Effect of Nitrogen and Phosphorus Application, and Rhizobium and Mycorrhiza Inoculation on the Performance of Soybean (*Glycine max.* L. merr) in a humic *Nitisol*

By

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DECLARATION

I Ndivo, declare that this thesis is my original work and has not been submitted for a degree in any university.

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This thesis has been submitted for examination with my approval as university supervisor.

Signed .....

Dr. Amos Musandu

Date 28/07/03

DEDICATION

To my late husband (Martin Mutua), children (Ndunge, Muendo, Musyoni) and parents.

2005/65515

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ABSTRACT

The demand for soybean grain and other soybean products in Kenya outstrips supply. Low soil fertility has been identified as the major cause of poor soybean yields in Kenya. Soybean production costs could be reduced by partial substitution of inorganic N and P fertilizers with biologically fixed N and enhanced P uptake by use of mycorrhizas. A greenhouse study was conducted to determine the effects of N and P and Rhizobium and mycorrhiza inoculation on the performance of soybean (Glycine max) grown in a humic Nitisol from Kaguru Farmers Training Centre in Meru District of Kenya. The four factors: Nitrogen, Phosphorus and Rhizobium and Mychorrhiza inoculations each at two levels; were combined to give 16 treatments which were allocated to pots in the greenhouse in a randomised complete block design with 4 replications. The results showed that P and mycorrhiza inoculation did not individually affect any of the measured variables significantly. However, significant ($p=0.05$) interaction effects were observed between N by P for the dry weights of nodules, shoot, root and seed, and between Rhizobium inoculation by mycorrhiza inoculation for seed and nodule numbers. Rhizobium inoculation significantly ($p=0.05$) increased the nodule number and the root contents of N and P, while N significantly ($p=0.05$) increased the seed number and the dry weights of seeds and shoots. The observed responses, showed that the factors: N, P and Rhizobium and mycorrhiza inoculations influence the production of soybeans in humic Nitisols and should therefore be further studied in field experiments in order to determine the optimum rates for increased soybean production in different soil types of Kenya.

CHAPTER ONE

1.0 INTRODUCTION

1.1 General background to the Study

Soybean production is a new concept in Kenyan farms and households. Small scale soybean growing has been practiced since the early sixties in Nyanza and Western Provinces (Njuguna, 1985). Soybean was also grown as a fodder crop by large scale farmers on a limited scale in Trans Nzoia, Uasin Gishu, Nakuru and Laikipia districts. In 1982, the East African Industries Company in Kenya showed interest in soybean mainly for oil extraction. They successfully introduced commercial soybean production to small scale farmers in western Kenya.

Protein malnutrition is a widespread form of deficiency disease today (MOA, 1996). Soybean seed contains considerably high nutritional quality protein (40 %) and therefore assumes an important role as a potential source of low cost, and readily available protein in a developing country such as Kenya where animal protein is in short supply and quite expensive for low income people. Soybean is also an important oil crop and is the most widely cultivated oilseed crop in the world (Iruria *et al.*, 1997). There is an increased demand for the soybean grain in many industries in Kenya. According to Ephanto (1994), soybean production in the country was 1000 metric tons while the demand was 30,000 metric tons. The country therefore imported about 99.5 % of its soybean requirement. The demand is projected at 150,000 metric tons by 2010. Kenyan farmers need to intensify soybean production per unit area.

Soybean variety evaluation has been done in different parts of Kenya and the possibility of a large scale production has been demonstrated in Kisii District (Thairu and Shakoor, 1985). The varieties developed and now being used in Kenya include; Duiker, E A 13600, Nyala, Gazelle, Sable and SCSI. The variety Nyala is largely grown in marginal rainfall areas while varieties Duiker, Sable and Gazelle are recommended for the warm and moderate temperature areas found in Nyanza and Western Provinces, in Kenya.

Low soil fertility has been identified as one of the constraints to crop production in Kenya (Karanja and Kibunja, 1989). The problem currently facing the soybean industry in Kenya is how to increase its yield to meet the demand. Production costs have been on the increase especially the use of chemical inputs such as nitrogen and phosphorus fertilizers leading to use of sub-optimal rates of fertilizers by farmers. Other constraints to soybean production include lack of production technologies, poor seed quality, limited research, limited on-farm utilization, lack of awareness at producer and consumer levels, and the secondary status the crop is given in the crop rotation system (MoA, 1987).

Nitrogen constitutes about 78 % of the atmosphere, yet it is one of the elements which, in addition to P, often limit agricultural production in tropical soils which are generally highly weathered and deficient of both elements (Karanja and Kibunja, 1989). Mikhail (1976), observed that soybean inoculation with Abuscular Mycorrhiza Fungi (AMF) increased P-uptake and that it also plays a role in the uptake of other plant nutrients as well as aiding in the symbiotic nitrogen fixation by legumes, biological control of root pathogens and drought resistance of plants.

Meru District is one of the districts in Kenya experiencing marginal rainfall in most areas. The people of Meru rely on agriculture for their livelihood. The main sources of vegetable proteins in Meru District include beans, pigeon peas, cowpeas, green grams and dolichos. Soybean are relatively more drought tolerant compared to other grain legumes. The high value of soybean both as vegetable protein source and as an oilseed crop necessitated the need to introduce soybean to the Meru District cropping systems. However, due to low soil fertility in most soils of Meru District, it is important that the nutritional requirement of soybean be determined.

1.2 Statement of the Problem

The demand for soybean grain is on the increase in Kenya both at the domestic and the industrial level. Kenya's demand for vegetable oil and protein rich foods continues to increase due to a fast growing population estimated at 3.5 % per annum. However, there is a decline in the production of soybean which could be attributed to high production costs and hence the use of suboptimal rates of fertilizers.

1.3 Justification of the Study

In Kenya, the demand for soybean and soybean products outstrips supply. Low soil fertility has been identified as the major cause of poor soybean yields in Kenya. There is therefore a need to investigate some of the soil fertility factors which would be expected to influence the production of soybean. The exploratory experiment done in the greenhouse is expected to give information upon which future field trials will be based in order to determine the optimum rates of the factors for optimum production of soybean by resource poor farmers.

1.4 Objectives

The general broad objective of this study was to investigate some soil fertility factors which influence soybean performance in a humic *Nitisol.* The specific objective were:

1) To isolate, screen rhizobial strains for their N-fixing potential, and determine the effect of rhizobial inoculation on the performance of soybean.

2) To isolate, screen arbuscular mycorrhizal (AM) strains for their p-uptake efficiency and determine the effect of AM inoculation on the efficiency of N and P uptake in soybean.

3) To determine the effect of fertilizer application on the performance of soybean.

1.5 Hypotheses

The hypotheses postulated for this study were:

1) The effectiveness of the *Rhizobia*l strains in the soil is the same as that of the commercial strain.

2) The effectiveness of the mycorrhizal strains in the soil is the same as that of the commercial strain.

3) Inoculation of soybean with Rhizobia and mycorrhiza will have no effect on its performance.

4) Application of fertilizer N and P will have no effect on the performance of soybean.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Soybean Production

2.1.1 Trends in Production

According to FAO (1990), 88% of world soybean production originates from four countries; the United States, Brazil, the People's Republic of China and Argentina. Soybean yields in Africa vary from 158 kg/ha in Tanzania, 341 kg/ha in Nigeria and 800 kg/ha in Uganda to 2658 kg/ha in Zimbabwe. According to the Republic of Kenya (1987), total soybean production in Kenya in 1987 was 4,000 metric tonnes. In subsequent years, production declined due to lack of a streamlined marketing system but the situation improved in 1994 with an increased demand for vegetable oil with an increasing population. By 1997, the production was almost 3000 metric tonnes. The production was mainly from the Kakamega, Bungoma, and Meru districts.

Soybean has been fitted into different cropping systems. It has been planted in pure stands continuously or in rotation, or as a intercrop. When grown in rotation, it is used as a break crop by small scale farmers who presently grow maize continuously in pure stands. As an inter-crop, the soybean is planted in the same row with the second crop (usually maize or sorghum) or they can be strip-cropped.

2.1.2 Soybean Agronomy and Management

According to the MoA (1996), soybean can be grown in a wide range of agro-ecological zones. It performs optimally in the temperature range of 25 to 32 °C, though the requirement depends on the variety. It can be grown from sea level to over

1800 metres above sea level. Rainfall requirement is between 350 to 700 mm per annum but the crop grows best in a humid climate with plenty of rain during the growing period. The maturity period is 110 to 130 days depending on the variety and climate. The soils should be fertile and well drained with a high water holding capacity and a pH range of 5.5-6.8.

The land intended for soybean growing should be cleared of the preceding crop or any other vegetation cover after which ploughing is done (MoA, 1996). The residues should be incorporated into the soil and allowed to decompose before sowing. The seeds for planting should preferably have a germination percentage greater than 75 %. Early planting is recommended with an inter-row spacing of either 45 or 60 cm and intra-row spacing of 5 cm. The crop has been shown to respond to direct fertilization when the soil fertility is low. In low N soils, 20 to 40 kg N/ha or 5 t/ha of well rotted manure is applied at planting. Although the soybean can make use of atmospheric nitrogen, inoculation with the right type of *Rhizobium* is important especially in fields where soybean has not been grown before.

2.1.3 Role of Soybean in Nutrition and in Industry

Soybean is a rich source of protein, fat, energy, vitamins and minerals and it has become the standard to which other vegetable food ingredients are compared (Nwokolo, 1996).

Soybean has double the protein and amino acids present in most dry beans, lentils, peas and cowpeas used as human food (MoA, 1996). It supplies essential amino acids such as lysine that are not present in sufficient quantities in staple cereal crops. A

balanced diet can hence be maintained even in the absence of a high intake of animal products due to economic or religious reasons. In situations where the majority of the population is strictly vegetarian, soybean can provide upto 70 % of dietary protein intake (Smart, 1990).

The MoA (1996) reported that except when soybean is fried as nuts, it is not eaten alone. The soybean are usually mixed with other grain cereals and then cooked. Soybean may be consumed whole or processed into various products, like flour, grits, milk, okara, oil, soya protein concentrates, soya protein isolates and textured soya proteins. These products are very versatile food ingredients, primarily due to their functional properties which serve to enhance the value of finished foods. These can be incorporated into a wide range of Kenyan dishes like *githeri*, *ugali*, *porridge*, *chapati* etc. According to Ephanto (1994), some of the soybean products made in Kenya include: pure extruded soya flour, soya oil, soya meal, soya cake, soya biscuits, soya/meat sausages, soya cheese (tofu), soya milk, and soya flour blends like soya/millets, soya/sorghum and soya/corn.

Ephanto (1994) observed increased utilization of soybean at the ordinary households level especially by the Seventh Day Adventists who consume the seeds green, dry or sprouted. The green seeds are used as vegetables. In schools, hospitals and prisons, soybean flour is used in supplementary feeding. Soybean protein is used in weaning formulas for infants and in special supplement formulation for undernourished preschool and school-age children. For full-term infants, it has been noted that whereas mother's milk is best for infants, artificial infant formulas based on cow's milk and soybean protein permit optimum growth and development for term-sized

infants (Leleiko, 1981). Despite the importance of soybean in diets, Bressani (1981) pointed out that in human beings, animal proteins have a higher digestibility coefficient than soybean protein in general, although the more the degree of processing of soybean products, the higher the digestibility coefficient.

Soybean is also an important feed for livestock. The use of soybean meal in animal feeds accounts for almost 98 % of the processed products (Ephanto,1994). The utilization of the defatted meal products such as soybean meal and soy protein concentrates is an important feed ingredient because of its high quality protein (44 – 47 %). There has also been a widespread utilization of the soybean meal in poultry after the realization that heat inactivation of antinutritional factors in raw beans improves its feed efficiency.

Industrially, refined soybean oil is used in the manufacture of mayonnaise, salad dressings, margarine and shortenings complexing. The lecithin, a by product of vegetable oil refining is used in food wetting, colloidal dispersion, lubrication and release, control of crystallization and starch complexing. The oil is also used in paints and varnishes, surface coatings, printing inks, soaps, greases, lubricants, emulsifiers, solvents, biodegradable plastics and biodiesel fuels. In the phamaceutical industry, the soybean provides a cheap source of nitrogen and oil for the culture medium (Ephanto, 1994).

2.1.4 Role of Soybean in Soil Fertility

Nitrogen fixation by legumes is an ancient source of N (Peoples *et al.*, 1995), and it can contribute at least 7 million metric tonnes of N per year worldwide. The values

estimated for various leguminous crops and pasture species fall in the range of 200 to 300 kg N/ha (Peoples *et al.*, 1995). A soybean crop that is well nodulated can be independent of inorganic fertilizer N. Bergersen *et al.* (1985) found that a crop of soybean fixes 234 kg N/ha while Rennie *et al.* (1988) recorded a value of 643 kg N/ha. Such crops may not respond to N fertilization (Jat *et al.*, 1998). Due to the high nitrogen fixing ability by the soybean, its cultivation is of great economic importance as production costs are low.

The soybean crop intercropped with a cereal, can improve the N economy of the cereal both by contributing N to the soil for uptake by the cereal (nitrogen transfer) or the soybean may remove less N than if the cereal was grown in a pure stand, that is, sparing effect (Vallis *et al.*, 1967) which means that if the legume removes only a small amount of the soil N, more is available for use by the companion crop. Sain and Jauregui (1993) found that the N economic optimum was lower for wheat after soybean than for wheat after maize. Legume/non-legume inter-cropping can actually increase the level of symbiotic nitrogen fixation by the legume and the total N uptake by the joint components of the system, as demonstrated by Martin *et al.* (1991) in a soybean-maize inter-crop. In lowland rice-based cropping systems, it has been found that the high requirement for N fertilizer can be replaced by fixed N from rotational crops of food legumes such as cowpea, mungbean, pigeonpea, groundnut and soybean (George *et al.*, 1992). The Bradyrhizobium for soybean can tolerate flooding for prolonged periods (Roughley *et al.*, 1995). Kavamahanga *et al.* (1995) evaluated the effects of inter-cropping on grain sorghum hybrid Agritec with common beans (*Phaseolus vulgaris*) cv. Dark Red and soybean (*Glycine max* L.) cv. Essex showed

that grain sorghum yield when inter-cropped with common beans and soybean was higher than the sorghum crop in mono-culture.

Soybean residues can be used as organic manures due to their large content of N and also because this N is more likely to become readily available for uptake by other plants than N in many other crop residues. Legumes have a C:N ratio of less than 30:1 and therefore are likely to decompose more rapidly with a net mineralization of N occuring right from the time of application (Frankenberger and Abdelmagid, 1985). The main residues from soybean are mainly from roots and nodules and any leaves which fall to the soil during the growth of the crop.

Legumes, including soybean, can also be used as green manures where the legume crop is ploughed into the soil whilst the plant material is still green. Legumes used as green manures supply N to the soil, and contribute to the build up of soil organic matter which lowers the soil bulk density, increases soil microbial biomass, improves the soil structure and hence root penetration, nutrient retention, water holding capacity and water infiltration (Harris *et al.*, 1994). With long term soil incorporation of green manures, mineralization of organic N might be sufficient to adequately sustain wheat yields. This was observed by Badaruddin and Meyer (1990) who found that grain yields following legume green manures were similar to fertilized wheat in 3 out of 4 years. McGuire *et al.* (1998) found that the N supplied by a legume used as green manure was sufficient to obtain grain yields higher than those of the fertilized plots. The main problem of using legume residues as green manures is to synchronize nutrient release from organic matter with the optimum timing for uptake by crops (Woomer and Ingram, 1990).

In many parts of the tropics, land is left fallow between cropping seasons for grazing of cattle (Humphreys, 1991). MacColl, (1990) showed that the yield of maize was improved by planting legumes than leaving the field fallow or planting non-legume crops while Huang *et al.* (1996) observed that in irrigated or high rainfall production regions, soybean-maize rotations have high Nitrogen Use Efficiency and can reduce the amount of residual N available for leaching when compared with continuous corn.

Crop rotation has been recommended for control of plant diseases and pests although the rotation crop has not always been economic for the farmer (Baker and Cook, 1974). The soybean has been found to be of economic importance when included in rotations as it reduces diseases and insects in both itself and other crops (Hu *et al.* 1983).

2.2 *Rhizobia*l Microsymbiont

2.2.1 *Rhizobia* Taxonomy

The most important criterion used in early classification of *Rhizobia* was host range. Fred *et al.* (1932) classified *Rhizobia* into 6 groups with specific names being derived from homologous hosts (in brackets): *Rhizobium leguminosarum* (pea), *Rhizobium trifolii* (clover), *Rhizobium phaseoli* (bean), *Rhizobium meliloti* (alfalfa), *Rhizobium japonicum* (soybean) and *Rhizobium lupinii* (lupin). This classification system remained more or less unchanged until the early 1980s. Woese (1987) used bacterial morphology but the much simpler morphologies of bacteria were of little use in establishing phylogeny and hence little progress in defining the taxonomy.

The first major step in *Rhizobial* classification of recent times was the division of *Rhizobia* into slow- and fast-growing species and the naming of the former as a new genus, *Bradyrhizobium* (Jordan, 1982). Since then classification of *Rhizobia* has greatly advanced as a consequence of new and improved techniques. Increasing use of molecular methods has enabled comparisons among *Rhizobia* and between *Rhizobia* and other bacteria.

The polyphasic approach is increasingly being used in classifying and naming new bacterial genera and species (Vandamme *et al.*, 1996). De Lajudie *et al.* (1994) used the polyphasic approach to describe and name two species of *Rhizobium* isolated from acacia and sesbania. Jarvis *et al.* (1982) used plant specificity, soluble protein pattern and DNA sequence homology to illustrate differences between *Rhizobium loti* and other fast growing species. Since then, 16s rRNA sequencing, protein electrophoresis (SDS-PAGE), biolog metabolic profiling, genetic hybridisation (Dupuy *et al.* 1994), phenotypic and genotypic characters (Chen *et al.* 1988), restriction fragment length and polymorphism (RFLP) analysis (Eardly *et al.* 1990) have revealed other species closely related to *Rhizobium loti*.

The family *Rhizobiaceae* lies within the alpha subdivision of the Proteobacteria, which also includes the genus Agrobacterium (Young *et al.*, 1991). *Rhizobium* is currently divided into 6 genera (*Allorhizobium, Azorhizobium, Bradyrhizobium, Mesorhizobium, Rhizobium, Sinorhizobium*). The *Rhizobia* species that have been known to nodulate the soybean are the *Bradyrhizobium* species (*B. Elkanii.*, *B. Japonicum* and *B.liaoningense*) and *Sinorhizobium fredii*.

2.2.2 *Rhizobia* Symbiosis

The symbiotic association of *Bradyrhizobium japonicum* and soybean shows great complexity in the infection process, nodule development, and host-strain specificities that determine the N_2-fixing capacity in the nodules (Vest *et al.*, 1973). Vest *et al.* (1973) attributed the distinct feature of the symbiosis to the appearance of the N_2-fixing enzyme nitrogenase in the *Bradyrhizobium* bacteria which neither the soybean plant nor the *Rhizobia* alone can produce, but only the symbiosis can induce the synthesis of nitrogenase. Dilworth and Parker (1969) suggested that genetic information for the nitrogenase system resides partly in the plant and partly in the *Bradyrhizobium* bacterium. According to Vest *et al.* (1973), complementation between the protein parts synthesized by the plant and by the *Bradyrhizobium* bacterium could explain the degree of effectiveness found in symbiosis of one plant with several *Bradyrhizobium japonicum* strains or one strain with several different plants.

The infection of a legume by *Rhizobium* is an intimate association which depends on the total specific mutual recognition for effective nitrogen fixation. Steps in establishing the symbiosis involves colonization of the rhizosphere, entrance (through the root hair or during the emergence of lateral roots or otherwise) resulting in the formation of infection threads, commencement of a persisting nodule meristem, release of *Rhizobia* from the infection thread, their multiplication within membrane envelopes of the nodule host cell, conversion to nodule bacteroids, the establishment and continuance of a shared metabolism between plant and bacterium (Vincent, 1982).

Under favourable temperature and moisture conditions, soybean seedlings have visible nodules at about 9 days after planting (Bergersen, 1958). Soybean plants grown in sand culture showed that the number of nodules increased from 40 days after sowing onwards, reaching peak values at early pod filling, 100 days after sowing (El-Kady *et al.*, 1982). When temperatures and moisture conditions are favourable N_2-fixation in the nodules begins at 14 days after sowing (Hardy *et al.*, 1971). According to Imsande and Ralston (1981) N_2-fixation increased 100 fold during a 3 week growth reaching 50 mol C_2H_4/hr. Awai (1981) reported nitrogenase activity of 12.057 mol C_2H_4 per plant/hr for soybean plants inoculated with SN2(S) strain of *Bradyrhizobium japonicum* at 50 days after sowing. On the contrary, Chiu *et al.* (1990) observed that the N_2-fixation up to 36 days after sowing was negligible while Ruschel *et al.* (1979) showed that by 35 and 55 days after sowing, hardly any N_2-fixation had taken place. This contradiction on the time of onset of N_2-fixation is probably due to varietal, temperature and moisture differences prevailing during growth period.

Not only do soybean genotypes differ in their susceptibility to nodulation but *Bradyrhizobium japonicum* strains also differ in their ability to induce nodulation and to fix nitrogen. Inoculation with single strains or mixtures of *Bradyrhizobium japonicum* led to genotype x culture interactions for different characteristics indicating that *Bradyrhizobium* strains have specifities for different varieties (Khurana, 1986). In pot and field trials conducted by Liang *et al.* (1991) strain 61A71 was found to have a wide adaptability to cultivars and environments while strain RB-9 was suited to early maturity cultivars and strain E45 to medium maturity cultivars. Strain 123 was ineffective at all locations tested using cv. Lee while strain 135 was

very poor and did not form nodules at one of the locations (Al-Rashidi and Damirgi, 1978).

2.2.3 *Rhizobia* Inoculation

The role of legume inoculant is to provide sufficient and effective root nodule bacteria to allow the legume host to form nodules, which at least improve and at best maximise nitrogen fixation. This improvement is reflected in better crop or forage yield, and a reduction in the plants dependence on available soil N. Responses to inoculation are expected in soils in which the specific *Rhizobia* are absent or sparse, where indigenous *Rhizobia* are ineffective or partially effective in N_2-fixation (Vincent, 1965). Volkora and Chernova (1988) observed that the effect of inoculation decreased as the population of indigenous *Rhizobia* in the soil gradually increased. Soybean is a new crop in the Kenya farming systems and it is therefore necessary that the seeds be inoculated with *Bradyrhizobium* bacteria where no soybean crop has previously been grown in the field (Kang, 1975). Semu and Hume (1979) showed that inoculation increased seed yields only when soybean were introduced into new areas. Ham *et al.* (1971) and Nelson *et al* . (1978) revealed little or no yield advantage for inoculated over uninoculated plots using different commercial inoculants on several established soybean production areas in Minnesota. The general lack of yield response to inoculation was attributed to the competitiveness of introduced strains and the poor recovery of the nodules with 5 to 10 % recovery being common (Ham *et al.*, 1971).

Chilimba (2000) found that beans nodulated freely with the indigenous strains of *Rhizobia* in the soil. Karanja *et al.* (2000) observed the presence of nodules in the

plants sampled from the control treatment. Gitonga *et al.* (2000) found that inoculation of pigeon peas was not useful since there were adequate populations of pigeon pea *Rhizobia* in the soils of semi-arid areas of Kenya where the crop is commonly grown. The presence of nodules in uninoculated plants is an indicator of the probable presence of effective indigenous *Rhizobial* populations. In contrast, legumes introduced to an area for the first time show poor nodulation and needed inoculation with an effective rhizobial strain (Anyango *et al.* 2000).

Increases in yield of soybean due to *Bradyrhizobium* inoculation have been reported (Kucey *et al.*, 1988; Chowhury, 1977). Olufajo (1990) reported increased seed yield and number of pods per plant of inoculated promiscuous soybean in 1 out of 3 years. In field trials, dry matter accumulation at all growth stages were higher in the inoculated plants than in the control (Liang *et al.*, 1991). According to Essa and Dulaimi (1985), inoculated plants were taller and produced higher root dry matter weight than uninoculated plants. Inoculation of soybean in two field experiments increased plant dry matter weight, seed yield and 100 seed weight in all six cultivars used (Ibrahim and Mahmoud, 1989). They also noted that inoculation had no effect on K, Na, and Ca contents of the plant tops but decreased the plant P content. In seeds, the P content was increased and Ca content decreased with *Rhizobium* inoculation. Inoculated plants were capable of absorbing adequate amounts of micronutrients.

The benefits of *Rhizobium* inoculation have been reported in other legume and non-legume crops. Inoculation of clusterbean significantly increased nodulation, shoot dry weight, seed production and fixed N (Abdelgani *et al.*, 2000). Matiru *et al.* (2000)

realized significant increase in shoot fresh/dry weight, root length and root tissue P concentration of inoculated sorghum over the uninoculated treatment. The test strains were shown to produce active amounts of the growth promoting molecule, Indole Acetic Acid.

2.2.4 *Rhizobia* and Fertilizer Interactions

The level of P supply affects nodulation and nodule growth much more than root or shoot growth (Cassman *et al.*, 1980). In soybean, the minimum concentration of P required for nodulation is ~0.5g/litre in the external solution. In particular, an increase in the concentration from 200 to 500 g/litre leads to a relatively greater increase in the nodule dry weight than in the shoot and especially, the root dry weight.

When legumes dependent on symbiotic nitrogen recieve an inadequate supply of P, they suffer from N deficiency. Under these conditions, N-deficiency symptoms are dominant and can be alleviated by the application of P fertilizers. Correspondingly, in soybean grown in a soil low in available P, a similar yield increase has been obtained with mineral fertilizer supply of either 90 kg P or 120 kg N per ha (Dadson and Acquaah, 1984). The high specific P requirement for nodulation is responsible, at least in part, for the interactions in certain legume species between the infection of roots with mycorrhizae and nodulation. With plants growing in P-deficient soil, nodulation could be induced either by the application of P fertilizer or by root infection with mycorrhizal strains.

The N status of soil infuences the quantity of N_2-fixed. An average of 317 kg N/ha was fixed when soybean were planted on infertile soil while about 123 kg N/ha was

fixed on fertile soil (Herridge, 1981). For plants grown in a greenhouse on nitrogen deficient soil, N estimates showed a high level of N_2-fixation while results of a field experiment conducted on a fertile soil showed a modest level of N_2-fixation using the N-difference method (Kohl *et al.*, 1980).

Haque *et al.* (1980) found that uninoculated soybean receiving no N showed deficiency symptoms throughout the field trials and inoculated but unfertilized plants showed N deficiency during flowering owing to poor nodulation. Yield increases due to inoculation have been improved by use of fertilizer N. Abdalla *et al.* (1985) found that with inoculation, dry matter production of soybean increased with increasing N applications up to 50 kg N/ha while dry matter yield of uninoculated plants was highest with 36 kg N/ha. In some cases, inoculation alone has produced soybean yields equivalent to or higher than those produced by high rates of nitrogen. Keya *et al.* (1981) found increased seed yield of soybean with inoculation over application of 90kgN/ha

Several workers have demonstrated the possibility of obtaining high yields of soybean by using nitrogen in conjunction with *Bradyrhizobium* inoculation. Mahmoud *et al.* (1979) reported that inoculation plus 75 kg N/ha resulted in higher nodulation and seed yields than 100 kg N/ha applied alone. With 120 kg N/ha, Bishnoi and Dutt (1983) reported higher dry matter, seed size and seed yields in inoculated treatments compared with non-inoculated treatments recieving same nitrogen rates. According to Dubetz and Rennie (1981), the mean yields of inoculated soybean was higher than for non-inoculated with application of 90 kg N/ha.

Inverse relationship of fertilizer N and inoculation has been reported in several experiments. Application of 100 kg N/ha decreased the yields of nodulating soybean (Vasilas and Ham, 1985). Nitrogen fertilizer at 120 kg N/ha in conjunction with inoculation increased the yield of green and dry matter but decreased the activity of symbiotic N_2 fixation and yield of soybean (Karyagin, 1980). Tracer experiments with $^{15}NH_4$-N in pot trials showed that as fertilizer N was increased, absorption of fixed N from the atmosphere over a period of 40 days decreased (Yoshida, 1979). Allos and Bartholomew (1959) clarified the extent to which N_2 fixation can be reduced by application of N fertilizer. Low rates of mineral N under N-deficient conditions increased growth and N absorption more than they diminished N_2 -fixation in solution cultures. When N was applied at the rate of 10-15 % of the total soybean requirement, N_2 -fixation contributed 83 % of the total N. This percentage decreased to 42 when more N was supplied than absorbed by the crop.

2.3 Arbuscular Mycorrhiza Fungi Microsymbiont

2.3.1 Arbuscular Mycorrhiza Fungi Taxonomy

The taxonomy of Arbuscular Mycorrhiza Fungi (AMF) is largely based on morphological (arrangement, colour, shape, ornamentation) and subcellular (spore wall, spore contents) features of soil-borne asexual spores, spore germination, soil hyphae and associated structures, infection characteristics, developmental patterns and biochemical properties (Morton, 1993). Morphological characters of the mycelium and spores of glomalean organisms contain enough information to hierachically order species in a phylogenetic plant (Morton, 1990) and define taxonomic groups (Morton and Benny, 1990). Approximately 150 species (Schenck and Perez, 1990) have been described based on morphological features. Currently 8 genera of AMF are

recognized and taxonomy is based in part on results of morphological characters of somatic (hyphae, arbuscules, vesicles) and reproductive (resting spores) stages of known arbuscular species plus additional considerations of spore ontogeny and modes of spore germination (Morton and Benny, 1990).

2.3.2 AMF Symbiosis

Arbuscular mycorrhiza fungi are an important group of soil-borne microorganisms that contribute substantially to the establishment, productivity, and longevity of natural or man-made ecosystems (Harley and Smith, 1983). These fungi form obligate mutualistic associations in roots of most terrestrial plant families (Trappe, 1987). The fungi have a wide host range (Gerdemann, 1975). Consequently due to their polycyclic habit and their characteristic of growing through soil to colonise other roots, the fungi may develop an extensive network linking colonies within and among root systems (Francis *et al.* 1986). Fungal isolates probably have the same host range as the species, although the degree of compatibility between fungal isolates and host may vary (Tommerup, 1988). Those plants whose roots resist infection by AMF, such as the Brassicaceae and Lupinus, could be more easily recognised through differences among isolates in their interaction phenotypes, which indicate fungus strain-host cultivar specificity. Although some crop plants may be resistant, in natural ecosystems where resistant and susceptible plants are interspersed, differentials in susceptibility to the fungi may prejudice the outcome of competitive interactions amongst them. Many AMF can simultaneously colonise a root segment (Tommerup, 1988). Hence, hypha-hypha interactions within a root, at the root surface or in the soil may be between hyphae of the same isolate, the same species or different species.

The AMF colonization originates from hyphae arising from soil-borne propagules or from a AMF plant growing nearby. On arrival of the fungal hyphae at the root surface, an appresorium is formed on the cortical cells. The infection units grow as the hyphae spread between and through cells of the cortical root layers (Wilson, 1984). On reaching the inner cortex, the hyphae can grow into cells and , by repeated dichotomic branching, form some tree-like structures called arbuscules. When the internal colonization is spreading, the extramatricial hyphae ramify. These may grow along the root surface, forming more penetration points and also out into the sorrounding soil, to form an extensive tridimensional network of mycelium. When the colonization process is well established, the fungus may form vesicles, which have a storage function. In intracellular colonizations, the fungus is surrounded by the intact host-cell plasmalemma. Therefore, arbuscule formation represents a large surface of cellular content between the two symbionts. This facilitates the interchange of metabolites between host and fungus, hence the arbuscule is the main site of transfer of mineral nutrients from the fungus to the plant.

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Mycorrhizal infection enhances plant growth by increasing nutrient uptake via increases in the absorbing surface area, by mobilizing sparingly available nutrients sources, or by excretion of chelating compounds or ectoenzymes. Mycorrhizal infection may also protect roots from soil pathogens (Perrin, 1990) and thereby increase root growth and nutrient acquisition of the host root. A higher nutrient uptake in mycorrhizal plants can therefore not exclusively be attributed to uptake by the fungal hyphae. For these beneficial effects, mycorrhizal plants have to pay a price – between 10 to 20 % of the net photosynthates are required for formation, maintainance and function of mycorrhizal structures (Jakobsen and Rosendahl, 1990).

Competition between the fungus and the roots for photosynthates is the main factor responsible for the typical larger shoot:root dry weight ratio in favour of the shoots in mycorrhizal plants (Berta *et al.*, 1990). The impact of this shift for host plant growth depends on various soil and environmental factors and in particular on the capacity of the fungus to fully compensate for or even surpass the root functions in nutrient and water uptake.

AMF are obligatorily dependent on host plant carbon for their source of energy. In AMF symbiosis, growth of both partners is influenced by the host plant genotype, AM fungal species and ecotype, and the soil type (Bethlenfalvay *et al.*, 1985) or the percentage of root infection (Vierheilig and Ocampo, 1991). At a given infection level, nutrient uptake efficiency can be affected by the parameters of nutrient exchange at the fungus-root interface (arbuscules), and the extension, viability and transport capacity of the external hyphae.

Mycorrhiza are composed of an external hyphal matrix and an exchange surface between plant and fungus. Nutrients move via the hyphae from one host to the fungus to another host. As the fungus approaches the root, internal hyphae are formed which connect the external absorbing hyphae with the tranfer structures. At the exchange interfaces, nutrients are converted from forms designed for internal transport in one organism to forms more readily utilised and protected in the other e.g. glucose and sucrose sugars readily utilised by the plant, are taken up by the fungus and converted to trehalose and mannitol, sugar forms readily utilised and transported by the fungus (Lewis and Harley, 1965). Phosphorus is transported through the fungus in polyphosphate granules. At the interface, alkaline phosphatases are concentrated and

these granules are broken down, releasing locally high concentrations of inorganic phosphate (Gianinazzi-Pearson and Gianinazzi, 1983). In AMF, there is a decreasing concentration from arbuscule through the xylem sap (Schoknecht and Hattingh, 1976). As the rate of photosynthates often increases in the mycorrizal plants, increasing the conversion rates of inorganic phosphate, this pathway actually decreases the free energy necessary to transport phosphate in the host and has been hypothesized to be important in the increased P uptake associated with mycorrhizal infection (Allen *et al.*, 1981). Most large growth enhancement effects of root infection with mycorrhizal fungi are caused by increases in P absorptionn, particularly from sparingly soluble P sources (Bolan *et al.*, 1987). When root exploration is restricted, upto 80 % of the plant P can be delivered by the external AMF hyphae to the host plant over a distance of more than 10 cm from the root surface (Li *et al.*, 1991). Therefore with respect to P nutrition, the growth response to AMF infection depends on soil and plant factors which determine the P acquisition of the host plants. Mycorrhizal fungal hyphae can also transport other mineral nutrients like N, Ca, Zn, S, K (Harley and Smith, 1983) and water from soil (Auge *et al.*, 1986).

Mycorrhiza also affect morphological responses that ultimately improve plant survival and growth. Huang *et al.*, (1985) demonstrated that AMF altered the leaf folding and orientation of *Leucaena leucocephala*. This morphology regulated light interception and leaf energy balance hence determining the plant growth rate. Morphological and hormonal changes related to mycorrhizal infection alter the ability of the plants both to survive stress and to gain access to resources that ultimately improve the fitness of the plant (Allen, 1985).

A variety of resources provided by the fungus may or may not be important in specific habitats such as water, nitrogen and micronutrients (Trent *et al.*, 1989). By increasing access to resources, mycorrhiza increases C-fixation through increased photosynthetic rates. Trent *et al.* (1989) using CO_2 flux measurements demonstrated greater instantaneous photosynthesis in mycorrhizal than nonmycorrhizal plants.

Mycorrhizal plants can also take up more carbon in drought cycles than nonmycorrhizal plants. Mycorrhizal *Bouteloua gracilis* maintained more open stomates at lower soil water potentials than did nonmycorrhizal plants (Allen *et al.*, 1984) and wheat plants infected by *Glomus fasciculatum* had stomates open for upto 3 days longer during drought than nonmycorrhizal wheat plants (Allen and Boosalis, 1983). Trent *et al.* (1989) demonstrated greater photosynthesis and stomatal conductance while the plants maintained equal to lower leaf water stress during grain filling of mycorrhizal versus nonmycorrhizal winter wheat with no significant differences. Mycorrhizae can also increase total seasonal carbon gain. Flower phenology was delayed with drought when plants were mycorrhizal versus when nonmycorrhizal and leaf mortality was reduced with mycorrhizal infection (Allen and Allen, 1986).

2.3.3 AMF Ecology

Mycorrhizal fungi are a major microbiological component of plant and soil ecology. They uder-pin plant function in most natural habitats and may have a similar role in many agricultural and land rehabilitation sites. Any interactions among hyphae of AMF will influence their functional relationships with roots, the soil-root interface and the wider soil matrix. To survive, these biotrophic fungi must form an association

with a host plant which enables them to produce propagules throughout periods when living roots are absent (Tommerup, 1988). The life styles of individual AMF associated with susceptible hosts are influenced by three major facets of their environment, namely; the incidence of competition, stress and disturbance (Tommerup, 1988). In the absence of a susceptible host, the nature and importance of stress factors are increased. AM fungi are indigenous to soils throughout the world. Many AM fungal species are represented on most continents. Trappe (1977) proposed that AM fungi were disseminated intercontinentally prior to continental drift. AM fungi can be dispersed through active dissemination by growth of mycelium through soil (Powell, 1979) and passive dissemination where AMF are moved by wind, water, or by soil microogarnisms (Ponder, 1980). Spore germination is influenced by the presence of a host, soil microoganism and the physical and chemical environments (Daniels and Trappe, 1980).

Root colonization and subsequent spore germination by AMF are influenced by fungal, environmental and host effects. Ferguson (1981) showed that increased inoculum dosage resulted in increased % root infection though Carling *et al.* (1979) found that an upper limit of root colonization is reached and greater inoculum dosages had no further increase in root colonization. The effect of combined fungal species inoculation may be synergistic or competitive. Ocampo *et al* (1980) found out that there was less AMF colonization in barley, lettuce, potato or onion with mixed endophyte population in nonsterile soil than occurred when *Glomus fasciculatum* alone was inoculated onto these crops in sterilized soil. On the other hand, Ross and Ruttencutter (1977) found that less colonization occurred in *Glomus macrocarpum*

inoculated peanuts and *soybean* than when these hosts were inoculated with *Giagspora gigantea* and *Gigaspora macrocarpum* together.

Higher temperatures (Hayman, 1974), increased light intensity (Furlan and Fortin, 1977) and longer daylengths (Johnson *et al.*, 1982) have been shown to increase root AMF colonization and sporulation. The presence or absence of a host plant obviously plays a large role in whether or not colonization and subsequent colonization will occur. Nonhost plants can become minimally colonized by AMF (Kruckelmann, 1975) particularly when grown in the presence of host plants (Ocampo *et al.* 1980). Hayman *et al.* (1975) found out that the presence of nonmycorrhizal plants resulted in reduced colonization of mycorrhizal host plants possibly because of toxic nonhost-root exudates or seed coat components. The affinity of host plants to AM fungal endophytes will also determine the degree of colonization or sporulation which occurs. Schenck and Kinloch (1980) observed that the incidence of AM fungal species depended upon the plant species which was colonized. They found that spore numbers of *Gigaspora* spp. were most numerous around soybean plants, but *Glomus* and *Acaulospora* spp. predominated around monocotyledonous crops. Although AMF have extremely wide host ranges (Mosse, 1973), the existence of host preference has been suggested by Mosse (1975).

Both spores of AMF and colonized root pieces can be effective propagules, initiating typical AM fungal colonization in host plants (Warner and Mosse, 1982) although the ability of AMF to persist in soil may depend partly on the type of propagule formed. It is clear that hyphal fragments and root pieces colonize host roots more rapidly than spore inoculum, thus producing a growth response faster (Hall, 1979) but the ability

of this inoculum form to survive in the absence of an actively growing host is questionable. The survival of propagules of AMF is directly influenced by the cropping schedule, soil condition, presence of other soil organisms and the season. The number of growing seasons in the absence of a host plant apparently influences AMF survival whether the soil is fallowed or cropped to a nonhost. Since new spores are not produced in these situations, the population of AMF can only decline. Soil storage or fallowing appears to reduce the inoculum potential level in soil. Gould and Liberta (1981) demonstrated reduced inoculum potential in stored topsoil compared with undisturbed soil even greatly, though spore populations did not differ. Similarly, Black and Tinker (1979) found that spore levels generally decreased by the end of a barley growing season, but a greater rate of decrease was evident in fallowed or nonhost-cropped soil. A year of fallow or nonhost-cropping reduced by half the colonization level obtained in the subsequent barley crop. Ocampo *et al.* (1980) suggested that AMF may be able to derive some benefit from nonhost plants and that nonhost cropping may favour the development of AMF more than fallowing. After many years of deleterious cropping schedules, however, AMF populations may be markedly reduced (Kruckelmann, 1975). In such cases, more than one host cropping season is necessary to restore AMF inoculum potential to levels which benefit plant growth. If AMF inoculum is eliminated from soil by over-fertilization or deleterious cropping schedule, re-inoculation with AMF is necessary.

The soil temperature influences AMF root colonization and sporulation (Hayman, 1974). It also affects the activity of soil microflora (Griff, 1972) which may affect the rate of AMF hyphal lysis or hyperparasitism of spores. Though soil conditions can influence the survival of AMF in general, Lambert *et al.* (1980) demonstrated that

indigenous fungi were often more efficient at increasing plant growth than introduced fungi in soils to which they had become adapted.

Interaction of AMF with other soil organisms may also affect its survival. Mites and worms can eat spores while nematodes pierce and suck contents of fungal spores (Griffin, 1972). Bacteria and other fungi can also penetrate spores or produce degrading enzymes to fungal cellwall and disintegrate fungal protoplasm (Griffin, 1972). Based on the spore numbers isolated, Black and Tinker (1979) documented seasonal variations within AM fungal populations. They realized that peak spore production is related to climate and the crop, hence there is a significant decline in winter while spore levels increase during the growing season.

2.3.4 AMF Inoculation

Phosphorus uptake under natural conditions is limited by the diffusion impedance of the soil (Nye and Tinker, 1981). The AMF has been found to reduce the diffusion path of poorly mobile ions by producing an extensive network of external hyphae thereby increasing the effective absorptive surface of the root (Read, 1984). The AM fungi stimulate plant growth primarily through their enhancing effect on P-uptake and any other nutrient that moves by diffusion into the root region in nutrient poor soils (Abbott and Robson, 1984). The AM fungi infect the roots and extend hyphae beyond the P-depletion zone of the roots to absorb and translocate additional P (Sanders *et al.*, 1977). Bolan *et al.* (1984) found that hyphae from mycorrhizae may absorb labile P unavailable to non mycorrhizal plants. Nitrogen fixation has been found to require upto 3 times more P than that required for root growth alone (Mosse, 1981). The AMF may increase phosphate uptake of crops by solubilizing P. Seed

inoculation with AMF increased the dry matter production , P uptake and grain yield of beans, peas and lentil by solubilizing otherwise unavailable forms of P (Gleddie, 1993). In addition, AMF infection may also improve plant water relations, with mycorrhizal plants having less resistance to water flow and improved drought resistance than non mycorrhizal plants (Cooper, 1984).

The major impacts of AMF inoculation are likely to be in soils where native mycorrhizal populations are ineffective or have low numbers of propagules (Shepherd *et al.*, 1996). AMF enhances growth and yield of many field crops (Bolan, 1991). The AMF infection of soybean roots has been reported to stimulate both nodulation and nitrogen fixation (Singh, 1994), while Chang *et al.*(1992) found enhanced P absorption and hence increased plant P content. Sreeramulu *et al.* (1996) observed that *Amaranthus viridis* and *Trigonella foenuni* recorded significant increases in height, leaf number, shoot and root biomass at 40 days after sowing when unsterilized sandy loam soil was inoculated with *Glomus fasciculatum* over the control. Increased number of nodules per plant, shoot and root dry weight, seed yield, percent infection, nitrogen fixation, tissue N and tissue P was reported by Talukdar and Germida (1995) and Rao and Rao (1996) working with lentils and green grams. The AMF vetches had significantly more nodules, better growth and higher nitrogen fixation than nonmycorrhizal plants (Niemi amd Eklund, 1988).

2.3.5 AMF Interaction with Fertilizer

It is widely accepted that maximum root colonization and sporulation occur in soil of low fertility. Both phosphorus (Ross, 1971) and nitrogen (Hayman, 1975) may significantly reduce root colonization if present at high levels, and a delicate balance

between these two elements appears to exist. Bevege (1972) found that root colonization increased as N content increased if P levels were moderate. At higher levels of P however, N applications were inhibitory. A similar balance has been observed for zinc and phosphorus (McIlveen and Cole, 1979). The effect of high soil fertility on root colonization depends on the host plant grown. S trezemska (1975) observed that root colonization of rye, wheat, barley and oats was reduced after years of cropping in highly fertilized soils, but colonization of bean roots was not similarly reduced under these conditions. Gerdemann (1970) observed that many crops in the midwest US remained highly mycorrhizal despite high soil fertility.

P application significantly influences mycorrhizal infection in roots (Hayman, 1982). Generally, P fertilizer depresses infection by AMF (Schubert and Hayman, 1986) but Salinas *et al.* (1985) working with *Phaseolus phaseoloides* reported that fertilizer may not have any influence on mycorrhizal infection. This was supported by *Arias et al.* (1991) who found that P fertilization inhibited fungal colonization in only one out of 3 legumes.

Menge *et al.* (1978) have shown that much of the influence of soil fertility on root colonization is plant mediated. they observed high levels of root colonization and sporulation in roots exposed to high levels of soil P if the bulk of the root system was exposed to lesser amounts of P. Thus the initial P concentration of the plants controls the level of root colonization and sporulation by AMF which will occur. Ratnayake *et al.* (1978) and Graham *et al.* (1981) have further demonstrated that the net leakage of root exudates is significantly greater under low P levels. High levels of exudation were correlated with decreased phospholipid levels and increased permeability of root

membranes. They suggested that root colonization by AMF is inhibited at high P levels because of the decreased root exudation. Thus, the content of P in the root can mediate root colonization by AM fungi.

Although the level of root colonization has been related to the quantity of P fertilizer added to the soil (Sanders and Tinker, 1973), Plenchette *et al.* (1983) showed that the internal P status of the host plant could control the rate of spread and the intensity of colonization by the endophyte. Nelsen and Safir (1982) observed that greater root colonization occurs in plants receiving adequate water, though, they also realized that high levels of root colonization can occur in drought-stressed plants even in highly fertile soils because low moisture levels can reduce the diffusion rate of nutrients such as P and decrease the availability of these nutrients in the plants.

With respect to P nutrition, the growth response of AMF infection depends on soil and plant factors which determine the P acquisition of the host plants. With increasing soil P, the enhancement effect of AMF declines and may either be abolished or lead to growth depressions if the carbon costs of the symbiosis are not compensated by other beneficial effects (Raju *et al* ., 1990). Depression of AMF infection levels by high soil P depends on the fungal species and the level of N supply especially NH_4-N (Baath and Spokes 1989), in relation to both lower carbohydrate levels in the roots and lower total root length per plant.

Application of organic material to soil also affect the productivity of AMF. Roldan and Albaladejo (1993) reported decreased AMF populations in a highly-eroded semiarid soil the first year after amendment with non-composted urban refuse. Lambert and Weidensaul (1991) found that sewage sludge depresses AMF in soybean

crops probably due to the presence of toxic compounds. Ryan *et al.* (1994) demonstrated that plants in organic farming systems have higher percentages of AMF colonization than in conventional farming systems. This could be attributed to vermicompost having a higher water soluble P than the surrounding soil (Van der Werff *et al.*, 1995). When the soil has no P, growth AMF is inhibited (Bethlenfalvay and Yoder, 1981). When P availability is low, enhanced growth of the host occurs. At intermediate levels of P, fungal proliferation may be at the expense of the host without enhancing P-uptake (Brown, 1978), while at the high levels of P, fungal growth is inhibited (Schubert and Hayman, 1986).

2.4 Triapartite Symbionts

Carpenter and Allen (1988) observed that neither *Rhizobium* nor mycorrhizal inoculation altered the growth and reproduction of Hedysarum in the field, but together they both contributed N to the plant and increased growth and reproduction. There is probably little competition for infection sites between *Rhizobium* and AMF as *Rhizobia* invade root hair cells, while AMF invades the cortical cells of roots. Rose and Youngberg (1981) suggest that interaction between Frankia and AMF is synergistic in *Ceanothus velutinus* as both growth and nutrition concentrations in the dual-inoculated plants are greater than the responses when each endophyte is added separately.

Inoculation of legume seed with *BradyRhizobium* to ensure effective nodulation and biological nitrogen fixation is a standard agricultural practice. The co-inoculation of legume crops with both *Rhizobium* and AMF is gaining interest but raises questions about the compatibility of the two organisms. Kucey (1987) found no detrimental

effect of either organism on the other, whereas, Downey and van Kessel (1990) suggests that the organic acids produced by the AMF may inhibit the activity of *Rhizobium*.

Kucey and Paul (1982) have indicated that AMF may stimulate nitrogen fixation independently of any influence on the growth of the host plant, but this effect may be restricted to situations where nutrient supply, especially P is limited. Synergistic effects of dual inoculation with AMF fungi and *Rhizobia* in soybean (Kumutha and Santhana - Krishinan, 1994), Lucerne (Smith and Daft, 1977), French beans (Lin *et al.*, 1993), groundnuts (Krishna and Bagyaraj, 1984)), and black gram and green gram (Rao and Rao, 1993) have been observed. Asai (1944) reported that nodulation of various legumes was dependent on AMF formation. Mycorrhizal inoculation of nodulated soybean plants can increase yield and N content of above ground parts (Ross, 1971) and nodulation (Ganry *et al.*, 1982). Manjunath *et al.*, 1984 observed increased mycorrhizal colonization in plants inoculated with AMF together with *Rhizobium* compared to single inoculation.

Barea and Azcon-Aguilar (1983) and Daft *et al.* (1985) demonstrated positive interactive effects of AMF and *Rhizobium* infections on legume productivity. Hayman (1986) indicated that the main effect of AMF on *Rhizobium* activity is phosphate mediated. Arias *et al.* (1991) observed that non-mycorhizal plants failed to nodulate and growth was poor, but additions of small amounts of P fertilizer to the soil improved growth and nodulation of such plants. Mycorrhizal inoculation stimulated plant growth, nodule numbers and nodule weight. Barea *et al.* (1992) attributed this stimulation to improved plant nutrient uptake and drought resistance,

but non-nutritional interactions have also been implicated (Ross and Harper, 1970). When AMF improve the P nutrition of the host plant there may be a corresponding increase in nodulation, nitrogen fixation and growth (Robson *et al.*, 1981). In view of the high P requirement for nodulation, many legume species growing on low P soils are highly dependent of VAM infection.

The existence of extra-radical hyphal bridges between individual plants permits transfer of nutrients such as N This transfer has been observed from soybean to maize only when the legume is supplied with mineral N but not when relying on nitrogen fixation (Bethlenfalvay *et al.* 1991). Enhancement effects of AMF on the N content in nodulated legumes is derived from better nitrogen fixation and also higher uptake of soil N (Azeon and Barea, 1992). However, Singh (1994), working with soybean found that dual inoculation did not affect nodule number, dry weight and seed yield.

Dual infection of legume roots improves the supply of P and N to the host plant thus reducing the requirements on both elements from input of synthetic fertilizer. However, there is a cost to the host plant for these infections. Kucey and Paul (1982) showed in *Vicia faba* that AM fungi used about 4% of the carbon fixed in photosynthesis, that nodules in non-mycorrhizal plants used 6% and that in mycorrhizal plants, the combined drain was 12% of the fixed carbon. However, while this additional carbon drain may limit the early growth of the host, it appears that the rates of CO_2 fixation increase in plants infected with both symbionts, and so does the compensation. Harris *et al.* (1985) working with *soybean* showed that AMF colonization stimulated the early development of root nodules and that the rate of CO_2 fixation by nodulated mycorrhizal plants was up to 47% greater than in plants without

these symbionts. Competition between AM fungi and *Rhizobium* has not been studied extensively, but available evidence suggests that antagonistic interactions can develop if infection by one symbiont precedes the other (Bethlenfalvay *et al.*, 1985). Under conditions of photosynthate loss, mycorrhizae can develop at the expense of *Rhizobia* (Bayne *et al.*, 1984).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Description of the Soil Sampling Site

The soil used in the study was obtained from Kaguru Farmers Training Centre (FTC), Meru District (latitude 0.2^0S and longitutide 37.6^0E). According to the legend to the Soil Map of the World (FAO-UNESCO, 1974), the soil is classified as a humic *Nitisol*. The soils are extremely deep (>180 cm), dusky red to reddish dark brown in colour, and consist of friable to firm clay, with an acid humic topsoil. They have a moderate, sub-angular to angular blocky structure and a very high bioporosity, apart from the somewhat compacted AB horizon which has a moderate high bioporosity. The physiographic position of the centre is undulating terrace with a slope of 4 – 6 % while the topography of the surrounding country is undulating to hilly (steepest slopes 16-30 %). The landform is volcanic footridges. The centre is at an altitude of 1460 m above sea level. The area receives mean annual rainfall of 1726 mm. According to Jäetzold and Schmidt (1983), the area lies in the agro-ecological zone UM2 which is mainly a coffee zone. The mean annual temperature is 18 to 21 ^{0}C, with mean minimum of 11 to 14 ^{0}C and maximum 25 to 28 ^{0}C. The annual average precipitation is 65 to 80 % of the potential evaporation (Eo). The sub-zone has two medium cropping seasons, the 1st season from early March –early August and the 2nd from mid October- early February. The major activities in this agro-ecological zone include maize growing and dairy keeping.

3.2 Soil Sampling

The soil used in the study was randomly collected from uncultivated field which had been left fallow for 6 years. Four locations 3 m apart were selected and prior to sampling, all the vegetation was removed from the surface using a hoe. Bulk top (0 to 15 cm) soil samples were dug out using a hoe. A spade was used to transfer the dug out soil into gunny bags and sealed. The soil was then transported to the Kenya Forestry Research Institute (KEFRI), Muguga.

From the freshly sampled bulk top soil, 500 g of soil was put in a polythene bag and kept in a fridge at 4 °C to await mycorrhizal spore density determination and *Rhizobia*l count. The rest of the soil sample was poured on to a large polythene paper and mixed thoroughly. This ensured that a representative sample was obtained. Large clods were broken into smaller aggregates with the aid of a stick and the debris removed. A representative sample was separated for laboratory analysis and ground in a mortar with a pestle. Soil for soil texture and pH determinations was sieved through a 2 mm sieve while that for determining organic C, total N, total P and available P was sieved through a 0.5 mm sieve. The bulk of the soil was mixed with sand in the ratio of 3:1 (Odee, unpublished data) to improve infiltration and put in the glasshouse for filling the pots.

3.3 Soil Analysis

3.3.1 Soil Reaction (pH)

Following the procedure outlined by Anderson and Ingram (1993), 20 grams of soil, sieved through 2 mm was mixed with 50 ml of distilled water or 0.01M $CaCl_2$

solution (ratio of 1:2.5 mass by volume). In both cases, the soil-solvent mixture was shaken for 30 minutes using a rotary shaker at a speed of about 200 rpm. It was left to stand for 10 minutes. Soil pH was then determined using a Corning ion analyzer pH-meter.

3.3.2 Organic Carbon

Organic carbon was determined by the method of Walkley and Black (Nelson and Sommers, 1982). 0.1 gram of soil was weighed into a conical flask. The carbon was oxidized at a temperature of approximately 120 ^{0}C by adding 10 ml of 1 N potassium dichromate solution and 20 ml of concentrated sulphuric acid to the sample. After settling for 30 minutes, 250 ml of distilled water and 5.0 ml of orthophosphoric acid were added. The excess potassium dichromate not reduced by organic matter of the soil was determined by titration with 0.5 N ferrous sulphate using diphenylamine as indicator. Percent carbon was then calculated using the following formula:

$$\%C = \frac{(B - T) \times 0.3V}{W \times B}$$

Where: C = carbon
B = blank titre
T = sample titre
W = weight of oven dry soil
V = volume of $K_2Cr_2O_7$

3.3.3 Total Nitrogen

Total nitrogen in soil was determined using Kjeldahl method (Bremner and Mulvaney, 1982). One gram of soil was weighed into the digestion tube and the nitrogen compounds in the soil samples were digested for 30 minutes with a mixed catalyst comprising of 6 ml of concentrated Sulphuric acid, 2.5 gm of potassium sulphate, and 0.5 gm Copper sulphate. During digestion, nitrogen was converted to

ammonia that was fixed by excess acid as ammonium sulphate. The digest was made alkaline with 40 ml of saturated (40 %) sodium hydroxide and the ammonia distilled and collected into conical flasks containing 25 ml of boric acid-indicator solution. The amount of ammonia held in boric acid was then determined by titration with 0.05 M HCl, and the percent nitrogen calculated according to the formula;

$$\%N = 14.01 \times \frac{(T - B) \times 0.1}{W \times 10}$$

Where: T = titre reading

B = blank reading

W = weight of soil taken

3.3.4 Total Phosphorus

0.3 g of soil was digested using the Kjeldahl digestion chamber as described by Olsen and Sommers (1982). Five ml of the digestion mixture containing 0.42 g selenium powder, 14 g lithium sulphate, 350 ml hydrogen peroxide and 420 ml concentrated sulphuric acid was added to the soil samples in digestion tubes and these were digested for 3 hours at 360 ^{0}C until any remaining sand was white. 20 ml of distilled water was added to the digests and then transferred into 100 ml volumetric flasks. The flasks were made to the mark with distilled water and then allowed to cool. One millilitre of the clear suspension of the soil sample and the standards was pipetted into test tubes and the colour developed using 4.0 ml of 1 % Ascorbic acid dissolved in 3.0 ml molybdate reagent. The intensity of the blue colour, which depends on the phosphate content of the samples (remains stable for at most 24 hours) was measured by reading the absorbance at 880 nm after one hour using a Spectrophotometer (Model–Spectronic 21). Phosphorus concentration in ppm was then read from the calibration curve.

3.3.5 Available Phosphorus

Available P was determined following the procedure by Watanabe and Olsen (1965). A 2.5 g soil sample was weighed into plastic shaking bottles and 50 ml of sodium hydrogen bicarbonate solution at pH 8.5 added. The bottles were then shaken for 30 minutes at a speed of 200 revolutions per minute using a rotary shaker. The mixture was then filtered through a Whatman No. 42 filter paper and the filtrate collected into conical flasks. One millilitre of the filtrate and the standards was pipetted into test tubes and the colour developed using 4.0 ml of 1 % Ascorbic acid dissolved in 3.0 ml molybdate reagent. The intensity of the blue colour which depends on the phosphate content and remains stable for at most 24 hours was measured by reading the absorbance at 880 nm after one hour using a Spectrophotometer (Model–Spectronic 21). Phosphorus concentration in ppm was then read from the calibration curve.

3.3.6 Exchangeable Bases

Exchangeable K, Na, Ca and Mg were extracted using the Ammonia saturation method (Anderson and Ingram, 1993). Five grams of soil was weighed into a beaker and 20 ml of ammonium acetate solution at pH 7. The soil solution suspension was thoroughly mixed by swirling and allowed to stand for 30 minutes with occasional swirling. The soil-solution mixture was then transferred into a filter funnel fitted with a filter paper and 180 ml of ammonium acetate solution added slowly, collecting the leachate in a 250 ml volumetric flask. The volumetric flask was then made to the mark with Ammonium acetate solution and then mixed well. Twenty millilitres of the leachate was pipetted into a 100 ml volumetric flask, 20 ml of lanthanum chloride solution added and then made to the mark with distilled water. The amounts of exchangeable Na and K in the extract were determined on a flame photometer while

Ca and Mg were determined by atomic absorption spectrophotometer, both of model Hitachi 180-330.

3.3.7. Soil Texture

Soil texture was determined by the hydrometer method according to Bouyoucos (1951). Fifty grams of air-dried soil (2 mm) was weighed into a plastic shaking bottle and 300 ml of distilled water added. 10 ml of Hydrogen peroxide was added to destroy the organic matter present in soil. The soil was then dispersed mechanically by shaking in a rotary shaker overnight and chemically by addition of 50 ml calgon solution. The soil suspension was transfered into 1 000 ml graduated cylinder and made to the mark with distilled water. The first hydrometer reading was done after 40 seconds to get the concentration of silt and clay, and the second reading after 2 hours, from the time stirring ceased to get the concentration of clay. The hydrometer readings were corrected using the blank reading according to the equation:

$$C = R - Rb$$

Where: C= actual concentration of soil suspension in g/litre

R= hydrometer reading in the soil suspension

Rb = hydrometer reading in the blank

The amount of sand, silt and clay in the soil were calculated according to the formula:

$$\% P = \frac{C}{Co} \times 100$$

Where Co = weight of soil taken

Therefore, the amount of silt + clay in the soil suspension is given by:

$$\% (\text{silt} + \text{clay}) = \frac{C}{Co} \times 100$$

Hence, % sand = 100 - %(silt + clay)

The amount of Clay in the soil was calculated according to the formula:

$$\% \text{ clay} = \frac{C}{Co} \times 100$$

Therefore, % silt=100-(%sand + %clay)

The textural class of the soil was read from the Textural triangle.

3.3.8 Determination of Field Capacity the Soil

The soil's field capacity was determined according to the procedure by Somasegaran and Hoben (1985). The soil-sand mixture (3:1 v/v) to be used in the pot experiment was filled into a 250 ml transparent measuring cylinder with a hole at the bottom to allow air to escape in the process. The cylinder was tapped to a similar consistency as that in the pots. Water was poured gently into the cylinder until two thirds of the soil volume was wetted. The water column was allowed to equilibrate for 48 hours. The middle 5 cm of the soil column was removed using a spatula into a pre-weighed crucible, and the wet weight taken. The soil sample was then dried at 110 ^{0}C to a constant weight and the gravimetric soil water content at field capacity (%) calculated using the formula;

$$\% \text{ M.C} = \frac{\text{weight of wet soil} - \text{weight of oven dry soil}}{\text{weight of dry soil}} \times 100$$

3.3.9 Spore Isolation

Spores were extracted from soil by sucrose centrifugation method of Jenkins (1964) with modifications by Walker *et al.* (1982). Fifty grams of the soil were weighed into

a 1 L plastic beaker, tap water added and the mixture thoroughly strirred and then allowed to settle for 40 seconds. The 710 μm and 45 μm sieves were then assembled and the suspension poured into the 710 μm sieve. The residue trapped in the 45 μm sieve was washed clean, then transfered into centrifuge tubes. Centrifuging was done at 3000 rpm for 4 minutes. The tubes were removed and the centrifugation solution poured. Sixty percent sucrose solution was added upto three quarters full, the tubes shaken and centrifuged again for 40 seconds. The solution was then poured into the 45□m sieve and washed clean of the sucrose. The residue was transfered into a shallow beaker and observed under the microscope at high magnification (x50). The spores were picked with a forceps and separated according to genus for culturing.

3.3.10 *Rhizobia*l Counts Using L-Jars

Autoclavable plastic bottles were used to make modified L-jars (Leonard, 1943). Vermiculite enough to pack 150 L-jars was soaked in a basin for two days. The vermiculite was then washed clean with tap water and the pH adjusted to 6.8 by adding 1 % NaOH. The neck and shoulder part of the jars was packed full with vermiculite. A minus nitrogen nutrient solution was prepared by mixing compounds which contain macro and micro nutrients as shown in Table 1.

Table 1: Compounds used in the preparation of the nutrient solution.

Compound	Element	Concentration (μM)
$CaCl_2.2H_2O$	Ca	1000
KH_2PO_4	P	500
Fe-Citrate	Fe	10
$MgSO_4.7H_2O$	Mg	250
K_2SO_4	K	250
$MnSO_4.H_2O$	Mn	1
H_3BO_3	B	2
$ZnSO_4.7H_2O$	Zn	0.5
$CuSO_4.5H_2O$	Cu	0.2
$CoSO_4.7H_2O$	Co	0.1
$Na_2MoO_4.2H_2O$	Mo	0.1
KNO_3	K	2.5

Ten ml of each of the nutrient stock solutions ($CaCl_2.2H_2O$, KH_2PO_4, Fe-Citrate, $MgSO_4.7H_2O$, K_2SO_4 and the micronutrients) was put into 20 litres of distilled water, thoroughly mixed and about 600 ml of the nitrogen free solution placed in the reservoir. The top was then placed firmly on the rim of the reservoir. The jar assembly was then placed in a brown moisture proof paper, the open end of the assembly capped with a petri-dish and the complete assembly was sterilized by autoclaving for 1 hour at 121 ^{0}C and 15 bars. The test seeds used were; soybean of variety Nyala, obtained from the GTZ-Soybean Project in Kenya, *Acacia polyacantha* (Provenance-Kitui) and *Faidherbia albida* (Provenance-Kainuk) obtained from KEFRI- Biotechnology division, Muguga, Kenya.

Fifty seeds of each of the plant species were sterilized and pre-treated by immersing in warm water (soybean and *Feidherbia albida*) and hot water (*Acacia polyacantha*) and then adding 95 % alcohol and 3 % NaOCl and the seeds then pre-germinated in sterilised water agar as described by Somasegaran and Hoben (1985). Two germinated seeds with a straight radicle were planted aseptically with a sterilized

forceps in each L-jar and thereafter, the L-jars left covered with the petri-dish bottoms in the preparation room.

A strain of Bradyr*hizobium* japonicum, USDA 110 was obtained from the Biotechnology Division of KEFRI which had been stored in vials at –70 °C. A loopfull was picked from the vials with a sterilized loop and streaking done on congo red plates following the procedure by Somasegaran and Hoben (1985). The plate was incubated at 25 °C for 7 days. A well grown colony was picked with a sterile loop and inoculated into 9 ml of sterile broth in a Mcarteny bottle to act as the starter culture. This was incubated in a rotary shaker at 100 rpm until late exponential phase ($\sim 10^9$ cells ml^{-1}). One ml of the starter culture of USDA 110 was used to inoculate 9.0 ml of sterilized broth dispensed in Mcarteny bottles. The bottles were shaken for 7 days until the cultures were in late exponential phase. On the fifth day after planting, the plumules had touched the surface of the inverted petri-dishes and hence were ready for inoculation. Ninety ml of quarter strength was dispensed into two conical flasks which was then corked with cottonwool and the mouth wrapped with an aluminium foil. Nine ml of the quarter strength was also dispensed into 12 Mcarteny bottles for serial dilution. The flasks and bottles were then sterilized with a clinical autoclave for 11 minutes at 126 °C. After cooling, 10.0 g of the soil was weighed into the labelled conical flask and then shaken for 10 minutes in the rotary shaker at 100 rpm. The soil suspension was then serially diluted from 10^0 to 10^6. One ml of the culture and the twelve soil dilutions was used to inoculate the respective labelled plants. Two non-inoculated controls, +N containing 2.5 N as 0.059 KNO_3 and -N were also included for each plant species. The plants were then moved to the glasshouse and then uncovered. After two weeks, a complete nutrient solution was

added to the +N controls. The plants were then allowed to grow for a total of six weeks (*soybean*) and 8 weeks (*Feidherbia albida* and *Acacia polyacantha*), harvested by washing off the vermiculite from the roots and then examined for nodulation and leaf colour. The results were coded as (+) for nodulated plants and (-) for non-nodulated plants. The number (M) of *Rhizobia* in the soil was found following the procedure by Vincent, (1970). Calculations were done using the formula:

$$M = \frac{m \times d}{v}$$

Where, m = most likely number (from table)
d = lowest dilution
v = volume of inoculant used per plant

This procedure aimed at determining the level of *Rhizobia*l populations in the humic *Nitisol* from Kaguru Farmers Training Centre and then comparing the indigenous and commercial *Rhizobia*l strains for their efficiency in N-fixation.

3.4 Pot Experiment: Effect of N and P application, and *Rhizobia* and AMF Inoculation on the Performance of Soybean

The four factors under study were Nitrogen and Phosphorus fertilization, and *Rhizobium* and mycorrhiza inoculation. There were two levels of each factor :N_0=0 kg N/ha, N_1= 22.5 kg N/ha; P_0= 0 kg P/ha, P_1=50 kg P/ha; R_0=No inoculation, R_1=inoculation with *Rhizobium*; M_0=No inoculation, M_1=Inoculation with VAM; which were combined to give 16 treatments. The 16 treatments were allocated to pots using randomized complete block design (RCBD) and replicated four times in the greenhouse at KEFRI-Muguga.

One hundred and twenty eight polythene pots of size 15 x 22.5 inches were used. Each pot received 1.5 kg of the soil-sand mixture (3:1 v/v). For those treatments which required mycorrhiza inoculation, a layer of 20 g of the soil-sand-spore inoculum (Glomus eticulantum species)obtained from the National Museums of Kenya, was spread uniformly on the surface of a two-thirds filled pot and this layer was covered with the potting mixture. All the pots were then watered to field capacity with tap water. The pots were then placed on a table in the greenhouse at a spacing of 50 by 50 cm. Two pots per treatment were placed in positions next to each.

Four hundred seeds of *soybean* were sterilized by immersing in 95 % alcohol for 10 minutes and in 3 % NaOCl for 3 minutes and then pre-germinated in 0.8 % water agar. Two pre-germinated seeds were then planted per pot. On the fourth day after planting, one ml of the fully grown *Rhizobium* inoculum (3.13×10^8 cells/ml) was placed around the base of the seedlings that required *Rhizobium* inoculation. Fertilization was done two weeks after planting by dissolving 0.08 g $(NH_4)_2SO_4$ or 0.11 g K_2HPO_4 or a combination of both chemicals depending on the treatment in 50 mls of distilled water and then pouring into the pots. The plants were maintained by watering the soil to field capacity and removing the weeds.

The first harvest was done six weeks after planting. The root system of each plant was washed free of soil by gently soaking it in water, taking care not to detach the nodules during the process. The nodules were then detached, counted and together with the roots and shoots, were dried in an oven at 60 °C for two days. The dry weights were then recorded. These plant components were then ground and the total N and P determined as described by Bremner and Mulvaney (1982) and Olsen and

Sommers (1982) respectively. The second harvest was done after 20 weeks from sowing when all the pods were brown and the leaves yellow. The pods were detached and their numbers per plant recorded. The pods were dried at 30 °C in an oven for 3 days, the seeds extracted, counted and the total seed dry weight per plant determined. The dried seeds were ground and the total N and P determined as for the other plant materials.

Soybean roots from the first harvest were assessed for mycorrhiza infection using the Staining Technique according to Koske and Gemma (1989). Roots were cut into 1 cm fragments and about 100 fragments randomly sampled. The roots were then placed in a container according to Claassen and Zasoski, (1991) and covered with 2.5 % KOH solution overnight. They were then rinsed and alkaline hydrogen peroxide added. These were allowed to stand for 20 minutes. The H_2O_2 was emptied out and the roots rinsed after which 1 % HCl was added and allowed to stand for 1 hour. The HCl was emptied out and the stain added (0.05 % trypan blue in acidic glycerol). The roots were left to stain for 12 hours and then observed under the microscope.

The root length infected by mycorrhiza was estimated using the gridline intersect method (Tennant, 1975). The method involved spreading out a root sample onto a petri-dish marked with a 1.2 cm x 1.2 cm grid. A thin film of water was added to aid the dispersion and uniform spread of the fragments so that no root obscured another. The total root length was estimated by scanning the horizontal and vertical gridlines under a microscope (NIKON,SMZ-2B Japan) and counting the fragments that crossed the gridlines. This was repeated with the same sample but counting only the infected fragments which were identified by blue stained hyphae within or without the root

cortices. The percentage root infection was calculated from the ratio of the length of the infected fragments to the total length of the root.

3.5 Data Analysis

The tissue biomass, tissue N and P concentration, extent of mycorrhizal infection, and nodule components were subjected to Analysis of Variance (ANOVA) and means separated by Least Signifinance Difference (LSD) Test at p=0.05 using the General Linear Models procedure (SAS Inst., 1992).

CHAPTER FOUR

4.0 RESULTS

4.1 Soil Analyses

Characteristics of the humic *Nitisol* sampled from Kaguru FTC, Meru are presented in Table 2. The results show that the soil was slightly acidic and low in P. The soil had a texture of clay. The soil's content of organic carbon and exchangeable bases was moderate and the total N content low.

4.2 Microbial Populations and Performance of *Rhizobial* Strains

The level of *Rhizobial* population in the soil, determined using three leguminous plant species is shown in Table 3. *Glycine max* and *Faidherbia albida* picked the indigenous *Rhizobium* strains from the soil but *Acacia polyacantha* did not. However, *Faidherbia albida* was more susceptible to the indigenous *Rhizobium* strains than *Glycine max* as indicated by the higher *Rhizobial* counts.

The performance of the three leguminous plant species in terms of nodulation, inoculated with a commercial strain compared with the uninoculated plants is presented in Table 4. *Glycine max* and *Faidherbia albida* both nodulated in the presence of the commercial strain, USDA 110 while *Acacia polyacantha* did not. The uninoculated control plants did not nodulate.

Table 2: Physical and chemical characteristics of the humic *Nitisol* from Kaguru FTC in Meru District.

Soil Property	Determination
Texture	
Clay (%)	42
Sand (%)	32
Silt (%)	26
Class	Clay
pH- H_2O	6.72
pH- $CaCl_2$	5.58
Organic C (%)	1.05
Total N (%)	0.15
Olsen P (mg P/g soil)	2.4
C/N Ratio	7:1
Ca (meq $100g^{-1}$)	3.3
Mg (meq $100g^{-1}$)	1.2
K (meq $100g^{-1}$)	1.0
Na (meq $100g^{-1}$)	0.2

Table 3: *Rhizobial* counts estimated by Most probable Number assay with 3 trap host species

Host species	No. of *Rhizobial* cells/g soil
Glycine max	5.8
Acacia polyacantha	0
Faidherbia albida	5.8×10^5

Table 4: Effect of N and Inoculation with *Rhizobium* strain USDA 110 on nodulation of three legumes.

	Treatment		
	Inoculation with	Controls	
Host Species	USDA 110	with N	without N
Glycine max	+	-	-
Faidherbia albida	+	-	-
Acacia polyacantha	-	-	-

Key: (+ = Presence of nodules; - = Absence of nodules; N= 70 ppm KNO_3)

Plants inoculated with the commercial *Rhizobial* strain, USDA 110 were greener than plants inoculated with the soil dilutions (Plate 1) and were comparable to those plants supplied with nitrogen. Plate 2 shows the root system of the soybean plant grown in potted soil and inoculated with the commercial strain, USDA 110 (R), while plate 3

shows the root system of the soybean plants growing in the potted soil but not inoculated with USDA 110 (So). The root system in plate 2 was well nodulated while that in plate 3 was poorly nodulated.



Plate1: Plants growing in L-jars in the greenhouse supplied with nitrogen (+N=70 pmm as 0.059 KNO_3) and inoculated with 7 soil dilutions (0 – 6) and a commercial strain, USDA 110 (ZBE).

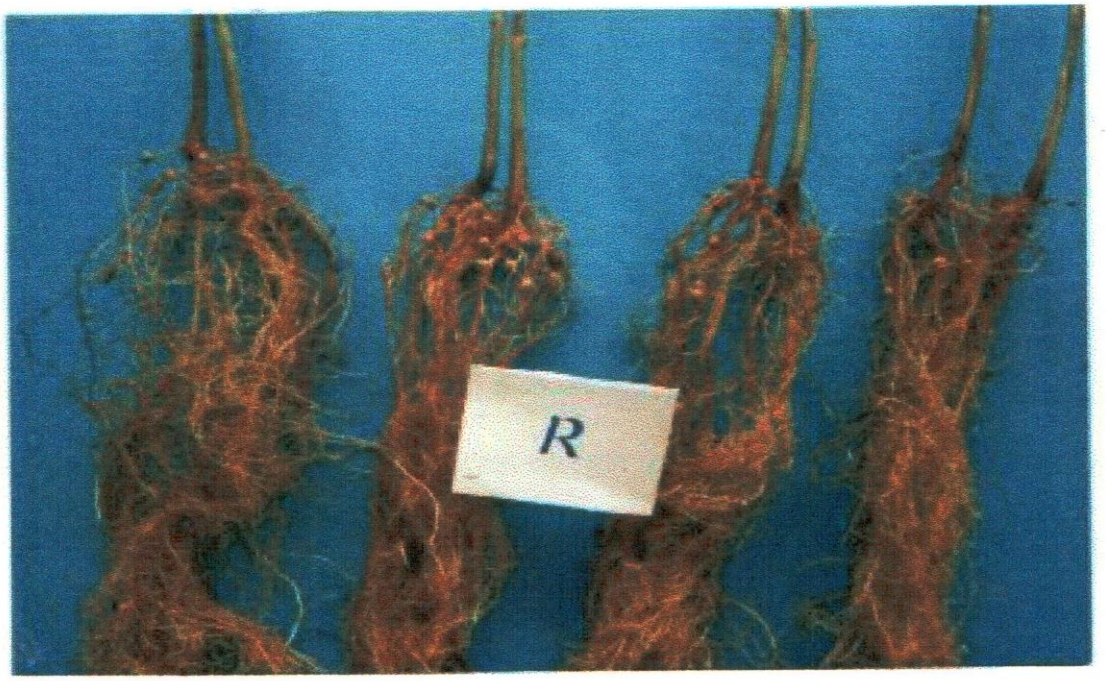


Plate 2:Root system of well nodulated potted plants inoculated with USDA 110.



Plate 3: Root system showing poorly nodulated potted plants.

The humic *Nitisol* was found to have a variety of AMF spores. The three genera identified were: *Glomus*, *Scutellospora* and *Gigaspora*. The spores varied in colour and size. They were also at different growth stages.

4.3 Main Treatment Effects

Rhizobium inoculation significantly ($P \leq 0.05$) increased nodule number (Figure 1), root N (Figure 2) and root P (Figure 3). Nitrogen significantly ($p=0.05$) increased the number of seeds per plant (Figure 4), and also the dry weights of shoots (Figure 5) and seeds (Figure 6). Small and non-significant changes were observed for N and P inputs and also for *Rhizobium* and mycorrhiza inoculations with respect to the % root mycorrhizal infection, nodule and root dry weights, seed N and P contents, shoot N and P contents and the pod number.

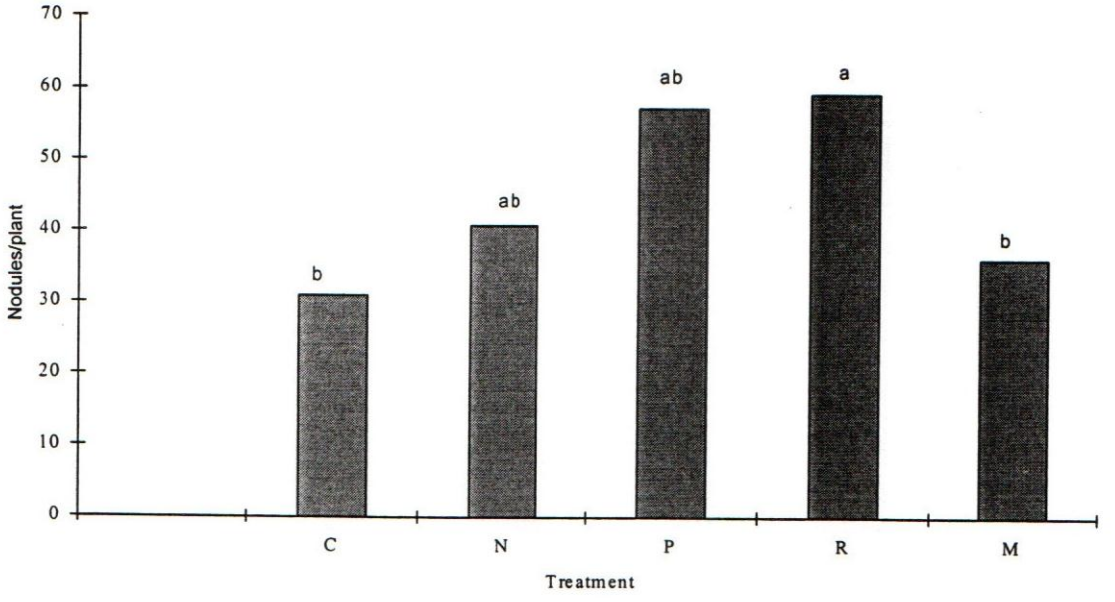


Fig. 1. Effect of Nitrogen (N), Phosphorus (P), rhizobium (R) and mycorrhiza (M) on nodulation at 6 weeks after planting. Column bars followed by the same letter(s) are not significantly different at 5 %.

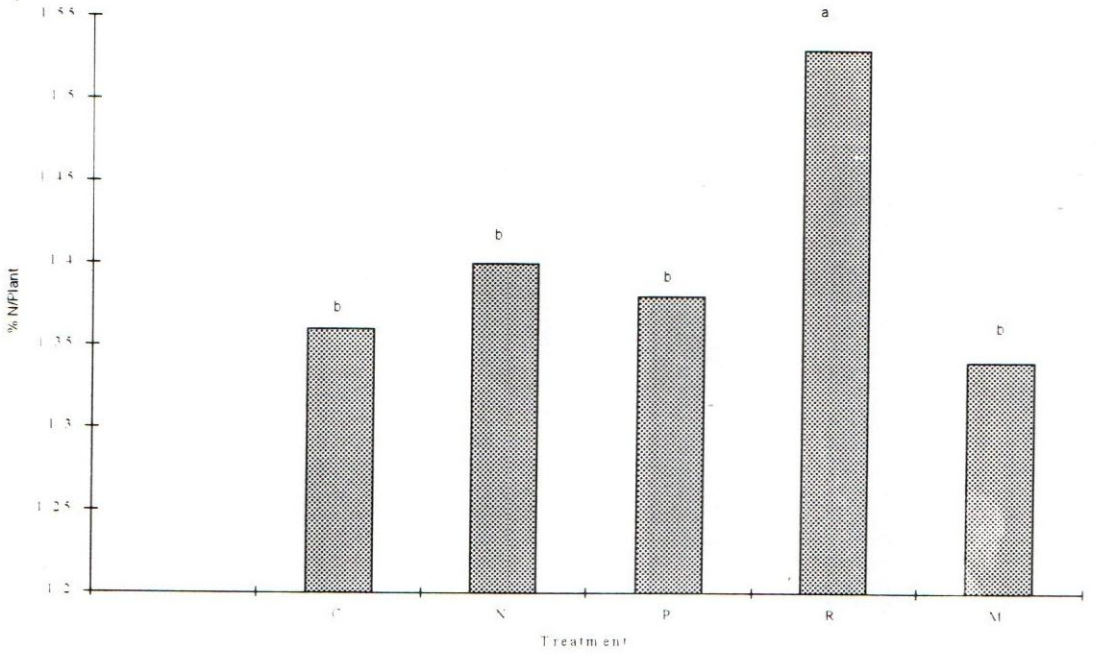


Fig. 2. Effect of Nitrogen (N), Phosphorus (P), rhizobium (R) and mycorrhiza (M) on root nitrogen content at 6 weeks after planting. Column bars followed by the same letter(s) are not significantly different at 5 %.

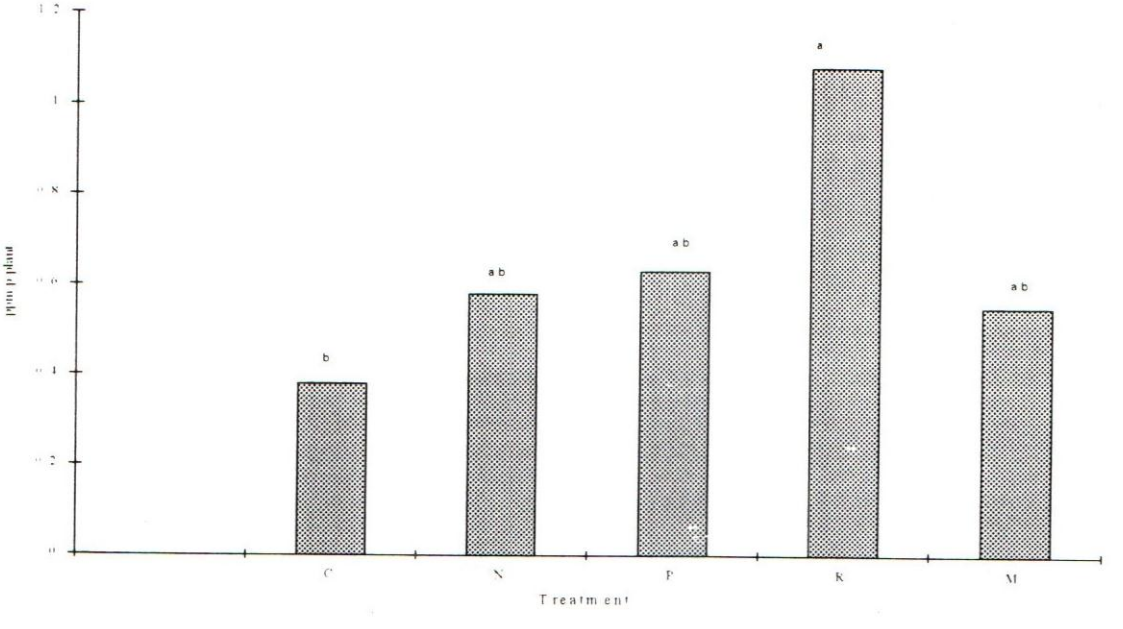


Fig. 3. Effect of Nitrogen (N), Phosphorus (P), rhizobium (R) and mycorrhiza (M) on root nitrogen content at 6 weeks after planting. Column bars followed by the same letter(s) are not significantly different at 5 %.

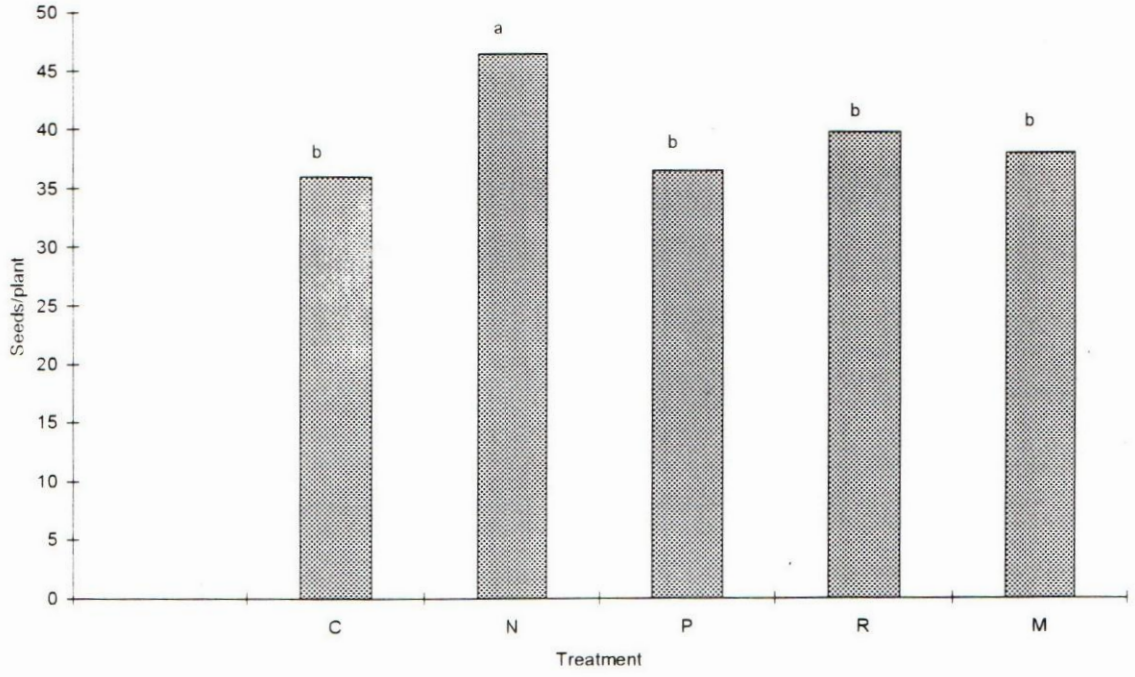


Fig. 4. Effect of Nitrogen (N), Phosphorus (P), rhizobium (R) and mycorrhiza (M) on seed number at 20 weeks after planting. Column bars followed by the same letter(s) are not significantly different at 5 %.

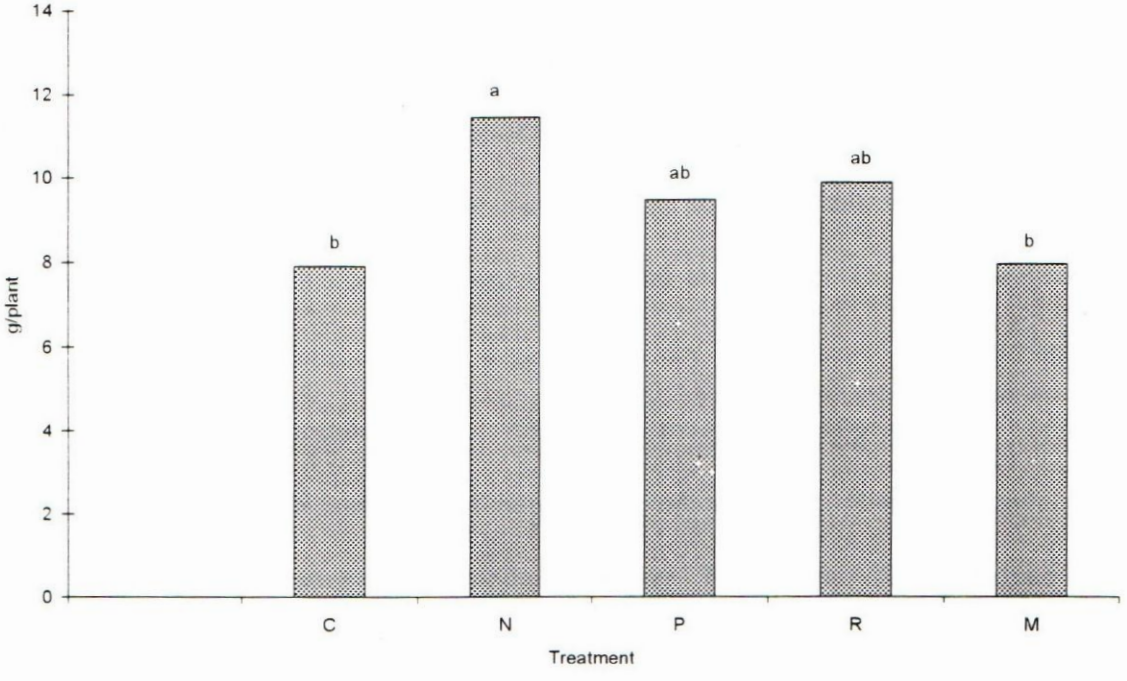


Fig. 5. Effect of Nitrogen (N), Phosphorus (P), rhizobium (R) and mycorrhiza (M) on shoot dry weight 6 weeks after planting. Column bars followed by the same letter(s) are not significantly different at 5 %.

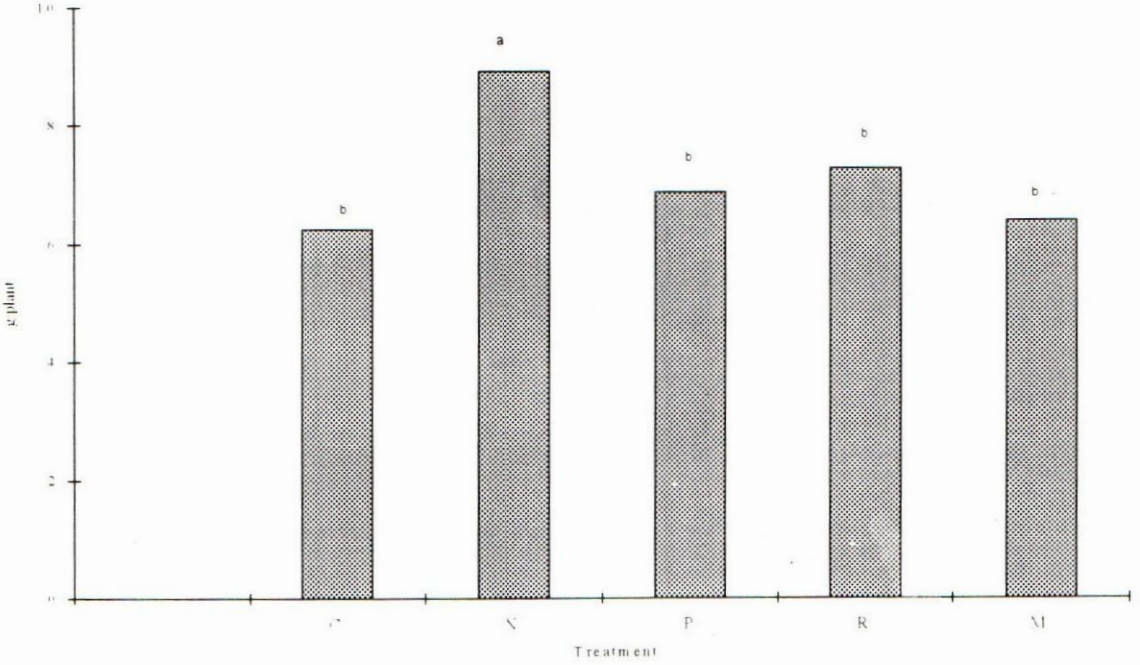


Fig. 6 Effect of Nitrogen (N), Phosphorus (P), rhizobium (R) and mycorrhiza (M) on seed dry weight at 20 weeks after planting. Column bars followed by the same letter(s) are not significantly different at 5 %.

4.4 Interaction Effects

Effects of N, P, *Rhizobium* and mycorrhiza on soybean are shown in Table 5. Shoot dry weight, seed number and dry weight increased by dual fertilization with mycorrhiza inoculation or dual fertilization combined with dual inoculation. Dual fertilization also increased nodule dry weight, root dry weight, shoot dry weight, seed dry weight and root N. Dual fertilization combined with *Rhizobium* inoculation improved the shoot dry weight, seed number, N and P, pod number. Combined *Rhizobium* with mycorrhiza inoculations increased seed P and nodule number. Nodule number improved by P fertilization with *Rhizobium* inoculation or dual inoculation with N fertilization. Combination of dual fertilization and dual inoculation increased the nodule dry weight, number and the root mycorrhiza infection. Increases were significant ($p=0.05$).

Table 5: Interaction effects on the Performance of Soybean

Measurement/ Treatment	Pod No.	Seed No.	Seed Dry Wt.	Seed N	Seed P	Shoot Dry Wt.	Shoot N	Shoot P	Root Dry Wt.	Root N	Root P	Root Infection (%)	Nodule No.	Nod. Dry Wt.
$N_0P_0R_0M_0$	18.30bc	41.00cdef	6.25e	6.00b	1.50c	7.90de	2.93ab	1.43abc	5.53bcd	1.36bc	0.55bc	4.28bc	31.00d	0.12c
$N_1P_1R_0M_0$	20.50bc	44.25bcde	8.88abc	6.43a	2.86bc	16.98a	2.31b	1.23abc	9.48a	1.54a	0.40c	6.97abc	59.25bcd	0.35a
$N_1P_0R_1M_0$	24.75ab	45.75bcde	6.95de	6.21ab	3.09abc	11.05bcd	2.68ab	0.85cd	3.83de	1.34bc	0.50bc	5.77bc	64.25bcd	0.24abc
$N_1P_0R_0M_1$	20.00bc	36.75ef	6.98de	6.33ab	1.61c	7.40e	2.50ab	0.80cd	2.73e	1.22c	1.08ab	7.77abc	60.75bcd	0.19bc
$N_0P_1R_1M_0$	18.75bc	39.00def	7.18cde	6.21ab	2.36bc	11.00bcd	2.37ab	0.88cd	4.63bcde	1.32bc	0.40c	5.18bc	93.75abc	0.22abc
$N_0P_1R_0M_1$	19.00bc	33.50f	6.40e	6.23ab	1.93c	11.45bcd	3.17a	0.98bcd	6.70b	1.24c	0.60abc	6.82abc	47.50cd	0.17c
$N_0P_0R_1M_1$	19.25bc	35.00f	6.23e	5.95b	4.04a	11.00bcd	2.95ab	0.80cd	5.15bcd	1.51ab	0.37c	9.47ab	94.00abc	0.19abc
$N_1P_1R_1M_0$	27.00a	56.00a	10.68a	6.44a	3.79ab	11.85bc	2.77ab	0.93bcd	4.28cde	1.45ab	1.48a	6.98abc	51.50cd	0.18bc
$N_1P_1R_0M_1$	20.20bc	48.75ab	8.58bc	6.26ab	1.57c	12.75bc	2.74ab	0.83cd	5.20bcd	1.34abc	0.73abc	5.35bc	74.50abcd	0.23abc
$N_0P_1R_1M_1$	20.95bc	47.00abc	8.58bcd	6.32ab	2.91bc	11.10bcd	2.78ab	1.53ab	3.75de	1.47ab	0.58bc	5.27bc	68.25bcd	0.25abc
$N_1P_0R_1M_1$	18.25c	37.25def	7.10cde	5.97b	3.18abc	10.83cde	3.02ab	0.83cd	6.48bc	1.44ab	0.53bc	2.29c	98.25ab	0.21abc
$N_1P_1R_1M_1$	20.25bc	49.25ab	9.33ab	6.28ab	1.67c	12.15bc	3.18a	1.83a	4.33cde	1.41abc	0.63abc	12.90a	150.00a	0.34ab
LSD	6.34	9.68	1.54	0.47	1.83	3.54	0.85	0.52	2.36	0.19	0.49	6.65	59.43	0.16
CV	21.03	16.03	13.59	5.33	51.36	35.16	21.47	31.88	52.59	9.80	53.84	62.48	61.57	53.70

CHAPTER FIVE

5.0 DISCUSSION

The study showed that the Kaguru soil had low indigenous *Rhizobia*l counts (<10.0 cells per gram of soil) that could nodulate soybean. The few *Rhizobia* could only form few nodules that were ineffective and hence inefficient in nitrogen fixation as indicated by the yellowing of the soybean plants. However, the soil was found to have high *Bradyrhizobium* populations as indicated by the high counts with *Faidherbia albida.* Ninety eight percent of the plants inoculated with the soil inocula were nodulated and most of the plants were green in colour which was a sign of efficient nitrogen fixation. *Faidherbia albida*, a leguminous tree species, has been shown to nodulate with slow growing *Rhizobia* (Dupuy *et al.*, 1994). This was confirmed in this study whereby there was nodulation when *Feidherbia albida* was inoculated with USDA 110, a slow growing *Rhizobium* strain (Table 3). The legume has a very high natural ability to nodulate across a number of sites (Odee *et al.* 1995) and can also nodulate with fast-growing *Rhizobia* (Odee *et al.*,1997). The humic *Nitisol*, however, was found to be completely devoid of fast growing *Rhizobia*, as indicated by the absence of nodules on *Acacia polyacantha.* This is also a leguminous tree species which nodulates only with fast growing *Rhizobia* (Duhoux and Dommergues, 1985).

Lack of effective nodulation of the soybean plants, variety Nyala, growing in the humic *Nitisol* could have been due the variety specificity to *Rhizobia*l strains. Although the humic Nitisol had a high indigenous Bradyr*hizobium* population, none of the strains was compatible with the soybean plants. Host specificity for *Rhizobia*l strains has been

reported in plant species by Turk and Keyser (1992). Specificity of *soybean* for the microsymbiont *Bradyrhizobium japonicum* has also been observed in tropical soils (Brooks *et al.*,1994, Thies *et al.*,1995, Anyango *et al.*, 2000). Lack of effective nodulation on soybean could also be attributed to the small population of compatible indigenous *Rhizobia* in the humic *Nitisol*. Thies *et al* (1991) observed that the population of indigenous *Rhizobia* affects the likelihood and magnitude of a legume response to a certain *Rhizobium* strain while the success of the *Rhizobium*-legume symbiosis in the soil depended on both the number of *Rhizobia* and their effectiveness in fixing nitrogen (Vincent, 1982). Soybean is a 'new' crop in Kenya and therefore would be expected to nodulate only when inoculated with a compatible specific commercial strain. The absence or little nodulation of soybean in most soils of the tropics with introduced plant species has been reported by Odee *et al* (1995) and Shepherd *et al* (1996) due to low compatible native populations. Thies *et al.*, (1991) found that soybean frequently responded to inoculation with *Bradyrhizobium japonicum* when first introduced into an area where the indigenous populations were low or absent. This has been confirmed in this study where the native *Rhizobia*l population in the humic *Nitisol* had a poor ability to nodulate the soybean of variety Nyala. Only the soybean inoculated with the commercial strain (USDA 110) had a well nodulated root system. This showed that the commercial strain was a better competitor for nodule sites and in N fixation with the soybean than the indigenous strains.

With reference to NARL, 1981, soil analysis results showed that soil N and P were below levels for optimum soybean growth. However, fertilizer N significantly ($p=0.05$)

increased seed number and seed dry weight. This concurred with the results by Mahdi *et al* (1992) who found that the application of N to soybean improved all the measured parameters, and also with the results by Mochoge (1992) who showed that an addition of inorganic N (75 kg N/ha) improved bean (*Phaseolus vulgaris*) yields. Lack of significant yield response to applied nitrogen was also observed in this study. This could be due to the fact that all the treatments had effective nodulation and hence were fixing nitrogen. The effect of the applied nitrogen could therefore not be separated statistically. Failure to realize a positive response to applied N was also observed by Teresia (1994) who found no advantage to applied nitrogen fertilizer on soybean performance. Fertilizer P did not significantly improve the performance of soybean. This finding is in agreement with the observation of Howard *et al.*(1990; 1998). Nitrogen and phosphorus combined significantly ($p=0.05$) increased seed, shoot, root and nodule dry weights. According to Hussein *et al.*, (1991) and Smithson *et al.* (1993), a combination of N and P improves yields of legumes since both the elements are essential for plant growth and development.

Inoculation of soybean with AMF did not significantly improve its performance. The soil was low in available P and as a positive significant response to AMF inoculation is always expected in nutrient-deficient soils (Dodd *et al.*, 1990, Arias *et al.*, 1991). This could have been due to the relatively slow infection rate of the soybean plants by the AMF. Root staining showed that most of the root infection process was at the initial stages (Plate 4). The stained root segments observed under the microscope showed swollen hyphae at the point of contact with the root, just about to penetrate the root. Arbuscules (site of nutrient exchange) were also seen in the process of formation (Plate

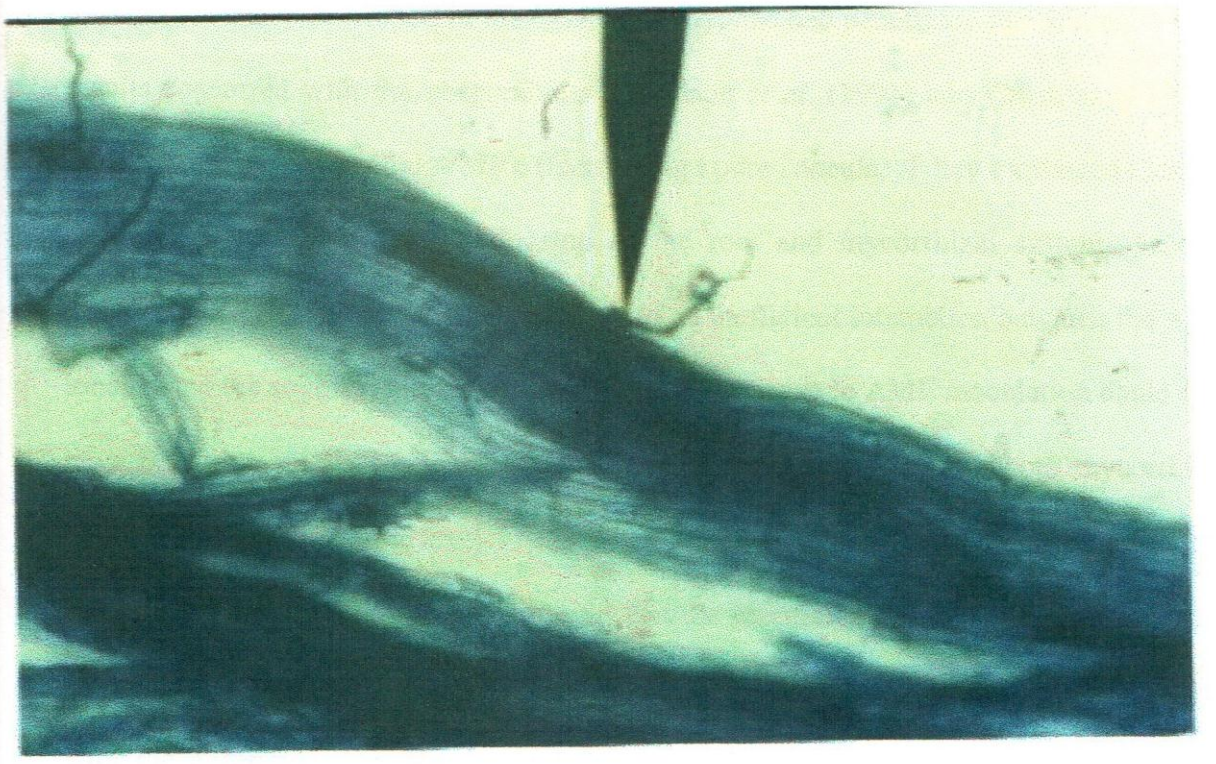


Plate 4: An hyphae about to penetrate a root at 6 weeks after planting

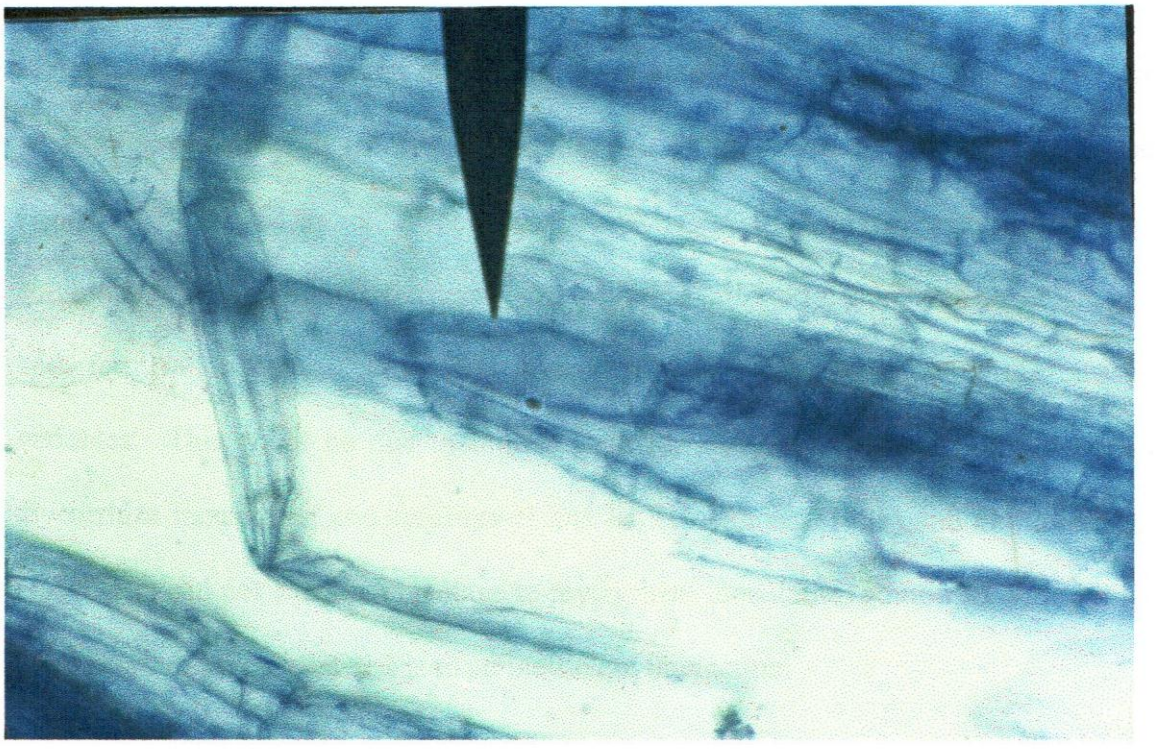


Plate 5: An arbuscule in the process of formation inside the root cells at 6 weeks after planting

5), with few fully developed in the case where hyphae had penetrated the root cells. The results concurred with the observations of Wilson *et al.* (1991) and Crush *et al* (1995) who showed no or limited AMF effects using spore inocula. They explained that the response usually take time to develop as the root and mycorrhizal systems become established. Sylvia *et al* (1993) related this to an inefficient interaction between the AMF inocula and the soybean plants. Lack of positive response to AMF inoculation could also have been due to the soybean variety used in this experiment not being mycorrhizal dependant. The infection level of the inoculated plants was comparable to that of the uninoculated control plants which showed that the inoculation of the soybean plants with the mycorrhizal species, *Glomus eticulantum* was not beneficial. These results concurred with the observation by Nwoko and Sanginga (2000) who realized that most of the soybean lines they were working with did not respond to mycorrhiza inoculation as they were not mycorrhizal dependent.

Significant ($p=0.05$) increases observed in seed number, and seed and shoot dry weights were in agreement with the findings by Medina *et al.* (1990) and, Mahdi and Atabani, (1992) who realized increased performance of legumes inoculated with mycorrhiza in combination with inorganic fertilizers. However, in this study, there was no advantage to both fertilization with N and P and mycorrhiza inoculation for most of the measured variables. This could be due to limited time for an efficient interaction between the mycorrhiza inoculation and fertilizer N and P. Rosemeyer (1991) and Guillemin *et al* (1995) also did not find an advantage to mycorrhiza inoculation combined with fertilizer application and they related this to an inefficient interaction.

Soybean grown in potted soil in which no soybean had been grown before and inoculated with the *Rhizobium* commercial strain (USDA 110) had the nodule number and root P significantly increased compared to the control plants. This positive response to *Rhizobium* inoculation could be due to the humic *Nitisol* having a low population of ineffective indigenous *Rhizobia*. The inoculant therefore had no competition from the indigenous *Rhizobia* in the soil. Allen and Allen (1981) reported that soybean was relatively a specific host and could not nodulate effectively when grown in the field for the first time. The humic *Nitisol* was found to be low in total N and therefore *Rhizobium* inoculation enhanced nitrogen fixation and hence uptake by the soybean plants (Dou *et al.*, 1993). *Rhizobium* inoculation did not have a significant effect on most of the measured parameters. This could be due to efficient N fixation in all the treatments which were also well nodulated, hence the benefit to *Rhizobium* inoculation could not be established. Lack of a significant response has also been observed by Milev (1998) and Voss *et al* (1998).

Nodule number was significantly increased by *Rhizobium* inoculation combined with phosphorus fertilization. The humic *Nitisol* was low in both N and P. This environment favoured the *Rhizobium* activity (Arias *et al.*, 1991). Musandu and Ogendo (2000) and Jan *et al.*,(1994) also recorded benefits due to *Rhizobium* inoculation by P interaction effects. Dual fertilization and *Rhizobium* inoculation significantly ($p=0.05$) increased the pod number, seed number, seed dry weight, seed P, shoot dry weight and root P. This could be related to the low content of soil nutrients and the low and ineffective indigenous *Rhizobia*l population of the humic *Nitisol*. Jat *et al* (1998) observed improved

soybean performance due to dual fertilization and *Rhizobium* inoculation. The combined effect of dual fertilization with *Rhizobium* inoculation had no benefit to soybean performance whereby most of the measured parameters were comparable to the control.

Dual inoculation did not improve soybean performance. The humic *Nitisol* was limited in nutrient supply and hence a stimulatory effect of nitrogen fixation could be attributed to mycorrhiza inoculation (Kucey and Paul, 1982). Lack of a positive response to dual inoculation was also found by Singh (1994) working with soybean. Dual inoculation by dual fertilization interaction significantly increased nodule number, nodule dry weight and % root infection. This could have been due to the combined starter fertilizer effect which is important in the early stages of growth when the seedlings have not started fixing nitrogen since the process of nodule formation by *Rhizobium* is energy demanding.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATION

6.1 Conclusion

1) *Rhizobium* inoculation is necessary to ensure adequate N supply to soybean.
2) Applied N increases the yield components of soybean grown in humic nitisols deficient in nitrogen.
3) The N by P interaction effect increases the yields and yield components of soybean.
4) The *Rhizobium* inoculation by mycorrhiza inoculation effects cause increased P uptake and nodulation of soybean.

6.2 Recommendation

The factors: N, P and *Rhizobium* and mycorrhiza inoculations should be studied in field experiments in order to determine the optimum rates for increased soybean production in different soil types in Kenya.

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