

**INTERACTION BETWEEN VA MYCORRHIZA AND PHOSPHATE
SOLUBILIZING FUNGI AND THEIR EFFECTS ON RHIZOSPHERE
MICROFLORA AND GROWTH OF FINGER MILLET**

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**Department of Agricultural Microbiology
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BANGALORE
1980**

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MICROFLORA AND GROWTH OF FINGER MILLET**

By

N. N. GOPALA KRISHNA

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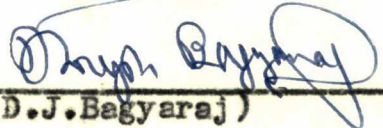
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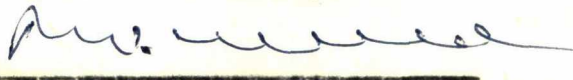
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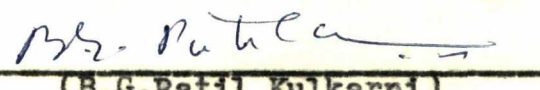
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INTRODUCTION

CHAPTER I

INTRODUCTION

The uptake of inorganic nutrients by plants is influenced by microorganisms in the rhizosphere. Symbiotic endophytes such as Rhizobium and mycorrhiza are examples of microorganisms that are involved in the uptake of two vital plant nutrient elements, nitrogen and phosphorus.

There are many recent reports providing evidence that most of the taxonomically higher order plants are infected with mycorrhizal fungi (Mosse, 1973) which assists in the uptake of nutrients like phosphorus (Gerdemann, 1970), sulphur (Rhodes and Gerdemann, 1978), potassium (Powell, 1975a) and micronutrients like zinc, copper, manganese, and iron (Ross, 1971).

Relatively few studies have been conducted to investigate the effect of vesicular-arbuscular (VA) mycorrhiza on other microorganisms in the rhizosphere (Bagyaraj and Menge, 1978). Some of the specific organisms for which detailed study has been done are, nematode (Sikora, 1978), phytopathogens (Schonbeck, 1978), Rhizobium (Bagyaraj et al., 1979b) and phosphorus solubilising bacteria (Barea et al., 1975; Azcon et al., 1976; Azcon G.de Angilar and Barea, 1978a; Azcon et al., 1978b). Results of the experiment conducted by Barea et al. (1975) and Azcon et al. (1976) show that VA mycorrhiza and phosphorus solubilising bacteria interacted synergistically increasing crop growth and nutrition.

It has been pointed out by earlier workers that VA mycorrhiza do not solubilise unavailable forms of phosphorus, but help the plant take up more phosphorus from the readily available source mainly through the increased surface area of absorption. However, there are certain fungi like Aspergillus niger and Penicillium funiculosum which are known to solubilise insoluble forms of phosphorus to forms available to plants growth (Raj, 1980). There is no information on the effect of dual inoculation with VA mycorrhiza plus phosphate solubilising fungi on plant growth and nutrition and also their interaction with each other and their influence on other microorganisms in the rhizosphere. Hence, the present study was undertaken to know the possible interaction between mycorrhiza and two phosphorus solubilising fungi and their effect on crop growth and rhizosphere microflora.

REVIEW OF LITERATURE

CHAPTER II

REVIEW OF LITERATURE

Mycorrhiza meaning "fungus root" are a group of fungi which form a symbiotic association with the roots of higher plants. Depending on the type of association formed, they have been divided into three categories, Ectomycorrhiza, Ectendomycorrhiza and Endomycorrhiza.

The fungi forming ectomycorrhiza mostly belong to the class Basidiomycetes particularly the families Agaricaceae and Boletaceae. In addition, the fungus of the class Deuteromycetes (Coenococcum graminiformis) also form ectomycorrhiza. The ectomycorrhizal fungi form a unique net like structure called hartignet. The net comprises of closely knit fungal mycelium covering the entire root surface. The mycelium penetrate only the epidermal layer. The net forms 20-30 per cent of dry weight of the infected root. The infected root is also known to contain 85 per cent moisture (Harley, 1969). The ectomycorrhizal association is found to be obligatory in some trees like pines and conifers. The fungal hyphae are known to form a bridge between soil and the plant for the translocation of nutrients.

Ectendomycorrhiza also known as orchid mycorrhiza belong to the classes Basidiomycetes and Mycelia sterilia. In this case the fungus penetrates the root and spread in the epidermal and outer cortical region and does not form a hartignet. The

fungus is obligatory for orchids to grow since it helps in carbon and phosphorus nutrition.

Endomycorrhiza also known as VA mycorrhiza are the fungi belonging to the class Phycomycetes. These fungi infect the roots of a wide variety of plants. The fungal hyphae penetrate the root and spread in the cortical region. The hyphae ramify intra or inter cellularly sometimes ending in a vesicular structure. The hyphae inside the cell break into fine branched structures called arbuscules. The arbuscules are rich in phosphorus and believed to be the site of exchange of nutrients between the fungus and the plant.

Occurrence of VA mycorrhiza

Even though the occurrence of VA mycorrhizae was recorded as far back as 1809 by Link, the exact role it plays in association with plant was not known until the middle of twentieth century. This long gap to study its role could be attributed to the fact that in the present times due to the shortage of chemical fertilizers, the scientists are trying to know and improve the natural processes by which plants fulfil their nutritional requirement. Another importance for which the scientists are trying to study mycorrhiza is because of its occurrence in a wide ecological condition ranging from sand dunes (Khan, 1974; Sutton and Sheppard, 1976; and Clough and Sutton, 1978), and coal mines (Daft and Nicolson, 1974; and

Khan, 1978) to aquatic environments (Sondergaard and Laegard, 1977; and Bagyaraj et al., 1979a). The main interest in mycorrhiza is in its occurrence in agriculturally important crops like maize (Gerdemann, 1968), soybean (Gerdemann, 1968; and Ross and Harper, 1970), cotton (Gerdemann, 1968), citrus (Gerdemann, 1968; and Mengé, 1978), grape (Gerdemann, 1968; and Deal et al., 1972), tomato (Gerdemann, 1968), grasses (Nicolson, 1959), peanuts and other legumes (Butler, 1939; Daft and Giahmi, 1976; Graw and Rehm, 1977; and Bagyaraj et al., 1979b).

In India, the occurrence of VA mycorrhiza was reported as early as 1939 by Butler in peanuts. There was a large gap in the study of mycorrhiza from 1939 till Godse et al. (1976) who conducted a survey of crop plants grown in and around Bangalore for the presence of mycorrhiza and reported its occurrence in soybean, cow pea, finger millet, chillies, maize, pearl millet and napier grass. A study conducted in U.A.S. under the Indian Council of Agricultural Research Project on Mycorrhiza showed the presence of mycorrhiza in most of the commonly cultivated crop plants.

Effect of VA mycorrhiza on plant growth and plant nutrient uptake

Mosse (1957) showed that mycorrhizal apples grew better and absorbed more P, K, Fe and Cu than non-mycorrhizal apples.

Mn was found to be less in mycorrhizal than in non-mycorrhizal plants. Daft and Nicolson (1966) studied the growth effects of three mycorrhizal *Endogone* endophyte on tobacco, tomato and maize. They found that the extent of the stimulus obtained depended on nutrient conditions and subsequent levels of infection developed in root system. Marked stimulus occurred at low P levels.

Gray and Gerdemann (1969) compared the uptake and accumulation of phosphorus by mycorrhizal and non-mycorrhizal onion plants. They found that mycorrhizal onion plants accumulated significantly more phosphorus in the roots and tops than non-mycorrhizal plants. They concluded that VA mycorrhizae were the sites of increased phosphorus accumulation compared to non-mycorrhizal roots and a fungi toxicant (Parachloronitrobenzene) reduced phosphate accumulation by mycorrhiza but did not significantly affect phosphate accumulation by non-mycorrhizal roots.

Baylis (1970) found that Coprosoma robusta grew well in unsteamed soil forming mycorrhizae with or without phosphorus. In steamed soil it grew well with phosphorus. He suggested that mycorrhizae entered through extensive root hair (or rhizoids) and that Coprosoma robusta lacked root hair and this explained their need for added phosphorus when mycorrhizae were not formed. Gilmore (1971) found that inoculation with mycorrhizae completely corrected Zn deficiency in peach plants.

Out of the six Endogone species tested, all were found to be beneficial, although two of them completely removed Zn deficiency symptoms. He also found that peach seedlings grown with one of these two strains had 2-3 times more Zn in their leaves compared to control plants. Ross (1971) found that at low, medium and high phosphates in the soil, yield increase due to endomycorrhiza in soybean were 122, 67 and 12 per cent respectively. Concentrations of N, P, Ca and Cu in foliage of mycorrhizal plants were greater than those from non-mycorrhizal plants. P concentration in foliage of mycorrhizal plants at the lowest P level was greater than that in non-mycorrhizal plant at the highest phosphate level. Hayman and Mosse (1971) comparing four irradiated and unsterilized soils containing little soluble phosphorus for the benefit of mycorrhizal infection, concluded that mycorrhizal inoculation may have practical significance in some low phosphate soils containing either few indigenous mycorrhizal fungi or infective strains.

Safir et al. (1971) found that the mycorrhizae produced by Endogone mossae decreased the resistance to water transport in soybean (Glycine max L.). The decrease was associated with an increase in shoot growth but not root growth.

Hayman and Mosse (1972a) found in onion plants grown in a range of soils labelled with ^{32}P that mycorrhizal plants had

taken up more phosphorus and grew larger, but the proportion of ^{32}P to total P (specific activity) taken up by mycorrhizal and non-mycorrhizal plants after 10 weeks was not significantly different. They finally concluded that the mycorrhizal plant roots used the same source of labile P but explored a greater volume of soil beyond the root zone of P depletion.

Hayman and Mosse (1972b) concluded that with ^{32}P studies that mycorrhizal roots obtained their extra P from the soluble source. They further brought out that the major role of VA mycorrhiza in increasing the uptake of soil P by root is a physical one namely the provision of extra absorbing surface. Khan (1972) reported that mycorrhizal maize seedlings when transplanted into field grew better than non-mycorrhizal maize seedlings. Mycorrhizal plants removed more phosphorus from soil and had greater dry matter and phosphorus. There was a positive correlation between spore number in the soil and mycorrhizal development and between the extent of root infection and increased plant growth. Root infection decreased with increase in phosphorus content of the soil. The onset of reproductive process in non-mycorrhizal plants growing without added fertilizer was delayed and even in those cases where ears did form, their quality was poor. The mycorrhizal fungus increased the number of grains per ear and grain weight almost 12 times.

Mosse (1972a) studied the interaction of different strains of Endogone with different soil types on the growth of onion,

Paspalum notatum and rye grass and concluded that effects on plant growth was more dependent on interaction between a fungal strain and the soil rather than between a fungal strain and its host.

Mosse (1972b) compared different strains of Endogone in stimulating the growth of Paspalum notatum var. batatai in two Brazilian soils. This study brought out that some mycorrhizal strains of Endogone were more beneficial than others.

Gray and Gerdemann (1973) using ^{35}S found more uptake of S by mycorrhizal red clover and maize than the non-mycorrhizal plants. Hatting et al. (1973) showed that the ^{32}P labelled phosphate incorporated 27 mm from the root surface were better absorbed by mycorrhizal plants than non-mycorrhizal plants. They have shown through autoradiography that there was diffusion of 7.5 mm or less from the point of application. Their evidence indicated that the mycelial network of endomycorrhizal fungi enabled plants to remove phosphate from a larger volume of soil extending beyond the immediate vicinity of the root surface.

Ross and Gilliam (1973) compared the seed yields of mycorrhizal soybeans with non-mycorrhizal soybeans in a P deficient soil fertilized with various P sources. Mycorrhizal plants out-yielded non-mycorrhizal plants by 79, 53 and 50 per cent when both the plants were fertilized with Al, Fe rock or mono-calcium phosphate respectively. Yield from mycorrhizal plants fertilized with Fe phosphate were 1/6th and 1/5th of those fertilized with

monocalcium phosphate and Al phosphate respectively. They concluded that the increased P absorption by mycorrhizal soybean plant grown under P stress is principally from those phosphate sources most available under non-mycorrhizal condition.

LaRue et al. (1975) compared the peach nursery inoculated with mycorrhiza with that of the standard nursery practice of side dressing with P and Zn. They found that mycorrhizae helped peach seedlings extract Zn from the soil and the fungi were equally or more efficient in overcoming soil fumigation nutrient deficiency.

Powell (1975b) observed that P uptake was greatest in most mycorrhizal onion and red clover plants than in control. Specific activity of absorbed P was almost identical in all mycorrhizal and control plants.

Crush (1976) observed that Acaulospora laevis a VA mycorrhizal fungus behaved as a symbiont with Trifolium repens and T. pratense, but was parasitic on T. hybridum and Medicago sativa reducing growth by 3 to 16 per cent. The result indicated that the endophyte host relationship in symbiosis changed from mutualism to parasitism as P content increased.

Graham et al. (1976) studied the effect of two mycorrhizal types Glomus fasciculatus and G. mosseae on growth and tuberisation of potatoes. They found that the 3-year mean number of tubers formed on G. fasciculatus inoculated plants was nearly

twice than that of non-inoculated control and the dry weights of tops, roots and total plants were 1.4, 1.9 and 1.4 times greater respectively. G. mosseae inoculated plants did not differ significantly from that of non-inoculated control in tuber number, but differed in dry weight of tops, roots and total plants (1.5, 2.5 and 1.5 times respectively). Haines and Best (1976) found that compared with controls of soil alone and soil + Styraciflua seedlings alone, treatments with the mycorrhiza showed a reduction of ammonia loss from 5 and 25 cm depths and of nitrite loss at 5 cm. No significant effect was observed on nitrate loss.

Iqbal and Qureshi (1976) observed that wheat grown alone became heavily infected with VA endophyte but the infection reduced when grown with Brassica campestris. Higher mycorrhizal infection was accompanied by more numerous tillers, and larger ear producing greater number of grains. In mixed sowing of wheat with B. campestris, the mycorrhizal infection in wheat was reduced by B. campestris which was non-mycorrhizal. The mycorrhizal infection of wheat grown in soil containing roots of B. campestris was reduced by 25 per cent. They finally concluded that reduction in VA endophyte infection in wheat when grown with B. campestris could be due to fungitoxic substance present in the root exudates of B. campestris.

Koucheki and Read (1976) observed that members of Graminae

were particularly heavily mycorrhizal and this was found to be true even in Cyperaceae and Junaceae which were once considered non-mycorrhizal. They also found that infection spread from root to root when the plants are close rather than from the spore. Infection levels were high throughout the year and highest in nutrient stressed condition.

Pichot and Trung Binh (1976) found a marked effect on the start of Agrotis growth and phosphorus uptake when inoculated with endomycorrhiza. The L-value determination did not show the effect of endomycorrhiza on the pool of the mobile phosphorus ions in the soil or the solubilisation of rock phosphate.

Powell (1976) working with 37 hill country soils with P content ranging from 8-120 ppm found that in 3 infertile soils from North Island, while clover roots showed 12-28 per cent infection after 15 days and it rose to 40 per cent after 40 days. In 2 forest soils only 6 to 11 per cent infection was observed even after 43 days. In all the other 32 soils tested including those from well developed pasture, 30 to 90 per cent infection occurred only after 15 days. He also conducted a pot culture experiment with clover seedling and found that indigenous mycorrhizal fungi greatly increased growth over that in the non-mycorrhizal controls, especially in infertile forest soils where response to mycorrhizal fungi were upto 38-fold. However, in

two forest soils the indigenous mycorrhizal fungi were particularly inefficient and inoculation with Glomus E₃ from Rothamsted led to massive increase in shoot dry matter of the test plants. Eventhough the mycorrhizal response decreased as soil fertility increased the indigenous mycorrhizal fungi were still found beneficial in most fertile soil. In 24 soils E₃ was more efficient than the indigenous mycorrhizal fungi in increasing plant growth whereas in 11 soils the indigenous fungi were more efficient than E₃.

Rhodes and Gerdemann (1976) observed that mycorrhizal onion plants grown in soil chambers showed significantly greater uptake of ³⁵S in a five-day period than non-mycorrhizal plant. More volatile ³⁵S was collected from mycorrhizal plants on a dry weight basis than from non-mycorrhizal plants. Movement of ³⁵S from the application area to the root (approximately 3-6 cm) was similar for all treatments, indicating that mobility of ³⁵S in soil was not rate limiting for accumulation of ³⁵S by plants.

Sanni (1976a) showed a positive correlation between the presence of mycorrhizal and the amount of P and N in the tissues of cowpea, tomato and maize. The infected roots grew more vigorously than those of control.

Sanni (1976b) observed in rice, a semi-aquatic plant, positive correlation between VA mycorrhiza, the spore number

in individual root system and plant growth. Better growth was observed in mycorrhizal plants than in control or in plants treated with $\text{Fe}(\text{OH})_2\text{H}_2\text{PO}_4$ alone.

Abbot and Robson (1977) studied the effect of two mycorrhizal types on growth of subterranean clover (Trifolium subterraneum). They found that one of the two types identified as Glomus mosseae was more efficient in increasing growth and phosphorus content of clover than the other type, G. fasciculatus. The greater growth response in plants inoculated with the fungus G. mosseae was associated with a greater number of mycorrhizal roots. Response in nodulation closely paralleled responses in growth.

Barrow et al. (1977) studied the effect of VA mycorrhiza in improving the availability of P in two different soil conditions. One of the condition where the phosphorus was fixed in the soil by heating the soil and phosphorus to 70°C. Second condition was soil with freshly added phosphorus. The results showed that in Trifolium subterraneum and Allium cepa the inoculation with VA mycorrhiza doubled the availability of P, but the improvement was not bigger for firmly held P than for labile P.

Black and Tinker (1977) studied the effect of inoculation of mycorrhiza on potatoes under field condition and compared it with P treatment. They found that inoculation with mycorrhiza and added P (0.3 kg/m²) gave a large increase in yield (6.15 to

7.39 kg/plot). The yield increase due to inoculation with mycorrhiza alone without added P was comparable with only P fertilizer treatment (9.49 kg/plot in treatment with P compared to 8.8 kg/plot with only inoculum).

Cooper (1977) reported an increased growth of saprophytes accompanied with mycorrhizal infection in Dryopteris filix mass. D. filix benefitted from mycorrhizal, only when available P was low and C-P nutrients were added to fertile soil. The development of sex organs and sporophytes from gametophytes were unaffected with mycorrhizal infection.

Fitter (1977) observed that when two grasses Lolium perenne and Holcus lavatis were grown together with mycorrhizal infection and root competition, both gave a slight advantage to H. lavatus but, in combination, they produced considerable suppression of L. perenne. Root length of L. perenne was reduced by mycorrhizal infection. The competitive effects could be explained solely in terms of mycorrhizal infection.

Graw and Rehm (1977) observed that peanut pegs were very intensively infected with mycorrhizal fungus. They assumed that the mycorrhiza in pegs also enhanced phosphate uptake.

Iqbal and Qureshi (1977) observed that sunflower plants became mycorrhizal as early as the two leaf stage in P deficient soil. The mycorrhizal plants at the time of harvest were taller, stouter, with more numerous and larger leaves and bore bigger

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discs producing viable seeds. They attributed the increase in growth to greater uptake of P from the rhizosphere.

Janos (1977) observed that Bactris graspieas seedlings survived better with mycorrhiza. He found that 7 out of 9 seedlings survived when mycorrhizal whereas all without mycorrhiza died. The mycorrhizal seedlings also grew taller with larger leaves and roots.

Mojaloi and Weed (1977) showed that when soybean plants were grown with mycorrhiza in presence of 3 types of micas produced more growth and increased P uptake compared with non-mycorrhizal treatment. No K deficiency was observed in both the cases. Mycorrhiza was found to accelerate weathering of micas particularly biotite and the phlogopite.

Mosse (1977) observed that VA endophyte established in 11 out of 12 soils tested and inoculated plants of Stylosanthes guyanensis and maize with added phosphate made the best growth. Added rock phosphate was utilized considerably by inoculation, but there was no evidence of increased availability. There was no clear relationship between percentage plant P and nodulation.

Powell (1977) observed an increased shoot growth of clover when pre-inoculated with E_3 (a sporocarp species of Glomus) and transplanted into soil compared with pre-inoculation with indigenous strains. He suggested that E_3 can be introduced into soil in order to increase uptake of residual and fertilizer P from soil.

Swaminathan and Verma (1977) showed that in an infertile soil mycorrhizal plants took up more P and had more growth than non-mycorrhizal plants. They also carried out a survey of all potato fields in and around Simla and found their presence in only 23 per cent of the fields surveyed.

Trimick (1977) showed that Lupinus angustifolius, L. consentinii and L. leutens were weakly infected (10% of root length) with VA mycorrhiza. Vesicles were present in approximately 30 per cent of the field plants and arbuscules were observed in only one species of L. consentinii. Infection was further reduced when soil moisture was high and by small additions of phosphorus to the soil. VA mycorrhizal infection was stimulated in L. consentinii when grown with red clover. He finally concluded that the main reason for less mycorrhizal infection could be due to the proteoid like rootlets that were observed on Lupinus which had specialized root-soil surface properties which helped in improving phosphorus uptake.

Abbot and Robson (1978) tested the ability of 3 VA endophytes isolated and identified as isolates of Glomus monosporus and G. fasciculatus to increase the growth of subterranean clover Trifolium subterraneanum when introduced into a freshly collected field soil. Four indigenous endophytes also infected the roots in both control and the treatments which were previously inoculated by the 3 VA endophytes. Inoculation of the 3 VA endophytes did not reduce initial infection by the four

indigenous endophytes. The mycorrhiza formed by 2 isolates of G. monosporus and one G. fasciculatus were distinguished from those formed by indigenous endophytes on the basis of morphology. Plants inoculated with isolates of G. monosporus had a greater proportion of their dry weight and P content in the tops than in plants inoculated with isolate of G. fasciculatus or the control plants.

Azcon et al. (1978b) while examining the intensity of mycorrhizal infection as affected by the interaction of different levels of P in soil and in host, observed that in soil with no added P, mycorrhizal infection was not affected by the initial P content of the plants. When 0.8 and 1.5 kg K_2PO_4 was given per kg soil, both the internal and the external P negatively influenced the infection. P content that was optimal for mycorrhizal infection was not optimal for plant growth. They concluded that the critical P concentration in plants depended on the age of the host.

Cooper and Tinker (1978) studied the uptake and transfer of ^{32}P , ^{65}Zn and ^{35}S by mycorrhiza in white clover and onion. They observed a lag phase of 2-3 days in the appearance of ^{32}P in the shoot which was caused by the delay in the uptake and translocation of P by hyphae rather than transfer from fungus to host. They calculated the maximum translocation rate of P/onion as 6.4×10^{-16} moles $cm^{-2}s^{-1}$ and the P flux in the

hyphae as about 2×10^{-10} moles $\text{cm}^{-2}\text{s}^{-1}$. They finally concluded that molar amount of P, S and Zn translocated were in the ratio of 35:5:1.

Daft and ElGiahmi (1978) while studying the effect of defoliation, time of harvest and light duration on the growth of host and mycorrhiza, observed that defoliation of maize (Zea mays) and tomato (Lycopersicum esculentum) reduced the mycorrhizal growth response, and the development of the endophyte, estimated as percentage infection, root pigmentation and spore production. In case of grasses more fresh and dry matter was produced in the presence of mycorrhiza when harvested on 3 separate occasions than from a single or final harvest. The total yield from 2 harvests and the yield from a single harvest of alfalfa (Medicago sativa) were similar although nodulated and mycorrhizal plants were larger at each harvest. In both grasses and alfalfa, periodic harvesting reduced the mycorrhizal infection by about 50 per cent. Reduction in day length and irradiance depressed the growth more in mycorrhizal than in uninfected maize plants. In Rhizobium and Glomus infected alfalfa plants, a day length of 16 hours produced the highest carbon content and C/N ratios. Plants exposed to short days produced more, smaller nodules and had higher nitrogen contents than plants given longer days.

Gaunt (1978) studied the growth of plants in sterile soil

from seed pelleted with endotrophic mycorrhizal fungi comparing with non-inoculated plants and plants grown in sterile soil to which mycorrhizal spores were added. The dry weight of onion (Allium cepa) and tomato (Lycopersicum esculentum) plants grown with mycorrhizal fungi was greater than the non-mycorrhizal control treatments, in a range of soil-vermiculite mixtures. There was no significant difference between the methods of mycorrhizal inoculation.

Hall (1978a) showed that mycorrhizal clover was better equipped than non-mycorrhizal clover to compete with rye grass for soil P and that inter and intra-specific competition for soil P resulted in VA mycorrhiza stimulating clover more and at higher P levels. Rye grass responded poorly to both P and VA mycorrhiza which was attributed to N deficiency.

Hall (1978b) studied the effect of 4 mycorrhizal fungi on 2 varieties of maize Px610 and W415 and one sweet corn variety. He found that Px610 outgrew the other two cultivars and was well infected with mycorrhiza but failed to respond to VA mycorrhiza. In the non-mycorrhizal state the superiority of Px610 over the other two cultivars was attributed to its ability to produce extensive root systems and low laminar P concentration. VA mycorrhiza also depressed the root dry weight per total plant weight ratio of Px610.

Hughes et al. (1978) found that strawberry inoculated

with Glomus fasciculatus had the highest P and N concentration in the serial parts of the plant compared to Gigaspora calospora and control. Inoculation with G. fasciculatus resulted in greatest length of roots and the highest percentage mycorrhizal infection in the root length.

Menge et al. (1978a) observed that Avocado (Persea gratissima) inoculated with beneficial mycorrhizal fungi (Glomus fasciculatus) had upto 250 per cent greater growth rate than non-mycorrhizal Avocados. Mycorrhizal Avocados resisted transplant shock because of better water absorption.

Menge et al. (1978b) observed that citrus root stocks exhibited the greatest mycorrhizal dependancy of citrus cultivars at the medium fertilizer regime. They also observed an increased correlation between the dry weight of non-mycorrhizal dependancy of citrus cultivars.

Menge et al. (1978c) have evidenced that it is the concentration of the phosphorus within plants and not the soil phosphorus which leads to reduction in colonization, infection and spore production of G. fasciculatus.

Powell and Daniel (1978) studied the effect of crop rotation on the development of mycorrhizae and utilization of rock phosphate. They observed that when a crop of clover (Trifolium repens) followed by 3 crops of rye grass were infected with several mycorrhizal fungi and grown in sterilized soil which

had received soluble phosphate or Nauru rock phosphate. Plant growth response to mycorrhizal infection was longer in later crops. Glomus tenuis was successfully introduced into the soil which already had indigenous mycorrhizal fungi and was the most efficient fungus stimulating phosphate uptake. In all four crops Nauru rock phosphate was available to mycorrhizal plants but unavailable to non-mycorrhizal plants. Plants infected with G. tenuis and the indigenous mycorrhizal fungi recovered 10-27 per cent of the phosphate fertilizer applied to the soil, while non-mycorrhizal plants recovered only 0.4-3.0 per cent.

Powell and Sithamparamanathan (1978) observed that indigenous mycorrhizal fungi infected white clover throughout the year except during winter when soils were water logged. Mycorrhizal infection rate was slower and dependence on infection for growth and P uptake was usually less in lotus and grasses than in clover. Gigaspora margarita was most efficient at stimulating growth of the white clover and grasses among the fungi tested, but was less efficient than the indigenous fungi in stimulating growth of red clover.

Rhodes and Gerdemann (1978) observed that mycorrhizal and non-mycorrhizal onion plants from high P treatment had significantly higher ³⁵S compared to non-mycorrhizal low P plants. They concluded that increased S uptake by mycorrhizal

plants can result from increased S absorbing power of its roots with enhanced P status.

Timmer and Leyden (1978) found that stunted citrus seedlings were frequently devoid of mycorrhiza and vigorous seedlings in seed beds and orchards contained high mycorrhizal infection. Inoculation with G. fasciculatus increased seedling height by more than 300 per cent and increased leaf phosphorus concentration. In some orange seedlings grown with complete nutrient solutions, per plant application of P at 200 mg per plant did not increase growth or P concentration under the same condition. Application of higher rates of P induced Cu deficiency symptoms, more severe in non-mycorrhizal plants.

Interaction of VA mycorrhiza and other Rhizosphere microorganisms

Mycorrhiza being a root zone microorganisms has to compete with so many other microorganisms within the root and also in the soil. From the time the work on mycorrhiza started, investigations regarding its effect on some of the beneficial as well as pathogenic organisms also started. Some of the organisms on which the mycorrhizal effect has been studied are, Rhizobium, Azotobacter, phosphorus solubilisers, total bacteria and fungi in the rhizosphere. Plant parasitic nematode and some of the root pathogens like Phytophthora, Thielaviopsis, etc.

Interaction with phytopathogens and plant parasitic nematodes

Schonbeck and Dehne (1977) observed that black root rot

caused by Thielaviopsis basicola occurred on mycorrhizal and non-mycorrhizal roots to the same extent, but the severity of damage was more in non-mycorrhizal plants.

Matare and Hatting (1978) observed that mycorrhizal fungus G. fasciculatus stimulated growth of Avocadoes seedling in the presence or absence of Phytophthora cinnamomi. They also observed that G. fasciculatus had no effect upon infection by P. cinnamomi or subsequent disease development.

Schonbeck (1978) observed more local lesions of TMV in mycorrhizal tobacco plants compared to non-mycorrhizal plants. Mycorrhizal plants had an adverse effect on the establishment of Meloidogyne incognita in their roots. Fewer larvae developed into adults. Resistance to T. basicola root rot in tobacco and cotton and fusarial wilt of tomato was shown in mycorrhizal plants.

Sikora (1978) showed that the attractiveness of the root system to M. incognita larvae is altered by the presence of the endotrophic mycorrhizal fungi G. mosseae. There was a negative relationship between the distribution of M. incognita galls and the levels of G. mosseae in different areas of root system. G. mosseae adversely affected M. incognita during several phases of its life cycle, most probably by altering the physiological make up of the host.

Interaction with Rhizobium

It is of great interest to study the interaction between these two organisms since one is a nitrogen fixer and the other phosphorus absorber.

Schenck and Heison (1973) showed that inoculation with Endogone significantly increased seed yield and other traits of nodulating but not the non-nodulating isolate of Hardee soybean (Glycine max L.). They suggested that there is an indirect evidence to show that Endogone did not significantly increase nitrogen absorption of the non-nodulating isolate.

Daft and ElGiahmi (1974) found that the dual inoculation with mycorrhiza and Rhizobium in Phaseolus gave an increased growth, reproduction, number and weight of nodules, acetylene reduction rates, leg haemoglobin content, phosphorus and total protein content compared to Rhizobium alone. The root and shoot ratio was reduced in dual inoculation treatments. The application of P stimulated the effect of the fungus.

Daft and ElGiahmi (1976) observed that yields of fruits, plants size and chemical content of shoot, root and seeds were higher in peanut infected with G. mosseae. Infection with G. mosseae and Rhizobium stimulated nodulation and acetylene reduction rates. Plants inoculated with both the organisms produced more fruit, dry matter, nodules and contained more P and N.

Mosse et al. (1976) studied the interaction between VA mycorrhiza, utilization of rock phosphate and nodulation in 3 legumes (Trifolium sp, Stylosanthes sp, and Centrosema sp) and in onion grown in eight P deficient soils ranging from pH 8.1-5.3. They observed that irrespective of pH, inoculation with VA mycorrhiza increased P uptake in all host plants in all the soils, when the indigenous endophyte had been removed by irradiation. In acid soils, addition of rock phosphate generally improved growth of the non-mycorrhizal plants and inoculation with VA endophyte greatly improved its utilization. In neutral and alkaline soils rock phosphate was unavailable to both non-mycorrhizal and mycorrhizal plants. In case of legumes they found that inoculation with specific Rhizobium strains with VA endophytes in the presence of rock phosphate greatly improved nodulation and nitrogen fixation.

Godse et al. (1978) showed that inoculation of cowpea with VA mycorrhiza increased nodulation, root and shoot development, not only in plants inoculated with Rhizobium but also in naturally nodulating plants. The increased shoot and root development was due to improved P uptake.

Bagyaraj et al. (1979) observed that dual inoculation with G. fasciculatus and Rhizobium japonicum on soybean in a P deficient soil increased nodulation, nitrogen fixation and P content significantly under field condition.

Interaction with Azotobacter

Bagyaraj and Menge (1973) found larger population of bacteria and actinomycetes in the rhizosphere of tomato plants inoculated with the mycorrhizal fungus Glomus fasciculatus and Azotobacter chroococcum than the uninoculated plants. The plants inoculated with both G. fasciculatus and A. chroococcum had greater number of bacteria and actinomycetes in the rhizosphere remained unaffected by inoculation with A. chroococcum. Inoculation of G. fasciculatus increased the population of A. chroococcum in the rhizosphere and maintained it for a long time. A. chroococcum inoculation increased the infection and sporulation of G. fasciculatus. The dry weight of tomato plants inoculated with both organisms was significantly greater than the uninoculated plants.

Interaction with phosphate solubilising organisms

Barea et al. (1975) have shown that when plants inoculated with mycorrhiza plus P solubilising bacteria in the presence of rock phosphate utilized more phosphate than the uninoculated control. They used two P solubilising bacteria, a Pseudomonas sp, and a Agrobacterium sp and two crops, maize and lavender. They also showed that population of P solubilizing bacteria was high in mycorrhiza inoculated treatment than in the treatment where only bacteria was inoculated. The lack of inhibition of VA infection which could occur only when P concentration was high also suggested limited solubilization.

Azcon et al. (1976) showed that the plants inoculated with VA mycorrhiza and P solubilising bacteria in the presence of rock phosphate and under alkaline condition, took up more phosphorus than those with only mycorrhiza and bacteria. In this experiment they used two P solubilising bacteria and lavender as the crop. Experiment was conducted in alkaline P deficient soil. Rock phosphate was used at the rate of 0, 0.1 and 0.05 per cent and found that in all concentrations maximum P uptake took place when both mycorrhiza and P solubilising bacteria were inoculated.

Azcon G. de Angilar and Barea (1978a) compared the growth and infection levels in mycorrhizal lavender Lycopersicum and Medicago plants after treatment with preparation from Rhizobium, Azotobacter and phosphate solubilising bacteria (a Pseudomonas sp). Cell free supernatants and in some cases the whole bacterial culture behaved as the pure hormone in improving the dry weight and infection levels compared with mycorrhizal only control plants.

Azcon et al. (1978b) working with Medicago found that cell free supernatants of Rhizobium and phosphobacteria improved plant growth, nodulation and mycorrhiza formation. They also found that the best positive effect was achieved by a treatment which consisted of whole culture of Rhizobium, phosphobacteria and the mycorrhizal fungi applied together.

MATERIAL AND METHODS

CHAPTER III

MATERIAL AND METHODS

A pot culture experiment was conducted under green house condition to know the effect of interaction between phosphorus solubilising fungi and mycorrhiza on the growth of finger millet (Eleusine coracana).

Soil-physical and chemical properties

The soil used was red sandy loam collected from the Gandhi Krishi Vignana Kendra farm of the University of Agricultural Sciences, Bangalore, having 48.45 per cent fine sand, 24.33 per cent coarse sand, 4.4 per cent silt, 22.76 per cent clay, 0.75 per cent organic carbon, pH 4.8, CEC 4.2 me per 100 g, 0.14 per cent available nitrogen, 0.002 per cent P_2O_5 and 0.003 per cent K_2O . The soil had a native VA mycorrhizal population of 35 spores per 25 ml.

Experimental set up

Nursery: Finger millet (Eleusine coracana L.) var. Indaf-5 nursery was raised in two seed pans filled with sterilized sand. To inoculate finger millet seedlings with VA mycorrhiza a thin layer of mycorrhizal inoculum was placed 2 cm below the soil surface in one of the seed pan before sowing seeds. Inoculum consisted of roots and soil from a pot culture of Sudan grass (Sorghum vulgare Pers.) which was infected with Glomus fasciculatus (Thaxt.) Gerd. and Trappe and grown for $3\frac{1}{2}$ months.

The inoculum contained hyphae, vesicles, chlamydospores and arbuscules of G. fasciculatus.

The two fungi Aspergillus niger and Penicillium funiculosum were used as effective phosphorus solubilisers in soil. One month old culture of these fungi grown separately in Richard's broth under static conditions were used as inoculum. At the time of transplanting the broth containing the fungal mat was mixed thoroughly in an electric mixer to a uniform suspension. Ten ml of this suspension was added into the pot and the seedling planted above it. Initial population of the inoculum was determined by plating on Rose bengal agar. The count was 20×10^6 cells per ml for P. funiculosum and 25×10^6 cells per ml in case of A. niger.

There were six inoculation treatments with four replications. C = Control (plant alone), G = Plant + G. fasciculatus, P = Plant + P. funiculosum, A = Plant + A. niger, GP = Plant + G. fasciculatus + P. funiculosum and GA = Plant + G. fasciculatus + A. niger. 21 day old finger millet seedlings from both mycorrhizal and non-mycorrhizal nursery were transplanted into pots holding 3 kg sandy loam unsterilized soil with 2 per cent organic matter and fertilizer dosage of 120 mg, 80 mg and 40 mg (which works out to 40:20:20 $\frac{1}{2}$ NPK/ac) urea, rock phosphate and muriate of potash. Treatments C, P and A received non-mycorrhizal seedling and treatments G, GP and GA received mycorrhizal seedlings.

There were 12 replications in each treatment. At intervals of 30, 50 and 70 days after transplanting plants from 4 pots in each treatment were removed, soil and plant samples were used for various analyses.

Estimation of rhizosphere microflora

Samples of rhizosphere soils were plated for total bacteria, fungi, actinomycetes and phosphorus solubilising fungi. Bacterial plate count was taken at 10^{-6} dilution on soil extract agar whose composition is given below:

Soil extract agar

Glucose	..	1.0 g
Yeast extract	..	0.5 g
K ₂ HPO ₄	..	0.5 g
Soil extract	..	100 ml
Water	..	900 ml
Agar	..	18.0 g
pH	..	7.0

Soil extract - 1000 ml water added to 1000 g soil autoclaved for 15 minutes at 15 lb pressure. CaCO₃ is added and filtered. The filtrate is used as soil extract.

Fungal plate count was taken at 10^{-4} dilution on Martin's Rose Bengal Agar whose composition is as given below:

Martin's Rose Bengal Agar

Dextrose	..	10 g
Peptone	..	5 g

K ₂ HPO ₄	..	1 g
MgSO ₄	..	0.5 g
Rose Bengal	..	0.03 per cent
Agar	..	18 g
Water	..	1000 ml
pH	..	5-6

At the time of pouring streptomycin sulphate at 3 ml per lit was added.

Actinomycete count was taken at 10⁻⁴ dilution on Kuster's Agar whose composition is as given below:

<u>Kuster's agar</u>		
Starch	..	10 g
Casein	..	0.3 g
KNO ₃	..	2 g
NaCl	..	2 g
K ₂ HPO ₄	..	2 g
MgSO ₄	..	0.05 g
CaCO ₃	..	0.02 g
FeSO ₄	..	0.01 g
Agar	..	18 g
Water	..	1000 ml
pH	..	7-7.2

The phosphate solubilising fungi were plated at 10⁻⁴ dilution on a special medium known as Sperber's medium (Sperber, 1957) having the following composition.

Sperber's medium

Glucose	..	10 g
Yeast extract	..	0.5 g
CaCl ₂	..	0.1 g
MgSO ₄	..	0.25 g
Soil extract	..	250 ml
Water	..	750 ml
pH	..	7.0

100 ml of the above media was taken in 250 ml flasks and 1.8 g agar was added. Ten per cent CaCl₂ and 10 per cent K₂HPO₄ were prepared separately and all the three sterilized. At the time of pouring into plates 3 ml of CaCl₂ and 2 ml of K₂HPO₄ were added 100 ml media at 60°C. Calcium phosphate precipitated and then the media was poured into plates at 45°C. Solubilisation was detected by the halo formation around the colonies.

All the plate counts were expressed per gram of dry rhizosphere soil.

Mycorrhizal spore count and percentage infection

The mycorrhizal assessment was done by taking the spore count from the soil. Soil wet sieving procedure of Gerdemann and Nicolson (1963) was followed. Water suspension of 25 ml of representative soil was passed through sieves of sizes 1000, 300, 250, 105 and 45 μ m kept one below the other in the same order. The soil and spores on the bottom two sieves were transferred onto

a nylon mesh of same size as the last sieve. The nylon mesh was placed in a petridish and the spores counted under a stereomicroscope.

The percentage mycorrhizal infection of the plant root was done according to the procedure given by Phillips and Hayman (1970). The root samples from each treatment was cut into 1 cm bits, fixed in formalin acetic acid (formalin 5 ml, acetic acid 5 ml and ethanol 90 ml) for atleast four hours. The roots were then cleared by autoclaving with 10 per cent KOH at 15 lb pressure for 15 minutes. The alkalinity due to KOH was neutralized by adding 1 per cent HCl for 5 minutes. Staining was done by simmering the roots in 0.05 per cent trypan blue in lacto phenol for 10 minutes. Excess stain was removed by keeping the roots in lacto phenol. (Lactic acid 20 ml, phenol 20 ml, Glycerol 40 ml, distilled water 40 ml). The stained root bits were arranged on a slide, covered by a cover slip and observed under microscope for VA mycorrhizal mycelium, vesicles and arbuscules. The percentage infection was calculated as follows:

$$\text{Per cent mycorrhizal infection} = \frac{\text{Number of root bits having infection}}{\text{Number of root bits taken for observation}} \times 100$$

Plant dry weight and estimation of nutrient content

The plants were dried in an oven at 60°C to a constant weight and the dry weights of the root and shoot were recorded.

The dried root and shoot samples were powdered in a grinder. The powdered plant material was digested in triacid mixture (concentrated nitric acid 6 vol, perchloric acid 2 vol, concentrated sulphuric acid 1 vol). The digested residue was diluted to 100 ml by distilled water in a volumetric flask. 50 ml of the above diluted residue was taken for phosphorus estimation and the remaining 50 ml was used to estimate potassium, zinc and manganese.

Estimation of phosphorus

Total phosphorus content was estimated by procedure given by Jackson (1967). 50 ml of the triacid digested residue was taken in a 100 ml volumetric flask. 10 ml of Vanadomolybdate reagent was added to develop yellow colour due to phospho-vanado molybdate complex. The yellow colour was read at 490 mμ (blue filter) in a Klett Summerson Colorimeter.

Estimation of potassium

The total potassium was estimated by the flame photometric method (Jackson, 1967). One to two ml of the digested and diluted residue was fed into the flame photometer (EliCo) with potassium filter in it. Galvanometer reading was recorded and further calculated.

Estimation of zinc and manganese

Total zinc and manganese were estimated by the atomic absorption spectrophotometric method (Issac and Kerber, 1971).

The atomic absorption spectrophotometer (Hitachi 508) was first set to analyse zinc by putting a cathode tube specific for zinc and adjusting the wave length to 2138 Å. The digested and diluted plant sample was then fed into the instrument. The galvanometer reading was recorded and the amount of zinc in the sample was determined by comparing with the standard curve.

Manganese was also estimated in a similar way. The atomic absorption spectrophotometer was set to analyse manganese but putting in a cathode tube specific for manganese and adjusting the wave length to 2795 Å. The digested and diluted sample was fed into the instrument. The galvanometer reading was recorded and compared with the standard graph to calculate the amount of manganese.

RESULTS

CHAPTER IV

RESULTS

Estimation of rhizosphere microflora

The results of the rhizosphere microflora estimated at 3 different intervals viz., 30, 50 and 70 days after transplanting are given in the first four Tables.

Bacteria:

Fig 1 Table 1 shows the total bacterial count at the three intervals. The differences in the bacterial count amongst the various treatments was significant on day 30. Inoculation with G. fasciculatus alone resulted in greater number of bacteria compared to uninoculated control but the difference was not statistically significant. Inoculation with the two P solubilising fungi did not have any effect on the bacterial population. Inoculation with either of the two P solubilising fungi plus G. fasciculata increased the bacterial population significantly over control and the treatment with P. funiculosum alone. In general, the trend changed after 30 days with the treatment with G. fasciculatus alone showing an increased count, followed by uninoculated control. The two dual inoculation treatments showed a drastic reduction in the bacterial counts by day 70.

Fungi:

The total fungal count in the rhizosphere as influenced

Table 1. Bacterial population in finger millet rhizosphere as influenced by inoculation with G. fasciculatus and the two P solubilising fungi P. funiculosum A. niger

Treatments	Days after transplanting		
	30	50	70
	(Population in 10^6 /g dry rhizosphere sample)		
Control	5.37	19.45	42.39
<u>G. fasciculatus</u>	28.65	33.86	61.13
<u>P. funiculosum</u>	5.62	31.58	30.92
<u>A. niger</u>	17.10	14.09	15.40
<u>G. fasciculatus</u> + <u>P. funiculosum</u>	55.33	23.00	16.07
<u>G. fasciculatus</u> + <u>A. niger</u>	63.55	49.85	16.25
C.D. at P=0.05	44.81	NS	NS

NS = Not significant

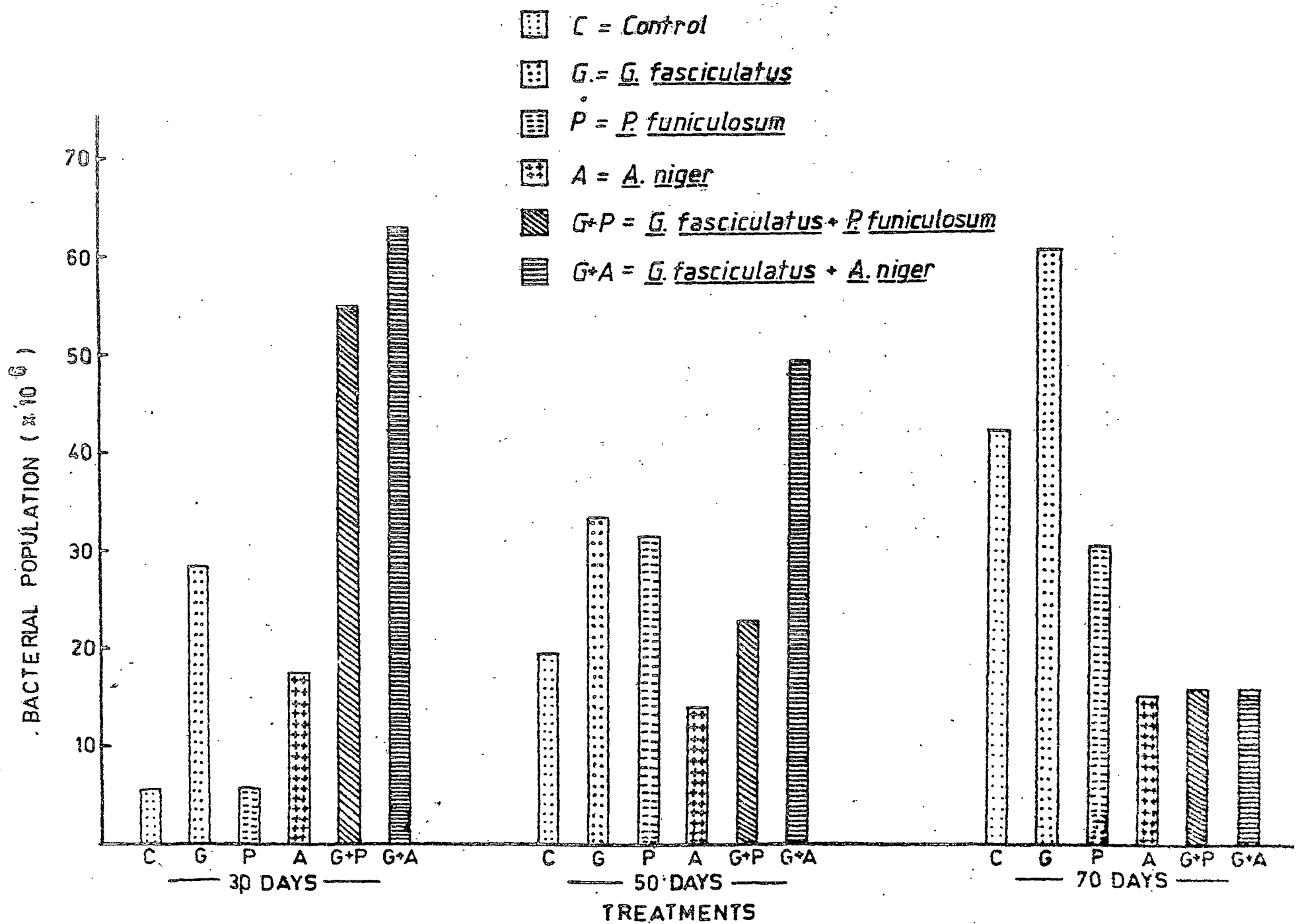


FIG.1. BACTERIAL POPULATION IN THE RHIZOSPHERE OF FINGER MILLET AS INFLUENCED BY MYCORRHIZA AND P SOLUBILIZING FUNGI

by the various treatments is given in Table 2 & Fig. 2. The differences in count amongst the various treatments was significant only on day 70. In general, it could be seen that plants inoculated with G. fasciculatus harboured more fungi while those treated with A. niger harboured least fungi in their rhizosphere. The differences in the fungal counts in the rhizosphere of non-mycorrhizal and mycorrhizal plants when inoculated with P. funiculosus or A. niger were not statistically significant.

Actinomycetes:

The differences in total actinomycete count was significant on 30th day only (Table 3 & Fig. 3). Inoculation with G. fasciculatus resulted in increased count compared to uninoculated control and A. niger alone treatment which showed the least counts. The two dual inoculation treatments even though showed an increase in population towards 70th day did not exceed that of G. fasciculatus alone treatment.

Phosphate solubilising fungi:

The rhizosphere count of phosphate solubilising fungi is given in Table 4 & Fig. 4. The differences in the count was significant at all the three intervals. Inoculation with G. fasciculatus alone did not have any effect on P solubilising fungi. The dual inoculation treatment G. fasciculatus + A. niger showed higher counts of P solubilising fungi compared to A. niger alone treatment.

Table 2. Fungal population in finger millet rhizosphere as influenced by inoculation with G. fasciculatus and two P solubilising fungi P. funiculosum and A. niger

Treatments	Days after transplanting		
	30	50	70
	(Population in 10^4 /g dry rhizosphere sample)		
Control	4.54	4.74	14.13
<u>G. fasciculatus</u>	7.19	5.31	27.11
<u>P. funiculosum</u>	6.08	5.58	12.25
<u>A. niger</u>	1.70	5.01	9.71
<u>G. fasciculatus</u> + <u>P. funiculosum</u>	6.68	7.75	43.31
<u>G. fasciculatus</u> + <u>A. niger</u>	4.68	3.98	7.83
C.D. at P=0.05	NS	NS	15.52

NS = Not significant

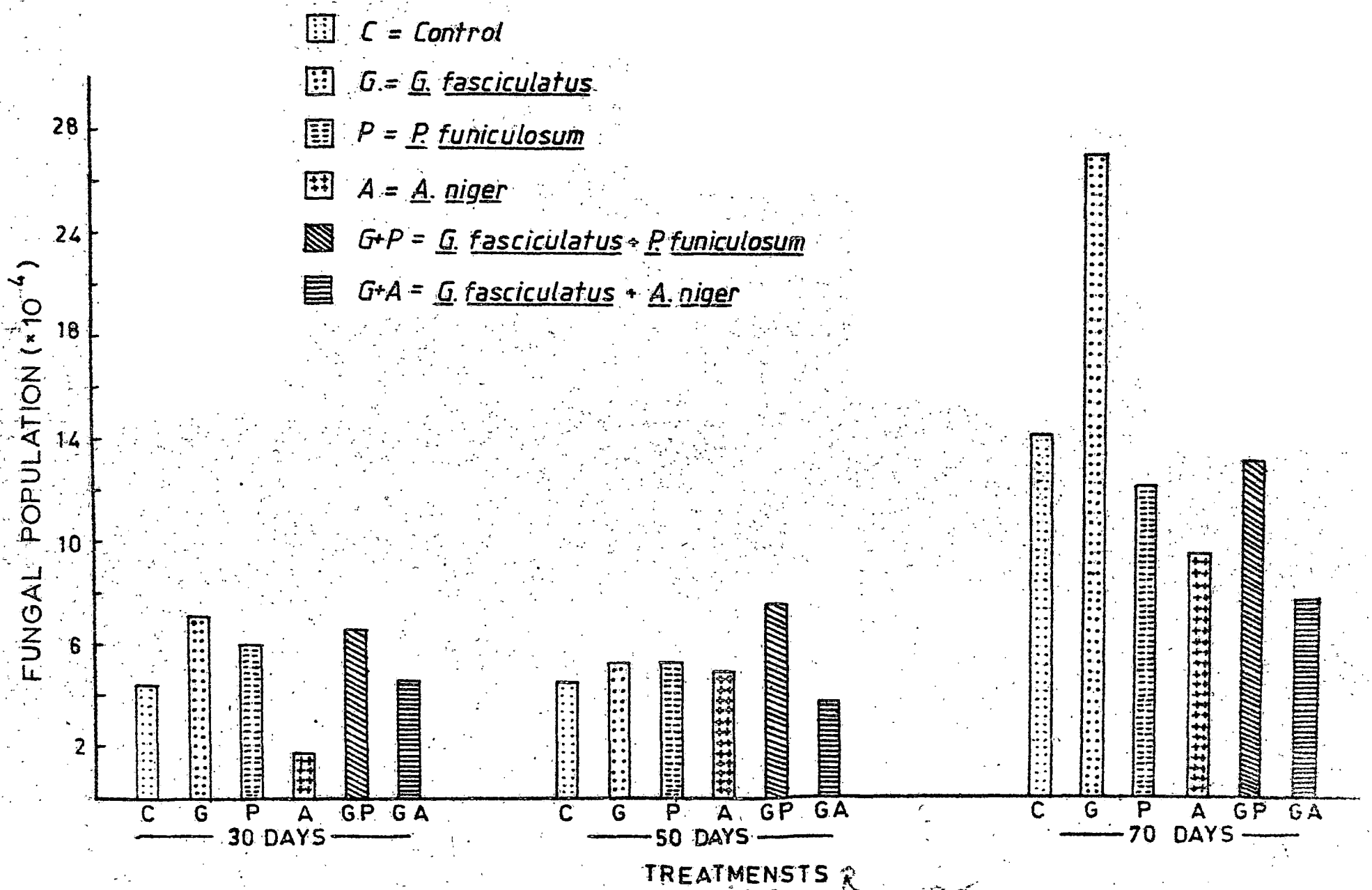


FIG. 2 FUNGAL POPULATION IN THE RHIZOSPHERE OF FINGER MILLET AS INFLUENCED BY MYCORRHIZA AND P SOLUBILISING FUNGI.

Table 3. Actinomycete populations in finger millet rhizosphere as influenced by inoculation with G. fasciculatus and two P solubilising fungi P. funiculosum and A. niger

Treatment	Days after transplanting		
	30	50	70
	(Population in 10^4 /g dry rhizosphere sample)		
Control	4.27	8.39	8.96
<u>G. fasciculatus</u>	11.34	19.36	14.13
<u>P. funiculosum</u>	8.22	8.31	14.23
<u>A. niger</u>	0.83	10.27	5.07
<u>G. fasciculatus</u> + <u>P. funiculosum</u>	6.68	9.87	12.96
<u>G. fasciculatus</u> + <u>A. niger</u>	6.61	5.46	10.26
C.D. at P=0.05	6.61	NS	NS

NS = Not significant

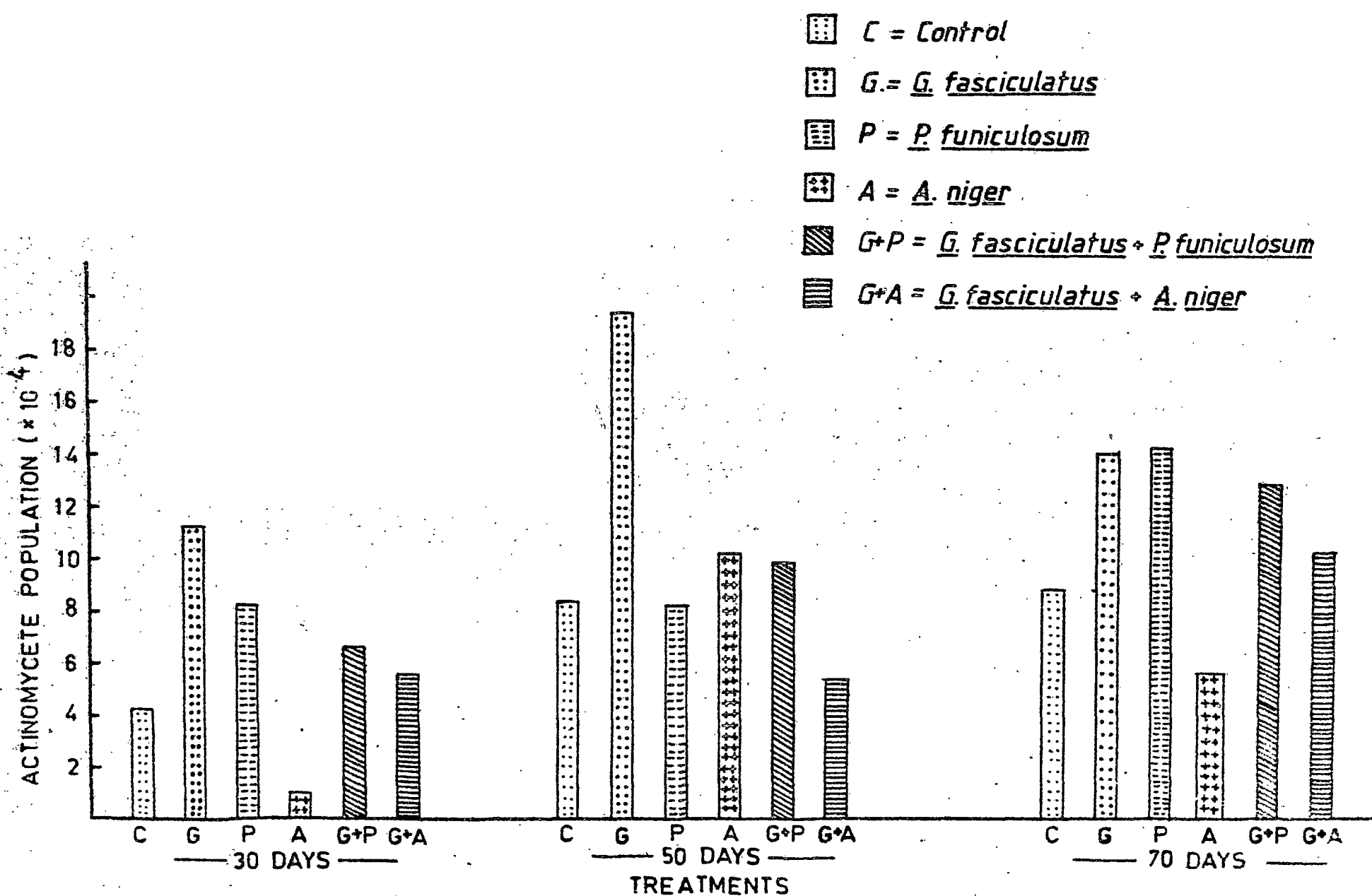


FIG. 3 ACTINOMYCETE POPULATION IN THE RHIZOPHERE OF FINGER MILLET AS INFLUENCED BY MYCORRHIZA AND P SOLUBILISING FUNGI

Table 4. PSF populations in finger millet rhizosphere as influenced by the inoculation with G. fasciculatus and two P solubilising fungi P. funiculosus and A. niger

Treatments	Days after transplanting		
	30	50	70
	(Population in 10^4 /g dry rhizosphere sample)		
Control	0.71*	0.71	0.71
<u>G. fasciculatus</u>	0.71	0.71	0.71
<u>P. funiculosus</u>	1.43	1.13	1.68
<u>A. niger</u>	1.06	2.94	1.28
<u>G. fasciculatus</u> + <u>P. funiculosus</u>	1.97	1.40	1.29
<u>G. fasciculatus</u> + <u>A. niger</u>	3.23	4.19	2.10
C.D. at P=0.05	1.18	2.07	1.03

PSF = Phosphorus solubilising fungi

*Square root transformed figures

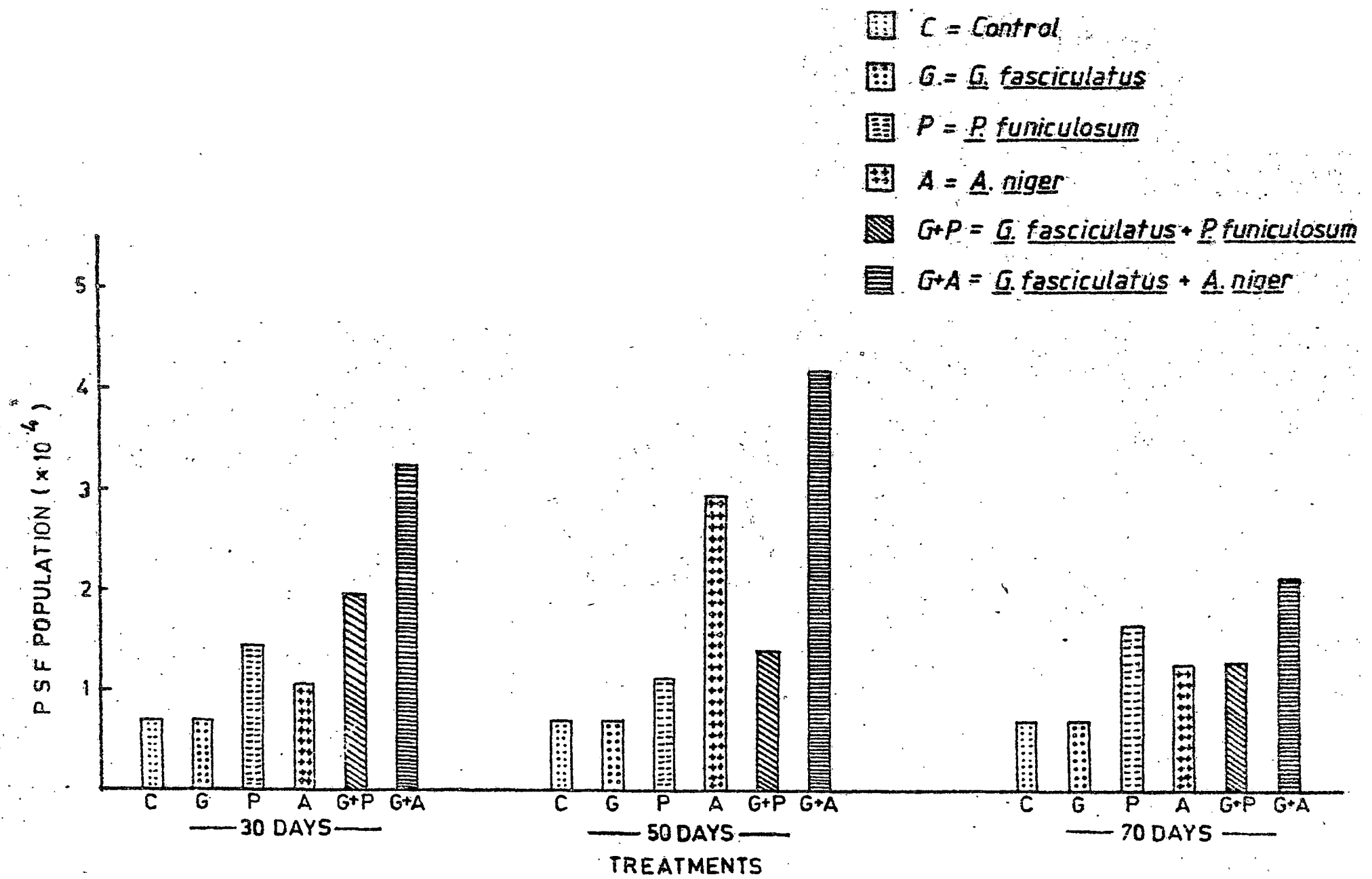


FIG. 4. PHOSPHOROUS SOLUBILISING FUNGAL POPULATION IN THE RHIZOSPHERE OF FINGER MILLET AS INFLUENCED BY MYCORRHIZA & P SOLUBILISING FUNGI.

Mycorrhizal spore count in the soil and percentage mycorrhizal infection of plant roots.

The variation in the mycorrhizal spore count encountered in different treatments was significant at all the three intervals (Table 5 & Fig. 5 & 6). On day 30 mycorrhizal seedlings showed a high spore count compared to those treatments which received non-mycorrhizal seedlings. Maximum spore count was recorded in G. fasciculatus + A. niger treatment. At 50th day those treatments which did not receive mycorrhizal seedlings also showed an increase in the spore number of the native mycorrhiza. The treatments with mycorrhiza showed an increase and there was no difference between mycorrhiza alone treatment and the dual inoculation treatments recorded increase spore numbers over G. fasciculatus alone treatment. The native mycorrhizal spore number in control nearly equalled that of introduced G. fasciculatus alone treatment. The only treatment where the spore number did not show an increase over period of time was P. funiculosum alone treatment.

The percentage mycorrhizal infection is also given in Table 5 & Fig. 5 & 6. In general, the infection trend shows that the mycorrhizal treatments showed a good percentage infection over the non-mycorrhizal treatments, although the difference narrowed over a period of time. The native mycorrhizal infection in the three non-mycorrhizal treatments showed a uniform increase with advancement in age. In case of the three treatments inoculated with G. fasciculatus initially the two dual inoculation treatments showed less infection compared to G. fasciculatus alone treatment. Later all the three recorded equal percentage infection.

Table 5. VA mycorrhizal spores in soil and percentage infection of roots in unsterilized soil as influenced by inoculation with G. fasciculatus and two P solubilising fungi P. funiculosum and A. niger

Treatments	Days after transplanting					
	Number of spores/ 25 ml soil			Percentage infection*		
	30	50	70	30	50	70
Control	34	138.5	189.0	17	58.5	53.3
<u>G. fasciculatus</u>	60	201.7	205.7	84	85.3	94.8
<u>P. funiculosum</u>	34.7	130.7	143.2	17.6	74.3	83.2
<u>A. niger</u>	39.2	133.5	343.2	29.4	75.2	70.8
<u>G. fasciculatus</u> + <u>P. funiculosum</u>	66.2	203.2	323.7	52.4	93.7	94.7
<u>G. fasciculatus</u> + <u>A. niger</u>	104.2	199.2	334.2	30.9	87.2	98.7
C.D. at P=0.05	42.11	44.95	125.45			

*Statistical analysis for percentage infection is not calculated

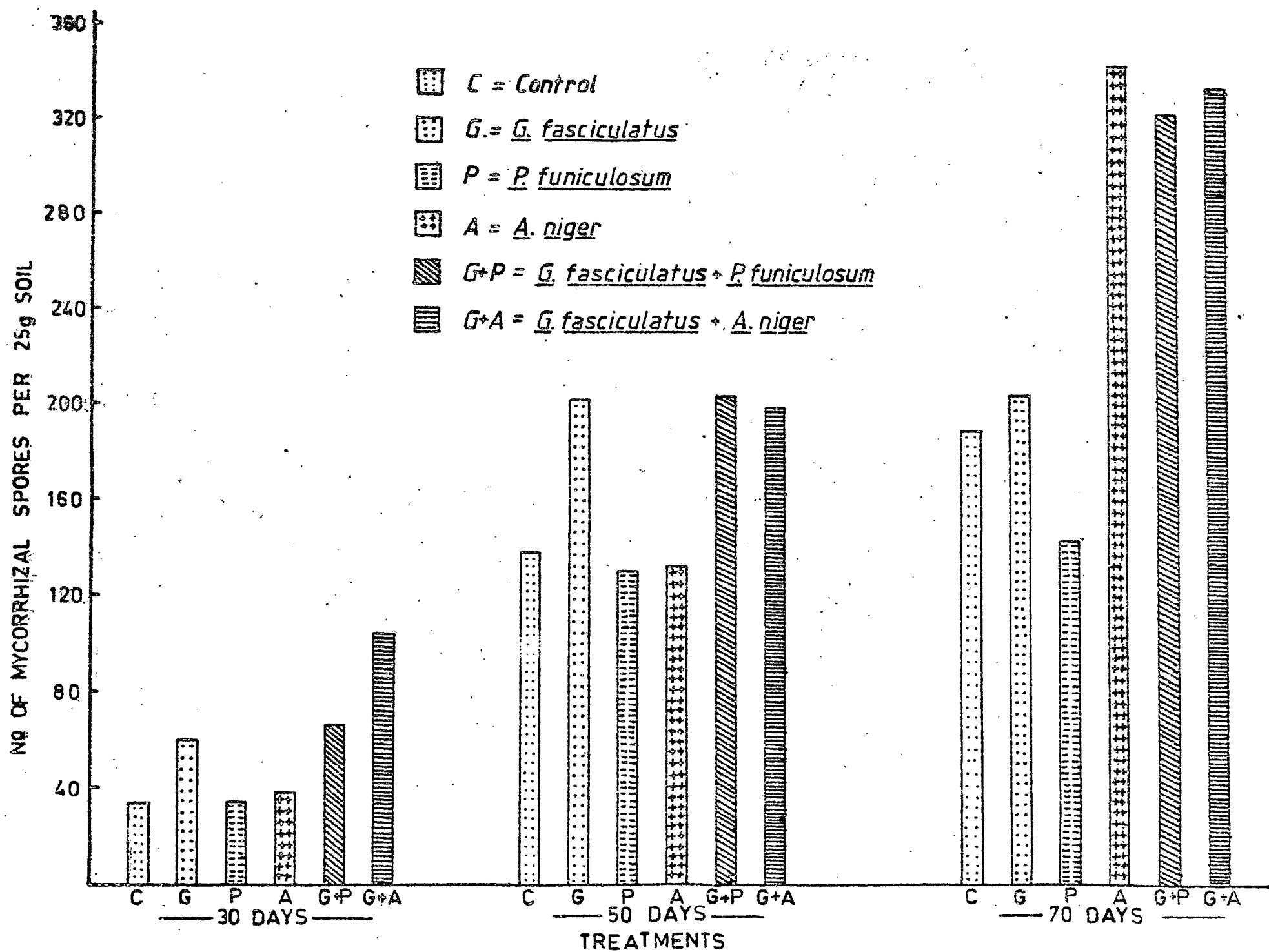


FIG. 5 VA MYCORRHIZAL SPORE COUNTS IN THE RHIZOPHERE OF FINGER MILLET AS INFLUENCED BY MYCORRHIZA AND P SOLUBILISING FUNGI

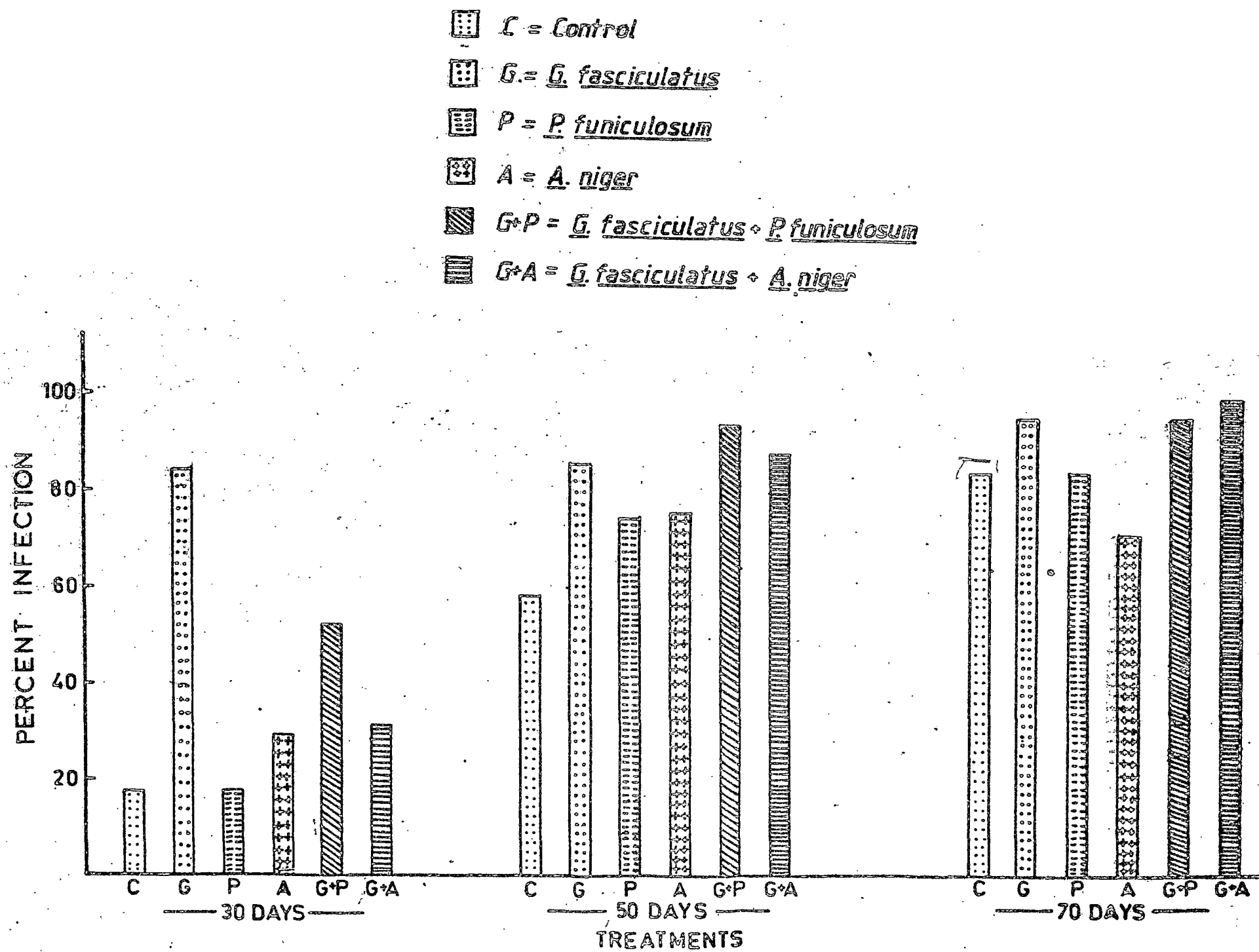


FIG. 6 PERCENT VA MYCORRHIZAL INFECTION OF FINGER MILLET ROOTS AS INFLUENCED BY MYCORRHIZA AND P SOLUBILISING FUNGI

Plant root and shoot dry weight.

The dry weight of root and shoot of finger millet inoculated with G. fasciculatus and phosphate solubilising fungi is presented in Table 6 & Fig. 7 & 8. The difference in root dry weight was significant only on day 70. The first four treatments viz., control, G. fasciculatus alone, P. funiculosum alone and A. niger alone treatment did not show any difference in root dry weight. Although the two dual inoculation treatments recorded increased dry weight, the increase was significant only in G. fasciculatus + A. niger treatment.

The differences in the dry weight of shoot as influenced by the treatments was found significant on days 30 and 50. The four treatments, control, G. fasciculatus alone, P. funiculosum alone and A. niger alone did not show any significant difference among themselves. The two dual inoculation treatments recorded the increased shoot weight with G. fasciculatus + P. funiculosum showing significant difference at both intervals and G. fasciculatus + A. niger at only day 50.

Estimation of nutrient content.

Table 7 & Fig. 9 shows the phosphorus content of root and shoot. The root phosphorus content was found non-significant at all the three intervals. The overall trend showed that the dual inoculation treatments contained more phosphorus at all the three intervals. The difference in phosphorus content of shoot was found significant at all the three intervals. At day 30, G. fasciculatus alone treatment showed a higher phosphorus

Table 6. Finger millet root and shoot dry weight as influenced by inoculation with G. fasciculatus and two P solubilising fungi P. funiculosum and A. niger

Treatments	Dry weight of plant (g)					
	Root			Shoot		
	Days after transplanting			Days after transplanting		
	30	50	70	30	50	70
Control	0.854	3.647	4.215	1.228	7.697	15.923
<u>G. fasciculatus</u>	1.226	4.062	4.306	1.356	8.812	17.655
<u>P. funiculosum</u>	0.979	3.650	4.756	1.009	7.403	18.811
<u>A. niger</u>	0.546	4.177	4.364	1.253	8.854	17.412
<u>G. fasciculatus</u> + <u>P. funiculosum</u>	1.048	4.715	5.550	2.045	10.870	19.291
<u>G. fasciculatus</u> + <u>A. niger</u>	1.142	4.707	5.717	1.763	10.094	18.280
C.D. at P=0.05	NS	NS	1.50	0.82	1.98	NS

NS = Not significant

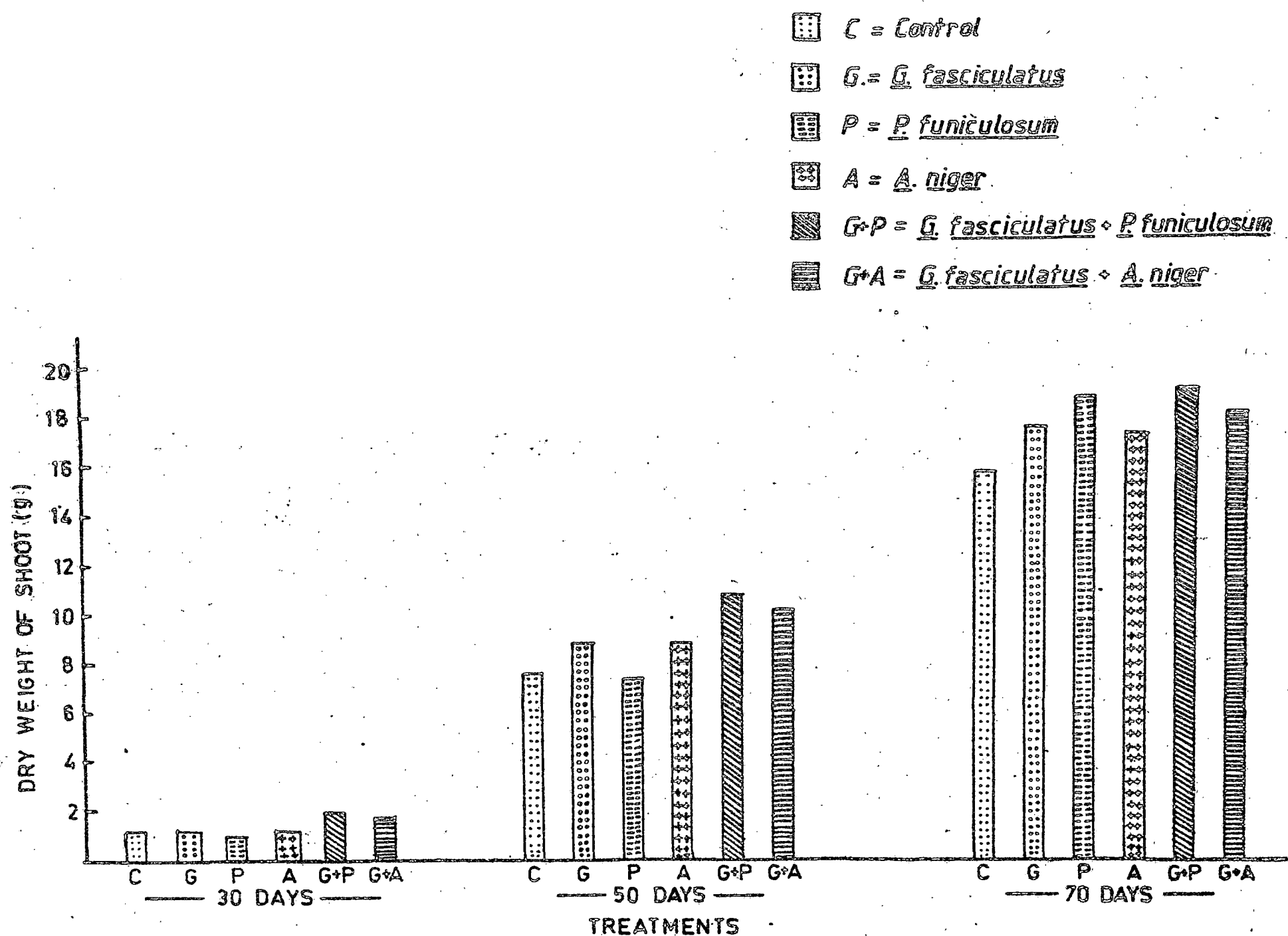


FIG. 7. SHOOT DRY WEIGHT OF FINGER MILLET AS INFLUENCED BY MYCORRHIZA AND P SOLUBILISING FUNGI

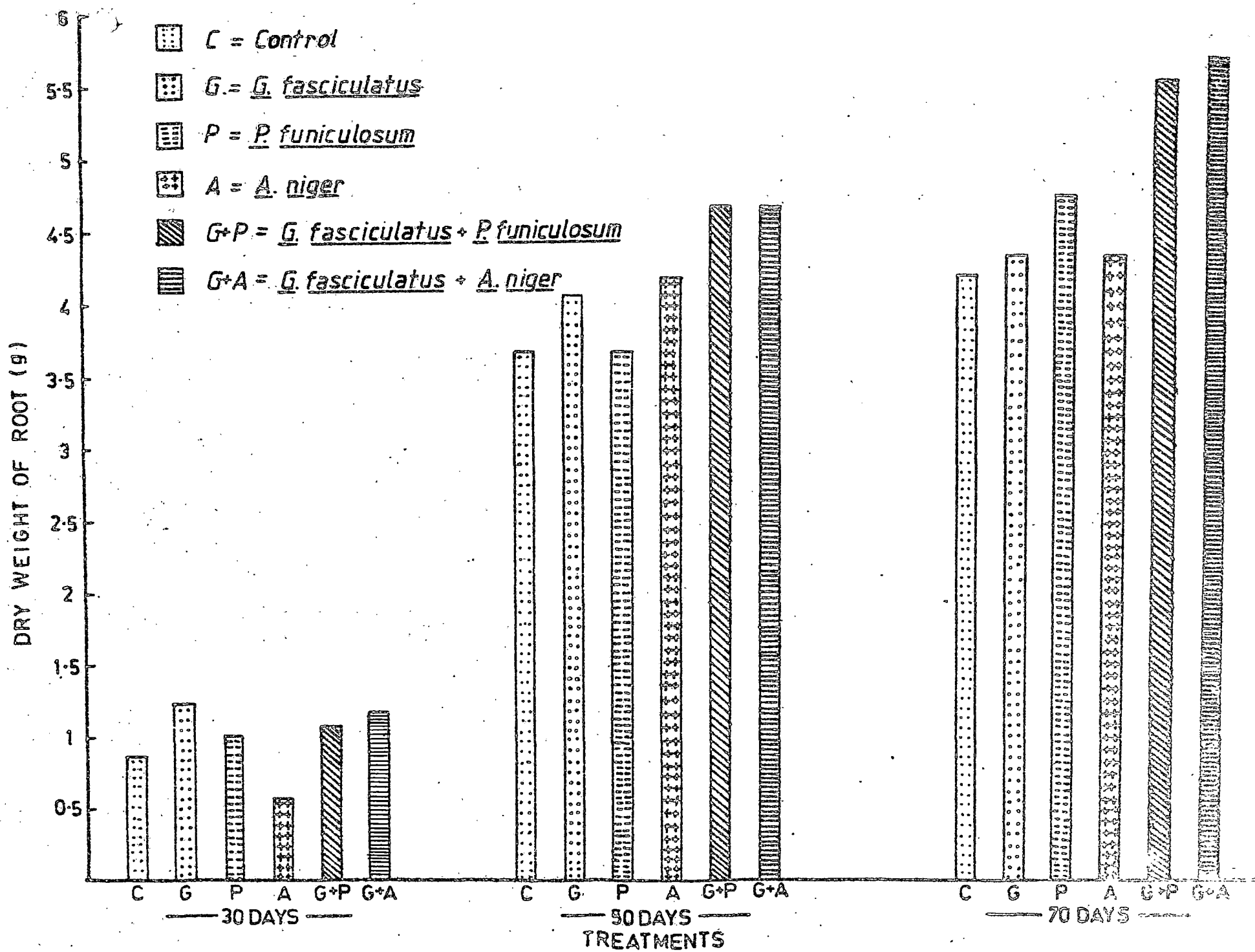


FIG. 8. ROOT DRY WEIGHT OF FINGER MILLET AS INFLUENCED BY MYCORRHIZA AND P SOLUBILISING FUNGI.

Table 7. Phosphorus content of finger millet root and shoot as influenced by inoculation with G. fasciculatus and two P solubilising fungi P. funiculosus and A. niger

Treatments	Phosphorus content (mg)									
	Roots					Shoots				
	Total P (mg/plant)					Total P (mg/plant)				
	Days after transplanting		Days after transplanting		Per cent P (mg/100 g)	Days after transplanting		Days after transplanting		Per cent P (mg/100 g)
	30	50	70	70		30	50	70	70	
Control	0.49	1.40	1.77	1.60	4.87	4.31	0.125	0.057	0.027	
<u>G. fasciculatus</u>	0.67	1.52	1.92	2.10	5.44	5.29	0.165	0.062	0.030	
<u>P. funiculosus</u>	0.45	1.12	1.89	1.68	5.17	7.52	0.175	0.070	0.040	
<u>A. niger</u>	0.41	1.15	1.96	2.13	6.12	6.15	0.172	0.070	0.035	
<u>G. fasciculatus</u> + <u>P. funiculosus</u>	0.81	1.30	2.40	3.16	9.77	6.78	0.155	0.090	0.035	
<u>G. fasciculatus</u> + <u>A. niger</u>	0.74	1.70	2.12	2.75	8.34	6.86	0.157	0.082	0.037	
C.D. at P=0.05	NS	NS	NS	1.26	2.96	2.09	NS	NS	0.0085	

NS = Not significant

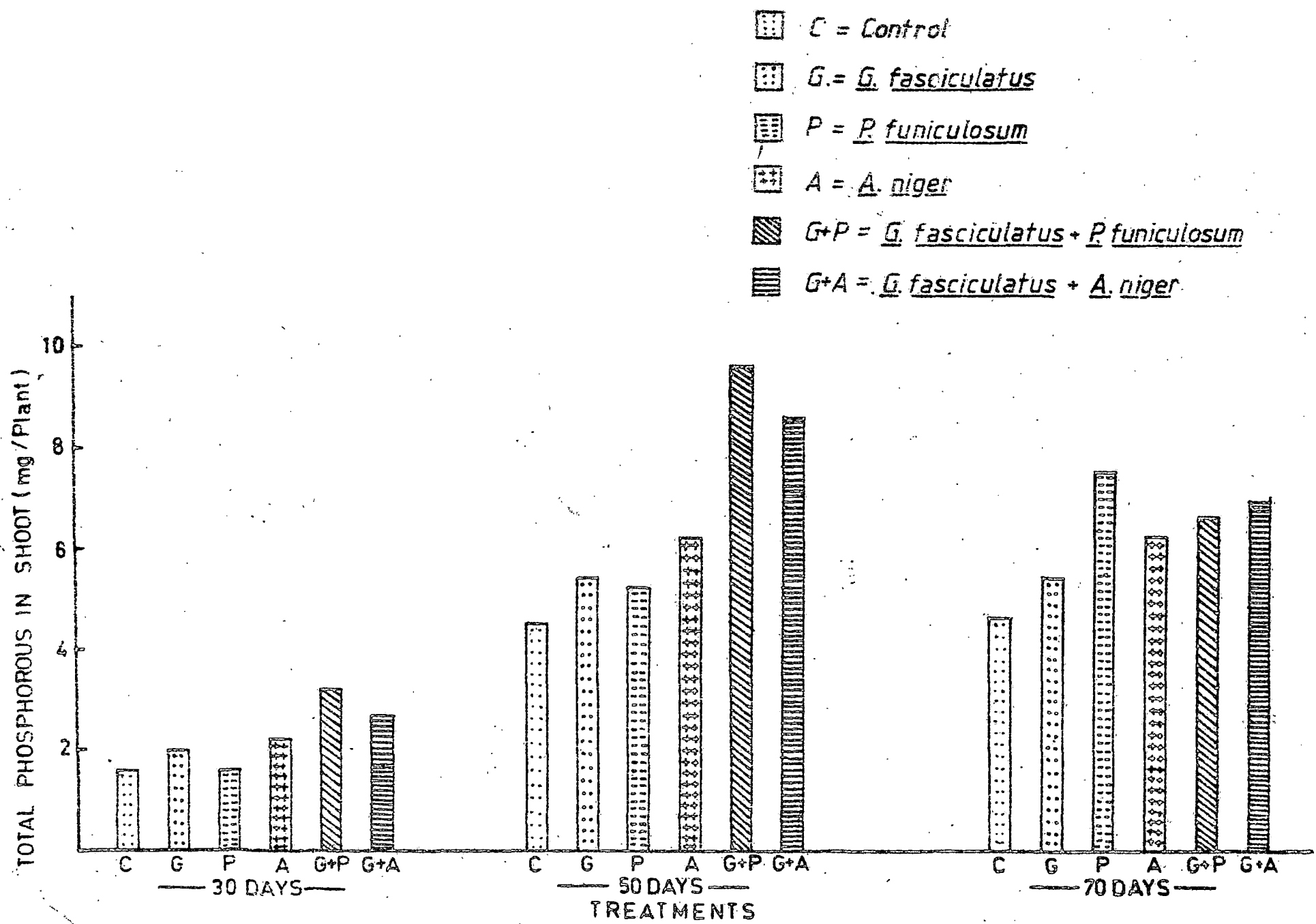


FIG.9. PHOSPHOROUS CONTENT OF FINGER MILLET SHOOT AS INFLUENCED BY MYCORRHIZA AND P SOLUBILISING FUNGI.

content in shoot than control or P. funiculosum alone treatment but the difference was not significant. A. niger alone treatment showed a phosphorus content equal to that of G. fasciculatus + A. niger. The only treatment which showed a high phosphorus content which was statistically significant was G. fasciculatus + P. funiculosum. At 50th day the same trend was observed. At 70th day, except the treatment P. funiculosum alone which showed a steady increase in phosphorus content and A. niger alone which maintained its phosphorus content, rest of the treatments showed a reduction in the phosphorus content of the shoot.

The potassium content of the finger millet shoot as influenced by different treatments is given in Table 8 & Fig 10. The difference in potassium content was found significant only on days 30 and 50. On day 30, G. fasciculatus alone treatment did not show any difference in potassium content compared to control. The two treatments P. funiculosum alone and A. niger alone also did not show any difference in their potassium contents compared to control. Only the two dual inoculation treatments recorded higher potassium content at all the intervals. The difference in the potassium content of plants inoculated with G. fasciculatus + P. funiculosum and P. funiculosum alone was statistically significant. The same trend was observed on day 50 also.

Zinc content of the shoot is given in Table 9 & Fig 11. The

Table 8. Potassium content of finger millet shoot as influenced by inoculation with G. fasciculatus and two P solubilising fungi P. funiculosum and A. niger

Treatments	Total K (mg/plant top)		
	Days after transplanting)		
	30	50	70
Control	0.60	1.96	3.18
<u>G. fasciculatus</u>	0.51	1.76	3.52
<u>P. funiculosum</u>	0.43	1.47	2.84
<u>A. niger</u>	0.53	1.98	3.05
<u>G. fasciculatus</u> + <u>P. funiculosum</u>	0.97	2.16	3.85
<u>G. fasciculatus</u> + <u>A. niger</u>	0.84	2.01	3.65
C.D. at P=0.05	0.33	0.55	NS

NS = Not significant

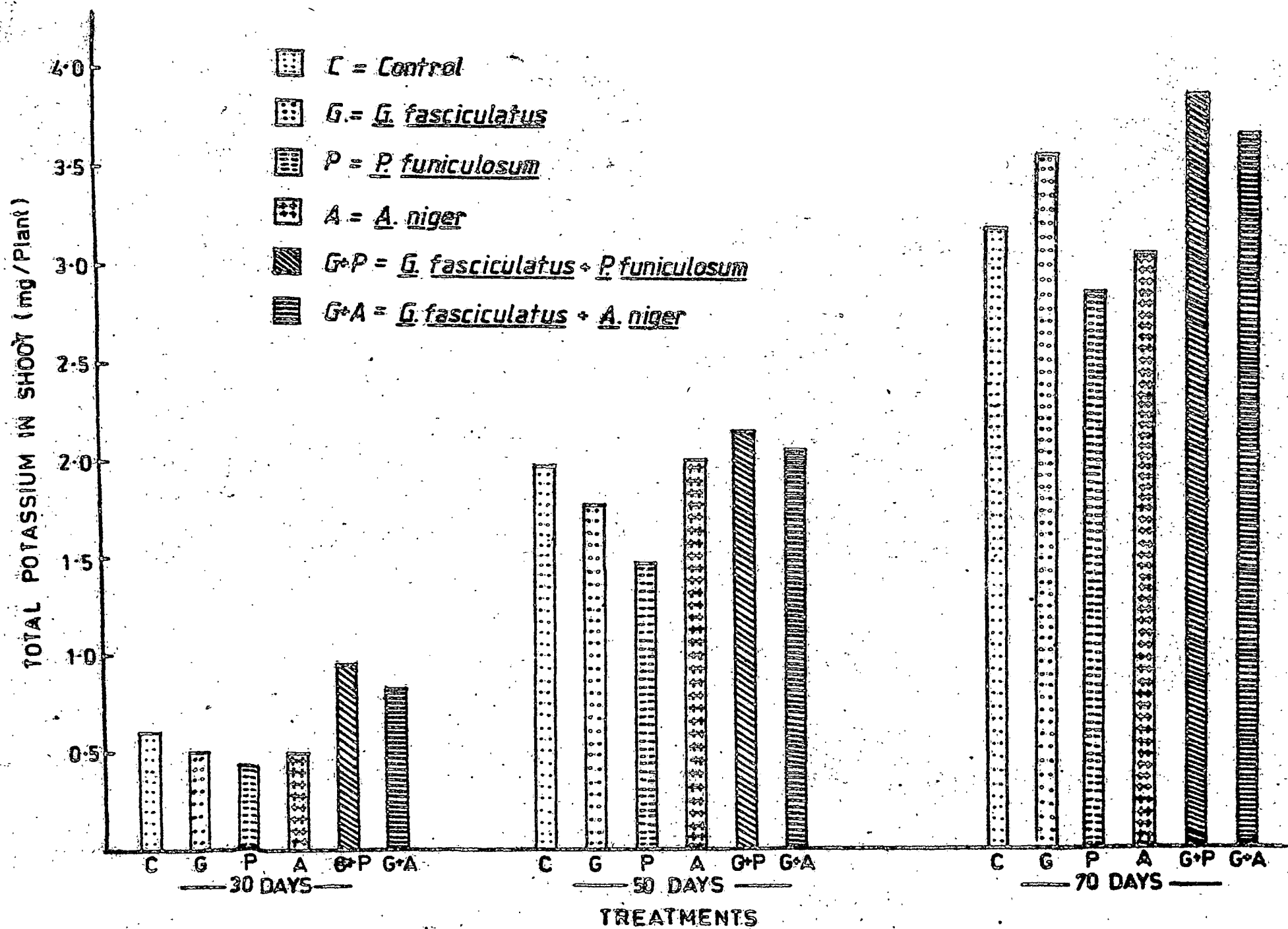


FIG.10. POTASSIUM CONTENT OF FINGER MILLET AS INFLUENCED BY MYCORRHIZA AND P SOLUBILISING FUNGI.

Table 9. Zinc content of finger millet shoot as influenced by inoculation with G. fasciculatus and two P solubilising fungi P. funiculosum and A. niger

Treatments	Total Zn (mg/plant top)		
	Days after transplanting		
	30	50	70
Control	0.004	0.015	0.026
<u>G. fasciculatus</u>	0.004	0.017	0.040
<u>P. funiculosum</u>	0.002	0.024	0.065
<u>A. niger</u>	0.002	0.013	0.021
<u>G. fasciculatus</u> + <u>P. funiculosum</u>	0.005	0.021	0.039
<u>G. fasciculatus</u> + <u>A. niger</u>	0.007	0.054	0.042
C.D. at P=0.05	NS	0.029	NS

NS = Not significant

difference in zinc content was found to be significant only on day 50. The difference in the zinc content between control and G. fasciculatus alone inoculated plants were statistically not significant. P. funiculosum and A. niger alone treatments also did not influence the zinc content of the plant tops. Plants inoculated with G. fasciculatus + A. niger however recorded significantly more zinc in the shoot compared to uninoculated control, G. fasciculatus inoculated and A. niger alone treatments.

The manganese content of the finger millet shoot as influenced by various treatments is given in Table 10 & Fig 12. The difference in manganese content was found significant only on day 30. Treatments G. fasciculatus alone, P. funiculosum alone, A. niger alone did not have any influence on manganese content of the shoot compared with the uninoculated control treatment. Dual inoculation treatments recorded more manganese in the plant tops, the highest being in G. fasciculatus + P. funiculosum inoculated plants.

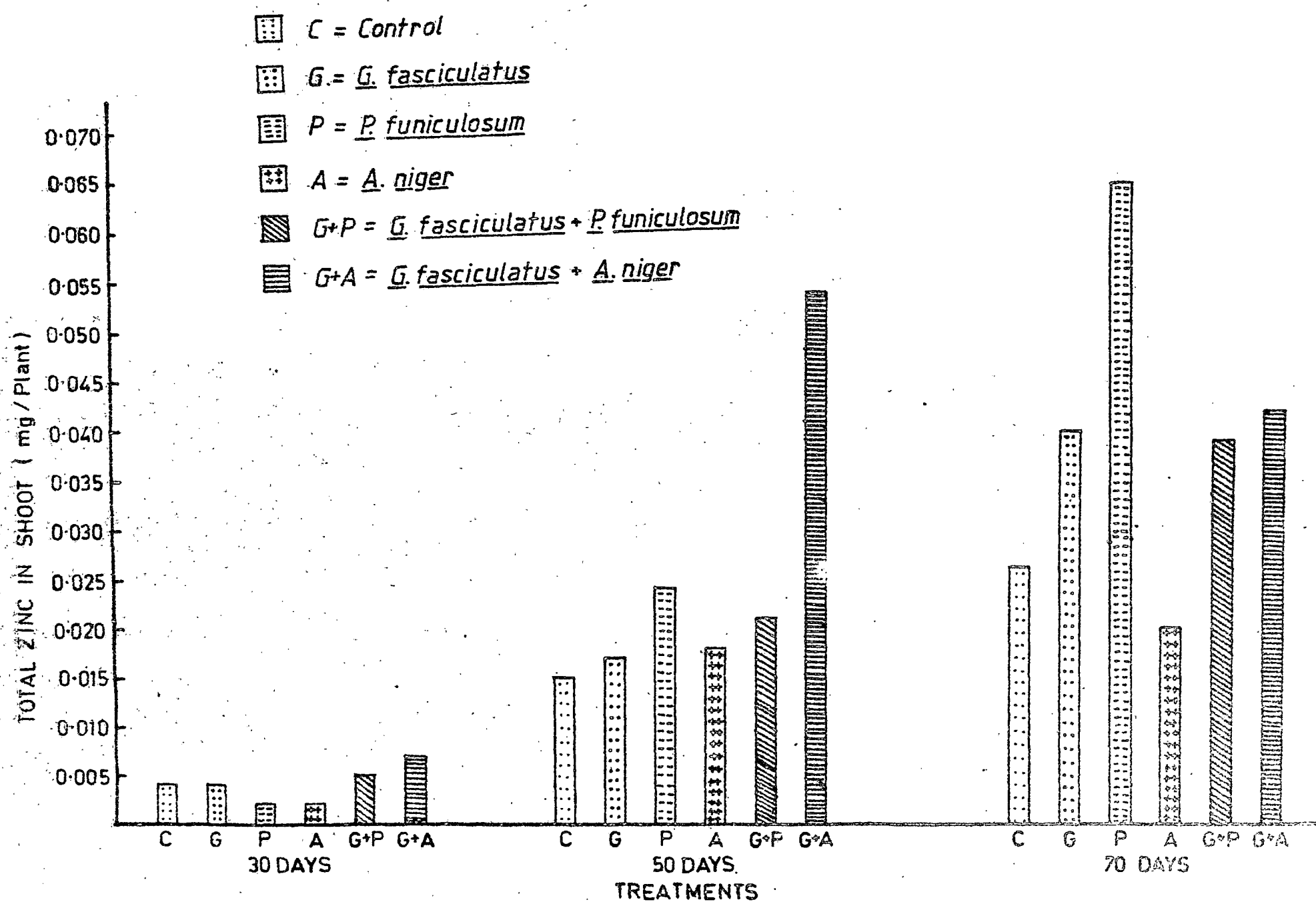


FIG.11. ZINC CONTENTS OF FINGER MILLET SHOOT AS INFLUENCED BY MYCORRHIZA AND P SOLUBILISING FUNGI.

Table 10. Manganese content of finger millet shoot as influenced by inoculation with G. fasciculatus and two P solubilising fungi P. funiculosum and A. niger

Treatments	Manganese (mg/plant top)		
	Days after transplanting		
	30	50	70
Control	0.003	0.028	0.063
<u>G. fasciculatus</u>	0.005	0.032	0.087
<u>P. funiculosum</u>	0.003	0.033	0.093
<u>A. niger</u>	0.007	0.055	0.073
<u>G. fasciculatus</u> + <u>P. funiculosum</u>	0.012	0.048	0.074
<u>G. fasciculatus</u> + <u>A. niger</u>	0.009	0.050	0.102
C.D. at P=0.05	0.005	NS	NS

NS = Not significant

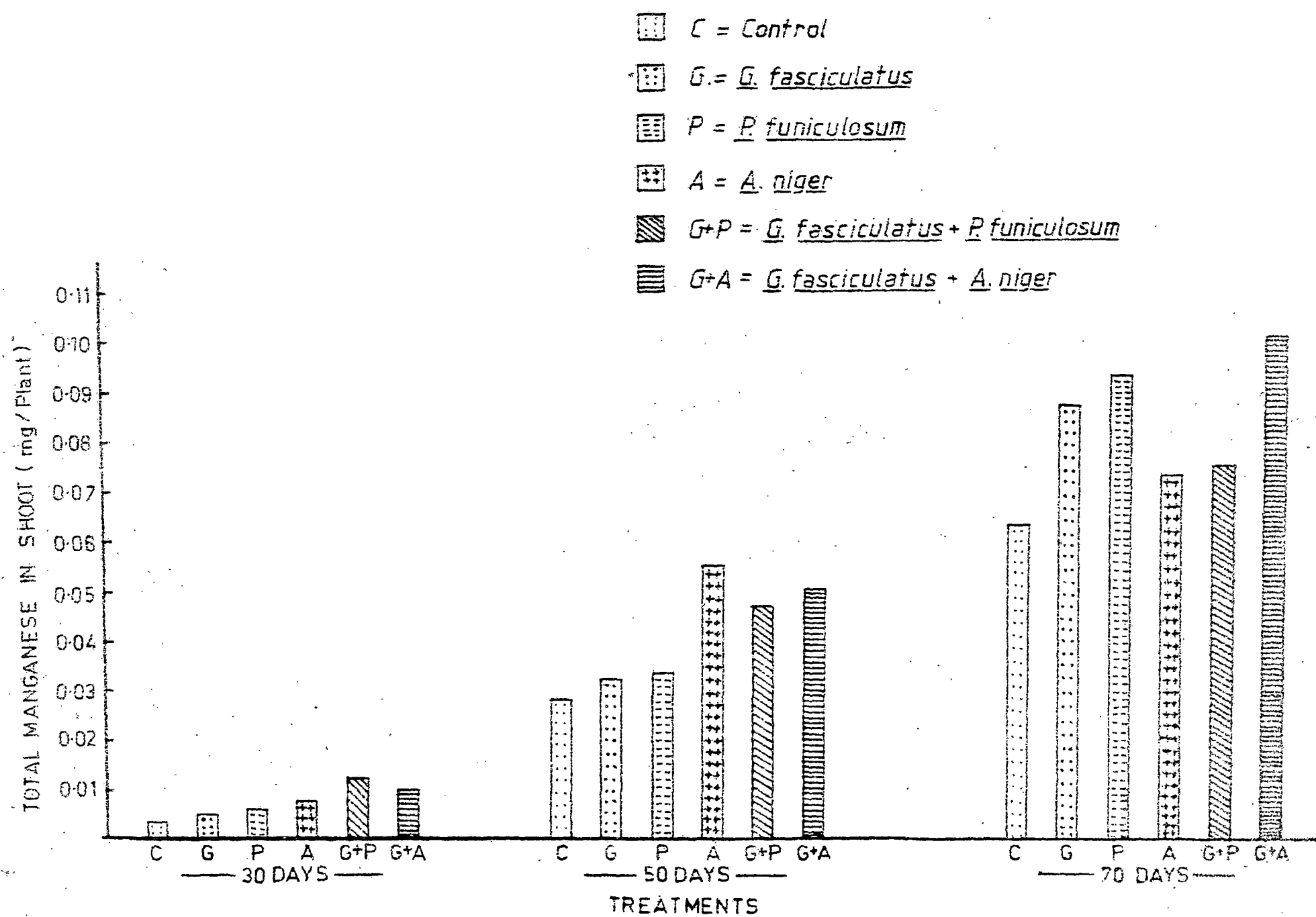


FIG.12. MANGANESE CONTENT OF FINGER MILLET SHOOT AS INFLUENCED BY MYCORRHIZA AND P SOLUBILISING FUNGI.

DISCUSSION

CHAPTER V

DISCUSSION

It is already a well known fact that many agriculturally important plants are infected with VA mycorrhiza, and some have responded favourably to it for their phosphorus nutrition requirement (Mosse, 1973). The occurrence and response of finger millet to VA mycorrhizal infection has also been reported (Bagyaraj ^{and Manjunath} *et al.*, 1980).

The ability of the two fungi *P. funiculosum* and *A. niger* to solubilise rock phosphate and improve plant growth also has been studied (Raj, 1980).

Some studies have been made to know the interaction between VA mycorrhiza and P solubilising microorganisms. The synergistic interaction between VA mycorrhiza and phosphate solubilising bacteria and their beneficial effect on growth and phosphorus nutrition of maize and lavender has been reported (Barea *et al.*, 1975; and Azcon *et al.*, 1976).

It is a well known fact that high microbial activity and nutrient concentration are found in the rhizosphere region (Brown, 1975). Any change in this region might affect the rhizosphere microflora and in turn the plant growth. In ectomycorrhiza the stimulation of the microbial activity around it has lead to the recognition of the region called mycorrhizosphere (Oswald and Ferchau, 1968). Such studies are very few in case of VA mycorrhiza.

Barea et al. (1975) pointed out that since root growth and metabolism are altered by infection with VA mycorrhiza, it is likely that the root exudates and rhizosphere population could be affected. This was found to be true in case of Azotobacter and other microorganisms (Bagyaraj and Menge, 1978). The present study was conducted to reveal the interaction between the VA mycorrhizal fungus, G. fasciculatus and the P solubilising fungi, A. niger and P. funiculosus and their effect on growth and nutrition of finger millet and the microflora in the rhizosphere. In this experiment initial high bacterial population in the rhizosphere of the finger millet inoculated with both G. fasciculatus plus either of the two P solubilising fungi suggests a synergistic or additive effect of these two organisms. Rapid reduction in the bacterial population in these two treatments in the later stages of plant growth could be due to increase in the fungal and actinomycete population during that period. High population of fungi in the rhizosphere of finger millet inoculated with G. fasciculatus was reduced when the two P solubilising fungi were inoculated along with it. This reduction could be due to the presence of large population of these two fungi which might have upset the population of other rhizosphere fungi by competing for the same nutrient source. Large actinomycete population in the rhizosphere of finger millet inoculated with G. fasciculatus observed in the present study is in conformity with the earlier findings by Bagyaraj and Menge (1978).

The population of phosphorus solubilising fungi introduced was very much influenced by the mycorrhizal infection. The mycorrhizal fungus enabled particularly A. niger to maintain a high population till the end of plant growth compared with A. niger alone treatment. This is in agreement with the result obtained by Barea et al. (1975).

Azcon et al. (1978a) pointed out that whole culture or cell free culture preparation of phospho bacteria Rhizobium and Azotobacter can also behave as pure hormone and improve mycorrhizal infection. In this experiment also the introduced two P solubilising fungi due to their presence alone or perhaps by producing growth promoting hormones have increased the spore count and infection not only of G. fasciculatus but also of native mycorrhiza as seen in A. niger alone treatment.

Improved plant growth due to inoculation with mycorrhiza has already been studied. Studies conducted under sterilized soil condition have shown good responses to inoculation with VA mycorrhiza (Hayman and Mosse, 1971 and 1972; and Mosse, 1972). However, responses to inoculation were small or non-significant in unsterilized soil conditions (Gerde mann, 1964; and Ross, 1971), although some workers could get significant increase in plant growth under unsterile field conditions due to VA mycorrhizal inoculation (Khan, 1972 and 1975).

Since this experiment was conducted under unsterilized condition, inoculation with G. fasciculatus did not result

in significant increase in growth of finger millet compared to uninoculated control where native mycorrhiza had infected. It has also been suggested by Powell (1976) that in some soils native mycorrhiza were found equal or superior over the inoculated exotic strain. Since VA mycorrhizal strains in our soils have not been isolated, identified and studied, possibilities of a superior strain being present in our soil cannot be ruled out. Increased dry weight of root and shoot in the two dual inoculation treatments over G. fasciculatus alone treatment could be due to the production of growth hormones by these two P solubilising fungi.

Mosse et al. (1976) have shown that rock phosphate when added to acidic soil is available to plants with or without mycorrhiza, but the presence of mycorrhiza helps in better absorption than without mycorrhiza. Therefore, in this experiment less difference in the phosphorus content between G. fasciculatus inoculated treatment and the uninoculated control treatment could be due to the availability of added rock phosphate in the acidic soil used and also due to the presence of native mycorrhiza in the uninoculated control treatment. This also proves that native mycorrhiza are also as effective in absorption of phosphorus as G. fasciculatus. High phosphorus content in two dual inoculation treatments could be mainly due to extra rock phosphate solubilized by these two fungi in addition to solubilization due to soil

acidity and subsequent absorption by G. fasciculatus. High phosphorus content in the treatments with only P. funiculosum and A. niger towards the end further confirms the ability of native mycorrhiza in absorption of phosphorus.

Difference in K, Ca, Na, Mg, Fe, Mn, Cu, B, Zn and Al concentration of mycorrhizal and non-mycorrhizal plants have been reported (Ross and Harper, 1970; Ross, 1971; and Kleinshmidt and Gerdemann, 1972). However, the results are highly inconsistent. Holveas (1966) and Mosse (1973) found less potassium in uninoculated plants than in inoculated plants. In the present study also, G. fasciculatus inoculated plants contained less potassium than control. Therefore, high potassium content in dual inoculation treatments might perhaps be due to extensive root growth produced by the interaction of G. fasciculatus and two P solubilizing fungi which might have given extra absorption surface for the plant.

High zinc and manganese content in two dual inoculation treatments again could be due to extra absorption surface got by extensive root growth. High manganese content particularly in A. niger alone and G. fasciculatus plus A. niger could be due to the ability of A. niger to solubilize the insoluble MnO_2 (Barea et al., 1971a and 1971b) and subsequent effective absorption by mycorrhizal roots.

The present study has brought out that VA mycorrhizal

fungus G. fasciculatus could interact favourably with the two P solubilizing fungi P. funiculosum and A. niger in increasing the dry weight of root and shoot and also the phosphorus content of finger millet plant. The interaction favoured each other through the increased spore count of G. fasciculatus and population count especially in case of A. niger, in addition to their influence in increasing the rhizosphere microbial population especially of bacteria and actinomycete.

SUMMARY

CHAPTER VI

SUMMARY

A pot culture study was done to know the interaction between VA mycorrhizal fungus G. fasciculatus and the two P solubilizing fungi A. niger and P. funiculosum and its effect on growth and nutrition of finger millet and also on the microbial population in the rhizosphere. The interaction effect was assessed at three intervals of crop growth by enumerating the bacterial, actinomycete and fungal load and mycorrhizal spore count from the rhizosphere soil. Root and shoot weight of finger millet and the nutritional content of finger millet shoot at the three intervals was also determined.

The results of the experiment clearly established a positive interaction between VA mycorrhizal and the two P solubilizing fungi. The effect of interaction has also been fairly good. The microbial population in the rhizosphere especially bacteria and actinomycete was increased by the presence of these two organisms. The fungal population in the rhizosphere however was reduced by their presence. In the case of two P solubilizing fungi introduced, only A. niger population was found to be increased by the presence of mycorrhiza.

Both the P solubilizing fungi stimulated the extra-matrical chlamydospore production by the VA mycorrhizal fungus

in the soil around root zone.

Dual inoculation with G. fasciculatus and either of the two P solubilizing fungi A. niger or P. funiculosum increased significantly the dry weight of the root and shoot of finger millet at the initial stages of plant growth. Dual inoculation also increased the nutrient content like P, K, Zn and Mn in the shoot especially P which was found in the higher amounts at all the three intervals.

This work brings out the fact that the dual inoculation with VA mycorrhizal fungus G. fasciculatus and two P solubilizing fungi A. niger and P. funiculosum have synergistic beneficial effect on each other and also stimulatory effect on the growth and nutrition of finger millet and also on the rhizosphere microflora.

REFERENCES

CHAPTER VII

REFERENCES

- Abbot, L.K. and Robson, A.D., 1977. Growth stimulation of subterranean clover with vesicular-arbuscular mycorrhiza. Aust. J. Agric. Res., 28:639-649.
- Abbot, L.K. and Robson, A.D., 1978. Growth of subterranean clover in relationship to the formation of endomycorrhiza by introduced and indigenous fungi in a field soil. New Phytol., 81:575-585.
- Azcon, R., Barea, J.M. and Hayman, D.S., 1976. Utilization of rock phosphate in alkaline soils by plants inoculated with mycorrhizal fungi and P solubilizing bacteria. Soil Biol. Biochem., 8:135-138.
- Azcon, R., Marin, A.D. and Barea, J.M., 1978. Comparative role of P in soil or inside the host on the formation and effect of endomycorrhizas. Pl. Soil., 49:561-567.
- Azcon, R., Azcon, G. de Angilar, C. and Barea, J.M., 1972. Effects of plant hormones present in bacterial cultures on the formation and responses to VA endomycorrhiza. New Phytol., 80:359-364.
- Azcon G. de Angilar, C. and Barea, J.M., 1978. Effects of interaction between different culture fractions of phosphobacteria and Rhizobium on mycorrhizal infection, growth and nodulation of Medicago sativa. Can. J. Microbiol., 24:520-524.
- Bagyaraj, D.J. and Menge, J.A., 1978. CInteraction between a VA mycorrhiza and Azotobacter and their effect on rhizosphere microflora and plant growth. New Phytol., 80:567-73.
- Bagyaraj, D.J. and Manjunath, A., 1980. Response of crop plants to mycorrhizal inoculation in an unsterile Indian soil. New Phytol., 84: ?

- Bagyaraj, D.J., Manjunath, A. and Reddy, D.D.R., 1978. Interaction of vesicular-arbuscular mycorrhiza with root knot nematode in tomato. Pl. Soil, 51:397-403.
- Bagyaraj, D.J., Manjunath, A. and Patil, R.B., 1979a, Occurrence of vesicular-arbuscular mycorrhiza in some tropical aquatic plants. Trans. Brit. Mycol. Soc., 72:164-167.
- Bagyaraj, D.J., Manjunath, A. and Patil, R.B., 1979b, Interaction between a VA mycorrhiza and Rhizobium and their effect on soybean in the field. New Phytol., 82:141-145.
- Barea, J.M., Azcon, B. and Hayman, D.S., 1975. Possible synergistic interaction between Endogone and phosphate solubilising (Ed. F.E. Sanders, B. Mosse and P.B. Tinker), Academic Press, London, pp.409-417.
- Barea, J.M., Olivares, J., Aguilar, A. and Callao, V., 1971a, Microbial reduction of MnO_2 and its application to agricultural soils deficient in assimilable Mn. I. Microbial and analytical studies of a mould of the soil capable of Mn solubilisation. Agrochemica., 15:472-479.
- Barea, J.M., Olivares, J., Aguilar, A. and Callao, V., 1971b. Microbial reduction of MnO_2 and its application to agricultural soils deficient in assimilable Mn. II. Use of Aspergillus niger as microbial fertilizer. Agrochemica., 15:480.
- Barrow, N.J., Malajeznk, N. and Shaw, T.C., 1977. A direct test of the ability of vesicular-arbuscular mycorrhiza to help plants take up fixed soil phosphate. New Phytol., 78:269-276.
- Baylis, G.T.S., 1970. Root hairs and phycomycetous mycorrhizae in phosphate deficient soils. Pl. Soil., 33:713-716.
- Black, R.L.B. and Tinker, P.B., 1977. Interaction between effects of vesicular-arbuscular mycorrhizae and fertilizer phosphorus on yields of potato in the field. Nature, 267:510-511.

Brown, M.E., 1975. Rhizosphere microorganisms - opportunists bandits or benefactors. In: "Soil Microbiology" (Ed. N. Walker), Halsted Press, New York, pp.21-38.

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

Clough, K.S. and Sutton, T.C., 1978. Direct observation of fungal aggregates in sand dune soil. Can. J. Microbiol., 24:333-335.

Cooper, K.M., 1977. Endomycorrhizas affect growth of Dryopteris filix Mass. Trans. Brit. Mycol. Soc., 69:161-164.

Cooper, K.M. and Tinker, P.B., 1978. Translocation and transfer of nutrients in VA mycorrhizae. II. Uptake and translocation of phosphorus, zinc and sulfur. New Phytol., 81:43-52.

Crush, J.R., 1976. Entomycorrhizas and legume growth in some soils of Mackenzie basin, Canterbury, New Zealand. N.Z. J. Agric. Res., 19:473-476.

Daft, M.J. and Nicolson, J.H., 1966. The effect of Endogone mycorrhiza on plant growth. New Phytol., 65:343-350.

Daft, M.J. and El Giahmi, A.A., 1974. Effect of Endogone mycorrhiza on plant growth. VII. Influence of infection on growth and nodulation in French beans (Phaseolus vulgaris). New Phytol., 73:1139-1147.

Daft, M.J. and El Giahmi, A.A., 1976. Studies on nodulated and mycorrhizal peanuts. Ann. Appl. Biol., 82:273-276.

Daft, M.J. and El Giahmi, A.A., 1978. Effect of arbuscular-mycorrhiza on plant growth. VIII. Effect of defoliation and light on selected hosts. New Phytol., 80:365-372.

- Deal, M.J., Boothroyd, C.W. and Mai, W.F., 1972. Replanting of vine yard and its relationship to VA mycorrhiza. Phytopathology, 62:172-175.
- Fitter, A.H., 1977. Influence of mycorrhizal infection on competition for phosphorus and potassium by two grasses. New Phytol., 79:119-125.
- Gaunt, R.E., 1978. Inoculation of vesicular-arbuscular mycorrhizal fungi on onions and tomato seeds. N.Z. J. Bot., 16:69-71.
- Gerdemann, J.W., 1964. The effect of mycorrhiza on the growth of maize. Mycologia, 56:342-394.
- Gerdemann, J.W., 1968. Vesicular-arbuscular mycorrhiza and plant growth. Ann. Rev. Phytopathol., 6:397-418.
- Gerdemann, J.W., 1970. The significance of vesicular-arbuscular mycorrhiza in plant nutrition. Root Diseases and Plant Pathogens (Ed. T.A. Toussoun, R.V. Bega and P.E. Nelson). Berkley Univ. Calif. Pub., p 125-129.
- Gerdemann, J.W. and Nicolson, T.H., 1963. Spores of mycorrhizal Endogone species extracted from soil by zer sieving and decanting. Trans. Brit. Mycol. Soc., 46:235-244.
- Gilmore, A.E., 1971. The influence of endotropic mycorrhiza on the growth of peach seedlings. J. Am. Soc. Hort. Sci., 96:35-38.
- Godse, D.B., Madhusudhan, Bagyaraj, D.J. and Patil, R.B., 1976. Occurrence of vesicular-arbuscular mycorrhiza in crop plants in Karnataka. Trans. Brit. Mycol. Soc., 67:169-171.
- Godse, D.B., Wani, S.P., Patil, R.B. and Bagyaraj, D.J., 1978. Response of cowpea (Vigna unguiculata L. Walp) to Rhizobium VA mycorrhizal dual inoculation. Curr. Sci., 47:784-785.

- Graham, S.O., Green, N.E. and Hendrix, J.W., 1976. The influence of vesicular-arbuscular mycorrhizal fungi on growth and fertilization of potatoes. Mycologia, 68:925-929.
- Graw, D. and Rehm, S., 1977. Vesicular-arbuscular mycorrhiza in the pegs of Arachis hypogaea. J. Agron. Crop Sci., 145:75-78.
- Gray, L.E. and Gerdemann, J.W., 1969. Uptake of phosphorus-32 by vesicular-arbuscular mycorrhiza. Pl. Soil., 30:415-422.
- Gray, L.E. and Gerdemann, J.W., 1973. Uptake of sulphur-35 by vesicular-arbuscular mycorrhiza. Pl. Soil., 39:687-689.
- Hains, B.L. and Best, G.R., 1976. Glomus mosseae endomycorrhiza with Liquidambar styraciflua L. seedlings retards NO_3 , NO_2 and NH_4 nitrogen loss from a temperate forest soil. Pl. Soil., 45:257-261.
- Hall, I.R., 1978a, Effect of endomycorrhiza on the competitive ability of White clover. N.Z. J. Agric. Res., 21:509-515.
- Hall, I.R., 1978b. Effect of VA mycorrhiza on two varieties of maize and one sweet corn. N.Z. J. Agric. Res., 21:517-519.
- Harley, J.L., 1969. The Biology of Mycorrhiza. Leonard Hill Books, London.
- Hatting, M.J., Gray, L.E. and Gerdemann, J.W., 1973. Uptake and translocation of ^{32}P labelled phosphate to onion roots by endomycorrhizal fungi. Soil Sci., 116:383-387.
- Hayman, D.S. and Mosse, B., 1971. Plant growth response to vesicular-arbuscular mycorrhizae. I. Growth of endogone inoculated plants in phosphate deficient soils. New Phytol., 70:

- Hayman, D.S. and Mosse, B., 1972a. The role of vesicular-arbuscular mycorrhiza in the removal of phosphorus from the soil by plant roots. Rev. Ecol. Biol. Sol., 9:463-470.
- Hayman, D.S. and Mosse, B., 1972b. Plant growth responses to vesicular-arbuscular mycorrhiza. II. Increased uptake of labelled P from soil. New Phytol., 71:41-47.
- Halveas, C.D., 1966. The effect of vesicular-arbuscular mycorrhiza on uptake of soil phosphorus by strawberry (Fragaria sp var. Cambridge Favourite). J. Hort. Sci., 41:57-64.
- Hughes, M., Martin, L.W. and Black, P.J., 1978. Mycorrhizal influence on the nutrition of strawberries. J. Am. Soc. Hort. Sci., 103:179-181.
- Iqbal, S.H. and Qureshi, K.S., 1976. The influence of mixed sowing (cereals and crucifer) and crop rotation on the development of mycorrhiza and subsequent growth of crops under field condition. Biologia, 22:287-298.
- Iqbal, S.H. and Qureshi, K.S., 1977. The effect of vesicular-arbuscular mycorrhizal association on growth of sunflower (Helianthus annuus) under field conditions. Biologia, 23:189-196.
- Issac, R.A. and Kerber, J.D., 1971. Atomic absorption and flame photometry. Techniques and uses in soils, plants and water analysis. In "Instrumental Methods for Analysis of Soils and Plant Tissue" (Ed. L.M. Walsh). Soil Sci. Soc. Am., Inc. Madison, Wisconsin, U.S.A., pp.17-37.
- Jackson, M.L., 1967. Soil Chemical Analysis. Prentice Hall of India Pvt. Ltd., New Delhi.
- Janos, D.P., 1977. Vesicular-arbuscular mycorrhiza affect the growth of Bactris griseopices.
- Khan, A.G., 1972. The effect of vesicular-arbuscular mycorrhizal association on growth of cereals. I. Effect on maize growth. New Phytol., 71:613-619.

Khan, A.G., 1974. The occurrence of mycorrhizas in halophytes, hydrophytes and xerophytes and of endogone spores in adjacent soils. J. Gen. Microbiol., 81:7-14.

Khan, A.G., 1975. The effect of VA mycorrhizal association on growth of cereals. II. Effect on wheat growth. Ann. Appl. Biol., 80:27-36.

Khan, A.G., 1978. VA mycorrhizas in plants colonizing black wastes from bituminous coal mines in the Illawara region of New South Wales. New Phytol., 81:53-63.

Kleinschmidt, G.D. and Gerdemann, J.W., 1972. Relationship of endomycorrhizas to citrus stunting in fumigated soils. Phytopathology, 62:769.

Koucheki, H.K. and Read, D.J., 1976. VA mycorrhiza in natural systems. II. Relationship between infection and growth in Festula ovina. New Phytol., 77:655-666.

LaRue, J.H., Maclean, W.D. and Peakcock, W.L., 1975. Mycorrhizal fungi and peach nursery nutrition. Calif. Agric.,

Link, H.F., 1809, Observatione in ordine plantarum naturales. Ges. Naturforsch. Freunde. Berlin, 3: 3-42.

Matare, R. and Matting, M., 1975. status of Avocado seedlings on rootrot caused by Phytophthora cinnamomi. Pl. Soil., 49:433-435.

Menge, J.A., Johnson, E.L.V. and Platt, R.G., 1978. Mycorrhizal dependency of several citrus cultivars under 3 nutrient regimes. New Phytol., 81:553-559.

Menge, J.A., Steirle, D., Bagyaraj, D.J., Johnson, E.L.V. and Leonard, R.D., 1978. Phosphorus concentration in plants responsible for inhibition of mycorrhizal infection. New Phytol., 80:575-578.

Menge, J.A., Davis, R.M., Johnson, E.L.V. and Zentmeyer, G.A., 1978c. Mycorrhizal fungi increases growth and reduce transplant injury in avocados. Calif. Agric., 32:6-7.

- ✓ MoJaloi, H. and Weeds, S.B., 1978. Weathering of micas by mycorrhizal soybean plants. Soil Sci. Soc. Am. J., 42:367-372.
- ✓ Mosse, B., 1957. Growth and chemical composition of mycorrhizal and non-mycorrhizal apples. Nature, 179:922-924.
- ✓ Mosse, B., 1972a. The influence of soil type and Endogone strain on the growth of mycorrhizal plants in phosphate deficient soils. Rev. Ecol. Biol. Sol., 9:529-537.
- ✓ Mosse, B., 1972b. Effect of different Endogone strain on growth of Paspalum notatum. Nature, 239:221-223.
- ✓ Mosse, B., 1973. Advances in the study of vesicular-arbuscular mycorrhiza. Ann Rev. Phytopath., 11:171-196.
- ✓ Mosse, B., 1977. Plant growth responses to VA mycorrhiza. X. Responses of Stylosanthes and maize to inoculation in unsterile soils. New Phytol., 78:277-288.
- ✓ Mosse, B., Powell, C.L. and Hayman, D.S., 1976. Plant growth responses to VA mycorrhiza. IX. Interaction between VA mycorrhiza, rock phosphate and symbiotic nitrogen fixation. New Phytol., 76:331-342.
- ✓ Nicolson, J.H., 1959. Mycorrhizas in the Graminae. I. VA endophytes with special reference to the external phase. Trans. Brit. Mycol. Soc., 42:421-438.
- ✓ Oswald, E.T. and Ferchau, H.A., 1968. Bacterial association of coniferous mycorrhizae. Pl. Soil, 28:187.
- ✓ Phillips, J.M. and Hayman, D.S., 1970. Improved procedure for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Trans. Brit. Mycol. Soc., 55:158-161.

Pichot, J. and TrungBinh, 1976. Action of endomycorrhiza on growth and phosphorus nutrition in Agrotis in pots and on isotopically exchangeable phosphorus in soil (Lvalue). Agrin. Trop., 31:375-378.

Powell, C.L., 1975a. Potassium Uptake by Endotropic Mycorrhizas. In: "Endomycorrhizas" (Ed. F.E.Sanders, B.Mosse and P.B. Tinker). Academic Press, London, pp.461-468.

Powell, C.L., 1975b. Plant growth responses to vesicular-arbuscular mycorrhiza. VII. Uptake of P by onion and clover infected with different Endogone spore types in ^{32}P labelled soils. New Phytol., 75:563-566.

Powell, C.L., 1976. Mycorrhizal fungi stimulate clover growth in New Zealand hill country soils. Nature, 264:436-438.

Powell, C.L., 1977. Mycorrhizas in hill country soils. III. Effect of inoculation on clover growth in unsterile soils. N.Z. J. Agric. Res., 20:343-348.

Powell, C.L. and Daniel, J., 1978. Mycorrhizal fungi stimulate uptake of soluble and insoluble phosphate fertilizer from a phosphate deficient soils. New Phytol., 80:351-358.

Powell, C.L. and Sithamparanathan, J., 1978. Mycorrhizas in hill country soils. IV. Infection rate in grass and legume species by indigenous mycorrhizal fungi under field conditions. N.Z. J. Agric. Res., 20:489-494.

Raj, J., 1980. Role of soil microorganisms in the release of soil phosphate to plants. Ph.D. Thesis (Unpublished), University of Agricultural Sciences, Bangalore.

Rhodes, L.H. and Gerdemann, J.W., 1976. Effect of vesicular-arbuscular mycorrhizas on sulphate uptake by onion plants. (Abstract) Proc. Am. Phytopath. Soc., 3:274-275.

Rhodes, L.H. and Gerdemann, J.W., 1978. 1978. Influence of phosphorus nutrition on sulphur uptake by VA mycorrhiza on onions. Soil Biol. Biochem., 10:361-364.

- Ross, J.P., 1971. Effect of phosphate fertilization on yield of mycorrhizal and non-mycorrhizal soybean. Phytopathology, 61:1400-1403.
- Ross, J.P. and Harper, J.A., 1970. Effect of Endogone mycorrhizae on soybean yield. Phytopathology, 60:1552-1556.
- Ross, J.P. and Gilliam, J.H., 1973. Division S-4 soil fertility and plant nutrition. Effect of Endogone mycorrhiza on phosphorus uptake by soybean from inorganic phosphates. Soil Sci. Soc. Am. Proc., 37:237-239.
- Safir, G.R., Boyer, J.S. and Gerdemann, J.W., 1971. Mycorrhizal enhancement of water transport in soybean. Science, 172:581-583.
- Sanni, S.O., 1976a. VA mycorrhiza in some Nigerian soils and their effect on the growth of cowpea, tomato and maize. New Phytol., 77:667-671.
- Sanni, S.O., 1976b. VA mycorrhiza in some Nigerian soils. The effect of Cigaspora gigantea on the growth of rice. New Phytol., 77:673-674.
- Schenck, N.C. and Heison, K., 1973. Response of nodulating and non-nodulating soybeans to a species of Endogone mycorrhiza. Agron. J., 65:849-850.
- Schonbeck, F. and Dehne, H.W., 1977. Damage to mycorrhizal and non-mycorrhizal cotton seedlings by Thielaviopsis basicola. Pl. Dis. Reprtr., 61:266-267.
- Schonbeck, F., 1978. Effect of the endotrophic mycorrhiza on disease resistance of higher plants. Z. Pflanzenker Pflanzenschutz, 85:191-196.
- Sikora, R.A., 1978. Effect of the endotrophic mycorrhiza on the host parasite relationship Meloidogyne incognita in tomato. Z. Pflanzenker Pflanzenschutz, 85:197-202.

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- Sondergaard, M. and Laegard, S., 1977. VA mycorrhiza in some aquatic crop plants. Nature, 268:232-233.
- Sperber, J.J., 1957. The incidence of apatite solubilizing organism in the rhizosphere and soil. Aust. J. Agric. Res., 9:778-781.
- Sutton, J.C. and Sheppard, B.R., 1976. Aggregation of sand dune soil by endomycorrhizal fungi. Can. J. Bot., 54:326-333.
- Swaminathan, K. and Verma, B.C., 1977. Symbiotic effect of vesicular-arbuscular mycorrhizal fungi on the phosphate nutrition of potatoes. Proc. Ind. Acad. Sci., B25:310-318.
- Timmer, L.W. and Leyden, R.F., 1978. Stunting of citrus in Texas and its correction by phosphorus fertilization and inoculation with mycorrhizal fungi. J. Am. Soc. Hort. Sci., 103:533-537.
- Trinick, M.J., 1977. Vesicular-arbuscular mycorrhizal infection and soil phosphorus utilization in Lupinus sp. New Phytol., 78:297-304.

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