EFFECT OF INOCULATION OF VESICULAR-ARBUSCULAR MYCORRHIZA ON NODULATION AND PHOSPHORUS UPTAKE IN COWPEA

By

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THESIS

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D E C L A R A T I O N

I hereby declare that this thesis entitled "Effect of inoculation of vesicular-arbuscular mycorrhiza on nodulation and phosphorus uptake 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, associateship, fellowship, or other similar title, of any other University or Society.

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Vellayani,

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C E R T I F I C A T E

Certified that this thesis, entitled "Effect of inoculation of vesicular-arbuscular mycorrhiza on nodulation and phosphorus uptake in cowpea" is a record of research work done independently by Smt. MEENA KUMARI. K.S. under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship, or associateship to her.

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INTRODUCTION

INTRODUCTION

The term mycorrhiza meaning a 'fungus-root' was first used by the German botanist, Albert Bernhard Frank in the year 1885 to describe a beneficial association between certain fungi and roots of higher plants. In fact, many plants depend on such an association for an adequate supply of various nutrients, especially from low fertile soils. The four common types of mycorrhizae are the ecto-mycorrhiza, ericoid mycorrhiza, orchidaceous mycorrhiza and the vesicular-arbuscular mycorrhiza. However, among cultivated crops, the most predominant type is that of vesicular-arbuscular mycorrhiza. This is characterized by the presence of one or more parallel strands of fungal mycelia in the host root cortex. They frequently branch dichotomously forming the typical arbuscular structures within the cortical cells. The hyphal branches also bear many characteristic oval to spherical shaped vesicles which may be either inter or intra cellular. These vesicles and arbuscules along with the associated mycelia in the host root system constitute a typical vesicular-arbuscular mycorrhiza. The fungi that form this type of mycorrhiza mainly belong to the genera of Acaulospora, Endogone, Gigaspora, Glaziella, Glomus, Modicella and Sclerocystis of family Endogonaceae and class Zygomycetes.

It is now well established that vesicular-arbuscular mycorrhiza enables many crop plants to absorb more effectively some of the nutrient elements like P, Zn, Mn, Cu, Fe and Mg from the soil. However, in legumes there is another type of association, a sort of dual symbiosis, comprising Rhizobium and VA-mycorrhiza. This is not only beneficial in enhancing the uptake of various nutrients from the soil but also favours better nodulation and nitrogen fixation by Rhizobium. But, in Kerala, not much work is done to understand about this phenomenon in detail and to exploit the same for a more economic cultivation of pulses. Therefore, the present investigation was taken up with cowpea as a mutual host for a specific strain of Rhizobium and two known cultures of VA-mycorrhizae.

Three separate pot culture experiments were conducted for this purpose. Initially, 10 cowpea varieties were screened for the extent of VA-mycorrhizal infection under natural condition. Based on this, a variety which had the maximum infection was chosen for further studies. This in fact consisted of two separate experiments to find out the effect of age of host plant on VA-mycorrhizal infection and a more detailed investigation on the effect of combined inoculation of Rhizobium and VA-mycorrhiza on nodulation, phosphorus uptake and yield in cowpea. The different methods used for these experiments and the results obtained therein are presented in this thesis.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Endomycorrhizae which have been know to mankind for over 100 years, probably existed in our planet some 300 million years ago. In fact, some of the early fungal infections observed in fossilized roots of Devonian and carboniferous periods were similar to the mycorrhizal infections studied today (Osborn, 1909; Kidston and Lang, 1921). In 1885, a typical VA-mycorrhizal association was first reported by Treub in Sugarcane. Extensive studies by Janse (1896) in Java also revealed the presence of VA-mycorrhiza in nearly 69 of the 75 plant species belonging to 56 families comprising of Bryophytes, Pteridophytes, Gymnosperms and various Angiosperms. Since 1896, a number of reports were published with regard to the occurrence of VA-mycorrhiza in plants like poplar (Dangeard, 1900), pigeon pea (Jones, 1924), *Leucaena*, *Phaseolus*, *Stylosanthes* (Butler, 1939), rubber (Wastie, 1965), grapes, citrus and tomato (Gerdemann, 1968), coconut (Lilly, 1975), cowpea, capsicum, *Eleusine coracana*, *Pennisetum typhoides*, soybean (Godse *et al*., 1976), potato (Thomazini, 1979) and in upland rice, olive, onion, cacao, tobacco, sugarcane, pineapple, lettuce, strawberries, apple, coffee, tea, papaya and oilpalm (Hayman, 1982). Similar incidences of VA-mycorrhiza in a wide variety of commonly cultivated crops in Kerala were reported by Potty (1978) in tuber crops such as cassava, sweet potato and coleus, Sivaprasad et al. (1984) in cacao, Girija and Nair (1985) in upland rice, cassava, cashew, coconut, rubber, pepper, betel vine, green gram, red gram, groundnut, stylosanthes, centrosema, subabul, siratro, para grass, guinea grass, dinanath grass, congo-signal grass, hybrid napier, chillies, brinjal, mango, citrus, pineapple, tomato, banana, jack, nutmeg and clove and by Nair and Girija (1986) in mango, custard apple, silk cotton, avocado, cinnamon, tamarind, bread fruit, guava, eucalyptus, arecanut, coffee, sapota and rambutan.

Gallaud, as early as in 1905 reported a staining technique with cotton blue in lactic acid for observing root infection by VA-mycorrhiza. Acid fuchsin in picric acid was used by O'Brien and McNaughton (1928) for this purpose. However, it was Phillips and Hayman (1970) who developed a proper staining technique for VA-mycorrhiza by using 0.05 per cent trypan blue in lactophenol after clearing the roots initially in 10 per cent KOH at $90^{\circ}C$ for one hour. In 1955, Gerdemann used a method of wet sieving and decanting for the isolation of VA-mycorrhizal spores from soil. Later, Mosse and Jones (1968) found that these spores could also be collected from soil by differential sedimentation in a column of gelatin.

The possibility of surface sterilization of VA-mycorrhizal spores for establishing a known mycorrhizal infection was first reported by Mosse (1962). But it was Nicolson (1967) who developed a viable technique for this purpose. In this method, individual spores of VA-mycorrhiza were placed initially in the collar region of a funnel filled with sterilized sand and seeded with a suitable host plant like sudan grass. These seedlings were allowed to grow for three or four weeks in order to enable the proper establishment of mycorrhizal infection in the host root system. They were then transferred to fresh pots containing a mixture of sterilized sand and soil in the ratio of 1 : 1 for further multiplication of the inoculum. Hayman (1982) reported that a number of alternate crops like onion, strawberry, white clover, black pepper, citrus, Nardus stricta, Coprosoma robusta and Stylosanthes sp. could also be successfully used for the mass production of VA-mycorrhizal inoculum.

Mosse (1977) observed that in unsterilized soil, an exotic culture of VA-mycorrhiza was more stimulatory for nodulation and phosphorus uptake in Stylosanthes. Similarly, Powell (1977) also reported an increase in shoot growth in clover pre-inoculated with an E_3 strain of Glomus sp. when compared to plants inoculated with only an indigenous strain of VA-mycorrhiza. Barea *et al*. (1980) made a comparative study on the effect of introduced and indigenous VA-mycorrhizal fungi on nodulation, growth and nutrient uptake in *Medicago sativa* and found that the introduced culture was more beneficial to the crop plant.

Influence of host variety and age of host plant on VA-mycorrhizal infection

Bertheau *et al*. (1980) first reported an effect of host variety on infection by VA-mycorrhiza in wheat. They observed that the response of 20 wheat cultivars to infection by *Glomus mosseae* varied considerably, probably due to an influence of host variety. Azcon and Ocampo (1981) also noticed such a variation for mycorrhizal infection in 13 different cultivars of wheat. Ollivier *et al*. (1983) studied the influence of host variety in cowpea for the expression of three vesicular-arbuscular endomycorrhizal associations and found that in two cowpea cultivars, inoculation with *Glomus* E_3 and *Glomus mosseae* considerably stimulated the plant growth. A similar result was also reported by Pandher *et al*. (1986) in mung bean.

Sutton (1973) observed a three-phase pattern for mycorrhizal development in several crops grown under controlled conditions. There was an initial lag phase of 20 to 25 days for mycorrhizal infection mainly due to the time taken for spore germination, germtube growth and penetration of host root system by the Endogone sp. During the second phase, lasting for 30 to 35 days, there was an extensive root colonisation coinciding with most of the shoot growth and the development of external Endogone mycelium leading to multiple infections. However, in the third and final phase, the proportion of mycorrhizal to non-mycorrhizal roots remained more or less constant. Saif and Khan (1975) found that both season as well as age of host plant had a significant effect on colonisation by Endogone sp. in wheat grown under field condition. Smith and Daft (1977) also stressed the importance of time factor in the proper development of a tripartite symbiosis between Medicago sativa, Rhizobium and mycorrhiza. They further noted that the mycorrhizal plants showed more extensive nodulation along with higher rates of nitrogenase activity from two weeks onwards. However, at the time of harvest, although significant differences were observed in plant dry weight, nitrogen and phosphorus contents, the nitrogenase activity of root nodules remained more or less constant. An early increase in mycorrhizal infection up to 35 days in onion was also reported by Manjunath and Bagyaraj (1981).

<u>Effect of VA-mycorrhizal infection on nodulation by *Rhizobium* in legumes.</u>

Asai (1944) made a detailed study on the effect of mycorrhizal association in legumes. He found that the formation of adequate number of root nodules by *Rhizobium* in different legumes such as *Trifolium*, *Melilotus* and *Medicago* spp. in sterilized soil depended on the addition of 5 to 10 g of unsterilized garden soil which in fact lead to the development of VA-mycorrhiza in their root system. Ross and Harper (1970) observed that the growth and yield of nodulating soybean were increased after inoculation with *Glomus mosseae* in fumigated soil. Schenck and Hinson (1973) grew two soybean isolines in small field plots and found that VA-mycorrhizal inoculation increased the growth of nodulating line by 53 per cent but had no significant effect on the non-nodulating line. A strong stimulation of nodulation and growth was also noted by Crush (1974) in *Centrosema pubescens*, *Stylosanthes guanensis*, *Trifolium repens* and *Lotus pedunculatus* due to mycorrhizal association.

Daft and Giahmi (1974) found that infection of *Phaseolus* with *Endogone* and *Rhizobium* in comparison to *Rhizobium* alone significantly increased the growth, number and weight of root nodules, phosphorus and total protein content of plants. Daft and Giahmi (1976) also reported that plant size, fruit yield and the chemical content of shoot, root and seeds were superior in groundnut infected with *Glomus mosseae*. Mosse *et al*. (1976) examined the interaction between VAM, utilization of rock phosphate and nodulation in three legumes such as clover, *Stylosanthes* and Centrosema and found that these plants nodulated in P-deficient soil only when they were also mycorrhizal and that added rock phosphate greatly improved nodulation and nitrogen fixation of such mycorrhizal plants. Bagyaraj *et al*. (1979) reported that inoculation with *Glomus fasciculatus* greatly improved nodulation and nitrogen fixation in field grown soybean along with *Rhizobium japonicum*. Carling *et al*. (1979) observed that the colonization rate and growth response of soybean plants inoculated with VA-mycorrhizal fungi improved with an increasing quantity of *G. fasciculatus* inoculum. Hayman and Mosse (1979) also got improved growth in white clover under field conditions due to mycorrhizal inoculation.

Owusu - Benhoah and Mosse (1979) reported a four fold increase in the growth of lucerne due to inoculation with *Glomus caledonicum* in low phosphate soils. Smith *et al*. (1979) observed that a rapid establishment of VA-mycorrhiza in clover was associated with improved nodulation and increased nodule efficiency. Such a result was also reported by Azcon et al. (1979) in Medicago sativa. Krishna et al. (1982) found that in groundnut, there was an increase in dry matter content and yield up to three fold in sterilized soil and 1.2 fold in unsterilized soil due to mycorrhizal inoculation.

Green et al. (1983) studied the influence of VA-mycorrhizal fungi on nodulation and growth of subterranean clover and found that subterranean clover plants inoculated with Glomus fasciculatus, Glomus mosseae or both had 2, 1.4 to 1.9 times as many nodules as in uninoculated control plants. Sivaprasad et al. (1983) reported that inoculation with Rhizobium and mycorrhiza increased the growth, nodulation and phosphorus content of Leucaena leucocephala. Bala and Singh (1983) observed that in lentil (Lens esculenta) growth, dry matter production, nodulation and nitrogen fixation were improved with VA-mycorrhizal inoculation. Pandher et al. (1986) also obtained a higher level of nodulation in mung bean when inoculated with Rhizobium and VA-mycorrhiza.

Godse et al. (1978) found that VA-mycorrhiza increased nodulation, root and shoot development of cowpea not only in plants inoculated with Rhizobium but also in naturally nodulated plants. La Torraca (1979) also reported an increase in nodulation and yield in cowpea due to inoculation of VA-mycorrhiza. Bagyaraj and Manjunath (1980) got significant increases in root and shoot dry weights of cowpea inoculated with Glomus fasciculatus. Islam et al. (1980) found that among transplanted seedlings of cowpea, with and without Glomus fasciculatus inoculation, the growth parameters were higher in plants inoculated with VA-mycorrhiza in combination with rock phosphate application. Islam and Ayanaba (1981) also obtained higher yield in cowpea inoculated with Glomus mosseae. Manjunath and Bagyaraj (1984) studied the response of cowpea to phosphate and dual inoculation with VA-mycorrhiza and Rhizobium and observed that plants inoculated with both the organisms and supplemented with phosphorus recorded higher shoot dry weight, nitrogen and phosphorus content.

Effect of VA-mycorrhizal infection on nutrient uptake in crop plants

The importance of mycorrhiza in the absorption of phosphorus and other nutrient elements was first reported by Mosse (1957). She observed that mycorrhizal apple absorbed more P, K, Fe and Cu than non-mycorrhizal plants. Gray and Gerdemann (1967), Bowen and Mosse (1969) also found that endomycorrhizal association in many plants greatly increased the uptake of P and Zn from a nutrient solution. Ross and Harper (1970) reported that mycorrhizal soybean plants accumulated greater amounts of N, P, Ca, Cu and Mn in their foliage than non-mycorrhizal plants. Gilmore (1971) found that inoculation with two Endogone mycorrhizal fungi completely corrected the Zn deficiency in peach plants. However, Hayman and Mosse (1972) reported that although mycorrhizal onion plants had taken up more P and grown larger, the proportion of ^{32}P to total P taken up by the mycorrhizal and non-mycorrhizal plants after ten weeks was not significantly different. In an experiment conducted to study the effect of VA-mycorrhiza on sulphur uptake, Gray and Gerdemann (1973) noticed an increased uptake of ^{35}S by mycorrhizal red clover and maize plants.

Sheriff O. Sanni (1976) reported a positive correlation between VA-mycorrhizal infection and the amount of phosphorus and nitrogen in tissues of cowpea, tomato and maize. Smith and Daft (1977) recorded a higher percentage of nitrogen in mycorrhizal Medicago sativa at the time of harvest. The phosphate content of such plants was also greater than non-mycorrhizal plants at the age of seven weeks. Further, by 10 or 12 weeks when significant mycorrhizal enhancement of growth was apparent, the total nitrogen and phosphorus contents per mycorrhizal plant were also higher. However, the phosphate content measured on a dry weight basis showed no significant differences between mycorrhizal and non-mycorrhizal plants. Cooper and Tinker (1978) investigated the uptake and translocation of ^{32}P, ^{65}Zn and ^{35}S supplied to white clover with mycorrhizal infection and observed that all the three radioactive elements were translocated through the external hyphae to the host plant. Asimi et al. (1980) while studying the influence of soil phosphorus levels on interactions between VA-mycorrhiza and Rhizobium in soybean, observed an improved uptake of phosphorus by dually inoculated and mycorrhiza alone plants.

Gray and Gerdemann(1969) reported that the presence of an extensive mycelial net work of VA-mycorrhizal fungi on the infected root enabled the host plant to absorb more phosphorus from larger volume of soil. In an attempt to find out the actual mechanism of increased phosphorus uptake by mycorrhizal plants, Sanders and Tinker (1971) found that the increased surface area due to mycelial net work was primarily responsible for the enhanced uptake of phosphorus. However, Hayman and Mosse (1972) observed that eventhough the mycorrhizal roots used the same source of labile phosphorus, since it explored a greater volume of soil beyond the zone of phosphate depletion near the root surface, there was a greater uptake of phosphorus by the root system. A similar observation was also made by Hattingh et al. (1973) who reported that the external hyphae of VA-mycorrhiza transported an appreciable amount of ^{32}P to host root across a distance of 27 mm within three days.

Effect of application of phosphate fertilizer on the incidence of VA-mycorrhiza

Murdoch et al. (1967) observed a significant growth difference in maize plants with and without mycorrhiza grown in soil amended with rock phosphate when compared to plants grown in unamended soil. Several other workers have also reported that VA-mycorrhiza could greatly improve the growth of host plants when supplied with relatively insoluble form of phosphates such as bone meal, tricalcium phosphate and apatite (Daft and Nicolson, 1966) and rock phosphate (Jackson et al., 1972). But Mosse (1973) suggested that with the addition of soluble phosphate in the soil, there was an increase in the concentration of phosphorus in the plant tissues which ultimately affected the spread of the endophyte. However, Menge et al. (1978) suggested that it was the phosphorus concentration of the root and not the amount of phosphorus applied to the soil which actually determined the extent of root colonisation by VA-mycorrhiza.

Many workers have reported a beneficial effect due to rock phosphate application on phosphorus uptake by VA-mycorrhizal plants. Thus Mosse et al. (1976) found that the combination of rock phosphate and VA-mycorrhiza acted synergistically in increasing the plant dry weight in several crop plants. Mosse et al. (1976) also examined the interaction between VA-mycorrhiza, utilization of rock phosphate and nodulation in three legumes such as clover, Stylosanthes and centrosema and observed that these legumes inoculated with Rhizobium alone nodulated in most P-deficient soils only when they were also mycorrhizal and that the added rock phosphate greatly enhanced such nodulation and nitrogen fixation. Further, Mosse (1977) reported that in some cerrado soils, rock phosphate application particularly when coupled with mycorrhizal inoculation served as a better source of phosphorus than the more soluble forms of phosphates.

Waidyanatha et al. (1979) found that in mycorrhizal Pueraria and Stylosanthes, the application of rock phosphate greatly stimulated nodulation and nodule activity. Jalali and Thareja (1985) also observed a significant growth response in chickpea grown in phosphorus deficient soils when inoculated with VA-mycorrhiza. Manjunath and Bagyaraj (1986) have recently reported that inoculation with _Glomus fasciculatus_ along with super phosphate at the rate of 22 kg P/ha in a P-deficient soil did not affect the percentage of root colonization by VA-mycorrhiza in black gram, green gram and chickpea.

MATERIALS AND METHODS

MATERIALS AND METHODS

The present study on the effect of vesicular-arbuscular mycorrhiza and *Rhizobium* inoculation on nodulation, phosphorus uptake and yield in cowpea was conducted at College of Agriculture, Vellayani, Trivandrum, during 1983-85. Three separate pot culture experiments were done for this purpose.

1.1. Mycorrhiza

Two cultures of VA-mycorrhizae, *Glomus microcarpus* (standard culture) obtained from the Central Tuber Crops Research Institute, Sreekaryam, Trivandrum and *Glomus* sp. (local culture) isolated from the College of Agriculture, Vellayani, were used for various experiments.

1.2. Isolation of *Glomus* sp.

The spores of the local culture of VA-mycorrhiza, *Glomus* sp., were isolated by the modified wet sieving and decanting method of Gerdemann (1955). For this, 250 g of cowpea rhizosphere soil was initially suspended in 1000 ml of tap water in a measuring cylinder and after the heavier particles had settled, the supernatant was passed through a set of sieves 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 of fresh 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 material present on each sieve was transferred to 100 ml beakers in small volume of water and filtered through Watman No. 1 filter paper. The contents of each filter paper were carefully examined under a stereomicroscope for the typical spores of VA-mycorrhiza. Spores of uniform size and shape which were predominant in number were selected and transferred to moistened filter paper in petri dishes with the help of a fine capillary pipette. These were either used for the mass production of VA-mycorrhizal inoculum or preserved at 4°C in a refrigerator. The spores of Glomus microcarpus were also isolated by this method. However, this was done only from plants which were previously inoculated with this culture and grown under aseptic conditions.

1.3. Identification of local culture of VA-mycorrhiza

The spores of the local culture of VA-mycorrhiza were identified mainly on the basis of their size, colour and the nature of basal attachment. These were then compared with the key prepared by Gerdemann and Trappe (1974) for the identification of Glomus spp.

1.4. Mass production of VA-mycorrhizal inoculum

Mass production of both the standard as well as the local culture of VA-mycorrhiza was done by inoculating seeds of sorghum (*Sorghum vulgare*) with single spores of *Glomus microcarpus* or *Glomus* sp. The plants were grown in steam sterilized sand in small pots of 11 x 14 cm size for 21 days for the proper development of VA-mycorrhiza. The roots from these plants were then harvested for using as the starting material for the mass production of VA-mycorrhizal inoculum. This was done in large pots of 35 x 35 cm size containing 10 kg of steam sterilized sand and soil in the ratio 1 : 1 and seeded with fifty grams each of the appropriate mycorrhizal inoculum in the form of infected roots cut into small bits of approximately 1 cm size and soil from the pot where these plants were grown initially. The inoculum was initially placed in the centre of each pot at a depth of about 5 cm over which seeds of sorghum were sown and grown for 60 days. The plants were irrigated regularly with sterilized tap water. The infected roots from these plants along with 50 spores of the respective VA-mycorrhizae were then used as the mycorrhizal inoculum for various experiments conducted during this investigation.

1.5. Staining of root samples of VA-mycorrhiza

The method of Phillips and Hayman (1970) was used for observing VA-mycorrhizal infection in various root samples. One hundred root bits of approximately 1 cm length were examined, segment-wise, for this purpose. The root bits were initially washed in tap water and softened by simmering in 10 per cent KOH at 90°C for 1 hour. After cooling, the excess of alkali was removed by repeated rinsing in tap water and then acidified with 2 per cent HCl before staining with 0.05 per cent trypan blue in lactophenol at 90°C for three minutes.

Preparation of trypan blue

Trypan blue (Romali)	50 mg
Lactophenol	100 ml

Preparation of lactophenol

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

The excess stain from the root tissue was removed by clearing overnight in fresh lactophenol. Ten root bits were examined at a time for the typical VA-mycorrhizal infection under a light microscope. Each root bit was divided into four equal segments for recording the presence or absence of VA-mycorrhiza and based on this, different grades from 0 to 4 were given depending on the extent of mycorrhizal infection. The average value thus obtained for 100 root bits examined was taken as the mycorrhizal index.

2. Rhizobium

A specific strain of *Rhizobium*, KAU-11 developed by the Microbiology Section of College of Agriculture, Vellayani, was used for inoculation of cowpea seeds. This culture was maintained on yeast extract mannitol agar of following composition

Yeast extract mannitol agar (YEMA) (Allen, 1953)

Mannitol	10.0 g
K_2HPO_4	0.5 g
$MgSO_4 \cdot 7H_2O$	0.2 g
NaCl	0.1 g
$CaCO_3$	3.0 g
Yeast extract	1.0 g
Congo red (1% aqueous solution)	2.5 ml
Agar	15.0 g
Distilled water	1000.0 ml
pH	7.0

Seeds of cowpea were initially surface sterilized with 0.1 per cent mercuric chloride solution and washed throughly in sterilized tap water before inoculation with the above Rhizobium culture.

3. Influence of host variety on the natural incidence of VA-mycorrhiza in cowpea

A pot culture experiment was conducted for screening different varieties of cowpea (*Vigna unguiculata*) (L) Walp for the natural incidence of VA-mycorrhiza. The experiment was laid out in completely randomized design with the following ten varieties of cowpea.

Number	Variety	Source
1	C-152	Regional Agrl. Research Station, Kerala Agrl. University, Pilicode.
2	CG-11	,,
3	HG-22	,,
4	New Era	,,
5	PTB-1	,,
6	PTB-2	,,
7	RC-25	,,
8	S-488	,,
9	U-16	,,
10	V-38	,,

The potting mixture consisted of unsterilized sand, soil and cowdung in the ratio of 1 : 1 : 0.5. The seeds were uniformly inoculated with the Rhizobium culture prior to sowing. There were two phosphate treatments, with and without rock phosphate application at the rate of 30 kg P_2O_5 per hectare. Muriate of potash was added uniformly to all pots at the rate of 10 kg K_2O per hectare. Three replications were maintained for each variety. The plants were grown for 45 days when the extent of mycorrhizal infection for each variety was determined by the method described earlier.

4. Effect of age of host plant on mycorrhizal infection in cowpea

A pot culture experiment was conducted to study the effect of age of host plant on VA-mycorrhizal association by using the cowpea variety which had the maximum mycorrhizal infection under natural condition. The experiment was laid out in completely randomized design with three replications each. The potting mixture consisted of steam sterilized sand, soil and cowdung in the ratio 1 : 1 : 0.5. The seeds were uniformly inoculated with the Rhizobium culture prior to sowing. There were two phosphate treatments with and without rock phosphate application at the rate of 30 kg

P_2O_5 per hectare. Muriate of potash was added uniformly to all pots at the rate of 10 kg K_2O per hectare. The standard culture of VA-mycorrhiza, Glomus microcarpus was used as the mycorrhizal inoculum. The plants were irrigated regularly for 90 days with sterilized tap water. The extent of mycorrhizal infection on 15th, 30th, 45th, 60th, 75th and 90th day of plant growth was determined by the method described earlier.

5.1. Effect of VA-mycorrhizal inoculation on nodulation, phosphorus uptake and yield in cowpea

A pot culture experiment was conducted to study the effect of VA-mycorrhizal inoculation on nodulation, phosphorus uptake and yield in cowpea using the aseptic sand culture technique. The cowpea variety which had the maximum mycorrhizal infection under natural condition was used for this experiment also which was laid out in completely randomised design with six replications each. The potting material consisted of washed river sand which was initially steam sterilized in an autoclave at 121°C for two hours. Both the standard as well as the local cultures of VA-mycorrhizae, Glomus microcarpus and Glomus sp., respectively, were used as the mycorrhizal inocula. There were two Rhizobium treatments, with and without seed inoculation prior to sowing.

The three phosphate treatments were with and without the application of rock phosphate and super phosphate at the rate of 30 kg P_2O_5 per hectare. Muriate of potash was added uniformly to all pots at the rate of 10 kg K_2O per hectare. The different treatment combinations were as follows:-

Mycorrhiza treatments	With Rhizobium (R^+)			Without Rhizobium (R^-)		
	Rock phosphate (RP)	Super phosphate (SP)	Without phosphorus (P^-)	Rock phosphate (RP)	Super phosphate (SP)	Without phosphorus (P^-)
With <u>Glomus microcarpus</u> (MS)	MSR^+RP	MSR^+SP	MSR^+P^-	MSR^-RP	MSR^-SP	MSR^-P^-
With <u>Glomus</u> sp. (ML)	MLR^+RP	MLR^+SP	MLR^+P^-	MLR^-RP	MLR^-SP	MLR^-P^-
Without mycorrhiza (M^-)	M^-R^+RP	M^-R^+SP	$M^-R^+P^-$	M^-R^-RP	M^-R^-SP	$M^-R^-P^-$

The plants were irrigated regularly with sterilized distilled water and twice a week with a modified phosphate free quarter strength plant nutrient solutions of following composition for 110 days.

Composition of plant nutrient solution

A. Macronutrient solution

KCl	0.0745	g
$CaSO_4$	0.344	g
$MgSO_4$	0.246	g
Distilled water	1000.0	ml

B. Trace element solution

$CuSO_4$	0.78	g
$ZnSO_4$	2.22	g
$MnSO_4$	2.03	g
Sodium molybdate	0.01	g
Boric acid	1.43	g
Distilled water	1000.0	ml

0.5 ml of the trace element solution was added to 1000 ml of the macronutrient solution prior to sterilization in an autoclave at 121°C for 20 minutes. This was diluted four times with sterilized distilled water whenever necessary for preparing the quarter strength plant nutrient solution.

Observations were taken by using three replications each at two stages of plant growth, on 45th day and at the time of harvest. These were on nodule number, nodule fresh and dry weight, plant height, fresh and dry weight of shoot, root length, mycorrhizal infection and the percentage nitrogen and phosphorus contents of shoot. However, at the time of harvest, few additional observations on crop yield and on the residual nitrogen and phosphorus content of soil were also taken. The number of nodules formed were counted after carefully removing each plant with its intact root system from the pots with the help of a mild jet of water. The separated nodules were weighed in a chemical balance for determining their fresh weight and then dried to a constant weight at 60°C in a drying oven for taking its dry weight. The root length and the plant height were measured in cm from the base of the shoot to the maximum growing tip with the help of a metre scale. The extent of mycorrhizal infection was determined by the method described earlier. The nitrogen and phosphorus contents of plant and soil samples were estimated by the methods described below.

5.2. Estimation of total nitrogen by microkjeldahl method (Jackson, 1967)

Five hundred mg of powdered plant sample and 1 g of digestion mixture consisting of potassium sulphate, cupric sulphate and selenium powder in the ratio of 10 : 1 : 0.1 were initially digested with 10 ml of concentrated H_2SO_4 of specific gravity 1.84 for 2 hours in 100 ml Kjeldahl's digestion flasks. These flasks were allowed to cool down to room temperature before the contents were carefully transferred to 100 ml volumetric flasks for making up the volume with distilled water. Ten ml of this sample along with 40 per cent NaOH solution were then steam distilled till about 30 ml of the distillate was collected in a receiver flask containing 10 ml of 4 per cent boric acid solution and a drop of mixed indicator.

Preparation of mixed indicator solution

Bromocresol green	0.5 g
Methyl red	0.1 g
Ethanol	100.0 ml

The ammoniacal nitrogen of the distillate was estimated by titration against 0.01 N HCl and from the titre value, the percentage nitrogen content was calculated as follows:-

$$\text{Percentage nitrogen content of shoot} = \frac{V \times N \times V_1 \times 0.014 \times 100}{V_2 \times W}$$

where V = titre value - blank value

V_1 = total volume of plant sample made up

V_2 = total volume of plant sample distilled

N = Normality of HCl and

W = Weight of plant sample used for digestion.

5.3. Estimation of total phosphorus by Vanadomolybdo-phosphoric acid method (Jackson, 1967)

Preparation of Vanadate molybdate reagent

Solution A

Ammonium molybdate	25.0 g
Distilled water	400.0 ml

Solution B

Ammonium meta vanadate	1.25 g
Boiling water	300.0 ml
Conc. HNO_3	250.0 ml

Solution A was added to solution B before the final volume was made up to one litre with distilled water.

Five hundred milligrams of powdered plant sample was digested with 10 ml of conc. H_2SO_4 of specific gravity 1.84 for two hours in 100 ml Erlenmeyer flasks. These flasks were allowed to cool down to room temperature, before the contents were carefully transferred to 100 ml volumetric flasks for making up the volume with distilled water. Five ml of this solution was then pipetted into a 50 ml volumetric flask. Two to four drops of 2,4, dinitrophenol indicator and 4 N Na_2CO_3 were added drop by drop to the above sample till an yellow colour was developed. This was decolourised with 6 N HCl in such a way that the final pH of the solution was 4.8. Ten ml of vanadate molybdate reagent was then added to the above solution before the final volume was made up to 50 ml with distilled water. After 30 minutes, the intensity of the yellow colour developed was measured in a Spectronic-20 spectro photometer (Bausch and Lomb, Rochester, U.S.A.) at 470 nm. A standard curve was also prepared in a similar manner using 0, 1, 2, 4, 6, 8 and 10 ppm solution of KH_2PO_4. The concentration of phosphorus in the plant extract was determined from the standard curve. The percentage phosphorus content of shoot was calculated as follows:-

$$\text{Percentage phosphorus content of shoot} = \frac{X \times 50 \times V_1}{10000 \times V_2 \times W}$$

where X = ppm concentration of P from the standard curve

V_1 = Volume made up

V_2 = Volume taken for colour development.

5.4. <u>Estimation of available soil nitrogen by alkaline permanganate method</u> (Subbiah and Asijaa, 1956)

Twenty grams of air dried soil were initially taken in a round bottom distillation flask to which 20 ml of water, 100 ml of 0.32 per cent potassium permanganate solution and 100 ml of 2.5 per cent NaOH solution were added. This was steam distilled till about 30 ml of the distillate was collected in a receiver flask containing 10 ml of 4 per cent boric acid solution and a drop of mixed indicator. The ammoniacal nitrogen of the distillate was estimated by titration against 0.01 N HCl and from the titre value the percentage nitrogen content of various soil samples were calculated as follows:-

$$\text{Percentage nitrogen content of soil} = \frac{V \times N \times 0.014 \times 100}{W}$$

where V = titre value - blank value

N = normality of the acid

W = weight of the soil sample taken.

5.5. Determination of available phosphorus in soil by Bray's extraction method (Jackson, 1967)

Five grams of air dried soil passing through 0.25 mm sieve were mixed with 50 ml of Bray's solution in a glass stoppered bottle.

Preparation of Bray's solution

Ammonium fluoride	1.11 g
Hydrochloric acid (6 N)	4.16 ml
Distilled water	1000 ml

The mixture was shaken for five minutes before the supernatant was filtered through a moistened Whatman No.42 filter paper. Five ml of this filtrate was then transferred to a 50 ml volumetric flask to which 5 ml of ammonium molybdate reagent and 1 ml of freshly prepared stannous chloride solution were added. After 10 minutes, the intensity of blue colour developed was measured in a Klett summerson photoelectric colorimeter (Arthur, H. Thomas Company, U.S.A.) at 660 nm using the red filter.

Preparation of ammonium molybdate reagent

Ammonium molybdate	15 g
Warm distilled water	300 ml
HCl (10 N)	350 ml

A standard curve was also prepared in a similar manner by using 0, 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 ppm solution of KH_2PO_4. The concentration of phosphorus in the filtrate was determined from the standard curve. The percentage of phosphorus content of various soil samples were calculated as follows:-

$$\text{Percentage phosphorus content of soil} = \frac{X \times V_1 \times V_3}{W \times V_2 \times 10000}$$

where X = ppm concentration of phosphorus in the filtrate

V_1 = Volume of Bray's extractant

V_2 = aliquot of soil extract used

V_3 = volume to which the aliquot was made up

W = Weight of soil taken.

Statistical methods of analysis

The data on various observations were analysed by the methods described by Snedecor and Cochran (1967) for the analysis of variance of completely randomised design.

RESULTS

RESULTS

1. Identification of VA-mycorrhiza

The local culture of VA-mycorrhiza was identified as *Glomus* sp. on the basis of size, colour and the nature of basal attachment of spores. The spores were typical of *Glomus* sp. with an average size of 100 - 200 micrometers. They were yellowish brown in colour under incident light in a stereo-microscope with a simple basal attachment. Typical root infection by the standard as well as the local culture of VA-mycorrhiza and a spore of *Glomus microcarpus* are shown in Plates I to III.

2. Influence of host variety on the natural incidence of VA-mycorrhiza in cowpea

The result of the pot culture experiment to study the influence of host variety on the natural incidence of VA-mycorrhiza in cowpea is given in Table 1, Figure 1. There were significant differences between varieties in their mean mycorrhizal index. The infection was maximum for the variety C-152 which had an average index of 1.19. This was significantly higher than that of varieties such as CG-11, HG-22, PTB-1, RC-25, S-488, U-16 and V-38 (Table 1). However, the mean mycorrhizal index of two other varieties, New Era

Plate I. Root infection by the standard culture of VA-mycorrhiza (*Glomus microcarpus*) in cowpea.

Plate II. Root infection by the local culture of VA-mycorrhiza (*Glomus* sp.) in cowpea.

Plate I

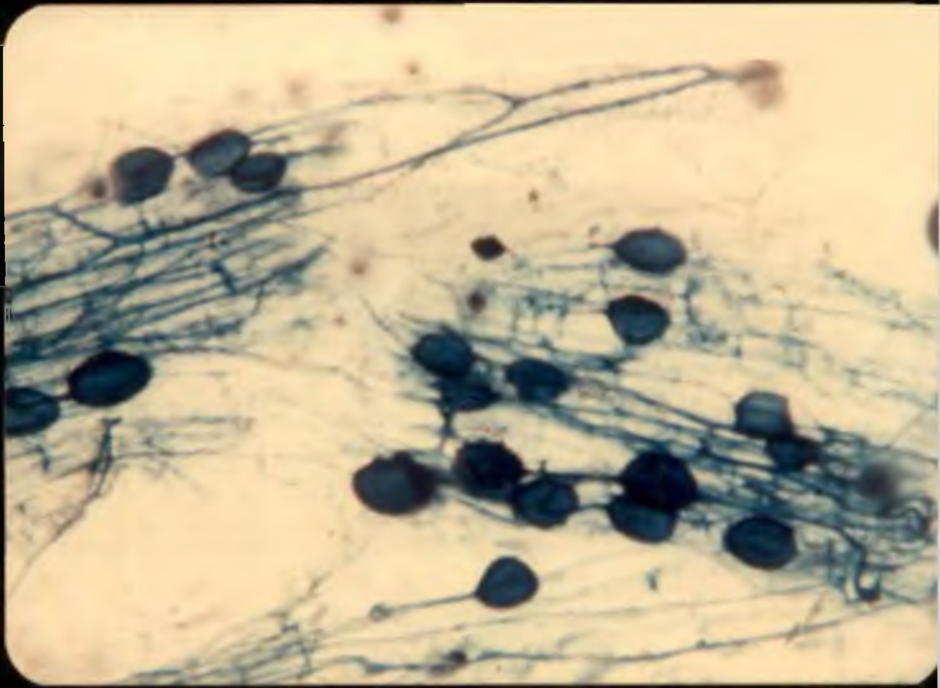


Plate II

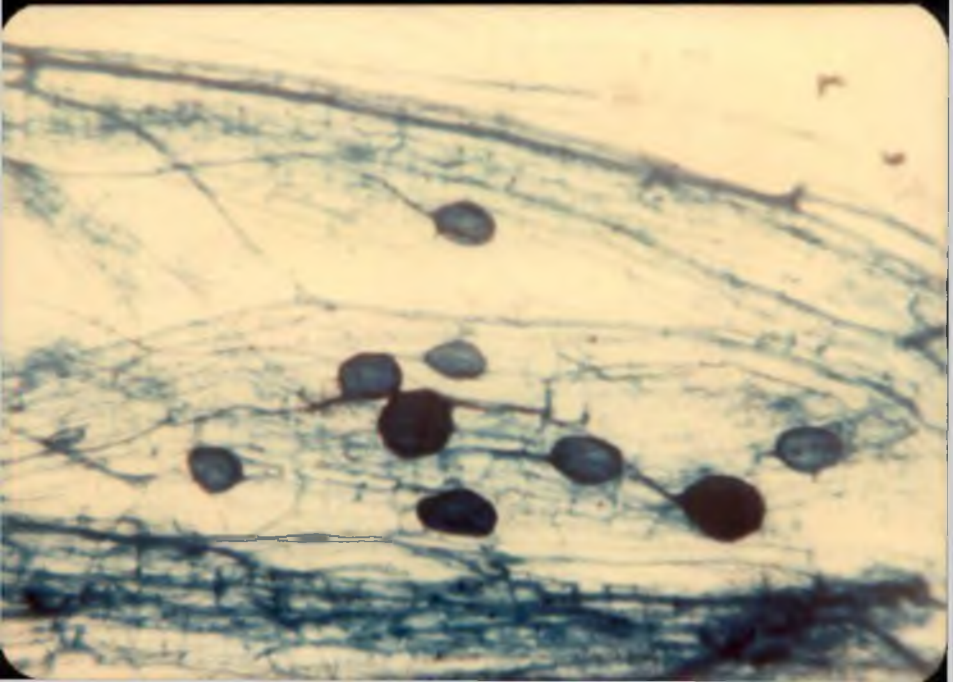


Plate III. A typical spore of *Glomus microcarpus*.

Table 1. Influence of host variety on the natural incidence of VA-mycorrhiza in cowpea

Host variety	Mycorrhizal Index*		
	With rock phosphate	Without rock phosphate	Mean for each variety
C-152	1.79 (1.67)	0.64 (1.28)	1.19 (1.48)
CG-11	0.90 (1.38)	0.02 (1.01)	0.44 (1.20)
HG-22	0.88 (1.37)	0.00 (1.00)	0.42 (1.19)
New Era	1.76 (1.66)	0.30 (1.14)	0.96 (1.40)
PTB-1	0.77 (1.33)	0.19 (1.09)	0.46 (1.21)
PTB-2	1.79 (1.67)	0.37 (1.17)	1.02 (1.42)
RC-25	0.28 (1.13)	0.06 (1.03)	0.17 (1.08)
S-488	0.08 (1.04)	0.04 (1.02)	0.06 (1.03)
U-16	0.56 (1.25)	0.04 (1.02)	0.30 (1.14)
V-38	0.90 (1.38)	0.02 (1.01)	0.44 (1.20)
Mean	0.99 (1.41)	0.17 (1.08)	--

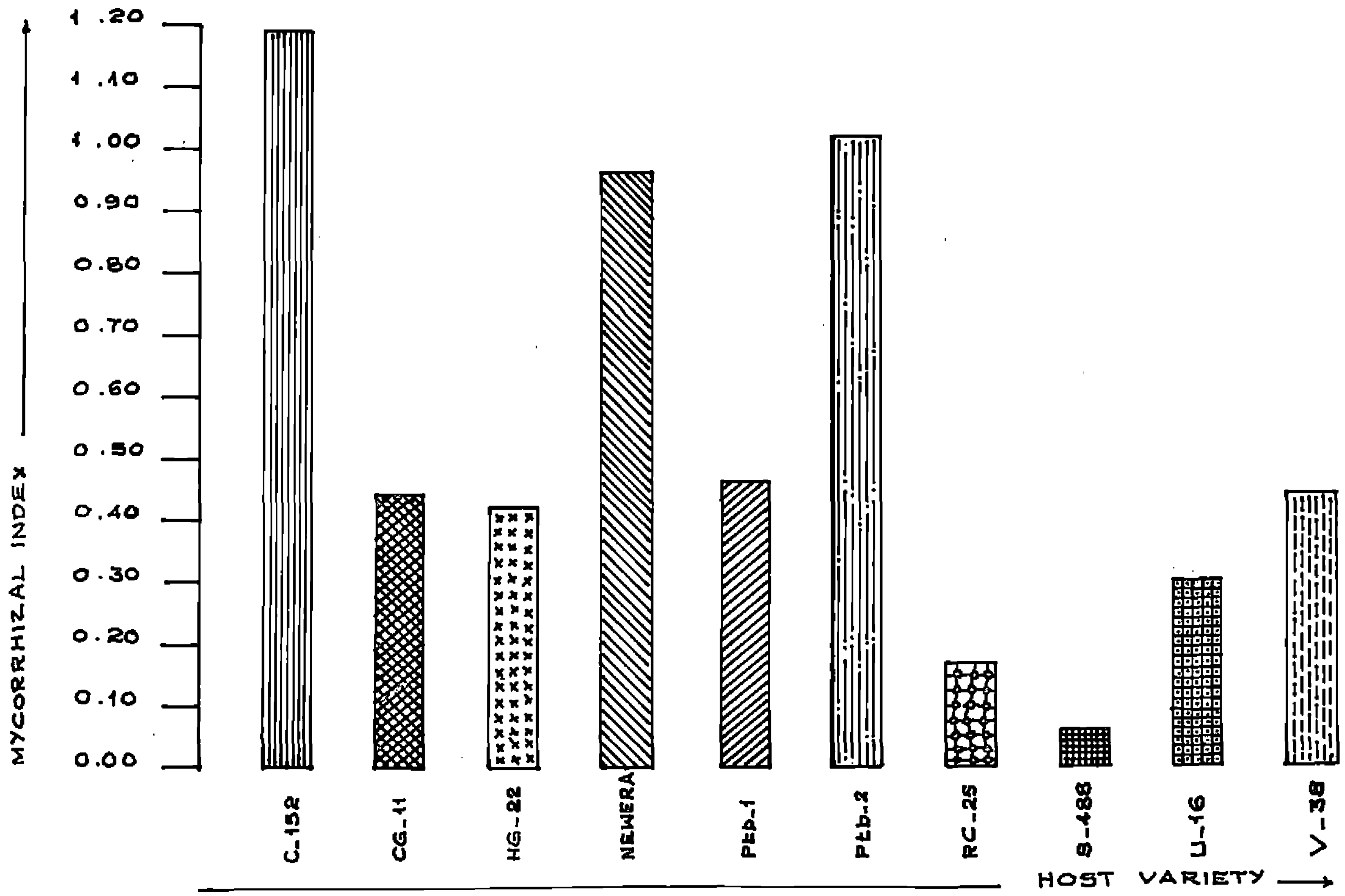
* Mean of 3 replications

Figures in parentheses are after $\sqrt{x + 1}$ transformation

CD (0.05) for comparison between varieties = 0.17

CD (0.05) for comparison between phosphate treatments = 0.08

FIG-1. INFLUENCE OF HOST VARIETY ON THE NATURAL INCIDENCE OF VA-MYCORRHIZA IN COWPEA



(0.96) and PTB-2 (1.02) was statistically on par with that of C-152. The application of rock phosphate at the rate of 30 kg P_2O_5/ha had a significant effect in enhancing the mycorrhizal infection in all varieties except in S-488 where such an effect due to phosphate application on mycorrhizal infection was not observed.

3. <u>Effect of age of host plant on VA-mycorrhizal infection in cowpea</u>

The result of the pot culture experiment to study the effect of age of host plant on VA-mycorrhizal infection in cowpea is given in Table 2, Figure 2 and Plates IV to VI. The infection was maximum on 45th day with an average index of 1.62. This was significantly higher than that observed at all other stages of plant growth (Table 2). On 15th, 30th, 60th, 75th and 90th day of plant growth, the mean mycorrhizal index was 0.30, 0.77, 0.82, 0.74 and 0.49, respectively. When compared to 15th day, the extent of mycorrhizal infection on 60th day was also significantly higher. The relative incidence of VA-mycorrhiza at the three critical stages of plant growth, on 15th, 45th and 90th day is shown in Plates IV to VI. The application of rock phosphate at the rate of 30 kg P_2O_5/ha had a uniform significant effect in enhancing the mycorrhizal infection (Table 2) at all stages of plant growth.

Table 2. Effect of age of host plant on VA-mycorrhizal infection in cowpea

Age of host plant (days)	Mycorrhizal index*		
	With rock phosphate	Without rock phosphate	Mean for each stage
15	0.51 (1.23)	0.08 (1.04)	0.30 (1.14)
30	1.46 (1.57)	0.17 (1.08)	0.77 (1.33)
45	3.37 (2.09)	0.30 (1.14)	1.62 (1.62)
60	1.79 (1.67)	0.04 (1.02)	0.82 (1.35)
75	1.62 (1.62)	0.04 (1.02)	0.74 (1.32)
90	0.96 (1.40)	0.06 (1.03)	0.49 (1.22)

* Mean of three replications

Figures in parentheses are after $\sqrt{x + 1}$ transformation

C.D. (0.05) for comparison between different stages = 0.20

C.D. (0.05) for comparison between phosphate treatments = 0.12

FIG. 2. EFFECT OF AGE OF HOST PLANT ON MYCORRHIZAL INFECTION IN COWPEA

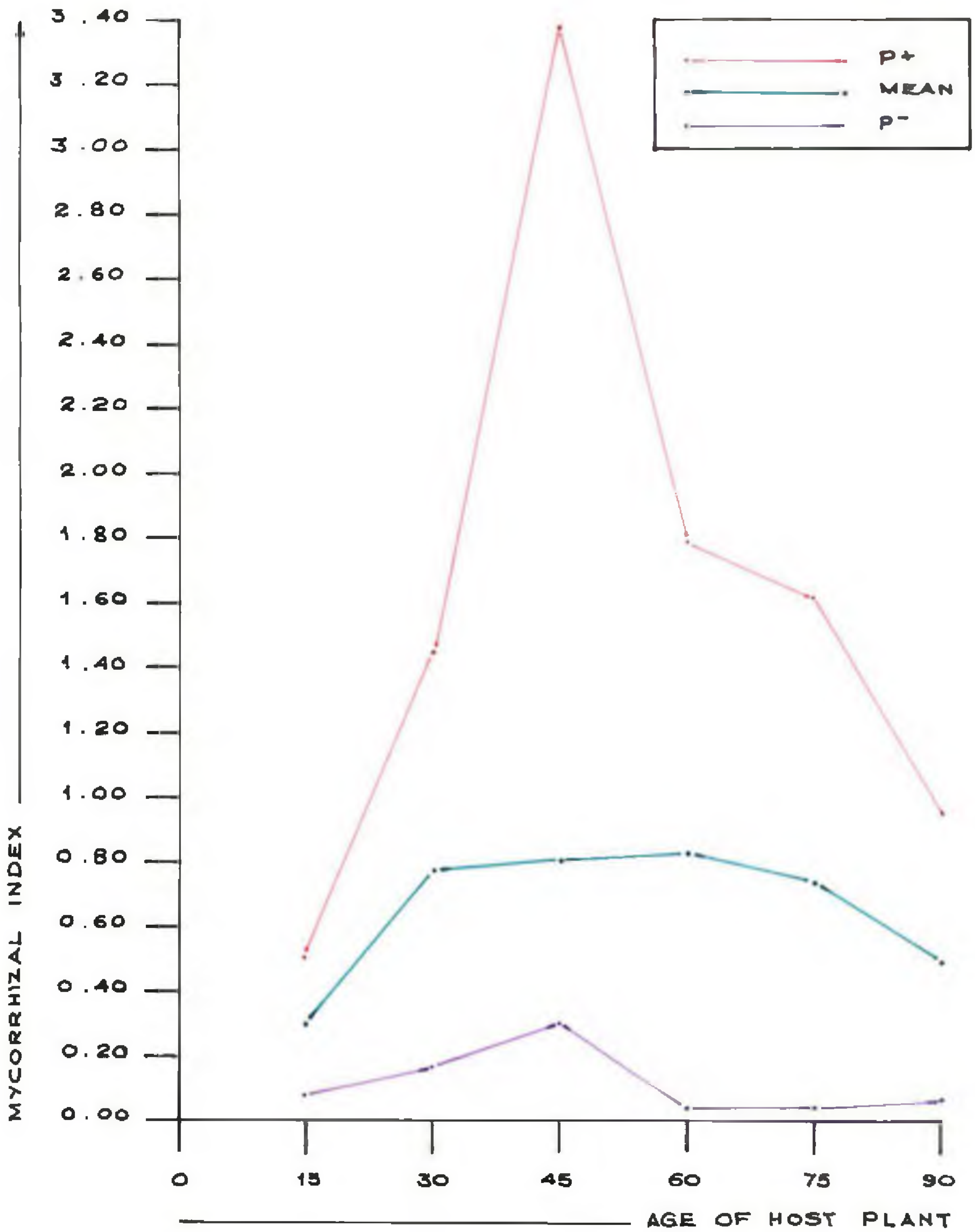


Plate IV. Extent of root infection by *Glomus microcarpus* on 15th day in cowpea.

Plate V. Extent of root infection by *Glomus microcarpus* on 45th day in cowpea.

Plate V

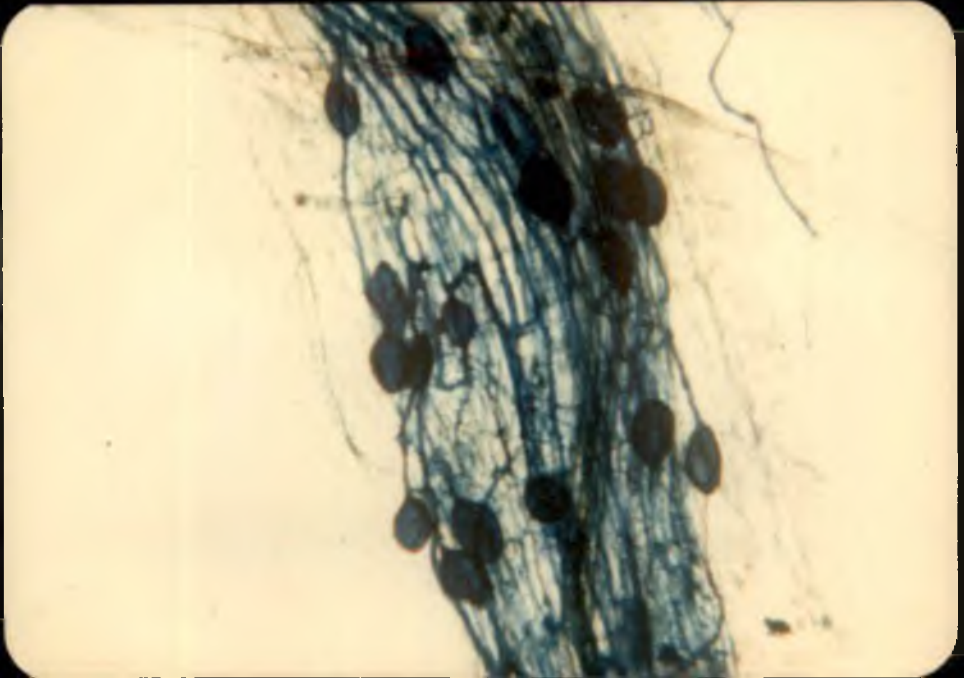


Plate VI. Extent of root infection by *Glomus microcarpus* on 90th day in cowpea.

Plate VI

4. Effect of VA-mycorrhiza and *Rhizobium* inoculation on nodulation, phosphorus uptake and yield in cowpea

The results of the pot culture experiment to study the effect of VA-mycorrhiza and *Rhizobium* inoculation on nodulation, phosphorus uptake and yield in cowpea are given in Tables 3 to 15, Figures 3 to 6 and Plates 7 to 10. The observations were taken at two stages of plant growth, on 45th day and at the time of harvest.

A uniform treatment effect on various plant characters studied was not observed on 45th day. The number of nodules were maximum in the treatment combination consisting of the standard culture of VA-mycorrhiza, *Rhizobium* inoculation and the application of super phosphate (Table 3). In this treatment (MSR^+SP), 27.72 nodules were formed per plant. These were significantly higher than the control ($M^-R^-P^-$) and various other treatments except the MLR^+P^- and MSR^+P^- treatments, where the number of nodules formed, 22.81 and 20.62 respectively were statistically on par with the above (MSR^+SP) treatment. The fresh and dry weights of nodules were also maximum in this treatment. But the root length and plant height were significantly higher in the MSR^+P^- and $M^-R^-P^-$ treatments respectively.

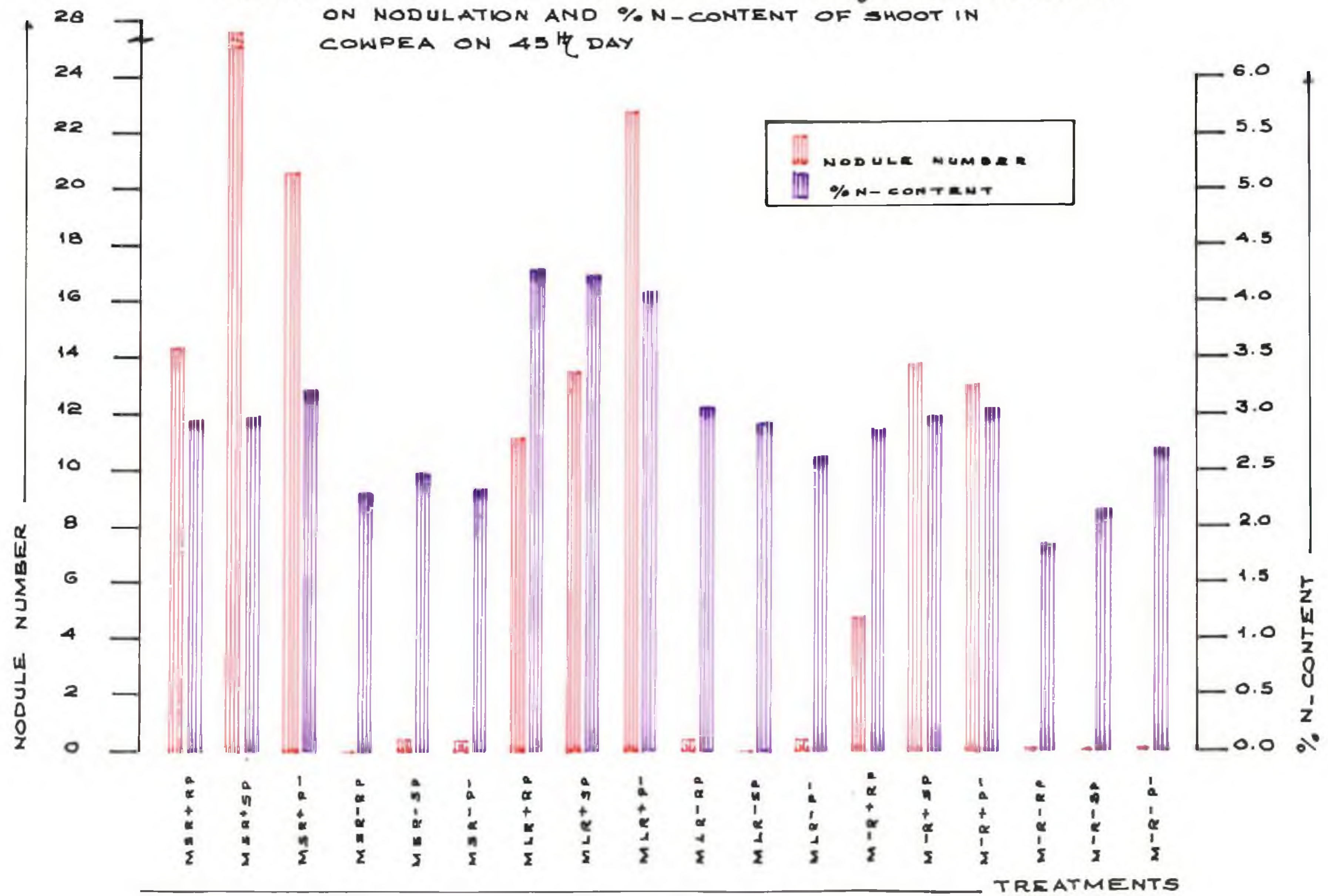
Table 3. Effect* of VA-mycorrhiza and *Rhizobium* inoculation on nodulation and other characters in cowpea on 45th day.

Treatment combinations	Nodule number		Nodule fresh wt. (mg)	Nodule dry wt. (mg)	Root length (cm)	Plant height (cm)
MS R^+RP	14.37	(3.92)	102.17	47.67	15.33	39.67
MS R^+SP	27.72	(5.36)	158.00	67.83	22.67	44.58
MS R^+P^-	20.62	(4.65)	102.50	47.00	26.33	37.67
MS R^-RP	0.00	(1.00)	0.00	0.00	16.17	31.46
MS R^-SP	0.46	(1.21)	0.50	0.36	15.33	33.50
MS R^-P^-	0.46	(1.21)	0.50	0.32	21.50	28.83
ML R^+RP	11.04	(3.47)	64.33	26.67	20.67	41.02
ML R^+SP	13.52	(3.81)	146.83	62.50	21.83	38.00
ML R^+P^-	22.81	(4.88)	107.00	48.17	22.56	34.33
ML R^-RP	0.46	(1.21)	0.50	0.22	25.47	33.82
ML R^-SP	0.00	(1.00)	0.00	0.00	15.33	35.83
ML R^-P^-	0.46	(1.21)	0.50	0.23	18.13	29.72
M^-R^+RP	4.76	(2.40)	41.00	19.33	23.83	31.17
M^-R^+SP	13.98	(3.87)	109.17	48.50	22.67	37.56
$M^-R^+P^-$	13.14	(3.76)	134.17	56.33	25.53	48.03
M^-R^-RP	0.17	(1.08)	1.17	0.50	16.16	34.33
M^-R^-SP	0.14	(1.07)	2.83	0.83	15.17	34.00
$M^-R^-P^-$ (Control)	0.17	(1.08)	0.50	0.17	17.95	48.20
C.D. (0.05)		0.93	38.65	18.22	7.53	10.64

* Mean of three replications

Figures in parentheses are after $\sqrt{x+1}$ transformation

FIG. 3. EFFECT OF VA-MYCORRHIZA AND *Rhizobium* INOCULATION ON NODULATION AND % N-CONTENT OF SHOOT IN COWPEA ON 45th DAY



The fresh and dry weights of plants, 8.17 and 3.20 g respectively were maximum in the MSR^+RP treatment (Table 4). These were significantly higher than the control and various other treatments. However, the dry weight of plants in the MSR^+SP treatment was also statistically on par with the above treatment. In general, there was a significant positive correlation between nodule number and plant dry weight (Table 5). The percentage nitrogen content of shoot was uniformly higher in all Rhizobium inoculated treatments. The nitrogen content of 4.29 per cent was maximum in the MLR^+RP treatment (Table 4). This was significantly higher than the control and other treatments except in the MLR^+SP and MLR^+P^- treatments where the percentage nitrogen content of the shoot obtained was statistically on par with the above (MLR^+RP) treatment. In general, there was a significant positive correlation (Table 5) between nodule number and percentage nitrogen content of shoot.

The mean mycorrhizal index of 3.50 was maximum in the MSR^+RP treatment (Table 4). This was significantly higher than the control and other treatments except in the MSR^-RP and MLR^+RP treatments where the mean mycorrhizal index obtained, 3.25 and 2.83 respectively, were statistically on par with the above (MSR^+RP) treatment. In general, there

Table 4. Effect* of VA-mycorrhiza and *Rhizobium* inoculation on phosphorus uptake and other characters in cowpea on 45th day

Treatment combinations	Plant fresh wt. (g)	Plant dry wt. (g)	N-content of shoot (%)	Mycorrhizal index	P-content of shoot (%)
MS R$^+$RP	8.17	3.20	2.92	3.50 (2.12)	0.12
MS R$^+$SP	5.67	3.02	2.95	2.57 (1.88)	0.14
MS R$^+$P$^-$	3.67	1.37	3.18	1.49 (1.57)	0.02
MS R$^-$RP	3.17	1.63	2.26	3.25 (2.06)	0.18
MS R$^-$SP	3.17	1.42	2.49	2.63 (1.90)	0.13
MS R$^-$P$^-$	1.67	0.74	2.32	1.64 (1.62)	0.01
ML R$^+$RP	4.33	1.69	4.29	2.83 (1.96)	0.18
ML R$^+$SP	4.56	2.50	4.23	1.85 (1.67)	0.17
ML R$^+$P$^-$	2.83	0.93	4.06	0.98 (1.39)	0.03
ML R$^-$RP	2.50	0.83	3.05	2.22 (1.79)	0.11
ML R$^-$SP	2.67	0.52	2.90	2.07 (1.75)	0.23
ML R$^-$P$^-$	1.83	0.50	2.60	1.59 (1.61)	0.02
M$^-$R$^+$RP	2.67	0.86	2.83	0.01 (1.00)	0.19
M$^-$R$^+$SP	3.17	1.03	2.95	0.01 (1.00)	0.18
M$^-$R$^+$P$^-$	2.67	0.68	3.02	0.02 (1.01)	0.02
M$^-$R$^-$RP	1.67	0.68	1.81	0.01 (1.00)	0.19
M$^-$R$^-$SP	1.83	0.70	2.12	0.00 (1.00)	0.11
M$^-$R$^-$P$^-$ (Control)	2.17	0.50	2.65	0.01 (1.00)	0.03
C.D. (0.05)	1.35	0.58	0.97	0.20	0.07

* Mean of three replications

Figures in parentheses are after $\sqrt{x + 1}$ transformation

Table 5. Correlation between nodule number, plant dry weight, mycorrhizal index and percentage of nitrogen and phosphorus content of shoot in cowpea on 45th day

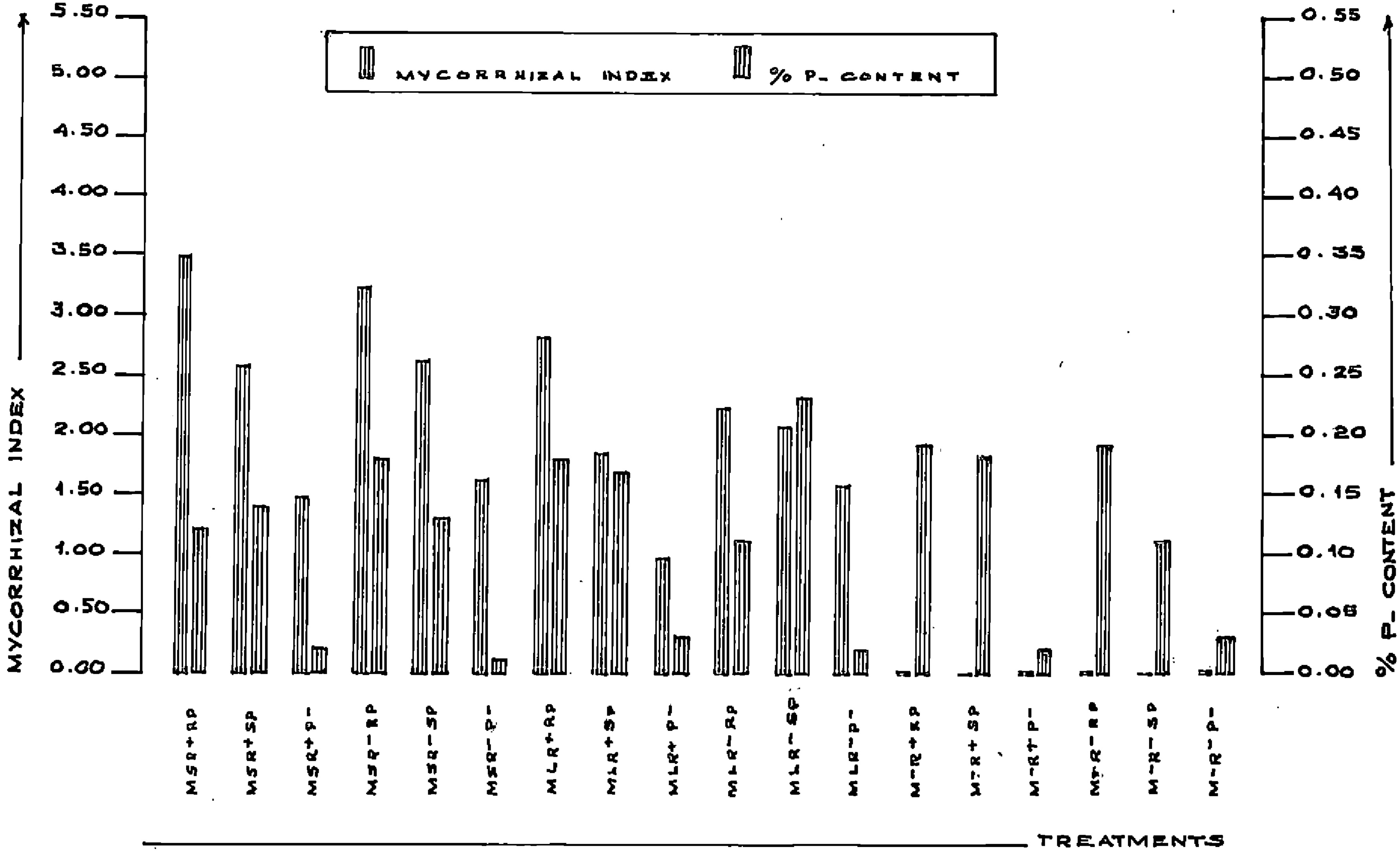
	Nodule number	Plant dry wt.	N-content of shoot	Mycor-rhizal index	P-content of shoot
Nodule number	1.	+0.58	+0.59	0.16	-0.13
Plant dry weight	+0.58	1	0.34	+0.64	0.29
N-content of shoot	+0.59	0.34	1	0.23	0.02
Mycorrhizal index	0.11	+0.64	0.22	1	0.27
P-content of shoot	-0.13	0.29	0.02	0.27	1

was significant positive correlation between the mycorrhizal index and plant dry weight (Table 5). However, there was no correlation between the mean mycorrhizal index and plant nitrogen content.

The percentage phosphorus content of shoot was uniformly higher in both the phosphate treatments. The phosphorus content of 0.23 per cent was maximum in the MLR$^-$SP treatment (Table 4). This was significantly higher than that in the control and other treatments except in the MSR$^-$RP, MLR$^+$RP, MLR$^+$SP, M$^-$R$^+$RP, M$^-$R$^+$SP, M$^-$R$^-$RP treatments where the percentage phosphorus content of the shoot obtained was statistically on par with the above (MLR$^-$SP) treatment. But there was no positive correlation between the mean mycorrhizal index and percentage phosphorus content of shoot (Table 5). Besides, a significant negative correlation between percentage phosphorus content of shoot and nodule number was also observed.

The treatment effects on various plant characters studied were more or less uniform at the time of harvest. The number of nodules formed per plant and their fresh and dry weights were significantly higher in the treatment combination consisting of the standard culture of VA-mycorrhiza, <u>Rhizobium</u> inoculation and the application of

FIG-4. EFFECT OF VA-MYCORRHIZA AND *Rhizobium* INOCULATION ON % P-CONTENT OF SHOOT IN COWPEA ON 45th DAY



rock phosphate at the rate of 30 kg P_2O_5/ha. In this treatment (MSR^+RP), 96.02 nodules were formed with a fresh and dry weights of 231.33 and 126.83 mg respectively (Table 6). The root length of 61.83 cm was however, maximum in the MSR^-RP treatment. This was significantly higher than the control and other treatments except in the MSR^+RP, MSR^+SP and MSR^-SP treatments where the root length measured was statistically on par with the above (MSR^+RP) treatment (Table 6). The plant height was also significantly higher in this treatment.

The fresh and dry weight of plants, 37.00 and 15.83 g, respectively, were maximum in the MSR^+RP treatment (Table 7). In general, there was a significant positive correlation between nodule number and plant dry weight (Table 9). The percentage nitrogen content of shoot was uniformly higher in all <u>Rhizobium</u> inoculated treatments. The nitrogen content of 3.75 per cent was maximum in the MSR^+RP and MSR^+SP treatments. This was significantly higher than the control and various other treatments except in the MLR^+SP and MLR^+RP treatments where the percentage nitrogen content of shoot obtained was statistically on par with the above two treatments. In general, there was a significant positive correlation (Table 8) between nodule number and percentage nitrogen content of shoot.

FIG-4. EFFECT OF VA-MYCORRHIZA AND *Rhizobium* INOCULATION ON % P-CONTENT OF SHOOT IN COWPEA ON 45th DAY

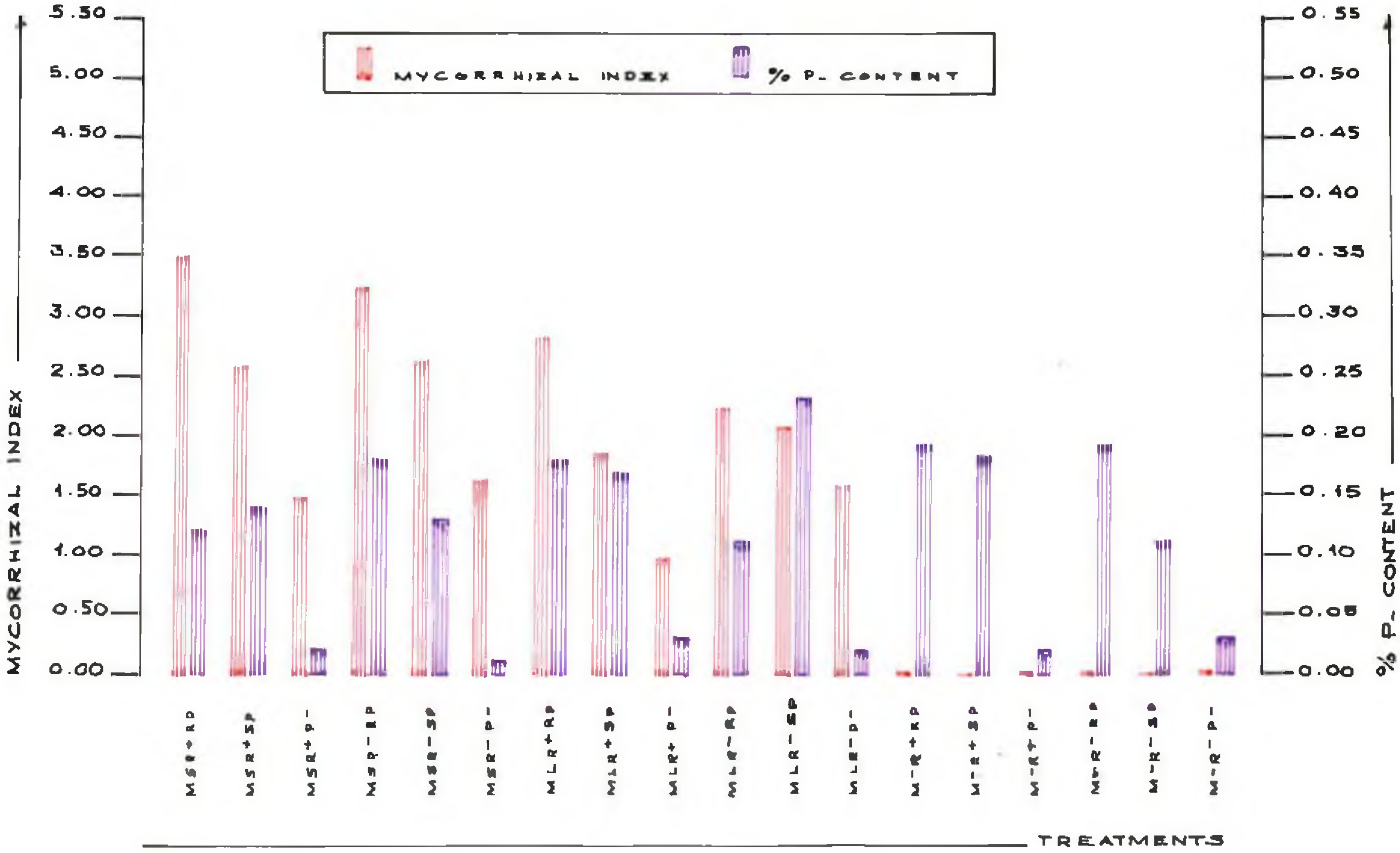


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Table 6. Effect* of VA-mycorrhiza and *Rhizobium* inoculation on nodulation and other characters in cowpea at the time of harvest

Treatment combinations	Nodule number		Nodule fresh wt. (mg)	Nodule dry wt. (mg)	Root length (cm)	Plant height (cm)
MS R^+RP	96.02	(9.85)	231.33	126.83	60.50	215.50
MS R^+SP	56.76	(7.60)	157.67	64.50	59.67	162.50
MS R^+P^-	28.81	(5.46)	124.00	42.33	32.50	99.50
MS R^-RP	1.46	(1.57)	45.00	2.83	61.83	191.17
MS R^-SP	2.50	(1.87)	11.50	4.00	56.17	121.57
MS R^-P^-	2.92	(1.98)	13.00	5.00	26.50	80.17
ML R^+RP	50.12	(7.15)	170.67	65.17	33.17	139.83
ML R^+SP	41.12	(6.49)	154.33	48.83	30.17	127.00
ML R^+P^-	28.38	(5.42)	80.27	52.83	30.56	95.50
ML R^-RP	5.30	(2.51)	27.17	15.00	26.83	129.83
ML R^-SP	5.86	(2.62)	28.17	13.83	29.13	123.50
ML R^-P^-	3.97	(2.23)	20.33	6.83	24.83	77.83
M^-R^+RP	25.21	(4.92)	121.83	33.00	33.40	50.83
M^-R^+SP	24.60	(5.06)	111.17	27.83	29.20	40.60
$M^-R^+P^-$	17.92	(4.35)	87.83	28.67	29.17	73.83
M^-R^-RP	0.66	(1.29)	6.67	4.67	32.50	72.17
M^-R^-SP	2.31	(1.82)	11.67	5.17	28.33	35.83
$M^-R^-P^-$ (Control)	3.28	(2.07)	18.17	6.00	29.20	65.27
C.D. (0.05)		1.05	45.32	14.24	6.13	21.03

* Mean of three replications

Figures in parentheses are after $\sqrt{x+1}$ transformation

FIG. 5. EFFECT OF VA-MYCORRHIZA AND *Rhizobium* INOCULATION ON NODULATION AND % N-CONTENT OF SHOOT IN COWPEA AT THE TIME OF HARVEST

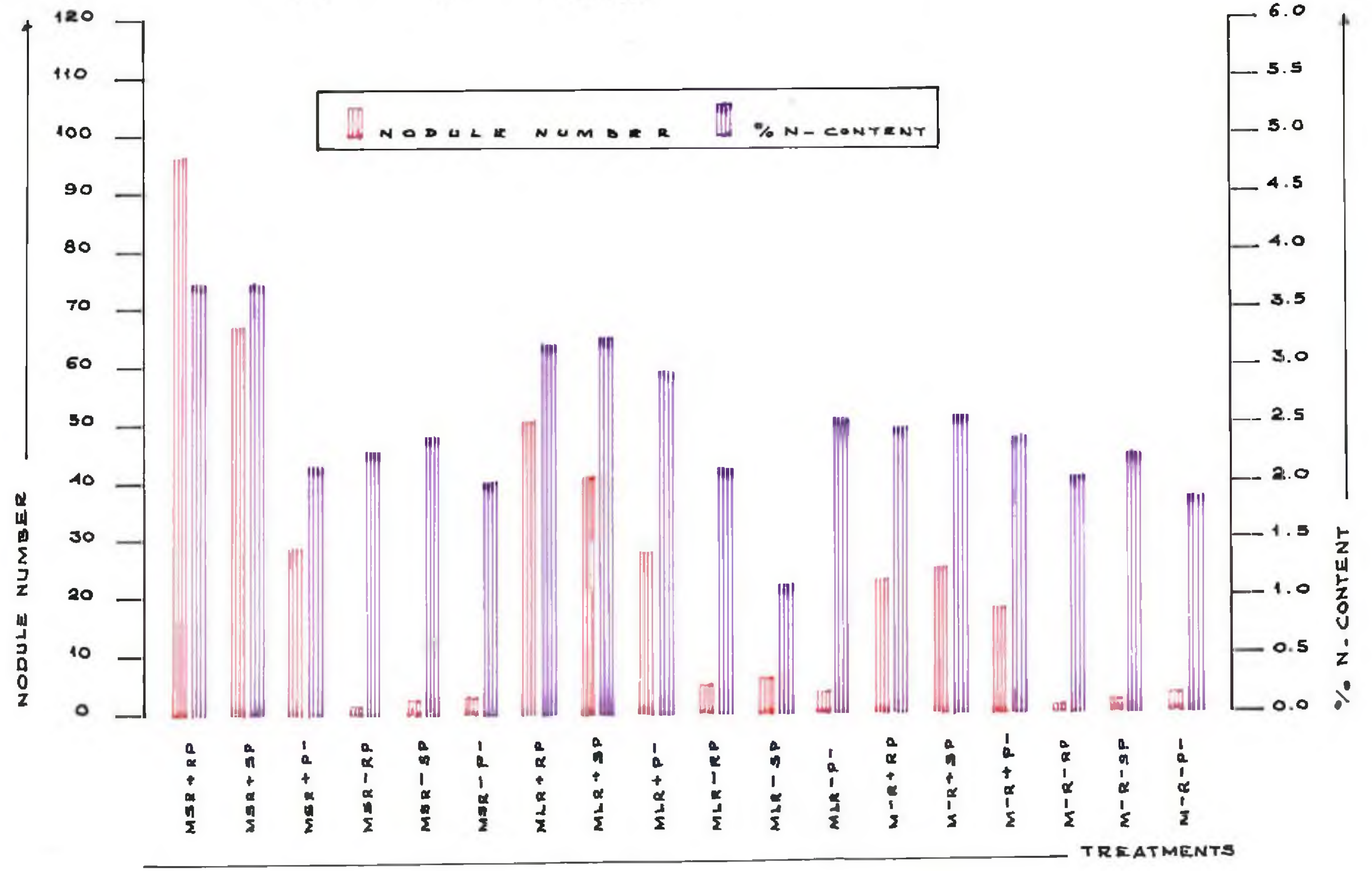

Table 7. Effect* of VA-mycorrhiza and *Rhizobium* inoculation on phosphorus uptake and other characters in cowpea at the time of harvest.

Treatment combinations	Plant fresh wt. (g)	Plant dry wt. (g)	N-content of shoot (%)	Mycorrhizal index	P-content of shoot (%)
MS R^{+}RP	37.00	15.83	3.75	2.92 (1.98)	0.32
MS R^{+}SP	24.33	9.50	3.75	1.66 (1.63)	0.29
MS $R^{+}P^{-}$	15.50	5.50	2.14	0.96 (1.40)	0.10
MS R^{-}RP	32.33	13.00	2.29	2.76 (1.94)	0.27
MS R^{-}SP	21.50	8.00	2.44	1.59 (1.61)	0.22
MS $R^{-}P^{-}$	14.33	5.17	2.02	1.02 (1.42)	0.10
ML R^{+}RP	28.17	12.83	3.22	2.13 (1.77)	0.24
ML R^{+}SP	24.67	9.50	3.27	1.92 (1.71)	0.13
ML $R^{+}P^{-}$	16.50	5.83	2.99	1.02 (1.42)	0.02
ML R^{-}RP	23.00	9.50	2.14	1.92 (1.71)	0.18
ML R^{-}SP	24.56	10.67	1.40	0.99 (1.41)	0.13
ML $R^{-}P^{-}$	13.17	5.17	2.55	1.62 (1.62)	0.02
$M^{-}R^{+}$RP	11.50	6.17	2.48	0.00 (1.00)	0.17
$M^{-}R^{+}$SP	14.17	8.00	2.58	0.02 (1.01)	0.12
$M^{-}R^{+}P^{-}$	15.00	5.33	2.35	0.02 (1.01)	0.02
$M^{-}R^{-}$RP	12.17	5.00	2.03	0.00 (1.00)	0.10
$M^{-}R^{-}$SP	13.33	5.33	2.20	0.02 (1.01)	0.10
$M^{-}R^{-}P^{-}$ (Control)	13.20	3.17	1.82	0.00 (1.00)	0.02
C.D. (0.05)	3.93	2.55	0.66	0.17	0.06

* Mean of three replications.

Figures in parentheses are after $\sqrt{x + 1}$ transformation.

Table 8. Correlation between nodule number, plant dry weight, mycorrhizal index and percentage of nitrogen and phosphorus content of shoot in cowpea at the time of harvest.

	Nodule number	Plant dry wt.	N-content of shoot	Mycorrhizal index	P-content of shoot
Nodule number	+0.99	+0.61	+0.85	0.45	0.53
Plant dry weight	+0.61	1	0.50	+0.80	+0.85
N-content of shoot	+0.85	0.50	1	0.51	0.54
Mycorrhizal index	0.45	+0.80	0.51	1	+0.65
P-content of shoot	0.53	+0.85	0.54	+0.65	1

FIG-6. EFFECT OF VA-MYCORRHIZA AND *Rhizobium* INOCULATION ON % P-CONTENT OF SHOOT IN COWPEA AT THE TIME OF HARVEST

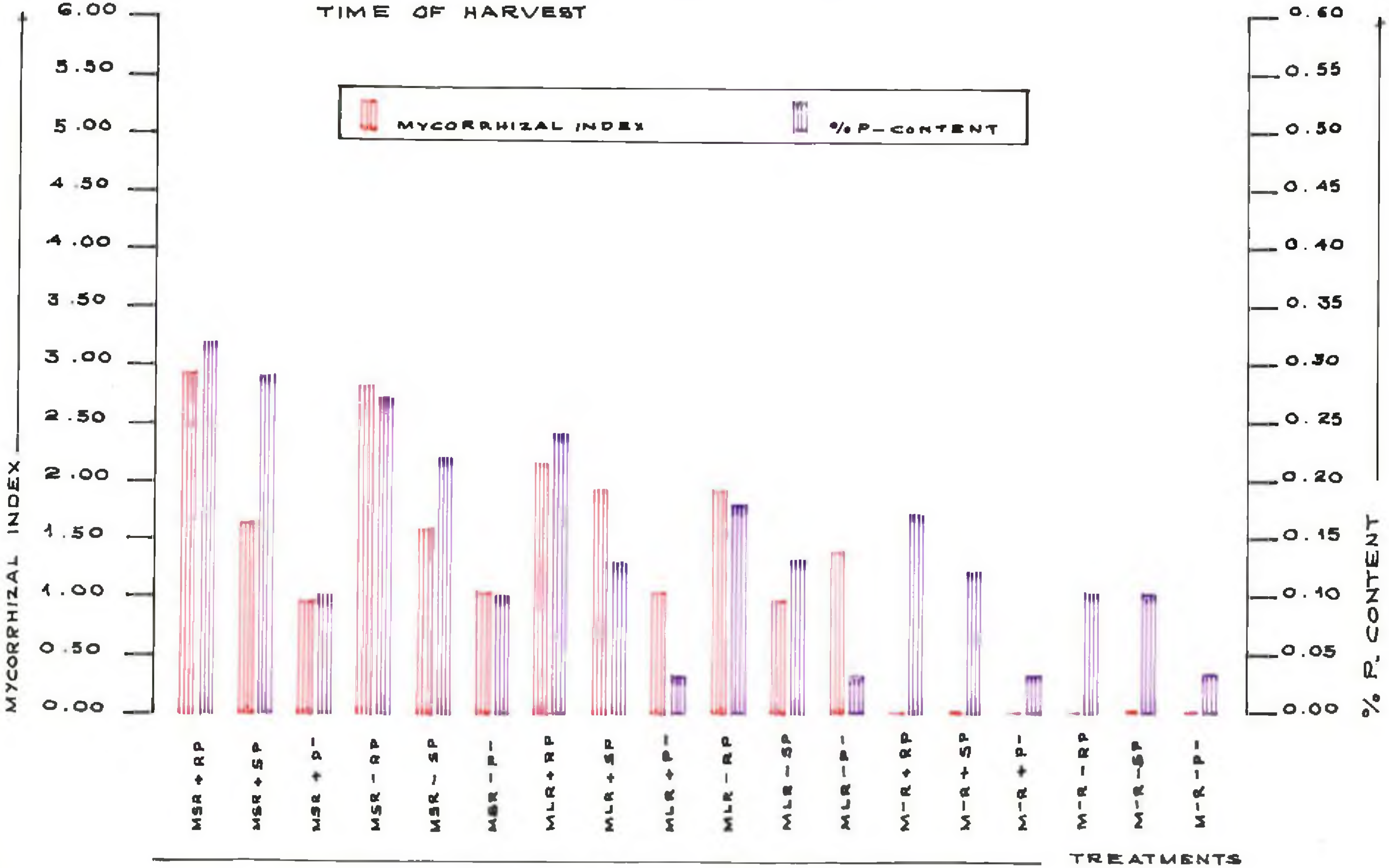

Table 9. Effect of VA-mycorrhiza and *Rhizobium* inoculation on yield in cowpea

Treatment combinations	Grain yield* g/pot	% increase over control
MS R^+RP	6.44	109.77
MS R^+SP	6.18	101.30
MS R^+P^-	4.64	51.14
MS R^-RP	5.82	89.58
MS R^-SP	4.65	51.47
MS R^-P^-	4.13	34.53
ML R^+RP	5.43	76.87
ML R^+SP	5.68	85.02
ML R^+P^-	3.52	14.66
ML R^-RP	3.94	28.34
ML R^-SP	4.92	60.26
ML R^-P^-	3.31	7.82
M^-R^+RP	3.63	18.24
M^-R^+SP	4.58	49.19
$M^-R^+P^-$	3.50	14.01
M^-R^-RP	3.67	19.54
M^-R^-SP	4.53	47.56
$M^-R^-P^-$ (Control)	3.07	0.00
C.D. (0.05)	1.91	

* Mean of three replications.

The mean mycorrhizal index of 2.92 was maximum in the MSR^+RP treatment (Table 7). This was significantly higher than the control and various other treatments except in the MSR^-RP treatment where the mean index of 2.76 was statistically on par with the above treatment. In general, there was a significant positive correlation between the mean mycorrhizal index and plant dry weight (Table 8). But there was no correlation between the mean mycorrhizal index and plant nitrogen content.

The percentage phosphorus content of shoot was significantly higher in both the phosphate treatments. The phosphorus content of 0.32 per cent was maximum in the MSR^+RP treatment (Table 7). This was significantly higher than the control and various other treatments except in MSR^+SP and MSR^-RP treatments where the percentage phosphorus content of shoot obtained was statistically on par with the above (MSR^+RP) treatment. In general, there was a significant positive correlation between the mean mycorrhizal index and phosphorus content of shoot (Table 8). Such a positive correlation also existed between plant dry weight and percentage phosphorus content of shoot.

The average grain yield of 6.44 g per pot was also maximum in the MSR^+RP treatment (Table 9). This was significantly higher than the control ($M^-R^-P^-$) treatment. However, the grain yields in most of the treatment combinations involving mycorrhiza and phosphate application were also higher and statistically on par with the above treatment (Table 9). But, in three of the treatments where there were either no mycorrhizal inoculation (M^-R^-SP and M^-R^+SP) or phosphate application (MSR^+P^-) there was a similar increase in yield.

In general, inoculation with VA-mycorrhiza had a beneficial effect on various plant characters studied. However, between the two mycorrhizae treatments involving Glomus microcarpus and Glomus sp., there were no significant differences in nodule number, nodule fresh weight, plant dry weight, percentage nitrogen content of shoot and the mean mycorrhizal index (Table 10). But, the dry weight of nodules, root length, plant height, plant fresh weight, percentage phosphorus content of shoot and grain yield were significantly higher in plants inoculated with the standard culture of VA-mycorrhiza (Table 10). Similarly, out of the two Rhizobium treatments, seed inoculation with Rhizobium had a significant effect in enhancing nodule number, fresh and dry weight of nodules, root length, plant height, fresh and dry weight of plants, percentage nitrogen and phosphorus content of shoot and grain yield (Table 10). But, there were no significant differences in the mean mycorrhizal index

Table 10. Individual effects of VA-mycorrhiza, *Rhizobium* and phosphate application on various plant characters in cowpea at the time of harvest.

Treatments	Nodule number	Nodule fresh wt. (mg)	Nodule dry wt. (mg)	Root length (cm)	Plant height (cm)	Plant fresh wt. (g)	Plant dry wt. (g)	N content of shoot (%)	Mycorrhizal index	P content of shoot (%)	Grain yield g/pot
MS	(4.70)	97.08	40.92	49.52	145.06	24.16	9.50	2.73	(1.67)	0.19	5.31
ML	(4.40)	80.16	33.75	29.10	115.58	21.66	8.91	2.59	(1.61)	0.12	4.46
M^-	(3.25)	59.56	17.88	30.30	56.42	13.22	5.50	2.24	(1.00)	0.09	3.82
R^+	(6.26)	137.68	54.44	37.58	111.67	20.75	8.72	2.94	(1.44)	0.15	4.84
R^-	(1.98)	20.19	7.26	35.03	99.70	18.61	7.22	2.09	(1.41)	0.12	4.22
RP	(4.54)	100.44	41.58	41.37	133.22	24.02	10.39	2.65	(1.57)	0.21	4.81
SP	(4.23)	79.08	27.36	38.77	101.83	20.41	8.50	2.60	(1.40)	0.16	5.09
P^-	(3.58)	57.27	23.61	28.78	82.01	14.61	5.02	2.30	(1.31)	0.02	3.69
C.D.(0.05) M/P =	0.43	18.50	5.81	2.50	11.03	1.60	1.44	0.27	0.07	0.02	3.69
C.D.(0.05) R =	0.35	15.11	4.75	2.04	9.01	1.30	0.87	0.22	0.05	0.02	0.34

Figures in parentheses are after $\sqrt{x + 1}$ transformation

Plate VII. Effect of inoculation with the standard culture of VA-mycorrhiza, _Glomus microcarpus_ and _Rhizobium_ on plant growth in cowpea at the time of harvest.

2 - $M^-R^-P^-$

4 - MSR^+SP

Plate VII

Plate VIII. Effect of inoculation with the local culture of VA-mycorrhiza (<u>Glomus</u> sp.) and <u>Rhizobium</u> on plant growth in cowpea at the time of harvest.

1 - $MLR^{+}SP$

2 - $M^{-}R^{-}P^{-}$

Plate VIII

due to Rhizobium inoculation. As regard to different phosphate treatments, application of phosphate fertilizer either in the form of rock phosphate or super phosphate had a beneficial effect on various plant characters studied at the time of harvest. But, there were no significant differences between the two phosphate treatments in increasing the nodule number and grain yield (Table 10). However, in the remaining characters studied, rock phosphate application had a significant effect in enhancing the fresh and dry weight of nodules, root length, plant height, fresh and dry weight of plants, percentage nitrogen and phosphorus content of shoot and the mean mycorrhizal index.

The effect of interactions between VA-mycorrhiza and Rhizobium, VA-mycorrhiza and phosphate application and between phosphate application and Rhizobium inoculation on various plant characters studied in cowpea at the time of harvest are given in Tables 11 to 13. A significant increase in nodule number, nodule fresh and dry weights were obtained due to the combined inoculation of the standard culture of VA-mycorrhiza (Glomus microcarpus) and Rhizobium (Table 11). Such an effect on nodulation was also observed with the use of Glomus microcarpus and rock phosphate in the M x P interaction (Table 12) and between

Table 11. Interaction between VA-mycorrhiza and *Rhizobium* inoculation on various plant characters in cowpea at the time of harvest

Treatments	Nodule number	Nodule fresh wt. (mg)	Nodule dry wt. (mg)	Root length (cm)	Plant height (cm)	Plant fresh wt. (g)	Plant dry wt. (g)	N content of shoot (%)	Mycorrhizal index	P content of shoot (%)	Grain yield g/pot
MSR^+	(7.64)	171.00	77.89	50.88	159.16	25.61	10.28	3.21	(1.67)	0.21	5.75
MSR^-	(1.77)	23.17	3.94	48.16	130.96	22.72	8.72	2.24	(1.66)	0.17	4.86
MLR^+	(6.36)	135.09	55.61	31.27	120.77	23.11	9.38	3.15	(1.63)	0.13	4.87
MLR^-	(2.45)	25.22	11.89	26.93	110.38	20.22	8.44	2.03	(1.58)	0.11	4.05
M^-R^+	(4.78)	106.94	29.83	30.58	55.08	13.85	6.50	2.47	(1.00)	0.10	3.90
M^-R^-	(1.72)	12.17	5.94	30.01	57.75	12.90	4.50	2.01	(1.00)	0.07	3.75
C.D. (0.05)	0.60	26.16	8.22	3.54	15.60	2.26	1.47	0.38	0.10	0.04	0.58

Figures in parentheses are after $\sqrt{x+1}$ transformation

Table 12. Interaction between VA-mycorrhiza and phosphate application on various plant characters in cowpea at the time of harvest.

Treatments	Nodule number	Nodule fresh wt. (mg)	Nodule dry wt. (mg)	Root length (cm)	Plant height (cm)	Plant fresh wt. (g)	Plant dry wt. (g)	N content of shoot (%)	Mycor-rhizal index	P content of shoot (%)	Grain yield g/pot
MSRP	(5.71)	138.17	64,83	61.16	203.33	34.66	14.42	3.02	(1.96)	0.30	6.12
MSSP	(4.69)	84.58	34.25	57.91	142.03	22.91	8.75	3.09	(1.62)	0.25	5.42
MSP^-	(3.72)	68.50	23.67	29.50	89.83	14.91	5.33	2.07	(1.41)	0.02	4.39
MLRP	(4.83)	98.92	40.08	30.00	134.83	25.58	11.17	2.68	(1.74)	0.21	4.68
MLSP	(4.56)	91.25	31.33	29.65	125.25	24.58	10.08	2.34	(1.56)	0.13	5.29
MLP^-	(3.83)	50.30	29.83	27.66	86.66	14.83	5.50	2.76	(1.52)	0.02	3.42
M^-RP	(3.10)	64.25	19.83	32.95	61.50	11.83	5.58	2.25	(1.00)	0.14	3.65
M^-SP	(3.44)	61.42	16.50	28.76	39.21	13.75	6.66	2.39	(1.00)	0.02	3.28
M^-P^-	(3.21)	53.00	17.33	29.18	69.55	14.10	4.25	2.08	(1.00)	0.02	3.28
C.D. (0.05)	0.74	32.04	10.07	4.33	19.11	2.77	1.80	0.46	0.12	0.04	0.71

Figures in parentheses are after $\sqrt{x+1}$ transformation

Table 13. Interaction between phosphate application and Rhizobium inoculation on various plant characters in cowpea at the time of harvest.

Treatments	Nodule number	Nodule fresh wt. (mg)	Nodule dry wt. (mg)	Root length (cm)	Plant height (cm)	Plant fresh wt. (g)	Plant dry wt. (g)	N content of shoot (%)	Mycorrhizal index	P content of shoot (%)	Grain yield g/pot
RPR^{+}	(7.31)	174.61	75.00	42.35	135.38	25.55	11.61	3.15	(1.58)	0.24	5.17
RPR^{-}	(1.78)	26.28	8.17	40.38	131.05	22.50	9.16	2.15	(1.54)	0.19	4.47
SPR^{+}	(6.39)	141.06	47.06	39.67	110.03	21.05	9.00	3.20	(1.45)	0.18	5.48
SPR^{-}	(2.07)	17.11	7.67	37.87	93.63	19.77	8.00	2.01	(1.35)	0.15	4.70
$P^{-}R^{+}$	(5.08)	97.37	41.28	36.72	89.61	15.66	5.50	2.48	(1.28)	0.02	3.88
$P^{-}R^{-}$	(2.09)	17.17	5.94	26.84	74.42	13.56	4.50	2.12	(1.35)	0.02	3.80
C.D. (0.05)	0.60	26.16	8.22	3.54	15.60	2.26	1.47	0.38	0.10	0.04	0.58

Figures in parentheses are after $\sqrt{x + 1}$ transformation

rock phosphate application and Rhizobium inoculation in the P x R interaction (Table 13). The above interactions were also significant in enhancing plant height and fresh and dry weight of plants at the time of harvest.

The increase in root length was maximum in the interactions between G. microcarpus and Rhizobium and both the types of phosphate fertilizers (Table 11, 12). Such an increase was also obtained with the use of rock phosphate along with Rhizobium culture, in the P x R interaction (Table 13). However, the increase in root length due to inoculation of the standard culture of VA-mycorrhiza alone (MSR^-) in the M x R interaction and the application of rock phosphate alone (RPP^-) in the P x R interaction were also significant.

There was a significant increase in the percentage nitrogen content of shoot in the interactions between the G. microcarpus and Rhizobium (MSR^+) and both the types of phosphate fertilizers (Table 11, 12). Such an increase was also obtained with the use of either rock phosphate or super phosphate along with Rhizobium inoculation (Table 13). The interactions between the local culture of VA-mycorrhiza, Glomus sp. and Rhizobium (MLR^+) in the M x R interaction and rock phosphate application (MLRP) in the M x P interaction

(Tables 11, 12) were also significant in enhancing the plant nitrogen content.

The use of both the cultures of mycorrhizae, either with or without Rhizobium inoculation, had a significant effect in increasing the mean mycorrhizal index of cowpea at the time of harvest (Table 11). In the interactions between mycorrhiza and phosphate application, maximum increase in mycorrhizal infection was obtained with the use of G. microcarpus and rock phosphate (Table 12). A similar effect of rock phosphate application was also observed with or without Rhizobium inoculation in the P x R interaction.

The use of the standard culture of VA-mycorrhiza either with or without Rhizobium inoculation also had a significant effect in enhancing the percentage phosphorus content of shoot at the time of harvest (Table 11). Similar positive effects were also observed in the interactions between this culture and rock phosphate (Table 12) and in the use of rock phosphate and Rhizobium culture (Table 13) in the P x R interaction.

The increase in yield was maximum with the dual inoculation of Glomus microcarpus and Rhizobium (Table 11).

Plate IX. Comparative effect of inoculation with the standard and local cultures of VA-mycorrhizae on plant growth in cowpea at the time of harvest.

3 - MLR+RP

4 - MSR+RP

Plate IX

Table 14. Effect of plant growth on the percentage nitrogen content of soil.

Treatments	Initial	Harvest	
		R^+	R^-
MS RP	0.0030	0.0037	0.0033
MS SP	0.0032	0.0030	0.0030
MS P^-	0.0030	0.0030	0.0030
ML RP	0.0032	0.0034	0.0042
ML SP	0.0032	0.0038	0.0026
ML P^-	0.0027	0.0035	0.0027
M^-RP	0.0032	0.0032	0.0032
M^-SP	0.0034	0.0025	0.0029
M^-P^-	0.0032	0.0032	0.0034
C.D. (0.05)	NS	NS	NS

NS. Not Significant.

Table 15. Effect of plant growth on the percentage phosphorus content of soil

Treatments	Initial	Harvest	
		R^+	R^-
MS RP	0.0012	0.0010	0.0008
MS SP	0.0015	0.0008	0.0010
ML RP	0.0015	0.0010	0.0008
ML SP	0.0012	0.0008	0.0010
M^-RP	0.0015	0.0003	0.0010
M^-SP	0.0015	0.0008	0.0011
C.D. (0.05)	NS	NS	NS

NS- Not significant.

Plate X. Comparative effect of rock phosphate and super phosphate application along with VA-mycorrhizal inoculation on plant growth in cowpea at the time of harvest.

14 - $MSR^{+}RP$

15 - $MSR^{+}SP$

Plate X

The interaction between this culture and both the type of phosphate fertilizers (MSRP and MSSP) were also significant in enhancing the yield (Table 12). However, between the two phosphate treatments, the yield increase was maximum with the use of Glomus microcarpus and rock phosphate (Table 12). In the interaction between phosphate application and Rhizobium inoculation, maximum increase in yield was obtained with the use of super phosphate and Rhizobium culture (Table 13). The interaction between rock phosphate and Rhizobium inoculation (RPR^{+}) was also significant in improving the yield of cowpea.

The percentage nitrogen and phosphorus content of soil, both before and after plant growth were also estimated (Table 14 and 15). However, there were no significant differences between various treatments. The increase in the percentage nitrogen content of soil was maximum in the $MLR^{-}RP$ treatment followed by the $MLR^{+}RP$ treatment. In general, there was a decrease in the percentage phosphorus content of soil after plant growth.

DISCUSSION

DISCUSSION

The symbiosis between microorganisms and higher plants has fascinated mankind for several decades. In fact, man was conscious about the benefits of crop rotation with legumes right from the dawn of 'Agriculture'. However, a systematic study to elucidate the mechanisms of root nodulation in legumes by Rhizobium began only with the work of Martinus Willem Beijerinck. Around the same period, another group of workers started investigating about mycorrhiza, a type of beneficial association by certain fungi with higher plants. In the beginning such studies were conducted more or less independently. But later it was found that a sort of dual symbiosis by Rhizobium and VA-mycorrhiza existed in legumes under natural conditions and that it was more beneficial to the host plant than when these associations occurred alone. Currently several research workers are actively engaged in understanding about this phenomenon of dual symbiosis in legumes. However, in Kerala, not much work is done in this direction and hence the present investigation on the "Effect of inoculation of vesicular-arbuscular mycorrhiza on nodulation and phosphorus uptake in cowpea" was taken up.

It is generally found that in many plant microbial relationships, apart from the microorganism, the host variety also exerts its influence on such a system. Therefore, in the first part of the present investigation, a pot culture experiment was conducted to study the effect of host variety on the natural incidence of VA-mycorrhiza in ten different varieties of cowpea. It was found that there were significant differences between varieties in their mean mycorrhizal index. The infection was maximum for the cowpea variety C-152 (1.19) followed by PTB-2 and New Era (Table 1). However, the mean mycorrhizal indices of the remaining seven varieties like CG-11, HG-22, PTB-1, RC-25, S-488, U-16 and V-38 were significantly lower than that of C-152. The application of rock phosphate at the rate of 30 kg P_2O_5/ha had a more or less uniform significant effect in enhancing the mycorrhizal infection in all varieties except in S-488 where such an effect due to phosphate application was not observed.

The occurrence of host varietal variations on the natural incidence of VA-mycorrhiza was reported earlier also in the case of wheat (Bertheau et al., 1980; Azcon and Ocampo, 1981), cowpea (Ollivier et al., 1983) and mung bean (Pandher et al., 1986). Although this is a well documented phenomenon, it is not yet clear what exactly leads to this type of varietal variations for infection by

VA-mycorrhiza. The presence or absence of certain stimulatory substances in the host root exudate favouring early spore germination and its subsequent root infection may be a reason for this type of natural differences in infection by VA-mycorrhiza even under identical soil and environmental conditions. It is also possible that since VA-mycorrhizal fungi are primarily dependent on their host for their carbon requirement in the form of simple sugars, the availability of such sugars in adequate quantity in the root exudate of a particular host variety will also influence the extent of root infection. However, one will have to conduct detailed studies in order to understand about the nature of such stimulatory substances in the root exudates of different host varieties. The effect of application of phosphate fertilizer on mycorrhizal infection is discussed later in this chapter.

An interesting observation made during this experiment was that the presence of VA-mycorrhiza under natural conditions was maximum in some of the popular varieties of cowpea such as C-152, PTB-2 and New Era. The higher incidence of VA-mycorrhiza in these varieties will be advantageous in that it will enable them to uptake more effectively the available phosphorus and some of the minor elements from the soil, when compared to varieties which normally have a lesser degree of mycorrhizal infection. This is especially important when one takes into consideration the fact that the soils of Kerala are generally P-limiting and P-fixing with relatively less amount of available phosphorus in the soil. Therefore, in future one may also include the natural ability of a particular pulse variety to form VA-mycorrhizal association, apart from nodulation and other desirable characters, as an important criterion for its selection for large scale cultivation. This is important for a crop like cowpea which is mostly cultivated as a supplementary crop in rice fallows or in small areas of garden land where the stress is often for using minimum quantity of fertilizers in order to increase the return from such cultivation practices.

Once a cowpea variety was identified for having maximum mycorrhizal infection, it was further used to study the effect of age of host plant on VA-mycorrhizal infection. This experiment was also done in pots but under aseptic conditions using the standard culture of VA-mycorrhiza, Glomus microcarpus and rock phosphate at the rate of 30 kg P_2O_5/ha. The observations on mycorrhizal infection were taken at 15 days interval up to 90 days. The infection was maximum on 45th day with a mean index of 1.62 (Table 2 and Plate 5). This was significantly higher than that observed at all other stages of plant growth (Plates 4 and 6). The extent of mycorrhizal infection on 30th, 60th and 75th day were also higher when compared to the infection on 15th and 90th day of plant growth. Such variations in mycorrhizal infection with respect to age of host plant were also reported earlier in the case of wheat (Saif and Khan, 1975), lucerne (Smith and Daft, 1977) and onion (Manjunath and Bagyaraj, 1981). As in the previous experiment, the application of rock phosphate at the rate of 30 kg P_2O_5/ha had a significant effect in enhancing the mycorrhizal infection at all stages of plant growth (Table 2). The possible effect of phosphate application on mycorrhizal infection is discussed later in this chapter.

When one critically examines the degree of mycorrhizal infection at different stages of plant growth in cowpea, a definite pattern consisting of three different phases similar to the one reported earlier by Sutton (1973) and Smith and Daft (1977) may be observed. During the early stages of plant growth lasting for about 20 to 25 days, the mycorrhizal infection was relatively low. This lag phase probably resulted from the time taken by the added inoculum mainly in the form of fungal spores and mycelia to make the necessary initial contact with the host root system prior to rapid proliferation in the cortex. Besides, the photosynthetic efficiency of the host will also be less at this stage affecting the actual availability of some of the nutrients especially sugars from the host system on which the VA-mycorrhiza is mainly dependent for its growth and infection. Further, there can also be a certain degree of competition with Rhizobium for such nutrients. Another limiting factor can be the total root surface area available for infection by the fungal mycelium. All these factors together may contribute to the observed lag phase in mycorrhizal infection during the early stages of plant growth.

Once the lag phase came to an end, there was an active period of root colonisation by VA-mycorrhiza. This stage lasted for another 20 to 25 days and reached a peak around the 45th day of plant growth. This in fact can be considered as the exponential phase of mycorrhizal infection in cowpea which incidentally corresponded with the peak photosynthetic phase of the host plant. Therefore, during this stage the availability of nutrients to VA-mycorrhiza from the host will also be adequate favouring a higher degree of root infection. Besides, nitrogen fixation by Rhizobium also attains an optimum level by this time. However, during this stage there can be greater competition between VA-mycorrhiza and Rhizobium for the available host carbon. But, this effect may be nullified to a great extent by the higher photosynthetic activity of the host plant itself associated with its maximum vegetative growth.

The mycorrhizal index on 60th, 75th and 90th day of plant growth showed a gradual decline. This can be due to the fact that the degree of mycorrhizal infection on new roots produced by the host after its peak vegetative growth is rather low or even absent. This in turn can lead to a gradual increase in the ratio of non-infected to infected roots. Further, there will also be a reduction in the availability of carbon for initiating fresh infection by VA-mycorrhiza because of its greater requirement by the host for seed formation. Therefore, it is quite possible that at this stage of plant growth, the host may induce a sort of 'host restriction' for new infection by VA-mycorrhiza. In fact, one usually observes a similar phenomenon in legumes - Rhizobium symbiosis where there is practically no fresh nodule formation in most of the annuals soon after the phase of peak vegetative growth.

Having observed that there is a varietal variation in cowpea for the natural incidence of VA-mycorrhiza and that the extent of mycorrhizal infection is influenced by the age of host plant, a detailed investigation was conducted to study the effect of combined inoculation of VA-mycorrhiza and Rhizobium on nodulation, phosphorus uptake and yield in cowpea. The observations were taken at two different stages of plant growth, on 45th day corresponding approximately with the maximum vegetative growth and at the time of harvest.

A uniform treatment effect on various plant characters studied was not observed on 45th day. The number of nodules formed and their fresh and dry weights were maximum in the treatment combination consisting of the standard culture of VA-mycorrhiza, Rhizobium inoculation and the use of super phosphate at the rate of 30 kg P_2O_5/ha (Table 3). However, the nodule number in two of the treatments without phosphate application (MSR^+P^- and MLR^+P^-) was also higher and statistically on par with the super phosphate treatment. The root length and plant height were maximum in the MSR^+P^- and $M^-R^-P^-$ treatments respectively. But the fresh and dry weights of plants and mycorrhizal index were significantly higher in the (MSR^+RP) treatment (Table 4). The percentage of nitrogen and phosphorus content of shoot were not correspondingly higher in the treatment combinations where the nodule number and the mean mycorrhizal index were significantly higher. As a result of this, it is rather difficult to point out any particular treatment combination as more beneficial for cowpea at this stage of plant growth. This can be due to the fact that it takes nearly 40 days for both micro symbionts to establish well in the host root system and then attain an optimum level of activity. Besides, the photosynthetic efficiency of the host also reaches a peak level only around this time enabling it to meet the increased carbon requirement of both the micro symbionts. This will in turn favour a greater expression of their physiological activities like the increased uptake of available phosphorus and other elements from the soil and also the enhanced reduction of atmospheric nitrogen to ammonia in the nodule cytosol. If one accepts the above hypothesis, then the beneficial effects of VA-mycorrhiza and Rhizobium inoculation will be more evident at the time of harvest. In fact, the results obtained during the present investigation lead to such a conclusion.

In general, the treatment combination consisting of the standard culture of VA-mycorrhiza, Rhizobium inoculation and rock phosphate application at the rate of 30 kg P_2O_5/ha produced more beneficial effects in cowpea at the time of harvest than all other treatments. Certain exceptions were also observed as in the case of root length which was maximum in the MSR$^-$RP treatment (Table 6) and in plant nitrogen content of the MSR$^+$SP treatment which was the same as that of MSR$^+$RP treatment (Table 7). The root length in the MSR$^+$RP, MSR$^+$SP and MSR$^-$SP treatments, percentage nitrogen content of shoot in the MLR$^+$SP and MLR$^+$RP treatments, percentage phosphorus content of shoot in the MSR$^+$SP and MSR$^-$RP treatments (Table 7) and the grain yield in the MSR$^+$P$^-$, M$^-$R$^-$SP and M$^-$R$^+$SP treatments (Table 9) were also statistically on par with the above MSR$^+$RP treatment. Although such variations in treatment effects are possible to some extent, it is rather difficult to explain the increase in yield obtained in the MSR$^+$P$^-$ treatment where there was no phosphate fertilizer application. But in two of the remaining treatments (M$^-$R$^-$SP and M$^-$R$^+$SP) without any mycorrhizal inoculation, the increase in yield may be due to the use of super phosphate. Therefore, in order to understand the actual effects of combined inoculation of VA-mycorrhiza and Rhizobium along with phosphate fertilizers, the data was further analysed for their individual effects as well as for various interactions.

There were no significant differences between the two mycorrhizae treatments involving the standard culture of Glomus microcarpus and the local culture of Glomus sp. in increasing the nodule fresh weight, plant dry weight, mycorrhizal index and the percentage nitrogen content of shoot when compared to treatments without any mycorrhizal inoculation (Table 10). However, in the remaining characters studied especially with regard to the percentage phosphorus content of shoot and final grain yield, the benefits were maximum with the inoculation of Glomus microcarpus. This indicated that the introduced inoculum of VA-mycorrhiza was more superior than the local culture of Glomus sp. In fact several of the earlier reports published by Mosse (1977), Powell (1977) and Barea et al. (1980) substantiate this point. As regard to the individual effects of the two Rhizobium treatments, seed inoculation with an appropriate culture had a uniform beneficial effect on various plant characters studied. Such effects of Rhizobium inoculation are well documented.

The combined use of Glomus microcarpus and Rhizobium also had a beneficial effect on nodulation and other related characters in cowpea. However, if one measures the efficiency of nitrogen fixation in terms of the percentage nitrogen content of shoot instead of the final grain yield, one can see that the interaction between the local culture of VA-mycorrhiza and Rhizobium was also significant (Table 11). It therefore appears that the inoculation of VA-mycorrhiza is beneficial for nodulation by Rhizobium in cowpea. In fact as early as in 1944, Asai had clearly demonstrated the importance of VA-mycorrhizal association for an effective nodulation in various legumes like *Trifolium*, *Melilotus* and *Medicago* spp. grown in sterilised soil. Such effects due to mycorrhizal inoculation on nodulation in sterilised or pre-fumigated soil for eliminating soil borne fungal pathogens were also reported in the case of soybean (Ross and Harper, 1970; Schenck and Hinson, 1973; Bagyaraj *et al*., 1979), *Centrosema pubescens*, *Stylosanthes guanensis* and *Trifolium repens* (Crush, 1974), *Phaseolus* (Daft and Giahmi, 1974), groundnut (Daft and Giahmi, 1976), cowpea (Godse *et al*., 1978 and LaTorraca, 1979), *Medicago sativa* (Azcon *et al*., 1979), clover (Smith *et al*., 1979; Green *et al*., 1983), *Leucaena Leucocephala* (Sivaprasad *et al*., 1983), lentil (Bala and Singh, 1983) and mung bean (Pandher *et al*., 1986).

The benefits of VA-mycorrhizal association on nodulation may be indirect in that it may be favouring an early nodule initiation process and later its activity by absorbing more phosphorus and some of the minor elements like Ca, Mg, Mo and Fe from the soil. All these elements influence the nodulation process to a great extent. A number of reports were already published with regard to the ability of VA-mycorrhiza to absorb various elements such as P, K, Cu and Fe (Mosse, 1957), Zn (Bowen and Mosse, 1969; Gilmore, 1971), N, P, Ca, Cu and Mn (Ross and Harper, 1970) and S (Gray and Gerdemann, 1973) from the soil. Interestingly, Rhizobium inoculation did not have any effect on mycorrhizal infection (Table 11) or on the percentage phosphorus content of shoot in cowpea.

The results on the relative benefits of the three phosphate treatments showed that the combination of rock phosphate and VA-mycorrhiza was more beneficial to cowpea than the use of super phosphate. The mean mycorrhizal index and percentage phosphorus content of shoot were significantly higher in various rock phosphate treatments (Table 7). However, there were no significant differences between the application of rock phosphate and super phosphate in the number of nodules formed and in the final grain yield at the time of harvest (Table 10). Therefore, the combination of VA-mycorrhiza and super phosphate was not that harmful to cowpea, but rather it was only less beneficial when compared to the use of VA-mycorrhiza and rock phosphate. The effect of super phosphate appears to be on the infection process by VA-mycorrhiza. This can be deduced from the interaction between VA-mycorrhiza and phosphate application.

It was found that when no phosphate fertilizer was used even with artificial inoculation of VA-mycorrhiza, the extent of root infection was very low (Table 12). However, with the use of rock phosphate or super phosphate at the rate of 30 kg P_2O_5/ha there was an increase in infection by the standard as well as the local culture of VA-mycorrhiza.

These effects were more pronounced in the interaction between Glomus microcarpus and both the types of phosphate fertilizers. Therefore, a certain quantity of phosphate in any form should be available during the early stages of plant growth for initiating an effective VA-mycorrhizal infection. Now, the question that arises here is that between the two phosphate fertilizer treatments, which one is more useful for inducing a higher degree of root infection by VA-mycorrhiza? It was found that it will be more beneficial to use rock phosphate to get such an effect eventhough, the use of super phosphate at lower doses will not significantly affect the mycorrhizal infection. This may be due to the fact that it is not exactly the type of the phosphate fertilizer which is used along with the mycorrhizal inoculum that is important, but what matters is the resultant concentration of phosphate ions within the root system.

When super phosphate is used, there will be a rapid absorption of phosphate ions both by the host root system and the developing VA-mycorrhiza leading to an early increase in the root concentration of phosphate ions. This in turn affects the infection process by VA-mycorrhiza as pointed out by Mosse (1973) and Menge et al. (1978). However, detailed studies will be required to find out the exact level of phosphate ions required within the root system to produce such an effect. But when rock phosphate is used, a rapid increase in the internal concentration of phosphate ions does not take place. This is because, the extent of phosphate ions available for absorption by the host root system as well as the VA-mycorrhiza will be relatively low since both can uptake only those phosphate ions which are normally dissociating from rock phosphate into an available form by various natural processes. Besides, it is already well established that VA-mycorrhiza does not directly solubilize rock phosphate as is observed with various phosphate solubilising fungi and bacteria. Therefore, with the use of rock phosphate, there will not be a rapid increase in the concentration of phosphate ions within the root system which can influence the infection by VA-mycorrhiza. But since VA-mycorrhizae are good scavengers of phosphate ions from soil because of their ability to explore greater volume of soil even beyond the rapidly developing phosphate depletion zone around host root system, plants with VA-mycorrhizal association will be able to absorb over a period of time more phosphate ions even when rock phosphate is used as a source of phosphatic fertilizer, when compared to plants without any VA-mycorrhizal association. Thus, the net amount of phosphate ions absorbed in this manner by the host plant with the help of VA-mycorrhiza will be comparable to that resulting from the application of super phosphate and as observed during the present investigation, it was even superior to the use of super phosphate itself.

In conclusion, the interactions between the standard culture of VA-mycorrhiza Glomus microcarpus and Rhizobium and Glomus microcarpus and rock phosphate application, (Tables 11 and 12) were significant and superior to all other interactions. Thus, maximum increases in nodule number, nodule fresh and dry weights, root length, plant height, fresh and dry weights of plant, mycorrhizal index, percentage nitrogen and phosphorus contents of shoot and grain yield were obtained in the above interactions. This also explained why the treatment combination consisting of these three factors together ($MSR^{+}RP$) was more beneficial to cowpea than various other treatments tested during this investigation.

The percentage nitrogen and phosphorus content of soil both before and after plant growth were also estimated (Tables 14 and 15). However, the results obtained here did not indicate any definite treatment effect and therefore they could not be correlated with the various beneficial effects obtained due to the use of VA-mycorrhiza, Rhizobium inoculation and phosphate application on plant growth in cowpea.

SUMMARY

SUMMARY

A study on the effect of inoculation of vesicular-arbuscular mycorrhiza and *Rhizobium* on nodulation, phosphorus uptake and yield in cowpea was conducted at College of Agriculture, Vellayani, Trivandrum, during 1983-85.

Three separate pot culture experiments were conducted during this investigation. Initially, ten cowpea varieties were screened for the natural incidence of VA-mycorrhiza. There were significant differences between varieties in their mean mycorrhizal indices. The infection was maximum for the variety C-152 which had an average index of 1.19. This was significantly higher than that of varieties such as CG-11, HG-22, PTB-1, RC-25, S-488, U-16 and V-38. The mycorrhizal indices of two other varieties, New era and PTB-2 were also on par with that of C-152. The application of rock phosphate at the rate of 30 kg P_2O_5/ha had a significant effect in enhancing the mycorrhizal infection in all varieties except in S-488 where such an effect due to phosphate application on mycorrhizal infection was not observed.

The cowpea variety which had the maximum mycorrhizal index, C-152, was further used to study the effect of age of host plant on mycorrhizal infection. The infection was

maximum on 45th day with an average index of 1.62. This was significantly higher than that observed at all other stages of plant growth. On 15th, 30th, 60th, 75th and 90th day of plant growth, the mean mycorrhizal indices were 0.30, 0.77, 0.32, 0.74 and 0.49 respectively. The application of rock phosphate at the rate of 30 kg P_2O_5/ha had a uniform significant effect in enhancing the mycorrhizal infection at all stages of plant growth.

In the last part of the present investigation, a detailed study on the combined effect of VA-mycorrhiza and Rhizobium on nodulation, phosphorus uptake and yield in cowpea was undertaken. Observations were taken at two stages of plant growth, on 45th day and at the time of harvest.

A uniform treatment effect on various plant characters studied was observed only at the time of harvest. The number of nodules formed per plant and their fresh and dry weights were significantly higher in the treatment combination consisting of the standard culture of VA-mycorrhiza, Rhizobium inoculation and the application of rock phosphate at the rate of 30 kg P_2O_5/ha. In this treatment (MSR$^+$RP), 96.02 nodules were formed with a fresh and dry weights of 231.33 and 126.83 mg respectively. The average fresh and dry weight of plants, 37.00 and 15.83 g respectively were also maximum in the MSR$^+$RP treatment. In general, there was a significant positive correlation between nodule number and plant dry weight. The percentage nitrogen content of shoot was uniformly higher in all Rhizobium inoculated treatments. The nitrogen content of 3.75 per cent was maximum in the MSR$^+$RP and MSR$^+$SP treatments. There was also a significant positive correlation between nodule number and percentage nitrogen content of shoot.

The mean mycorrhizal index of 2.92 was maximum in the MSR$^+$RP treatment. Besides, there was a significant positive correlation between the mean mycorrhizal index and plant dry weight. But there was no correlation between the mycorrhizal index and plant nitrogen content. The percentage phosphorus content of shoot was significantly higher in both the phosphate treatments. The phosphorus content of 0.32 per cent was maximum in the MSR$^+$RP treatment. Further, there was a significant positive correlation between the mean mycorrhizal index and phosphorus content of shoot. Such a positive correlation also existed between plant dry weight and percentage phosphorus content of shoot. The average grain yield of 6.44 g per pot was maximum in the MSR$^+$RP treatment. This was significantly higher than the control (M$^-$R$^-$P$^-$) treatment.

The percentage nitrogen and phosphorus content of soil both before and after plant growth were also estimated. However, there were no significant differences between various treatments. The increase in the percentage nitrogen content of soil was maximum in the MLR⁻RP treatment followed by the MSR⁺RP treatment. In general, there was a decrease in the percentage phosphorus content of soil after plant growth.

The interactions between the standard culture of VA-mycorrhiza, *Glomus microcarpus* and *Rhizobium*, *Glomus microcarpus* and rock phosphate application, and rock phosphate application and *Rhizobium* inoculation were also significant and superior to all other interactions. Thus maximum increase in nodule number, nodule fresh and dry weights, root length, plant height, fresh and dry weights of plant, mycorrhizal index, percentage nitrogen and phosphorus contents of shoot and grain yield were obtained in the above interactions. This also explained why the treatment combination consisting of these three factors together (MSR⁺RP) was more beneficial to cowpea than various other treatments tested during this investigation.

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(* Original not seen)

APPENDICES

APPENDIX I

Analysis of Variance Table

Influence of host variety on the natural incidence of VA-mycorrhiza in cowpea

Source	df.	S.S.	M.S.S.	F
Total	59	4.006		
Treatment	19	3.183	0.168	7.81**
Variety	9	1.220	0.135	6.31**
Phosphorus	1	1.570	1.570	73.16**
Variety x phosphorus	9	0.390	0.044	2.04
Error	38	0.820	0.021	

** Significant at 0.01 level

APPENDIX II

Analysis of Variance Table

Effect of age of host plant on VA-mycorrhizal infection in cowpea

Source	df.	S.S.	M.S.S.	F.
Total	35	4.599		
Treatment	11	3.936	0.359	12.27**
Age	5	0.812	0.162	5.57**
Phosphorus	1	2.620	2.620	89.83**
Age x phosphorus	5	0.505	0.101	3.46*
Error	22	0.642	0.029	

* Significant at 0.05 level

** Significant at 0.01 level

APPENDIX III

Analysis of Variance Table

Effect of VA-mycorrhiza and *Rhizobium* inoculation on nodulation and other characters in cowpea on 45th day.

Source	df.	Mean square				
		Nodule number	Nodule fresh wt. (mg)	Nodule dry wt. (mg)	Root length (cm)	Plant height (cm)
Total	53					
Treatment	17	7.716**	10978.900**	1912.246**	47.16**	83.20**
Error	36	0.314	544.921	120.857	20.65	41.22

** Significant at 0.01 level

APPENDIX IV

Analysis of Variance Table

Effect of VA-mycorrhiza and <u>Rhizobium</u> inoculation on phosphorus uptake and other characters in cowpea on 45th day

Source	df	Mean Square				
		Plant fresh weight (g)	Plant dry weight (g)	N-content of shoot (%)	Mycorrhizal index	P-content of shoot (%)
Total	53					
Treatment	17	7.924**	2.158**	1.417**	0.513	0.016**
Error	36	0.671	0.125	0.341	0.015	0.002

** Significant at 0.01 level

APPENDIX V

Analysis of Variance Table

Effect of VA-mycorrhiza and *Rhizobium* inoculation on nodulation and other characters in cow pea at the time of harvest.

Source	df	Mean square				
		Nodule number	Nodule fresh wt. (mg)	Nodule dry wt. (mg)	Root length (cm)	Plant height (cm)
Total	53					
Treatment	17	18.945**	14239.950**	3111.783**	508.291**	7558.661**
Error	36	0.598	748.019	73.861	13.680	266.186

** Significant at 0.01 level

APPENDIX VI

Analysis of Variance Table

Effect of VA-mycorrhiza and *Rhizobium* inoculation on phosphorus uptake and other characters in cowpea at the time of harvest.

Source	df	Mean square					
		Plant fresh weight (g)	Plant dry weight (g)	N-content of shoot (%)	Mycorrhizal index	P-content of shoot (%)	Grain yield g/pot
Total	53						
Treatments	17	171.66**	35.38**	1.240**	0.360**	0.032**	3.192**
Error	36	5.62	2.37	0.160	0.010	0.004	0.368

** Significant at 0.01 level

APPENDIX VII

Analysis of variance Table

Effect of VA-mycorrhiza and Rhizobium inoculation on nodulation and other characters in cowpea at the time of harvest.

Source	df	Mean square				
		Nodule number	Nodule fresh wt. (mg)	Nodule dry wt (mg)	Root length (cm)	Plant height (cm)
Total	53					
Treatments	17	18.950**	14239.950**	3111.783**	508.29**	7558.61**
M	2	10.620**	6357.750**	2499.650**	2364.61**	36681.38**
P	2	4.330**	8389.770**	1618.010**	795.30**	11999.91**
R	1	246.420**	186360.900**	30056.990**	87.66*	1935.56*
M x P	4	1.710**	1949.100	622.980**	533.45**	6427.08**
P x R	2	7.240**	5362.891**	1321.420**	6.00	198.69
M x R	2	9.310**	3363.000**	2859.230**	16.06	1080.38*
M x P x R	4	1.450	243.750	898.710**	13.88	232.95
Error	36	0.398	748.020	73.860	13.68	266.19

* Significant at 0.05 level

** Significant at 0.01 level

APPENDIX VIII

Analysis of variance Table

Effect of VA-mycorrhiza and Rhizobium inoculation on phosphorus uptake and other characters in cowpea at the time of harvest.

Source	df	Mean square: Plant fresh weight (g)	Plant dry weight (g)	N-content of shoot (%)	Mycorrhizal index	P-content of shoot (%)	Grain yield g/pot
Total	53						
Treatments	17	171.660**	35.380**	1.239*	0.360**	0.0317**	3.192**
M	2	591.370**	84.040**	1.140**	2.410**	0.0478**	9.940**
P	2	405.750**	113.160**	0.640*	0.302**	0.1831**	9.850**
R	1	62.080**	30.380**	9.730**	0.007	0.0125**	5.130**
M x P	4	203.470**	28.140**	0.880**	0.121**	0.0351**	1.430*
P x R	2	3.560	3.010	0.840*	0.036*	0.0036	0.190*
M x R	2	7.480	1.260	0.540*	0.003	0.0003	0.748
M x P x R	4	6.480	3.940	0.380	0.031*	0.0007	0.490
Error	36	5.620	2.370	0.160	0.010	0.0014	0.368

* Significant at 0.05 level

** Significant at 0.01 level

APPENDIX IX

Analysis of Variance Table

Effect of plant growth on the percentage of nitrogen content of soil

Source	df	S.S.	M.S.S.	F
Total	53	1.120833E-05		
Treatment	17	2.708337E-06	1.593139E-07	0.6747418
M	2	3.333371E-07	1.666685E-07	0.7058906
P	2	5.277798E-07	2.638899E-07	1.117652
R	1	3.750065E-07	3.750065E-07	1.588264
M x P	4	8.888819E-07	2.222205E-07	0.9411696
P x R	2	3.611058E-07	1.805529E-07	0.764695
M x R	2	1.111039E-07	5.555194E-08	0.2352789
M x P x R	4	1.111221E-07	2.778052E-08	0.1176587
Error	36	8.499996E-06	2.36111E-07	

APPENDIX X

Analysis of Variance Table

Effect of plant growth on the percentage of phosphorus content of soil

Source	df	S.S.	M.S.S.	F
Total	53	1.721655E-05		
Treatments	17	5.302981E-06	3.119400E-07	1.4190624
M	2	4.157804E-07	2.079890E-07	0.9461735
P	2	2.584653E-07	1.292327E-07	0.5878959
R	1	2.015149E-07	2.016147E-07	0.9167218
M x P	4	1.300941E-06	3.252353E-07	1.479545
P x R	2	4.012836E-07	2.006418E-07	0.9127499
M x R	2	3.546041E-07	1.770205E-07	0.8052931
M x P x R	4	0.244204E-05	0.11051E-06	0.277976
Error	36	7.913565E-06	2.198212E-07	

EFFECT OF INOCULATION OF VESICULAR-ARBUSCULAR MYCORRHIZA ON NODULATION AND PHOSPHORUS UPTAKE IN COWPEA

By

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ABSTRACT OF A THESIS
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ABSTRACT

A study on the effect of inoculation of vesicular-arbuscular mycorrhiza and *Rhizobium* on nodulation, phosphorus uptake and yield in cowpea was conduoted at College of Agriculture, Vellayani, Trivandrum, during 1983-85.

Three separate pot culture experiments were conducted during this investigation. Initially, ten cowpea varieties were screened for the natural incidence of VA-mycorrhiza. There were significant differences between varieties in their mean mycorrhizal index. The infection was maximum in C-152 whioh had an average index of 1.19. This was significantly higher than that of varieties such as CG-11, HG-22, PTB-1, RC-25, S-488, U-16 and V-38. The application of rock phosphate had a significant effect in enhancing the mycorrhizal infection in all varieties except in S-488. The cowpea variety which had the maximum mycorrhizal index under natural conditions was further used to study the effect of age of host plant on mycorrhizal infection. This was significantly higher on 45th day. The application of rock phosphate at the rate of 30 kg P_2O_5/ha had a uniform significant effect in enhancing the mycorrhizal infection at different stages of plant growth.

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A study on the effect of inoculation of vesicular-arbuscular mycorrhiza and Rhizobium on nodulation, phosphorus uptake and yield in cowpea was conducted at College of Agriculture, Vellayani, Trivandrum, during 1983-85.

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In the last part, a detailed study on the combined effect of VA-mycorrhiza and _Rhizobium_ inoculation on nodulation, phosphorus uptake and yield in cowpea was conducted. Observations were taken at two stages of plant growth, on 45th day and at the time of harvest. A uniform treatment effect on various plant characters studied was observed only at the time of harvest. The number of nodules formed per plant and their fresh and dry weights were significantly higher in the treatment combination consisting of the standard culture of VA-mycorrhiza, _Rhizobium_ inoculation and the application of rock phosphate at the rate of 30 kg P_2O_5/ha. The fresh and dry weights of plants and the mean mycorrhizal index were also maximum in this (MSR^+RP) treatment.

The percentage phosphorus content of shoot was significantly higher in both the phosphate treatments and was maximum in the MSR^+RP treatment. The percentage nitrogen content of shoot was uniformly higher in all _Rhizobium_ inoculated treatments. The nitrogen content was same in the MSR^+RP and MSR^+SP treatments. The average grain yield per pot was also maximum in the MSR^+RP treatment. This was significantly higher than the control. However, the mean grain yield obtained in most of the treatments involving mycorrhiza and phosphate application was also higher and statistically on par with the above (MSR+RP) treatment.

The percentage nitrogen and phosphorus content of soil both before and after plant growth were also estimated. However, the result obtained here did not indicate any definite treatment effect.

The interactions between the standard culture of VA-mycorrhiza, Glomus microcarpus and Rhizobium, Glomus microcarpus and rock phosphate, and rock phosphate application and Rhizobium inoculation were also significant and superior to all other interactions. Thus, maximum increase in nodule number, nodule fresh and dry weights, root length, plant height, fresh and dry weights of plants, mycorrhizal index, percentage nitrogen and phosphorus contents of shoots and grain yield were obtained in the above interactions. This also explained, why the treatment combination consisting of these three factors together (MSR+RP) was more beneficial to cowpea than various other treatments tested during this investigation.