik *et al.* (1995) conducted a study to find the effect of vesicular arbuscular mycorrhizal inoculation in horsegram. The plants were inoculated with *Glomus fasciculatum* or *Glomus mosseae* or not inoculated. They observed that *Glomus fasciculatum* was most effective than *G. mosseae* in increasing the plant growth and nodulation. It increased the shoot height by 36.03 per cent, plant dry weight by 94.44 per cent, nodule dry weight by 100 per cent and individual seed dry weight by 27.45 per cent compared with the uninoculated control. Ortas (1996) inoculated sorghum cultivar SSV_2 and leek plants with four mycorrhizal fungi and recorded maximum spore population with *Glomus mosseae* in sorghum and with *Glomus etunicatum* in leek plants. He observed maximum plant dry weight, phosphorus uptake and percent VAM infection with inoculum rate of 18 g pot^{-1} in soil with low phosphorus content.

Singh *et al.* (1992) correlated the variation in spore density and VA mycorrhizal root colonisation of kinnow and rough lemon seedlings with change of season. The infection of VA mycorrhizal fungi was observed maximum in June and minimum in November. The maximum and minimum spore population of endophytes in soil were recorded in June and October respectively. They recorded the maximum root colonisation of 72 and 73 per cent by VAM fungi for kinnow and rough lemon seedlings in the month of June, gradually it declined and minimum was 24 per cent for both in November. Similarly maximum spore population of 518 and 508 per 50 g soil was observed from soil samples of kinnow and rough lemon seedlings respectively in June and gradually reduced to 370 and 360 per 50 g soil in both plants in October.

A seasonal variation in the percentage of root colonisation with VAM fungi was reported by Mago and Mukerji (1994). They observed that the lowest colonisation was during winter and the highest during late summer and autumn. Shamim *et al.* (1994) also recorded seasonal variation in VAM colonisation on perennial plant species. They found the maximum colonisation in spring. Then it gradually decreased in the following season reaching minimum in winter.

Bhaskaran and Selvaraj (1997) recorded *Glomus aggregatum* and *Sclerocystis pakistanica* as dominant VAM fungi in ten major plant species collected from four different coastal locations of Tamil Nadu. They observed a relatively high fungal spore density during the summer season at all sites.

2.2 Microscopical studies of mycorrhizal roots

Trappe (1982) prepared a key for the identification of VAM cultures. He reported that the spores of *Glomus* sp. could be identified based on their characters, viz. size, shape, colour, surface texture, wall thickness and colour, nature of spore attachment to the hyphae and presence or absence of sporocarp.

Grundwaldt-Stocker and Dehne (1989) conducted a fluorescence microscopy and scanning electron microscopy to study the structure of *Glomus etunicatum*. They found that the mycelia and chlamydospores on clay surface did not differ morphologically from those in soil. They also reported that the chlamydospores are of minor importance for survival and infection, but the mycelial fragments play a greater role as a source of inoculum.

Maia *et al.* (1993) described an efficient technique for the fixation of spores of *Glomus* sp. to conduct ultra structural electron microscopic studies. This method included fixation of spores with glutaraldehyde in a microwave oven and breaking the spores under liquid nitrogen before post fixation with osmium tetroxide.

The morphological and ultra structural characters of spore wall of *Acaulospora morrowiae* and *A. scrobiculata* were studied by Maia and Kimbrough (1993). They observed three zones for the spore wall of *A. morrowiae* while the other species had only two zones. They also reported that the spores of *Acaulospora* were similar to those of certain other glomalean fungi and that indicated the uniqueness of spore walls in the Glomales.

Baird and Caruso (1994) examined the root and nodule sections under a light microscope and observed abundant VAM hyphal infection in the nodule cortex after five weeks of inoculation. They observed vesicles in root and nodule tissues and also structures similar to degenerating arbuscules in the central region of nodules and in the nodule cortex. They also found VAM hypha in nodules particularly in regions where senescence of bacteria filled cells had begun.

Schmid and Oberwinkler (1995) studied the VAM host fungus interactions under light and electron microscope in Gleicheniaceae. They found that the aseptate hyphal coils produced arbuscules in young part of root midrib while in older midrib, arbuscules disintegrated and vesicles produced. They also noticed that the primary roots of < 2 mm were not infected, where a complete intracellular development of fungus occurred in primary roots of > 2 mm thickness.

2.3 Effect of VA mycorrhiza on growth improvement of crops

VA mycorrhizal fungi played an important role in improving the nutrient uptake, plant growth and yield. Inoculation of plants with suitable VAM fungus has been commercially utilised to stimulate the crop production. The beneficial effects of VAM on plants have been studied by various workers.

A study was conducted by Powell (1981) in barley, inoculated with a mixture of *Glomus mosseae, G. fasciculatum* and *Gigaspora margarita* or with the indigenous mycorrhizal fungi. He found that the introduced fungi stimulated the seed yield by 27 per cent. Kuo and Huang (1982) reported an increased plant dry weight and seed yield of soybean inoculated with *Glomus fasciculatus, G. mosseae, G. etunicatus* or *Acaulospora scrobiculata*.

A pot culture experiment was conducted in phosphorus deficient soil by Manjunath and Bagyaraj (1986) to study the response of blackgram, chickpeas and mung beans to inoculation with *Glomus fasciculatus* along with superphosphate at the rate of 22 kg P ha^{-1}. They observed an increase in the dry weight of plants and also phosphorus content of shoot and root due to VAM inoculation. They recorded a shoot and root phosphorus content of 9.33 mg and 4.54 mg in black gram, 18.52 mg and 7.28 mg in chickpea and 7.80 mg and 3.35 mg in mung bean respectively. They noted that the application of phosphorus did not reduce the percentage of root colonisation by VA mycorrhiza, but it increased the number of extramatrical chlamydospores in the soil. Endomycorrhizal spores 50 ml^{-1} soil recorded were 125, 117 and 172 in blackgram, chickpea and mung bean respectively. They suggested the need for phosphorus application to obtain maximum benefit from VAM in phosphorus fixing soils.

Rajapakse (1987) reported higher shoot dry weight in cowpea inoculated with *Glomus fasciculatum*. Rajapakse and Miller (1987) reported significant interaction between the VAM fungi *Glomus mosseae* or *G. fasciculatum* and cowpea cultivars on shoot dry weight. Here the inoculation increased the shoot dry weight by 40 per cent. They also obtained an increased plant height, but a decreased root length due to VAM inoculation.

In a pot culture experiment with phosphorus deficient soil, Champawat (1989) found that shoot and root dry weight and phosphorus uptake were greater in chick pea plants inoculated with *Glomus fasciculatum* in unsterilized soil than in sterilized uninoculated soil. The plants also recorded the highest colonisation of 85.7 per cent with *G. fasciculatum*.

Kumari and Nair (1989) compared ten cowpea varieties on the natural incidence of vesicular arbuscular mycorrhiza. They observed that the average mycorrhizal index was differed among varieties. The maximum being in the variety C-152 (1.91) followed by Ptb-2 and New Era. They also opined the addition of rock phosphate at the rate of 30 kg P_2O_5 ha^{-1} to the soil for the enhancement of mycorrhizal formation.

Lin and Hao (1989) studied the effect of phosphorus fertilizers on VA mycorrhizal response under sterilized soil condition. The results of their experiment showed that 60 kg P_2O_5 ha^{-1} applied as superphosphate was the optimum phosphorus level for VA mycorrhizal infection in the soil studied. They observed that phosphorus fertilizer at this rate along with VAM inoculation, increased the plant growth.

Amijee *et al.* (1990) inoculated the leek (*Allium porrum*) plants raised in sterilized soil, amended with $Ca(H_2PO_4)_2H_2O$, with *Glomus mosseae* to test the hypothesis that high concentration of soil phosphorus inhibited the formation of VA mycorrhiza by reducing the concentration of soluble carbohydrate in the root. They found that at the concentration of soil phosphorus, at which VAM infection was reduced, the concentration of soluble carbohydrate was at its maximum. So they discounted the hypothesis and suggested that the high concentration of soil phosphorus reduces the VAM infection by changing the anatomy of the root by making it resistant to fungal penetration.

Increase in plant height, total dry matter of root and shoot were reported by Chhabra *et al.* (1990) in cowpea incoulated with VA mycorrhiza. They also reported an extensive mycorrhizal development of 53-78 per cent in inoculated plants.

The results of the experiment conducted by Hao *et al.* (1991) in mung bean showed that infection by indigenous VAM fungi in most soils were low. Usually there was a lag phase for infection by indigenous VAM fungi. But for the introduced mycorrhizal fungi, the lag phase was decreased and the infection was increased to 50-60 per cent after 25-40 days of inoculation. They also found that phosphorus application encouraged mycorrhizal infection in most soils deficient in available phosphorus. But in a report from Western Nebraska, USA, Yocom and Boosalis (1991) stated that wheat plants colonized either by indigenous VAM fungi (*Glomus fasciculatum*) or by introduced VAM fungi, produced more grains than the non-mycorrhizal wheat.

Costa *et al.* (1992a) reported the best biomass production in *Cajanus cajan* inoculated with *Acaulospora muricata* along with the addition of 80 kg P_2O_5 ha^{-1}, while the best mycorrhizal colonisation was obtained at lower phosphorus concentrations of 60 and 40 kg P_2O_5 ha^{-1}. In another experiment, when Leucaena was inoculated with VAM fungi *Scutellospora heterogama*, the highest level of VAM colonisation was found to occur in the presence of 40 kg P_2O_5 ha^{-1} (Costa *et al.*, 1992b).

Isobe *et al.* (1992) worked out the infection rate of VAM fungi in soybean and stated that it was strongly influenced by root diameter. They observed the highest infection in roots with a diameter of 333.3-366.7 μm.

The response of chilli to VAM inoculation was investigated by Sreenivasa *et al.* (1993) and reported that the application of soluble phosphorus fertilizers could be reduced by 50 per cent if chilli plants were inoculated with

VAM fungus. They observed that inoculation with *Glomus macrocarpus* had a greater effect than with *Glomus fasciculatum* in chilli.

Edathil *et al.* (1994) reported the maximum root colonisation by native VA mycorrhiza at 45, 50 and 60 days after germination of brinjal, tomato and chilli respectively under field conditions and on 60th day in pot culture.

Geethakumari *et al.* (1994) reported that the recommended level of 30 kg P_2O_5 ha^{-1} can be reduced to 22.5 kg P_2O_5 ha^{-1} for grain type cowpea by the combined application of mussorie rock phosphate and mycorrhiza.

Lingaraju (1994) conducted an experiment in soybean inoculated with *Glomus fasciculatum* and *Gigaspora margarita* at different levels of phosphorus fertilizer. They found that soil inoculation with mycorrhiza along with the application of 60 kg P_2O_5 gave similar seed yield as 80 kg P_2O_5 application alone without inoculation of VAM.

Combined inoculation of Azotobactor and VAM fungi in maize and wheat was found to improve the plant growth and increase phosphorus, nitrogen and zinc content in plants (Elgala *et al.*, 1995). A pot culture experiment was conducted by Santhi and Sundarababu (1995) with cowpea inoculated with VA mycorrhiza and the nematode, *Meloidogyne incognita* at different levels of phosphorus. They reported a negative correlation between phosphorus levels and the VAM spore population and colonisation in roots.

Plenchette and Morel (1996) reported that the growth of soybean was stimulated due to the mycorrhizal inoculation. They worked out the external requirement of phosphorus as 0.11 $\mu g\ ml^{-1}$ to obtain 80 per cent of maximum yield in soybean, which in terms of phosphorus fertilization corresponds to a saving of 22.2 kg P_2O_5 ha^{-1}.

A field study was conducted by Tarafdar and Rao (1997) to determine the effect of *Glomus mosseae* and *Glomus fasciculatum* on cluster bean, mung bean and moth mean. All the legumes showed similar effects up on inoculation with the VAM fungi. But nodulation, nitrogenase activity, per cent root infection by VAM fungi and the number of VAM spores in soil were increased significantly up on inoculation. They also reported an increased dry matter production of 20-38 per cent and grain yield of 15-22 per cent up on inoculation.

2.4 Effect of VA mycorrhiza in improving the nutrient uptake of crops

VAM fungi was found to improve the plant nutrition mainly through increased uptake of phosphorus and many micronutrients from soil. Uptake of phosphate by mycorrhizal plants has received more attention than many other aspects in VA mycorrhizal symbiosis.

The first study on the uptake of phosphorus and other nutrient elements by VAM infected plants was carried out by Mosse in 1957. She reported that mycorrhizal apple absorbed more phosphorus, potassium, iron and calcium than non mycorrhizal plants. From the trial conducted by Sanders and Tinker (1971) it was found that increased surface area due to mycelial network was the reason for better phosphorus uptake by the plants.

Increased uptake of zinc and iron was also reported by LaRue *et al.* (1975) in mycorrhizal peach. Bagyaraj and Manjunath (1980) also reported an increased concentration of phosphorus and zinc in mycorrhiza inoculated cowpea, cotton and finger millet. But the manganese content of these plants did not show any significant differences.

Powell (1981) reported an increased seed phosphorus content by 35 per cent in barley inoculated with a mixture of *Glomus mosseae*, *G. fasciculatum* and *Gigaspora margarita* or with the indigenous mycorrhizal fungi.

Rajapakse and Miller (1987) reported a similar increase in the phosphorus and nitrogen content of the shoot in cowpea cultivars due to inoculation with *Glomus mosseae* or *G. fasciculatum*. But, Rajapakse (1987) found a negative correlation between root colonisation by *Glomus fasciculatum* and phosphorus content of growth medium. Phosphorus content of the shoot was also found less in cowpea. The phosphorus uptake of VAM inoculated plants was promoted by the application of superphosphate at the rate of 60 kg P_2O_5 ha^{-1} (Lin and Hao, 1989).

Significant increase in nitrogen, phosphorus and potassium content of cowpea plants inoculated with VA mycorrhiza was reported by Chhabra *et al.* (1990). They also recorded 53-78 per cent mycorrhizal colonisation in the roots of inoculated cowpea plants.

Costa *et al.* (1992b) observed the highest VAM colonisation and an increased phosphorus uptake by Leucaena inoculated with VAM fungus, *Scutellospora heterogama*, in the presence of phosphorus fertilizers at and above 40 kg P_2O_5 ha^{-1}.

Elwan (1993) reported the greatest increase in the uptake of phosphorus, potassium, calcium, manganese, iron, magnesium, copper and zinc in maize plants inoculated with VA mycorrhiza along with phosphorus fertilizer. The behaviour of VA mycorrhizal fungi was studied by Isobe *et al.* (1993) and found that VAM infection increased phosphoric acid content of soybean at 50 days after emergence. They also reported the adverse effect of the application of superphosphate fertilizer on mycorrhizal infection.

Increase in the phosphorus content of shoot and changes in the uptake of zinc, copper, manganese and iron by chilli inoculated with VAM fungi (*Glomus macrocarpus* and *G. fasciculatum*) were reported by Sreenivasa *et al.* (1993). Urzua *et al.* (1993) observed improved phosphorus, potassium, calcium, magnesium, copper, zinc, iron and manganese uptake in white clover inoculated with *Glomus* sp. They also found enhanced nitrogen fixation in VAM inoculated plants as a result of improved phosphorus uptake.

Eranna and Parama (1994) investigated the effect of liming on VAM inoculation in soybean. They found that the liming decreased the phosphorus uptake, but it had no significant effect on the dry weight of plants. Whereas mycorrhizal inoculation alone increased both the dry weight and phosphorus uptake in soybean. They also revealed that the phosphorus content of the shoot was increased in response to soil phosphorus content up to 20 g P kg^{-1} soil whereas root phosphorus content was increased with up to 40 g P kg^{-1} soil.

Heggo and Barakah (1994) conducted a study on the interaction between phosphorus and zinc in maize inoculated with VA mycorrhiza. They found that the shoot dry weight was about 1.5 times that of the control at the high rate of phosphorus and zinc, whereas at low rates, the dry matter yield was about four fold. They also noticed that the high rate of phosphorus application decreased the zinc, copper and iron content of plant.

Pai *et al.* (1994) investigated on the role of VA mycorrhizal colonisation in the uptake of calcium by cowpea plants. They found high ^{45}Ca levels in all parts of cowpea plants inoculated with *Glomus fasciculatum* compared with non mycorrhizal plants.

Increased phosphorus, sulphur, zinc and copper concentrations in sorghum inoculated with VA mycorrhiza was reported by Medeiros *et al.* (1995).

They observed a negative correlation between shoot dry matter and manganese content of shoot and root in VAM treatments.

Rathore and Singh (1995) also reported increased dry matter accumulation and uptake of nitrogen, phosphorus and potassium by maize plants due to VAM inoculation and phosphorus fertilizer application. But they noticed a significant reduction in the mycorrhizal root colonisation with the increased levels of phosphorus. They opined that mycorrhizal inoculum may substitute phosphatic fertilizer equivalent to 30 kg P ha^{-1}.

Bermudez and Azcon (1996) worked out a positive correlation between calcium content of alfalfa plants and nodulation in mycorrhizal treatment. They concluded that mycorrhizal mechanism which alter the nutrient to calcium ratio, may be the reason for increased nodulation.

Tarafdar and Rao (1997) reported significantly higher concentration of nitrogen, phosphorus, copper and zinc in cluster bean, mung bean and moth bean inoculated with VAM fungi (*Glomus mosseae* and *G. fasciculatum*). But the concentrations of potassium, calcium, magnesium, sodium, iron and manganese remain unaffected due to the inoculation.

2.5 Effect of *Bradyrhizobium* on plant growth, nodulation and nutrient uptake in legumes

Biological nitrogen fixation is one of the major process by which soil nitrogen status is maintained. In this, *Rhizobium* legume symbiosis plays an important role. Inoculation of efficient *Rhizobium* is often essential for an efficient symbiotic nitrogen fixation by legumes.

Hartwell and Pember (1911) reported that by nitrogen fixing phenomenon of legumes, nearly one tonne of soil nitrogen $acre^{-1}$ was gained in pot culture experiment with cowpea and soybean. Sen and Rao (1953) investigated the fixation of nitrogen under favourable conditions by cowpea and reported that the quantity fixed was 251 lb annum^{-1} acre^{-1}.

Alexander (1961) revealed that nitrogen fixation by cowpea varied between 64 and 131 kg ha^{-1} year^{-1}. In soybean, the quantity fixed was between 64 and 106 kg ha^{-1} year^{-1}. Chhonkar and Negi (1971) inoculated different strains of *Rhizobium* to soybean in rhizobia free soil and found that seed inoculation increased the dry weight as well as the yield of plant.

Nutman (1976) enlisted the amount of nitrogen fixed by different legumes and he pointed out that cowpea fixed an average of 198 kg nitrogen ha^{-1}. Rai *et al.* (1977) observed an increase in yield in bengal gram due to *Rhizobium* inoculation, but they did not find any correlation among yield, number of nodules and the dry weight of nodules.

Sivaprasad and Shivappashetty (1980) reported that cowpea cv. Pusa Phalguni when inoculated with *Rhizobium* strain IS-8 and IS-12, significantly increased the yield, plant top dry weight and leghaemoglobin content of nodule. They also obtained a significant correlation between leghaemoglobin content of nodules, plant top dry weight and final grain yield. But there was no significant correlation between nodule number and nitrogen content of plant top and final grain yield.

Nair and Sivaprasad (1981) observed the beneficial effects of inoculation with different isolates of rhizobia in cowpea. They also reported a correlation between symbiotic efficiency and increase in dry weight of cowpea plants.

Girija (1982) reported a favourable response for all plant characters studied, when an isolate of *Rhizobium* was used for inoculating its own homologous host variety. She also observed that the host varietal specificity of rhizobia was not absolute, but it was a host determined phenomenon.

Seripong and Masayna (1984) studied the effect of phosphorus and potassium on the growth, nodulation and nutrient status of cowpea and recorded the best growth with 50 to 100 kg P ha^{-1} without potassium. They also observed a decrease in the total and individual weights of nodules due to the potassium application.

Manel and Chahal (1987) reported that nitrogenase and nitrate reductase activities in *Vigna radiata* increased with up to 20 ppm N applied as KNO_3 along with *Rhizobium* inoculation. At higher levels, the nitrogenase activity was decreased and the leaf nitrate reductase activity was increased.

Shaktawat (1988) evaluated the response of cowpea to *Rhizobium* inoculation and phosphorus application. He reported that application of phosphorus fertilizer at the rate of 0, 30 and 60 kg P_2O_5 ha^{-1} to three cowpea varieties recorded an average seed yields of 546, 763 and 816 kg ha^{-1} respectively.

Rahman and Sanoria (1990) reported a positive correlation between the growth rate and nitrogen fixation at flowering and mid pod formation stage in soybean. Stamford *et al.* (1990) conducted a study for the selection of *Bradyrhizobium* for cowpea in acid soil in semi arid Brazil and found that shoot nitrogen was greatest when the acid soil was amended with $CaCO_3$ at the rate of 2 t ha^{-1}. They also noted that shoot dry matter was decreased with increasing lime rate. Shoot nitrogen concentration was positively correlated with nodule dry weight.

Mand *et al.* (1991) reported that inoculation of fast and slow growing cowpea rhizobia in guar (*Cyamopsis tetragonoloba*) cv. Ha 75 increased the nodule number, nodule fresh weight, plant biomass and plants nitrogenase activity. They observed that the nitrogenase activity was doubled by applying a basal dose of 40 kg P_2O_5 ha^{-1} in slow growers.

Raghava *et al.* (1993) recorded the maximum nodule number and dry weight in inoculated *Vigna unguiculata* cv. Pusa Barsati on 50 days after germination and there after the values decreased due to the senescing of nodules. They obtained the maximum leghaemoglobin content and total soluble protein at 40-45 days after germination.

Soybean plants inoculated with *Bradyrhizobium japonicum* recorded a maximum nodule number of 349 $plant^{-1}$ and nodule mass of 3.9 g wet weight $plant^{-1}$ than those inoculated with wild type strain which recorded a nodule number of 159 $plant^{-1}$ and a nodule mass of 2.2 g wet weight $plant^{-1}$. This was reported by Hunter (1994).

Mahmoud *et al.* (1994) recorded the highest seed yield and nodule dry weight in soybean when the seeds were inoculated with *Bradyrhizobium* along with the application of nitrogen fertilizer at the rate of 20 kg $feddan^{-1}$. They noted that nodule dry weight was decreased with increased rate of nitrogen application.

Senaratne and Ratnasinghe (1995) in a trial on sequential cropping, found that groundnut showed a significantly higher nitrogen fixation and residual nitrogen effect on the succeeding rice crop than cowpeas, black gram, mung beans and pigeon peas. They also observed a positive correlation between the growth and nitrogen yield of rice and the quantity of nitrogen fixed by the preceeding legume crop.

Takankhar *et al.* (1998) conducted an experiment on bengal gram cv. BDN 9-3 inoculated with *Rhizobium* and given 0, 75 kg P_2O_5 and 25 or 50 kg N ha^{-1}. They observed a significant increase in phosphorus uptake when the seeds were inoculated with *Rhizobium* along with the application of nitrogen and phosphorus. They reported that the application of 75 kg P_2O_5 with *Rhizobium* inoculation produced the highest seed yield of 1.25 t ha^{-1}.

2.6 Dual inoculation of VA mycorrhiza and *Bradyrhizobium* on improving the growth of legumes

In legumes, often, a tripartite association is usually seen which consists of the mycorrhizal fungus, the host plant and the *Rhizobium*. Inoculation of soil with an efficient strain of VA mycorrhizal fungus and *Rhizobium* is known to improve nodulation, nitrogen fixation, phosphorus nutrition and dry matter yield of legumes.

Asai (1944) reported that sufficient number of root nodules were formed by *Rhizobium* inoculation in *Trifolium, Medicago* and *Melilotus* spp when 5-10 g of unsterilized garden soil was also added. The added garden soil might have acted as VAM inoculum. Later, increased growth and yield of nodulating soybeans after inoculation with *Glomus mosseae* in fumigated soil was reported by Ross and Harper (1970). This was confirmed by Schenck and Hinson (1973) by inoculating the nodulated soybean line with VA mycorrhiza and obtained 53 per cent increased growth over non-inoculated line.

Increased root and shoot weight and also the nodulation of cowpea plants were reported by Godse *et al.* (1978) as a result of the combined inoculation of plants with *Rhizobium* and VA mycorrhiza in a pot culture experiment. Plants with combined inoculation recorded a shoot dry weight of 19.11 g $plant^{-1}$ compared to plant with *Rhizobium* alone (5.70 g $plant^{-1}$). They reported 67 to 71 per cent infection by mycorrhiza in plants received the VAM fungus alone and the fungus with *Rhizobium*. They also suggested that the increased plant development and nodulation with VAM might be due to the increased uptake of phosphorus by the plants.

Bagyaraj *et al.* (1979) also confirmed the above observation by inoculating soybean with *Glomus fasciculatus* and *Rhizobium japonicum*. He stated that the combined inoculation greatly improved nodulation and nitrogen fixation in field grown soybean.

Sivaprasad *et al.* (1983) found increased growth, nodulation and phosphorus content in *Leucaena leucocephala* after the combined inoculation of the plants with *Rhizobium* and VA mycorrhiza.

Bethlenfalvay *et al.* (1985) from their experiments observed that inoculation of soil with any one endophyte at the time of sowing inhibits the development of the endophyte inoculated later. They suggested the importance of time of inoculation. This influences the outcome of the symbiosis. In mung bean, a high level of nodulation was reported by Pandher *et al.* (1986) as a result of combined inoculation with *Rhizobium* and VA mycorrhiza.

Rajapakse (1987) from his experiments with cowpea cultivars inoculated with VA mycorrhiza, reported a negative correlation between percentage root colonization by VAM and phosphorus content of the growth medium and shoot phosphorus concentration.

Satizabal and Saif (1987) found that *Centrosema macrocarpum* plants inoculated with *Rhizobium* strains and *Glomus manihotis* or *Acaulospora longula* had the highest yield of dry matter, mineral absorption, nodulation and infection by VAM. They also noticed the highest growth in plants inoculated with VAM along with a phosphorus level of 40 kg P_2O_5 ha^{-1}.

The highest total nitrogen production was reported by Vejsadova *et al.* (1988) in the biomass of nodulated soybean plants inoculated with VAM fungi. This stressed the fact that the inoculation with VAM fungi positively influenced nitrogenase activity of nodules, biomass production and shoot-root ratios in nodulating plants. Pillai (1989) conducted a study on the seedlings of subabul and reported that inoculation of the local isolate of *Rhizobium* R8 and mycorrhizal fungus M2 had great influence in increasing all the growth parameters. Standard mycorrhizal cultures and local isolates performed equally well.

Champawat (1990) reported that the combined inoculation of *Gigaspora calospora* and *Rhizobium* enhanced the plant growth, root nodulation and uptake of phosphorus and nitrogen in chickpea.

In pigeon pea also Costa *et al.* (1990) recorded a significant improvement on dry matter yield, nodulation and nitrogen and phosphorus uptake as a result of dual inoculation with *Rhizobium* and VA mycorrhiza. They did not get any nodulation in treatments without *Rhizobium* inoculation. Similarly, there was no mycorrhizal infection in treatments without mycorrhizal inoculation.

Guzman Plazola *et al.* (1990) obtained the best results in the establishment of *Leucaena leucocephala* when the seedlings were inoculated with *Glomus intraradices* and *Rhizobium loti* at the time of transplanting to the acid soils. Where as importance of inoculation of both VAM fungi and *Rhizobium* at the time of sowing was stressed by Mallesha and Bagyaraj (1990). They reported that inoculation of *Leucaena* plants with the microsymbionts at the time of sowing significantly increased the nodulation, mycorrhizal colonisation, plant biomass and nitrogen and phosphorus uptake by the plants.

Interaction of vesicular-arbuscular mycorrhiza and *Rhizobium* in cowpea was studied by Ramaraj *et al.* (1990) and found that dual inoculation with the microsymbionts in sterilized and unsterilized soil conditions increased the nodules, mycorrhizal colonisation and the biomass to a greater extent than when either was inoculated alone. But Singh (1990) reported that the dual inoculation with *Bradyrhizobium japonicum* and VAM fungi or phosphate solubilizers in soybean did not produce a significant increase in nodule number, dry weight of nodule and organic matter accumulation in plants.

Alten *et al.* (1991) in their experiments with soybean inoculated with *Bradyrhizobium* and VA mycorrhiza, recorded significant increase in number of nodules in plants with both the symbionts. But they could not record a correlation between growth promotion and increased nodulation. Ames *et al.* (1991) suggested the importance of selection for effective rhizobia in non-sterilized soil and pairing that with specific co-selected VAM fungi can significantly improve the legume growth response.

In chickpea also Chaturvedi and Kumar (1991) observed an increased nodulation and nitrogenase activity when inoculated with *Rhizobium* and *Glomus caledonium*.

Ianson and Linderman (1991) suggested the inter-endophyte compatability between *Rhizobium* and VAM fungi which was very critical to optimize the nitrogen fixation in legumes. They also reported a high positive correlation between the growth and nitrogen fixation in pigeon pea.

The synergistic association between *Glomus fasciculatum* and *Rhizobium* in pigeon pea was studied by Sivaprasad and Rai (1991). They found that the combined inoculation increased the nodulation by 178 per cent and nitrogenase activity by 185 per cent, compared to inoculation with *Rhizobium* sp. alone. They also observed that the response of dual inoculation was less in the field than in the green house.

Cabello (1992) reported an increased nitrogen and phosphorus content at the rate of 102 and 233 per cent respectively in soybean inoculated with *Bradyrhizobium* and *Glomus epigaens*. Chang *et al.* (1992) also reported increased yield, protein content, rate of nitrogen fixation and phosphorus uptake in soybean cultivar Ludon 2 dually inoculated with *Rhizobium* and *Glomus epigacum*.

Smolin and Shabaev (1992) observed that the dual inoculation of soybean with *Rhizobium* and VAM fungi increased yield as a result of increased uptake of major and trace elements. But they observed a significant reduction in the iron concentration.

Increased pod yield, mycorrhizal infection, nodule formation and shoot phosphorus and nitrogen content was reported by Thyagarajan *et al.* (1992) in cowpea inoculated with *Rhizobium* and VAM fungi. They suggested the appropriate pairing of VAM fungus and *Rhizobium* for better performance in field inoculation. They reported that cowpea Rhizobium strain JRC-14 when paired with *Glomus pallidum* or *Sclerocystis microcarpa* showed maximum increase in yield of 45 per cent.

Sorghum plants were inoculated with VAM fungi and nitrogen fixing bacteria individually or in combination by Aboul-Nasar (1993) and the result of this experiment showed a positive response between nitrogen fixing bacteria and VAM fungi. This depended on the VAM species. He reported that the nitrogen fixing bacteria helped to increase the percent VAM infection in the roots of plants. But the treatments had no significant effect on shoot and root dry weight. He also reported the maximum increase in phosphorus content (90.2 per cent) in plants inoculated with *Glomus etunicatum*.

But Ianson and Linderman (1993) in their experiments with pigeon pea nodulated with *Rhizobium* in the presence of *Glomus* sp. found that the level of VAM formation was not related to nitrogen fixation under nitrogen limiting conditions, because same fungi formed extensive VAM infection but did not affect the nodulation.

A field experiment was conducted by Mane *et al.* (1993) in groundnut inoculated either with *Glomus fasciculatum* or *Rhizobium* and reported increased root length, root weight, biomass production, nodulation, nitrogen fixation and phosphorus uptake. Seed yield ranged from 235-330 kg ha^{-1}.

Thyagarajan and Ahmed (1993) from their experiments observed that competitiveness of rhizobia can be enhanced by co-inoculating with a selected strain of a VAM fungus. Cowpea Rhizobium strain JRC-29 occupied 59.2 per cent of the total nodules when inoculated alone, but this was increased to 71.2 per cent when JRC-29 was used in dual inoculation with VAM fungus. They found that the introduced *Bradyrhizobium* strain became more competitive than native rhizobia in cowpea, in the presence of VAM fungus in non-sterilized soil.

Kumutha and Santhanakrishnan (1994) studied about the enhancement of nitrogen fixation in soybean by dual inoculation of VAM and *Bradyrhizobium* and found that the dual inoculation approximately doubled the nitrogen fixation compared with *Rhizobium* alone. An experiment was conducted by Singh (1994) in soybean dually inoculated with *Bradyrhizobium japonicum* and *Glomus fasiculatum* or phosphate solubilizing microbes to study the inoculation response in terms of nodulation, plant growth and seed yield. He found that the dual inoculation did not record any significant increase in any of the parameters studied.

Soddi *et al.* (1994) conducted a field trial with cowpea cultivar C-152 to study the effect of seed treatment with *Rhizobium* and soil inoculation with *Glomus fasciculatum*. The plants were also given 0, 50 or 100 per cent of the recommended nitrogen and phosphorus rates of 25 kg N and 50 kg P ha^{-1}. The results showed that the number and dry weight of nodules were highest with seed and soil inoculation along with 50 per cent nitrogen and phosphorus. Seed yield also showed a similar trend. But the shoot and root nitrogen and phosphorus contents were highest with seed and soil inoculation along with the recommended dose of nitrogen and phosphorus.

Khan *et al.* (1995) reported increased nitrogen fixation as well as nitrogen and phosphorus content in groundnut as a result of dual inoculation with *Glomus* sp. and *Bradyrhizobium* sp. They observed a significant increase in nitrogen fixation in dually inoculated plants at 100 days after planting. They also observed that the dually inoculated plants derived 75 per cent of nitrogen from fixation whereas plants inoculated with *Bradyrhizobium* derived only 68 per cent nitrogen from fixation.

Thakur and Panwar (1995) also reported higher dry matter production and seed yield in mung bean dually inoculated with *Bradyrhizobium* and *Glomus fasciculatum*, compared with either single inoculant.

Das *et al.* (1997) studied the effect of VA mycorrhiza and *Rhizobium* inoculation on nutrient uptake, growth and yield of green gram. They observed increased shoot and root length, number of nodules, number of pods plant^{-1}, dry weight of pods and uptake of nitrogen and phosphorus in plant with dual inoculation compared with the uninoculated control.

Singh (1990) reported that the grain and shoot yield in pigeon pea was increased due to dual inoculation with *Rhizobium* and *Glomus fasciculatum* at all levels of nitrogen and phosphorus fertilizers applied. He opined that the greatest benefit to the crop was obtained when both the organisms were inoculated together with the fertilizer levels of 50 kg P ha^{-1} and 20 kg N ha^{-1}. Arafat *et al.* (1995) inoculated *Vicia faba* with *Rhizobium* and VAM fungus and applied superphosphate or rock phosphate as a phosphorus source. They found that the application of phosphorus as superphosphate gave higher plant dry weight and uptake of phosphorus and nitrogen than rock phosphate. Singh (1996) reported maximum nitrogen fixation in pigeon pea dually inoculated with *Rhizobium* and VAM (*Glomus fasciculatum*) in the presence of phosphorus at the rate of 50 kg P_2O_5 ha^{-1} and nitrogen at the rate of 20 kg N ha^{-1} in the form of superphosphate and ammonium sulphate respectively. He also recorded a significant increase in nodule number and grain yield in dually inoculated plants along with 50 kg P_2O_5 ha^{-1}. He also observed that the total nitrogen yield recorded by the plants when inoculated with VAM along with nitrogen at the rate of 20 kg N ha^{-1} was equivalent to *Rhizobium* sp. inoculation.

Materials and Methods

3. MATERIALS AND METHODS

The study on the `Interaction between VA mycorrhiza and *Bradyrhizobium* in cowpea' was conducted during 1994-97 at College of Horticulture, Vellanikkara, Thrissur. Three separate pot culture experiments and one field experiment were carried out for this purpose. The details of the materials used and the techniques adopted in this investigation are presented below:

3.1 Estimation of natural occurrence of VAM in cowpea

A preliminary survey was conducted to find out the extent of natural VA mycorrhizal colonisation in cowpea [*Vigna unguiculata* (L.) Walp.] plants in different locations of Thrissur district. The locations selected were the following:

Sl.No.	Locations
1	Vegetable Research Farm of College of Horticulture, Vellanikkara (COH, Vellanikkara)
2	Vegetable garden of KHDP, Vellanikkara
3	Banana Research Station, Kannara (BRS, Kannara)
4	Farmer's field at Nadathara
5	Farmer's field at Moorkanikkara

The intensity of VAM colonisation in tap root and lateral roots and also the spore count in soil in these locations were estimated in rainy (July) and summer (March) seasons in 1995.

3.2 Estimation of VA mycorrhizal colonisation

Twenty plants grown in each location were selected at random to estimate the VAM colonisation. The plants were uprooted without disturbing its root system and the roots were taken for observing the infection. Five hundred gram of rhizosphere soil was also taken for counting the number of spores of VAM. The roots were observed for the mycorrhizal colonisation as suggested by Phillips and Hayman (1970). The roots were first washed in tap water to remove all adhering soil particles and then cut into small bits of approximately one cm in length. They were then transferred into clean labelled bottles and fixed with formalin: acetic acid : alcohol mixture (FAA) (Appendix-I).

For staining, the root bits taken from FAA were initially softened by simmering in 10 per cent KOH at 90°C for one hour. After cooling, the excess alkali was removed by repeated rinsing in tap water and then neutralized by 2 per cent HCl. The root bits were then stained with 0.05 per cent trypan blue in lactophenol for three minutes (Appendix-II).

The excess stain from the root tissues was removed by clearing overnight in fresh lactophenol. The root bits were examined for the typical VAM colonisation under a light microscope. The percentage of VAM colonisation was calculated by the following formula.

$$\text{Percent VAM colonisation} = \frac{\text{Number of positive root segments} \times 100}{\text{Number of root segments observed}}$$

3.3 Isolation of VA mycorrhizal spores from soil

Modified wet sieving and decanting method of Gerdemann and Nicolson (1963) was used for the isolation of VAM spores from the soil. Hundred gram of rhizosphere soil collected from each location was initially suspended in 1000 ml of tap water. This was agitated vigorously to disperse all soil clumps. After the heavier particles had settled, the supernatant was filtered through a set of sieves (Jayant test sieves, Jambalwadi, Kalbadevi, Bombay) of B.S.S.No.60 (250 microns), 150 (150 microns) and 350 (45 microns). The residue left behind in the measuring cylinder was resuspended in 1000 ml tap water and passed through the same set of sieves.

This procedure was repeated three to four times in order to collect maximum number of spores from the soil. Finally, the materials present on each sieve was transferred to 100 ml beakers in a small volume of water and filtered through Whatman No.I filter paper. The content of each filter paper was carefully examined under a stereo microscope for the typical spores of VAM. Spores of uniform size and shape which were predominant in number at a given location were selected and transferred to moistened filter paper in petri dishes with the help of fine capillary pipette. The number of spores present in 100 g soil was recorded.

3.4 Measurement of VAM spores

The spore size in micron was recorded with the help of an ocular micrometer precalibrated with a stage micrometer.

3.5 Identification of VAM spores

The spores were identified on the basis of their shape, colour, surface texture and the nature of spore attachment by observing under a stereo microscope. The characters were then compared with the key prepared by Trappe (1982).

3.6 Mass multiplication of the VAM inoculum

Mass multiplication of the isolates of VA mycorrhizae was done by inoculating them in maize (*Zea mays* L.). Fifty spores of each culture of VAM were used for inoculation into maize. The maize plants were grown in steam sterilized potting mixture containing sand and soil in 1:1 ratio in small pots of 11 x 14 cm size for one month for the proper development of VAM. The roots from these plants were then collected for using as the starting material for the mass production of VAM inoculum. The inoculum was further multiplied by inoculating this mycorrhizal roots in large pots of 35 x 35 cm size containing 10 kg of steam sterilized potting mixture of the above composition (Plate 1a and 1b). The

Plate 1a
Maize plants inoculated with VAM for mass multiplication.

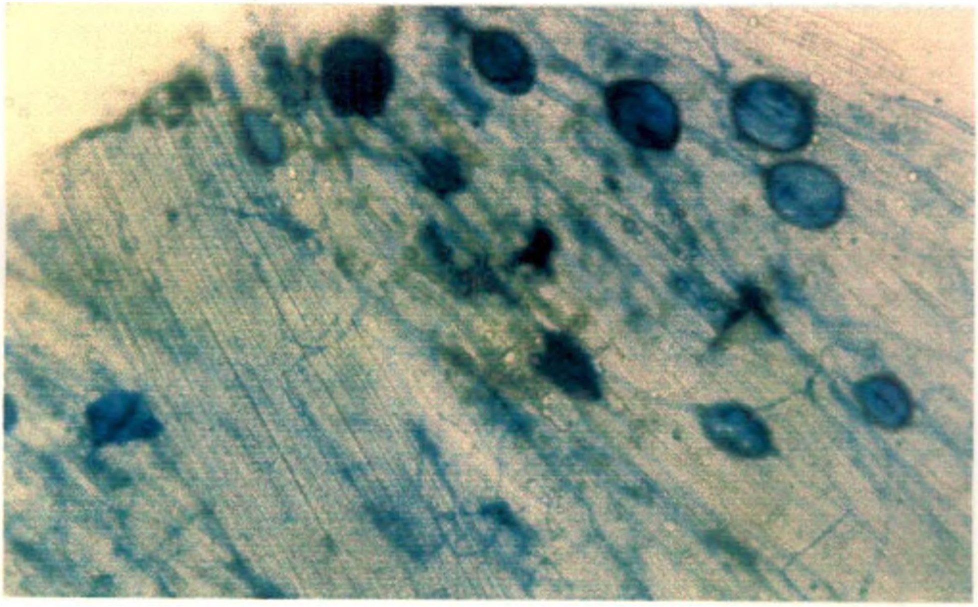


Plate 1b
VAM infected maize root (100 X)

mycorrhizal roots were cut into small bits of about one cm length and 50 g each of this root bit inoculum was used for inoculating each pot.

This inoculum was placed at a depth of about five cm in the centre of the large pot. Over this the seeds of maize were sown. The maize plants were grown for 60 days for the proper development of infected roots. Infected roots of these plants along with 50 spores of the respective VAM were used as the mycorrhizal inoculum for various experiments conducted during this investigation.

3.7 Screening of VA mycorrhiza for efficiency in cowpea

This experiment was conducted in pot culture. Five isolates of VAM obtained from five different locations were used. Along with these local isolates, two isolates [*Glomus fasciculatum* (Thaxter Sensu Gerd.) Gerd. & Trappe and *Glomus mosseae* (Nic. & Gerd.) Gerd. & Trappe] procured from University of Agricultural Sciences(U.A.S.), Bangalore were also used. The experiment was laid out in completely randomised design with four replications.

The different treatments were the inoculation of the following mycorrhizal isolates.

T_1 - Isolate from BRS, Kannara

T_2 - Isolate from farmer's field at Moorkanikkara

T_3 - Isolate from farmer's field at Nadathara

T_4 - Isolate from vegetable research farm, COH, Vellanikkara

T_5 - Isolate from vegetable garden, KHDP, Vellanikkara

T_6 - *Glomus fasciculatum* from U.A.S., Bangalore

T_7 - *Glomus mosseae* from U.A.S., Bangalore

T_8 - Control

The experiment was conducted in earthen pots containing a potting mixture of sand, soil and cowdung in 2:2:1 ratio. The potting mixture was initially steam sterilized in an autoclave for two hours at 1.05 kg cm^2. Inoculation with VAM was done as described earlier.

3.7.1 Cowpea variety

Cowpea variety 'Pusa Komal' was used throughout this investigation. Seeds were obtained from the Department of Olericulture, College of Horticulture, Vellanikkara.

3.7.2 Irrigation and cultural practices

The pots were irrigated regularly with sterilized water. The cultural operations were done as per Package of Practice Recommendations of KAU, 1993.

3.7.3 Observations

Biometric observations of the plants, viz. height of plant, number of leaves, fresh and dry weights of plant, root length, fresh and dry weights of roots, nodule number, fresh and dry weights of nodules and per cent VAM infection were recorded on the 45th day after sowing.

3.7.3.1 Height of plant

Height of plant was measured from the soil level to the top most part of the plant.

3.7.3.2 Number of leaves

Total number of leaves was taken by counting from oldest to youngest leaves of plant.

3.7.3.3 Fresh weight of plant

Fresh weight of plant was taken immediately after uprooting and removing the soil particles adhered to the root system by gentle wash with water.

3.7.3.4 Length of root

This was taken from the collar region to the tip of the longest root.

3.7.3.5 Fresh weight of root

Root system of the plant was separated, washed with water, allowed to drain and the weight was taken in an electronic balance.

3.7.3.6 Nodule number

The number of nodules formed were counted after carefully uprooting each plant and removing adhering soil particles with the help of mild jet of water.

3.7.3.7 Fresh weight of nodule

Fresh weight of nodule was taken in an electronic balance.

3.7.3.8 Dry weight of shoot, root and nodules

The dry weights of shoot, root and nodules were determined after drying the samples to a constant weight at 60°C in a drying oven.

Similarly in all other experiments, the biometric observations were taken as described above.

3.8 Procurement of *Bradyrhizobium* culture

Bradyrhizobium culture developed by the Kerala Agricultural University for cowpea was used for this study. The culture was maintained on yeast extract mannitol agar (Appendix-III).

3.8.1 Purity of *Bradyrhizobium* culture

A loopful of the culture was suspended in five ml of sterilized water taken in test tube. This suspension was streaked on YEMA medium in petridishes and incubated at 28±2°C in a B.O.D. incubator for four days. Individual colonies showing the typical characteristics of *Bradyrhizobium* were then transferred to sterilized YEMA slants for further studies.

3.8.2 Primary characterisation of *Bradyrhizobium*

Bradyrhizobium culture was characterized by their cell morphology, Gram staining, growth on YEMA and glucose peptone agar medium.

3.8.2.1 Growth on glucose peptone agar

A loopful of the culture was streaked on glucose peptone agar medium (Appendix-IV) and then incubated at 28±2°C for seven days in B.O.D. incubator. The extent of growth and change of colour of the medium if any were noted.

3.8.3 Inoculation of *Bradyrhizobium*

A thick suspension of four days old culture of *Bradyrhizobium* in YEMA slants was initially prepared by adding approximately 10 ml of sterilized water to each slant. This suspension was mixed thoroughly. Cowpea seeds were disinfected with 70 per cent ethyl alcohol and then washed with sterilized water. The bacterial suspension was mixed with the seeds. The treated seeds were sown immediately. This was the procedure followed for the bacterial inoculation of cowpea seeds in all the experiments.

3.9 Study on the infection pattern of VA mycorrhiza and *Bradyrhizobium* inoculants

This study was conducted in a pot culture experiment. It was laid out in completely randomized design with four replications. The potting mixture consisted of sand, soil and cowdung in the ratio 2:2:1. This mixture was steam sterilized as described earlier. Multiple plants were maintained to accommodate for destructive sampling.

Treatments

B_0M_0 - No inoculants

B_0M_1 - VA mycorrhizal inoculation

B_1M_0 - *Bradyrhizobium* inoculation

B_1M_1 - VA mycorrhiza + *Bradyrhizobium* inoculation

3.9.1 VA mycorrhiza

The most efficient VAM isolate obtained from the screening trial was used as VAM inoculum. Inoculation and cultural operations were done as described earlier.

3.9.2 Observations

The extent of mycorrhizal colonisation and the number of nodules developed were observed at 10 days interval upto 60th day after sowing. The percentage of mycorrhizal colonisation was determined by the method described earlier. Nodule number was recorded after carefully exposing the root system.

3.10 Anatomical studies of VAM colonisation pattern and nodulation

3.10.1 Scanning electron microscopy of VAM colonised roots

VAM colonised roots were removed from the plant and washed in tap water to remove all adhering soil particles. They were then cut into small bits and fixed in 2.5 per cent glutaraldehyde in phosphate buffer for 12 hours. They were then passed through three changes of phosphate buffer at 15 minutes interval. Osmium oxide was added to root sample and kept in refrigerator for 1½ to 2 hours. It was washed with phosphate buffer for three times and passed through acetone series of 50, 60, 70 and 80 per cent for 25 minutes each. The sample was then kept overnight in 90 per cent acetone. The sample was then passed through three changes of 95 per cent acetone for 30 minutes and then immersed in 100 per cent acetone for absolute dehydration. The root bits were taken from the acetone and the cut surface was coated with gold vapour. They were then observed in Hitachi S-530 scanning electron microscope.

3.10.2 Examination of VA mycorrhiza under a light microscope

The root samples were stained by the method described earlier (Philips and Hayman, 1970) and examined under a light microscope for VAM colonisation.

3.10.3 Scanning Electron Microscopy of root nodules

Healthy root nodules were initially detached along with a bit of root and washed in tap water to remove all adhering soil particles. They were fixed in 2.5 per cent glutaraldehyde in phosphate buffer for 12 hours and prepared for examination in SEM by the method described earlier.

3.11 Pot culture experiment on the efficiency of the microsymbionts on the uptake of nutrients under different levels of nitrogen and phosphorus

A pot culture experiment was conducted to study the efficiency of cowpea plants inoculated with VAM and *Bradyrhizobium* under different levels of added nitrogen and phosphorus fertilizers. The experiment was laid out as 4 x 6 factorial in CRD with three replications. The treatment details are given below:

Factors:

Inoculants

I_0 - No inoculants

I_1 - VAM inoculant

I_2 - *Bradyrhizobium* inoculant

I_3 - VAM + *Bradyrhizobium*

Fertilizer levels

F_0 - No added fertilizer

F_1 - 20:30:10 kg NPK ha^{-1} (Package of Practice Recommendations of KAU, 1993)

F_2 - 10:30:10 kg NPK ha^{-1} (½ N+P+K)

F_3 - 5:30:10 kg NPK ha^{-1} (¼ N+P+K)

F_4 - 20:15:10 kg NPK ha^{-1} (N+½P+K)

F_5 - 20:7.5:10 kg NPK ha^{-1} (N+¼P+K)

The treatment combinations were as follows

I_0F_0	I_1F_0	I_2F_0	I_3F_0
I_0F_1	I_1F_1	I_2F_1	I_3F_1
I_0F_2	I_1F_2	I_2F_2	I_3F_2
I_0F_3	I_1F_3	I_2F_3	I_3F_3
I_0F_4	I_1F_4	I_2F_4	I_3F_4
I_0F_5	I_1F_5	I_2F_5	I_3F_5

The experiment was conducted in earthen pots containing a potting mixture of sand, soil and cowdung in the ratio 2:2:1, which was initially steam sterilized in an autoclave for two hours at 1.05 kg cm^2 pressure.

3.11.1 Application of inoculum

Inoculation with *Bradyrhizobium* and VAM was done wherever required by the methods described earlier.

3.11.2 Fertilizer application

The fertilizers such as Urea, Superphosphate and Muriate of Potash were used to supply nitrogen, phosphorus and potassium respectively.

The nitrogen fertilizer was given in two split applications; half at the time of sowing and the other half after 20 days of sowing.

3.11.3 Observations

Biometric observations of the plant, viz. height of plant, number of leaves, fresh and dry weights of plant, length of root and fresh and dry weights of roots were recorded at 45th day after sowing. Nodule number, fresh and dry weights of nodules and per cent mycorrhizal infection were also recorded. Biometric observations were recorded as described earlier.

3.11.4 Nutrient analysis of plant

The plant top was dried under sun for two days and then dried in an oven at 65°C for two days. They were then powdered in a laboratory mill and this powder was used for the analysis of nutrients.

3.11.4.1 Nitrogen content

The nitrogen content of plant samples was determined by digesting 0.1 g of the sample in five ml of concentrated H_2SO_4 using digestion mixture (K_2SO_4, $CuSO_4$ and Selenium powder in 100:10:1 ratio) and the nitrogen in the digest was determined by microkjeldahl method.

3.11.4.2 Phosphorus and potassium content

One gram of the powdered sample was digested in diacid (Nitric acid and Perchloric acid in 2:1 ratio) and the digest was made up to 50 ml. Two ml of the sample was taken to determine the phosphorus content colorimetrically by vanadomolybdate phosphoric yellow colour method. The colour intensity was read in a photoelectric colorimeter using blue filter. An aliquot (0.5 ml) from the diacid extract was taken to read potassium using flame photometer.

3.11.4.3 Micronutrients

For the estimation of calcium, magnesium, iron, copper and manganese in the plant sample, two ml of the diacid extract was taken and read directly in

Atomic Absorption Spectrophotometer. The analysis of nitrogen, phosphorus, potassium and micronutrients were done by the methods given in Table 1.

Table 1. Methods of plant analysis

Nutrient	Method of estimation	Reference
1. Nitrogen	Microkjeldahl method	Jackson, 1958
2. Phosphorus	Vanadomolybdophosphoric yellow colour method	Jackson 1958
3. Potassium	Flame photometry	Jackson, 1958
4. Calcium	Estimated in Atomic Absorption Spectrophotometer	Page, 1982
5. Magnesium	,,	Page, 1982
6. Manganese	,,	Page, 1982
7. Copper	,,	Page, 1982
8. Iron	,,	Page, 1982

3.11.5 Soil Analysis

Soil samples were analysed after the experiment, for the available nitrogen, phosphorus and potassium content. Soil samples were collected from each pot for the estimation of nutrients status.

Ten gram of the air dried soil was used for the estimation of available nitrogen by alkaline permanganate method. Bray's extraction method was used to determine the available phosphorus in the soil. Five grams of air dried soil, passing through 0.25 mm sieve was mixed with 50 ml of Bray's solution in a glass stoppered bottle. One ml of this extract was used to read the phosphorus content by ascorbic acid blue colour method. To determine the potassium content of soil, five grams of air dried soil was extracted with neutral normal ammonium acetate and two ml of the extract was used to read the potassium content by EEL Flame photometer. The methods adopted are given in Table 2.

Table 2. Methods of chemical analysis of soil

Nutrient	Method of estimation	Reference
1. Available Nitrogen	Alkaline permanganate method	Subbiah and Asija, 1956
2. Available Phosphorus	Ascorbic acid blue colour method (Bray-1 extraction)	Watanabe and Olsen, 1965
3. Available Potassium	Flame photometry (Neutral normal ammonium acetate extraction)	Jackson, 1958

3.12 Field experiments on the efficiency of the microsymbionts on the uptake of nutrients under different levels of nitrogen and phosphorus

This field experiment was laid out in randomized block design with three replications. The plot size was 2 x 2 m.

Treatment combinations were as follows:

T_1 - Control - No inoculants, no added NPK

T_2 - 20:30:10 kg NPK ha^{-1} (Package of Practice Recommendations of KAU, 1993)

T_3 - VAM + No NPK

T_4 - VAM + 20:15:10 kg NPK ha^{-1} (VAM+N+½P+K)

T_5 - VAM + 20:7.5:10 kg NPK ha^{-1} (VAM+N+¼P+K)

T_6 - *Bradyrhizobium* + No NPK

T_7 - *Bradyrhizobium* + 10:30:10 kg NPK ha^{-1} (B+½N+P+K)

T_8 - *Bradyrhizobium* + 5:30:10 kg NPK ha^{-1} (B+¼N+P+K)

T_9 - VAM + *Bradyrhizobium* (VAM + B)

T_{10} - VAM + *Bradyrhizobium* + 10:15:10 kg NPK ha^{-1} (VAM+B+½N+½P+K)

T_{11} - VAM + *Bradyrhizobium* + 5:7.5:10 kg NPK ha^{-1} (VAM+B+¼N+¼P+K)

3.12.1 Preparation of field

The experimental area was tilled, levelled and laid out into blocks and plots.

3.12.2 Collection of soil samples

Soil samples were collected from each plot for the estimation of available nitrogen, phosphorus and potassium. Samples were taken as per the standard method.

3.12.3 Application of inoculum

Seed treatment with *Bradyrhizobium* and soil application of VA mycorrhiza culture were done as described earlier.

3.12.4 Fertilizer application

Fertilizers were applied as described in the earlier experiment.

3.12.5 Observations

Biometric observations of the plants, viz. height of plant, number of leaves, fresh and dry weights of plant, length of root and fresh and dry weights of root were taken at 45th day after sowing. These observations were also recorded at the time of harvest. Nodule number, fresh weight and dry weight of nodule were recorded both at 45th day after sowing and at the time of harvest. Mycorrhizal colonisation of roots was recorded at 50 per cent flowering of plants. At the time of harvest yield was recorded.

3.12.6 Nutrient content of plant

The nitrogen, phosphorus, potassium, calcium, magnesium, manganese, copper and iron content in the plant samples were analysed by the methods described earlier.

3.12.7 Soil analysis

The available nitrogen, phosphorus and potassium content of soil samples after the experiment were analysed by the methods described earlier.

3.13 Statistical analysis

Data pertaining to each experiment were tabulated separately and subjected to appropriate statistical analysis using the MSTAT package available at the Central Computer Facility of the College of Horticulture, Vellanikkara, Thrissur.

Results

4. RESULTS

4.1 Estimation of natural occurrence of VA mycorrhizal colonisation in cowpea

A survey on the occurrence of natural VA mycorrhizal colonisation in five cowpea growing locations in Thrissur district was conducted during the rainy and summer seasons of 1995. The pH of the soil in these locations was acidic and it ranged from 5.2 to 5.8.

The results of the survey are presented in Tables 3 to 6. The results showed that the natural VAM colonisation in all the locations was fairly good and it ranged from 26.09 to 83.40 per cent. VAM colonisation was found high during the rainy season than in summer.

The results showed that during rainy season, mean VAM colonisations on the lateral roots of cowpea plants were the maximum at farmer's field at Nadathara (80.08%) and the minimum colonisation (61.72%) was found in plants grown at BRS, Kannara. At Nadathara, the VAM colonisation percentage on lateral roots ranged from 75.00 to 93.75 per cent. At Kannara, the maximum colonisation was 89.06 and the minimum, 17.18 per cent (Table 3).

The results on the percentage of VAM colonisation on the tap roots during rainy season are presented in Table 4. The maximum tap root colonisation was found in cowpea plants at farmer's field at Nadathara (83.4%) and minimum colonisation at farmer's field, Moorkanikkara (26.09%). At Nadathara, the colonisation percentage ranged from 68.75 to 100. Tap root colonisation of VAM was minimum and almost similar at farmer's field at Moorkanikkara (26.09%) and at BRS, Kannara (26.12%).

Table 3. Percentage of VAM colonisation of lateral roots of cowpea plants at survey locations in rainy season

Plant No.	BRS Kannara	COH Vellanikkara	KHDP Vellanikkara	Farmer's field Moorkanikkara	Farmer's field Nadathara
1	42.18	100.00	81.25	98.43	90.62
2	70.31	54.68	76.56	85.93	85.93
3	67.18	76.56	70.31	78.12	75.00
4	79.68	62.50	73.43	92.18	89.06
5	62.50	62.50	69.16	76.56	78.13
6	82.81	76.56	85.93	76.56	81.25
7	35.93	87.50	71.87	54.68	90.62
8	35.93	57.81	62.50	39.06	89.06
9	45.31	71.86	69.16	65.62	93.75
10	59.37	65.62	65.62	78.12	81.25
11	17.18	68.75	73.43	75.00	76.56
12	67.18	65.62	71.87	78.12	84.35
13	84.37	82.81	84.37	79.68	76.56
14	89.06	73.43	75.00	82.81	79.68
15	87.50	70.31	75.00	84.37	76.56
16	37.50	87.50	71.87	84.37	92.18
17	46.87	87.50	73.43	84.37	85.93
18	81.25	89.06	71.87	85.93	84.37
19	65.92	84.37	84.37	75.00	82.81
20	78.12	75.00	67.18	79.68	78.12
Mean	61.79	74.99	73.70	74.23	80.08

Table 4. Percentage of VAM colonisation of tap root of cowpea plants at survey locations in rainy season

Plant No.	BRS Kannara	COH Vellanikkara	KHDP Vellanikkara	Farmer's field Moorkanikkara	Farmer's field Nadathara
1	42.50	50.00	90.62	37.50	87.50
2	20.80	43.75	81.25	43.75	100.00
3	32.60	53.12	90.62	28.12	100.00
4	25.00	21.87	59.37	28.12	71.87
5	21.80	21.87	56.25	21.87	87.50
6	20.16	37.50	90.62	25.00	78.12
7	40.50	25.00	81.25	21.87	87.50
8	25.00	21.87	90.62	21.87	90.62
9	34.38	21.87	56.25	15.62	87.50
10	26.10	46.87	68.75	21.87	75.00
11	18.65	25.00	68.75	18.75	75.00
12	32.56	40.62	50.00	25.00	100.00
13	27.16	18.75	71.87	34.37	71.87
18	24.40	25.00	56.25	25.00	68.75
15	20.50	34.37	62.50	25.00	78.12
16	22.60	21.87	62.50	25.00	81.25
17	18.50	25.00	65.62	28.12	87.50
18	24.67	21.87	62.50	15.62	75.00
19	25.12	25.00	65.62	31.25	71.87
20	19.50	28.12	62.50	28.12	93.75
Mean	26.12	30.47	69.68	26.09	83.40

The percentage of VAM colonisation on the lateral roots of cowpea plants during summer season is presented in Table 5. Here also the maximum colonisation (65.43%) was found in plants grown at farmer's field at Nadathara where, the colonisation ranged from 46.87 to 89.06 per cent. The minimum colonisation on lateral roots was found in plants grown at BRS, Kannara where the colonisation was only 44.37 per cent. Here colonisation on tap root varied from 18.75 to 62.5 per cent.

The results on tap root colonisations of VAM during summer season is presented in Table 6. The tap root colonisation was maximum at farmer's field at Nadathara (48.7%) and the minimum was in the plants grown at COH, Vellanikkara (27.34%). In the farmer's field at Nadathara, the tap root colonisation ranged from 16.66 to 78.12 per cent, whereas at COH, Vellanikkara, it ranged from 6.25 to 50 per cent.

4.1.1 Spore count of VAM fungi in rhizosphere soil

Spore count of VAM fungi was taken from the rhizosphere soil of cowpea plants at each location during rainy and summer seasons. The results are presented in Table 7. There was not much differences among the locations in the mean number of VA mycorrhizal spores. On comparing the seasons, more number of VAM spores were seen in the rainy season in all locations except in farmer's field at Moorkanikkara. A maximum of 87 spores were recorded from the soils of COH, Vellanikkara; KHDP, Vellanikkara and Farmer's field at Nadathara during the rainy season.

In summer season, the maximum number of 87 spores was recorded from farmer's field at Moorkanikkara and farmer's field at Nadathara. The lower spore count of 53 and 60 were obtained from the soil sample collected from BRS,

Table 5. Percentage of VAM colonisation of lateral roots of cowpea plants at survey locations in summer season

Plant No.	BRS Kannara	COH Vellanikkara	KHDP Vellanikkara	Farmer's field Moorkanikkara	Farmer's field Nadathara
1	51.56	56.25	73.43	67.18	89.06
2	59.37	51.56	67.18	56.25	79.18
3	51.56	68.75	78.12	62.50	87.50
4	56.25	56.25	70.31	81.25	67.18
5	46.87	43.75	43.75	70.31	64.06
6	56.25	64.06	42.18	81.25	73.43
7	53.12	67.18	67.18	48.43	85.93
8	35.93	45.31	56.25	46.87	53.12
9	42.18	65.62	62.50	60.93	85.93
10	29.68	54.68	57.81	37.50	70.31
11	32.93	40.62	51.56	62.50	62.50
12	39.06	32.81	59.37	48.43	56.25
13	18.75	28.12	50.00	53.12	67.18
14	37.50	43.75	51.56	59.37	60.93
15	43.75	53.12	32.81	48.43	56.25
16	42.18	57.81	37.50	45.31	46.87
17	50.00	59.37	42.18	45.31	50.00
18	32.81	65.62	57.81	54.68	57.81
19	62.50	54.68	50.00	42.18	46.87
20	42.18	40.62	54.68	48.43	48.43
Mean	44.37	52.49	55.31	56.06	65.43

Table 6. Percentage of VAM colonisation of tap root of cowpea plants at survey locations in summer season

Plant No.	BRS Kannara	COH Vellanikkara	KHDP Vellanikkara	Farmer's field Moorkanikkara	Farmer's field Nadathara
1	37.50	25.00	31.25	28.12	75.00
2	31.25	28.12	28.12	46.87	50.00
3	15.62	31.25	50.00	31.25	78.12
4	28.12	37.50	46.87	25.00	68.75
5	40.62	40.62	37.50	40.62	50.00
6	34.37	15.62	43.75	43.75	65.62
7	62.50	31.25	40.62	53.12	46.87
8	46.57	6.25	15.62	43.75	16.66
9	56.25	21.87	25.00	40.62	46.87
10	53.12	25.00	15.62	50.00	37.50
11	37.50	15.62	28.12	21.87	37.50
12	46.78	28.12	12.50	15.62	56.25
13	56.25	28.12	28.12	37.50	34.37
14	50.00	31.25	37.50	12.50	40.62
15	56.25	25.00	46.87	15.62	50.00
16	15.62	50.00	15.62	16.66	73.12
17	28.12	12.50	37.50	25.00	43.75
18	50.00	15.62	31.25	31.25	46.87
19	15.62	37.50	28.10	12.50	18.75
20	12.50	40.62	15.62	50.00	37.50
Mean	38.75	27.34	30.78	29.58	48.70

Table 7. VAM spore count in the rhizosphere soil at the survey locations

Location	No. of spores/100 g rhizosphere soil	
	Rainy season	Summer season
1. Banana Research Station, Kannara	60	53
2. Vegetable research farm of College of Horticulture, Vellanikkara	87	73
3. Vegetable garden of K.H.D.P., Vellanikkara	87	80
4. Farmer's Field, Moorkanikkara	80	87
5. Farmer's Field, Nadathara	87	87

Kannara during the summer and rainy seasons respectively. There was no seasonal effect for the spore count at the farmer's field at Nadathara.

4.2 Identification of VA mycorrhiza

VA mycorrhizal spores collected from the rhizosphere soils of cowpea plants at the survey locations were identified on the basis of their size, shape, colour, surface texture and mode of hyphal attachment. Most of the spores were globose with simple basal attachment. The colour showed variations of yellow, brown and reddish brown under incident light in a stereomicroscope. The surface texture was smooth. The spores were typical of *Glomus* sp. with an average size of 131.02 µm. It was found that the VAM spores found in all locations were identical to that of *Glomus* (Plate 2a and 2b).

4.3 Screening of VA mycorrhizal isolates for efficiency in cowpea

The results of the experiment are presented in Table 8. The results showed that plant height, number of leaves and fresh and dry weights of the plant were not significantly different among the treatments. But the fresh weight of root and percentage of VAM colonisation showed significant differences.The maximum mean fresh weight of root (3.05 g) was recorded by the plants treated with native culture of VAM (T_3) isolated from farmer's field at Nadathara. The same native VAM culture recorded the maximum mean percentage of VAM colonisation (67.93) in cowpea plants.

In all other observations, eventhough this isolate did not attain statistical significance, ranked either second or third. For selecting the best among the different isolates, VAM colonisation per cent was given more weightage. Considering the results on other parameters and giving more importance on VAM

Table 8. Screening for efficiency of VA mycorrhizal isolates - Effect of VAM from survey locations on plant characters and percentage of VAM colonisation

Treatments	Height of plant (cm)	No. of leaves	Fresh weight of plant (g)	Dry weight of plant (g)	Fresh weight of of root (g)	VAM colonisat-ion (%)
T_1	26.53	10.50	11.11	4.88	1.16	60.80
T_2	27.50	13.77	15.00	7.14	1.24	64.67
T_3	31.27	16.53	20.55	7.43	3.05	67.93
T_4	31.63	15.30	18.33	5.90	1.23	33.97
T_5	29.80	15.80	20.72	7.22	2.16	40.50
T_6	31.37	17.13	21.39	7.59	2.87	32.67
T_7	32.20	15.87	19.25	7.31	1.91	38.57
T_8	30.73	15.30	15.99	5.87	1.49	18.93
CD	NS	NS	NS	NS	0.90**	7.08*

**Significant at 1 per cent level
* Significant at 5 per cent level
NS - Non significant

colonisation, T_3 (isolate from farmer's field at Nadathara) was selected for further experiments in this study.

4.4 Characterisation of *Bradyrhizobium* culture

The cowpea *Bradyrhizobium* culture procured from College of Agriculture, Vellayani was tested for its purity. Microscopic observations showed rod shaped cells. Gram reaction was negative. Colonies appeared after 48-72 h in YEMA medium. On glucose peptone agar, the growth was scanty without any change in colour of the medium even after seven days of incubation.

4.5 Infection pattern of VA mycorrhiza and *Bradyrhizobium* in cowpea plants

The results of this experiment are presented in Table 9.

4.5.1 Infection pattern of *Bradyrhizobium*

The results showed that nodulation was started 10 days after sowing. Plants inoculated with *Bradyrhizobium* alone and in combination with VAM recorded a nodule number of 35.25 and 45.75 respectively on the 10th day of plant growth. Later a gradual increase in nodule number was observed till 40th day. On 40th day *Bradyrhizobium* treatment yielded 91 and its combination with VAM inoculation yielded 106.5 nodules. After that there was a gradual reduction in nodule number. Thus the maximum nodulation in both the treatments were found to be at 40th day (Plate 3). Except at 20th day, at all other stages the *Bradyrhizobium* inoculation combined with VAM inoculation produced more number of nodules than when *Bradyrhizobium* alone was inoculated. At 40th day control plants produced only 12.25 nodules.

Table 9. Infection pattern of VA mycorrhiza and *Bradyrhizobium* in cowpea - Effect of inoculation of microsymbionts on nodule number and percentage of VAM colonisation

Age of the plant (days)	Number of nodules				Per cent VAM colonisation			
	B_0M_0	B_0M_1	B_1M_0	B_1M_1	B_0M_0	B_0M_1	B_1M_0	B_1M_1
10	9.50	16.75	35.25	45.75	0	50.28	3.92	51.19
20	10.75	21.00	84.75	69.50	0	51.37	8.85	43.57
30	10.75	25.00	87.50	100.25	3.12	48.25	5.38	59.71
40	12.25	33.50	91.00	106.50	2.95	65.97	3.47	65.80
50	8.25	16.25	70.75	96.25	6.77	52.25	4.51	64.05
60	9.50	25.75	90.00	91.50	5.90	47.22	5.21	58.50

4.5.2 Infection pattern of VAM

In control plants VAM infection was noticed only from 30th day onwards, whereas in treated plants infection was found to start from 10th day onwards (Table 9, Plate 4). The percentage of VAM colonisation was also found to be the maximum on 40th day of plant growth in both treatments, viz. inoculation with VAM alone and VAM along with *Bradyrhizobium*. The colonisation percentage were 65.97 and 65.80 respectively. Plants inoculated with VAM showed about 50 per cent colonisation of roots even on 10th day of plant growth. Afterwards colonisation per cent reached the maximum on 40th day. Observations on 50th and 60th day did not show much variation from the 40th day of plant growth. In control plants the maximum colonisation percentage of 6.77 was found at 50th day.

4.6 Anatomical studies of nodulation and VAM colonisation pattern

4.6.1 Light microscopy of VA mycorrhizal roots

Light microscopy of VAM colonised roots showed the presence of hyphae and vesicles only in the root cortex. The highly branched hyphae of VAM were found to grow almost parallel to the root axis, occupying much of the primary cortex. The hypha showed H-shaped and V-shaped branching (Plate 5). The vesicles formed were typically terminal and oval in shape. Under high power (400 x) magnification, oil globules were visible in the vesicle (Plate 6).

About 50 per cent root colonisation was obtained in plants inoculated with VAM on 10th day of plant growth (Table 9). At this stage VAM colonisation in the root cortex was clearly visible under the light microscope. The hypha of VAM spread along the walls of the host cells and formed intracellular hyphal coils. At ten days of plant growth, the hyphae gave rise to arbuscules in the cells (Plate 7a and 7b). Later the arbuscules gradually disintegrated and vesicles started to form. Number of vesicles on 10th day of plant growth was very less. But it gradually

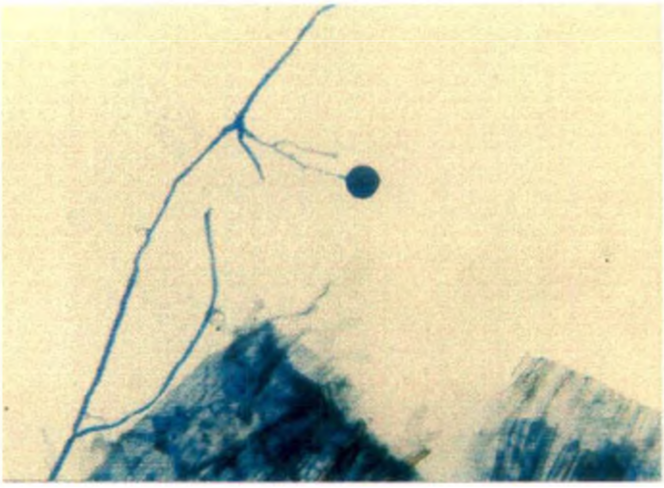


Plate 2a

Direct attachment of VAM spore at the hyphal tip. (100 X)

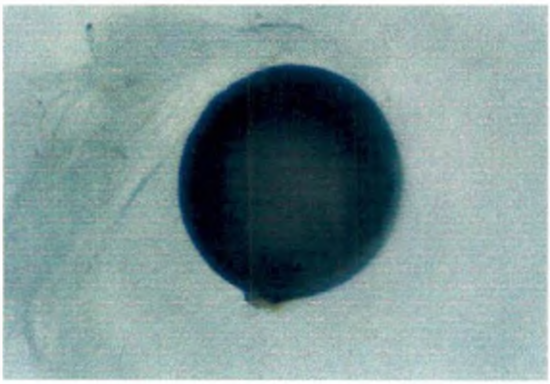


Plate 2b

A typical spore of Glomus sp. (200 X)

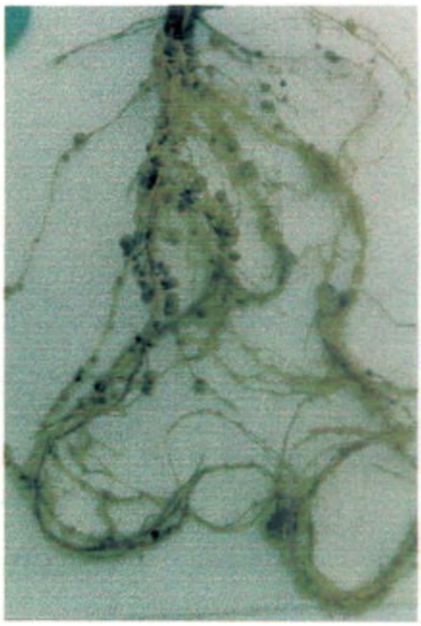


Plate 3

Nodulation of cowpea plant at 40th day after sowing

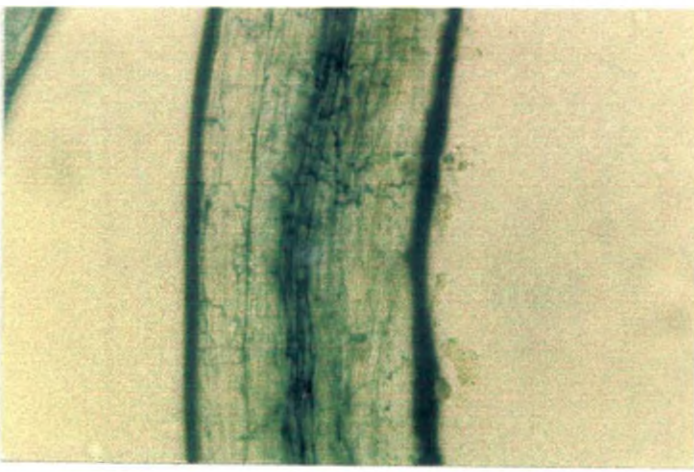


Plate 4

VAM colonisation of cowpea root at 10th day after sowing. (100 X) increased in the later stages of plant growth. Observation on 40th day of plant growth showed the maximum percentage of VAM colonisation in cowpea roots. Observations of spores under light microscope confirmed the presence of *Glomus* sp. Observations of the nodules under the light microscope showed the presence of VA mycorrhizal hyphae in the nodule tissues (Plate 8).

4.6.2 Scanning Electron Microscopy of VA mycorrhizal roots

Electron microscopic studies of the infected roots and nodules were carried out and the results are depicted in Plates 9 to 16. The electron micrographs showed considerable presence of VA mycorrhizal hyphae on the root surface (Plate 9). A magnified view revealed that the mycelium grew adhering to the root surface before penetrating in to the root cortex (Plate 10). The mycelium had grown along the contours of the root surface and gradually spread out in to the soil zone. The Plates 11 and 12 revealed the type of penetration of the VAM fungus to the root cortex. Colonisation of the root by the hyphae were found to occur in two ways as revealed in the plates. In one, the mycorrhizal fungus was found to penetrate the root directly almost at right angle with the root (Plate 11). In another, the hyphae grew along the root surface and gradually entered in to the root cortex (Plate 12).

Electron micrographs of the vesicle (Plate 13a and 13b) revealed its fine structures. The vesicles were found attached terminally on the hyphae. The vesicles were oval and the host tissue contents were found adhered on the vesicle surface. Arbuscules were composed of highly branching hyphal tips (Plate 14) and the branches were short, twisted and the tips were slightly bulged.

Observations of nodules under electron microscope revealed the rough nature of the nodule surface (Plate 15). The nodule surface was found relatively free of hyphal growth compared with root surface. However, a scanty growth of the hypha on the nodule surface was observed. The presence of coiled mycelium was

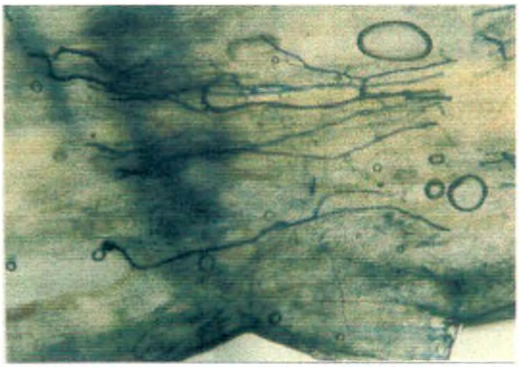


Plate 5
VAM hyphae showing 'H' and 'V' shaped branching in cowpea root. (400 X)

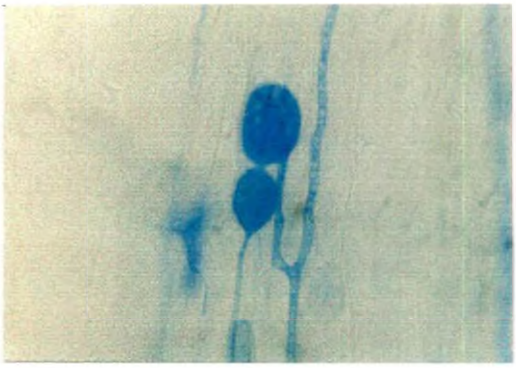


Plate 6
VAM vesicles showing terminal attachment and presence of oil globules. (400 X)

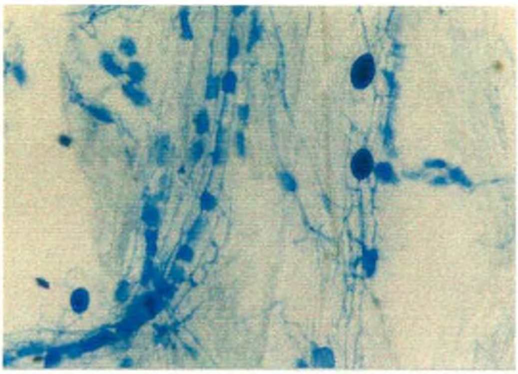


Plate 7a
VAM arbuscules and vesicles in cowpea root. (100 X)

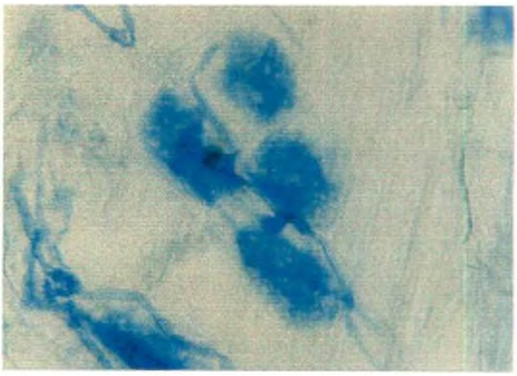


Plate 7b
VAM arbuscules under high power. (400 X)

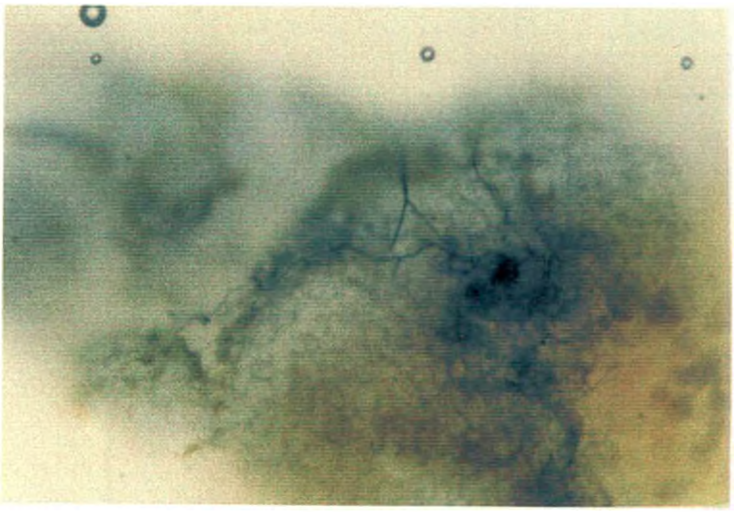


Plate 8
Presence of VAM hypha in root nodule tissue. (400 X)

seen in the inner tissues of nodules (Plate 16). But its growth and spread was very much restricted.

4.7 Pot culture experiment on the efficiency of the microsymbionts on the uptake of nutrients under different levels of nitrogen and phosphorus

All biometric observations recorded in this experiment were statistically analysed. The data are presented in Tables 10 to 20.

The individual and interaction effects of VA mycorrhiza, *Bradyrhizobium* and different levels of nitrogen and phosphorus fertilizers on growth parameters of the plant are detailed below.

4.7.1 Number of leaves

The results on number of leaves are presented in Table 10. The effect of inoculants on the number of leaves per plant was statistically significant. But the effect of different fertilizer levels and the interaction between inoculants and fertilizer levels were not significant. Among the inoculants, maximum number of leaves (10.81) was produced by the plants dually inoculated with VAM and *Bradyrhizobium*. Eventhough the interaction between inoculants and fertilizer application was not significant, maximum number of leaves (13.00) was recorded in plants treated with VAM + *Bradyrhizobium* along with N + ½P + K.

4.7.2 Height of plant

Only the effect of inoculants showed statistical significance in influencing the height of plants (Table 11). Fertilizer treatments and interaction between fertilizer and inoculants were not significant. Among the microsymbiont treatments maximum plant height (27.72 cm) was observed in plants treated with

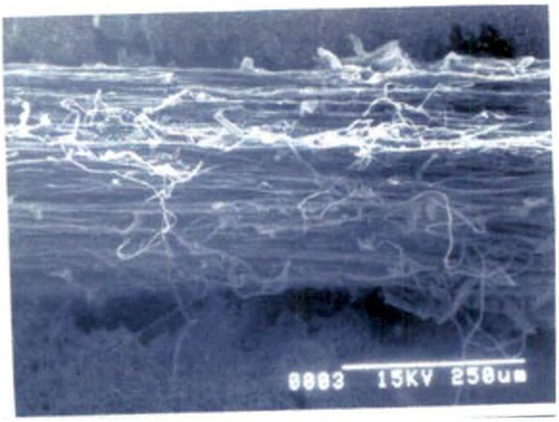


Plate 9
SEM of cowpea root surface showing the VAM hyphae.

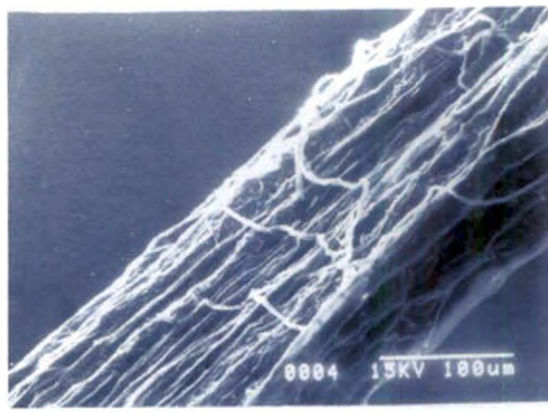


Plate 10
SEM showing VAM hyphae adhered on the root surface.

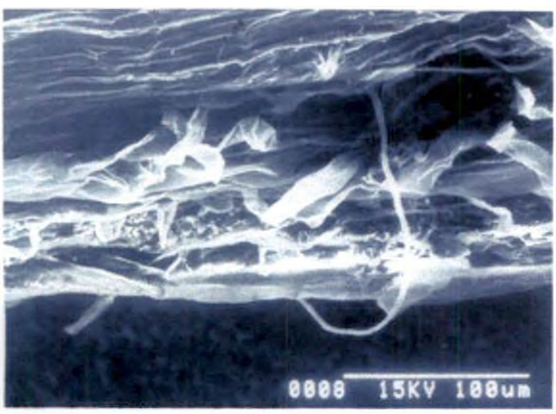


Plate 11
SEM showing VAM hyphal penetration into the root at right angle.

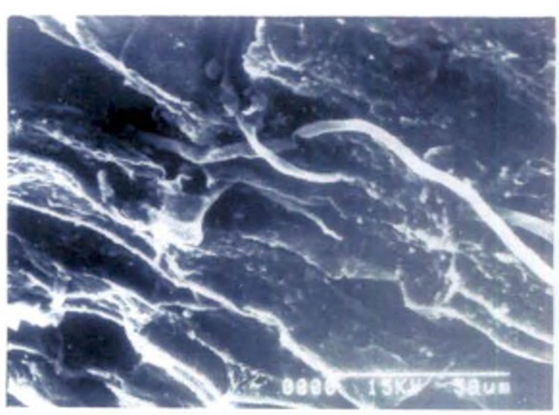


Plate 12
SEM showing gradual entry of VAM hyphae into the root.

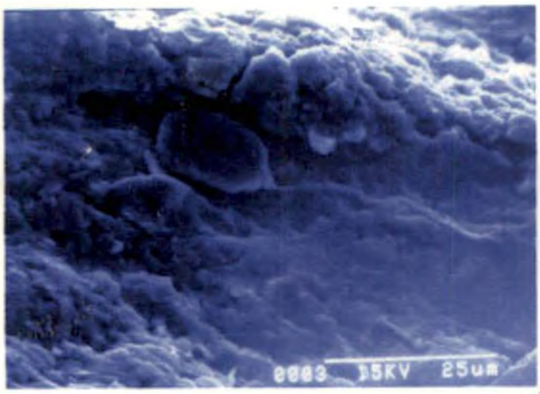


Plate 13
SEM of VAM vesicle in the root tissue.

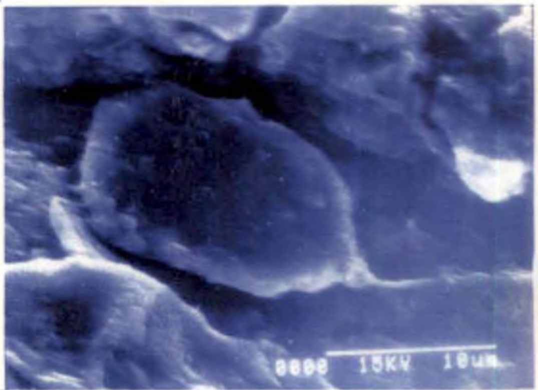


Plate 13b
A magnified view of VAM vesicle.

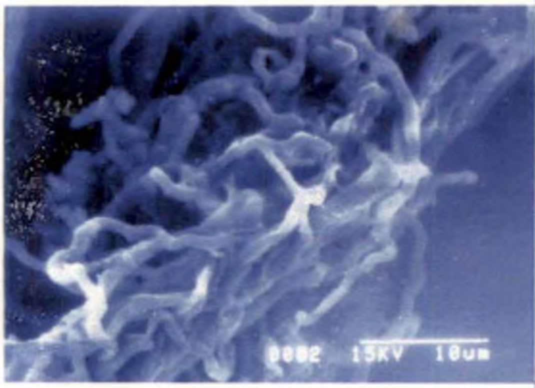


Plate 14
SEM of arbuscules in the root tissue.

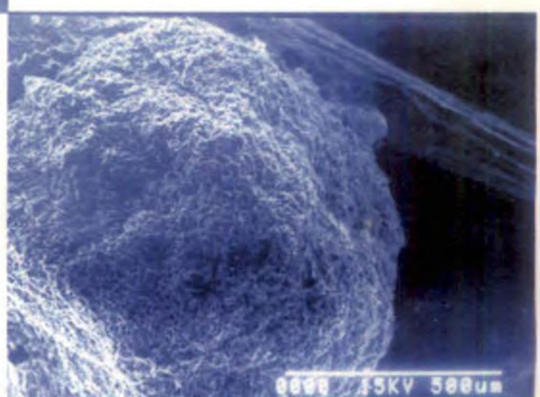


Plate 15
SEM of nodule surface free of VAM hypha.

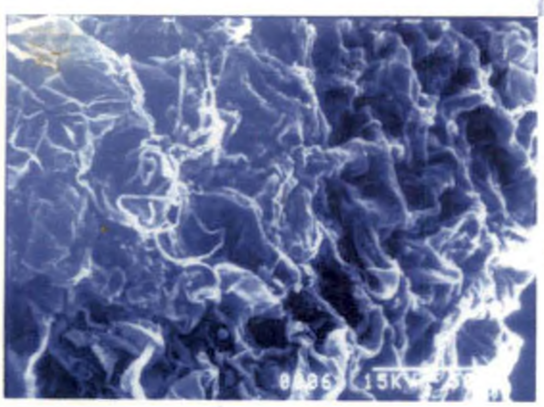


Plate 16
SEM of internal tissues of nodule showing the presence of VAM hypha.

Table 10. Pot culture experiment - effect of microsymbionts and fertilizer levels on number of leaves of cowpea

Treatments	No fertilizer	N+P+K	½N+P+K	¼N+P+K	N+½P+K	N+¼P+K	Mean
No inoculants	6.17	6.33	8.33	9.17	12.50	8.00	8.42
VAM	8.33	9.00	9.50	8.33	7.50	9.17	8.64
Bradyrhizobium	7.50	9.17	8.17	8.33	7.67	8.33	8.28
VAM + *Brady-rhizobium*	11.33	10.33	8.50	10.00	13.00	11.67	10.81
Mean	8.33	8.71	8.63	9.08	10.17	9.30	

CD(0.01) for comparison of inoculum 1.09
Fertilizer level NS
Inoculum x fertilizer level NS

Table 11. Pot culture experiment - effect of microsymbionts and fertilizer levels on height of cowpea (cm)

Treatments	No fertilizer	N+P+K	½N+P+K	¼N+P+K	N+½P+K	N+¼P+K	Mean
No inoculants	26.00	26.00	25.67	25.00	26.17	27.33	26.03
VAM	28.00	26.83	24.83	27.00	28.50	30.83	27.67
Bradyrhizobium	26.33	28.67	28.50	28.67	29.17	25.00	27.72
VAM + *Brady-rhizobium*	26.00	25.67	23.50	26.00	25.00	23.83	25.00
Mean	26.58	26.79	25.63	26.67	27.21	26.75	

CD(0.01) for comparison of inoculum 1.65
Fertilizer level NS
Inoculum x fertilizer level NS

Bradyrhizobium and was on par with VAM inoculation. Among the interactions, the maximum height of plant (30.83 cm) was recorded by the treatment VAM + N + ¼P + K, eventhough its effects were not significant.

4.7.3 Root length

The results on the root length are presented in Table 12. Only the effect of inoculants was statistically significant in influencing the length of root. Fertilizer application and the interaction between inoculants and fertilizer application were not significant in influencing the root length. The maximum root length recorded was 26.61 cm in the treatment where *Bradyrhizobium* alone was applied. Dual inoculation of the symbionts was also statistically on par with the above treatment. Eventhough the effect of fertilizer levels were not significant, N + ¼P + K produced the maximum root length of 25.75 cm. The treatment *Bradyrhizobium* alone without any fertilizer appliction recorded maximum root length (29.00 cm) eventhough its effects were not significant.

4.7.4 Fresh weight of plant

Fresh weight of plants are given in Table 13. The results showed that the effect of microbial inoculum and the interaction between inoculum and fertilizer levels were significant. Among the inoculants combined application of VAM and *Bradyrhizobium* was statistically superior in increasing the fresh weight of plants (22.00 g) and was on par with VAM inoculation alone. Among the fertilizer treatments, ½N + P + K was the first ranking treatment giving 19.5 g of fresh weight eventhough there was no significant difference. VAM combined with ½N + P + K was the statistically superior treatment combination which recorded 25.5 g of plant fresh weight.

Table 12. Pot culture experiment - effect of microsymbionts and fertilizer levels on length of roots (cm)

Treatments	No fertilizer	N+P+K	½N+P+K	¼N+P+K	N+½P+K	N+¼P+K	Mean
No inoculants	18.67	19.00	18.83	19.83	19.50	20.83	19.44
VAM	21.33	20.50	22.83	21.17	23.50	28.33	22.94
Bradyrhizobium	29.00	27.17	26.33	26.00	24.67	26.50	26.61
VAM + *Brady-rhizobium*	26.33	25.50	23.00	26.50	28.17	27.33	26.14
Mean	23.83	23.04	22.75	23.38	23.96	25.75	

CD(0.01) for comparison of inoculum 1.98
Fertilizer level NS
Inoculum x fertilizer level NS

Table 13. Pot culture experiment - effect of microsymbionts and fertilizer levels on fresh weight of cowpea (g)

Treatments	No fertilizer	N+P+K	½N+P+K	¼N+P+K	N+½P+K	N+¼P+K	Mean
No inoculants	6.83	9.33	12.33	12.50	13.67	11.18	10.97
VAM	16.83	21.50	25.50	22.17	18.50	24.83	21.56
Bradyrhizobium	15.50	19.00	18.00	16.00	19.50	15.00	17.17
VAM + *Brady-rhizobium*	24.67	20.17	22.17	25.33	21.67	18.50	22.00
Mean	15.96	17.50	19.50	19.00	18.21	17.38	

CD(0.01) for comparison of inoculum 2.06
Fertilizer level NS
CD(0.05) for comparison of inoculum x fertilizer level 5.05

4.7.5 Dry weight of plant

The effect of treatments on dry weight of plant is presented in Table 14. Different inoculants and different levels of fertilizer application had significant effect on plant dry weight. But there was no significant difference in the interaction between inoculants and fertilizer application on the dry weight of plant. Dual inoculation of VAM with *Bradyrhizobium* was the superior treatment giving 8.29 g of plant dry weight. VAM inoculation alone was also on par with this treatment. Among fertilizer treatments, except the application of N+P+K as per Package of Practice Recommendations of KAU, 1993, all other treatments were statistically superior to the control. N + ½P + K was the first ranking treatment which gave 7.68 g of plant dry weight.

4.7.6 Fresh weight of roots

The results on fresh weight of roots are presented in Table 15. The effect of inoculants and fertilizer treatments were statistically significant. VAM inoculation alone was the top ranking treatment producing 2.99 g of fresh weight of root. This was followed by dual inoculation treatment which was on par with VAM alone. Fertilizer level of ¼N + P + K was found to be the best among fertilizer treatments producing 2.68 g of root fresh weight. This was followed by three other treatment levels which were statistically on par. Interaction of inoculants with fertilizer levels was not significant.

4.7.7 Dry weight of roots

Table 16 shows the dry weight of roots as influenced by different treatments. Here also, the effect of inoculum and levels of fertilizer application showed statistical significance. Among the inoculants VAM was top ranking one

Table 14. Pot culture experiment - effect of microsymbionts and fertilizer levels on dry weight of cowpea (g)

Treatments	No fertilizer	N+P+K	½N+P+K	¼N+P+K	N+½P+K	N+¼P+K	Mean
No inoculants	3.38	4.02	6.17	5.50	6.58	4.80	5.11
VAM	6.78	7.78	8.10	8.05	8.12	9.00	7.97
Bradyrhizobium	6.87	8.00	7.55	7.63	7.68	7.38	7.52
VAM + *Brady-rhizobium*	8.82	7.72	8.07	8.88	8.35	7.92	8.29
Mean	6.46	6.93	7.47	7.52	7.68	7.28	

CD(0.01) for comparison of inoculum 0.62
CD(0.05) for comparison of fertilizer level 0.77
Inoculum x fertilizer level NS

Table 15. Pot culture experiment - effect of microsymbionts and fertilizer levels on fresh weight of roots of cowpea (g)

Treatments	No fertilizer	N+P+K	½N+P+K	¼N+P+K	N+½P+K	N+¼P+K	Mean
No inoculants	1.90	2.08	2.45	2.25	2.10	2.10	2.15
VAM	2.26	3.05	3.34	3.38	2.65	3.25	2.99
Bradyrhizobium	1.60	2.22	1.83	1.95	1.89	1.59	1.85
VAM + *Brady-rhizobium*	2.53	2.81	2.74	3.15	2.58	2.49	2.72
Mean	2.07	2.54	2.59	2.68	2.30	2.36	

CD(0.01) for comparison of inoculum 0.30
CD(0.05) for comparison of fertilizer level 0.37
Inoculum x fertilizer level NS

Table 16. Pot culture experiment - effect of microsymbionts and fertilizer levels on dry weight of roots of cowpea (g)

Treatments	No fertilizer	N+P+K	½N+P+K	¼N+P+K	N+½P+K	N+¼P+K	Mean
No inoculants	1.16	1.29	1.46	1.31	1.35	1.32	1.32
VAM	1.23	1.56	1.61	1.58	1.46	1.83	1.55
Bradyrhizobium	1.36	1.79	1.52	1.53	1.58	1.42	1.53
VAM + *Brady-rhizobium*	1.34	1.48	1.55	1.70	1.39	1.52	1.50
Mean	1.27	1.53	1.54	1.53	1.44	1.52	

CD(0.01) for comparison of inoculum	0.15
CD(0.05) for comparison of fertilizer level	0.18
Inoculum x fertilizer level	NS

producing 1.55 g of root dry weight. *Bradyrhizobium* and dual inoculation with VAM and *Bradyrhizobium* were also on par with VAM treatment. Among the fertilizer levels, ½N + P + K was the best treatment giving 1.54 g of root dry weight. All other fertilizer levels except no fertilizer application were also statistically on par with this. Interaction of inoculants with fertilizer levels was not significant.

4.7.8 Nodule number

Table 17 shows the results on nodule number. It was observed that the treatment with inoculants had a significant effect on nodulation on cowpea. But the fertilizer treatment and interaction effects were not significant to influence the root nodulation. Among the inoculants, combined inoculation with VAM and *Bradyrhizobium* recorded maximum number of nodules (110.61). This was statistically superior to all other inoculants. Eventhough there was no significant difference among the interaction between inoculants and fertilizer levels, the plants treated with VAM + *Bradyrhizobium* along with N + ¼P + K recorded maximum nodule number (135.0).

4.7.9 Fresh weight of nodules

The results on fresh weight of nodules are presented in Table 18. The results showed that the treatments with inoculants and with different doses of fertilizers had a significant effect on the fresh weight of nodule. The effect of interaction between inoculants and fertilizer levels was not significant. The plants dually treated with VAM and *Bradyrhizobium* recorded maximum fresh weight of 2.04 g of nodules. The maximum fresh weight of nodules produced by fertilizer application of N + ¼P + K was 1.85 g and was on par with N+P+K as per Package of Practice Recommendations of KAU, 1993.

Table 17. Pot culture experiment - effect of microsymbionts and fertilizer levels on number of nodules in cowpea

Treatments	No fertilizer	N+P+K	½N+P+K	¼N+P+K	N+½P+K	N+¼P+K	Mean
No inoculants	8.33	16.50	38.83	46.30	36.50	34.00	30.08
VAM	54.33	59.00	61.17	43.83	58.50	62.67	56.58
Bradyrhizobium	59.83	75.00	54.33	56.33	86.00	82.33	68.97
VAM + *Brady-rhizobium*	85.33	120.67	88.50	113.00	121.17	135.00	110.61
Mean	51.96	67.79	60.71	64.87	75.54	78.50	

CD(0.01) for comparison of inoculum	16.93
Fertilizer level	NS
Inoculum x fertilizer level	NS

Table 18. Pot culture experiment - effect of microsymbionts and fertilizer levels on fresh weight of nodules of cowpea (g)

Treatments	No fertilizer	N+P+K	½N+P+K	¼N+P+K	N+½P+K	N+¼P+K	Mean
No inoculants	1.19	1.90	1.00	0.95	1.01	1.06	1.18
VAM	1.56	1.60	1.46	1.71	1.46	2.41	1.70
Bradyrhizobium	1.35	1.36	1.31	1.19	1.42	1.53	1.36
VAM + *Brady-rhizobium*	1.93	2.36	1.72	2.19	1.63	2.38	2.04
Mean	1.51	1.80	1.37	1.51	1.38	1.85	

CD(0.01) for comparison of inoculum	0.27
CD(0.01) for comparison of fertilizer level	0.33
Inoculum x fertilizer level	NS

4.7.10 Dry weight of nodules

The results on the dry weight of nodules are presented in Table 19. Inoculation with the microsymbionts had a significant effect on nodule dry weight. Among them treatment with *Bradyrhizobium* alone recorded the highest nodule dry weight of 1.03 g. This was also on par with the dual inoculation of VAM and *Bradyrhizobium*. The fertilizer treatment and interaction effects had no significant influence on nodule dry weight.

4.7.11 VAM colonisation

The effects of different treatments on percentage of VAM colonisation are presented in Table 20. VAM colonisation was maximum when it was inoculated along with *Bradyrhizobium* (76.38%) and was on par with VAM inoculation alone (74.84%). Among fertilizer treatments it was found that plants which received no fertilizers recorded maximum VAM colonisation (44.08%). Here also two other doses of fertilizers (N + ½P + K and N + ¼P + K) were statistically on par with the above treatments to increase the VAM colonisation. Among the interactions, VAM alone without any fertilizers recorded maximum colonisation (87.96%) and was on par with dual inoculation without fertilizers and dual inoculation with N + ¼P + K.

4.7.12 VAM spore count

The spore count of VAM fungus 100 g^{-1} soil under different treatments are presented in Table 21. It was observed that the effect of inoculants and the interaction of inoculants with fertilizers had significant effects on the spore count. The spore count was maximum when VAM was inoculated alone (134.89). This was statistically on par with the dual inoculation of VAM and *Bradyrhizobium*

Table 19. Pot culture experiment - effect of microsymbionts and fertilizer levels on dry weight of nodules of cowpea (g)

Treatments	No fertilizer	N+P+K	½N+P+K	¼N+P+K	N+½P+K	N+¼P+K	Mean
No inoculants	0.81	0.94	0.83	0.71	0.81	0.86	0.83
VAM	0.88	0.85	0.83	0.86	0.85	1.05	0.89
Bradyrhizobium	0.94	1.02	1.08	1.01	1.12	0.98	1.03
VAM + *Brady-rhizobium*	0.89	1.03	0.92	1.01	0.87	1.24	0.99
Mean	0.88	0.96	0.92	0.90	0.92	1.03	

CD(0.01) for comparison of inoculum 0.10
Fertilizer level NS
Inoculum x fertilizer level NS

Table 20. Pot culture experiment - effect of microsymbionts and fertilizer levels on VAM colonisation in cowpea (per cent)

Treatments	No fertilizer	N+P+K	½N+P+K	¼N+P+K	N+½P+K	N+¼P+K	Mean
No inoculants	5.55	10.23	1.99	3.87	10.45	7.01	6.52
VAM	87.96	69.44	67.27	68.52	78.39	77.46	74.84
Bradyrhizobium	0.22	8.02	1.61	2.61	4.70	3.77	3.49
VAM + *Brady-rhizobium*	82.60	76.21	61.71	75.31	80.39	82.07	76.38
Mean	44.08	40.98	33.15	37.58	43.48	42.58	

CD(0.01) for comparison of inoculum 3.61
CD(0.01) for comparison of fertilizer level 2.95
CD(0.01) for comparison of inoculum x fertilizer level 7.22

Table 21. Pot culture experiment - effect of microsymbionts and fertilizer levels on VAM spore count in rhizosphere soil (per 100 g)

Treatments	No fertilizer	N+P+K	½N+P+K	¼N+P+K	N+½P+K	N+¼P+K	Mean
Control	11.0	33.00	0.00	22.00	33.00	33.00	22.00
VAM	133.00	100.00	133.00	122.00	144.00	177.33	134.89
Bradyrhizobium	22.00	44.00	44.00	33.00	33.00	33.00	34.83
VAM + *Brady-rhizobium*	144.00	144.00	155.00	111.00	99.67	155.00	134.78
Mean	77.50	80.25	83.00	72.00	77.42	99.58	

CD(0.01) for comparison of inoculum	14.54
Fertilizer level	NS
CD(0.05) for comparison of Inoculum x fertilizer level	35.58

(134.78). However, VAM inoculation along with the application of N + ¼P + K resulted in maximum spore count (177.33).

4.8 Nutrient content in plant and soil

4.8.1 Nitrogen content in plant

Nitrogen content in plant under different treatments are presented in the Table 22. It was observed that the treatment with inoculants alone had a significant effect on the nitrogen content of plants. The maximum nitrogen content was recorded in plants treated with *Bradyrhizobium* (3.43%) and was on par with VAM + *Bradyrhizobium*. In control plants nitrogen content was only 2.83 per cent. Fertilizer levels and interaction effects were not significant. However, the interaction between dual microbial inoculation and ¼N + P + K resulted in increasing the nitrogen content of the plant to 4.03 per cent.

4.8.2 Phosphorus content in plant

The effect of inoculants, different levels of fertilizers and their interactions were statistically significant in influencing the phosphorus content of plant (Table 23). VAM inoculation increased the plant phosphorus content to a maximum level of 0.36 per cent. This treatment was on par with dual inoculation with VAM and *Bradyrhizobium*. Among fertilizer levels, ¼N + P + K was statistically superior (0.37%) in producing maximum phosphorus content than treatments except ½N+P+K and N+¼P+K. Interaction effect was also significant. Interaction of N + ½P + K alone and VAM with ½N + P + K were the better treatments.

Table 22. Pot culture experiment - effect of microsymbionts and fertilizer levels on nitrogen content of cowpea (per cent)

Treatments	No fertilizer	N+P+K	½N+P+K	¼N+P+K	N+½P+K	N+¼P+K	Mean
No inoculants	2.38	2.50	2.99	2.80	3.41	2.89	2.83
VAM	2.33	2.92	2.33	2.50	2.62	2.68	2.57
Bradyrhizobium	3.06	3.69	3.41	3.48	3.24	3.73	3.43
VAM + *Brady-rhizobium*	2.71	3.76	3.48	4.03	3.13	2.85	3.32
Mean	2.62	3.21	3.05	3.20	3.12	3.04	

CD(0.01) for comparison of inoculum 0.34
Fertilizer level NS
Inoculum x fertilizer level NS

Table 23. Pot culture experiment - effect of microsymbionts and fertilizer levels on phosphorus content of cowpea (per cent)

Treatments	No fertilizer	N+P+K	½N+P+K	¼N+P+K	N+½P+K	N+¼P+K	Mean
No inoculants	0.23	0.29	0.34	0.38	0.40	0.27	0.32
VAM	0.39	0.34	0.40	0.39	0.26	0.36	0.36
Bradyrhizobium	0.25	0.29	0.29	0.33	0.28	0.35	0.30
VAM + *Brady-rhizobium*	0.34	0.33	0.33	0.37	0.28	0.33	0.33
Mean	0.30	0.31	0.34	0.37	0.31	0.33	

CD(0.01) for comparison of inoculum 0.03
CD(0.01) for comparison of fertilizer level 0.04
CD(0.01) for comparison of inoculum x fertilizer level 0.07

4.8.3 Potassium content in plant

All the treatments and their interactions had significant effects on potassium content in plant (Table 24). Potassium content was maximum (2.48%) in untreated plants and was minimum (2%) in plants dually inoculated with VAM and *Bradyrhizobium*. Plants treated with ½N + P + K recorded significantly the maximum potassium content of 2.5 per cent. Among interactions, it was seen that N + ¼P + K without inoculants was the best recording 2.82 per cent potassium. While the minimum value was in the combination of VAM along with N+¼P+K.

4.8.4 Magnesium content in plant

The effects of all treatments and their interactions were significant in influencing the magnesium content of the plants (Table 25). Among inoculants, the treatment with *Bradyrhizobium* alone recorded the maximum magnesium content (0.409%). It was on par with the dual inoculation. Plants which received no fertilizers recorded the maximum of 0.407 per cent magnesium. Among interactions, dual inoculation along with N + ½P + K fertilizer dose recorded the maximum magnesium content of 0.423 per cent.

4.8.5 Calcium content in plant

The calcium content of plants under different treatments are presented in Table 26. Only the inoculants and interactions between inoculants and fertilizer levels showed statistical significance. Among inoculants, dual inoculation with VAM and *Bradyrhizobium* recorded the maximum calcium content of 0.92 per cent and was on par with VAM inoculation alone. Among the various interactions, both the inoculants along with N + ½P + K recorded the maximum calcium content (1.24%).

Table 24. Pot culture experiment - effect of microsymbionts and fertilizer levels on potassium content of cowpea (per cent)

Treatments	No fertilizer	N+P+K	½N+P+K	¼N+P+K	N+½P+K	N+¼P+K	Mean
No inoculants	2.28	2.40	2.62	2.42	2.32	2.82	2.48
VAM	2.15	2.65	2.68	2.40	2.20	1.73	2.30
Bradyrhizobium	1.92	2.77	2.50	2.43	2.33	2.52	2.41
VAM + *Brady-rhizobium*	1.93	2.03	2.20	1.97	1.78	2.10	2.00
Mean	2.07	2.46	2.50	2.30	2.16	2.29	

CD(0.01) for comparison of inoculum 0.18
CD(0.01) for comparison of fertilizer level 0.22
CD(0.05) for comparison of inoculum x fertilizer level 0.43

Table 25. Pot culture experiment - effect of microsymbionts and fertilizer levels on magnesium content of cowpea (per cent)

Treatments	No fertilizer	N+P+K	½N+P+K	¼N+P+K	N+½P+K	N+¼P+K	Mean
No inoculants	0.393	0.393	0.379	0.393	0.364	0.393	0.386
VAM	0.397	0.396	0.397	0.403	0.385	0.396	0.395
Bradyrhizobium	0.416	0.392	0.399	0.415	0.416	0.418	0.409
VAM + *Brady-rhizobium*	0.422	0.420	0.394	0.370	0.423	0.412	0.407
Mean	0.407	0.400	0.392	0.395	0.397	0.405	

CD(0.01) for comparison of inoculum 0.01
CD(0.01) for comparison of fertilizer level 0.01
CD(0.05) for comparison of inoculum x fertilizer level 0.02

Table 26. Pot culture experiment - effect of microsymbionts and fertilizer levels on calcium content of cowpea (per cent)

Treatments	No fertilizer	N+P+K	½N+P+K	¼N+P+K	N+½P+K	N+¼P+K	Mean
No inoculants	0.81	0.72	0.74	0.91	0.52	0.53	0.70
VAM	0.76	0.88	0.63	0.86	0.82	0.82	0.80
Bradyrhizobium	0.73	0.78	0.56	0.59	0.53	1.10	0.71
VAM + *Brady-rhizobium*	0.10	0.70	0.73	0.98	1.24	0.89	0.92
Mean	0.82	0.77	0.67	0.83	0.78	0.83	

CD(0.01) for comparison of inoculum 0.14
Fertilizer level NS
CD(0.01) for comparison of inoculum x fertilizer level 0.28

4.8.9 Iron content in plant

The effects of different treatments in the iron content of plants are presented in Table 27. The results revealed that treatments with inoculants had no significant influence on the iron content of plants. Whereas, the application of different levels of fertilizers and interaction of inoculants and fertilizers had significant effects on the iron content. Eventhough non significant, *Bradyrhizobium* inoculation alone recorded the maximum iron content in plants (612.74 ppm). Fertilizer dose of ¼N + P + K recorded the maximum iron content of 799.32 ppm. This was significantly higher than other fertilizer treatments. Among interactions, ¼N + P + K without inoculants recorded the maximum iron content (864.79 ppm).

4.8.10 Zinc content in plant

Table 28 shows the results on zinc content of plants. Only the treatments involving inoculation with microsymbionts alone and interaction between microsymbionts and fertilizer doses had significant effect on zinc content in plants. Among the inoculants, VAM alone recorded the maximum zinc content of 26.77 ppm in plants. VAM along with *Bradyrhizobium* was also on par with this. In the interactions, both the inoculants along with N + P + K as per Package of Practice Recommendations of KAU, 1993 recorded the maximum zinc content of 37.04 ppm in plants.

4.8.11 Manganese content in plant

The different levels of both the treatments and their interactions were significant in influencing the manganese content of cowpea plants (Table 29). The treatments with *Bradyrhizobium* alone showed significantly higher manganese content of 278.02 ppm. Dual inoculation was also statistically on par with this.

Table 27. Pot culture experiment - effect of microsymbionts and fertilizer levels on iron content of cowpea (ppm)

Treatments	No fertilizer	N+P+K	½N+P+K	¼N+P+K	N+½P+K	N+¼P+K	Mean
No inoculants	475.83	796.67	471.04	864.79	387.92	661.67	609.65
VAM	612.92	469.38	609.38	692.08	439.17	352.50	529.24
Bradyrhizobium	807.50	477.92	611.25	837.08	402.50	540.21	612.74
VAM + *Brady-rhizobium*	539.58	491.88	493.96	803.33	817.50	416.46	593.79
Mean	608.96	558.96	546.41	799.32	511.77	492.71	

Inoculum	NS
CD(0.01) for comparison of fertilizer level	95.99
CD(0.01) for comparison of inoculum x fertilizer level	235.14

Table 28. Pot culture experiment - effect of microsymbionts and fertilizer levels on zinc content of cowpea (ppm)

Treatments	No fertilizer	N+P+K	½N+P+K	¼N+P+K	N+½P+K	N+¼P+K	Mean
No inoculants	20.83	15.00	21.88	27.29	9.58	26.25	20.14
VAM	31.25	33.13	22.29	27.08	28.96	17.92	26.77
Bradyrhizobium	14.38	12.50	17.50	21.08	27.10	20.56	18.85
VAM + *Brady-rhizobium*	24.00	37.04	26.88	20.00	26.63	23.17	26.29
Mean	22.62	24.42	22.14	23.87	23.07	21.97	

CD(0.01) for comparison of inoculum	6.60
Fertilizer level	NS
CD(0.05) for comparison of inoculum x fertilizer level	13.19

Table 29. Pot culture experiment - effect of microsymbionts and fertilizer levels on manganese content of cowpea (ppm)

Treatments	No fertilizer	N+P+K	½N+P+K	¼N+P+K	N+½P+K	N+¼P+K	Mean
No inoculants	286.67	231.67	216.67	263.54	143.33	251.25	232.18
VAM	259.58	189.38	241.25	226.25	242.29	200.00	226.46
Bradyrhizobium	340.00	282.92	237.29	328.13	217.92	261.88	278.02
VAM + *Brady-rhizobium*	213.75	227.29	252.29	305.83	299.38	204.79	250.56
Mean	275.00	232.81	236.88	280.94	225.73	229.48	

CD(0.01) for comparison of inoculum 35.30
CD(0.01) for comparison of fertilizer level 28.82
CD(0.01) for comparison of inoculum x fertilizer level 70.60

Among fertilizer applications, ¼N + P + K recorded the highest manganese content of 280.94 ppm and this was on par with no fertilizer application. Among various interactions, *Bradyrhizobium* inoculation without any fertilizer application resulted in the maximum manganese content in plant (340.00 ppm).

4.8.12 Copper content in plant

The results on copper content in plants are presented in Table 30. The various inoculants and fertilizer levels were not significant in influencing the copper content of plants. But, their interactions were significant. Eventhough not significant, VAM inoculation alone gave the maximum copper content in plants (3.06 ppm). Similarly, among fertilizer levels, plants which received no fertilizers recorded the maximum copper content (3.39 ppm). Among interactions, the copper content was maximum (5.42 ppm) in VAM inoculated plants without any fertilizers and was on par with eight other treatment combinations.

4.8.13 Nitrogen content in soil

Table 31 shows that all treatments involving inoculants, different levels of fertilizers and their interactions had a significant effect on the nitrogen content of soil. Among the inoculants, VAM inoculation was significantly superior to other treatments. Among different fertilizer levels, N + P + K applied as per Package of Practice Recommendations of KAU, 1993 and also ½N + P + K were similar in contributing to the soil nitrogen content (250 ppm). Among interactions, VAM and ½N + P + K was found to be the best combination giving 370 ppm of soil nitrogen content.

Table 30. Pot culture experiment - effect of microsymbionts and fertilizer levels on copper content of cowpea (ppm)

Treatments	No fertilizer	N+P+K	½N+P+K	¼N+P+K	N+½P+K	N+¼P+K	Mean
No inoculants	1.46	1.88	2.08	2.92	2.08	5.00	2.57
VAM	5.42	1.88	2.08	3.54	3.75	1.67	3.06
Bradyrhizobium	3.33	2.29	1.46	4.79	1.04	2.71	2.60
VAM + *Brady-rhizobium*	3.33	4.38	1.88	1.46	2.56	1.04	2.43
Mean	3.39	2.60	1.88	3.18	2.34	2.60	

Inoculum	NS
Fertilizer level	NS
CD(0.05) for comparison of inoculum x fertilizer level	2.65

Table 31. Pot culture experiment - effect of microsymbionts and fertilizer levels on available nitrogen content in soil (ppm)

Treatments	No fertilizer	N+P+K	½N+P+K	¼N+P+K	N+½P+K	N+¼P+K	Mean
No inoculants	80	230	240	150	160	130	170
VAM	210	330	370	280	220	240	270
Bradyrhizobium	210	200	210	280	200	200	220
VAM + *Brady-rhizobium*	240	250	190	220	110	270	210
Mean	190	250	250	240	170	210	

CD(0.01) for comparison of inoculum	30
CD(0.01) for comparison of fertilizer level	40
CD(0.01) for comparison of inoculum x fertilizer level	80

4.8.14 Phosphorus content in soil

The results on the phosphorus content in soil are presented in Table 32. Effects of inoculants, different fertilizer levels and their interactions had no significant effect on the phosphorus content of soil. The phosphorus content in soil was found to be the highest in plants inoculated with VAM alone (110 ppm). Interactions showed the maximum phosphorus content of 140 ppm in treatments VAM + ½N + P + K and *Bradyrhizobium* alone with no fertilizer application.

4.8.15 Potassium content in soil

The results on potassium content in soil are presented in Table 33. The data revealed that the effect of inoculants, different fertilizer levels and their interactions were significant. Among the inoculants, plants which were not inoculated with microsymbionts recorded the maximum content of potassium (80 ppm). Among different fertilizer levels, treatment with ½N + P + K recorded the maximum content of potassium (79 ppm). Interaction effects recorded maximum values for the treatments ¼N+P+K, N+½P+K, N+¼P+K, all without inoculants.

4.9 Field experiment on the efficiency of the microsymbionts on the uptake of nutrients under different levels of nitrogen and phosphorus

The data on the above experiment are presented in Table 34 to 41.

Observations were recorded at 50 per cent flowering and at the time of harvest.

4.9.1 Number of leaves

Table 34 shows that there were no significant differences among treatments in the number of leaves at the time of harvest, but significant differences

Table 32. Pot culture experiment - effect of microsymbionts and fertilizer levels on available phosphorus content in soil (ppm)

Treatments	No fertilizer	N+P+K	½N+P+K	¼N+P+K	N+½P+K	N+¼P+K	Mean
No inoculants	110	110	100	100	100	90	100
VAM	110	90	140	120	90	120	110
Bradyrhizobium	140	80	130	60	100	110	100
VAM + *Bradyrhizobium*	90	90	70	100	100	80	90
Mean	110	90	110	90	100	100	

Inoculum	NS
Fertilizer level	NS
Inoculum x fertilizer level	NS

Table 33. Pot culture experiment - effect of microsymbionts and fertilizer levels on available potassium content in soil (ppm)

Treatments	No fertilizer	N+P+K	½N+P+K	¼N+P+K	N+½P+K	N+¼P+K	Mean
No inoculants	85	43	86	88	88	88	80
VAM	72	53	83	72	64	67	68
Bradyrhizobium	49	75	73	69	63	82	69
VAM + *Bradyrhizobium*	75	64	75	65	56	58	65
Mean	70	59	79	74	68	74	

CD(0.05) for comparison of inoculum	9
CD(0.05) for comparison of fertilizer level	12
CD(0.05) for comparison of inoculum x fertilizer level	24

were observed at the time of 50 per cent flowering. The maximum mean number of leaves, (12.25) was recorded in plants treated with fertilizers as per Package of Practice Recommendations of KAU, 1993 at 50 per cent flowering. But at the time of harvest, *Bradyrhizobium* along with ½N + P + K showed the maximum number of leaves (12.58). The treatment, VAM+N+½P+K ranked second in number of leaves both at 50 per cent flowering and at harvest. Number of leaves were the lowest in control plants at 50 per cent flowering. At harvest the leaves were minimum in dually inoculated plants.

4.9.2 Height of plant

There were significant differences among treatments on the height of plants at 50 per cent flowering and also at harvest (Table 34). The maximum mean height of 34.83 cm was recorded in plants treated with both the inoculants along with a fertilizer dose of ½N + ½P + K at 50 per cent flowering. Three other treatments were also statistically on par with this treatment. At the time of harvest, the maximum plant height was recorded by *Bradyrhizobium* inoculation with ¼N + P + K (48.50 cm).

4.9.3 Length of root

The results on the length of roots are presented in Table 34. The results revealed that there were significant differences among treatments in the length of roots at 50 per cent flowering, but not at harvest. Treatment with N + P + K as per the Package of Practice Recommendations of KAU, 1993 recorded the maximum length of root (16.67 cm) at 50 per cent flowering which was on par with nine other treatments. At the time of harvest, dual inoculation along with ¼N + ¼P + K showed the maximum length of root (31.67 cm).

Table 34. Field experiment - effect of microsymbionts and fertilizer levels on number of leaves, plant height and root length of cowpea

Treatments		No. of leaves		Plant height (cm)		Root length (cm)	
		at 50% flowering	at harvest	at 50% flowering	at harvest	at 50% flowering	at harvest
T_1	Control	6.50^{c}	9.92^{a}	21.50^{c}	34.43^{b}	11.87^{b}	25.92^{a}
T_2	N+P+K	12.25^{a}	10.75^{a}	32.58^{ab}	40.67^{ab}	16.67^{a}	28.25^{a}
T_3	VAM	9.50^{abc}	9.58^{a}	22.50^{c}	39.17^{ab}	12.67^{ab}	26.67^{a}
T_4	VAM+N+½P+K	12.08^{ab}	12.00^{a}	29.17^{abc}	42.17^{ab}	16.42^{a}	25.92^{s}
T_5	VAM+ N+¼P+K	9.34^{abc}	10.08^{a}	24.92^{bc}	30.75^{b}	14.67^{ab}	26.67^{a}
T_6	*Bradyrhizobium*	6.67^{c}	11.25^{a}	24.92^{c}	37.50^{ab}	13.08^{ab}	26.17^{a}
T_7	B+½N+P+K	10.33^{abc}	12.58^{a}	26.67^{bc}	37.58^{ab}	15.92^{a}	27.00^{a}
T_8	B+¼N+P+K	8.77^{abc}	10.17^{a}	24.58^{c}	48.50^{a}	14.67^{ab}	27.28^{a}
T_9	VAM+B	7.75^{bc}	8.58^{a}	23.67^{c}	34.25^{b}	12.58^{ab}	25.50^{a}
T_{10}	VAM+B+½N+½P+K	7.92^{abc}	11.25^{a}	34.83^{a}	42.58^{ab}	15.50^{ab}	28.25^{a}
T_{11}	VAM+B+¼N+¼P+K	9.75^{abc}	10.58^{a}	28.56^{abc}	41.67^{ab}	15.08^{ab}	31.67^{a}

Mean values with different superscripts differ significantly at 5% level

4.9.4 Fresh and dry weights of plant

In the fresh weight of plant, there were significant differences among the treatments at 50 per cent flowering as well as at the time of harvest (Table 35). The maximum fresh weight of 31.67 g was recorded by dual inoculation along with ½N + ½P + K at the time of 50 per cent flowering. This was on par with seven other treatments. At the time of harvest also, the maximum mean fresh weight (66.17 g) was recorded by the same treatment. This was also statistically on par with inoculation of *Bradyrhizobium* along with a fertilizer dose of ¼N + P + K.

There were significant differences among treatments in the dry weight of plants at 50 per cent flowering and at the time of harvest. At 50 per cent flowering VAM along with a fertilizer dose of N + ½P + K recorded the maximum mean dry weight of 9.47 g. It was significantly higher than the control, inoculation with *Bradyrhizobium* alone and with dual inoculation. But at the time of harvest, dual inoculation with ½N + ½P + K recorded the maximum mean dry weight of plant (18.21 g). It was statistically on par with the treatment, *Bradyrhizobium* with ¼N + P + K.

4.9.5 Fresh and dry weights of root

Table 36 shows the data on the fresh and dry weights of roots. There were significant differences among the treatments in the fresh and dry weights of root at 50 per cent flowering and at harvest. VAM + N + ½P + K recorded maximum root fresh weight of roots at 50 per cent flowering (1.80 g). The maximum mean fresh weight of root (7.19 g) at the time of harvest was recorded by the plants treated with *Bradyrhizobium* alone. This was on par with the treatments VAM + N + ½P + K and VAM + *Bradyrhizobium* + ¼N + ¼P + K and was significantly superior to the control and other treatments.

Table 35 Field experiment - effect of microsymbionts and fertilizer levels on fresh and dry weights of cowpea

Treatments		Fresh weight of plant (g)		Dry weight of plant (g)	
		at 50% flowering	at harvest	at 50% flowering	at harvest
T_1	Control	14.75^{c}	30.25^{d}	3.95^{c}	9.41^{c}
T_2	N+P+K	30.08^{a}	39.33^{cd}	9.13^{a}	10.57^{c}
T_3	VAM	28.83^{ab}	38.42^{cd}	7.76^{ab}	11.35^{bc}
T_4	VAM+N+½P+K	31.42^{a}	35.75^{cd}	9.47^{a}	9.48^{c}
T_5	VAM+ N+¼P+K	24.25^{abc}	35.00^{cd}	6.71^{abc}	9.86^{c}
T_6	*Bradyrhizobium*	18.00^{bc}	43.42^{bcd}	5.63^{bc}	12.78^{bc}
T_7	B+½N+P+K	30.25^{a}	47.33^{bc}	8.32^{ab}	13.28^{bc}
T_8	B+¼N+P+K	20.00^{abc}	55.08^{ab}	6.20^{abc}	14.78^{ab}
T_9	VAM+B	16.58^{c}	43.83^{bcd}	5.48^{bc}	12.59^{bc}
T_{10}	VAM+B+½N+½P+K	31.67^{a}	66.17^{a}	9.09^{a}	18.21^{a}
T_{11}	VAM+B+¼N+¼P+K	22.42^{abc}	45.25^{bc}	7.42^{ab}	13.52^{bc}

Mean values with different superscripts differ significantly at 5% level

Table 36. Field experiment - effect of microsymbionts and fertilizer levels on fresh and dry weights of roots of cowpea

Treatments		Fresh weight of root (g)		Dry weight of root (g)	
		at 50% flowering	at harvest	at 50% flowering	at harvest
T_1	Control	0.76^{b}	3.00^{d}	0.34^{b}	1.30^{e}
T_2	N+P+K	1.46^{ab}	5.10^{c}	0.73^{ab}	2.44^{bcd}
T_3	VAM	1.03^{ab}	4.96^{c}	0.49^{ab}	1.73^{de}
T_4	VAM+N+½P+K	1.80^{a}	6.90^{ab}	0.93^{a}	3.65^{a}
T_5	VAM+ N+¼P+K	1.37^{ab}	4.55^{c}	0.71^{ab}	1.80^{de}
T_6	*Bradyrhizobium*	0.83^{b}	7.19^{a}	0.46^{ab}	3.16^{ab}
T_7	B+½N+P+K	1.28^{ab}	4.88^{c}	0.67^{ab}	2.25^{bcde}
T_8	B+¼N+P+K	1.07^{ab}	4.41^{c}	0.58^{ab}	2.05^{cde}
T_9	VAM+B	0.85^{b}	5.28^{c}	0.54^{ab}	2.20^{bcde}
T_{10}	VAM+B+½N+½P+K	1.37^{ab}	5.60^{bc}	0.63^{ab}	2.92^{abc}
T_{11}	VAM+B+¼N+¼P+K	0.96^{b}	6.82^{ab}	0.67^{ab}	2.98^{abc}

Mean values with different superscripts differ significantly at 5% level

At 50 per cent flowering, significantly maximum dry weight of roots (0.93 g) was recorded by plants treated with VAM + N + ½P + K. The maximum mean dry weight of roots (3.65 g) at harvest was recorded by the treatment VAM + N + ½P + K. This was also on par with three other treatments.

4.9.6 Nodule number

There were significant differences among the treatments in the number of nodules at 50 per cent flowering and also at harvest (Table 37). The maximum number of nodules (17.58) was recorded by the dual inoculation with microsymbionts along with a fertilizer dose of ½N + ½P + K, which was significantly higher than the control. Four other treatments viz. VAM + N + ½P + K, *Bradyrhizobium* + ¼N + P + K, VAM + *Bradyrhizobium* + ¼N + ¼P + K and VAM + *Bradyrhizobium* were also on par with the above treatments in increasing the nodule number of plant. At harvest, the maximum mean number of nodules (29.67) was recorded by the plants treated with VAM + *Bradyrhizobium* along with a fertilizer dose of ½N + ½P + K and this was significantly higher than all other treatments.

4.9.7 Fresh and dry weights of nodules

Table 37 shows significant differences between treatments both at 50 per cent flowering and at harvest on the fresh weight of nodules. The maximum mean fresh weight of nodules at 50 per cent flowering (395 mg) and at harvest (700 mg) was recorded by the plants treated with VAM + *Bradyhizobium* and the application of ½N + ½P + K. This was significantly superior to all other treatments. At 50 per cent flowering the maximum mean dry weight of nodules (202 mg) was recorded by the treatment of dual inoculation along with ¼N + ¼P + K fertilizer. This was significantly superior to other treatments except two. The maximum mean dry

Table 37. Field experiment - effect of microsymbionts and fertilizer levels on nodule number, fresh and dry weights of nodules of cowpea

Treatments		Nodule number		Fresh weight of nodules (mg)		Dry weight of nodules (mg)	
		at 50% flowering	at harvest	at 50% flowering	at harvest	at 50% flowering	at harvest
T_1	Control	2.00^{g}	4.17^{e}	284^{f}	274^{b}	111^{d}	147^{c}
T_2	N+P+K	4.25^{fg}	8.92^{cde}	292^{ef}	362^{b}	113^{d}	214^{bc}
T_3	VAM	6.50^{efg}	13.08^{c}	299^{def}	440^{b}	143^{bcd}	248^{ab}
T_4	VAM+N+½P+K	16.08^{ab}	10.92^{cd}	304^{cde}	418^{b}	118^{cd}	213^{bc}
T_5	VAM+ N+¼P+K	7.25^{def}	5.83^{de}	289^{ef}	388^{b}	112^{d}	225^{b}
T_6	*Bradyrhizobium*	12.17^{bcd}	10.58^{cd}	302^{cdef}	348^{b}	112^{d}	208^{bc}
T_7	B+½N+P+K	10.42^{cde}	9.92^{cd}	295^{ef}	353^{b}	119^{cd}	192^{bc}
T_8	B+¼N+P+K	14.67^{abc}	9.92^{cd}	320^{bc}	420^{b}	101^{d}	212^{bc}
T_9	VAM+B	13.25^{abc}	18.75^{b}	315^{cd}	413^{b}	174^{abc}	225^{bc}
T_{10}	VAM+B+½N+½P+K	17.58^{a}	29.67^{a}	395^{a}	700^{a}	184^{ab}	318^{a}
T_{11}	VAM+B+¼N+¼P+K	14.08^{abc}	21.58^{b}	335^{b}	423^{b}	202^{a}	251^{ab}

Mean values with different superscripts differ significantly at 5% level

weight of nodules (318 mg) at the time of harvest was recorded by the treatment VAM + *Bradyrhizobium* + ½N + ½P + K and was on par with the treatments, VAM + *Bradyrhizobium* + ¼N + ¼P + K and VAM alone.

4.9.8 VAM colonisation

The statistical analysis on percentage of VAM colonisation recorded significant difference among treatments (Table 38). The maximum VAM colonisation of 76.96 per cent was recorded by the plants treated with VAM alone. This was statistically on par with five other treatments involving VAM inoculation

4.9.9 VAM spore count

The results are presented in Table 38. There were significant differences among the treatments in the VAM spore count. The plants treated with VAM + N + ¼P + K recorded the maximum spore count of 688.67. This was on par with the dual inoculation of VAM and *Bradyrhizobium* along with ¼N + ¼P + K. The control recorded the minimum spore count (110.67).

4.9.10 Yield

There were significant differences among the treatments in the yield of cowpea plants (Table 38). The maximum yield (550.33 g) was obtained from plants treated with *Bradyrhizobium* along with a fertilizer dose of ½N + P + K. This was significantly higher than the control and the treatments, VAM + *Bradyrhizobium* and *Bradyrhizobium* alone.

Table 38. Field experiment - effect of microsymbionts and fertilizer levels on percentage of colonisation, spore count of VAM and yield of cowpea

Treatments		VAM infection (%)	Spore count of VAM/100 g soil	Yield (g/plot)
T_1	Control	6.17^{c}	110.67^{e}	108.33^{d}
T_2	N+P+K	21.91^{bc}	166.33^{e}	382.67^{abc}
T_3	VAM	76.96^{a}	466.33^{cd}	434.00^{abc}
T_4	VAM+N+½P+K	66.97^{a}	366.33^{d}	491.67^{ab}
T_5	VAM+ N+¼P+K	68.82^{a}	688.67^{a}	371.67^{abc}
T_6	*Bradyrhizobium*	27.46^{bc}	121.67^{e}	271.67^{cd}
T_7	B+½N+P+K	27.46^{bc}	166.33^{e}	550.33^{a}
T_8	B+¼N+P+K	29.62^{b}	155.33^{e}	404.33^{abc}
T_9	VAM+B	59.27^{a}	421.67^{cd}	317.67^{bc}
T_{10}	VAM+B+½N+½P+K	70.05^{a}	522.07^{bc}	419.67^{abc}
T_{11}	VAM+B+¼N+¼P+K	70.06^{a}	611.00^{ab}	398.67^{abc}

Mean values with different superscripts differ significantly at 5% level

4.10 Nutrient content in plant and soil

4.10.1 Nitrogen content in plant

The data on the nitrogen content of plant is presented in Table 39. There were significant differences among treatments on the nitrogen content of plant. The maximum nitrogen content (5.18%) was recorded by the treatment *Bradyrhizobium* with ¼N + P + K. This was significantly superior to all other treatments except the treatment with fertilizer dose as per Package of Practice Recommendations of KAU, 1993.

4.10.2 Phosphorus content in plant

Analysis on the phosphorus content in plant showed significant difference among the treatments (Table 39). The maximum phosphorus content (0.46%) was showed by plants treated with *Bradyrhizobium* along with ¼N + P + K. This was significantly higher than the control. But this was on par with five other treatments also.

4.10.3 Potassium content in plant

Table 39 shows that there were significant differences among treatments on potassium content of plants. Treatment with dual inoculation of VAM and *Bradyrhizobium* along with a fertilizer dose of ½N + ½P + K recorded the maximum potassium content of 3.52 per cent. This was significantly higher than the control. Six other treatments were also on par with the above treatment.

4.10.4 Calcium content in plant

There were significant differences among the treatments in calcium content in plants. Plants inoculated with VAM alone recorded the maximum mean

Table 39. Field experiment - effect of microsymbionts and fertilizer levels on nitrogen, phosphorus and potassium content of cowpea (per cent)

Treatments		Nitrogen	Phosphorus	Potassium
T_1	Control	3.17[cd]	0.26[c]	2.92[b]
T_2	N+P+K	4.67[ab]	0.38[ab]	3.12[ab]
T_3	VAM	3.57[cd]	0.41[a]	3.13[ab]
T_4	VAM+N+½P+K	3.13[cd]	0.23[c]	2.83[b]
T_5	VAM+ N+¼P+K	3.55[cd]	0.26[c]	3.48[a]
T_6	*Bradyrhizobium*	3.90[bc]	0.40[a]	3.08[ab]
T_7	B+½N+P+K	2.78[d]	0.29[bc]	1.63[c]
T_8	B+¼N+P+K	5.18[a]	0.46[a]	3.00[ab]
T_9	VAM+B	3.48[cd]	0.37[ab]	3.13[ab]
T_{10}	VAM+B+½N+½P+K	3.91[bc]	0.45[a]	3.52[a]
T_{11}	VAM+B+¼N+¼P+K	4.15[bc]	0.29[bc]	3.08[ab]

Mean values with different superscripts differ significantly at 5% level

calcium content of 0.59 per cent (Table 40). This was significantly superior to all other treatments except VAM + N + ½P + K.

4.10.5 Magnesium content in plant

There were significant differences among the various treatments (Table 40) in magnesium content of the plant. The maximum magnesium content of 0.42 per cent was recorded by the plants in treatments dually inoculated with VAM and *Bradyrhizobium* along with a fertilizer dose of ½N + ½P + K and ¼N + ¼P + K. All other treatments except VAM alone, *Bradyrhizobium* alone and *Bradyrhizobium* + ½N + P + K were on par with the above treatment.

4.10.6 Copper content in plant

Copper content of the plants was not significantly affected by the various treatments (Table 40). However, the maximum copper content of 25 ppm was recorded by the plants dually inoculated with VAM and *Bradyrhizobium*.

4.10.7 Iron content in plant

Different treatments showed significant differences in the iron content of plants (Table 40). The maximum iron content of 0.15 per cent was showed by the plants treated with *Bradyrhizobium* alone. Five other treatments were also on par with this. In control plants also iron content was 0.14 per cent.

4.10.8 Zinc content in plant

Table 40 revealed significant differences among the treatments in zinc content of cowpea plants. Plants treated with VAM and *Bradyrhizobium* along with

Table 40. Field experiment - effect of microsymbionts and fertilizer levels on micronutrients of cowpea

Treatments		Ca (%)	Mg (%)	Cu (ppm)	Fe (%)	Zn (ppm)	Mn (ppm)
T_1	Control	0.39^{bcd}	0.40^{b}	12.29^{a}	0.14^{b}	39.04^{bcd}	426.88^{bc}
T_2	N+P+K	0.33^{cd}	0.40^{b}	8.96^{a}	0.07^{h}	37.31^{bcd}	513.96^{ab}
T_3	VAM	0.59^{a}	0.38^{d}	14.17^{a}	0.13^{c}	46.04^{ab}	429.38^{bc}
T_4	VAM+N+½P+K	0.48^{ab}	0.39^{c}	22.71^{a}	0.13^{d}	40.83^{bcd}	400.63^{c}
T_5	VAM+ N+¼P+K	0.45^{bc}	0.40^{b}	20.42^{a}	0.08^{g}	43.33^{bc}	384.79^{c}
T_6	*Bradyrhizobium*	0.29^{d}	0.37^{e}	17.92^{a}	0.15^{a}	50.00^{ab}	527.50^{a}
T_7	B+½N+P+K	0.31^{d}	0.38^{d}	7.50^{a}	0.13^{d}	26.46^{d}	411.04^{c}
T_8	B+¼N+P+K	0.37^{bcd}	0.40^{bc}	19.17^{a}	0.11^{e}	38.13^{bcd}	536.25^{a}
T_9	VAM+B	0.34^{cd}	0.40^{bc}	25.00^{a}	0.11^{e}	42.13^{bc}	441.67^{abc}
T_{10}	VAM+B+½N+½P+K	0.40^{bcd}	0.42^{a}	10.83^{a}	0.13^{d}	58.33^{a}	451.04^{abc}
T_{11}	VAM+B+¼N+¼P+K	0.39^{bcd}	0.42^{a}	11.85^{a}	0.10^{f}	30.46^{cd}	384.38^{c}

Mean values with different superscripts differ significantly at 5% level

a fertilizer dose of ½N + ½P + K recorded the maximum zinc content (58.33 ppm). This was on par with the treatments, VAM alone and *Bradyrhizobium* alone. The above treatments were significantly higher than the control and all other treatments in increasing the zinc content of cowpea.

4.10.9 Manganese content in plant

There were significant differences among the treatments in influencing the manganese content of plant (Table 40). Plants treated with *Bradyrhizobium* along with ¼N + P + K recorded the maximum mean manganese content (536.25 ppm). This was on par with the treatments, *Bradyrhizobium* alone, application of N + P + K as per Package of Practice Recommendations of KAU, 1993 and VAM + *Bradyrhizobium* along with a fertilizer dose of ½N + ½P + K. All other treatments were significantly lower than the above treatment.

4.10.10 Nitrogen content in soil

There were no significant differences among the treatments in nitrogen content of soil (Table 41). Thus, the inoculation of VAM, *Bradyrhizobium* and their combinations with different levels of fertilizers had no significant effect on the nitrogen content in soil.

4.10.11 Phosphorus content in soil

Significant differences were not found among the treatments in the phosphorus content in soil. All treatments were on par with each other (Table 41). Thus the soil inoculation of VAM, *Bradyrhizobium* and their combination with different levels of fertilizers had no effect on the phosphorus content in soil.

Table 41. Field experiment - effect of microsymbionts and fertilizer levels on available nitrogen, phosphorus and potassium content in soil (ppm)

Treatments		Nitrogen	Phosphorus	Potassium
T_1	Control	280[a]	4.3[a]	38[a]
T_2	N+P+K	200[a]	5.3[a]	31[a]
T_3	VAM	270[a]	8.3[a]	44[a]
T_4	VAM+N+½P+K	250[a]	6.3[a]	37[a]
T_5	VAM+ N+¼P+K	190[a]	4.1[a]	36[a]
T_6	*Bradyrhizobium*	270[a]	4.5[a]	50[a]
T_7	B+½N+P+K	230[a]	4.1[a]	38[a]
T_8	B+¼N+P+K	250[a]	4.5[a]	37[a]
T_9	VAM+B	220[a]	5.0[a]	33[a]
T_{10}	VAM+B+½N+½P+K	230[a]	4.8[a]	42[a]
T_{11}	VAM+B+¼N+¼P+K	240[a]	7.5[a]	32[a]

Mean values with different superscripts differ significantly at 5% level

4.10.12 Potassium content in soil

There were no significant differences among the treatments in potassium content in soil. All treatments including control were on par with each other (Table 41). Thus, the soil inoculation of VAM, *Bradyrhizobium* and their combination with different levels of fertilizers had no significant effect on potassium content in soil.

4.11 Correlation studies

Correlation of nodule number, nodule fresh weight, nodule dry weight and per cent VAM colonisation with other growth parameters were statistically analysed and the results are presented here.

4.11.1 Correlation between nodulation and VAM colonisation

Correlation coefficients between different nodulation parameters and VAM colonisation per cent are given in Table 42. The table shows that nodule number had significant positive correlation with the nodule fresh and dry weights and also with the percentage of VAM colonisation. Nodule fresh weight was having significant correlation with nodule dry weight and VAM colonisation per cent. Nodule dry weight was also having a significant positive correlation with percentage of VAM colonisation. The significant finding was that all the nodulation parameters viz., nodule number, fresh and dry weights of nodules had significant positive correlation with the percentage of VAM colonisation.

Table 42. Correlation between nodulation and percentage of VAM colonisation (correlation coefficient)

	Nodule No.	Nodule fresh weight	Nodule dry weight	VAM colonisation (%)
Nodule No.	1.00	0.69**	0.38**	0.42*
Nodule fresh weight	-	1.00	0.63**	0.37*
Nodule dry weight	-	-	1.00	0.42*
VAM colonisation (%)	-	-	-	1.00

4.11.2 Correlation of plant growth parameters with nodulation and percentage of VAM colonisation

The correlation coefficients of different growth parameters of the plant with nodulation and VAM colonisation per cent are given in Table 43. The results showed that nodule number had significant positive correlation only with the plant dry weight and yield. All other parameters could not be correlated with the nodule number. Another finding was that potassium content of the plant correlated negatively with the nodule number, however, this was not significant. Percentage of VAM colonisation was found to have no significant correlation with any of the plant growth parameters, NPK content of the plant and the yield. More over, it was found that nitrogen content of the plant was having a negative correlation with percentage of VAM colonisation, eventhough this was not significant.

4.11.3 Correlation between percentage of VAM colonisation and micronutrient status of the plant

The correlation coefficient on the above aspect are given in Table 44. Only two micronutrients, viz. calcium and manganese showed statistically significant correlation. Calcium showed a significant positive correlation (0.48) while manganese showed a significant negative correlation (-0.36) with percentage of VAM colonisation. Fe content of the plant was also negatively correlated (-0.12) with VAM colonisation per cent. There was no significant correlation between percentage of VAM colonisation and other micronutrient status also in the plant.

Table 43. Correlation between plant growth parameters with nodulation and percentage of VAM colonisation (correlation coefficient)

	Nodule number	VAM colonisation (%)
Plant height	0.32^{NS}	0.17^{NS}
Root length	0.14^{NS}	0.07^{NS}
Plant fresh weight	0.23^{NS}	0.25^{NS}
Root fresh weight	0.18^{NS}	0.12^{NS}
Plant dry weight	0.38*	0.32^{NS}
Root dry weight	0.09^{NS}	0.17^{NS}
N content	0.07^{NS}	$(-)0.07^{NS}$
P content	0.18^{NS}	0.01^{NS}
K content	$(-)0.03^{NS}$	0.32^{NS}
Yield	0.36*	0.33^{NS}

Table 44. Correlation between percentage of VAM colonisation and micronutrient status of plant (correlation coefficient)

Micronutrients	VAM colonisation (%)
Ca	0.48**
Mg	0.22^{NS}
Fe	$(-)0.12^{NS}$
Zn	0.22^{NS}
Cu	0.16^{NS}
Mn	(-)0.36*

Discussion

5. DISCUSSION

VA mycorrhizal association with roots of higher plants is a rule rather than an exception. Thus this type of fungal association is found in a wide variety of soils and crop plants in our ecosystem. The effect of VA mycorrhiza in enhancing the growth and performance of the plants and its effects in combination with *Bradyrhizobium* has been demonstrated by many workers (Bagyaraj *et al.*, 1979; Sivaprasad and Rai, 1991 and Thakur and Panwar, 1995). The effect of *Bradyrhizobium* in fixing atmospheric nitrogen in symbiotic association with leguminous plants is also well established (Vincent, 1970).

5.1 Natural occurrence of VAM in cowpea

As a preliminary investigation, a survey was conducted to study the natural incidence of VA mycorrhiza in cowpea. The results of the survey revealed a fairly good VAM colonisation in all the plant samples collected from the five locations. Always, the colonisation in the lateral roots was found to be higher than tap roots except in farmer's field at Nadathara.

There was differences in percentage of VAM colonisation among the survey locations. Maximum colonisation was found in plants from farmer's field at Nadathara. Plants from BRS, Kannara recorded the lowest colonisation. Differences in the soil type and microclimatic conditions might have attributed to the variations in VAM colonisation among different locations.

In all the five locations, colonisation was found to be more during the rainy season (Fig.1). Eventhough there was variations in percentage of VAM colonisation between seasons, these variations were meagre. This indicates that

VAM colonisation percentage is more or less static in the locations surveyed. This may be due to the higher moisture percentage in Kerala soils. Because of the high moisture level, the percentage of VAM colonisation remained more or less static during summer and rainy season. Frequent irrigation at the locations of survey also might have helped in maintaining a static VAM colonisation percentage both at summer and rainy seasons. Singh *et al.* (1992) reported maximum VAM root colonisation in June in kinnow and rough lemon seedlings. But Mago and Mukerji (1994) reported high VAM colonisation in summer.

5.1.1 Spore count of VAM

Spore count was also found to be less at BRS, Kannara compared to other locations. There seems to have a direct relation between the spore count and colonisation percentage. Spore count was more in rainy season in three locations, equal and less in each of the other two locations compared to summer season (Fig.2). The high moisture level of the soil during rainy season might have favoured the growth and sporulation of the VAM fungi. Farmer's field at Nadathara recorded the highest spore count of 87 in both summer and rainy seasons. Bhaskaran and Selvaraj (1997) recorded high spore count of VAM during summer season in ten major plant species infected with *Glomus aggregatum* and *Sclerocystis pakistanica*.

5.2 Identification of inoculants

The morphological characters of the VAM spores studied, viz. size, shape, colour, surface texture and mode of hyphal attachment were in conformity with those described for *Glomus* (Trappe, 1982). The spores were therefore identified as *Glomus* sp.

Fig.1. VA mycorrhizal colonisation of cowpea roots at survey locations

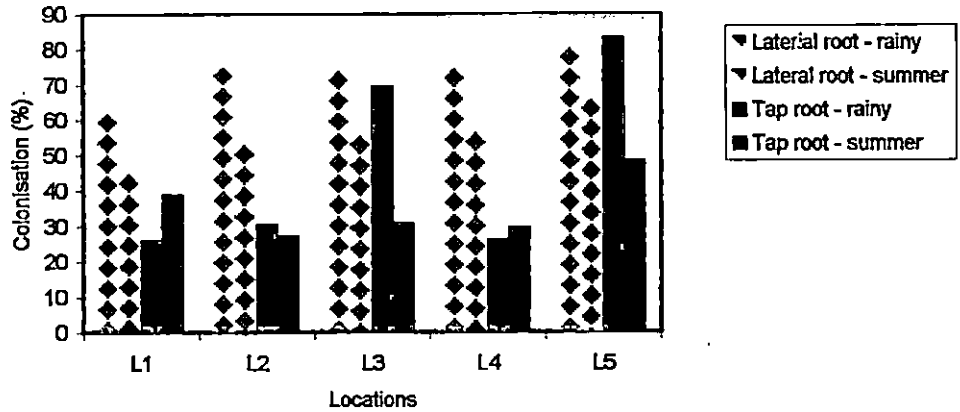
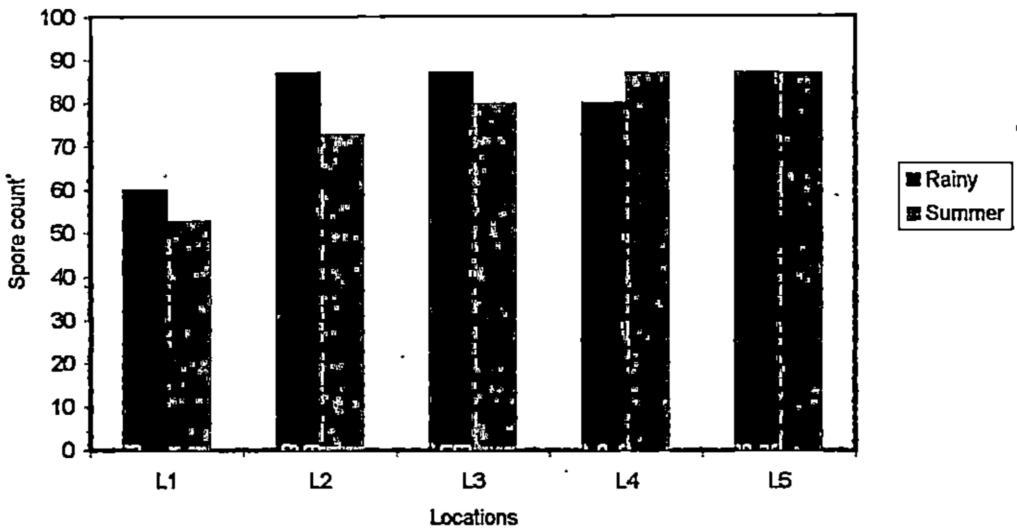


Fig.2. VA mycorrhizal spore count in the soils at the survey locations



L1-BRS, Kannara
L2-COH, Vellanikkara
L3-KHDP, Vellanikkara
L4-Farmers field at Moorkanikkara
L5-Farmers field at Nadathara

Bradyrhizobium was also purified and characterized as described by Vincent (1970). So the authenticity of both the organisms were confirmed.

5.3 Screening of VA mycorrhiza for efficiency in cowpea

Screening trial showed that the effect of VAM was mainly on fresh weight of roots since it was significant even at one per cent level. Also the efficiency of VAM varies as that can be seen in different values for the treatments in fresh weight. There were inefficient VA mycorrhizal fungus also like T_1 (isolate from BRS, Kannara), which showed fresh weight of root 1.16 g, which was even lesser than control. At the same time, it showed an colonisation percentage of 60.0. Similarly isolate T_6 (*Glomus fasciculatum* from U.A.S., Bangalore), the colonisation percentage was 32.67, while the fresh weight of root was 2.87 g. This also showed that per cent colonisation do not always relate with its efficiency. Only fresh weight of roots and percentage of VAM colonisation showed significant differences. Other parameters were not proportional with VAM colonisation per cent. Rajapakse and Miller (1987) also reported similar differential effects of VAM in cowpea. They reported increased shoot dry weight and plant height but decreased root length due to inoculation with *Glomus mosseae* or *Glomus fasciculatum* in cowpea. Thus in the selection of a suitable VAM, colonisation percentage alone cannot be taken as the criteria for efficiency of VAM.

However, there were significant differences between control plants and treatments in increasing VAM colonisation per cent. T_3 (isolate from farmer's field, Nadathara) was having higher percentage of VAM colonisation and fresh weight of root and ranked second in dry weight of plant and number of leaves. This isolate was therefore selected for further studies. This type of inconsistent performance in increasing all the growth parameters is common. Costa and Paulino in 1990 stated that the most efficient VAM fungi varied with the parameters measured. Similar selection based on percentage of VAM colonisation was done by Devi and Sitaramaiah (1991). They selected *Glomus constrictum* as the best VAM fungus in black gram, since it showed the maximum colonisation (89.1%). At the same time another culture, *Glomus fasciculatum* recorded the maximum root dry weight and *Glomus epigaeum* recorded the maximum shoot dry weight in black gram.

5.4 Infection pattern of VA mycorrhiza and *Bradyrhizobium* in cowpea plants

5.4.1 Infection pattern of *Bradyrhizobium*

Observations on nodulation pattern showed the presence of nodules on ten days after sowing. Dual inoculation showed more nodulation than *Bradyrhizobium* alone at the beginning and gradually it changed and from 30th day onwards dual inoculation showed more nodulation than *Bradyrhizobium* alone. On 60th day the nodulation was found more or less same for dual inoculation and *Bradyrhizobium* alone (Fig.3). Between 50th and 60th day dual inoculation had more number of nodules. In both cases, maximum number of nodulation was at 40th day.

The results showed that the nodulation pattern had a direct bearing with the growth stages of the plant. As the plant grows, the nodulation is also found to increase, reaching a peak at 50 per cent flowering stage of the plant. This showed that the vegetative phases of the plant influence positively the development of nodules. After 50 per cent flowering stage, when the photosynthates starts to accumulate in the pods, the nodulation was also found to decrease, probably due to the decreased supply of carbohydrate to the nodules.

Dual inoculation was found to have a synergistic effect on nodulation (Fig.3). VAM might have played a role in supplying more nutrients to the root system so as to influence the nodulation. Many workers support this finding that dual inoculation increase nodulation (Bagyaraj *et al.*, 1979; Sivaprasad and Rai, 1991). But Singh (1994) could not prove the superiority of dual inoculation with *Bradyrhizobium japonicum* and *Glomus fasciculatum* in increasing nodulation or any of the plant growth parameters in soybean.

5.4.2 Infection pattern of VAM

VAM infection pattern was also almost similar to that of nodulation pattern. Infection started at 10th.day reaching a peak at 40th day and gradually decreased till 60th day after sowing. From 30th day onwards dual inoculation was superior to VAM alone (Fig.4).

The Infection trend was found similar to nodulation trend. For both nodulation and for VAM infection, dual inoculation was found good. So it can be presumed that *Bradyrhizobium* and VAM do have a synergistic effect when inoculated in cowpea. This forms an efficient tripartite association. Many other workers also endorsed this view. Increased nodulation and growth parameters were reported by Pillai (1989) in subabul, Ramaraj *et al.* (1990) in cowpea, Thakur and Panwar (1995) in mung bean and Das *et al.* (1997) in green gram.

Unlike in nodulation, VAM infection was found to have a lag phase between 10^{th} and 20^{th} day before reaching the maximum colonisation. Similar lag phase for native strain was earlier reported by Hao *et al.* (1991) in mung bean.

Fig.3. Nodulation pattern of cowpea as influenced by the microsymbionts

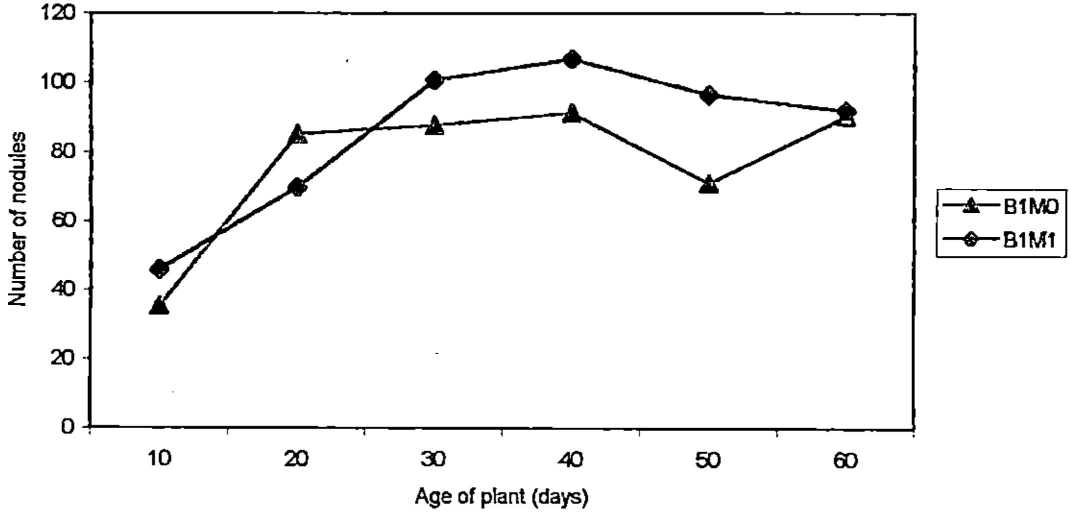
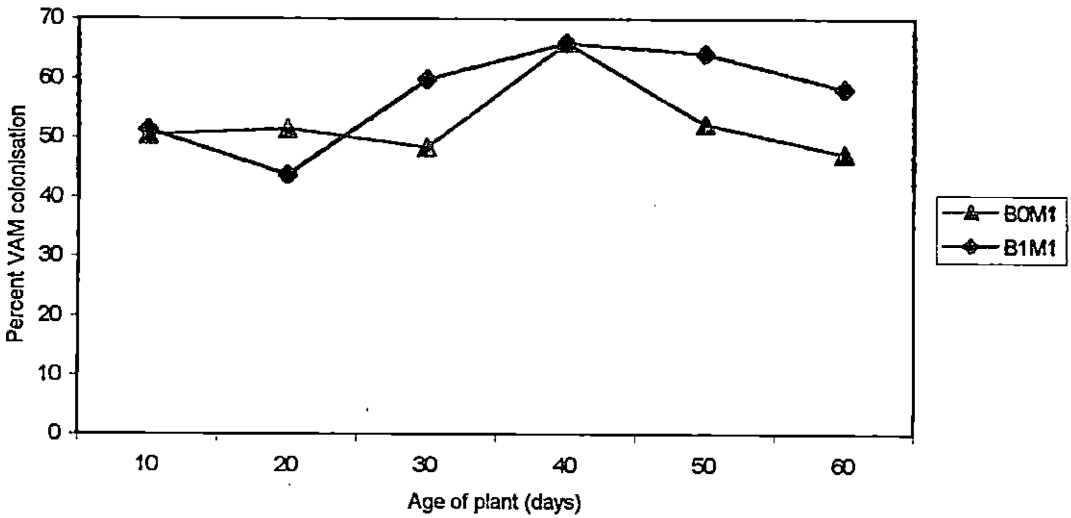


Fig.4. VAM colonisation pattern of cowpea as influenced by the microsymbionts



5.5 Anatomical studies of nodulation and VAM colonisation

Root sections showed the presence of vesicles only in the root cortex. Stele portion was comparatively free of colonisation. Schmid and Oberwinkler (1995) reported the presence of arbuscules in young part of stele portion of root.

Photomicrographs showed highly branched hyphae which occupy much of primary cortex and both H and V shaped branches were also seen. This showed a fairly good colonisation of the root. In most of the vesicles, oil globules were clearly seen which is characteristic of *Glomus* sp.

The roots of cowpea plants were found to get colonised by VAM at 10 days after inoculation. During this early growth stage, the fungus formed arbuscules in the roots and later observations showed that arbuscules were absent and vesicles were found to form. Schmid and Oberwinkler (1995) also recorded similar observations, where they found arbuscule formation in roots of less than two millimeter thickness. As the thickness of the roots increased, arbuscule formation was reduced and vesicle formation increased. These two observations are indicative of a possibility that the physiology of a young root may be condusive for the development of arbuscules. As the roots grow further and the thickness increases, the physiology of the root might have favoured the vesicle formation. Nodule tissues also showed the presence of mycorrhizal fungus. Baird and Caruso (1994) recorded mycorrhizal hyphae and vesicles, in the nodule tissues of plants at five weeks of inoculation. VAM do not seem to differentiate between the root tissues and nodule tissues.

The Electron microscope photomicrographs showed more details about the attachment and spread of the hypha on the root surface. The hyphal growth was seen running along the root surface for some distance before penetrating the root. In some other cases the hypha was found to penetrate almost perpendicularly into the root. Thus two types of hyphal penetration were found to occur in the roots. Bevage and Bowen (1975) found similar growth of mycorrhizal fungi in clover and onion where spreading of the fungus both along the surface of the root and within its tissues occurred in all VA mycorrhiza but the extent of the two processes vary according to the host as well as fungal species. The EM photomicrographs revealed the structure of vesicles formed in the root tissues. The vesicles were attached terminally on the hypha. Eventhough the vesicle surface appeared smooth under light microscope, the electron microscope showed the presence of some adhering material on its surface. EM also revealed the structure of arbuscules. Arbuscules were highly branched. The branches were short, twisted and the tips were slightly bulged. Similar arbuscular structures were recorded by Kinden and Brown (1975).

Electron microscopic study also revealed the surface features of nodules. Unlike that in root surface, the nodule surface was comparatively free of VAM hypha. The inner tissues revealed hyphal presence. Thus direct penetration of nodule tissue by VAM fungus was of less occurrence.

5.6 Pot culture experiment on the effect of microsymbionts and fertilizer levels

5.6.1 Biometric characters

An overall analysis of the influence of the microsymbionts on the biometric characters of the cowpea plants (Table 10 to 19) showed the beneficial effect of dual inoculation with VAM and *Bradyrhizobium* in placing five biometric characters, viz. number of leaves (10.81), fresh weight of plant (22.0 g), dry weight of plant (8.29 g), number of nodules (110.61) and fresh weight of nodules (2.04 g) to the top. For these characters individual inoculation was not found to be much beneficial. *Bradyrhizobium* inoculation alone was beneficial only in producing top ranks for height of plant (27.72 cm), root length (26.61 cm) and dry weight of nodules (1.03 g). VAM inoculation alone proved its efficiency in getting maximum values for fresh and dry weights of roots (2.99 g and 1.55 g respectively).

The results clearly revealed the superiority of dual inoculation over individual inoculation. Such results were endorsed by many workers. Sivaprasad and Rai (1991) reported 178 per cent increased nodulation in pigeon pea by the inoculation with *Glomus fasciculatum* and *Rhizobium*. Das *et al.* (1997) also reported increased shoot and root length and nodule number in green gram dually inoculated with VAM and *Rhizobium*. The control plants were always inferior to the plants inoculated with either a single microsymbiont or both. Thus it is proved that inoculation of either *Bradyrhizobium* or VAM was always better than uninoculated plants and dual inoculation was the best to increase the number of leaves, fresh and dry weights of the plant and number and fresh weight of nodules.

Fertilizer levels also showed variations in its effects on cowpea. Significant differences were seen only in the case of dry weight of plant, fresh and dry weights of roots and fresh weight of nodules. Generally, the results showed that application of full dose of NPK fertilizers did not give the maximum values for any of the biometric characters of the plant. Half of the recommended dose of nitrogen and full phosphorus and potassium (½ N + P + K) recorded the maximum fresh weight of plant (19.50 g) and dry weight of roots (1.54 g). But when nitrogen was further reduced to quarter of the recommended dose, the fresh weight of roots showed maximum value (2.68 g). When the phosphorus level was reduced to half (N + ½ P + K), number of leaves (10.17), height of plant (27.21 cm), and dry weight of plant (7.68 g) were found to increase. When the phosphorus level was further reduced to quarter dose (N + ¼ P + K), four characters, viz. root length (25.75 cm), nodule number (78.50), fresh weight of nodules (1.85 g) and dry weight of nodules (1.03 g) were having the maximum values. Thus from the above observations, it becomes clear that a judicious combination of fertilizer must be the N + ¼ P + K for enhancing the values of atleast four of the biometric characters.

Interaction effects showed that in microbial fertilizer interactions, where VAM is a partner, all the biometric characters except root length recorded the maximum value. It was observed that the presence of *Bradyrhizobium* in the system improved only the number of leaves (13.00), nodule number (135.00) and dry weight of nodules (1.24 g) and that too was along with VAM. *Bradyrhizobium* alone, without any fertilizer application increased only the root length (29.0 cm) of cowpea plants to the maximum. Thus, the influence of VAM was found to be much more compared to *Bradyrhizobium* in improving the biometric characters. The reason of such an enhanced performance of VAM could be attributed to the multifaceted activities of VAM. Increase in the absorption of phosphorus and micronutrients by VAM were already reported by many workers (Elwan, 1993; Eranna and Parama, 1994 and Medeiros *et al.*, 1995). The enhanced performance of VAM may be due to the increased supply of growth hormones including auxins, gibberellins, cytokinins etc. Stimulation of growth hormone production by endomycorrhizal fungi in sweet gum roots resulted in striking growth differences in this species (Marks and Kozlowski, 1973 and Barea and Azcon-Aguilian, 1982). The effect of *Bradyrhizobium* was only limited in nitrogen nutrition of the plant. So VAM should be the important partner in the dual inoculation system.

Among the interactions of fertilizers and microsymbionts, all the biometric characters except the fresh weight of plants did not show any significance between the treatments. Full dose of nitrogen could increase the values of seven characters out of ten to the maximum. They were the number of leaves (13.00), plant height (30.83 cm), dry weight of plant (9.0 g), dry weight of root (1.83 g), nodule number (135.0), fresh weight of nodule (2.41 g) and dry weight of nodule (1.24 g).

Among the doses of phosphorus, six characters out of ten were found to be the maximum at the quarter level of recommended dose. Thus the phosphorus level could be reduced to a quarter as against the Package of Practice Recommendations of KAU, 1993. This trend of low requirement of phosphorus fertilizer in a system where VAM is a partner could be easily explained based on the well proven ability of VAM in increasing the absorption of phosphorus by plants (Powell, 1981; Rajapakse, 1987; Isobe *et al.*, 1993 and Eranna and Parama, 1994). Considering all the above results it could reasonably assume that in the dual inoculation system, VAM plays a major role than *Bradyrhizobium* and the full dose of nitrogen as per Package of Practice Recommendations of KAU, 1993 and quarter dose of phosphorus along with *Bradyrhizobium* and VAM would be an efficient symbiotic system in cowpea.

5.6.2 Nutrient status and colonisation by VAM

A perusal of Tables 22 to 30 revealed that in general inoculation of the microsymbionts either alone or in combination was effective in improving the nutrient status of the plant when compared to control, except in the case of potassium. Another inference is that individual application of VAM and *Bradyrhizobium* was better than combined inoculation. VAM inoculation alone produced maximum values for phosphorus (0.36%), zinc (26.77 ppm) and copper (3.06 ppm), whereas *Bradyrhizobium* inoculation alone improved the content of nitrogen (3.43%), magnesium (0.409%), iron (612.74 ppm) and manganese (278.02 ppm) to the maximum. Dual inoculation was found to improve the calcium content (0.92%) alone to the maximum. There was about 31.4 per cent increase in calcium content in dually inoculated plants compared to control plants. Pai *et al.* (1994) reported high level of calcium in cowpea plants inoculated with *Glomus fasciculatum.*

Eventhough dual inoculation increased only calcium content of the plant, while considering the second rank also, the dual inoculation was found to improve the level of six nutrients, viz. nitrogen (3.32%), phosphorus (0.33%), magnesium (0.407%), calcium (0.92%), zinc (26.20 ppm) and manganese (250.56 ppm) proving the efficiency of this treatment. Superiority of dual inoculation in improving the various nutrients of plants have been well established. Sivaprasad *et al.* (1983) reported increased phosphorus uptake in Leucaena after the combined inoculation with VAM and *Rhizobium*. Smolin and Shabaev (1992) reported improved uptake of major and trace elements in soybean dually inoculated with *Rhizobium* and VAM. Thyagarajan *et al.* (1992) recorded increased shoot nitrogen and phosphorus in cowpea inoculated with *Rhizobium* and VAM fungi. Kumutha and Santhanakrishnan (1994) reported that dual inoculation with VAM and *Bradyrhizobium* in soybean doubled the nitrogen fixation compared with *Rhizobium* alone. A similar increased absorption by dual inoculation was not found in the case of potassium. In fact, dual inoculation decreased the potassium content.

The effect of a particular combination of fertilizer dose was not sufficient to improve the status of all the nutrients of the plant (Table 22 to 30). It was found that full dose N+P+K as per Package of Practice Recommendations of KAU, 1993 was required to improve the nitrogen (3.21%) and zinc (24.42 ppm) content to the maximum. Similarly quarter dose of nitrogen along with full dose of phosphorus was found effective in getting maximum value for phosphorus content (3.20%) of the plant. Considering the overall performance, the fertilizer dose of ¼ N + P + K was found the best in increasing the level of phosphorus (3.20%), calcium (0.83%), iron (799.32 ppm) and manganese (280.94 ppm). Magnesium and copper contents were maximum in control plants. The natural balance among the various nutrients in the plant system may be the reason for getting various values for different treatments.

The effect of interactions between the nutrients and symbionts were found similar to the biometric observations. Interactions involving VAM inoculation were found to improve the values of nutrients, viz. nitrogen (4.03%), phosphorus (0.40%), magnesium (0.423%), calcium (1.24%), zinc (37.04 ppm) and copper (5.42 ppm). But *Bradyrhizobium* was found to influence the values of only four nutrients to the maximum. Thus here also treatment combinations involving VAM are highlighted. The effect of different fertilizer doses along with the symbionts were not consistent. It was found that only quarter dose of nitrogenous fertilizer was necessary, when applied with the inoculation of both the microsymbionts to get maximum nitrogen content (4.03%) in plant. At quarter level of nitrogenous fertilizer, the plants inoculated with both microsymbionts showed 15.80 per cent increase in nitrogen content as compared to plants inoculated with *Bradyrhyzobium* alone. The efficiency of dual inoculation with VAM and *Rhizobium* in increasing the nitrogen content of plant was reported by several workers. Sivaprasad and Rai (1991) reported 185 per cent increase in nitrogenase activity in pigeon pea inoculated with *Glomus fasciculatum* and *Rhizobium* compared to inoculation with *Rhizobium* sp. alone. Khan *et al.* (1995) noted that groundnut plants inoculated with *Glomus* sp. and *Bradyrhizobium* sp. derived 75 per cent nitrogen from fixation, whereas plants inoculated with *Bradyrhizobium* alone derived 68 per cent nitrogen only. So by recommending the quarter dose of N as urea along with both the microsymbionts, we could save 15 kg N ha^{-1}. When ammonium was the nitrogen source, mycorrhizal plants seem to enhance nitrogen uptake (Read and Stribley, 1973). Increase in glutamate synthetase (GS) activity by VAM colonisation has shown that VA mycorrhizal fungi are able to assimilate ammonia, via. GS (Smith *et al.*, 1985). This ability would be important in stress conditions. Carling *et al.* (1978) reported that various VAM fungi have been found to contain higher values of nitrate reductase enzyme which is essential for the assimilation of nitrates. The effect of VAM as nitrate reductase are largely indirect and mediated through improved phosphorus nutrition.

Another observation was that interaction of VAM with ½ N + P + K and N + ½ P + K without any inoculants yielded maximum values for the phosphorus content (0.40%) of the plant. But it was on par with many other treatments including VAM alone, VAM+¼N+P+K, VAM+N+¼P+K and all the treatment combinations having VAM + *Bradyrhizobium* with fertilizer levels except N+½P+K. Usually VAM at low soil phosphorus level should be more efficient in the uptake of phosphate. But some of the above values showed the requirement of full dose of phosphorus with microsymbionts to maximise the phosphorus content of plant. The uptake of soil phosphate by mycorrhizal roots is attributed to extensive hyphae in the soil which absorb and translocate phosphate to the roots. The increase in absorbing sites provided by fungus may be a major factor contributing to the total increased phosphorus uptake. The restricted growth VAM hyphae in the pot could be the reason for the less efficiency of VAM in increasing the phosphorus content of plant in soil with low phosphorus level in the present study.

The VAM colonisation per cent in cowpea roots (Table 23) was found to be the maximum in treatment.. with VAM alone, without any fertilizer application (87.96%). But it was also on par with dual inoculation without any fertilizer dose

(82.60%) and with N+½P+K+dual inoculation of microsymbionts (82.07%). Perusal of Table 20 and 23 revealed that the above three treatments showed a higher VAM colonisation per cent and also gave higher phosphorus content of plant. But at the same time treatment combinations which recorded significantly lesser VAM colonisation per cent gave higher phosphorus content of plant. So the higher level of phosphorus in these treatments may not be due to the efficiency of VAM fungus. These observations led to the same conclusion that the low efficiency of VAM fungus may be due to the lack of extensive hyphal growth of VAM into the limited quantity of soil in the pot and reduced absorption sites of the organism.

Comparison of the spore count in the field (Table 38) with that in the pot culture experiment showed that the spore count in the field was much higher. This suggests that the limited quantity of soil in the pot hindered the growth of VAM fungus, phosphorus uptake and also the spore count in soil.

In the case of dual inoculation, only half dose of phosphorus with full nitrogen and potassium were required to increase the magnesium (0.423%) and calcium (1.24%) absorption to the maximum. But VAM inoculation alone without any fertilizer level was required to increase the copper content of plant (5.42 ppm). This proved the efficiency of VAM in influencing the uptake of these micronutrients. Similar observations were made by Pai *et al.* (1994), Medeiros *et al.* (1995) and Tarafdar and Rao (1997). Bermudez and Azcon (1996) worked out a positive correlation between calcium content of alfalfa plants and nodulation in mycorrhizal treatment. VAM did not seem to have much role in increasing the manganese content, but *Bradyrhizobium* inoculation alone without any fertilizer was sufficient to get the maximum value.

Table 31 to 33 shows the nitrogen, phosphorus and potassium content of soil. Among the inoculants, treatment with VAM alone showed maximum value of soil nitrogen (270 ppm) which was about 23 per cent increase when compared with *Bradyrhizobium* treatment. Among the interaction with microsymbionts and fertilizer levels, VAM with ½N+P+K ranked top in increasing the soil nitrogen to maximum (370 ppm). This showed the efficiency of VAM in increasing the soil nitrogen content in combination with half dose of nitrogenous fertilizer. The critical analysis of Table 32 revealed that there were no significant differences among treatments and its interactions in influencing the phosphorus status of soil. As far as potassium content of soil is concerned (Table 33), there was significant difference among treatment with microsymbionts with maximum in control plants (80 ppm). The results of the interaction between inoculants and fertilizer levels showed that most of the treatment combinations without inoculants had maximum value of potassium content in soil. This indicated the application of either VAM or *Bradyrhizobium* or its combination could reduce the potassium content of soil. A negative effect of potassium application to the total and individual weights of nodules was reported by Seripong and Masayna (1984).

The overall results of the experiment revealed that dual inoculation with VAM and *Bradyrhizobium* was found more beneficial than individual inoculations and was found to increase five biometric characters of plant to the maximum. Dual inoculation also improved the level of six nutrients, viz. nitrogen, phosphorus, magnesium, calcium, zinc and manganese.

In interaction between microsymbionts and different fertilizer levels, only fresh weight of plant recorded significant differences and the maximum value was recorded by VAM+½N+P+K. Other observations were not significant. Even then, those treatments where VAM was a partner, recorded the maximum values except in the case of length of root. In the case of nutrient status, the results of interactions showed inconsistent values. Even then, maximum nitrogen content in plant was recorded by VAM+B+N+½P+K and maximum calcium and magnesium content was recorded by VAM+B+N+½P+K.

5.7 Effect of microsymbionts and fertilizers at field level

5.7.1 Biometric characters

Results on the number of leaves, plant height and root length revealed that the values of all these parameters were more at the time of harvest than at 50 per cent flowering, except in the case of T_2 (application of fertilizer as per Package of Practice Recommendations of KAU, 1993), where the number of leaves was 12.25 at 50 per cent flowering and at harvest it was only 10.75 (Table 34). This is only normal that at 50 per cent flowering time the plants have not completed its full growth and naturally the number of leaves, plant height and root length will be less at 50 per cent flowering. At harvest time, maximum number of leaves was recorded by T_7 (B+½N+P+K). Comparing with the control plants, application of N+P+K as per Package of Practice Recommendations of KAU, 1993 and application of B+½N+P+K were required for getting maximum number of leaves at 50 per cent flowering and at harvest respectively. This also pointed out that nutrients given as fertilizers are easily utilized during the earlier stages of growth but the effect of inoculation of *Bradyrhizobium* is expressed only at a later stage of plant growth.

In the field, the plant height showed significant differences both at 50 per cent flowering and also at harvest. Here the treatment T_{10} (VAM+B+½N+½P+K) was the best giving maximum height of the plant both at 50 per cent flowering (34.83 cm) and at harvest (42.58 cm), eventhough at harvest it ranked second, but was on par with first ranked treatment (T_8). From this it could be derived that dual inoculation could effectively substitute half the dose of nitrogen and phosphorus fertilizers as far as plant height is concerned. Similar observation was made by Satizabal and Saif (1987) in *Centrosema macrocarpum* inoculated with *Rhizobium* strains and *Glomus manihotis* or *Acaulospora longula*. They reported the highest growth in plants inoculated with VAM alone with a phosphorus level of 40 kg P_2O_5 ha^{-1}. Rathore and Singh (1995) reported that mycorrhizal inoculum may substitute phosphatic fertilizer equivalent to 30 kg P ha^{-1} in maize.

The root length of plant were significantly different at 50 per cent flowering stage and after that at the time of harvest, the differences were not significant (Table 34). The effects of treatments on the root length were well exhibited during the active growth stage. Application of N+P+K as per Package of Practice Recommendations of KAU, 1993 (T_2) was the best treatment giving a root length of 16.67 cm. But this can well be substituted by microbial inoculants also (VAM+N+½P+K, 16.42 cm and *Bradyrhizobium* + ½N+P+K, 15.92 cm) which were on par with the treatment T_2. This means that the application of either VAM with half dose of phosphorus and full dose of nitrogen and potassium fertilizers or *Bradyrhizobium* with half dose of nitrogen and full dose of phosphorus and potassium fertilizers is equivalent to the application of N+P+K fertilizer as per Package of Practice Recommendations of KAU, 1993.

The fresh and dry weights of plants were the maximum in T_{10} (VAM+B+½N+½P+K) both at 50 per cent flowering and at harvest except the dry weight at 50 per cent flowering (Table 35). But it was on par with the first ranked treatment (T_4). This result indicates that the application of VAM and *Bradyrhizobium* in combination can effectively substitute half of the nitrogenous and phosphatic fertilizer requirement as far as the fresh and dry weight of the plants are concerned. Moreover, the weight gaining process was not uniform among the treatments. In the treatments T_2, T_3, T_4, T_5 and T_7, more than 60 per cent of the total fresh weight was gained by the time of 50 per cent flowering (45th day after sowing). Whereas in the other treatments (T_1, T_6, T_8, T_9, T_{10} and T_{11}) a uniform growth pattern in terms of fresh weight was observed. An average of 43.57 per cent of total fresh weight was gained by them by this time. Among them, the treatment T_{10} (VAM+B+½N+½P+K) recorded the highest fresh weight at 50 per cent flowering and also at harvest. When both VAM and *Bradyrhizobium* were inoculated together the use of nitrogen and phosphorus fertilizers could be reduced to half and at the same time a more or less uniform growth rate in terms of weight gaining was obtained, 47.86 per cent during the vegetative growth of the plant and the rest 52.14 per cent after flowering stage. Thus the application of microsymbionts not only reduce the use of nitrogenous and phosphatic fertilizers to the half, but also regulates the growth to a uniform pattern. Dry weight of plants also followed a similar pattern. Godse *et al.* (1978) reported increased shoot weight in cowpea inoculated with *Rhizobium* and VAM and suggested that it might be due to the increased uptake of phosphorus by plants. Satizabal and Saif (1987) also reported highest growth in *Centrosema macrocarpum* inoculated with VAM and *Rhizobium* at a phosphorus level of 40 kg P_2O_5 ha^{-1}.

The pattern of fresh and dry weight gaining process by the roots showed that the roots gained most of its weight after 50 per cent flowering stage (Table 36). Treatment with *Bradyrhizobium* alone (T_6) recorded the maximum fresh weight of root (7.19 g) and 88.5 per cent of this weight was gained only after 50 per cent flowering. This trend can be seen in all other treatments also. At 50 per cent flowering treatments involving VAM (T_3, T_4 and T_5) were on par with the best treatment (T_{10}) in increasing the fresh weight of the plants. Among them T_4 (VAM+N+½P+K) was the best treatment. This treatment ranked first in producing the maximum dry weight of roots also, both at 50 per cent flowering (0.93 g) and at harvest (3.65 g). This result emphasises the role of VAM inoculation in enhancing the root development of cowpea and also it shows that half the dose of phosphorus with full nitrogen and potassium could well be substituted with VAM inoculation as far as root development (fresh and dry weight) is concerned. Many workers (Champawat, 1989; Chhabra *et al.*, 1990 and Mane *et al.*, 1993) have reported that the VAM inoculation increases the root development by way of increased phosphorus uptake. At harvest the fresh weight of root was increased to 130 per cent and dry weight of root was increased to 180 per cent in the treatment T_4 (VAM+N+½P+K) over control.

The nodule number on treated plants showed that *Bradyrhizobium* inoculation alone was not sufficient to increase the nodule number per plant (Table 37). *Bradyrhizobium* inoculation alone (T_6) produced 12.17 nodules at 50 per cent flowering and 10.58 at harvest, while the combined inoculation with VAM (T_9) increased the nodule number to 13.25 and 18.75 respectively. However, the maximum nodulation (17.58 and 29.67) was found in T_{10} (VAM+B+½N+½P+K) (Fig.5). This shows that dual inoculation of both the microsymbionts increases the efficiency of nodulation and it can reduce the fertilizer requirement of nitrogen and phosphorus to half. The treatment T_{10} increased the nodulation to 180 per cent compared to treatment with *Bradyrhizobium* alone. VAM inoculation may have increased the nutrient availability to the plant, which resulted in increased nodulation. Sivaprasad and Rai (1991) reported 178 per cent increase in nodulation in pigeon pea inoculated with *Glomus fasciculatum* and *Rhizobium*. Thyagarajan and Ahmed (1993) also reported 71.2 per cent increase in nodulation when cowpea plants were dually inoculated with cowpea *Rhizobium* strain JRC-29 and VAM fungus. Similarly Soddi *et al.* (1994)reported highest nodulation in cowpea cultivar C-152 when dually inoculated with *Rhizobium* and *Glomus fasciculatum* along with 50 per cent of recommended nitrogen and phosphorus at the rates of 25 kg N and

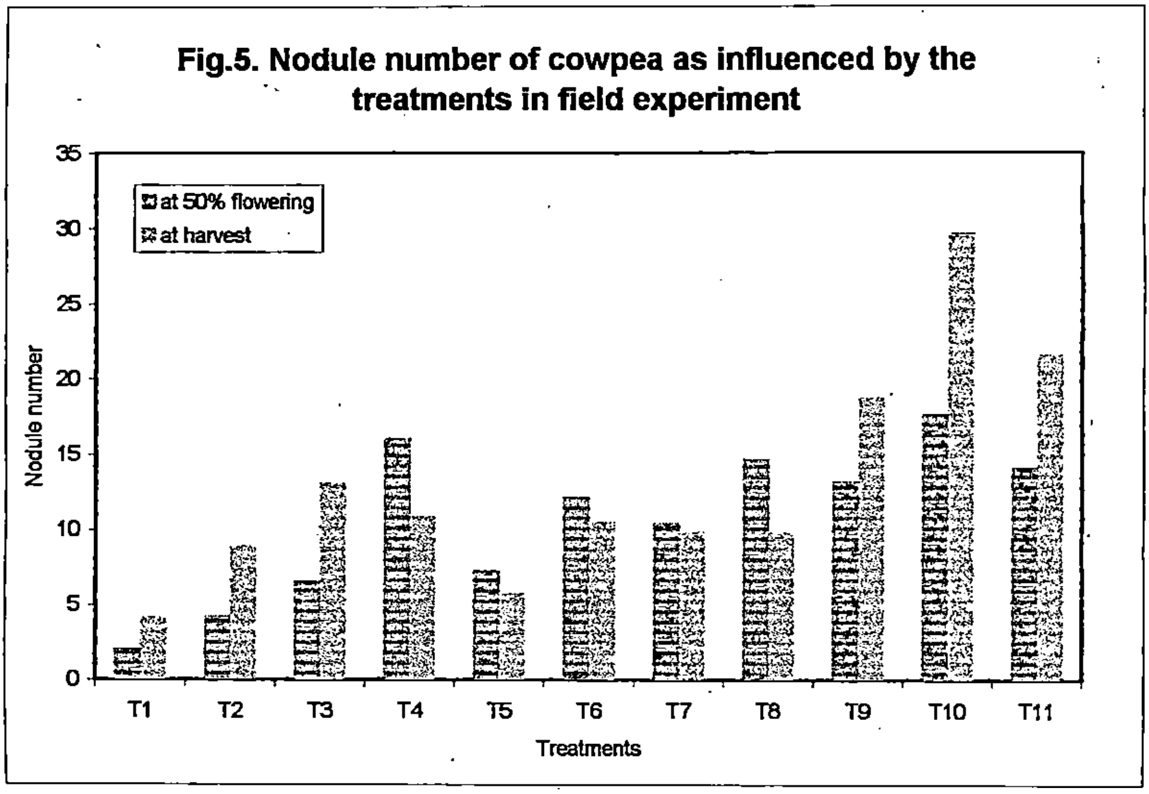


Fig.5. Nodule number of cowpea as influenced by the treatments in field experiment
at 50% flowering
at harvest
Nodule number
35
30
25
20
15
10
5
0
T1
T2
T3
T4
T5
T6
T7
T8
T9
T10
T11
Treatments

50 kg P ha^{-1}. Another finding was that in most combinations of VAM and *Bradyrhizobium* (T_9, T_{10} and T_{11}) nodule numbers were more at harvest than at 50 per cent flowering. This suggests that VAM supports nodule formation greatly after 50 per cent flowering stage.

A critical analysis of Table 37 reveals the superiority of the treatment T_{10} (VAM+B+½N+½P+K) in increasing the fresh and dry weight of nodules. Fresh weight of nodules showed maximum both at 50 per cent flowering (395 mg) and also at harvest (700 mg) in this treatment. The dry weight followed the same trend eventhough it ranked second at 50 per cent flowering. The increase in fresh weight over control at 50 per cent flowering stage was 39 per cent and at harvest it was 155 per cent. A similar trend was there for dry weight also.

From the data on number of nodules, fresh and dry weights of nodules, it becomes clear that T_{10} is a significantly superior over control. On comparison with the treatment T_2 (N+P+K) also, T_{10} showed its superiority. Many workers reported the superiority of dual inoculation in increasing the nodulation and nodule weight (Asai, 1944; Bagyaraj *et al.*, 1979; Allen *et al.*, 1991 and Das *et al.*, 1997). The well established fact that VAM improves the phosphorus availability to the plant (Sivaprasad *et al.*, 1983; Champawat, 1990a; Cabello, 1992; Khan *et al.*, 1995 and Das *et al.*, 1997) and *Bradyrhizobium* enhances nitrogen assimilation by the plant (Nair and Sivaprasad, 1981; Manel and Chahal, 1987; Mand *et al.*, 1991 and Senaratne and Ratnasinghe, 1995) made the plant to perform better over control.

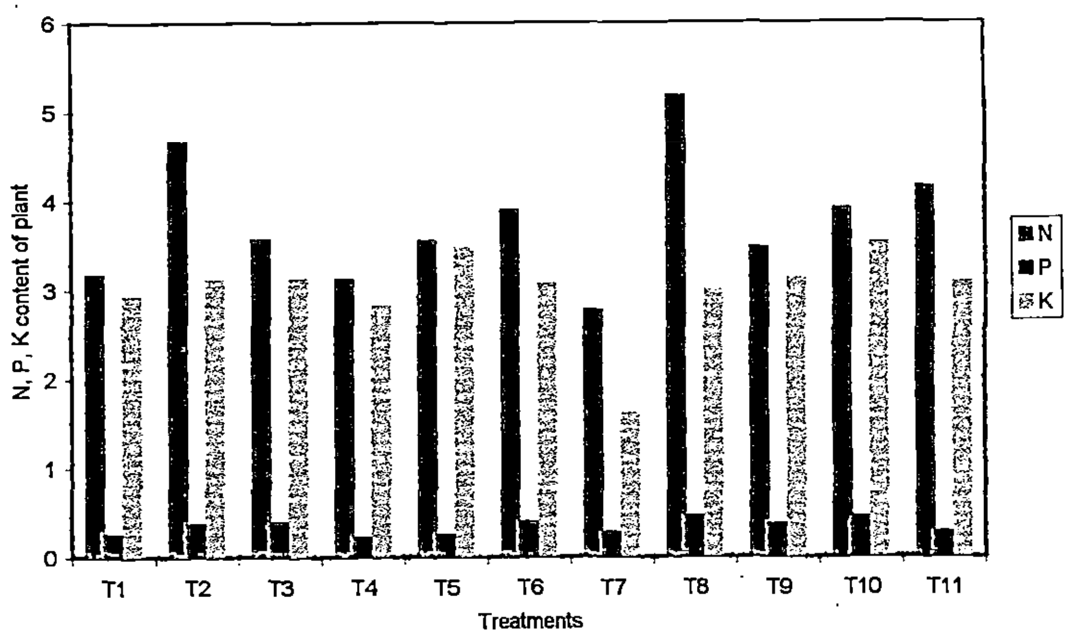
5.7.2 Nutrient status

The results on the NPK content of the plants (Table 39) showed that the nitrogen content was the highest (5.18%) in treatment T_8 (B+¼N+P+K).

Significantly higher phosphorus content was seen in four treatments viz. T_3 (VAM alone, 0.41%), T_6 (*Bradyrhizobium* alone, 0.40%), T_8 (B+½N+P+K, 0.46%) and T_{10} (VAM+B+½N+½P+K, 0.45%). Potassium content was the highest in T_{10} (VAM+B+½N+½P+K, 3.52%) and T_5 (VAM+N+¼P+K, 3.48%) (Fig.6). The treatment T_8 recorded 63.4 per cent increase in nitrogen content over control (T_1). Thus it becomes evident that inoculation by *Bradyrhizobium* is effective in increasing the nitrogen content of the plant to the maximum (Ezedinma, 1964 and Sahu and Bahara, 1972) and also it can reduce the fertilizer nitrogen requirement of cowpea to quarter dose. Among the treatments which ranked top for phosphorus content, the treatment T_{10} gave 0.45 per cent of phosphorus content of plant. The same treatment was found to give the maximum potassium content (3.52%) of plant, along with T_5. Thus the result indicated that B+¼N+P+K (T_8) was the best for improving nitrogen and VAM+B+½N+½P+K was the best for improving the phosphorus and potassium contents of plant. Mane and Chahal (1987) and Stamford *et al.* (1990) reported that *Bradyrhizobium* inoculation could reduce the nitrogen application in leguminous plants. The beneficial effects of individual inoculation of VAM (Rathore and Singh, 1995; Tarafdar and Rao, 1997) and dual inoculation with *Bradyrhizobium* were also well established. The data showed that dual inoculation can save half quantity of nitrogenous and phosphatic fertilizers. Singh (1996) reported maximum nitrogen fixation in pigeon pea dually inoculated with *Rhizobium* and *Glomus fasciculatum* in the presence of phosphorus and nitrogen @ 50 kg P_2O_5 ha^{-1} and 20 kg N ha^{-1} respectively.

The micronutrient content of plant showed that none of the control plants (T_1) or plants which received fertilizers as per Package of Practice Recommendations of KAU, 1993 (T_2) were top ranking as far as the five micronutrient elements were concerned. In this aspect also the treatment T_{10} (VAM+B+½N+½P+K) was superior in increasing the content of magnesium

Fig.6. NPK content of cowpea as influenced by the treatments in field experiment



(0.42%) and zinc (58.33 ppm). The treatment T_{11} (VAM+B+¼N+¼P+K) was also performing equally good as that of T_{10}, as far as magnesium content was concerned. The symbiotic nitrogen fixation activity is known to improve by the presence of magnesium as it is an integral constituent of leghaemoglobin. VAM inoculation might have improved the absorption of magnesium, thereby increasing the efficiency of *Bradyrhizobium*. VAM inoculation (T_3) greatly improved calcium content (0.59%) and *Bradyrhizobium* inoculation (T_6) improved the Fe content (0.15%) of the plants. The overall results showed that T_{10}, where dual inoculation was done with half quantity of nitrogen and phosphorus and full dose of potassium was the best treatment. The beneficial effects of dual inoculation were reported by many workers. Kumutha and Santhanakrishnan (1994) reported double nitrogen fixation in soybean inoculated with VAM and *Bradyrhizobium* compared to *Rhizobium* inoculation alone. Khan *et al.* (1995) recorded increased nitrogen and phosphorus content in groundnut as a result of dual inoculation with *Glomus* sp. and *Bradyrhizobium* sp.

As seen from the Table 41, the different treatments were not able to alter the available nitrogen, phosphorus and potassium content of soil to a significant level.

5.7.3 Percentage VAM colonisation, spore count of VAM and yield

A critical examination of the data on VAM colonisation percent, spore count of VAM and yield of plants (Table 38) revealed that the control plants were the poorest in expressing the above factors. The maximum VAM colonisation per cent was seen in plants inoculated with VAM alone (T_3, 76.96%). But on combination with *Bradyrhizobium* (T_9), there was no significant difference. The treatments, T_4, T_5, T_{10} and T_{11} were on par with the top ranking treatment (Fig.7).

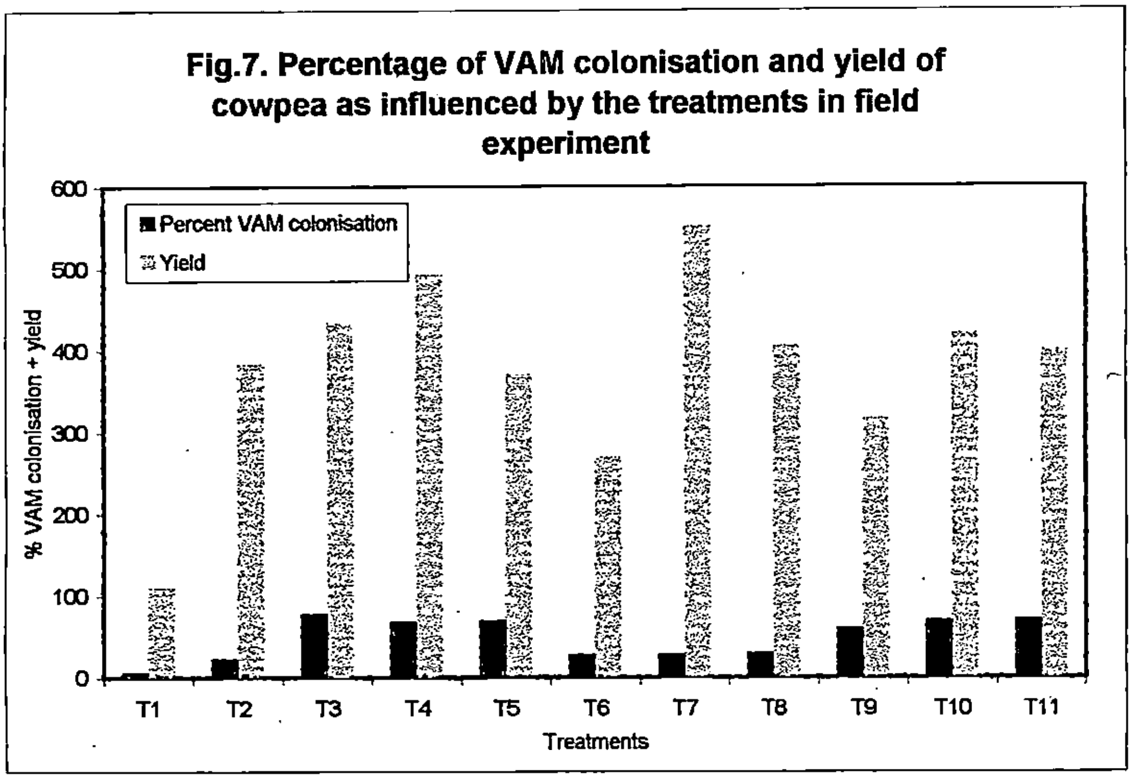


Fig.7. Percentage of VAM colonisation and yield of cowpea as influenced by the treatments in field experiment
600
500
400
300
200
100
0
% VAM colonisation + yield
Percent VAM colonisation
Yield
T1
T2
T3
T4
T5
T6
T7
T8
T9
T10
T11
Treatments

This showed that VAM colonisation was not significantly affected by dual inoculation and also by the application of fertilizers. Manjunath and Bagyaraj (1986) reported that application of phosphorus did not reduce the percentage of root colonization by VA-mycorrhiza, but it increased the extramatrical chlamydospores in the soil. They also suggested the need for application of phosphorus to obtain maximum benefit from VAM in phosphorus fixing soils. Hao *et al.* (1991) also made a similar observation in mung bean that phosphorus application encouraged mycorrhizal colonisation. Lee and Ryu (1992) made similar observation in sorghum inoculated with VAM fungi. They observed that the colonisation of roots with VAM was independent of phosphate level in the soil for sorghum.

But Rajapakse (1987) reported a negative correlation between root colonisation by *Glomus fasciculatum* and phosphorus content of growth medium in cowpea. Isobe *et al.* (1993) also reported the adverse effect of the application of superphosphate fertilizer in mycorrhizal colonisation in soybean.

The treatment T_5 (VAM+N+¼P+K) showed the maximum spore count of VAM (688.67 per 100 g soil). It was significantly higher than all other treatments except T_{11} (VAM+B+¼N+¼P+K, 611.00 per 100 g soil). In both these treatments only quarter dose of phosphorus was applied. In other treatments, wherever higher doses of phosphorus was applied, then spore count was less. This clearly indicates that the phosphorus level of soil has some influence on the spore count of VAM in soil. Santhi and Sundarababu (1995) reported a negative correlation between phosphorus levels and the VAM spore population in soil. From the table it was also clear that, VAM inoculation along with *Bradyrhizobium* has no influence on the spore count.

As far as the yield is concerned, T_7 (B+½N+P+K) was the top ranking treatment (550.33 g $plot^{-1}$). The treatment T_{10} which was found superior in many parameters was also statistically on par with T_7 (Fig.7). Thus for yield *Bradyrhizobium* along with ½N+P+K was the best treatment. Increase in yield by the inoculation of *Bradyrhizobium* have already been reported by many workers (Rai *et al.*, 1977; Sivaprasad and Shivappashetty, 1980). Mahmoud *et al.* (1994) reported the highest seed yield in soybean inoculated with *Bradyrhizobium* along with the application of nitrogen fertilizer.

The results of field experiment concluded that T_{10} (VAM+B+½N+½P+K) was the best treatment in improving six biometric characters of plant, viz. plant height, fresh and dry weights of plant, nodule number, fresh and dry weights of nodules and yield to the maximum value. T_{10} also improved the level of five nutrients, viz. phosphorus, potassium, magnesium, zinc and manganese in plant to the maximum value.

Thus the inoculation of VAM and *Bradyrhizobium* had a synergistic effect on the performance of cowpea. This might be due to the increased nutrient uptake and production of growth promoting substances like auxins, gibberellins and cytokinins. These substances alter the physiology of the plant. The results revealed that this dual inoculation could substitute the nitrogenous and phosphatic fertilizers to the half (10 kg N and 15 kg P_2O_5 ha^{-1}) of the recommended dose as per Package of Practice Recommendations of KAU, 1993.

The percentage of VAM colonisation was not affected by dual inoculation and different levels of fertilizer application. Spore count of VAM was found maximum in soil with low phosphorus level. But VAM inoculation along with *Bradyrhizobium* had no influence on the spore count.

5.8 Correlation studies on biometric characters and nutrient status of cowpea

Correlation studies (Table 42 and 43) revealed that percentage of VAM colonisation had significant positive correlation with nodule number and also fresh and dry weights of nodule. This showed a positive response between nitrogen fixing bacteria and VAM fungus. Aboul-Nasar (1993) also made a similar observation in sorghum and he reported that this response would depend upon VAM species. But Ianson and Linderman (1993) found that the level of VAM formation was not related to nitrogen fixation in pigeon pea inoculated with *Rhizobium* and *Glomus* sp. But percentage of VAM colonisation had no significant correlation with any of the biometric characters of the plant. There was significant positive correlation for the nodule number with the plant dry weight and also with the yield. This emphasis the need for *Bradyrhizobium* inoculation for increased yield. Sivaprasad and Shivappashetty (1980) also reported a significant correlation between leghaemoglobin content of nodules, plant top dry weight and final grain yield in cowpea. This can also reduce the fertilizer nitrogen dose to half (T_7). However, combined inoculation along with ½N+½P+K was found to improve many of the plant biometric characters.

Table 44 revealed that VAM colonisation per cent had a significant positive correlation with calcium content. Similar reports were made by Pai *et al.* (1994) and Bermudez and Azcon (1996). VAM also had a significant negative correlation with manganese content of the plant. Bagyaraj and Manjunath (1980) and Medeiros *et al.* (1995) also reported negative correlation for VAM with manganese content.

Summary

SUMMARY

An investigation was undertaken at the College of Horticulture, Vellanikkara during the period 1994-97 to study the interaction between VA mycorrhiza and *Bradyrhizobium* in cowpea. The results of this study are summarised below.

The survey on the natural occurrence of VAM in five locations revealed a fairly good VAM colonisation in all the samples collected. Colonisation in the lateral roots was found to be higher than that in tap roots. In all the five locations colonisations were found to be more during the rainy season than in summer. The VAM spore count was also found more during the rainy season. The predominant spores found in all the survey locations were that of *Glomus* sp.

The screening of VA mycorrhiza revealed that its effect was more conspicuous on fresh weight of root. Inoculation increased VAM colonisation per cent significantly. VAM isolate T_3 (isolate from farmer's field at Nadathara) was selected for further studies based on its performance in percentage of VAM colonisation (67.93), fresh weight of root (3.05 g), dry weight of plant (7.43 g) and number of leaves (16.53).

The colonisation pattern of *Bradyrhizobium* revealed that the initiation of nodulation occurs even at 10 days of plant growth. Dual inoculation with VAM and *Bradyrhizobium* showed more nodulation than that with *Bradyrhizobium* alone. In both cases maximum nodulation was found at 40^{th} day after sowing. So dual inoculation was found to have a synergistic effect on nodulation. The results also showed that the nodulation pattern had a direct bearing with the growth stages of the plant. Nodulation was found to reach at its peak at 50 per cent flowering stage (40^{th} day) and after that it decreased.

VAM infection was found to start at 10^{th} day after sowing, reaching the peak at 40^{th} day and gradually decreasing till 60^{th} day after sowing. Unlike in nodulation, VAM Infection was found to have a lag phase up to 20^{th} day to get adjusted with the environment.

For both nodulation and VAM colonisation, dual inoculation was found to have a synergistic effect.

Anatomical studies showed the presence of VAM vesicles only in the root cortex. Both H and V shaped branches of hyphae were seen in the stained roots. During the early stages of plant growth the fungus formed arbuscules in the roots and later the arbuscules were absent and vesicles were found to form. Nodule tissues also showed the presence of mycorrhizal fungus.

Electron microscopic study revealed two types of VAM fungal penetrations in the roots. In one, the hyphal growth was seen running along the root surface and gradually enter in to the root and in the other, hypha was found to penetrate almost perpendicularly in to the root. The EM photomicrographs revealed that the vesicles were attached terminally on the hypha and the arbuscules were highly branched, with short, twisted branches with slightly bulged tips.

Electron microscopic study of nodules revealed that the nodule surface was free of VAM hypha but the inner tissues had the hyphal presence.

The pot culture experiment showed that dual inoculation with VAM and *Bradyrhizobium* was beneficial than individual inoculations in placing five biometric characters, viz. number of leaves (10.81), fresh weight of plant (22.00 g), dry weight of plant (8.29 g), number of nodules (110.61) and fresh weight of nodules (2.04 g). Inoculation of either *Bradyrhizobium* or VAM is always better than uninoculated plants. Dual inoculation was found to have a synergistic effect in enhancing the above five biometric characters of the plant.

The results on the effect of fertilizer doses revealed that a judicious combination of fertilizer must be N+¼P+K for improving four of the biometric characters, viz. root length (25.75 cm), nodule number (78.50), fresh weight of nodules (1.85 g) and dry weight of nodules (1.03 g).

Among all the inoculant-fertilizer interactions, those in which VAM was a partner, recorded the maximum values for all the characters except root length. So VAM played a major role than *Bradyrhizobium* in improving the plant performance.

Analysis of the nutrient status of plant revealed that the dual inoculation with VAM and *Bradyrhizobium* improved the level of six different nutrients, viz. nitrogen (3.32%), phosphorus (0.33%), magnesium (0.407%), calcium (0.92%), zinc (26.20 ppm) and manganese (250.56 ppm) in cowpea. Among the fertilizer doses, ¼N+P+K was found best in increasing the four different nutrients, viz. phosphorus (0.37%), calcium (0.83%), iron (799.32 ppm) and manganese (280.94 ppm). The results on the interactions among the fertilizers and symbionts revealed that only quarter dose of nitrogenous fertilizer was necessary, when applied with both microsymbionts to get maximum nitrogen content of plant (4.03%). Another finding was that interaction of VAM with ½N+P+K, and N+½P+K alone yielded maximum phosphorus content of plant (0.40%).

Dual inoculation along with half the dose of phosphorus recorded the maximum magnesium (0.423%) and calcium (1.24%) content in plant.

Inoculation of microsymbionts along with the soil application of ½N+P+K ranked top in increasing the soil nitrogen to maximum. There were no significant differences among treatments and its combinations in influencing the phosphorus status of soil. Whereas in the case of potassium, the control plants showed maximum value (80 ppm) than either inoculants or its combinations. The application of either VAM or *Bradyrhizobium* or its combinations reduced the potassium content of soil.

The result of the field experiment showed that the fertilizer nutrients were easily utilized before 50 per cent flowering stage of plant and the effect of inoculation of *Bradyrhizobium* was expressed only after 50 per cent flowering stage. It was found that the treatment T_{10} (VAM+B+½N+½P+K) out perform other treatments for five characters, viz. plant height (34.83 cm), fresh weight of plant (31.67 g), dry weight of plant (9.09 g), nodule number (17.58) and fresh weight of nodules (395 mg) at 50 per cent flowering. At harvest this treatment (T_{10}) recorded maximum values in fresh weight of plant (66.17 g), dry weight of plant (18.21 g), nodule number (29.67), fresh weight of nodule (700 mg) and dry weight of nodule (318 mg).

As far as root length was concerned, T_2 (Package of Practice Recommendations of KAU, 1993) was the best treatment but, the combined inoculants also (VAM+N+½P+K and B+½N+P+K) could effectively substitute that treatment. This means that *Bradyrhizobium* and VAM could substitute half nitrogen and half phosphorus respectively as far as root length was concerned. Treatment T_{10} recorded the highest fresh weight at 50 per cent flowering and also at harvest. Another finding was that the inoculation of the microsymbionts not only reduce the use of nitrogenous and phosphatic fertilizers to half, but also regulated the plant growth to a uniform pattern.

Treatment with *Bradyrhizobium* alone (T_6) recorded maximum fresh weight of root at harvest (7.19 g) and 88.5 per cent of this weight was gained only after 50 per cent flowering stage. This trend can be seen in other treatments also. Thus the weight gaining process of the roots was found to occur more after 50 per cent flowering stage. Treatment T_4 (VAM+N+½P+K) was the best treatment in getting maximum fresh weight of root at 50 per cent flowering and dry weight both at 50 per cent flowering and at harvest. Thus half dose of phosphorus could be substituted by VAM inoculation.

Treatment T_{10} increased nodulation to 180 per cent over *Bradyrhizobium* inoculation alone. In most combinations of VAM and *Bradyrhizobium* (T_9, T_{10} and T_{11}) nodule number were more at harvest than at 50 per cent flowering. This suggests that VAM supports nodule formation greatly after 50 per cent flowering stage.

Analysis of the nutrient status of the plant showed that inoculation by *Bradyrhizobium* was effective in increasing the nitrogen content of the plant to the maximum and also it could reduce the fertilizer nitrogen to quarter dose. The data revealed that the treatment B+¼N+P+K (T_8) was the best for improving the nitrogen and VAM+B+½N+½P+K (T_{10}) was the best for improving the phosphorus and potassium content of the plant. T_{10} was the best treatment in increasing the magnesium and zinc content of the plant. The overall results showed that T_{10} (VAM+B+½N+½P+K), where dual inoculation was done and nitrogen and phosphorus fertilizers were reduced to half, was the best treatment in improving the over all performance of the plant. Another finding was that the different treatments involving the microsymbionts and different fertilizer doses were not able to alter the nitrogen, phosphorus and potassium content of soil to a significant level.

When VAM was inoculated and quarter dose of phosphorus was applied, the spore count was maximum (688.67). It was found that the phosphorus level of soil had some negative influence on the spore count of VAM in soil.

For getting maximum yield T_7 (B+½N+P+K) was found the best treatment giving 550.33 g pod yield per plot. The otherwise, best treatment T_{10} was on par with T_7.

There exists a positive response between nitrogen fixing bacteria and VAM fungus. In turn, there was a positive correlation for the nodule number with dry weight of nodules and also with yield of the plant. It is evident that both *Bradyrhizobium* and VAM inoculation positively influence most of the biometric characters and nutrient status of the plant and that they can reduce the use of nitrogen and phosphorus fertilizers. The percentage of VAM colonisation had a significant positive correlation with calcium content and significant negative correlation with manganese content of plant.

The entire study revealed that inoculation of *Bradyrhizobium* and VAM together was having a synergistic effect on the performance of the cowpea plant. The microsymbionts could substitute the fertilizers, especially nitrogen and phosphorus to the extent of half the recommendation. Thus a judicious treatment would be VAM+B+½N+½P+K, to get the best performance of cowpea.

References

REFERENCES

Aboul-Nasar, A. 1993. Effect of vesicular-arbuscular mycorrhiza and N_2 fixing bacteria on the growth and nutrient status in sorghum. *Alexandria J. agric. Res* **38**:427-437

Alexander, M. 1961. *Introduction to Soil Microbiology*. John Wiley, New York. p.472

Allen, O.N. 1953. *Experiments in Soil Bacteriology*. Burgees Publication Co., Minneapolis, U.S.A. pp.69-70

Allen, H.von., Tanneberg, A. and Schonbeck, F. 1991. Specific interaction of *Bradyrhizobium* and four VA mycorrhizal isolates in soybean. *The Rhizosphere and Plant Growth* (ed. Keister, D.L. and Cregan, P.B.) Universitat Hannover, Herrenhauser Str.2, Germany, p.376

Ames, R.N., Thyagarajan, T.R., Ahmad, M.H. and McLaughlin, W.A. 1991. Co-selection of compatible rhizobia and vesicular-arbuscular mycorrhizal fungi for cowpea in sterilized and non-sterilized soils. *Biol. Fertil. Soils* **12**:112-116

Amijee, F., Stribley, D.P. and Tinker, P.B. 1990. Soluble carbohydrates in roots of leek (*Allium porrum*) plants in relation to phosphorus supply and VA mycorrhizas. *Plant Nutrition: Physiology and Applications*. (ed. Beusichem, M.L. Van) Kluwer Academic Publishers, Netherlands. pp.161-164

Arafat, S.M., Sherif, M.A., Enany, M.H. and Saad, R.N. 1995. Effect of rhizobium and vesicular-arbuscular mycorrhizal (VAM) on growth, phosphorus and nitrogen uptake by *Vicia faba* (L.) in hydroponic culture. *Egyptian J. Soil Sci.* **35**:117-128

* Asai, T. 1944. *Uber die Mykorrhizen bildung der leguminosen pflanzen. Jap. J. Bot.* **13**:463-485

Bagyaraj, D.J. and Manjunath, A. 1980. Selection of a suitable host for mass production of VA-mycorrhizal inoculum. *Pl. Soil* **55**:495-498

Bagyaraj, D.J., Manjunath, A. and Patil, R.B. 1979. Interaction between a vesicular-arbuscular mycorrhiza and *Rhizobium* and their effects on soybean in the field. *New Phytol.* **82**:141-145

Baird, L.M. and Caruso, K.J. 1994. Development of root nodules in *Phaseolus vulgaris* inoculated with *Rhizobium* and mycorrhizal fungi. *Int. J. Pl. Sci.* **155**:633-639

Barea, J.H. and Azcon-Aguiliar, C. 1982. Quoted from Bhandari, S.C., Somani, L.L. and Gulati, I.J. 1990. Symbiotic Vesicular Arbuscular Mycorrhizas. *Biofertilizers* (ed. Somani, L.L., Bhandari, S.C., Saxena, S.N. and Vyas, K.K.) Scientific Publishers, Jodhpur, India. pp.295-311

Bermudez, M. and Azcon, R. 1996. Calcium uptake by alfalfa as modified by a mycorrhizal fungus and liming. *Symbiosis (Rehovot)* **20**:175-184

Bethlenfalvay, G.J., Brown, S.M. and Stafford, A.L. 1985. *Glycine-Glomus-Rhizobium* Symbiosis. II Antagonistic effects between mycorrhizal colonization and inoculation. *Pl. Physiol.* **79**:1054-1058

Bevege, D.I. and Bowen, G.D. 1975. Endogone strain and host plant differences in development of vesicular-arbuscular mycorrhizas. *Endomycorrhizas* (ed. Sanders, E.F., Mosse, B. and Tinker, P.B.) Academic Press, London and New York. pp.77-86

Bhaskaran, C. and Selvaraj, T. 1997. Seasonal incidence and distribution of VA-mycorrhizal fungi in native saline soils. *J. environ. Bot.* **18**:209-212

Butler, E.J. 1939. The occurrences and systematic position of the vesicular-arbuscular type of mycorrhizal fungi. *Trans. Br. mycol. Soc.* **22**:274-301

* Cabello, M.N. 1992. Interactions between VAM fungi and *Bradyrhizobium* and their effect on growth of soybean (*Glycine max*). *Boletin Micologico* 7:27-30

Carling, O.E., Richle, W.G., Brown, M.F. and Johnson, D.R. 1978. Effects of a vesicular-arbuscular mycorrhizal fungus on nitrate reductase and nitrogenase activities in nodulating and non-nodulating soybeans. *Phytopathology* **68**:1590-1596

Champawat, R.S. 1989. Effect of VAM fungi on growth and nutrition of chick pea. *Madras agric. J.* **76**:310

* Champawat, R.S. 1990a. Response of chickpea (*Cicer arietinum*) to *Rhizobium* and vesicular arbuscular mycorrhiza dual inoculation. *Acta Microbiologia Polonica* **39**:163-169

Champawat, R.S. 1990b. Effect of different VAM fungi under varying levels of phosphorus on growth and nutrition uptake of pigeon pea (*Cajanus cajan*). *Trends in Mycorrhizal Research*. (Proc. National Conference on Mycorrhiza). Haryana Agricultural University, Hisar, India. pp.141-143

Chang, C.Y., Lu, Z.H., Dai, S.J., Zhao, C.J. and Guo, D.X. 1992. Studies on selection of the best symbiotic system of high yielding and high protein content soybean, high efficient N fixation *Rhizobium* and mycorrhiza fungus. *Soybean Sci.* **11**:226-233

Chaturvedi, C. and Kumar, A. 1991. Nodulation and nitrogenase activity in gram (*Cicer arietinum* L.) as influenced by *Rhizobium* and VA-Mycorrhiza interation. *Nat. Acad. Sci. Lett.* 14:289-292

Chhabra, M.L., Singh, R.P. and Jalali, B.L. 1990. Studies on vesicular-arbucular (VA) mycorrhizal impact on growth and development of cowpea (*Vigna unguiculata* [L.] Walp). *Trends in Mycorrhizal Research*. (Proc. National Conference on Mycorrhiza). Haryana Agricultural University, Hisar, India. pp.150-151

Chhonker, P.K. and Negi, P.S. 1971. Response of soybean to rhizobial inoculation with different strains of *Rhizobium japonicum*. *Indian J. agric. Sci.* **41**:741-744

Costa, N.DEL. and Paulino, V.T. 1990. Response of leucaena to vesicular-arbuscular mycorrhizal inoculation and phosphorus fertilization. *Leuc. Res. Rep.* **11**:42-44

Costa, N.DEL., Paulino, V.T. and Rodrigues, A.N.A. 1990. Response of pigeon pea to *Rhizobium* and mycorrhiza inoculation. *Nitrogen Fixing Tree Res. Rep.* **8**:121-122

Costa, N.DEL., Paulino, V.T. and Vessey,E.A. 1992a. Phosphorus fertilization and mycorrhizal inoculation effect *Cajanus cajan* growth. *Nitrogen Fixing Tree Res. Rep.* **10**:125-126

Costa, N. DEL., Paulino, V.T. and Veasey, E.A. 1992b. Effect of phosphate fertilization and mycorrhizal inoculation on growth and phosphorus uptake of leucaena. *Leuc. Res. Rep.* **13**:8-9

* Dangeard, P.A. 1900. *Le 'Rhizophagus populinus'. Botaniste* 7:285-291

Das, P.K., Sahoo, P.N. and Jena, M.K. 1997. Effect of VA mycorrhiza and rhizobium inoculation on nutrient uptake, growth attributes and yield of green gram (*Vigna radiata* L.). *Environ. Ecol.* **15**:830-833

Devi, G.U. and Sitaramaiah, K. 1991. Influence of four species of endomycorrhizal fungi on root colonization and growth of black gram. *Indian J. Mycol. Pl. Pathol.* **21**:166-169

* Edathil, T.T., Maniam, S. and Udaiyan, K. 1994. Early establishment of native vesicular-arbuscular mycorrhizas in three vegetable crops of S. India - a comparative study. *Pertunika J. trop. agric. Sci.* **17**:157-161

* Elgala, A.M., Ishae, Y.Z., Abdel-Monem, M., El-Ghandour, I.A.I., Huang, P.M., Bollag, J.M., McGill, W.B. and Page, A.L. 1995. Effect of single and combined inoculation with Azotobacter and VA-mycorrhizal fungi on growth and mineral nutrient contents of maize and wheat plants. *Environmental Impact of Soil Component Interactions Vol.2. Metals, Other Inorganics and Microbial Activities*. (ed. Huang, P.M., Berthelin, J., Bollag, J.M. and McGill, W.B.) CRC Press Inc., Boca Raton, USA. pp.109-116

Elwan, I.M. 1993. Response of nutrient status of plants in calcareous soils receiving phosphorus fertilization and mycorrhizas. *Ann. agric. Sci.* **38**:841-849

Eranna, A. and Parama, V.R.R. 1994. Effect of liming, mycorrhizal inoculation and rock phosphate on phosphorus uptake and growth of soybean in an acid soil. *Mysore J. agric. Sci.* **28**:292-296

Ezedinma, F.O.C. 1964. Effect of nodulation with local isolates of cowpea *Rhizobium* and application of nitrate - nitrogen on the development of cowpea. *Trop. Agric.* **41**:243-249

* Frank, A.B. 1885. *Uber die auf Wurzel symbiose beruhende Ernahrung gewisser Baume durch unterir disch Pilze. Ber. deut. botan. Ges.* **3**:128-145

Geethakumari, V.L., Pushpakumari, R., Sheela, K.R. and Sivaprasad, P. 1994. Phosphorus - Mycorrhiza interaction studies in grain cowpea. *J. Soil Biol. Ecol.* **14**:25-28

Gerdemann, J.W. 1968. Vesicular-arbuscular mycorrhiza and plant growth. *A. Rev. Phytopath.* **6**:397-418

Gerdemann, J.W. and Nicolson, T.H. 1963. Spores of mycorrhizal endogone species extracted from soil by wet sieving. *Trans. Br. mycol. Soc.* **46**:234-235

Girija, V.K. 1982. Studies on host-varietal specificity for *Rhizobium* for nodulation in groundnut. M.Sc. thesis, Kerala Agricultural University, Vellanikkara, Thrissur p.84

Girija, V.K. and Nair, S.K. 1985. Occurrence of vesicular-arbuscular mycorrhiza in certain crop plants of Kerala. *Agric. Res. J. Kerala* **23**:185-188

Godse, D.B., Madhusudan, T., Bagyaraj, D.J. and Patil, R.S. 1976. Occurrence of vesicular-arbuscular mycorrhiza on crop plants in Karnataka. *Trans. Br. mycol. Soc.* **67**:169-171

Godse, D.B., Wani, S.P., Patil, R.B. and Bagyaraj, D.J. 1978. Response of cowpea (*Vigna unguiculata* (L.) Walp.) to *Rhizobium* - VA mycorrhiza dual inoculation. *Curr. Sci.* **47**:784-785

* Grundwaldt-Stocker, G. and Dehne, H.W. 1989. The use of vesicular-arbuscular mycorrhizal fungi in plant production. II. Characterisation of inocula on inorganic carrier material. *Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz* **96**:615-626

Guzman-Plazola, R.A., Ferrera-Cerrato, R. and Etchevers Barra, J.D. 1990. Effect of double inoculation *Glomus* + *Rhizobium* in early adaptation of *Leucaena leucocephala* on acid soils. *Leuc. Res. Rep.* **11**:81-83

* Hao, W.Y., Lin, X.G., Gu, X.X. and Niu, J.Q. 1991. Efficiency of VAM fungi and the prospect of their practical application in some soils. *Acta Pedologica Sinica* **28**:129-131

Hartwell, B.L. and Pember, F.R. 1911. The gain in nitrogen during a five year pot experiment with different legumes. *R.J. Agr. Expt. Sta. Bull.* 147

Heggo, A.M. and Barakah, F.N. 1994. A mycorrhizal role on phosphorus - zinc interaction in calcareom soil cultivated with corn (*Zea mays* L.). *Ann. agric. Sci.* **39**:595-608

Ho, I. 1993. Analysis of nutrient uptake by six species of vesicular-arbuscular mycorrhizal fungi in *Zea mays* L. *Indian J Mycol. Pl. Pathol.* **23**:64-69

Hogberg, P. and Kvarnstrom, M. 1982. Nitrogen fixation by the woody legume *Leucaena leucocephala* in Tanzania. *Pl. Soil* **66**:21-28

Hunter, W.J. 1994. Increased nodulation of soybean by a strain of *B. japonicum* with altered tryptophan metabolism. *Letters in appl. Microbiol.* **18**:340-342

Ianson, D.C. and Linderman, R.G. 1991. Variation in VA Mycorrhizal strain interaction with *Rhizobium* on pigeonpea. *The Rhizosphere and Plant Growth*. (ed. Keister, D.L. and Cregan, P.B.) Department of Botany and Plant Pathology, Oregon State University, Corvallis, USA. pp.371-372

Ianson, D.C. and Linderman, R.G. 1993. Variation in the response of nodulating pigeon pea (*Cajanus cajan*) to different isolates of mycorrhizal fungi. *Symbiosis (Rehovot)*. **15**:105-119

Isobe, K., Fujil, H. and Tsuboki, Y. 1992. Studies on infection characteristics of VA-mycorrhizal fungi in soybean plants. Tech. Bull. No.49 College of Agriculture and Veterinary Medicine, Nihon University. pp.1-6

Isobe, K., Fujii, H. and Tsuboki, Y. 1993. Studies on behaviour of VA-mycorrhizal fungi in soybean culture. *Japanese J. Crop. Sci.* **62**:351-358

Jackson, M.L. 1958. *Soil Chemical Analysis*. Prentice Hall of India Pvt. Ltd., New Delhi. p.498

* Janse, J.M. 1896. *Les endophytes radicaux de quelques plantes javanaises. Ann. Jard. Bot. Buitenz* **14**:53-212

KAU. 1993. Package of Practices Recommendations-Crops 1993. Directorate of Extension, Kerala Agricultural University, Mannuthy, Thrissur, Kerala

Kumari, K.S.M. and Nair, S.K. 1989. Influence of host variety on the natural incidence of VA mycorrhiza in cowpea. *Agric. Res. J. Kerala* **27**:59-60

Khan, M.K., Sakamoto, K. and Yoshida, T. 1995. Dual inoculation of peanut with *Glomus* sp. and *Bradyrhizobium* sp. enhanced the symbiotic nitrogen fixation as assessed by ^{15}N-technique. *Soil Sci. Pl. Nutr.* **41**:769-779

Kinden, D.A. and Brown, M.F. 1975. Electron microscopy of vesicular-arbuscular mycorrhizas of yellow poplar. II. Intracellular hyphae and vesicles. *Can. J. Microbiol.* **21**:1768-1780

Kumutha, K. and Santhanakrishnan, P. 1994. Enhancement of nitrogen fixation in soybean by the dual inoculation of VA mycorrhiza and *Bradyrhizobium*. *Madras. agric. J.* **81**:497-499

Kuo, C.G. and Huang, R.S. 1982. Effect of vesicular-arbuscular mycorrhizae on the growth and yield of rice-stubble cultured soybeans. *Pl. Soil* **64**:325-330

* La-Rue, J.H., Maclean, W.D. and Peakcock, W.L. 1975. Mycorrhizal fungi and peach nursery nutrition. *Calif. Agric.* **29**:7

Lee, S.S. and Ryu, C.N. 1992. Symbiosis of arbuscular mycorrhizae in the plant roots. *Korean J. Mycol.* **20**:126-133

* Lin, X.G. and Hao, W.Y. 1989. Effects of phosphorus fertilizers on VA mycorrhizal response under sterilized soil conditions. *Acta Pedologica Sinica* **26**:179-185

Lingaraju, B.S., Sreenivasa, M.N. and Babalad, H.B. 1994. Response of soybean to VA mycorrhizal fungi at varied levels of phosphorus. *J. Oilseed Res.* **11**:300-302

Mago, P. and Mukerji, K.G. 1994. Vesicular arbuscular mycorrhizae in Lamiaceae. I. Seasonal variation in some members. *Phytomorphology* **44**:83-88

Mahmoud, S.M., Badawy, F.A., Hassnein, H.G. and EL-Dosouky, M.M. 1994. N-fertilization on nodulation and yield of soybean. *Aust. J. agric. Sci.* **25**:191-198

Maia, L.C. and Kimbrough, J.W. 1993. Ultrastructural studies of spore wall of *Acaulospora morrowiae* and *A. scrobiculata. Mycol. Res.* **97**:1183-1189

Maia, L.C., Kimbrough, J.W. and Erdos, G. 1993. Problems with fixation and embedding of arbuscular mycorrhizal fungi. *Mycologia* **85**:323-330

Mallesha, B.C. and Bagyaraj, D.J. 1990. Effect of staggered inoculation of growth medium with *Glomus mosseae* and *Rhizobium* on *Leucaena. Mycorrhizal Symbiosis and Plant Growth.* (Proc. Second National Conference on Mycorrhiza held at Bangalore) Mycorrhiza Net Work of Asia and U.A.S., Bangalore. pp.104-106

Mand, S., Dahiya, B.N. and Lakshminarayana, K. 1991. Nodulation, nitrogen fixation and biomass yield by slow and fast growing cowpea rhizobia in guar under different environments. *Ann. Biol.* 7:31-38

Mane, S.S., Raut, R.S. and Kohire, O.D. 1993. Comparative performance of vesicular arbuscular mycorrhizal fungus and Rhizobium inoculation with groundnut. *Ann. Pl. Physiol.* 7:116-118

Manel, S. and Chahal, V.P.S. 1987. Effect of nitrate application and *Rhizobium* inoculation on symbiotic effectivity in *Vigna radiata* (L.) Wilezek. *Ann. Biol.* **3**:92-96

Manjunath, A. and Bagyaraj, J.D. 1986. Response of black gram, chick pea and mungbean to vesicular arbuscular mycorrhizal inoculation in an unsterile soil. *Trop. Agric.* **63**:33-35

Marks, G.C. and Kozlowski, T.T. 1973. *Ectomycorrhizae: Their Ecology and Physiology.* Academic Press, New York. p.444

Medeiros, C.A.B., Clark, R.B. and Ellis, J.R. 1995. Effect of excess manganese on mineral uptake in mycorrhizal sorghum. *J. Pl. Nutr.* **18**:201-217

Mosse, B. 1957. Growth and chemical composition of mycorrhizal and non-mycorrhizal apples. *Nature* **179**:922-924

Mosse, B., Powell, C.L. and Hayman, D.S. 1976. Plant growth responses to vesicular-arbuscular mycorrhiza. IX. Interactions between VA-mycorrhiza, rock phosphate and symbiotic nitrogen fixation. *New Phytol.* **76**:331-342

Nair, S.K. and Girija, V.K. 1986. Incidence of vesicular-arbuscular mycorrhiza in certain crop plants of Kerala. *Extended Summaries of Papers, Workshop on Beneficial Microbes in Tree Crop Management.* Central Plantation Crops Research Institute, Kasargode, Kerala. pp.24-25

Nair, S.K. and Sivaprasad, P. 1981. Preliminary study on nodulation in cowpea in acid soil. *Agric. Res. J. Kerala* **20**(1):98-100

Nutman, P.S. 1976. IBP Field experiments on nitrogen fixation by nodulated legumes. *Symbiotic Nitrogen Fixation in Plants.* (ed. Nutman, P.S.) Cambridge University Press, Cambridge. p.584

Ortas, I. 1996. The influence of use of different rates of mycorrhizal inoculum on root infection, plant growth and phosphate uptake. *Commun. Soil Sci. Pl. Anal.* **27**:2935-2946

Page, A.L. 1982. *Methods of Soil Analysis Part II.* 2nd ed. American Society of Agronomy. Inc., Madison, Wisconsin, USA. pp.301-334

Pai, G., Bagyaraj, D.J., Ravindra, T.P. and Prasad, T.G. 1994. Calcium uptake by cowpea as influenced by mycorrhizal colonisation and water stress. *Curr. Sci.* **66**:444-445

Pandher, M.S., Bhandari, S.C. and Gupta, R.P. 1986. Varietal response to dual inoculation of VA-mycorrhiza and *Rhizobium* in mung bean. *Abstracts of papers*. National Seminar on Microbial Ecology, Tamil Nadu Agricultural University, Coimbatore. p.22

Pattanaik, R., Sahu, S., Padhi, G.S. and Mishra, A.K. 1995. Effect of inoculation of vesicular arbuscular mycorrhiza on horse gram (*Macrotyloma uniflorum*) grown in soil in iron-mine area. *Indian J. agric. Sci.* **65**:186-190

* Paula, M.A., Siqueira, J.O. and Hoshika. E. 1990. Growth, mineral nutrition and production of soybeans inoculated with different populations of native vesicular-arbuscular mycorrhizal fungi. *Revista Brasileira de Ciencia do Solo* **14**:151-156

Phillips, J.M. and Hayman, D.S. 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Trans. Br. mycol. Soc.* **55**:158-161

Pillai, M.V.R. 1989. Symbiosis of *Rhizobium* and VA mycorrhiza in subabul. Ph.D. thesis, Kerala Agricultural University, Vellanikkara, Thrissur. p.116

Plenchette, C. and Morel, C. 1996. External phosphorus requirements of mycorrhizal and non-mycorrhizal barley and soybean plants. *Biol. Fertil. Soils* **21**:303-308

Potty, V.P. 1978. Occurrence of vesicular-arbuscular mycorrhiza in certain tuber crops. *J. Root Crops* 4:49-50

Powell, C.L. 1981. Inoculation of barley with efficient mycorrhizal fungi stimulates seed yield. *Pl. Soil* **59**:487-489

Raghava, N., Raghava, R.P. and Kumar, P. 1993. Protein and leghaemoglobin content in nodules in relation to flowering and pod formation in *Vigna unginculata* (L.) Walp. *Indian J. Pl. Physiol.* **36**:67-68

Rahman, M.M. and Sanoria, C.L. 1990. Effect of *Bradyrhizobium japonicum* strains on growth and N_2 fixation rate of soybean. *Ann. agric. Res.* **11**:205-207

Rai, R., Singh, S.N. and Murtuza, M.D. 1977. Different responses of *Rhizobium* strains of bengal gram grain yield. *Curr. Sci.* **46**:572-573

Rajapakse, S. 1987. Response of cowpea (*Vigna unguiculata* (L.) Walp) cultivars to vesicular-arbuscular mycorrhizal symbiosis. *Dissertation Abstracts International, B (Science and Engineering)*, Texas A & M University, USA. 23B-24B

Rajapakse, S. and Miller, J.C.Jr. 1987. Intraspecific variability of VA mycorrhizal symbiosis in cowpea (*Vigna unguiculata* (L.) Walp.). *Genetic Aspects of Plant Mineral Nutrition.* (ed. Gabelman, W.H. and Loughman, B.C.) Martinus Nijhoff, Netherlands. pp.523-536

Ramaraj, B., Sharmugam, N., Jalali, B.L. and Chand, H. 1990. Interaction of vesicular arbuscular mycorrhiza (*Glomus etunicatum*) and *Rhizobium* in cowpea. *Trends in Mycorrhizal Research.* (Proc. National Conference on Mycorrhiza) Haryana Agricultural University, Hisar, India. pp.107-108

Rathore, V.P. and Singh, H.P. 1995. Influence of vesicular-arbuscular mycorrhizal fungi and phosphate in maize. *J. Indian Soc. Soil Sci.* **43**:207-210

Read, D.J. and Stribley, D.P. 1973. Quoted from Bhandari, S.C., Somani, L.L. and Gulati, I.J. 1990. Symbiotic Vesicular Arbuscular Mycorrhizas. *Biofertilizers* (ed. Somani, L.L., Bhandari, S.C., Saxena, S.N. and Vyas, K.K.) Scientific Publishers, Jodhpur, India. pp.295-311

Ross, J.P. and Harper, J.A. 1970. Effect of *Endogone* mycorrhiza on soybean yields. *Phytopathology* **60**:1552-1556

Sahu, S.K. and Behera, B. 1972. Note on the effect of *Rhizobium* inoculation on cowpea, groundnut and green gram. *Indian J. Agron.* **17**:359-360

Sanders, F.E. and Tinker, P.B. 1971. Mechanism of absorption of phosphate from soil by *Endogone* mycorrhizas. *Nature* **233**:278-279

Santhi, A. and Sundarababu, R. 1995. Effect of phosphorus on the interaction of vesicular-arbuscular mycorrhizal fungi with *Meloidogyne incognita* on cowpea. *Nematologia Mediterranea* **23**:263-265

* Satizabal, E.J.H., Saif, S.U.R. 1987. Interaction between vesicular-arbuscular mycorrhiza and leguminous *Rhizobium* on an oxisol of the Eastern Plains of Colombia. *Acta Agronomica* **37**:7-21

Schenck, N.C. and Hinson, K. 1973. Response of nodulating and non-nodulating soybeans to a species of *Endogone* mycorrhiza. *Agron. J.* **65**:849-850

Schmid, E. and Oberwinkler, F. 1995. A light and electron-microscopic study on a VA-host fungus interaction in gametophytes and young sporophytes of the Gleicheniaceae. *New Phytol.* **129**:312-324

Sen, S. and Rao, W.V.B.S. 1953. Phosphate fertilization for legumes. *Rev. Series*, I.C.A.R., New Delhi. **3**:32

Senaratne, R. and Ratnasinghe, D.S. 1995. Nitrogen fixation and beneficial effects of some grain legumes and green manure crops on rice. *Biol. Fertil. Soils* **19**:49-54

Seripong, S. and Masayna, W. 1984. Effect of phosphorus and potassium on the growth, nodulation and nutrient status of cowpea in selected Thai soil. *Soil Science as a Tool for Rural Development Volume I.* (Proc. of the Fifth ASEAN Soil Conference held at the Department of Land Development, Khon Kaen University, Bangkok, Thailand). Khon Kaen University, Khon Kaen, Thailand, pp.1-12

Shaktawat, M.S. 1988. Response of cowpea to phosphorus and *Rhizobium* inoculation. *Indian J. Agron.* **33**:341-342

Shamim, D., Ahmed, T. and Ayub, N. 1994. Influence of seasonal variations on VAM infection in perennial plants. *Pakisth. J. Phytopath.* 6:77-86

Singh, C.S. 1990. Vesicular-arbuscular (VA) mycorrhiza in presence of *Rhizobium* sp. enhances nodulation, N_2 fixation, N utilization of pigeon pea (*Cajanus cajan*) as assessed with a ^{15}N technique. *Trends in Mycorrhizal Research.* (Proc. National Conference on Mycorrhiza) Haryana Agricultural University, Hisar, India. pp.152-154

Singh, C.S. 1996. Arbuscular mycorrhize (AM) in association with *Rhizobium* sp. improves nodulation, N_2 fixation and N utilization of pigeonpea (*Cajanus cajan*) as assessed with a ^{15}N technique in pots. *Microbiol. Res.* **151**:87-92

Singh, H.P. 1990. Response of dual inoculation with *Bradyrhizobium* and VA-Mycorrhiza or phosphate solubilizers on soybean in Mollisol. *Trends in Mycorrhizal Research.* (Proc. National Conference on Mycorrhiza) Haryana Agricultural University, Hisar, India. pp.120-121

Singh, H.P. 1994. Response to inoculation with *Bradyrhizobium.* Vesicular-arbuscular mycorrhiza and phosphate solubilizing microbes on soybean in a Mollisol. *Indian J. Microbiol.* 34:27-31

Singh, R.S., Hardip Singh and Kang, M.S. 1992. Effect of soil depth and seasonal change on spore population and mycorrhizal colonisation of kinnow and rough lemon seedlings. *Indian Phytopath.* **45**:337-343

Sivaprasad, P., Hedge, S.V. and Rai, P.V. 1983. Effect of *Rhizobium* and mycorrhizal inoculation on growth of *Leucaena. Leuc. Res. Rep.* 4:42

Sivaprasad, P., Jatinder Singh and Rai, P.V. 1984. Occurrence of vesicular-aubuscular mycorrhiza (VAM) in cocoa (*Theobromae cacao*) and its influence on the growth and phosphorus nutrition. *Proc. Sixth Symposium on Plantation Crops*. Oxford and IBH Publishing Co., New Delhi, pp.245-253

Sivaprasad, P. and Rai, P.V. 1991. Synergistic association between *Glomus fasiculatum* and *Rhizobium* sp. and its effects on pigeon pea (*Cajanus cajan*). *Indian J. agric. Sci.* **61**:97-101

Sivaprasad, P., Pillai, M.V.R. and Nair, M.C. 1982. Occurrence of endotropic mycorrhiza in rubber (*Hevea braziliensis* Muell. Arg.) *Agri. Res. J. Kerala* **20**:101-102

Sivaprasad, P. and Shivappashetty, K. 1980. Response of cowpea (*Vigna unginculata* (L.) Walp.) to *Rhizobium* seed inoculation. *Agric. Res. J. Kerala.* **18**:204-207

*Smith, S.E., St.John, B.J., Smith, F.A. and Nicholas, D.J.D. 1985. Quoted from Bhandari, S.C., Somani, L.L. and Gulati, I.J. 1990. Symbiotic Vesicular Arbuscular Mycorrhizas. *Biofertilizers* (ed. Somani, L.L., Bhandari, S.C., Saxena, S.N. and Vyas, K.K.) Scientific Publishers, Jodhpur, India. pp.295-311

Smolin, V.Yu. and Shabaev, V.P. 1992. Chemical composition of soybean plants inoculated with nodule bacteria and rhizosphere pseudomonads or endomycorrhizal fungi, with localized application of nitrogen fertilizer. *Agrokhimiya* **11**:73-79

Soddi, R.R., Sreenivasa, M.N., Chittapur, B.H. and Babalad, H.B. 1994. Field response of cowpea to the dual inoculation of VA mycorrhiza and *Rhizobium* at different fertilizer levels. *J. Maharashtra agric. Univ.* **19**:459-460

* Sreenivasa, M.N., Krishnaraj, P.U., Gangadhara, G.A. and Manjunathaiah, H.M. 1993. Response of chilli (*Capsicum annum* L.) to the inoculation of an efficienct vesicular-arbuscular mycorrhizal fungus. *Scientia Horticulturae* **53**:45-52

*Stamford, N.P., Vieira, I.M. DE M.B., Santos, D.R. Dos., Rosalia, C.E. DE and Santos, S. 1990. Selection of *Bradyrhizobium* for cowpea grown on acid soil (LUA) on semi arid Brazil. *Pesquisa Agropecuaria Brasileira* **25**:545-552

Subbiah, B.V. and Asija, G.L. 1956. A rapid procedure for estimation of available nitrogen in soils. *Curr. Sci.* **25**:259-260

Sulochana, K.K. and Nair,S.K. 1985. Occurrence of VAM in some local cultivars of tapioca. *Proc. of the National Symposium on TropicalTuber Crops Production and Utilization*: Central Tuber Crops Research Institute, Trivandrum, pp.27-28

Takankhar, U.G., Mane,S.S., Kamble, B.G. and Suryawanshi, A.P. 1998. Phosphorus uptake at different stages and yield attributes of gram crop as affected by P and N fertilization and *Rhizobium* inoculation. *J. Soils Crops* **8**:53-57

Tarafdar, J.C. and Rao, A.V. 1997. Response of arid legumes to VAM fungal inoculation. *Symbiosis (Rehovot)* **22**:265-274

Thakur, A.K. and Panwar, J.D.S. 1995. Effect of *Rhizobium* VAM interactions on growth and yield in mungbean (*Vigna radiata* [L.] Wilczek.). *Indian J. Pl. Physiol.* **38**:62-65

Thyagarajan, T.R. and Ahmad, M.H. 1993. Influence of a vesicular-arbuscular mycorrhizal fungus on the competitive ability of *Bradyrhizobium* sp. for nodulation of cowpea *Vigna unguiculata* (L.) Walp. in non-sterilized soil. *Biol. Fertil. Soils* **15**:294-296

Thyagarajan, T.R., Ames, R.N. and Ahmad, M.H. 1992. Response of cowpea (*Vigna unguiculata*) to inoculation with co-selected vesicular-arbuscular mycorrhizal fungi and *Rhizobium* strains in field trials. *Can. J. Microbiol.* **38**:573-576

Trappe, J.M. 1982. Synoptic keys to the genera and species of zygomycetous mycorrhizal fungi. *Phytopathology* **72**:1102-1108

* Treub, M. 1885. Onderzoekingen over sereh-zick Suikerriet. Meded. Pl. Tuin, Batavia., 2. Vander Pijl, L. 1934. *Die Mykorrhiza von Burmannia und Epirrhizanthes und die Fortflan zung ihres kndophyten. Rec. Trav. Bot. Nierl.* **31**:761-779

* Urzua, H., Munoz, P. and Borie, F. 1993. Effect of VA-mycorrhizae on N_2 fixation in white clover growing in soils in the southern region of Chile. *Ciencia e Investigacion Agraria* **20**:47-54

Vejsadova, H., Hrselova, H., Prilcryl, Z. and Vancura, V. 1988. Inter relationships between vesicular-arbuscular mycorrhizal fungi, *Bradyrhizobium japonicum* and soybean plants. *Interrelationships between Micro-organisms and Plants in Soil* (ed. Vancura, V. and Kunc, F.). Elsevier Science Publishers, Amsterdam, pp.115-123

Vincent, J.M. 1970. *A Manual for the Practical Study of Root Nodule Bacteria.* Brockwell Scientific Publishers, Oxford and Edinburg. p.164

Watanabe, F.S. and Olsen, S.R. 1965. Test of an ascorbic acid method for determining phosphorus in water and $NaHCO_3$ extracts from soil. *Proc. Soil Sci. Soc. Am.* **29**:677-678

Yocom, D.H. and Boosalis, M.G. 1991. Mycorrhizal fungi increase yields of winter wheat. *The Rhizosphere and Plant Growth.* (ed. Keister,D.L. and Cregan, P.B.) Department of Biology, Millersville University, Millersville, U.S.A. p.382

* Originals not seen

Appendices

APPENDIX-I

Composition of F.A.A.

Formalin (40%)	- 5 ml
Glacial acetic acid	- 5 ml
Ethanol (95%)	- 90 ml

APPENDIX-II

Composition of Trypanblue

Trypanblue	- 50 mg
Lacto phenol	- 100 ml

Lactophenol

Lactic acid	- 10 ml
Phenol	- 10 ml
Glycerol	- 20 ml
Water	- 20 ml

APPENDIX-III

Composition of Yeast Extract Mannitol Agar (Allen, 1953)

Mannitol	- 10.0 g
K_2HPO_4	- 0.5 g
$MgSO_4.7K_2O$	- 0.2 g
NaCl	- 0.1 g
$CaCO_3$	- 3.0 g
Yeast extract	- 1.0g
Congo red (1% aqueous solution)	- 2.5 ml
Agar	- 15.0 g
Distilled water	- 1000 ml
pH	- 7.0

APPENDIX-IV

Composition of Glucose Peptone Agar Medium

Glucose	- 10.0 g
Peptone	- 20.0 g
NaCl	- 5.0 g
Agar	- 15.0 g
Distilled water	- 1000 ml
Bromocresol purple (1.6% in ethanol)	- 10 ml
pH	- 7.2

INTERACTION BETWEEN VA MYCORRHIZA AND *BRADYRHIZOBIUM* IN COWPEA

By

S. BEENA

ABSTRACT OF THE THESIS

Submitted in partial fulfilment of the requirement for the degree of

DOCTOR OF PHILOSOPHY

(PLANT PATHOLOGY)

Faculty of Agriculture
Kerala Agricultural University

DEPARTMENT OF PLANT PATHOLOGY
COLLEGE OF HORTICULTURE
Vellanikkara, Thrissur-680 656
KERALA, INDIA
1999

ABSTRACT

An investigation on Interaction between VA mycorrhiza and *Bradyrhizobium* in cowpea was carried out at the College of Horticulture, Vellanikkara during the period 1994-97. The main objectives of the study were to explore the beneficial effects of dual inoculation of VA mycorrhiza and *Bradyrhizobium* in enhancing the uptake of nitrogen and phosphorus and to evolve specific recommendation on the use of these inoculants together in reducing the use of nitrogenous and phosphatic fertilizers.

The results of this investigation revealed the following conclusions. The survey on the natural occurrence of VAM in cowpea showed a fairly good VAM colonisation in all the plant samples collected from the five locations. VAM colonisation was found more in lateral roots than in tap root. The VAM colonisation and spore count were more during rainy season compared to summer. The predominant spores present in all the survey locations were identical to that of *Glomus* sp.

After the screening of VAM, the local isolate from farmer's field at Nadathara was selected for further studies based on its performance.

Observation of cowpea plants at 10 days after sowing showed the presence of nodules and VAM colonisation in the roots of inoculated plants. Both the nodulation and VAM colonisation were found to reach a peak at 40th day after sowing. Dual inoculation was found to have a synergistic effect in nodulation and VAM colonisation.

Light microscopy of VAM infected roots showed both 'H' and 'V' shaped branching of hyphae, arbuscules and vesicles of VAM fungus.

Electron microscopy revealed two types of fungal penetrations into the roots. It also showed the terminal attachment of oval shaped vesicles and highly branched arbuscules with short twisted branches with bulged tips in the root cortex.

Electron microscopic study of nodules revealed that the nodule surface was free of VAM hypha, but the inner tissues of nodules had the hyphal presence.

The pot culture experiment recorded synergistic effect in dual inoculation with VAM and *Bradyrhizobium* in enhancing five biometric characters, viz. number of leaves, fresh and dry weights of plant, number of nodules and fresh weight of nodules.

Among the inoculant-fertilizer interactions, eventhough all observations except fresh weight of plant recorded non significance between treatments, those in which VAM was a partner, recorded the maximum values for all the observations except root length.

Dual inoculation with VAM and *Bradyrhizobium* improved the level of six nutrients, viz. nitrogen, phosphorus, magnesium, calcium, zinc and manganese in cowpea.

The result of interaction with microsymbionts and fertilizer levels recorded inconsistent values. Treatment with VAM + B + ¼ N + P + K recorded maximum nitrogen, VAM + ½ N + P + K and N + ½ P + K alone recorded maximum phosphorus and dual inoculation along with ½N+P+K recorded maximum magnesium and calcium content of plant.

Inoculation of VAM + *Bradyrhizobium* along with ½ N + P + K ranked top in increasing the soil nitrogen to the maximum. There were no significant differences among treatments and its combinations in influencing the phosphorus status of soil. The application of either VAM or *Bradyrhizobium* or its combinations reduced the potassium content of soil.

The results of the field experiment revealed that the treatment T_{10} (VAM+B+½N+½P+K) out perform other treatments in five characters, viz. plant height, fresh and dry weights of plant, nodule number and fresh weight of nodules at 50 per cent flowering. At harvest this treatment recorded the maximum values in fresh and dry weights of plant, nodule number and fresh and dry weights of nodules. Treatment T_{10} increased nodulation to 180 per cent over *Bradyrhizobium* inoculation alone.

Bradyrhizobium+½N+P+K was found to be the best treatment in improving the nitrogen content of plant, whereas VAM+B+½N+½P+K was the best for improving the phosphorus, potassium, magnesium and zinc content of plant.

Another finding was that the different treatments involving microsymbionts and different fertilizer doses were not able to influence the nitrogen, phosphorus and potassium content of soil to a significant level.

The percentage of VAM colonisation was not significantly affected by dual inoculation and also by the application of different levels of fertilizers.

Dual inoculation had no influence on the spore count of VAM. The high level of phosphorus in soil had some negative influence on the spore count.

Treatment B+½N+P+K was the best in increasing the yield to the maximum, which was on par with T_{10}.

The overall results showed that T_{10} (VAM+B+½N+½P+K) where dual inoculation was done and nitrogen and phosphorus fertilizers were reduced to half of the recommended dose, was the best treatment in improving the biometric characters and nutrient status and yield of cowpea. Thus a judicious treatment would be VAM+B+½N+½P+K to get the best performance of cowpea.

The percentage of VAM colonisation recorded a significant positive correlation with nodule number and fresh and dry weights of nodule.

There was a significant positive correlation between nodule number and plant dry weight and also with yield. The percentage of VAM colonisation had a significant positive correlation with calcium content and significant negative correlation with manganese content of plant.