# EVALUATION OF RHIZOBIUM AND MYCORRHIZA INTERACTIONS IN SUSTAINABLE MAIZE (*Zea mays* L.) AND SOYABEAN (*Glycine max* L.) PRODUCTION

**BY**

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# ABSTRACT

Maize and soyabean are among the staples in the world. Their sustainable production through the use of synthetic fertilizer causes soil degradation and ground water contamination. An alternative means of boosting and sustaining the production of these staples through the use of rhizobium and mycorrhiza have been reported but there are contradictory reports on interactions between the two organisms. Therefore, the objective of this study was to assess the influence of *Rhizobium japonicum* and *Glomus etunicatum* on maize and soyabean production.

Three farmlands in Oyo town were purposively selected. In the first and second farmlands were planted sole maize and soyabean respectively and were rotated the following season while both crops were intercropped on the third farmland. The experimental layout was randomised complete block design in split plot with three replications. The treatments were mycorrhizal, rhizobium, combined inoculations and uninoculated (controls). Maize was inoculated with *Glomus etunicatum*, while soyabean was inoculated with *Glomus etunicatum* and broth of *Rhizobium japonicum*. The plants' population densities were 66,667 and 266,667 respectively. Growth parameters were measured using established methods at two weeks interval. The plants' biomass, yield, proximate and nutrient analyses were determined using standard methods. Data were analysed using descriptive statistics and ANOVA at $p = 0.05$.

The average heights (cm) of mycorrhiza, rhizobium and combined inoculated plants were 59.8±3.0, 68.3 ±1.8 and 63.7±1.2 respectively while that of their uninoculated counterparts was 52.7±3.4 in both sole and intercropped soyabean. Similarly, significant differences in biomass production were obtained between inoculated (3.7, 3.9, 3.9 g) and uninoculated (2.9 g) soyabean. Sole and rotated soyabean had significant biomass values which were 34.7 and 23.4 % respectively higher than the intercropped soyabean. There was no significant difference in nutrient contents of soyabean; its percentage proximate contents showed significant effects of rhizobium (4.5), mycorrhiza (4.0) or combined inoculation (4.2) in relation to uninoculated treatments (3.1). In intercropped and rotated maize, higher values of number of leaves and plant height were obtained in the mycorrhizal inoculated treatments compared with uninoculated counterparts. Biomass values of the intercropped and rotated maize were 15.5 and 12.5 % respectively higher than that of sole maize. There was significant main effect of mycorrhizal inoculation on nutrient contents of maize in intercropped and rotated farmlands. The grain yields of sole and combined inoculated soyabean ranged between 648.1–738.7 kgha$^{-1}$ and significantly outweighed that of the uninoculated counterparts (524.6–584.9 kgha$^{-1}$). There were no significant differences in grain yield of sole and intercropped maize, however, grain yield of rotated maize (585 kgha$^{-1}$) was significantly higher than that of sole maize (512.9 kgha$^{-1}$). Interactions between *R. japonicum* and *G. etunicatum* stimulated growth of both crops, while intercropping enhanced growth and biomass production of maize but had negative effects on soyabean.

Interaction between *Rhizobium japonicum* and *Glomus etunicatum* had synergistic effects on growth of maize and soyabean. The combination of the two organisms and the systems of farming favoured the yield of both crops. The use of both microsymbionts combined with the cultural farming systems is therefore recommended to boost soyabean and maize production.

**Keywords**: *Glomus etunicatum, Rhizobium japonicum,* Intercropping, Crop rotation

**Word count**: 499

# DEDICATION

To Almighty Allah for His mercies and blessing over me and to my parents Alhaji Hamzat, Gbadegesin Alakitan and Alhaja Hamzat, Fadhilat Aweni (Nee Daramola)

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# CERTIFICATION

I certify that this work is carried out by Mr. Mumini Adekunle AMUSAT in the Department of Botany, University of Ibadan.

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# TABLE OF CONTENTS

**CHAPTER 1**

**CHAPTER 2**

**CHAPTER 3**

**CHAPTER 4**

**CHAPTER 5**

**CHAPTER 6**

# LIST OF TABLES

# LIST OF FIGURES

Figure Page

Photograph of sole soyabean

| Treatment | Intercropped Maize | | | | Sole Maize | | | |
|---|---|---|---|---|---|---|---|---|
| | Leaf dry wt g plant$^{-1}$ | Shoot dry wt g plant$^{-1}$ | No of cob | Cob dry wt g plant$^{-1}$ | Leaf dry wt g plant$^{-1}$ | Shoot dry wt g plant$^{-1}$ | No of cob | Cob dry wt g plant$^{-1}$ |
| $M^+$ | 23.67a | 82.97a | 1.61a | 154.36a | 20.89a | 71.30a | 1.22a | 107.60a |
| $M^-$ | 22.70a | 77.76a | 1.17a | 25.95b | 20.14a | 66.58a | 1.17a | 106.64a |
| Main | | | | | | | | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| effect | ns | Ns | ns | * | Ns | ns | ns | ns |

| TMT | No of Leaves | Height (cm) | Total dry wt (g plant$^{-1}$) | Leaf Area (cm$^2$) | No of Nodules | No of Pods | Shoot dry wt (g plant$^{-1}$) | Root dry wt (g plant$^{-1}$) | Leaf dry wt (g plant$^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|
| M | 31.77a | 65.00b | 4.46ab | 11.53a | 6.44ab | 35.56a | 3.47ab | 1.02a | 1.80a |
| MR | 33.13a | 65.33b | 4.86ab | 12.06a | 7.56ab | 36.44a | 3.80ab | 1.06a | 1.90a |
| R | 33.89a | 71.78a | 5.11a | 12.96a | 10.56a | 39.67a | 4.02a | 1.09a | 2.19a |
| C | 27.21b | 60.89b | 3.86b | 11.28a | 3.22b | 24.33b | 2.96b | 0.89a | 1.20b |
| **Intercropped Soybeans** | | | | | | | | | |
| M | 29.56a | 54.67ab | 3.27ab | 11.80a | 3.89b | 27.33ab | 2.52ab | 0.76a | 1.13b |
| MR | 31.22a | 62.10ab | 3.71a | 11.86a | 8.78ab | 27.67ab | 2.99a | 0.813a | 1.68ab |
| R | 33.33a | 64.89a | 3.93a | 13.06a | 11.89a | 33.66a | 3.12a | 1.39a | 2.06a |
| C | 22.00b | 46.67b | 2.67b | 9.03a | 2.34b | 19.11b | 1.91b | 0.63a | 1.06b |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Main effect | * | * | * | ns | ns | * | * | ns | ns |
| M | * | * | * | ns | * | * | * | ns | * |
| R | * | * | * | ns | * | * | * | ns | * |
| MR | | | | | | | | | |
| Interactions | ns | ns | * | ns | * | ns | ns | ns | ns |
| R x M | ns | ns | ns | ns | * | ns | ns | ns | ns |
| R x MR | ns | ns | ns | ns | ns | ns | ns | ns | ns |
| M x RM | | | | | | | | | |

# CHAPTER 1

# INTRODUCTION

Crop production in sustainable manner is desirable in order to cater for continuous increasing population growth which subsequently demands for more food production. The quantity and quality of farm products obtained from a soil depend largely on the fertility of that soil. The immediate source of nutrients to the plant is the soil as it contains almost all the mineral requirements for plant growth and survival. However, the soil may be limited in some nutrients to support plant growth. For example, as a result of continuous cropping of land, some soil nutrients could be exhausted. When this happens, the plant yields obtained from such a soil or land will be comparably low both in quality and quantity. In order to boost plant yield, there is the need to device ways through which soil nutrients can be replenished and made available to the plant. Various methods had been adopted in the past, among which are the applications of chemical fertilizers, farm yard manure, bush fallowing, and planting of cover crops. Each of these methods has its own advantages and disadvantages. For instance, continuous use of nitrogen fertilizer results in soil acidification (Juo *et al.,* 1995) and has soil degradation. Despite the obvious advantages of the leguminous crops to the natural re-growth in fallow systems, use of cover crops for fallow in tropical Africa is still uncommon. This is mainly attributed to two shortcomings involved with cover crop-fallow: cost of cover crop establishment and the competition with food crops (Ashley, 2007).

The increase in pressure for food production, coupled with wide land requirement for land fallowing calls for the development of alternative agricultural practice which can boost plant production and retention of the same piece of land. Enhanced crop production can be achieved through the introduction of symbiotic non-pathogenic micro-organisms – rhizobium, nitrogen fixing bacteria and arbuscular mycorrhizal fungi recognized as soil biological resources in crop production which can increase nutrient uptake, particularly P, in nutrient deficient soil (Osonubi *et al;* 1991).

The beneficial effects of mycorrhiza on plant growth are generally attributed to nutritional factors but the fungi may also protect plants against path infections. Graham and Menge (1982) found a reduction in severity of take-all disease of wheat due to AMF colonization.

Rhizobium is a symbiotic bacterium usually associated with the root nodules of leguminous plants for nitrogen (N) fixation. Nitrogen is important to life because it is a component of proteins and nucleic acids (Roskoski, 1992). The reservoir of nitrogen (biological inactive element) is the atmosphere and can not be utilized directly in this form by living organisms. Nitrogen enters chemistry of life through nitrogen cycle as a result of activities of nitrogen fixing bacteria, which infect, nodulate, symbiotically fix atmospheric nitrogen and convert it to ammonia (Roskoski, 1992; Tamiru *et al*., 2012) when legume is inoculated with specific strain.

The benefit conferred on farmers is that inoculation helps to increase crop yields and reduce the input costs of N fertilizer. Yield responses to inoculation, even in fields with soil rhizobial populations sufficient to infect the particular legume host, are common for most grain legumes in field trials conducted in the northern Great Plains of Canada (Vessey, 2004). Generally, it had been reported McKenzie *et al*., (2001) and Bullied *et al*., (2002) with clear evidence that if a particular legume crop is being planted for the first time in a field which has never grown an inoculated legume crop of the same inoculation group, a crop can significantly respond to inoculation. However, even where yield responses are not evident, inoculation may still have benefits by increasing both seed and plant residues N levels and N levels in plant residues (McKenzie et al., 2001). Although higher residual N levels in soil and stubble may not be an immediate benefit to producers, nevertheless, it is an important component of the beneficial rotational effect of legumes in cropping systems (Kessel and Hartley, 2000).

Soyabean is a grain legume of high economic importance grown in diverse environments throughout the world. It is primarily essential as high protein meal and secondarily as oil crop and accounts for 30% of world’s processed vegetable oil (Graham and Vanace, 2003). It is being cultivated in South Africa and many other regions of the world as biofuel (Spark *et al*., 2010).

It has been observed that soyabean and other legumes form tripartite symbiotic association with nodule inducing bacteria of the genera *Rhizobia* (Caetano-Anolles and Greshoff, 1991) and with AM fungi (Bonfate -Fasolo, 1987; Koide and Schreiner, 1992).
Both the rhizobium and fungal micro-symbionts improve mineral nutrition of the host plant in exchange for assimilates provided by the latter. The nitrogenase enzyme of

rhizobia fixes atmospheric nitrogen in the nodule (Thorneley, 1992) and fungal hyphae facilitate the uptake of ions, mainly phosphate, in mycorrhizal roots (Hayman, 1983; Smith and Gianinazi-Pearson, 1988; Bowen *et al.,* 1995). In most cases investigated, especially when both nitrogen and phosphate are limiting factors, AM fungi and Rhizobium appear to act synergistically, since combined inoculation with mychorrhiza and rhizobia enhance plant growth and crop yield more than inoculation with either microsymbiont alone and also lead to a higher degree of colonization by the two microsymbionts (Daft and El-Ghiami, 1974; Cluett and Boucher, 1983; Kawai and Yamamoto, 1986; Pacovsky *et al.,* 1986; Chaturveli and Singh, 1989). Reports (Manjunath and Bagyaraj, 1984; Purcino *et al.,* 1986 and Benthlefalvay *et al.,* 1981) indicated that the AM increases P uptake, which under P stress condition would itself increase plant growth. Improved P nutrition in turn would favour the nitrogen fixation process by rhizobium. The combined effects of the two microsymbionts resulted in further growth enhancement. (Subba Rao and Tilak, 1986; Azco'n-Aguilar and Barea, 1981). However, factors that influence each symbiont separately may also affect the tripartite relationship. Different edaphic factors which influence the behaviour and effectiveness of the symbiosis would affect the combination as well.

Intercropping is considered as the practical application of ecological principles such as diversity, crop interaction and other natural regulation mechanisms. Intercropping is defined as the growth of two or more crops in proximity in the same field during a growing season to promote interaction between them (Whighaham and Bharati, 1986). Available growth resources, such as light, water and nutrients are more completely absorbed and converted to crop biomass by the intercrop as a result of differences in competitive ability for growth factors between intercrop components. Efficient utilization of growth resources could lead to yield advantages and increased stability compared to sole cropping (Makinde *et al*., 2011, Sani *et al*., 2011).

Soyabean and maize are commonly grown in intercropping with each other because of their similar time of maturity. Rao and Willey (1987) reported that time of maturity and growth habit of the component crops are important determinants of the productivity under intercropping systems.

The declining soil fertility is widely perceived and regarded as a major limitation to increasing yields, and a threat to sustainability of the maize based cropping systems. Maize is a staple food which dominates agriculture in many regions of the world

(Nkhuzenje *et al.,* 2002). Its productivity could be improved by use of high yielding varieties and application of inorganic nitrogen fertilizers. However, these are too expensive for most smallholder and resource-poor farmers. Alternatively, nitrogen-fixing system such as that of legume and rhizobium offer an economically attractive and ecologically stable means of reducing external inputs and improving internal resources. The incorporation of soyabean in maize cropping system will help supply nutrients especially nitrogen, which is critical in maize production (Nkhuzenje *et al.,* 2002).

Cereal/legume intercropping system is generally more productive than reference sole crops. The biological advantage of intercropping involves the complementarities of resources use by crops grown in combination. Such resources include nutrients, water and light (Willey, 1979). This complementarity can be regarded as temporary when the timing of peak resource need differ or spatial when differences in resources use arise from canopy or root dispersion (Willey, 1990). From ecological point of view, Vandermeer (1989) pointed out that yield advantages of intercropping are related to lack of competition between two species.

Cereal/legume intercropping involves partitioning of two major environmental resources: nitrogen and light. Nitrogen partitioning is of special interest, since legume has access to the atmospheric nitrogen pool through symbiotic nitrogen fixation. Legumes are likely to influence the nitrogen nutrition of intercropped cereal in two different manners: through excretion of fixed nitrogen in the rhizosphere (Sing *et al*., 1986; Ta *et al.,* 1986) and via competition with cereal for soil nitrogen. Direct nitrogen transfer from the legume to the cereal during the same season has been proposed (Eaglesham *et al*., 1981; Bandyopadhyay and De, 1986). Under field conditions, nitrogen absorption by cereals can stimulate nodulation of legumes (Thompson, 1977; Salez, 1986), but sometimes has little or no influence on nodulation (Nair *et al.,* 1979; Baker and Blamey, 1985).

Maize represents a highly compatible companion crop for short-statured legumes, because of its efficient C4-type photosynthetic apparatus of its leaf structure (Trenbath, 1986). In order to accommodate an under story legume, population density level of maize must be kept low to reduce light capture in upper canopy and low interception of light by tassel (Tetio-kagh and Gardner, 1988). Soyabean seems to be tolerant to light shading during the whole growth cycle (Wahua and Miller, 1978), but timing of peak light interception by maize is determinant, as growth stage of legumes are not equally tolerant to light intensity (Schout *et al.,* 1984).

Intercropping of soyabean with maize is an effective way of silage production comparable with monocropped corn. The advantage of the corn-soyabean intercropping is that protein concentration which affects the quantity of the silage is higher with the addition of the nitrogen rich soyabean (Ahamed and Rao, 1982; Herbert *et al.,* 1984; Toniolo *et al.,* 1987; Martin *et al.,* 1990).

An intercrop of corn and soyabean grown with narrow rows spacing would protect the soil during the growing season more effectively than a monocrop of either of these species. Martin *et al.* (1991a) intercropped corn and soyabean with 40 cm and 20 cm row spacing and found that narrower row spacing had no adverse effect on the yield of either crop. Intercropped corn has been shown to benefit from association with soyabean plant due to transfer of N from the legume to the corn (Martin *et al*., 1991b).

Intercropping is a method for simultaneous crop production and soil fertility building. It may also contribute to the interception of nitrogen leaching risks sometimes observed for sole crops such as grain legumes due to changes in incorporated residue chemical quality involving nutrient turnover. It is also an ecological method to manage pests, disease and weed via natural competitive principle that allow for more efficient resource utilization. The same competitive principles also contribute to an improved quality of intercrop products. The inclusion of nitrogen fixing crops in an intercrop leads to the utilization of the renewable resources of atmospheric nitrogen, which increases the sustainability of the agro system. Intercropping can also be regarded as a practice to increase the production of less stable crops such as grain legumes and thereby contribute to lowering the protein deficit in effective utilization (EU) at lower risk for the farmer (Moriola *et al*., 2014).

Intercropping perspective in arable systems and the potential area for intercrops in organic farming is large considering the possible economic benefits and future legal requirement in feed and food industry. Re-introducing intercropping in organic agriculture to a greater extent should not be reversion to old methodology, but rather considering the usefulness of this old and sustainable cropping practice in a modern, innovative and technology-oriented organic agriculture. Furthermore, intercropping constitutes a concrete means to increase the diversification of agricultural ecosystems, for which there is a worldwide appeal.

There is also increasing evidence that fertilizer alone cannot sustain yields for long periods. For example, in continuous rice or maize cropping with two to three crops grown annually, the use of N fertilizer increased with time but the yields often remained stagnant (Cassman and Pingali, 1995).

This reflects a higher fertilizer requirement to produce the same yields, implying a decline in yield response to nutrients, possibly because of an overuse of fertilizer. This is a reason for concern. As an alternative, tailoring plants to fit into the soil through the use of soil microorganisms such as symbiotic nitrogen fixing bacteria like rhizobium and mycorrhiza which can convert atmospheric nitrogen to soil nitrite and nitrate and can mobilize phosphorus respectively will make nutrient which may be otherwise unavailable for plant use and thus improving yield is considered more economical than changing the soil.

Different species of rhizobium are differentiated by their host preference. Six cross inoculation group of legumes are widely recognized and are being nodulated by six species of rhizobia (Jensen, 1967). There is no doubt that specificity exists between rhizobial strain and the legume, and compatibility between the two is essential for successful nodulation. This necessitates using specific cultures for different legumes. When growing a new legume species on a soil, it is necessary that the appropriate rhizobial culture be applied (Prasad and Power, 1997; Tamiru et al., 2012).The one that commonly nodulate soyabean and cowpea is known as *Rhizobium japonicum.*

Synergistic or antagonistic interactions among the components of the tripartite symbiotic associations have been suggested but with few documentations. This study is intended to look into various options of the tripartite associations with a view to recommending the farming system(s) that will increase crop productivity which will be sustainable. With these considerations in mind, the following objectives are desingned and they are:

1. To determine the impact of intercropping on dry matter and grain yields of intercropped maize and soyabean.
2. To determine the impact of residual Nitrogen fixed by soyabean (*Glycine max*) on maize growth through crop rotation.
3. To improve maize and soyabean yield through the introduction of arbuscular mycorrhizal fungi and rhizobium.

4. To examine the combined effects of the two microsymbionts and systems of farming on the yields of maize and soyabean.

# CHAPTER 2

# 2.0 LITERATURE REVIEW

## 2.1 Rhizobium and Nitrogen Fixation

Nitrogen is an essential plant nutrient. It is the nutrient that is most commonly deficient in soils, contributing to reduced agricultural yields throughout the world. Nitrogen can be supplied to crops by Biological Nitrogen Fixation (BNF), a process which is becoming more important for not only reducing energy costs, but also in seeking more sustainable agricultural production. Nitrogen fixing micro-organisms could therefore be an important component of sustainable agricultural systems (FAO, 2000).

The element nitrogen, or "azote," meaning "without life," as Antonie Lavoisier called it about 200 years ago, has proved to be anything but lifeless, since it is a component of food, poisons, fertilizers, and explosives (Schoot, 1990). The atmosphere contains about $10^{15}$ tonnes of $N_2$ gas, and the nitrogen cycle involves the transformation of some $3 \times 10^9$ tonnes of $N_2$ per year on a global basis (Postgate**,** 1982). However, transformations (e.g., $N_2$ fixation) are not exclusively biological. Lightning probably accounts for about 10% of the world's supply of fixed nitrogen (Sprent and Sprent**,** 1990). The fertilizer industry also provides very important quantities of chemically fixed nitrogen. World production of fixed nitrogen from dinitrogen for chemical fertilizer accounts for about 25% of the Earth's newly fixed $N_2$, and biological processes account for about 60%. Globally the consumption of fertilizer-N increased from 8 to 17 kgha$^{-1}$ of agricultural land in the 15-year period from 1973 to 1988 (FAO**,** 1990). Significant growth in fertilizer-N usage has occurred in both developed and developing countries (Peoples *et al.*, 1995). The requirements for fertilizer-N are predicted to increase further in the future (Subba-Rao**,** 1980); however, with the current technology for fertilizer production and the inefficient methods employed for fertilizer application, both the economic and ecological costs of fertilizer usage will eventually become prohibitive.

For more than 100 years, biological nitrogen fixation (BNF) has commanded the attention of scientists concerned with plant mineral nutrition, and it has been exploited extensively in agricultural practice (Burris, 1994; Dixon and Wheeler, 1986). However, its importance as a primary source of N for agriculture has diminished in recent decades as increasing amounts of fertilizer-N have been used for the production of food and cash crops (Peoples *et al.,* 1995). However, international emphasis on environmentally sustainable development with the use of renewable resources is likely to focus attention on the potential role of BNF in supplying N for agriculture (Dixon and Wheeler 1986; Peoples *et al.,* 1995). The expanded interest in ecology has drawn attention to the fact that BNF is ecologically benign and that its greater exploitation can reduce the use of fossil fuels and can be helpful in reforestation and in restoration of misused lands to productivity (Burris, 1994; Sprent and Sprent, 1990).

Currently, the subject of BNF is of great practical importance because the use of nitrogenous fertilizers has resulted in unacceptable levels of water pollution (increasing concentrations of toxic nitrates in drinking water supplies) and the eutrophication of lakes and rivers (Al-Sherif, 1998; Dixon and Wheeler, 1986; Sprent and Sprent, 1990). Further, while BNF may be tailored to the needs of the organism, fertilizer is usually applied in a few large doses, up to 50% of which may be leached (Sprent and Sprent, 1990). This not only wastes energy and money but also leads to serious pollution problems, particularly in water supplies.

### 2.2 Significance of Biological $N_2$ Fixation to Soil Fertility

Biological Nitrogen Fixation (BNF) is an efficient source of nitrogen (Peoples *et al.,* 1995). The total annual terrestrial inputs of N from BNF as given by Burns and Hardy (1975) and Paul (1988) ranged from 139 to 175 million tonnes, with symbiotic associations growing in arable land accounting for 25 to 30% (35 to 44 million) and permanent pasture accounting for another 30% (45 million tons). While the accuracy of these figures may be open to question (Sprent and Sprent, 1990), they do help illustrate the relative importance of BNF in cropping and pasture systems and the magnitude of the task necessary if BNF is to be improved to replace a proportion of the 80 to 90 million tonnes of fertilizer-N expected to be applied annually to agricultural land by the end of the decade (Peoples *et al.,* 1995a). Much land has been degraded worldwide, and it is time to stop the destructive uses of land and to institute a serious reversal of land degradation (Burris, 1994). BNF can play a key role in land remediation.

An examination of the history of BNF shows that interest generally has focused on the symbiotic system of leguminous plants and rhizobia, because these associations have the greatest quantitative impact on the nitrogen cycle. A tremendous potential for contribution of fixed nitrogen to soil ecosystems exists among the legumes (Brockwell, *et al*., 1995; Peoples *et al*., 1995b; Tate, 1995). There are approximately 700 genera and about 13,000 species of legumes, only a portion of which about 20% (Sprent and Sprent, 1990) have been examined for nodulation and shown to have the ability to fix $N_2$. It had been reported that rhizobial symbioses with greater than 100 agriculturally important legumes contribute nearly half the annual quantity of BNF entering soil ecosystems (Tate**,** 1995). Legumes are very important both ecologically and agriculturally because they are responsible for a substantial part of the global flux of nitrogen from atmospheric $N_2$ to fixed forms such as ammonia, nitrate, and organic nitrogen. Whatever the true figure, legume symbioses contribute at least 70 million tonnes of N per year, approximately half deriving from the cool and warm temperate zones and the remainder deriving from the tropics (Brockwell *et al*., 1995). Increased plant protein levels and reduced depletion of soil N reserves are obvious consequences of legume $N_2$ fixation. Deficiency in mineral nitrogen often limits plant growth, and so symbiotic relationships have evolved between plants and a variety of nitrogen-fixing organisms (Freiberg *et al.,* 1997).

Most of the attention in this review is directed toward $N_2$ fixation inputs by legumes because of their proven ability to fix $N_2$ to meet its requirements and those of subsequent crops (Aulakh *et al*., 2003) and their contribution to integral agricultural production systems in both tropical and temperate climates (Peoples *et al*., 1995a). Successful *Rhizobium*-legume symbioses will definitely increase the incorporation of BNF into soil ecosystems. *Rhizobium*-legume symbioses are the primary source of fixed nitrogen in land-based systems and can provide well over half of the biological source of fixed nitrogen (Tate**,** 1995).

Atmospheric $N_2$ fixed symbiotically by the association between *Rhizobium* species and legumes represents a renewable source of N for agriculture (Peoples *et al.,* 1995b). Values estimated for various legume crops and pasture species are often impressive, commonly falling in the range of 200 to 300 kg of N $ha^{-1}$ $year^{-1}$ (Peoples *et al*., 1995b). Yields increases of crops planted after harvesting of legumes are often equivalent to those expected from application of 30 to 80 kg of fertilizer-N $ha^{-1}$. Inputs of fixed N by alfalfa, red clover, pea, soyabean, cowpea, and vetch were estimated to be about 65 to 335 kg of N ha$^{-1}$ year$^{-1}$ (Tate, 1995) or 23 to 300 kg of N ha$^{-1}$ year$^{-1}$ (Wani *et al*., 1995). However, the measured amounts of N fixed by symbiotic systems may differ according to the method used to study $N_2$ fixation (Sellstedt *et al.,* 1993). Inputs into terrestrial ecosystems of BNF from the symbiotic relationship between legumes and their rhizobia amount to at least 70 million tons of N per year (Brockwell *et al*., 1995). This enormous quantity will have to be augmented as the world's population increases and as the natural resources that supply fertilizer-N diminish. This objective will be achieved through the development of superior legume varieties, improvements in agronomic practice, and increased efficiency of the nitrogen-fixing process itself by better management of the symbiotic relationship between plants and bacteria.

Two groups of plants are able to form nitrogen-fixing root nodule symbioses with soil bacteria: legumes (plus Parasponia in the Ulmaceae family) associate with rhizobia, while the so-called actinorhizal plants belonging to eight angiosperm families interact with Frankia. Inside root nodules, bacteria protected and nourished by the plant find a favorable environment for nitrogen fixation and, in exchange, provide the plant with fixed nitrogen (Sergio *et al.,* 2004). Molecular phylogeny studies based on the chloroplast gene *rbcL* indicate that plants entering rhizobial or actinorhizal symbioses belong to the same clade (Soltis *et al.,* 1995), suggesting that a predisposition to form nitrogen fixing root nodule symbioses originated once in the history of flowering plants. However, the nature of this predisposition remains unknown.

The symbioses between *Rhizobium* or *Bradyrhizobium* and legumes are a cheaper and usually more effective agronomic practice for ensuring an adequate supply of N for legume-based crop and pasture production than the application of fertilizer-N. The introduction of legumes into these pastures is seen as the best strategy to improve nitrogen nutrition of the grasses. Large contributions (between 75 and 97 kg of N ha$^{-1}$ in 97 days of growth) by *Stylosanthes guianensis* were reported (Viera-Vargas *et al*., 1995). $^{15}$N data suggested that over 30% of the N accumulated by the grass in mixed swards could be derived from nitrogen fixed by the associated legume. Other studies (Mandimba, 1995) revealed that the nitrogen contribution of *Arachis hypogaea* to the growth of *Zea mays* in intercropping systems is equivalent to the application of 96 kg of fertilizer-N ha$^{-1}$ at a ratio of plant population densities of one maize plant to four groundnut plants.The present challenge is to sustain soil fertility in cropping systems operating at high productivity levels.

Stresses (deficiencies and toxicities) due to availability, acquisition and utilization of nutrients are becoming increasingly widespread in many soils, leading to low crop productivity. For example, the yield potential of cropping systems on acid soils, which cover about 3.95 billion hectares of the earth's surface, is restrained by deficiencies of (P), calcium (Ca), magnesium (Mg), and potassium (K), and by toxicities of aluminum (Al), manganese (Mn), and iron (Fe) (Salazar *et al*., 1997).

Conventionally, fertilizers or soil amendments are used to counter such stresses. However, total dependence on fertilizers is neither economical nor pragmatic because of (a) the inability of many farmers to buy enough fertilizer, and (b) the capacity of many soils to fix applied nutrients into forms unavailable to plants (Sanchez and Uehara, 1980).

However, even a wet-dry rotation which includes legumes does not always conserve enough soil N. In Japan, more than 60% of soyabean is grown in paddy fields in rotation with rice, wheat or other crops. Soyabean yields have been slowly but steadily declining since the middle of the 1980s. Soyabean takes up a large amount of soil N for the production of grain, of which the N percentage is generally more than 8%. A sharp reduction in the level of mineralized soil N caused by the continuous cultivation of soyabean is suspected in many paddy fields with a wet-dry rotation. As good yields of grain legumes may exploit soil N more than rice, fertilizers may be required to replenish soil N fertility. Tirol-Padre *et al.* (1996) speculated that N use efficiency in rice was a more stable and suitable criterion than N uptake. Their studies suggested that root properties influencing rates of N absorption and assimilation can limit the rates of N acquisition from the soil. Therefore, genetic modification of roots to increase the efficiency of absorption and assimilation is a useful objective.

As far as P use efficiency is concerned, plants adopt at least four different mechanisms to increase their access to native or applied soil P. These include:

- Modification of soil exploration by roots (through increasing absorptive area);
- Better symbiosis with soil microbes (such as arbuscular mycorrhizal fungi);
- Modification of the rhizosphere to increase nutrient availability (through release of enzymes or other compounds capable of liberating P from metal-P compound or organic complexes); and
- Reduced tissue P requirements (Marschner, 1998).

All the above mentioned mechanisms could be facilitated through inoculation of plant with arbuscular mycorrhizal fungi.

## 2.3 Mycorrhiza

The fungi that are probably most abundant in agricultural soils are arbuscular mycorrhizal (AM) fungi (phylum Glomeromycota). They account for 5–50% of the biomass of soil microbes (Olsson *et al*., 1999). Biomass of hyphae of AM fungi may amount to 54–900 kg ha$^{-1}$ (Zhu and Miller, 2003), and some products formed by them may account for another 3000 kg (Lovelock *et al*., 2004). Pools of organic carbon such as glomalin produced by AM fungi may even exceed soil microbial biomass by a factor of 10–20 (Rillig *et al*., 2001). The external mycelium attains as much as 3% of root weight (Jakobsen and Rosendahl, 1990). Approximately 10–100 m mycorrhizal mycelium can be found per cm root (McGonigle and Miller, 1999). Almost all tropical crops are mycorrhizal, and many, if not most, are strongly responsive to arbuscular mycorrhizas. Norman and others (1995) treated 12 major food crop genera in detail, and listed further another 14. All these genera form AM symbioses. Only a few families and genera of plants do not generally form arbuscular mycorrhizas; these include Brassicaceae (their root exudates are possibly toxic to AM fungi), Caryophyllaceae, Cyperaceae, Juncaceae, Chenopodiaceae, and Amaranthaceae (although each of these families has some representatives that are usually colonized by AM fungi (Norman *et al.,* 1995).

The AM association has received attention as part of an increasingly popular paradigm that considers an active and diverse soil biological community as essential for increasing the sustainability of agricultural systems. The ability of AM fungi to enhance host–plant uptake of relatively immobile nutrients, in particular P, and several micronutrients, has been the most recognized beneficial effect of mycorrhiza. Rhizosphere interactions occur between AM fungi and other soil micro-organisms with effects on plant nutrient balances, such as nitrogen-fixing bacteria and plant growth-promoting rhizobacteria (Paula *et al*., 1993). AM colonization may furthermore protect plants against pathogens. AM fungi interact with heavy metals/micronutrients. They can restore the equilibrium of nutrient uptake that is misbalanced by heavy metals (Carneiro *et al*., 2001; Siqueira *et al*., 1999). AM fungi can alleviate Al toxicity. AM fungi improve water relations, especially under nutrient limitation. The extraradical hyphae of AM fungi contribute to soil aggregation and structural stability. Therefore, mycorrhizas are multifunctional in agroecosystems (Newsham *et al*., 1995), potentially improving physical soil quality (through the external hyphae), chemical soil quality (through enhanced nutrient uptake), and biological soil quality (through the soil food web).

Several reviews have dealt with the role of mycorrhizal associations in soil quality and sustainable agriculture (Dodd, 2000; Barea *et al*., 2002; Gianinazzi *et al*., 2002; Jeffries *et al*., 2002; Ryan and Graham, 2002; Harrier and Watson, 2003). These reviews generally focused on temperate soils. Reviewing the role of mycorrhizas in tropical soil fertility, more than two decades after the seminar book by Sieverding (1991), is important for two related reasons: (1) soils, major crops and possibly the species composition of AM fungal communities are different between the major climatic zones. Mycorrhizal functioning depends on the interplay between fungi, plants and the abiotic environment, different perspectives may arise from temperate and tropical views; (2) agriculture in temperate regions is often characterized by conditions of excess nutrients, whereas in tropical regions the problem of access to most soil nutrients is the case (Van Noordwijk and Cadish, 2002). The latter contrast is evident for both major macronutrients (phosphorus and nitrogen) and several micronutrients and heavy metals. The question whether mining P of saturated soils through mycorrhizal associations is desirable (Liu *et al*., 2003) is different from the question whether mining the unavailable P pools in P-fixing Oxisols is a useful strategy. Managing mycorrhizal associations for the remediation of heavy metal pollution in agricultural soils (Leyval *et al*., 1997; Entry *et al*., 2002) is different from the use of AM associations to prevent micronutrient deficiencies in crops. Whereas the increased interest in mycorrhizas in temperate cropping systems has received an impetus through the transformation towards organic farming (Ma¨der *et al*., 2002; Ryan and Graham, 2002), the situation in the tropics is very different. Resource poor farmers in the tropics are usually organic by default as a consequence of low prices for agricultural products and high prices for fertilizers and technical equipment. Consequently, an economic analysis as proposed by Miller *et al*., (1994), suggested that the prices for agricultural produce and costs of labour and fertilizers (as main variable factors) will result in different agricultural outputs and hence different management recommendations.

The role of mycorrhizal associations in enhancing nutrient uptake will mainly be relevant in lower input agro-ecosystems. The mycorrhizal role in maintaining soil structure is important in all ecosystems (Ryan and Graham, 2002). Mycorrhizal fungi contribute to soil structure by (1) growth of external hyphae into the soil to create a skeletal structure that holds soil particles together; (2) creation by external hyphae of conditions that are conducive for the formation of micro-aggregates; (3) enmeshment of microaggregates by external hyphae and roots to form macroaggregates; and (4) directly tapping carbon resources of the plant to the soils (Miller and Jastrow, 1990; 2000). This direct access will influence the formation of soil aggregates, because soil carbon is crucial to form organic materials necessary to cement soil particles. Hyphae of AM fungi may be more important in this regard than hyphae of saprotrophic fungi due to their longer residence time in soil, because fungivorous soil fauna prefers hyphae of the latter over those of AM fungi (Klironomos and Kendrick, 1996; Gange, 2000).

## 2.4 Arbuscular Mycorrhiza Fungi and Plant Nutrient Uptake

Researches have shown that AM Fungus (AMF) plays vital roles in plant nutrients uptake. The most prominent among the nutrients is the phosphorus which, is generally regarded as the most important benefit that AMF provide to their host plant, and plant P status is often the main controlling factor in the plant–fungal relationship (Thompson, 1987; Smith and Read, 1997; Graham, 2000). AMF can play a significant role in crop P nutrition, increasing total uptake and in some cases P use efficiency (Koide *et al*., 2000). This may be associated with increased growth and yield (Vosatka, 1995; Ibibijen *et al*., 1996; Koide *et al*., 2000). However, there are examples where crops fail to respond to colonisation by native AMF, e.g. Ryan *et al*. (2002). In many cases, this is due to a high concentration of (phyto) available soil P (Bethlenfalvay and Barea, 1994; Hetrick *et al*., 1996). Where colonisation by AMF is disrupted, uptake of P, growth and in some cases yield can be significantly reduced (Thompson, 1987; 1991; 1994; Thingstrup *et al*., 1998; Sorensen *et al*., 2005; Gosling *et al.,* 2006). Under such conditions, the colonisation of roots by AMF is often suppressed (Jensen and Jakobsen, 1980; Al-Karaki and Clark, 1999; Kahiluoto *et al*., 2001). Where strong AMF colonisation still occurs under conditions of high soil P concentrations it may reduce crop growth (Gavito and Varela, 1995; Kahiluoto *et al*., 2001).

## 2.5 Role of Mycorrrhizal in P Uptake

Low p availability limits plant growth in many acid soils of the tropics. P deficiency is mainly caused by strong adsorption of $H_2PO4^-$ to aluminium (Al) and iron (Fe) oxide, which turns large proportions of total P into forms that are unavailable to plants (Irene and Thomas, 2006). Researches have shown that P is readily available in the soil but at a very low percentage that can be mobilized and used by plants. Cardoso *et al*., (2004) in their experiment discovered that total P in the A and B horizons of oxisol in Brazil were about 270mg $kg^{-1}$ but the distribution among pools was different. For plant readily available and moderately available Pi constituted about 18% of all P in the A horizon and 14% in the B horizons. The improvement of P nutrition of plants has been the most recognized beneficial effect of mycorrhizas. The mechanism that is generally accepted for this mycorrhizal role consists of a wider physical exploration of the soil by mycorrhizal fungi than by roots.

Besides hyphae that extend beyond the root depletion zone, various subsidiary mechanisms have been proposed to explain P uptake by mycorrhizal fungi, such as (1) the kinetics of P uptake into hyphae differ from those of roots either through a higher affinity (lower Km) or a lower threshold concentration at which influx equals efflux (Cmin); (2) roots and hyphae explore microsites differently, especially small patches of organic matter (St John *et al*., 1983; Joner and Jakobsen, 1995); (3) plant roots and mycorrhizal hyphae affect chemical changes and P solubility in the mycorhizosphere differently. The last mechanism could lead to access to inorganic and organic P sources that are unavailable to non-mycorrhizal plants.

It has been suggested that mycorrhizas may benefit plant growth by increasing the availability of P from non-labile sources. In many studies, mycorrhizal and non-mycorrhizal plants appear to use the same labile P sources (Bolan, 1991; Herna´ndez *et al*., 2000), but other studies demonstrated that mycorrhizal plants obtained P from normally unavailable sources of Pi and Po (Bolan *et al*., 1987; Jayachandran *et al*., 1989, 1992; Koide and Kabir, 2000; Feng *et al*., 2003).

To address this controversy, Cardoso *et al*., (2006) used natural substrates, and analyzed the different P pools through P fractionation prior to and after a treatment with mycorrhizas. An Al-resistant maize (*Zea mays* L.) variety was grown for 3 months in pots with 200 g of the A horizon of an Oxisol. No significant changes occurred in the inorganic and organic P pools with non-mycorrhizal plants. Mycorrhizal plants, on the other hand, depleted the pools of Resin–Pi and NaHCO3–Pi completely, and the pool of NaOH–Pi by about 20%. Therefore, in the short term, mycorrhizas did more than simply shortening the distance that P ions must diffuse to plant roots, because mycorrhizas took up P that was not available in short terms to plants. In this study, Po was not used by the mycorrhizas and these pools even increased. These results confirmed uptake by mycorrhizal fungi from P pools not available to plants on a short term in a pot experiment.

Apart from P, AM fungus has been indicted in the uptake of other nutrients by the host plants. Zinc (Zn) nutrition is most commonly reported as being influenced by the AM association, though uptake of copper (Cu), iron, N, K, calcium (Ca) and Mg have been reported as being enhanced (Smith and Read, 1997; Clark and Zeto, 2000). In some cases, it is the availability of these other nutrients, which control the formation/initiation of the symbiosis (Ryan and Angus, 2003). AMF may also enhance plant uptake of N from organic sources (Hodge *et al*., 2001) though more work is required to fully understand the mechanisms involved (Read and Perez-Moreno, 2003).

Arbuscular mycorrhizae provide the plant with supplemental phosphorus (P), nitrogen (N), and micronutrients since the plant roots alone are not able to maximize the interception of nutrients (Allen *et al*., 2003; Adriana and Palle, 2008). Specically in soyabean, AM have been shown to improve the overall water status of the plant (Porcel and Ruiz-Lozano, 2004; Vejsadova *et al*., 1993), due to a reduced resistance to water transport (Safir *et al*., 1971), associated with an enhanced nutrient (Safir *et al*., 1972).

Dominique *et al*. (1997) recently reported that plants colonized with AM fungi showed a greater ability to take up soil nitrate than those that were not. They concluded that induced colonization of crop plants with AM inoculum in the field could help alleviate nitrate contamination of groundwater.

There are contradictory reports on the nutrient uptake of AMF. Researches have shown that it is possible under some conditions that AMF may cheat their host plant into supplying C with no apparent benefit to the plant. In some cases, this can cause a decline in growth (Lerat *et al*., 2003). However, proving that AMF are actually cheating is difficult (Fitter, 2001) because of the wide range of benefits to the host, which may only become obvious at specific times or under certain environmental conditions or stresses.

AMF also interact with a whole range of other microorganisms in soils. Bacterial communities and specific bacterial strains promote germination of AM fungal spores and can increase the rate and extent of root colonisation by AM (Johansson *et al*., 2004). Once the arbuscular symbiosis has developed, AM hyphae influence the surrounding soil, which has been termed the mycorrhizosphere (Linderman, 1988), resulting in the development of distinct microbial communities relative to the rhizosphere and bulk soil (Andrade *et al*., 1997). Within the mycorrhizosphere AMF interact with beneficial rhizosphere microorganisms including free living N fixing bacteria and general plant growth promoting rhizobacteria (PGPR) (Arias *et al*., 1991; Requena *et al*., 1997;

Galleguillos *et al*., 2000; Tsimilli-Michael *et al*., 2000; Biro *et al*., 2000). Large increases in yield over un-inoculated controls have been observed with some PGPR (Galleguillos *et al*., 2000) though the interaction with PGPR can be antagonistic as well as synergistic (Biro *et al*., 2000) and there seems to be a high degree of specificity between the plant, AMF and PGPR species involved in these interactions (Requena *et al*., 1997). The legume–rhizobium symbiosis is strongly influenced by AMF and there is some evidence to suggest that legume nodules contain AMF communities quite distinct from those found in the roots of legumes (Scheublin *et al*., 2004). The rhizobium symbiosis is dependent on high concentrations of P and so the enhanced P nutrition arising from the AM colonisation can result in an increase in nodulation and $N_2$ fixation (Ganry *et al*., 1985; Arias *et al*., 1991; Ibibijen *et al*., 1996; Va´zquez *et al*., 2002).

## 2.6 Intercropping System of Farming

Intercropping, which is intermingling growth of at least two crop species on the same piece of land at the same time, can increase grain yields greatly. Intercropping is considered as the practical application of ecological principles such as diversity, crop interaction and other natural regulation mechanisms. Intercropping is defined as the growth of two or more crops in proximity in the same field during a growing season to promote interaction between them. Available growth resources, such as light, water and nutrients are more completely absorbed and converted to crop biomass by the intercrop as a result of differences in competitive ability for growth factors between intercrop components. The more efficient utilization of growth resources leads to yield advantages and increased stability compared to sole cropping.

Legume-grass intercrops are known to overyield because of legume nitrogen fixation (Vandermeer, 1989; Stern, 1993; Li *et al*., 1999; Knudsen *et al*., 2004; Long Li *et al*., 2007). About a third of terrestrial soils have insufficient available phosphorus (P) for optimum crop production, with many tropical acid soils being highly P –deficient (Batjes, 1997; Vance, 2003). Some pot experiments have suggested that legume/cereal mixture can achieve greater P uptake on such soils than can either species by itself. In field conditions, similar greater P uptake by intercropped maize with faba bean also was observed (El Dessougi *et al*., 2003; Li, 2003). However, both pot and field experiments did not distinguish that the greater P uptake was derived from niche (rooting depth or seasonality) complementary direct interspecific facilitation. Li *et al*., (2007) hypothesized that overyielding of intercropped species on P-deficient soil may result from a plant's chemical alteration of the rhisosphere that mobilizes P and thus enhances its own productivity and that of another species. The phenomenon of plant's chemical alteration of the rhizosphere that mobilizes P and thus enhances the intercropped plants productivity is known as interspecific rhizosphere effect. The result obtained from the experiments showed that maize grain overyielded by 43% and faba beans overyielded by 26% compared with corresponding monoculture maize and faba bean.

Mixed culture or intercropping of legumes and cereals is an old practice in tropical agriculture that dates back to ancient civilization (Patrick 2006). The main objective of intercropping has been to maximise use of resources such as space, light and nutrients (Morris and Garrity, 1993; Li *et al*., 2003b), as well as to improve crop quality and quantity (Izaurralde *et al*., 1990; Mpairwe *et al*., 2002). Other benefits include water quality control through minimal use of inorganic nitrogen fertilisers that pollute the environment (Crew and Peoples, 2004).

Legumes contribute to maintaining the soil fertility via nitrogen fixation, which is increased in intercrops due to the more competitive character of the cereal for soil inorganic N. This leads to a complementary and more efficient use of N sources. Intercropping of grain legumes and cereals therefore offers an opportunity to increase the input of fixed nitrogen into agroecosystems without compromising cereal N use, yield level and stability

The agronomic advantages of intercropping are the result of differences in competitive ability for growth factors between intercropped components. In terms of competition, this means that the components are not competing for the same ecological niches and that interspecific competition is weaker than intraspecific competition for a given factor. The fact that the crops involved may have different resource requirements as well as different growth patterns makes it more complicated to define a proper methodology for the study of intercrops compared to studies involving one species – sole cropping (Zhang and Li, 2003).

Intercropping is a method for simultaneous crop production and soil fertility building and it may also contribute to the prevention of nitrogen leaching risks sometimes observed from sole crops such as grain legumes due to changes in incorporated residue chemical quality involving nutrient turnover. It is also an ecological method to manage pests, diseases and weeds via natural competitive principles that allow for a more efficient resource utilisation. These same competitive principles also contribute to an improved quality of intercrop products. The inclusion of $N_2$ fixing crops in an intercrop leads to the utilisation of the renewable resource of atmospheric nitrogen which increases the sustainability of the agroecosystem. Intercropping can also be regarded as a practice to increase the production of less stable crops such as grain legumes.

Gary and Charles (1999) evaluated the performance of maize and soyabean in intercropping system in their experiment conducted for three (3) consecutive years and discovered that corn yields were consistently higher in intercropping than monocropping when calculated across the entire strip while soyabean yields were consistently lower.

### 2.7 Significance of Intercropping

In developing countries, where the amount of arable land per capita is steadily decreasing, inherently sustainable local practices and knowledge adopted before the Green Revolution era have been systematically replaced. For instance, the subsistence agriculture of the pre-chemical era efficiently sustained the N status of soils by maintaining a balance between N lost with the grain harvest and N gain from biological N fixation. This was possible with less intensive cropping, adoption of rational crop rotations and intercropping systems, and the use of legumes as green manure.

Zeyaur *et al*., (2007) conducted experiment to assess the potential role of intercropping maize and sorghum with different food legumes. It was discovered that intercropping grain with legumes combined with other cultural methods significantly enhanced grain yield.

In another experiment conducted for a period of five years by Ghosh *et al*. (2006) on above and below ground evaluation of soyabean intercropped with sorghum, observed that above and below ground growth components as well as biological activities were greatly improved in intercropped sorghum while the values of these except nitrate reductase activity, soil microbial biomass and dehydrogenase activity were reduced in the intercropped soyabean indicating interspecies competition between component crops. The increased nitrate reductase activity, soil microbial biomass C and dehydrogenase activity in intercropped soyabean was attributed to interspecies facilitation in the system. They observed that yield and land equivalent ratio of both the intercrops increased over sole crops though based on aggressivity and relative crowding coefficient and that sorghum is more competitive than soyabean.

Interspecific facilitation on nutrient utilization is one of the most important mechanisms for intercropping yield advantage. Long and Fu-Suo, (2006) in their review reported among other things that the different P sources or forms in soil was utilized to a different extent by different associated species. For instance chickpea utilized well organic P (Phytate-P) by a large amount of acid phosphatase exudated from its root system and faba bean utilized sparingly –soluble inorganic P (Fe-P or Al-P) in soil more efficiently than maize did, which benefit P uptake by associated maize and reduced the interspecific competition on P in soil. Apart from this, the proportion of plant N derived from atmosphere, biological $N_2$ fixation by legumes, was enhanced by intercropping. The directly nitrogen transfer from legume to cereal is limited for N economical utilization in legume/cereals intercropping. However, main mechanism underlying efficient nitrogen utilization in the intercropping probably was through a stimulation of nodulation and nitrogen fixation of legumes, by soil nitrogen depletion that caused by more N competitive and more N uptake by associated cereals (Long and Fu-Suo, 2006).

Another way of improving sustainable crop production is the use of microorganisms as biofertilizer, an alternative approach for using of phosphate-solubilizers as microbial inoculants is the using of mixed cultures or co-inoculation with other microorganisms. On the other hand, it has been postulated that some phosphate solubilizing bacteria behave as mycorrhizal helper bacteria. Similarly, bacteria and their growth or activities are affected by fungi and their exudates in rhizosphere (Olsson *et al*., 1996). Biofertilizer provide the plant with the macro as well as micronutrients required for healthy growth, therefore, improve yield and quality of agricultural crops, and reduce the overall cost of chemical fertilizer (Shehata and Khawas, 2003). Clearly there is an urgent need for sustainable agricultural practices on a global level. To overcome the ecological problems resulting from the loss of plant nutrients and to increase crop yield, microorganisms that allow more efficient nutrient use or increase nutrient availability can provide sustainable solutions for present and future agricultural practices (Rai, 2006).

### 2.8 AM fungi and crop rotation

Crop rotation is one of the cornerstones of organic farming practice. Rotations generally consist of a period of fertility building lay followed by a period of cash cropping, before return to ley. A well-designed, diverse rotation, characteristic of many organic systems, can aid the management of crop nutrient requirements and pests and diseases (Gosling, 2006). It will also result in variations in AMF inoculum potential. Accounting for this is the design of rotations which could increase the benefits available from the AM association.

Soils used for agricultural production have limited species of AM fungi compared with natural ecosystem (Mene´ndez *et al*., 2001). One reason for this is the low diversity of hosts, which reaches its most extreme form in crop monoculture (An *et al*., 1993; Burrows and Pfleger, 2002; Oehl *et al*., 2003). Monoculture may select for AMF species that provide limited benefits to the host plant. Johnson *et al*. (1992) in their research conducted on maize and soyabean farming discovered that maize yielded higher and had higher nutrient uptake on soils that had grown continuous soyabean (*Glycine max*) for the previous five years than on soil that had grown continuous maize for the previous five years. Conversely, soyabean yielded higher and had higher nutrient uptake on soil which had grown five years of maize than five years of soyabean. The most abundant AMF species in the continuous maize soil was negatively correlated with maize yield, but positively correlated with soyabean yield; there was a similar effect with soyabean soil. They hypothesised that monocropping selects AMF species which grow and sporulate most rapidly and that these species will offer the least benefit to the plant because they divert more resources to their own growth and reproduction. The results bring smaller and smaller benefits of AM colonisation to the host plant while monocropping continues.

Researches have shown that there is reduced colonisation and crop yield of the subsequent plant when cropping with non-mycorrhizal plants. Miller (2000) examined AM colonisation of maize and showed that when maize followed non-mycorrhizal oilseed rape, AM colonisation was reduced along with early season P uptake, which in some cases resulted in yield reductions. Karasawa *et al*. (2002) also showed that AM colonisation and yield of maize decreased following non-mycorrhizal mustard (*Sinapis alba*) compared with mycorrhizal sunflower.

Bare fallow periods have a similar effect to nonmycorrhizal crops, reducing propagule numbers, colonisation, and nutrient uptake and in some cases yield of subsequent mycorrhizal crops. Troeh and Loynachan (2003) measured spore numbers after three years of continuous maize, soyabean or fallow. Maize and soyabean increased spore numbers, while under fallow, spore numbers declined during the first year, and then stabilised at a low level.

Bare fallows in the rotation is potentially highly detrimental, madeworse by the fact that these fallows are tilled regularly, further damaging AMF (Kabir *et al*., 1997). They should be avoided where possible, or if unavoidable kept short to minimise the negative impact on AMF (Kabir *et al*., 1999). In contrast to bare fallows, including green manures and cover crops in the rotation, a practice encouraged in organic systems, can increase AM inoculum potential and the growth and yield of subsequent AMF dependant crops, provided they are themselves mycorrhizal (Dodd and Jeffries, 1986; Galvez *et al*., 1995; Boswell *et al*., 1998).

### 2.9 Legume-Rhizobium-Mycorrhizal Tripartite Association

Series of researches have shown that inoculation with mycorrhiza fungi, rhizobium and dual inoculation with both microsynbionts have positive significant effects on the growth, nutrient uptake and biomass production of the leguminous plants. For instance El-Ghandor *et al.* (2005) conducted a research on the impact of arbuscular mycorrhiza fungi and rhizobium on the growth and N, P and Fe uptake of faba bean, the result indicated that, AM fungi and rizobial inoculation either alone or in combination increased dry matter yield of faba beans as compared to uninoculated plant. Also P, N, and Fe uptake were significantly increased due to inoculation, and that dual inoculation resulted in the highest.

Under low soil P concentrations, most plant species are dependent on a symbiotic association with arbuscular mycorrhizal fungi (AMF) for the acquisition of P (Smith and Read 1997). Under low N fertilizer and in the absence of AMF infection supplementary P fertilization is generally necessary for the maintenance of $N_2$-fixation rates by rhizobium at the levels required for economically viable crop production (Andrade *et al*., 1998). In legumes the positive synergistic interaction among the members of the tripartite symbiotic association (rhizobium AMF-legume) result in improved rates of P uptakes $N_2$-fixation and crop biomass production under conditions of reduced N and P fertilizer inputs (Azcon *et al*., 1991; Xavier and Germida, 2002; 2003). However, there is little information on the influence of P on N productivity or photosynthetic N use efficiency. Nitrogen productivity has been defined as the rate of biomass production per unit biomass N content (Agren 1985), whereas the photosynthetic N use efficiency is the amount of $CO_2$ fixed (mol $CO_2$) per unit biomass N content. In general, photosynthetic and specific growth rates increase with increasing plant tissue N concentration or N supply in a curvilinear fashion (Hirose and Werger, 1987; Sinclair and Horie, 1989; Jia and Gray, 2004).

Yinsuo *et al*. (2004), when studying the influence of rhizobium and arbuscular mycorrhiza fungi on nitrogen and phosphorus accumulation on *Vicia faba* hypothesized that the synergistic interactions among the members of the tripartite symbiotic association improve legume productivity through positive effects on the rates of photosynthetic $CO_2$ assimilation, $NO_2$ -fixation and P uptake. They also proposed that the three processes are interdependent or even tightly coupled. For example, the rate of photosynthetic $CO_2$ assimilation is influenced by the rate of N and P supply, and the rate of $N_2$-fixation is influenced by the rates of photosynthetic and P supply to the nodules. The result obtained from the experiment confirmed the assertion and indicated that the synergistic or additive interactions among the components of the tripartite symbiotic association (rhizobium-AMF- broad bean) increased plant productivity.

Depending on the plant species, bacteria infect the root either by root hair infection or through cellular spaces between epidermal cells (crack entry). Root hair infection is characteristic of most temperate legumes and of several actinorhizal genera like Alnus and Casuarina. In this case, bacteria induce a localized degradation of the cell wall of the root hair; the plasma membrane then invaginates leading to the formation of a tubular structure called the infection thread (IT). ITs are filled with bacteria and surrounded by newly deposited cell wall material and spread bacteria by growing inside plant cells and from one cell to another. Whereas actinorhizal ITs never release the bacteria, in most legume species, the ITs that reach the nodule release bacterial cells that then differentiate into bacteroids and start fixing nitrogen (Pawlowski and Bisseling, 1996).

In legume-rhizobia symbioses, secreted bacterial Nod factors play an essential role by mediating specific recognition between the two partners and activating a series of responses involved in nodule formation (Lerouge *et al.*, 1990; Denarié and Cullimore, 1993; Downie and Walker, 1999). Among these responses is the transcription of the so-called nodulin genes that are specifically transcribed in symbiotic tissues and may participate in the establishment of the symbiosis (Schultze and Kondorosi, 1998). Some of these nodulin genes are also activated in response to endomycorrhizal colonization of roots (Albrecht *et al*., 1998), and the analysis of plant mutants indicates that the signaling pathways involved in legume-rhizobia and mycorrhizal symbioses at least partially overlap (Duc *et al.*, 1989; Wegel *et al.*, 1998; Endre *et al.*, 2002; Stracke *et al.*, 2002). These results suggest that at least some of the molecular mechanisms of root nodule symbioses may have been recruited from the more ancient and widespread mycorrhizal symbiosis. As in legumes, several genes specifically induced during actinorhizal symbioses have been described, but little information concerning the signaling pathways involved in their regulation is available (Franche *et al*., 1998a).

The symbiotic association between certain plants and microorganisms plays important role in soil fertilization, and improves their growth and mineral nutrition. Microorganisms implicated in this symbiotic interaction are from two groups: bacteria and fungi .the bacteria group is implicated on nitrogen fixation (Pawlowski and Bisseling, 1996), while the fungi group is involved in the uptake of nutrients with low mobility (Diop, 1995 Gianinazzi-pearson, 1996). Among the bacteria which establish symbiotic association with dicotyledonous plants, nitrogen fixation is exclusively carried out by rhizobia and frankia in a specialized organ, the root nodule where atmospheric nitrogen is reduced to ammonium. Rhizobia and frankia are soil bacteria which are unicellular gram-negative and filamentous branching gram positive respectively. Rhizobia exist in symbiotic association with legumes and one species member of ulmaceae family, parasponia andersonii. In contrast, frankia can interact with diverse group of dicotyledonous plants which are called actinorhizal plants. Legume and actinorhizal plants can establish the same time a symbiotic association with the arbuscular mycorrhizal fungi the order Glomales. Recent studies conducted in Gymnostoma have shown the root nodules can also be colonized by arbuscuar mycorrhizae (Duhoux *et al*., 2001).

## 2.10 Significance of Legume-Cereal Rotation and Nutrient Utilization Efficiency

Conventionally, fertilizers or soil amendments are used to counter stresses involved in intercropping and rotation of legume and cereal crops. However, total dependence on fertilizers is neither economical nor pragmatic because of (a) the inability of many farmers to buy enough fertilizer, and (b) the capacity of many soils to fix applied nutrients into forms unavailable to plants (Sanchez and Uehara, 1980).

There is also increasing evidence that fertilizer alone cannot sustain yields for long periods. For example, in continuous rice cropping with two to three crops grown annually, the use of fertilizer N increased with time but the yields often remained stagnant (Cassman and Pingali, 1995). This reflects a higher fertilizer requirement to produce the same yields, implying a decline in yield response to nutrients, possibly because of an overuse of fertilizer. This is a reason for concern. As an alternative, tailoring plants to fit the soil through the use of soil microorganisms such as symbiotic nitrogen fixing bacteria like rhizobium and mycorhiza is considered more economical than changing the soil.

These two organisms can convert atmospheric nitrogen to soil nitrite and nitrate and can mobilize phosphorus respectively, thus making nutrients which may otherwise available for plant use and thus improving plant yield. It is believed that the use of microorganisms as biofertilizer reduces cost and ecology friendly; as such farmers can more easily adopt a genotype with useful traits, than crop and soil management practices that are associated with extra costs (Ruel and Bouis, 1997)

Legume-cereal rotations are traditionally practiced in agriculture throughout the world, and the role of legumes in maintaining soil fertility is well documented (Giller and Wilslon, 1991). Nitrogen application could be reduced in well selected rotation on fertile soil without reducing dry matter yields, particularly when leguminous crops are incorporated into the soil (Petrickova, 1992). Legumes reduced nitrogen fertilizer requirements of maize in a subsequent season by 18- 68 kgN/ha as compared to fallow (Peter et al., 2000).

Soyabean seems to make efficient use of soil N which other plants cannot take up easily. The better growth of maize following soyabean, compared to repeated crops of maize alone, has been mainly attributed to the residual effects of N fixed by soyabean nodules. Based on a long-term crop rotation experiment, Vanotti *et al*. (1995) speculated that growth enhancement of maize after soyabean was due to the stimulation of soil N mineralizing microbes by soyabean, which might gradually deplete readily available soil N. Also, alfalfa was reported to enhance soil N mineralization (Radke *et al*., 1988). It is suggested that some crops such as rice, flowering Chinese cabbage (*Brassica amperistris* L. spp. Chinensis (L.) and carrot absorb organic N directly, and/or solubilize insoluble forms of soil organic N (Yamagata *et al*., 1996; Matsumoto *et al*., 1999).

Soil organic N is continually lost through plant removal, leaching, denitrification, and ammonia volatalization. Continuous rice cropping under wetland conditions thus leads to a low level of soil N, unless it is replenished by biological N fixation. This has the overall effect of reducing the pool of available soil N in the lowlands (Kundu and Ladha, 1995). The decline in N supplying capacity of rice is attributed to degradation in the quality of soil organic matter under such a water regime (Cassman *et al*., 1995a; 1995b).

Nutrient Utilization Efficiency (NUE) is defined in several ways, such as efficiency of acquisition (plant nutrient content/available nutrient) or the physiological efficiency with which a nutrient is used to produce biomass (plant biomass/plant nutrient content) or grain (grain yield/plant nutrient content). It is also defined as the amount of additional grain yield per unit of fertilizer applied. Efforts to improve it must be guided by a thorough understanding of the soil and plant processes that govern NUE (Julie et al., 2008).

For example, an ideal and cost-effective approach to improving NUE in acid soils might be a combination of liming to neutralize soil acidity, coupled with selection for crops more tolerant to Al toxicity. In cropping systems where fertilizer use is already high, cost-effective technologies that improve NUE are necessary. In cropping systems with low fertilizer use, however, the most promising way of improving NUE is to add small amounts of high-quality organic matter and use crop varieties with a higher NUE.

### 2.11 Significance of Inoculation with Microorganisms over Chemical Fertilizer

The poor nutrition of organic crops during the early growth stage has frequently resulted in a low productivity (Aryal *et al*., 2006). Nutrients from organic fertilizers are not available to crops unless they are mineralized and a low microbial population can further delay the decomposition processes. It was hypothesized (Aryal *et al*., 2006) that increasing microbial population, such as rhizobia in the rhizosphere of the organic crops might offset the problem of low productivity by increasing the rate of mineralization of organic fertilizers. In the experiment conducted to evaluate the responses of potted beans to rhizobial inoculation in organically fertilized and chemically fertilized normal soils, inoculation resulted in a significant increase in the pod yield, shoot and root dry masses with organic fertilizer, yet not with chemical fertilizer. Inoculation positively influenced nodulation in both the organically fertilize (OF) and chemically fertilize (CF) plants, and the effect was more pronounced in the OF plants. The arbuscular mycorrhizal infection rates and frequency of arbuscule formation were always higher in the OF plants than in the CF plants, and moreover, the inoculation increased AM infectivity in the OF plants but not in the CF plants. The inoculated OF plants had more shoot N and P compared to un-inoculated plants, while the CF plants were unaffected. Interestingly, AM infection rates showed a significant positive correlation to the shoot P or N in the OF plants, yet the relation was not significant in the CF plants, indicating greater dependency of organic plants than the chemical plants on the symbiotic fungi (Jen-Hshuan, 2006).

It is well established that soil fauna play a major role in increasing nutrient availability and uptake, especially in nutrient-poor soils. N-fixing systems, including free-living, symbiotic or associative organisms, contribute significant amounts of fixed N to cropping systems. Rhizobia-legume systems fix N at rates of 50-300 kg N/ha/year. Cyanobacteria fix 15-25 kg N/ha/year and azospirillum-grass associations fix 10-30 kg N/ha/year. The interaction between mineral fertilizers and N-fixing systems needs further study as a way of achieving better integration of the nutrition systems of different crops.

It is also indicated that enhanced AM association of crops through cultivation of mycorrhizal crops in the previous season showed significant growth and yield promotion on soils of high P fixation capability (Thompson, 1991; Arihara *et al*., 2000).

AM inoculation is expensive, while indigenous AM fungi usually dominate inoculated AM fungi. Increasing indigenous AM fungi through proper cropping systems is a practical way to enhance growth and P uptake of mycorrhizal crops. Soil factors such as P status, soil type and pH, and climatic variables such as precipitation and temperature, determine the growth promoting effects of AM in a cropping system. AM fungal populations and colonization of roots by AM fungi, and their contribution to P uptake, were higher in soils with lower P availability. However, the effect of preceding crops on the growth of following crops varies in different soils, particularly when P availability was low. This means that differences in indigenous AM fungi in various soils may mediate the effect of preceding crops. High soil moisture also increases the colonization of AM, especially in soils with low populations of AM spores. This far outweighs the effect of previous crops on AM colonization (Karasawa *et al*., 2000). Mycorrhizal wheat plants had greater acquisition of P and other nutrients compared to non-mycorrhizal plants grown under water stress (Clark, 1996). Similarly, mycorrhizal corn could take up more Fe in alkaline soils than non-mycorrhizal corn. Further studies are, however, necessary to maximize the potential advantages from the mycorrhiza-crop symbiosis through a detailed understanding of mycorrhizal ecology in cropping systems.

## 2.12 Intercropping and Improved Nitrogen Uptake and Phosporus Mobilization

Researches have shown that legume-cereal intercropping does not only improve Plant-nitrogen uptake but also phosphorus mobilization. For instance, (Ae *et al*., 1991, Arihara *et al*., 1991a,b) in their studies in the semi-arid tropics of India revealed that the addition of pigeonpea, as a sole crop or as an intercrop in a cropping system, not only helps soil N fertility, but also makes more reserves available for subsequent crops. Based on studies of a 700-year-old practice of Egyptian clover-rice rotation, which covers about 60-70% of the entire rice acreage in Egypt, (Yanni *et al*., 1997) reported a unique natural endophytic association between *Rhizobium leguminosarum* bv. trifoli and rice roots. The

N supplied by this rotation replaces 25-33% of the recommended rate of fertilizer application to rice. Such benefits cannot be explained solely by the increased availability of fixed N through mineralization of N-rich clover crop residues. Yanni *et al*., (1997) found that clover-nodulating rhizobia naturally invade rice roots, and achieve an internal population density of up to 1.1 x $10^6$ endophytes per gram (fresh weight) of rice roots. They reported that inoculation with two endophytic strains (E11 and E12) of *R. leguminosarum* significantly increased grain yield, harvest index and fertilizer N use efficiency of field-grown (Giza 175) hybrid rice (Ladha, *et al*., 1993).

It is widely recognized that high yields resulting from heavy applications of fertilizer in modern cropping systems have been achieved at some cost to environmental quality. In places, soil nitrate concentrations had become so high that nitrate leached from agricultural fields had increased the concentration in groundwater to more than 10 ppm, to a level damaging to human health (Kross *et al*., 1993). This is especially important in areas where groundwater is used as drinking water. It is well documented that leaching of nitrate occurs during the period between fall and spring, when the downward flow of water exceeds evapo-transpiration.

## 2.13 Soyabean History

The Soyabean - *Glycine max* - has been used in China for 5 000 years in crop rotation, as a food and in medicines. It is an annual legume of the *Fabaceae* family and is believed to have derived from *Glycine nosuriensis*, a legume indigenous to the Manchurian region of China, not far from the Korean border.

Soyabean is one of the leguminous crops selected for active research, production and utilization (Atungwu and Afolami, 2001) and one of the common staple foods ranked the sixth among the ten staples that feed the whole world because of its high nutrient density.

The first successful soyabean production in Nigeria was reported in Benue state located in the southern guinea savannah agro-ecological zone of the country in 1937 (Egbe, 1995) and ever since has been integrated into the traditional systems of which most important component is soyabean - maize intercrop due to soyabean potential to improve human diet by supplying high quality protein as well as animal feed and serves as a source of raw material base for agro-industries (Atungu and Afolabi, 2001).

Economically, soyabean is the most important legume in the world, providing good quality vegetable protein for millions of people and animals, and ingredients for numerous products. In the late 20th Century to the present, soyabeans have played an important part in helping to alleviate world hunger. In recognition of the economic and nutritional importance of soyabean as grain legume and maize as important cereal crop, resource poor farmers prefer the cultivation of soyabean and maize in mixture as aginist sole cropping system (Alhasan, 2002).

Soyabean is often cultivated in rotation with maize or other cereal crops, because of its N-fixing capability. However, soyabean seems to reduce the nitrate concentration in the soil profile much more than maize does. The difference in the amount of nitrate in the soil profile at 60 cm depth between maize and soyabean plots is estimated to be more than 100 kg N/ha when a heavy dose of N fertilizer was applied. This surprisingly high figure suggests the possibility of using soyabean as a cleansing crop in fields with high nitrate levels, although the mechanisms involved in this phenomenon are still unclear.

Maize following soyabean in rotation had been reported to confer a great yield advantage to farmers. Lodewyckx (2008), conducted research on legumes and sunflower on maize production combined with other conventional agricultural practices for four seasons and observed that the yield of maize preceded by groundnut was 25 % higher than the yield of the monoculture maize, while the yield of maize preceded by sunflower was 31 % lower than the mean yield of maize after fallow and after groundnut. In addition, it was observed that the yield ranks amongst the rotation systems varied from season to season. Maize following maize had the worst in three of the seasons. Maize following soyabean had the highest rank in three seasons and maize following sunflower the highest in one but the lowest in two seasons. The yield of maize grown after dry beans was lower in two seasons and higher than that of maize after soyabean in three seasons.

Gary (2012), in his research conducted on the longer-run return impacts of soyabean-corn rotational systems observed that corn after soyabean had the highest yield advantage of 198 bushels which was 10 and 18 bushels higher than corn-after-corn and continuous corn respectively. The yield of soyabean-after-corn was estimated to be 56 bushels while that of soyabean-after-two year of corn had 3 bushels higher yield at 59 bushels per acre.

The rotation returns of the corn/soyabean were estimated for three rotations of corn -soyabean, corn-corn-soyabean and continuous corn. Gary (2012) submitted that corn-soyabean had the average return of $484 per acre, i.e $578 for corn-after-soyabeans and $390 for soyabeans after corn. Corn-corn-soyabeans has an average return of $504 per acre i.e. ($578 corn-after-soyabeans + $510 corn-after-corn + $425 soyabeans-after-corn) and continuous corn has a return of $467 per acre. This assumes that yield reductions relative to corn-after-soyabeans have occurred. Since their returns vary over time, this is a present value problem. Under current realistic discount factors, present value calculations suggest that corn-corn-soyabeans is the most profitable rotation over time.

### 2.14 Interspecific Competition among Intercropped Plants

Interspecific competition may occur when two crops are grown together (Van der Meer, 1989) such competition usually decreases survival, growth or reproduction of at least one species (Crawley, 1997). There is overlapping growth period that cause intense interpectific interaction between the intercropped species. The interactions frequently occured at the interface between two crop species where they were nearest in distance, and resulting in an increase or decrease of growth, development and even yield.

Interspecific root interaction between intercropped faba bean and maize played an important role in the yield advantage and nitrogen and phosphorus acquisition by the intercropping system (Li *et al.,* 1999, 2002a). When the roots of the two species intermingled, N and P uptake increased, however, when the roots of the two species were completely separated the uptake reduced intercropped plant.

Assessing the interaction between the intercropped species, Geno and Geno (2001), concluded that interspecific competition and facilitation occurs at the same time, Van der Meer (1989)l, noted that both competition and facilitation take place in many intercropping systems, and that it is possible to obtain the net result of land equivalent ratio, an indicator of intercropping advantage, where the complementary facilitation is contributing more to the interaction than the competitive interference.

### 2.15 Nitrogen Depletion through Continuous Cropping

Continuous rice cropping under wetland conditions thus leads to a low level of soil N, unless it is replenished by biological N fixation. This has the overall effect of reducing the pool of available soil N in the lowlands (Kundu and Ladha, 1995). The decline in N supplying capacity of rice is attributed to degradation in the quality of soil organic matter under such a water regime (Cassman *et al*. 1995a, 1995b). Changing from continuous wetland rice cropping to judiciously manage multiple cropping systems, with a wet-dry rotation which includes the cultivation of leguminous crops, may rectify such problems (Arihara, 2000).

# CHAPTER 3

# 3.0 MATERIALS AND METHODS

## 3.1 Methodology

Two crops were used – a legume (Soyabean - *Glycine max* L.) and a cereal (Maize - *Zea mays* L). The two crops were either intercropped or separately planted on different farmlands. The experiment was conducted in the biological garden of the Department of Biology, Emmanuel Alayande College of Education, Oyo, Nigeria. The physical and chemical properties of the soil were determined by established standard analytical method before and after planting. The soil organic matter (SOM), soil texture and pH were determined by with method of Nelson and Sommer, (1982). Soil nutrient analysis was carried out for essential macro elements by extractable method of Soltanpour and Schawab (1977).

## 3.2 Multiplication of AM Fungus Inoculum

The AM fungus (*Glomus etunicatum*) inoculum used for the experiment was collected from the Department of Botany, University of Ibadan, Ibadan. This was multiplied in the screenhouse using maize (*Zea mays*) (Ferguson and Woodhead, 1982) as the trap host. This was carried out on a sterilized sandy soil which had been sterilized by heating in the sterilizing machine ($200^0$C) for 2 hours after which it was allowed to cool for some minutes before taken out of the oven. The sterilized soil was filled into plastic pots, after which the maize grains were sown into each of the pots. AM fungus inoculum was placed directly beneath the maize grain in each pot to ensure that the germinating seedlings make contact with the inoculum layer. The pots were watered to field capacity. The maize plants were grown for three months after which they were allowed to dry to ensure maximum spore production, before they were harvested. The dried roots were cut into pieces.

## 3.3 Rhizobium Culture Preparation

The preserved slanting culture of Rhizobium (IRj 2180A) obtained from IITA, Ibadan was sub- cultured to obtain pure colonies. The culture medium used was Yeast

Extract Mannitol Agar (YEMA). This was prepared by dissolving 0.8 g $K_2PO_4$, 0.2 g $MgS0_4.7H_20$, 0.1 g $CaCl_2.7H_20$, 0.1 g NaCl, 10 g Mannitol sugar, 15 g Agar and 2.0 g Yeast Extract in 1000 mls of $H_20$. On getting pure culture of the strain, 500 g mls of Yeast Extract Manitol broth (containing all the constituents of Yeast Extract Manitol except Agar) was prepared as described above. The broth was dispensed into four 500 mls conical flaks and was sterilized by autoclaving at $121^0$C and 1.1 kg/cm for 15 minutes. On cooling, pure rhizobium colonies from prepared plates were picked with the aid of sterilized inoculating loop into the broth in the flask. The flasks and their contents were then incubated at $26^0$C for 8 days in incubator shaker.

The early maturing Soyabean (T*GX 1444 – 2E*) and maize (TZSR) grains used for the experiment were obtained from International Institute of Tropical Agriculture (IITA) Ibadan. These were surface sterilized by immersing in 0.1% Mercury Chloride for 5 minutes and then washed in several exchanges of distilled water.

The rhizobium inoculum broth was inoculated into the soil by placing the inoculum in furrow round the seed (Read, 1966). 5 mls of rhizobium broth was added at about 2cm below the soil surface with the aid of sterile syringe.

The root fragments of the host plant, the soil and the AM propagules (spores, arbuscules and hyphae) were mixed together and used as crude sources of inoculum for the experiment. The inoculation was carried out by placing 20 g of the prepared crude inoculum directly into the planting hole underneath maize grains and soyabean seeds during planting and were later covered with the top soil.

The experimental design was a randomized complete block design in split plot with three (3) replications. These comprised of four treatments; mycorrhiza, rhizobium, combined inoculation and uninoculated control treatments.

Three farmlands were used for the experiments. The size of each farmland was 15 by 11 $m^2$. Each of the farms was planted with either sole maize, soyabean or the two crops combined (intercrop). Each of the farmland was divided into 3 blocks with each of the blocks containing four plots which were 1m apart. The physico-chemical properties and nutrient analysis of soil were determined before planting.

The farmlands were ploughed and harrowed to facilitate seedling growth. The first farmland consisted of sole soyabean which was planted on a flat soil. The arrangement was a split plot with rhizobium and mycorrhizal inoculations as shown in Appendix 1. In each plot, two soyabean seeds per hole were planted 15 cm apart within rows and 50 cm between rows, giving a total population of 266,666.67 plant $ha^{-1}$. The second farmland contained soyabean intercropped with maize in alternating 2 rows of soyabean in between lines of maize in each plot. The soyabean was inoculated with either rhizobium, mycorrhiza or combined inoculation as applicable and sole mycorrhizal inoculation for maize. On the third farmland was sole maize planted with either mycorrhizal inoculation or uninoculation. The maize was 40 cm apart within row and 75 cm between rows with two seeds per hole as done in intercropped maize farmland, given a total population of was 66,667 plant $ha^{-1}$. The plants were monitored for eight weeks after which they were allowed to complete their life cycle.

The process was repeated the following season but the plants were rotated in such a way that soyabean was planted where maize was initially planted and vice versa and the mixture of the two crops (i.e. soyabean intercropped with maize) was maintained on the third farm.

### 3.4 Monitoring of Parameters

The plants height was measured from the soil surface to the tip of the uppermost leaf (Ekanayake, 1996) with the aid of meter rule at different stages of development for both plants.

Number of leaf was determined by physical counting of leaves on each replicate and the diameter of the stem was measured using digital Vernier caliper.

At maturity, the plants were harvested, partitioned into leaves, stem and roots. The fresh weights of leaves, stems and roots were determined after which they were oven dried to constant weight at $70^0$C for 2 days and dry weights were determined. The oven-dried shoots were milled and used for nutrient analysis. Also the nutrient qualities of the two plants were analysed using their seeds.

### 3.5 Estimation of AM Fungi Colonization

2 g of fresh root samples of soyabean were taken from each replicate; the roots were stored in McCartney bottles containing 50% ethanol for few days. At staining, the ethanol was drained off and rinsed thrice consecutively with water to remove traces of ethanol. The root samples were washed with distilled water and then cleared in 10% KOH at $90^0$ C (Oyetunji, 2001) for an hour in the oven after which they were bleached with alkaline $H_2O_2$ (3 ml 20% $NH_4OH$ in 30 ml of $H_2O_2$) for 30 minutes under room temperature. The roots were rinsed and acidified with 1% HCl for 3 minutes. Acidified roots were stained in acidic glycerol solution (prepared with 500 ml glycerol, 450ml $H_2O$,

and 50ml HCl) containing 0.05 trypan blue at $90^0$C for 60 minutes (Oyetunji, 2001). The excess stain was removed by soaking in glycerol. The stained roots were spread on a gridline plate and observed under dissecting microscope for mycorrhizal colonization. The percentage root colonization was evaluated according to grid line intersect method (Giovanetti and Mosse, 1980) thus:

$$\% \text{ root infection} = \frac{\text{Total no. of infected root}}{\text{Total no. of root observed}} \times 100$$

### 3.6 Mycorrhizal Spore Isolation

After harvest, 100 g soil sample from each treated plot were taken for spore counting using wet sieving and decanting method (Gerdeman and Nicolson, 1963). Each soil sample was suspended in water for 30 minutes. The suspension was decanted over a series of sieves with 200 – 45 µm. The content of 45 µm sieves was transferred into 10 ml centrifuge tube. Centrifugation was done at 3000 rpm for 5 minutes (Daniel and Skippers, 1982). The content was spread on counting dish and the spores were identified and counted at 3x under dissecting binocular microscope.

### 3.7 Proximate and Nutrient Analyses

The oven dried shoot of both soyabean and maize were milled into ash and used for proximate nutrient analysis. The milled sample was analyzed proximately and quantitatively to determine their crude protein, ether extract content, crude fibre, ash content and nitrogen, calcium, potassium, phosphorus and magnesium content of soyabean and maize plants.

### 3.7.1 Determinatinon of Crude Protein

Crude protein of the sample was determined using Kjeldal's method. Each sample (0.5 g) was weighed and digested in Kjeldahl digestion flasks with 10 mls concentrated Sulphuric acid using kjeldahl catalyst. Heating was done in fume cupboard until a colourless solution was obtained within four hours. The flasks were allowed to cool and content were transffered into 100 ml volumetric flask and the volume was made up to mark with distilled water.

Sodium hydroxide solution was added to 10 ml of the digest followed by steam distillation into 20 ml of boric indicator for 10 minutes. Twenty millimetre of the solution was later transferred into 1.0 litre standard flask containing 20 g of boric dissolved in 700 ml hot distilled water and 250 ml of 95% ethanol and made up to mark with distilled water. The distillated was later titrated with 0.01M HCl. Crude protein was obtained by multiplying the nitrogen content by a factor

$$\%N = \frac{\text{Vol. of acid X conc. of acid X 0.014 X 100}}{\text{Weight of sample}}$$

### 3.7.2 Determination of Crude Fibre

Two gram (2.0 g) of each milled sample was carefully transferred into a 500 ml conical flask and 200 ml of sulphuric acid was added. The suspension was refluxed for 30 minutes. It was allowed to cool and filtered under suction on a piece of coarse textured linen. The residue was returned to the conical flask using a clean spatula and 200 ml of 1.25% boiling sodium hydroxide solution was added. The suspension was reflux for 30 minutes. On cooling, it was filtered, and the residue was carefully transferred into a crucible. It was dried in the oven overnight at $100^{0}$C, cooled and weighed. The dried residue was then ashed in muffled furnace at $500^{0}$C for 3 hours, cooled and weighed. The loss in weight is the crude fibre content of the sample.

$$\%\ \text{Fibre} = \frac{\text{Loss in weight on ashing X 100}}{\text{Weight of sample}}$$

### 3.7.3 Determination of Moisture Content

Two gram (2.0 g) of each shoot sample was put inside a crucible and then dried in an oven at $105^{0}$C until constant weight was obtained. The difference in weight was recorded as the moisture content. While percentage moisture content was calculated as

$$\%\ \text{Moisture} = \frac{\text{Initial weight - Final weight X 100}}{\text{Initial weight}}$$

### 3.7.4 Determination of Ash Content

Two gram (2.0g) of each sample was weighed into the previously weighed crucible. The crucible and the content was reweighed and heated to constant weight at $500^{0}$C. The difference in weight between the ashed sample and crucible gave the weight of ash.

$$\%\ \text{Ash} = \frac{\text{Weight of Ash X 100}}{\text{Weight of sample}}$$

### 3.7.5 Fat Determination

Two gram (2.0 g) of each sample was weighed and extracted with petroleum ether for four hours using soxhlet extractor. The petroleum ether distilled off and the ether extract was weighed. The difference between weight of reaction flask before and after extraction gave the weight of ether extract.

$$\% \text{ Ether extract} = \frac{\text{Weight of ether} \quad \text{X } 100}{\text{Dry weight of sample}}$$

### 3.7.6 Determination of Phosphorus

Two gram (2.0 g) of each shoot sample was weighed into dry crucible, put inside a furnace set at $600^0$ C and allowed to ash for 2 hours. The ash was washed by pipetting 10 ml of 1N HCl into the ashed sample and placed on a hot plate. This was cooled and washed into 100 ml volumetric flask using filter paper and funnel and made to 60 – 100 ml level with diet water. Into a 500 ml volumetric flask, 10 ml of the solution from the volumetric flask was pipetted and 10 ml of vanadate yellow was added and made up to level with distilled water. It developed for 15 minutes and absorbent was read at 170 nm. Standard phosphorus was prepared and red first before the sample. Phosphorus level was determined using vanadate-molybdate colorimeter.

### 3.7.7 Determination of Calcium, (Ca), Potassium (K), and Sodium (Na)

From the washed sample, flame photometer was used to read the level of calcium, potassium and sodium after they have been standardized with respective minerals.

### 3.8 Statistical Analyses

All data obtained from the experiments were subjected to analysis of variance using SAS analytical programme (SAS Institutes, 1996) to test for treatment effects on all parameters measured. Mixed model ANOVAS, Duncan’s Multiple Range Test (DMRT) and least significant difference (LSD) or standard error in mixed model, as appropriate were used to separate the treatments’ means at P = 0.05.

# CHAPTER FOUR

## 4.0 RESULTS

### 4.1 Experiment 1

### 4.2 Soil modification through systems of farming

There was effect of systems of farming on the physical and chemical properties of the soil. Increase in the total organic matter, organic C and N were observed after the rotation and intercropping of the two crops. Except in the rotated maize farmland where there was reduction in available phosphorus content, percentage organic matter, sodium and potassium contents of the soil were also improved at the end of the experiment (Tables 4.1 and 4.2). There was a slight change in the pH of the soil from 6.5 to 6.8, at the end of the experiment, though the change was not significant but a modification and improvement making the soil to be less acidic. The Ca content of the soil was reduced at the end of the experiment.

### 4.3 Effects of inoculation on growth of soyabean

Significant differences in growth were observed ($P \leq 0.05$) between the inoculated and uninoculated treatments in both sole and intercropped soyabean from the second to eight weeks after planting (WAP) particularly in rhizobial inoculated plots (Figures 4.1 – 4.4). At 2 WAP, inoculation significantly increased the height of soybean by 20.0% compared with the inoculated plant. At 8 WAP in intercropped soyabean, rhizobium, mycorrhizal and dual inoculation significantly increased the height of soyabean by 17 – 39% compared to uninoculated plant (Fig. 4.2). Likewise in the sole soyabean at the 2 WAP, inoculation increased the height of the soybean by about 18.0% compared with uninoculated plant while in the 8th WAP the height of rhizobium, mycorrhizal or dual inoculated plant was between 6.0 – 17.0% higher than the uninoculated plant (Fig. 4.2). There were significant differences ($P \leq 0.05$) in the heights of inoculated plants with rhizobium, mycorrhizal, and combined inoculated and that of the control (Figs. 4.1 and 4.2).

The number of leaves at the end of the eighth week in the intercropped soyabean with rhizobium, mycorrhiza and dual inoculation was between 34.4 - 51.5% greater than the uninoculated plant (Fig. 4.3). In sole soyabean, the number of leaves for the single or combined inoculation was between 16.8 - 20.8% higher than those of the corresponding uninoculated counterpart (Fig. 4.4).

The results obtained in both sole and intercropped soybean showed that there were significant differences ($P \leq 0.05$) in plant heights between rhizobium inoculated and uninoculated plants.

### 4.4 Effect of mycorrhizal inoculation on growth of maize

The number of leaves of the intercropped mycorrhizal inoculated and uninoculated maize at the 8th week after planting was not significantly different from each other (Fig. 4.5). Similarly, in sole maize, there were no significant differences in the number of leaves between the inoculated and uninoculated maize plants (Fig. 4.6). Similar trend were also observed in the heights of mycorrhizal inoculated and uninoculated maize for both intercropped and sole maize (Figs. 4.7 and 4.8). Nevertheless, higher mean values of plant heights obtained in the mycorrhizal inoculated treatments in sole and intercropped maize were 4.49% and 2.47% respectively higher than that of uninoculated maize.

### 4.5 Effects of dual incoculation with rhizobium and mycorrhizal on biomass production of soyabean

In respect to dual inoculation with rhizobium and mycorrhiza on both sole and intercropped soyabean, the two microsymbionts had positive effects on the growth and biomass production of soyabean (Table 4.3). The number of leaves and leaf dry weight in sole soyabean whether dual or mono inoculated were significantly higher than those of the uninoculated control plants. Higher significant values of total dry weight, number of nodules, number of pods and shoot dry weight were also obtained in the treatments inoculated with either of the microsymbionts or both in sole soyabean. In contrast, the leaf area and root dry weight of the inoculated and uninoculated soyabean did not differ significantly (Table 4.3). The sole soyabean, inoculated with rhizobium showed higher and better growth in terms of height and number of pods than other treatments. In most of the parameters determined in sole cropping, inoculated plants showed better growth than the uninoculated ones (Table 4.3).

**Table 4.1: Pre-cropping soil analysis result**

| Properties | Value |
|---|---|
| **Physical composition** | |
| Sand | 73 |
| Silt | 06 |
| Clay | 21 |
| Textural class | Alfisol |
| **Chemical composition** | |
| Soil pH | 6.50 |
| **% Minerals** | |
| Total N (g/kg) | 0.6 |
| Available P. (mg kg$^{-1}$) | 11.46 |
| Organic C | 0.48 |
| Organic Matter | 0.83 |
| Na | 0.13 |
| K | 0.16 |
| Ca | 1.28 |
| Mg | 0.04 |
| $H^+$ | 0.09 |

CEC = cation exchange capacity

**Table 4.2: Post-cropping soil analysis**

| Properties | Value | Value | Value |
|---|---|---|---|
| **Physical composition** | **Rotated soybean** | **Rotated maize** | **Intercropped** |
| Sand | 73 | 73 | 73 |
| Silt | 06 | 06 | 06 |
| Clay | 21 | 21 | 21 |
| CEC | 1.70 | 1.69 | 1.71 |
| Textural class | Alfisol | Alfisol | Alfisol |
| **Chemical composition** | | | |
| Soil pH | 6.76 | 6.52 | 6.51 |
| **% Minerals** | | | |
| Total N (g/kg) | 0.11 | 0.06 | 0.08 |
| Available P. (mg kg$^{-1}$) | 16.92 | 9.82 | 12.35 |
| Organic C | 1.08 | 0.57 | 1.01 |
| Organic Matter | 1.56 | 0.83 | 0.74 |
| Na | 0.31 | 0.12 | 0.14 |
| K | 1.14 | 1.16 | 0.17 |
| Ca | 0.16 | 0.15 | 0.18 |
| Mg | 0.38 | 0.03 | 0.03 |
| $H^+$ | 0.09 | 0.08 | 0.08 |

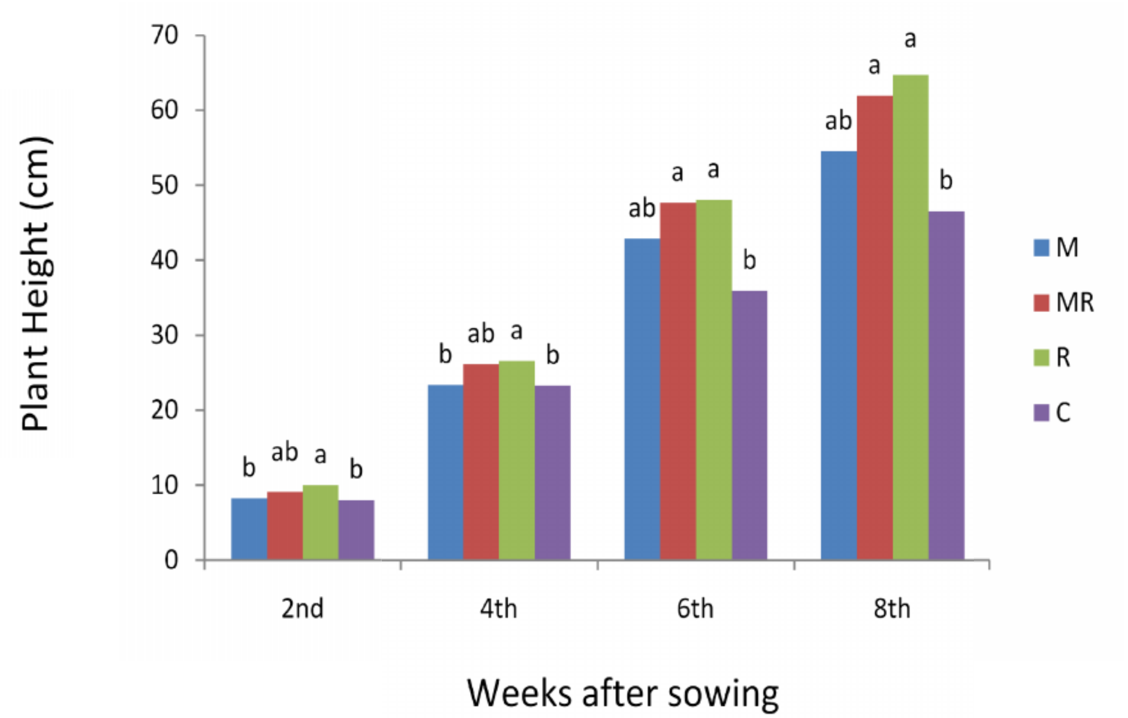

**Fig. 4.1: Height of the intercropped soyabean as affected by rhizobium and mycorrhizal inoculation.**

Bars are means of three replicates. Bars with different letters are significantly different at $P \leq 0.05$ according to Duncan's Multiple Range Test (DMRT).

M = Mycorrhizal inoculated

MR = Mycorrhizal and Rhizobium inoculated

R = Rhizobium inoculated

C = Uninoculated Plant

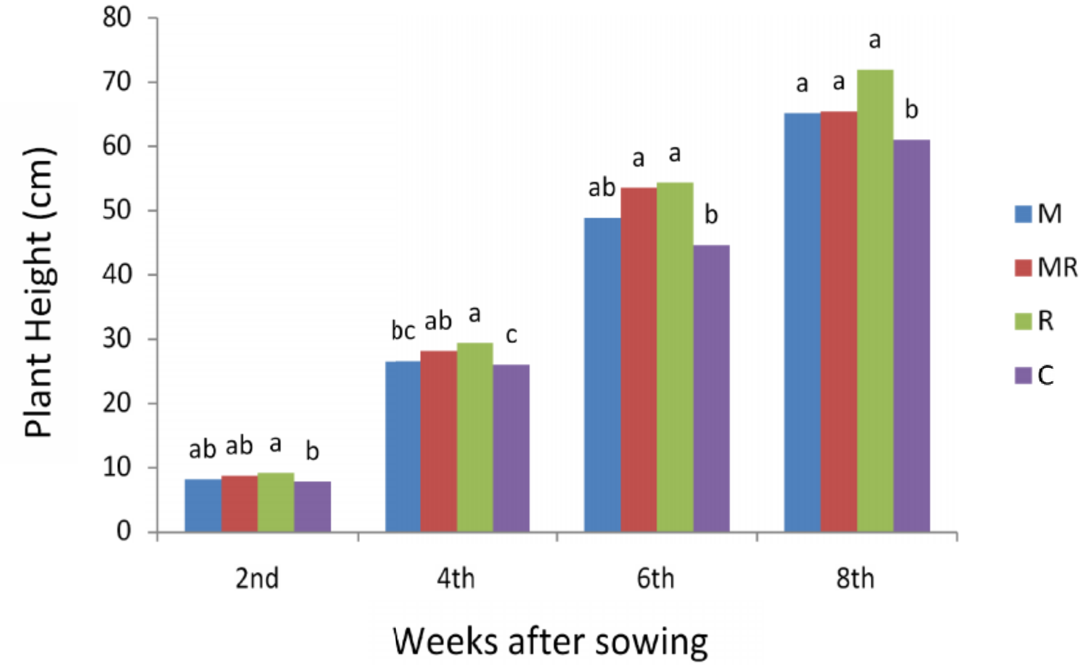

**Fig. 4.2: Height of sole soyabean as affected by rhizobium and mycorrhizal inoculation.**

Bars are means of three replicates. Bars with different letters are significantly different at $P \leq 0.05$ according to Duncan's Multiple Range Test (DMRT).

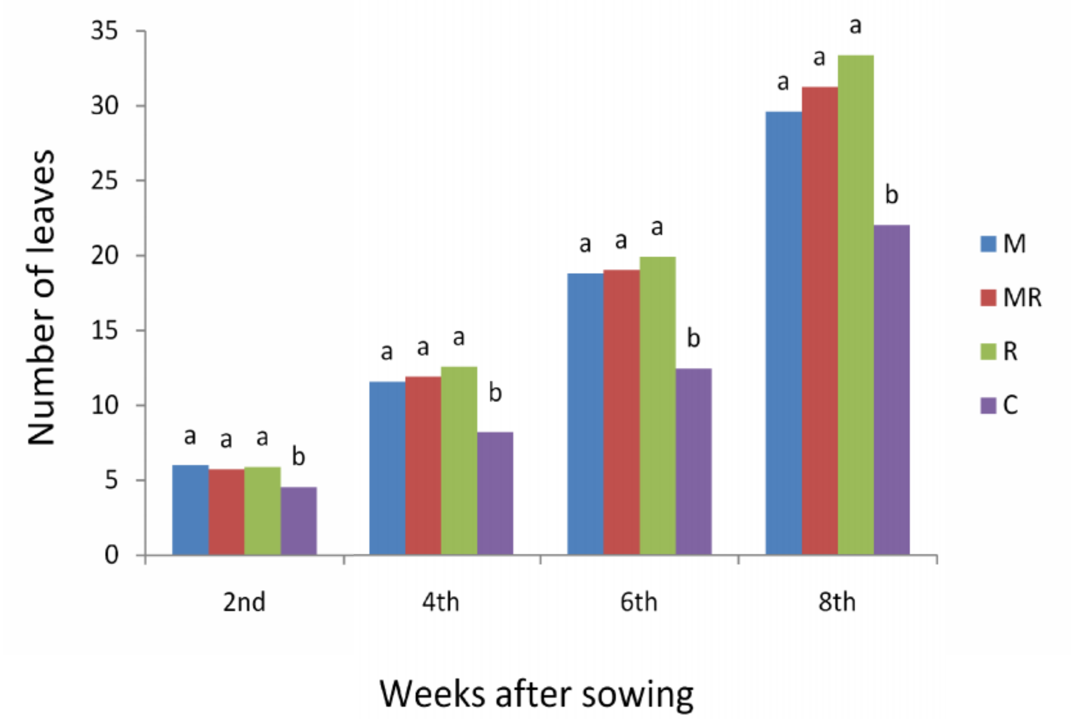

**Fig. 4.3: Number of leaves of the intercropped soyabean as affected by rhizobium and mycorrhizal inoculation.**

Bars are means of three replicates. Bars with different letters are significantly different at $P \leq 0.05$ according to Duncan’s Multiple Range Test (DMRT).

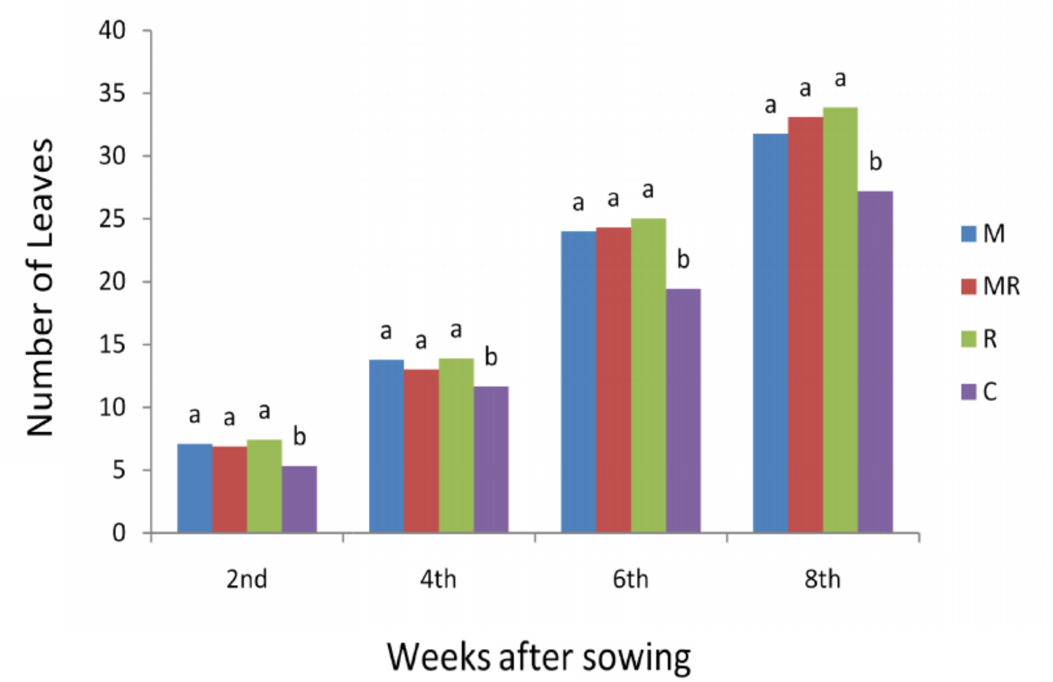

**Fig. 4.4: Number of leaves of the sole soyabean as affected by rhizobium and mycorrhizal inoculation.**

Bars are means of three replicates. Bars with different letters are significantly different at $P \leq 0.05$ according to Duncan's Multiple Range Test (DMRT).

Legend as in Figure 4.1

The total dry weight of sole soyabean inoculated with rhizobium, mycorrhizal and dual inoculated with both microsymbionts were 32.38%, 15.54%, and 25.91% respectively higher than uninoculated control treatments whereas in intercropped soyabean, the total dry weights of rhizobium, mycorrhiza dual inoculated plants were respectively 47.19%, 22.47% and 38.95% higher than their uninoculated counterparts (Table 4.3).

In intercropped soyabean, except for number of leaves, leaf area and root dry weight, the growth parameters did not follow any consistent pattern like sole soyabean and the later parameters were slightly higher than those of intercropped (Table 4.3).

### 4.6 Effects of inoculation on soyabean root nodulation

There was significant ($P \leq 0.05$) effect of rhizobium and mycorrhizal inoculation on root nodulation in both sole and intercropped soyabean. In sole soyabean, higher values of 227%, 100% and 134% root nodulation were obtained from the inoculated plants with rhizobium, mycorrhizal and combined inoculated soyabean respectively. Similarly, significant higher values of about 408%, 66% and 275% number of nodules for rhizobium, mycorrhizal and combined inoculated treatments respectively were obtained in intercropped soyabean (Table 4.3).

There was significant effect of inoculation on shoot dry weights of both sole and intercropped soyabean (Table 4.3). In sole soyabean, rhizobium, mycorrhizal and dual inoculated treatments were 42%, 17% and 28% respectively significantly higher than their uninoculated counterparts while in intercropped soyabean higher significant values of 52%, 23% and 46% respectively were obtained in the above mentioned treatments.

In both sole and intercropped soyabeans there were no significant effects of any of the inoculations and their interactions on leaf area and root dry weight. However, in both farming systems, there were significant interactions among inoculations in total dry weight and nodule production (Table 4.3).

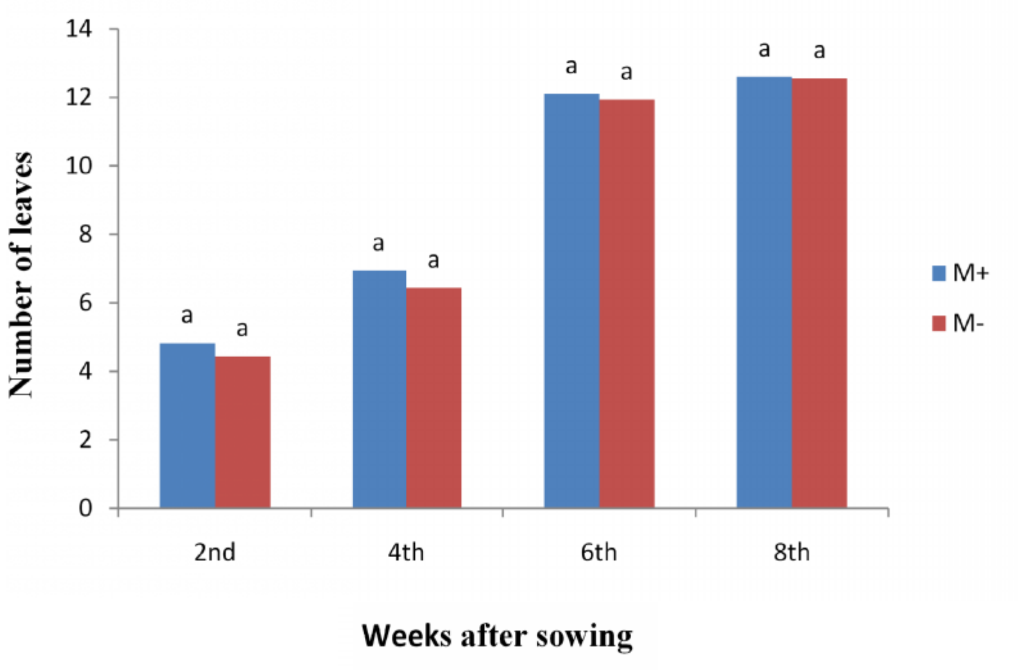

**Fig. 4.5:** **Number of leaves of the intercropped maize as affected by mycorrhizal inoculation**

Bars are means of three replicates. Bars with different letters are significantly different at $P \leq 0.05$ according to Duncan's Multiple Range Test (DMRT).

$M^+$ = Mycorrhizal inoculated

$M^-$ = Mycorrhizal uninoculated

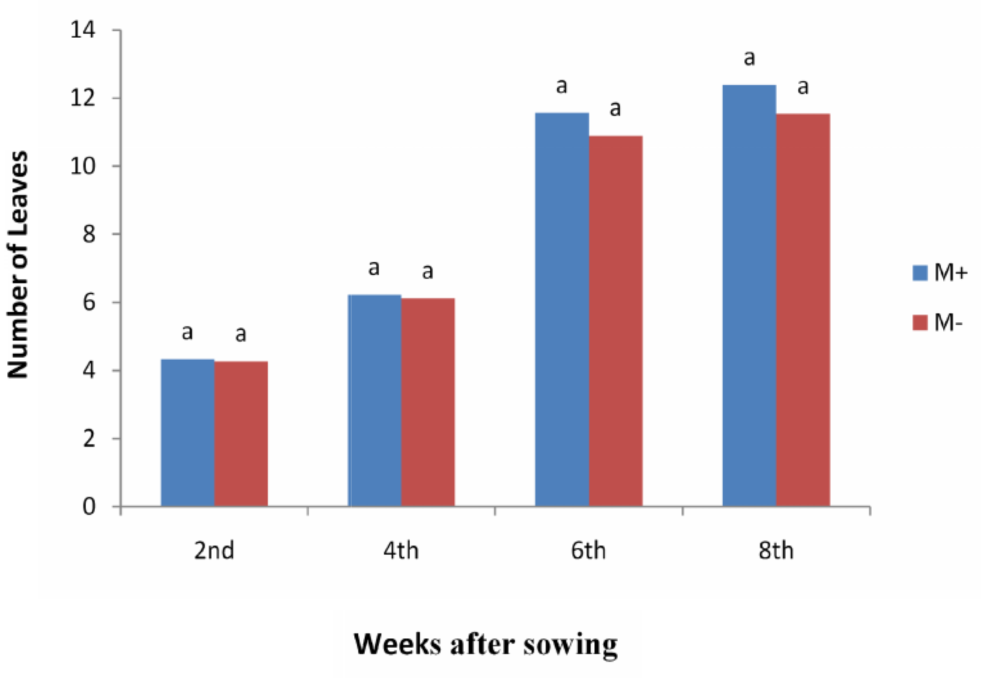

**Fig. 4.6:** **Number of leaves of the sole maize as affected by mycorrhizal inoculation**

Bars are means of three replicates. Bars with different letters are significantly different at $P \leq 0.05$ according to Duncan's Multiple Range Test (DMRT).

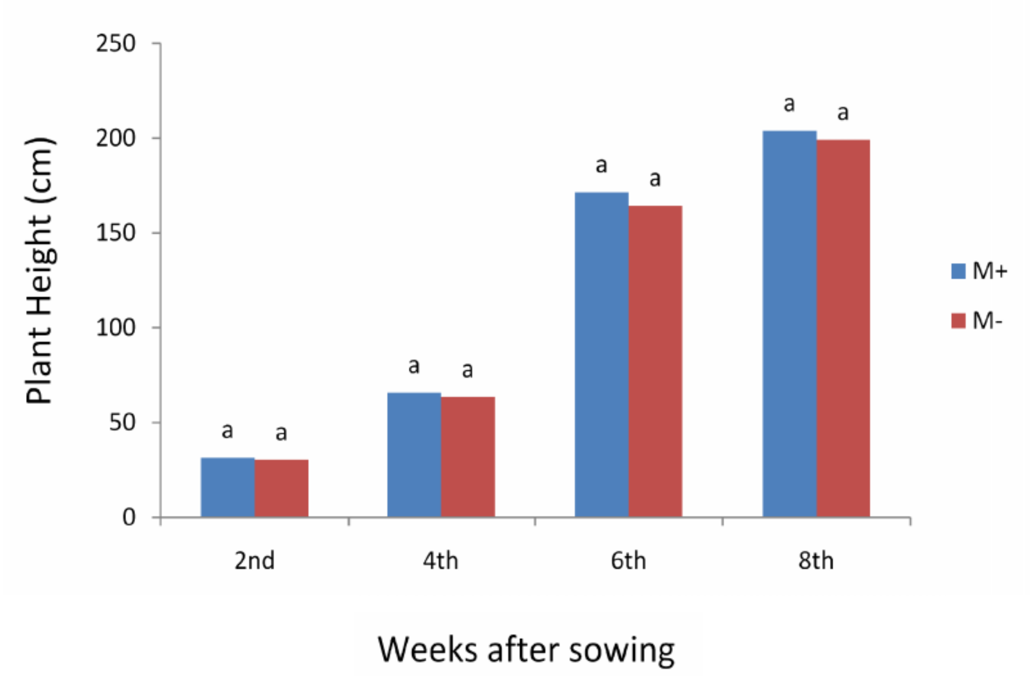

**Fig.4.7: Height of the intercropped maize as affected by mycorrhizal inoculation.**

Bars are means of three replicates. Bars with different letters are significantly different at $P \leq 0.05$ according to Duncan's Multiple Range Test (DMRT).

Legend as in Figure 4.5.

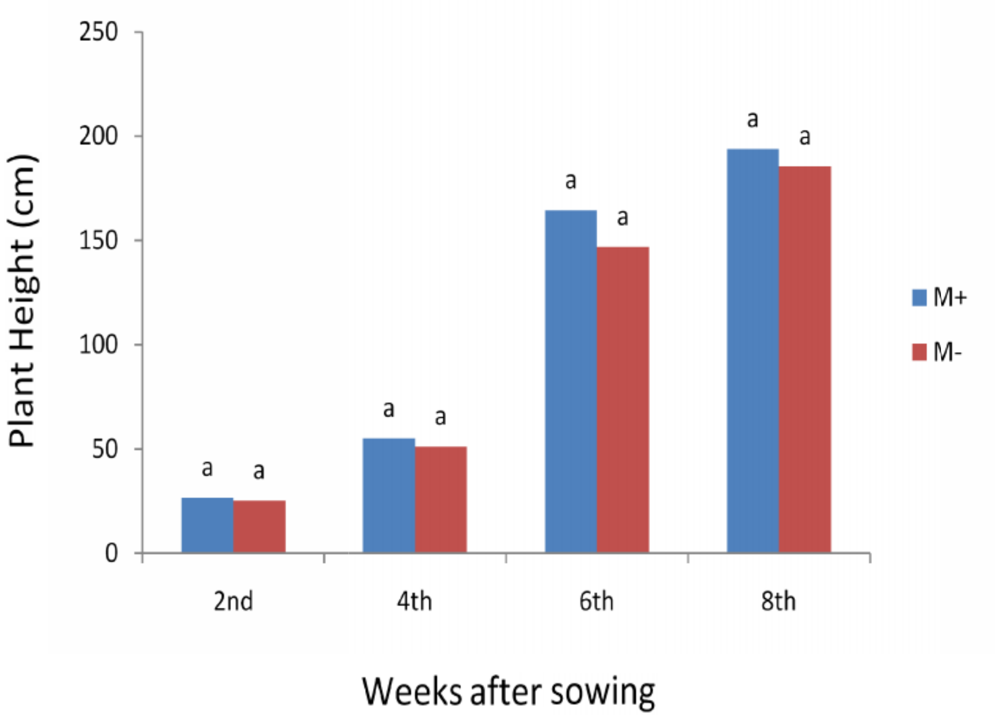

**Fig. 4.8: Height of sole maize as affected by mycorrhizal inoculation**

Bars are means of three replicates. Bars with different letters are significantly different at $P \leq 0.05$ according to Duncan's Multiple Range Test (DMRT).

See Figure 4.5 for abbreviation and legend.

## TABLE 4.3: Effect of Rhizobium and Mycorhizal Inoculation on root nodulation of Soyabean and Dry Matter Production

| Treatment | No of Nodules | No of Pods | Leaf Area (cm$^2$) | Leaf dry wt (g plant$^{-1}$) | Shoot dry wt (g plant$^{-1}$) | Root dry wt (g plant$^{-1}$) | Total dry wt (g plant$^{-1}$) |
|---|---|---|---|---|---|---|---|
| **Sole soyabean** | | | | | | | |
| M | 6.44ab | 35.56a | 11.53a | 1.80a | 3.47ab | 1.02a | 4.46ab |
| MR | 7.56ab | 36.44a | 12.06a | 1.90a | 3.80ab | 1.06a | 4.86ab |
| R | 10.56a | 39.67a | 12.96a | 2.19a | 4.02a | 1.09a | 5.11a |
| C | 3.22b | 24.33b | 11.28a | 1.20b | 2.96b | 0.89a | 3.86b |
| **Intercropped soyabean** | | | | | | | |
| M | 3.89b | 27.33ab | 11.80a | 1.13b | 2.52ab | 0.76a | 3.27ab |
| MR | 8.78ab | 27.67ab | 11.86a | 1.68ab | 2.99a | 0.813a | 3.71a |
| R | 11.89a | 33.66a | 13.06a | 2.06a | 3.12a | 1.39a | 3.93a |
| C | 2.34b | 19.11b | 9.03a | 1.06b | 1.91b | 0.63a | 2.67b |
| *Main effect* | | | | | | | |
| M | * | * | ns | ns | * | ns | * |
| R | * | * | ns | * | * | ns | * |
| MR | * | * | ns | * | * | ns | * |
| *Interactions* | | | | | | | |
| R x M | ns | ns | ns | ns | ns | ns | * |
| R x MR | ns | ns | ns | ns | ns | ns | ns |
| M x RM | ns | ns | ns | ns | ns | ns | ns |

The values are the mean of three replicates. Means with similar letters (within the same column) are not significantly different at P≤ 0.05 according to Duncan's Multiple Range Test (DMRT) = Mycorrhizal inoculated

R = Rhizobium inoculated

MR = Mycorrhizal and Rhizobium inoculated

C = Uninoculated Plant

* = significantly different at P≤ 0.05 level of significance

ns = Not significant

wt = Weight

## 4.7 Effects of mycorrhizal inoculation on dry matter production of maize

The results obtained from the maize experiment revealed non-significant differences ($P \leq 0.05$) between the mycorrhizal inoculated and uninoculated treatments (Table 4.4). However, the mycorrhizal inoculated treatment of intercropped maize was 6.7% higher than the uninoculated counterpart. In sole maize, similar results were obtained with mycorrhizal inoculated maize producing a shoot dry weight of 71.30g which was 7.08% higher than the uninoculated counterpart with a shoot dry weight of 66.58g (Table 4.4). Similarly, higher mean values of leaf dry weights (23.67g and 20.89g) which were 4.27% and 3.72% greater were obtained in mycorrhizal inoculated treatments in intercropped and sole maize respectively compared with that of their uninoculated counterparts.

Despite non significant differences ($P \leq 0.05$) statistically in the number of cob production between mycorrhizal inoculated and uninoculated treatments in both intercropped and sole maize, the number of cob of the intercropped mycorrhizal inoculated maize was 37.61% higher and significantly different from the uninoculated maize while in the sole maize the inoculated maize was 4.27% higher but not significantly different from its uninoculated counterpart (Table 4.4).

## 4.8 Effects of intercropping on growth of soyabean

It was observed that intercropping had significant effects on growth of soyabean (Fig. 4.9). The height of the sole soyabean was 15% significantly higher than that of the intercropped soyabean (Fig. 4.9). Although, there was no significant difference in the number of leaves between sole and intercropped soyabean at the end of eighth (8th) week of growth but the value obtained in sole soyabean was 10% higher than that of inercropped soyabean (Fig. 4.10).

There were significant differences in soyabean dry weight, number of pods and shoot dry weight of intercropped and sole soyabeans (Table 4.5). Higher significant ($P \leq 0.05$) value of 35% total dry weight, 39% number of pods and 33% shoot dry weight were obtained in sole soyabean compared with the intercropped soyabean. In contrast to number of pods and shoot dry weight, there were no significant differences in leaf area, leaf dry weight, and number of nodules between sole and intercropped soyabean. However, higher values of 7% leaf area, 3% number of nodules and 20% leaf dry weight were obtained in sole soyabean as against intercropped counterpart.

**TABLE 4.4: Effects of Mycorrhizal Inoculation on Biomass Production of Maize**

| Treatment | Leaf dry wt (g plant$^{-1}$) | Shoot dry wt (g plant$^{-1}$) | No of cob | Cob dry wt (g plant$^{-1}$) | Leaf dry wt (g plant$^{-1}$) | Shoot dry wt (g plant$^{-1}$) | No of cob | Cob dry wt (g plant$^{-1}$) |
|---|---|---|---|---|---|---|---|---|
| | **Intercropped Maize** | | | | **Sole Maize** | | | |
| $M^+$ | 23.67a | 82.97a | 1.61a | 154.36a | 20.89a | 71.30a | 1.22a | 107.60a |
| $M^-$ | 22.70a | 77.76a | 1.17a | 125.95b | 20.14a | 66.58a | 1.17a | 106.64a |
| | ns | ns | ns | * | ns | ns | ns | ns |

The values are the means of three replicates. Means with the same letter (within the same column) are not significantly different at $P \leq 0.05$ according to Duncan's Multiple Range Test (DMRT)

* = significantly different at $P \leq 0.05$ level of significance

ns = not significantly different

M+ = Mycorrhizal inoculated treatment

M- = Mycorrhizal uninoculated treatment

**TABLE 4.5: Effects of sole and intercropping farming systems on nodulation, leaf area and dry matter production of Soyabean**

| Soyabean | Leaf Area ($cm^2$) | Leaf dry wt (g/plant) | Shoot dry wt (g/plant) | Root dry wt (g/plant) | Total dry wt (g/plant) | No of Nodule | No of Pods |
|---|---|---|---|---|---|---|---|
| Intercropped | 11.44a | 1.48a | 2.67b | 0.87a | 3.40b | 6.72a | 26.13b |
| Sole | 12.25a | 1.77a | 3.56a | 1.01a | 4.58a | 6.95a | 36.50a |

The values are the means of three replicates. Means with the same letter in each column are not significantly different at $P \leq 0.05$ according to Duncan's Multiple Range Test.

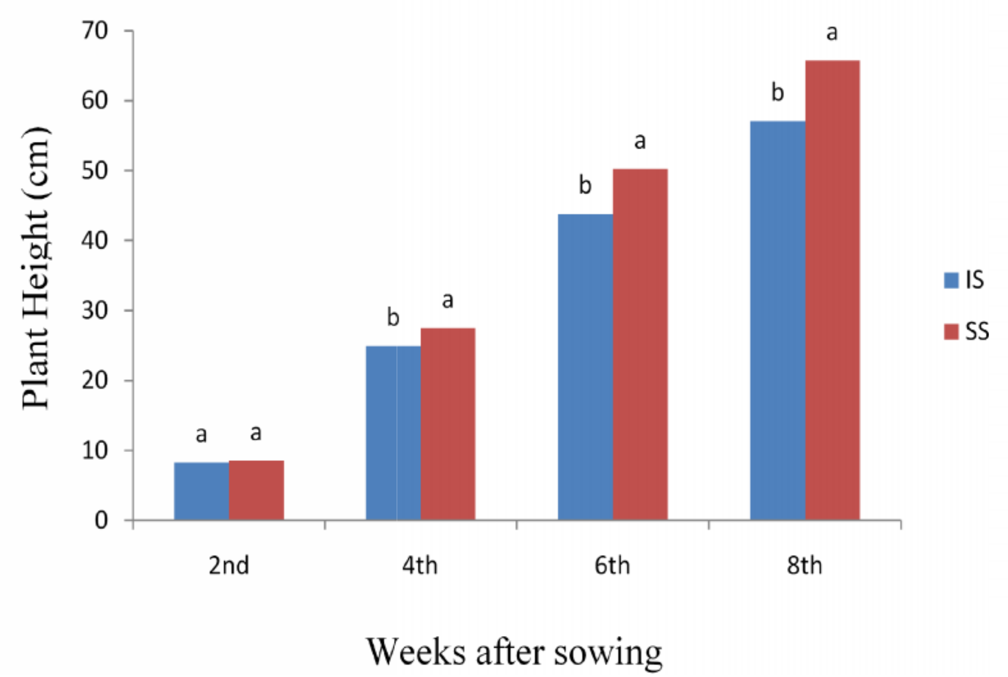

**Fig. 4.9: Effect of intercropping on height of soyabean**

Bars are means of three replicates. Bars followed with different letters are significantly different at $P \leq 0.05$ according to Duncan's Multiple Range Test (DMRT).

IS = Intercropped Soyabean
SS = Sole Soyabean

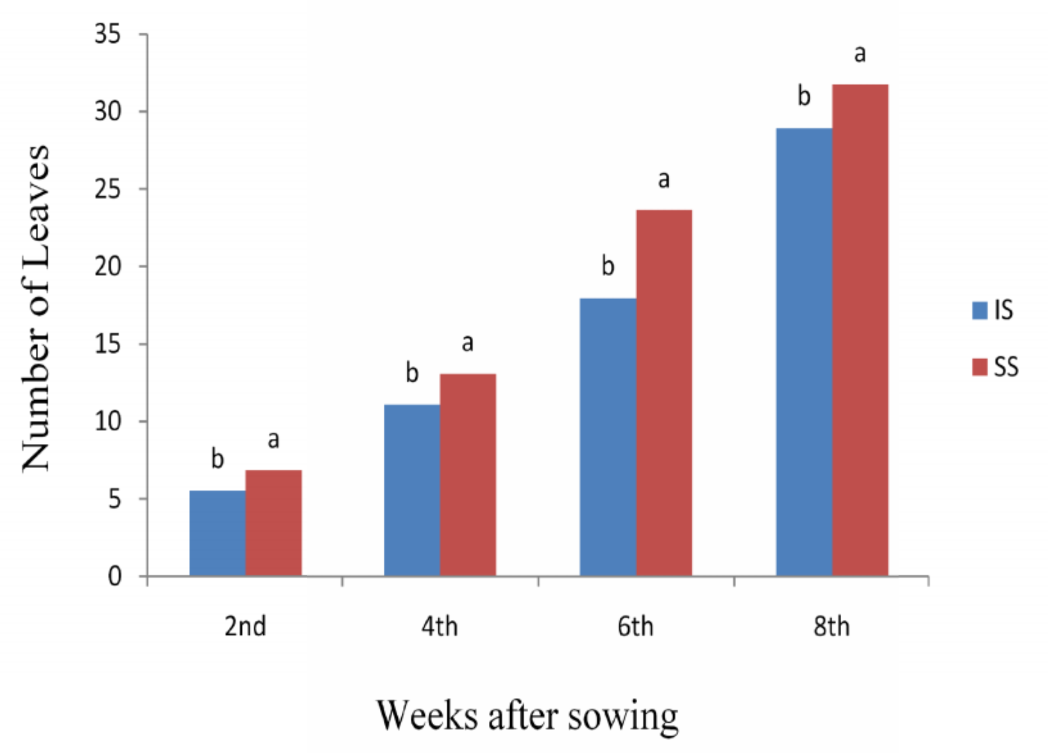

**Fig. 4.10: Effect of intercropping on number of leaves of soyabean**

Bars are means of three replicates. Bars followed with different letters are significantly different at $P \leq 0.05$ according to Duncan's Multiple Range Test (DMRT).

IS = Intercrop Soyabean

SS = Sole Soyabean

## 4.9 Effects of intercropping on growth of maize

There were significant differences between ($P \leq 0.05$) the plant height of intercropped and sole maize throughout the experimental period (Figure 4.11). The height of intercropped maize was 4% higher than its sole maize counterpart. Except for the (2nd) week of growth, the number of leaves between the intercropped and sole maize differ significantly (Figure 4.12). The number of leaves of the intercropped maize was 4% higher compared with sole maize.

There were similar significant effects of intercropping on dry matter (shoot, leaf and cob weights) production of maize (Table 4.6). Significant ($P \leq 0.05$) higher values of 17% shoot dry weight, 13% leaf dry weight and 27% cob dry weights were obtained in intercropped maize as against sole maize. Similarly, there were significant effects of intercropping on number of maize cobs produced per plant. The value obtained in intercropped maize was 16% higher than that of sole maize (Fig. 4.6).

## 4.10 Proximate and Nutrient Analyses

In sole soyabean, higher nutrient contents were obtained in the treatments inoculated with either of the two microsymbionts or both (Table 4.7). The soyabean inoculated with either rhizobium or mycorrrhiza have the values of crude protein which were 8%, and 7% respectively higher than their uninocculated counterparts (Table 4.7). Except for ash and moisture contents, inoculations did not show any significant difference in the proximate value over the uninoculated counterparts.

There were significant differences in moisture content of soyabean inoculated with either of the two or both microsymbionts and uninoculated soyabean. The percentage differences between the inoculated and uninoculated soyabean ranged between 30 and 36 with highest moisture content in the uninoculated soyabean (Table 4.7).

In the intercropped soyabean, higher values of nitrogen contents were obtained in inoculated soyabean over the uninoculated ones (Fig. 4.8). This showed that there were positive effects of inoculation with either or both microsymbionts on nitrogen contents of soyabean in comparism with their uninoculated counterparts in both sole and intercropped soyabean (Tables 4.7 and 4.8).

Except for plant moisture content and ether extract, the proximate contents of inculated soyabean were greater significantly than those of uninoculated counterparts (Table 4.8).

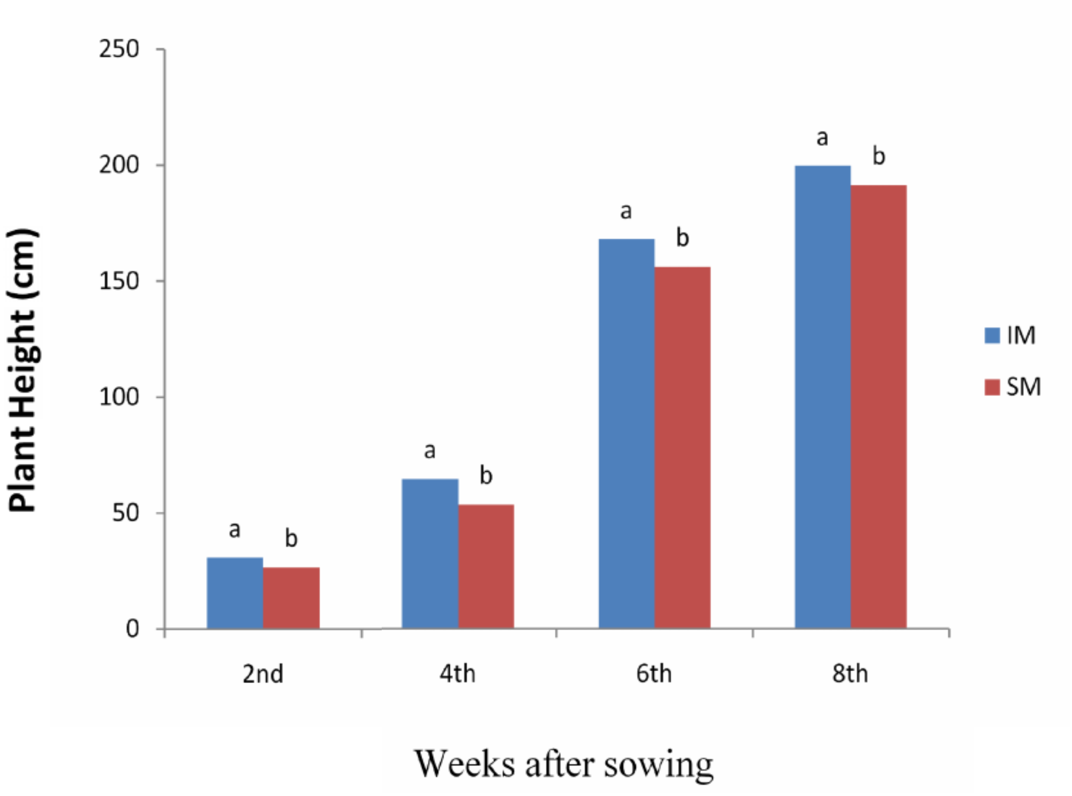

**Fig. 4.11: Height of Maize as affected by cropping systems**

Bars are means of three replicates. Bars followed by different letters are significantly different at $P \leq 0.05$ according to Duncan's Multiple Range Test (DMRT).

Im = Intercropped maize
Sm = Sole maize

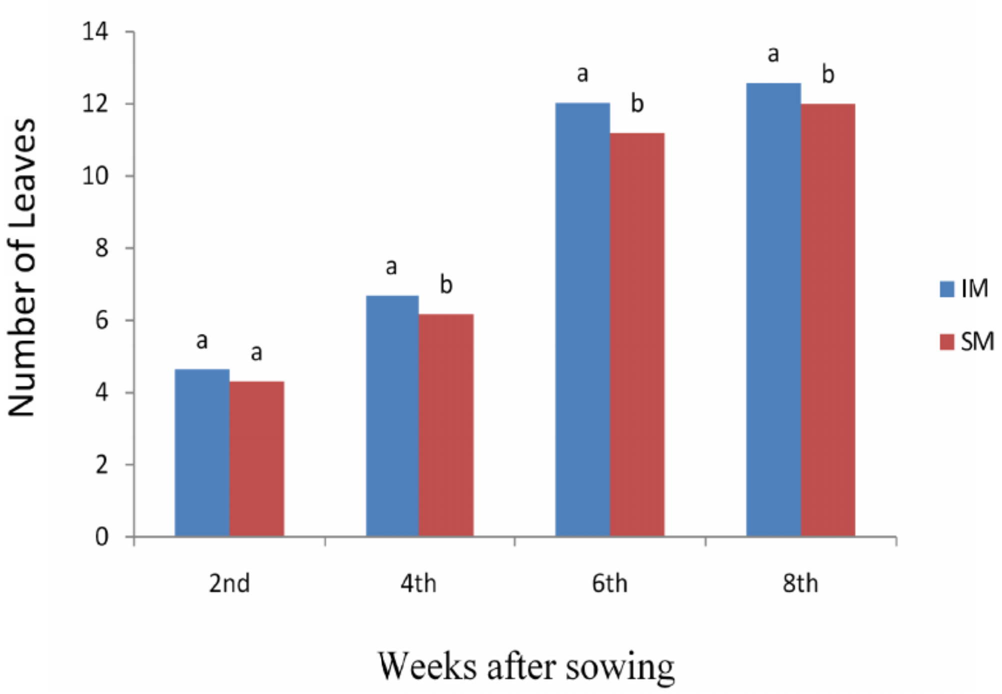

**Fig. 4.12: Number of leaves of maize as affected by cropping systems**

Bars are means of three replicates. Bars followed with different letters are significantly different at $P \leq 0.05$ according to Duncan's Multiple Range Test (DMRT).

**TABLE 4.6: Effects of Intercropping on Growth, Number of Cobs and Dry Matter (g/plant) Production of Maize**

| Treatment | Plant Height (cm) | No of Leaves | Leaf dry wt (g/plant) | Shoot dry wt (g/plant) | Total dry wt (g/plant) | No of Cobs | Cob Dry wt (g/plant) |
|---|---|---|---|---|---|---|---|
| Intcr Maize | 199.72a | 12.58a | 23.19a | 81.01a | 104.20a | 1.42a | 139.50a |
| Sole Maize | 191.39b | 12.00b | 20.52b | 69.08b | 89.60b | 1.22b | 109.51b |

The values are the means of three replicates. Means with the same letter within a column are not significantly different at P≤ 0.05 according to Duncan's Multiple Range Test

Intcr = intercropped

**TABLE 4.7: Proximate and Nutrient Contents of Sole Soyabean as Affected by Mycorrhiza and Rhizobium Inoculations**

| Tmt | % Crude protein | % Ether extract | % Crude fibre | % Ash | % Moisture content | N | Ca | K (ppm) | P | Mg |
|---|---|---|---|---|---|---|---|---|---|---|
| R | 37.23a | 20.66b | 3.40a | 6.99a | 5.63b | 0.20a | 0.25ab | 0.98a | 0.55a | 0.37ab |
| M | 36.70a | 19.73ab | 3.26a | 5.49ab | 5.76b | 0.16a | 0.20ab | 0.72ab | 0.57a | 0.47a |
| MR | 35.16b | 19.63ab | 3.16a | 5.16ab | 6.16b | 0.12a | 1.18a | 0.60b | 0.58a | 0.38ab |
| C | 34.23b | 18.52b | 2.69a | 4.63b | 8.80a | 0.07b | 0.13b | 0.58b | 0.54a | 0.24b |
| Main effect | | | | | | | | | | |
| R | ns | ns | ns | * | * | * | * | * | ns | * |
| M | ns | ns | ns | * | * | * | * | * | ns | * |
| MR | ns | ns | ns | * | * | * | * | * | ns | * |
| Intraction | | | | | | | | | | |
| R x M | ns | ns | ns | * | ns | * | ns | * | ns | * |
| R x RM | ns | ns | ns | * | ns | * | * | ns | ns | ns |
| M x RM | ns | ns | ns | ns | ns | * | * | ns | ns | * |

The values are the means of three replicates. Means with the same letter (within a column) are not significantly different at P≤ 0.05 according to Duncan's Multiple Range Test.

M = Mycorrhizal inoculated

R = Rhizobium inoculated

MR = Mycorrhizal and Rhizobium inoculated

C = Uninoculated Plant

* = significantly different at P≤ 0.05 level of significance

ns = Not significant

**TABLE 4.8: Proximate and Nutrient Contents of Intercropped Soyabean as Affected by Mycorrhiza and Rhizobium Inoculations**

| Treatment | % Crude protein | % Ether extract | % Crude fibre | % Ash | % Moisture content | N | Ca | K (ppm) | P | Mg |
|---|---|---|---|---|---|---|---|---|---|---|
| R | 38.48a | 20.40a | 4.15a | 6.86a | 5.96b | 0.18a | 0.20a | 0.85a | 0.66a | 0.37a |
| M | 38.45a | 20.34a | 4.01a | 6.87a | 6.98ab | 0.15a | 0.21a | 0.84a | 0.66a | 0.35a |
| MR | 38.32a | 20.16a | 4.57a | 6.99a | 6.67ab | 0.14a | 0.23a | 0.82a | 0.65a | 0.36a |
| C | 38.90a | 20.70a | 3.13b | 4.35b | 8.50a | 0.13a | 0.18a | 0.80a | 0.63a | 0.34a |
| Main effect | | | | | | | | | | |
| R | ns | ns | * | * | * | ns | ns | ns | ns | ns |
| M | ns | ns | * | * | * | ns | ns | ns | ns | ns |
| MR | ns | ns | * | * | * | ns | ns | ns | ns | ns |
| Intraction | | | | | | | | | | |
| R x M | ns | ns | ns | * | ns | ns | ns | ns | ns | ns |
| R x RM | ns | ns | * | * | ns | ns | ns | ns | ns | ns |
| M x RM | ns | ns | ns | * | ns | ns | ns | ns | ns | ns |

The values are the means of three replicates. Means with the same letter are not significantly different at $P \leq 0.05$ according to Duncan's Multiple Range Test.

Higher Calcium, Potassium and phosphorus contents were also obtained in the soyabean inoculated with either mycorrhiza or rhizobium or both inoculations while the least values were obtained in the uninoculated soyabean. These higher values of mineral elements contents range between 3% and 185% in sole soyabean (Table 4.7). Similar results of higher plant mineral nutrients were obtained in intercropped soyabean, these ranged between 8% and 36.0% higher than what was obtained in uninoculated soyabean (Table 4.8).

Except for ether extract and moisture content, inoculations with either or both microsymbionts showed significantly higher values of proximate analysis/contents than the uninoculated counterparts in intercropped soyabean (Table 4.8). However, for the nutrient contents, inoculations did not show any difference in the intercropped soyabean plants.

Generally, the cumulative performance of the two microsynbionts on the nutritive quality and mineral nutrients of soyabean were at high level.The results obtained showed high values of crude protein, ether extract and crude fibre in treatments inoculated with either or both microsynbionts. Although there were no significant differences in crude fibre, ash contents between the inoculated and uninoculated soyabean but for crude protein and ether extract, the treatments inoculated with mycorrhiza, rhizobium and combined inoculations were between 3 - 6% and 2 - 5% respectively higher than their uninoculated counterparts (Table 4.9). In the reverse, the moisture contents of the uninoculated treatments were significantly higher than that of their inoculated counterparts (Table 4.9).

With the exception of Magnesium where the inoculated treatements were between 14 – 41% significantly higher, there were no significant differences in mineral nutrient contents of the inoculated and uninoculated soyabean, though higher values of nitrogen, calcium, potassium and phosphorus contents were obtained in the treatments inoculated with either of the two or both microsymbionts (Table 4.9). There was no interactive effect of inoculation with both microorganisms and systems of farming on proximate and nutrient contents of soyabean

**TABLE 4.9: Proximate and Nutrient Contents of Soyabean as Affected by Mycorrhiza and Rhizobium Inoculations**

| Treatment | % Crude protein | % Ether extract | % Crude fibre | % Ash | % Moisture content | N | Ca | K (ppm) | P | Mg |
|---|---|---|---|---|---|---|---|---|---|---|
| R | 37.85a | 20.41a | 3.78a | 6.24a | 7.89a | 0.17a | 0.23a | 0.91a | 0.61a | 0.41a |
| M | 37.51a | 20.02a | 3.41a | 6.01a | 7.42a | 0.15a | 0.20a | 0.79a | 0.61a | 0.38a |
| MR | 36.81a | 19.91a | 3.35a | 5.74a | 7.13a | 0.13a | 0.19a | 0.71a | 0.62a | 0.33ab |
| C | 35.57b | 19.43a | 3.14a | 5.67a | 5.77b | 0.13a | 0.17a | 0.70a | 0.60a | 0.29b |
| Main effect | | | | | | | | | | |
| R | * | ns | ns | ns | * | ns | ns | ns | ns | * |
| M | * | ns | ns | ns | * | ns | ns | ns | ns | * |
| MR | * | ns | ns | ns | * | ns | ns | ns | ns | * |
| Intraction | | | | | | | | | | |
| R x M | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns |
| R x RM | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns |
| M x RM | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns |

The values are the means of the replicates in each treatment. Means with the same letter (within the same column) are not significantly different at $P \leq 0.05$ according to Duncan's Multiple Range Test.

## 4.11 Effects of intercropping on nutrient contents of soyabean

Comparing the effects of intercropping system on nutrient contents of both sole and intercropped soyabean, it could be seen in Table 4.10 that only higher crude protein and ash contents of the intercropped soyabean were significantly higher (6% and 19%) than the sole soyabean. The intercropping did not have significant effects on the remaining nutrient contents of soyabean. (Table 4.10).

Inspite of the enhanced plant growth obtained in sole soyabean, it did not translate to improve crude portein and ash contents but rather higher values of crude protein and ash contents were obtained in the intercropped soyabean.

## 4.12 Effects of rhizobium and mycorrhizal inoculation on yield of soyabean

The result obtained on the yield of soyabean showed that there were significant effects of rhizobium and mycorrhizal inoculation on the yield of both sole and intercropped soyabean (Table 4.11). Inoculations with mycorrhiza, rhizobium and combined microsymbionts produced higher yields than the uninoculated counterparts in either the sole or intercropped soyabean.

## 4.13 Effects of intercropping on yield of soyabean

Considering the effects of the two systems of farming on the yield of soyabean, the results obtained from the experiment showed that intercropping had negative effect by reducing the grain yield of soyabean (Table 4.12). The sole soyabean produced a greater grain yield of about 5% higher than the yield obtained in the intercropped soyabean.

## 4.14 Effects of Mycorrhizal Inoculation on Yield of Maize

The results obtained on the effects of mycorrhizal inoculation on yield of maize revealed that there were differences in the yields of mycorrhizal inoculated and uninoculated maize in both sole and intercropped farmlands (Table 4.13). In sole farmland, the mycorrhizal inoculated maize produced a yield of about 13% higher than that of uninoculated counterpart. While in the intercropped farmland a yield of 566.67 kg/ha was obtained in the mycorrhizal inoculated maize plot which, was about 18% higher than their uninoculated counterpart.

Table 4.14 shows the effects of sole and intercropping on the yield of the maize. There was no significant difference in the yield of sole and intercropped maize, although the intercropped maize had slightly higher yield of about 1.81% higher than what was obtained in the sole maize.

**TABLE 4.10: Nutrient Contents of Soyabean as Affected by Intercropping**

| Treatment | % Crude protein | % Ether extract | % Crude fibre | % Ash | % Moisture content | N ____ | Ca ____ | K (ppm) | P ____ | Mg ____ |
|---|---|---|---|---|---|---|---|---|---|---|
| Intercropped | 38.04a | 20.15a | 3.71a | 6.67a | 7.01a | 0.15a | 0.21a | 0.83a | 0.65a | 0.36a |
| Sole | 35.82b | 19.64a | 3.13a | 5.57b | 7.09a | 0.14a | 0.19a | 0.72a | 0.55a | 0.34a |

The values are the means of all replicates within the farmlands. Means with the same letter (within the same column) are not significantly different at $P \leq 0.05$ according to Duncan's Multiple Range Test

## TABLE 4.11: Yield ($kgha^{-1}$) of Soyabean as Affected by Rhizobium and Mycorrhizal Inoculations

| Treatment | Sole Soyabean Yield ($kgha^{-1}$) | Intercropped Soyabean Yield ($kgha^{-1}$) |
|---|---|---|
| M | 648.15a | 611.11a |
| MR | 674.07a | 629.63a |
| R | 666.67a | 607.41a |
| C | 433.33b | 522.22b |

The values are the means of the three plicates in each treatment. Means with the same letter (within the same column) are not significantly different at $P \leq 0.05$ according to Duncan's Multiple Range Test.

M = Mycorrhizal inoculated

R = Rhizobium inoculated

MR = Mycorrhizal and Rhizobium inoculated

C = Uninoculated Plant

**TABLE 4.12: Yield ($kgha^{-1}$) of Soyabean as Affected by Farming System**

| Treatment | Yield/hectare ($kgha^{-1}$) |
|---|---|
| Sole Soyabean | 624.07a |
| Intercropped. Soyabean | 592.59a |

The values are the cumulative products of soyabean replicates in the two farmlands.

**TABLE 4.13: Grain Yield (kgha$^{-1}$) of Maize as Affected Mycorrhizal Inoculation**

| Treatment | Sole Maize | Intercropped Maize |
|---|---|---|
| M$^{+}$ | 544.44 ± 8.70 | 566.67 ± 13.83 |
| M$^{-}$ | 481.48 ± 8.40 | 477.78 ± 7.71 |

M$^{+}$ = Mycorrhizal inoculated treatment

M$^{-}$ = Mycorrhizal uninoculated treatment

**TABLE 4.14: Grain Yield (kgha$^{-1}$) of Maize as Affected by Farming System**

| Treatment | Yield/hectare (kgha$^{-1}$) |
|---|---|
| Sole Maize | 512.96 ± 14.39 |
| Intercropped maize | 522.22 ± 21.10 |

The values are the cummulative products of maize in the two farmlands

## 4.15 Experiment 2

## 4.16 Effects of inoculation on growth of soyabean

The result obtained from the experiment two showed that there were significant differences in plant height, number of leaves and diameter of stem between inoculated treatments and their uninoculated (control) counterparts in both intercropped and sole/rotated soyabean (Figs. 4.13 – 4.18).

At 8th week after planting (WAP), the number of leaves of the mycorrhizal, rhizobium and combined inoculated treatments in the intercropped soyabean were 35, 40 and 43% respectively higher than what was obtained in the uninoculated treatments (Fig. 4.13). In sole/rotated soyabean, numbers of leaves of the inoculated treatments were 24, 30 and 28% higher than their uninoculated (control) treatment (Fig. 4.14).

The height of mycorrhizal, rhizobium and combined inoculated treatments were also 10, 21 and 27% respectively higher than the values obtained in the uninoculated treatment in the soyabean-maize intercrops (Fig. 4.15) while in the sole/rotated soyabean, the height of the inoculated treatments were between 15 to 64% higher than that of the uninoculated treatments (Fig. 4.16).

Diameter of stem of the mycorrhizal, rhizobium and dual inoculated treatments were 28, 28 and 30% respectively higher than the height of their uninoculated treatments in intercropped soyabean while in the rotated soyabean the percentage increase in stem diameter of the inoculated treatments were 22, 22 and 24% respectively higher when compared with uninoculated counterparts (Figs. 17 and 18).

## 4.17 Effects of inoculation on dry matter production of soyabean

There were significant effects of rhizobium and mycorrhizal inoculation on biomass production of both intercropped and rotated/sole soyabean (Table 4.15). In both inoculated intercropped and rotated soyabean, the growth parameters and mycorrhizal colonization were 22 – 200% significantly higher than their uninoculated control. Except for shoot dry weight and mycorrhizal colonization, the inoculations did not produce significant interaction in all the parameters determined (Table 4.15). Specifically, the number of nodules and AM root colonization of inoculated intercropped and rotated soyabean was about two to three fold of the uninoculated counterparts.

Except between mycorrhizal and combined inoculation with both microsymbioints where there was no interactive effect, other results showed that there was effect of interaction between rhizobium and mycorrhizal inoculation on AM root colonization.

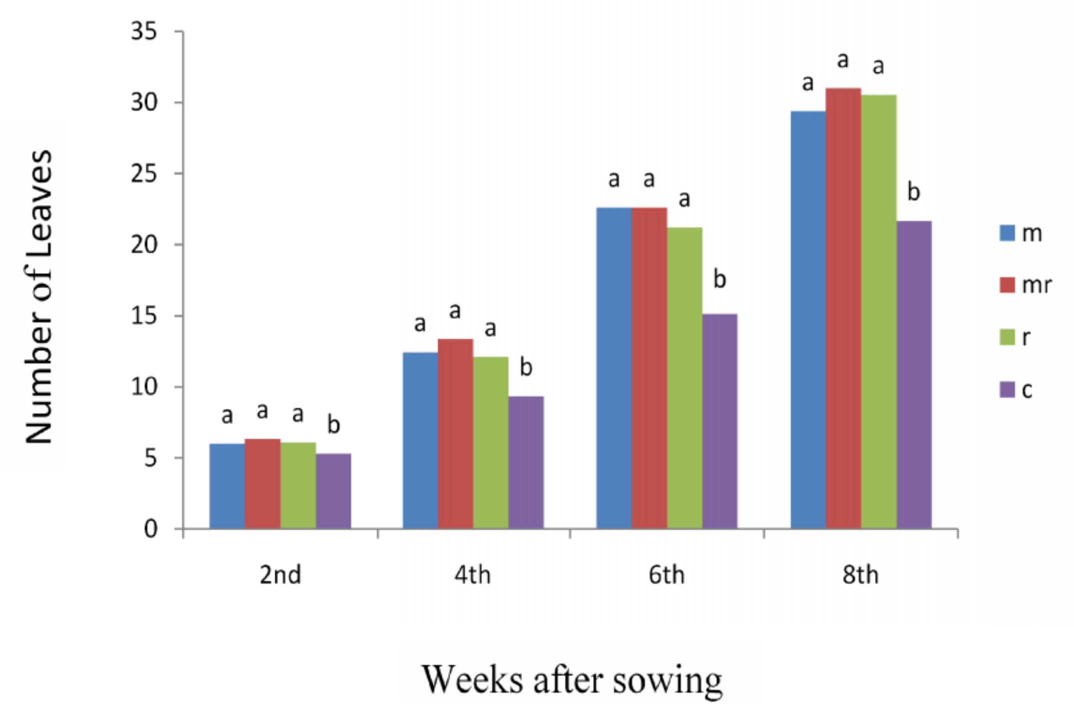

**Fig. 4.13: Number of leaves of the intercropped soyabean as affected rhizobium and mycorrhizal inoculation.**

Bars are means of three replicates. Bars with different letters are significantly different at P ≤ 0.05 according to Duncan’s Multiple Range Test (DMRT).

M = Mycorrhizal inoculated

R = Rhizobium inoculated

MR = Mycorrhizal and Rhizobium inoculated

C = Uninoculated Plant

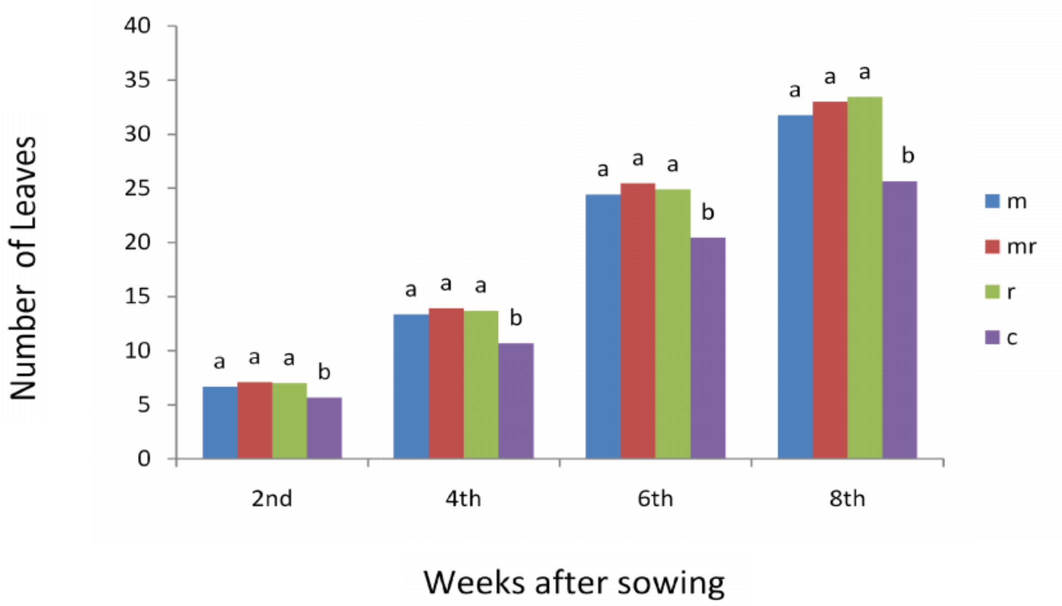

**Fig. 4.14: Number of leaves of the sole/rotated soyabean as affected rhizobium and mycorrhizal inoculation.**

Bars are means of three replicates. Bars with different letters are significantly different at $P \leq 0.05$ according to Duncan's Multiple Range Test (DMRT).

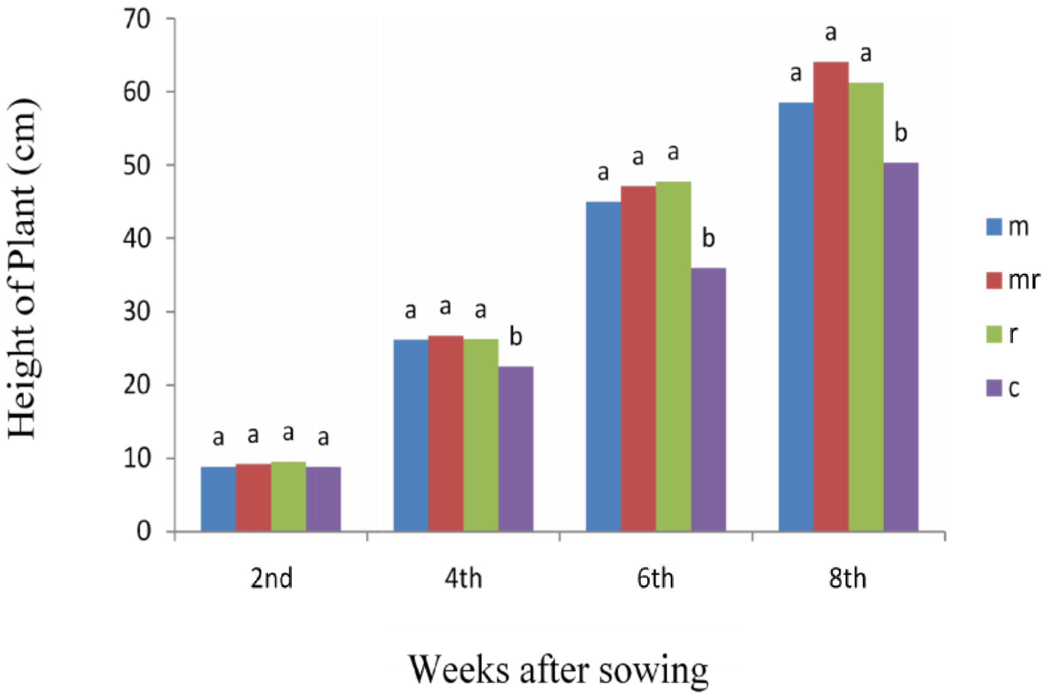

**Fig. 4.15: Height of the intercropped soyabean as affected by rhizobium and mycorrhizal inoculation.**

Bars are means of three replicates. Bars with different letters are significantly different at $P \leq 0.05$ according to Duncan's Multiple Range Test (DMRT).

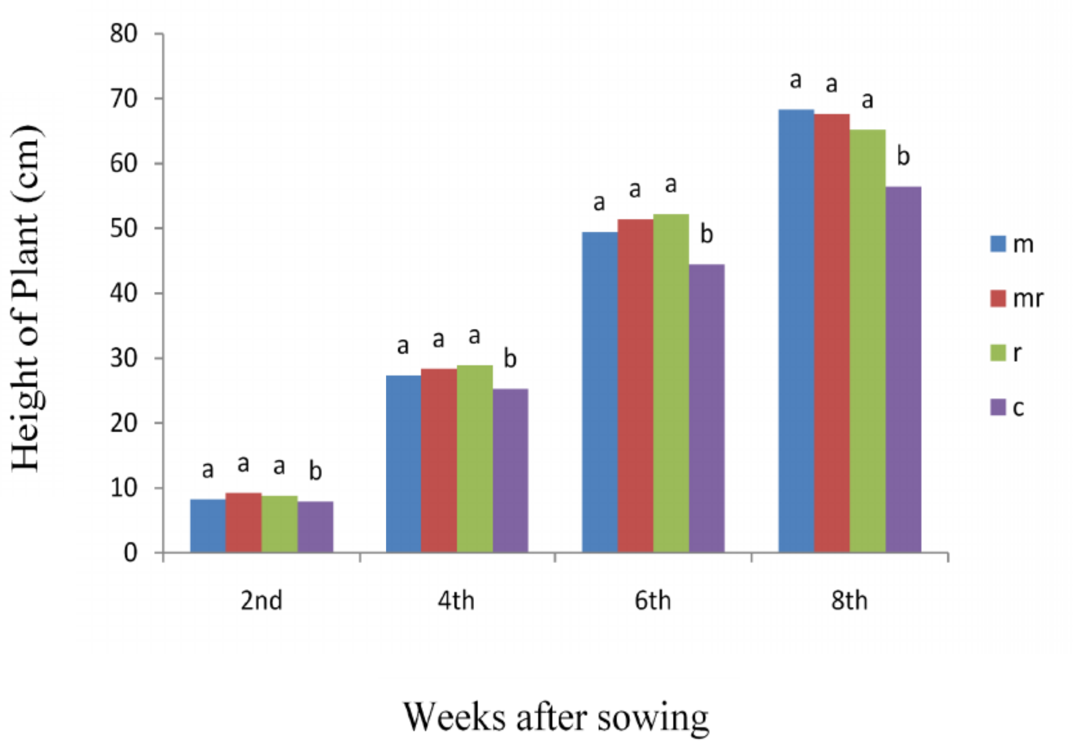

**Fig. 4.16: Height of sole/rotated soyabean as affected by rhizobium and mycorrhizal inoculation.**

Bars are means of three replicates. Bars with different letters are significantly different at $P \leq 0.05$ according to Duncan's Multiple Range Test (DMRT).

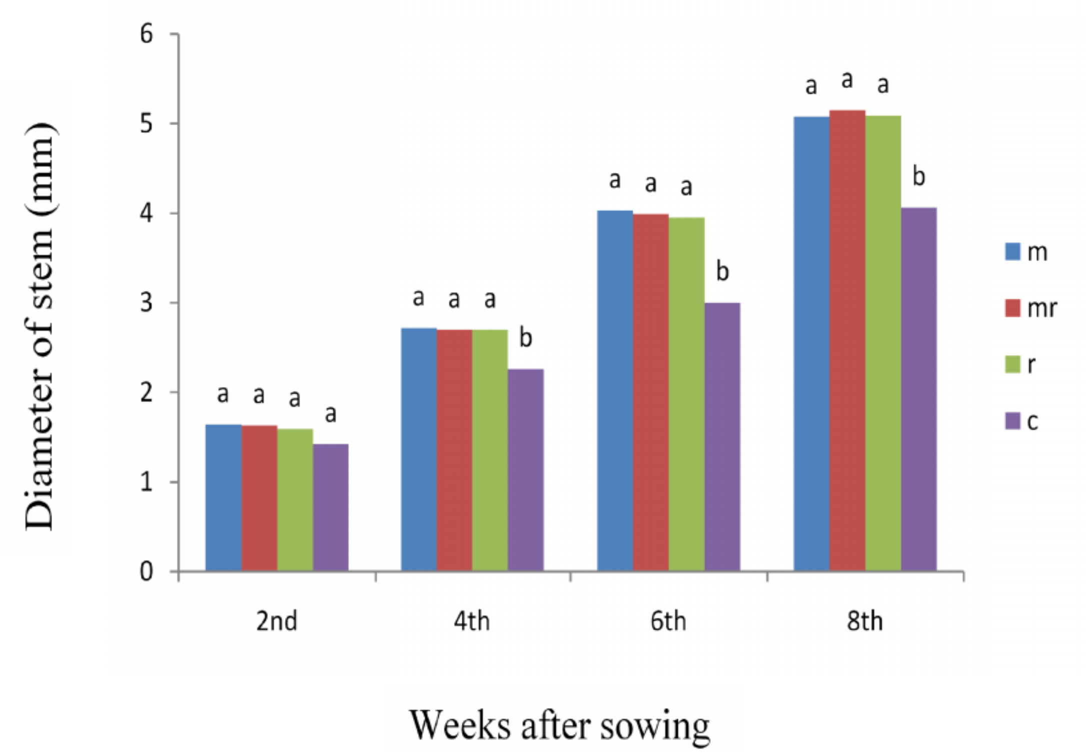

**Fig. 4.17: Diameter of stem of intercropped soyabean as affected by rhizobium and mycorrhizal inoculation.**

Bars are means of three replicates. Bars with different letters are significantly different at $P \leq 0.05$ according to Duncan’s Multiple Range Test (DMRT).

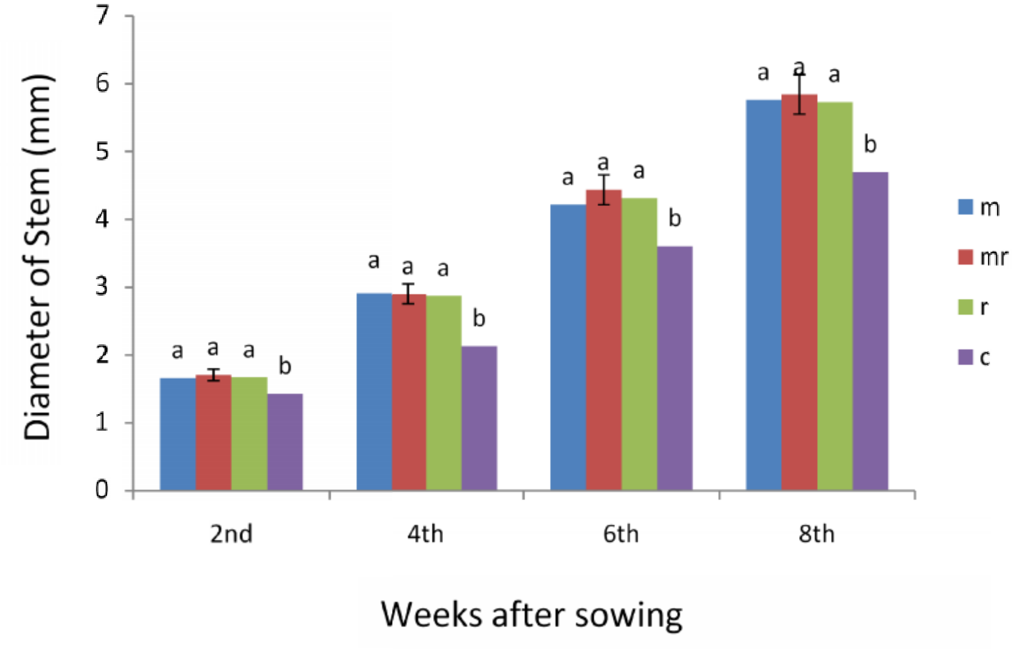

**Fig. 4.18: Diameter of stem of sole/rotated soyabean as affected by rhizobium and mycorrhizal inoculation.**

Bars are means of three replicates. Bars with different letters are significantly different at $P \leq 0.05$ according to Duncan's Multiple Range Test (DMRT).

Similarly, the results also revealed the effects of interactions between the treatements and systems of farming (Table 4.15)

## 4.18 Effects of farming systems on growth of soyabean

There were significant differences in plant height, number of leaves and stem diameter between intercropped and rotated soyabean from the second to the eighth week after planting (Table 4.16). The height of the rotated soyabean was between 11.04 – 11.58% higher than the values obtained in the intercropped soyabean. The number of leaves and stem diameter of the rotated soyabean were between 7 – 18% higher than those of intercropped soyabean.

## 4.19 Impact of intercropping and rotation systems of farming on soyabean biomass production

There were significant effects of crop rotation ($P \leq 0.05$) on biomass production of soyabean (Table 4.17). The rotated soyabean produced higher significant total dry weight of 4.80g which was about 23% higher than the value 3.89g obtained in intercropped soyabean. Leaf area, leaf dry weight, number of nodules, number of pods, shoot dry weight, root dry weight were between 4 - 24% significantly higher than the values obtained in the intercropped soyabean. The AM root colonization of the rotated soyabean and intercropped soyabean did not differ significantly from each other (Table 4.17).

## 4.20 Effects of mycorrhizal inoculation on growth of maize

Higher mean values of number of leaves and plant height were obtained in the mycorrhizal inoculated treatments in both intercropped and rotated maize compared with uninoculated (Figure 4.19 – 4.22).

There were significant effects of mycorrhizal inoculation on biomass and dry matter production of both intercropped and rotated maize (Table 4.18). The mean values of total dry weight, shoot dry weight, number of cobs and cob dry weights were higher in the inoculated treatments than the uninoculated counterparts in the intercropped maize. In the rotated maize however, the corresponding dry matter weights of mycrorrhizal inoculated treatments were similarly higher than their uninoculated counterparts (Table 4.18).

The result obtained on effects of farming systems showed that there were no significant differences in the biomass of intercropped and rotated maize (Table 4.19). Notwithstanding, the total dry matter production of both intercropped and rotated maize were higher than that of sole maize.

**Table 4.15: Effect of dual inoculation with rhizobium and mycorrhiza on soyabean Biomass production and AM colonization**

| Treatments | No of Nodules | No of Pods | Leaf Area g/plant | Leaf dry wt g/plant | Shoot dry wt g/plant | Root dry wt g/plant | Total dry wt g/plant | % AM root colonization |
|---|---|---|---|---|---|---|---|---|
| **Intercropped soyabean** | | | | | | | | |
| R | 13.55a | 26.67a | 13.20a | 1.41ab | 2.95b | 0.97a | 3.93ab | 73.75a |
| M | 14.44a | 32.56a | 13.14a | 1.80a | 3.32a | 1.09a | 4.45a | 75.15a |
| MR | 7.56ab | 31.89a | 12.68a | 1.74a | 3.19ab | 1.06a | 4.27a | 46.97ab |
| C | 2.33b | 18.44b | 10.18b | 1.04b | 2.24c | 0.65b | 2.90b | 28.60b |
| **Rotated soyabean** | | | | | | | | |
| R | 13.33a | 41.89a | 13.50a | 2.10a | 3.72a | 1.13a | 4.90a | 78.66a |
| M | 17.00a | 44.11a | 13.66a | 2.13a | 3.99a | 1.24a | 5.23a | 80.93a |
| MR | 14.77a | 47.00a | 13.37a | 2.22a | 3.92a | 1.26a | 5.22a | 50.43ab |
| C | 4.11b | 21.56b | 11.03b | 1.25b | 2.96b | 0.89b | 3.85b | 26.68 b |
| Main Effects | | | | | | | | |
| M | * | * | * | * | * | * | * | * |
| MR | * | * | * | * | * | * | * | * |
| R | * | * | * | * | * | * | * | * |
| Interactions | | | | | | | | |
| M x R | ns | ns | ns | ns | * | ns | ns | * |
| M x MR | ns | ns | ns | ns | * | ns | ns | ns |
| R x MR | ns | ns | ns | ns | * | ns | ns | * |
| M x R x MR x Sys | ns | ns | ns | ns | ns | ns | ns | * |

The values are means of three replicates. Means with the same letter (within the same column) are not significantly different at P = 0.05 according to Duncan Multiple Range Test (DMRT).

M = Mycorrhizal inoculated
R = Rhizobium inoculated
MR = Mycorrhizal and rhizobium inoculated
C = Uninoculated treatment
* = Significantly different at P = 0.05 level of significance
ns = Not significant
Sys = System of farming

**Table 4.16: Effects of farming systems on growth of sole and intercropped soyabean**

| Weeks after planting | Treatment | Plant Height (cm) | No of leaves | Stem diameter (mm) |
|---|---|---|---|---|
| 2nd Week | Int/c Soyabean | 9.14a | 5.75b | 1.52b |
| | Sole Soyabean | 8.53a | 6.62a | 1.62a |
| 4th Week | Int/c Soyabean | 24.72b | 10.89b | 2.30b |
| | Sole Soyabean | 27.45a | 12.89a | 2.70a |
| 6th Week | Int/c Soyabean | 46.05b | 19.96b | 3.22b |
| | Sole Soyabean | 49.39a | 23.81a | 4.13a |
| 8th Week | Int/c Soyabean | 59.17b | 28.14b | 4.62b |
| | Sole Soyabean | 65.92a | 30.97a | 5.49a |

The values are means of three replicates. Means with the same letter (within the same column) in each treatment are not significantly different at $P = 0.05$ according to Duncan Multiple Range Test (DMRT).

**Table 4.17: Effects of different farming systems on dry matter and biomass production of soyabean**

| Treatments | No of nodules | No of pods | Leaf area (cm) | Leaf dry wt. (g) | Shoot dry wt.(g) | Root dry wt. (g) | Plant Total dry wt (g) | AM root colonisation |
|---|---|---|---|---|---|---|---|---|
| Rot. Soyabean | 12.31a | 39.64a | 12.89a | 1.93a | 3.66a | 1.13a | 4.80a | 56.67a |
| Intc. Soyabean | 10.47b | 37.39a | 12.30a | 1.50a | 2.93b | 0.94b | 3.89b | 53.62a |
| Main Effect | * | ns | ns | ns | * | * | * | ns |

* = significantly different at P = 0.05 level of significance
ns = not significantly different at P = 0.05 level of significance

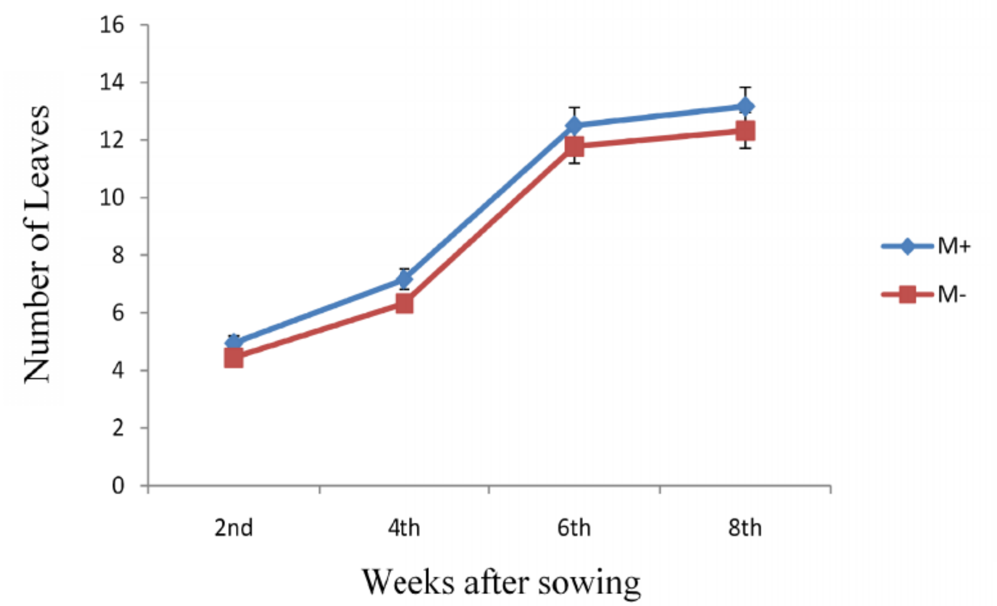

**Fig. 4.19: Number of leaves of the intercropped maize as affected by mycorrhizal inoculation**

$M^+$ = Mycorrhizal inoculated treatment
$M^-$ = Mycorrhizal uninoculated treatment

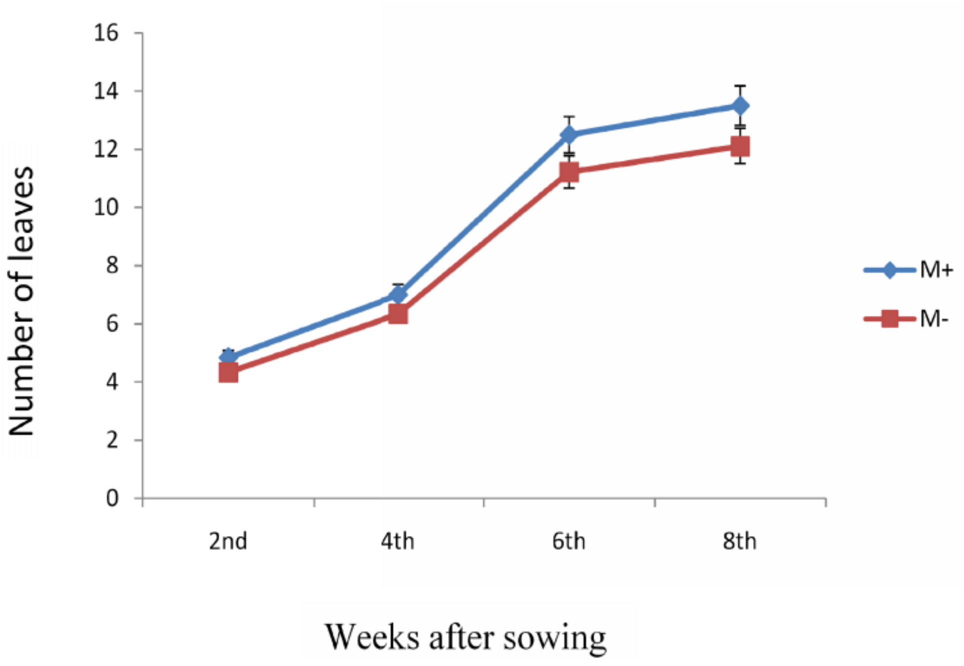

**Fig. 4.20: Number of leaves of the rotated maize as affected by mycorrhizal inoculation**

$M^+$ = Mycorrhizal inoculated treatment
$M^-$ = Mycorrhizal uninoculated treatment

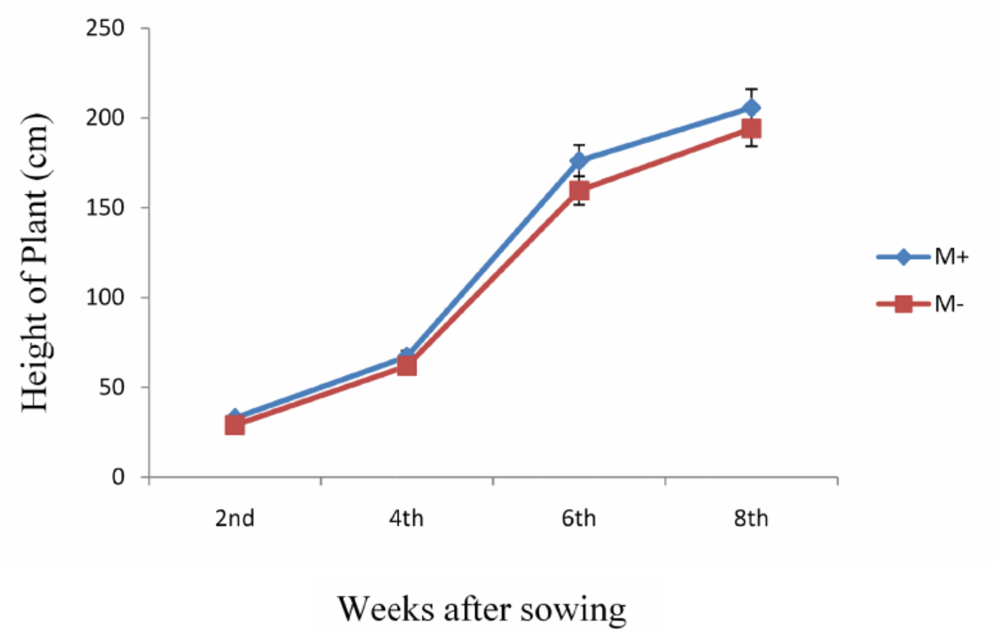

**Fig. 4.21: Height of the intercropped maize as affected by mycorrhizal inoculation**

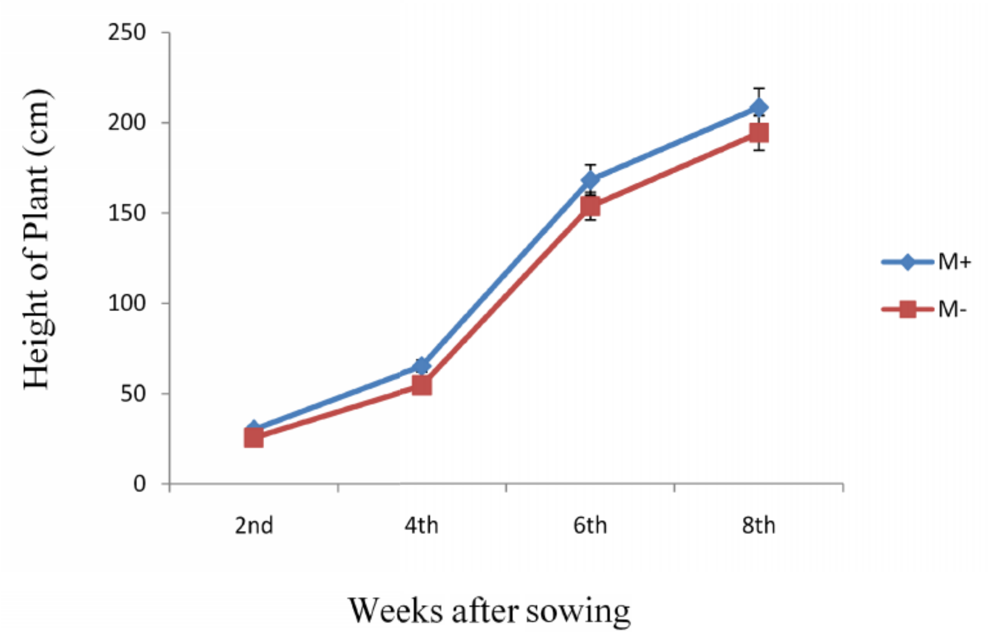

**Fig. 4.22: Height of the rotated maize as affected by mycorrhizal inoculation**

**Table 4.18: Effects of mycorrhizal inoculation on dry matter and biomass production of maize**

| Treatment | Plant height (cm) | No of leaves | Leaf dry wt. (g) | Shoot dry wt. (g) | Total dry wt (g) | No of cobs | Cob dry wt. (g) |
|---|---|---|---|---|---|---|---|
| **Intercropped maize** | | | | | | | |
| $M^+$ | 205.67±3.1 | 13.17±0.6 | 23.26±0.72 | 86.59±5.88 | 109.85±6.5 | 1.67±0.12 | 139.52±8.05 |
| $M^-$ | 194.01±3.25 | 12.33±0.24 | 20.86±0.45 | 74.63±3.26 | 95.49±3.31 | 1.17±0.11 | 106.42±7.80 |
| **Sole/rotated maize** | | | | | | | |
| $M^+$ | 208.12±5.52 | 13.50±0.14 | 22.62±0.72 | 89.93±5.30 | 112.55±6.2 | 1.67±0.12 | 133.73±6.50 |
| $M^-$ | 193.95±3.42 | 12.11±0.17 | 20.86±0.44 | 77.28±2.72 | 98.14±3.16 | 1.17±0.07 | 105.43±5.39 |
| | * | * | * | * | ns | * | * |

The values are the mean of three replicates. ± = Standard errors

* = Values are significantly different at P≤ 0.05 according to Duncan's Multiple Range Test (DMRT) for both intercropped and rotated maize.

ns = Values are not significantly different

**Table 4.19: Effects of systems of farming on dry matter weight and biomass production of maize**

| Treatment | Plant height (cm) | No of leaves | Leaf dry wt. (g) | Shoot dry wt. (g) | No of cobs | Cob dry wt. (g) |
|---|---|---|---|---|---|---|
| Interc. | 199.86±3.56 | 12.75±0.25 | 20.06±0.59 | 80.61±4.7 | 1.42±0.12 | 122.97±7.92 |
| Rotated | 201.03±4.47 | 12.81±0.16 | 21.74±0.58 | 83.61±4.01 | 1.42±0.10 | 119.58±5.95 |
| | ns | ns | ns | ns | ns | ns |

The values are the mean of three replicates. ± = Standard Errors

ns = Values are not significantly different at $P \leq 0.05$ according to Duncan's Multiple Range Test (DMRT).

## 4.21 Effect of rhizobium and mycorrhizal inoculation on proximate content of soyabean

There were significant effects of mycorrhizal and rhizobium inoculation on proximate contents of intercropped soyabean (Table 4.20). Except for P and Mg, the proximate and nutrient contents of inoculated soyabeans were significantly higher than those of uninoculated controls.

In the rotated plots, only the crude fibre and moisture contents of the inoculated soyabean showed significant difference over their uninoculated counterparts. However, for the nutrient contents, only P did not show significant difference between inoculated and uninoculated treatments. All other nutrients were significantly higher in the inoculated than uninoculated soyabeans (Table 4.20). The crude fibre and ash contents (Proximate) of intercropped soyabean were significantly lower than those from the rotated plots (Table 4.20), while it is only the N content in the nutrient analysis that was lower for the intercropped than for the rotated soyabean.

There was significant interactions between mycorrhizal and rhizobium in the proximate contents, while there were no significant interactions in the nutrient contents, except for N and P. (Table 4.20).

## 4.22 Effects of intercropping and crop rotation on proximate contents of soyabean

Considering the effects of the two systems of farming on the proximate content of soyabean, there was no significant differences in proximate contents between the intercropped and rotated soyabean, except for crude fibre, ash and contents which were higher in rotated than in intercropped soyabeans (Table 4.21). The proximate contents of the rotated soyabean were between 3.6 to 24.2% higher while its nutrient contents were between 2.9 - 3.8% higher than that of intercropped cropped soyabean.

**Table 4.20: Proximate and nutrient contents of soyabean as affected by rhizobium and mycorrhizal inoculation**

| Treatment | % Crude protein | % Ether extract | % Crude fibre | % Ash | % Moisture content | N | P | K | Ca | Mg |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | ____ | ____ | (ppm) | ____ | ____ |
| | | | | | **Intercropped soyabean** | | | | | |
| R | 38.07a | 20.05a | 3.34a | 5.21a | 5.90a | 0.18a | 0.65a | 0. 79a | 0.21a | 0.35a |
| M | 38.43a | 20.09a | 3.33a | 5.23a | 6.02a | 0.18a | 0.64a | 0. 84a | 0.25a | 0.34a |
| MR | 38.71a | 19.89a | 3.31a | 5.20a | 5.93a | 0.17a | 0.64a | 0. 82a | 0.22a | 0.35a |
| C | 36.60b | 18.55b | 3. 17b | 4.47b | 6.58b | 0.14b | 0.62a | 0.66b | 0.16b | 0.33a |
| | | | | | **Rotated soyabean** | | | | | |
| M | 38.56a | 20.53a | 4.09a | 6,14a | 5.91a | 0.23a | 0.66a | 0.85a | 0.22a | 0.37a |
| MR | 38.67a | 20.43a | 4.11a | 6.16a | 5,96a | 0.23a | 0.67a | 0.86a | 0.22a | 0.38a |
| R | 38.63a | 20.39a | 4.13a | 6.09a | 5.86a | 0.27a | 0.66a | 0.85a | 0.23a | 0.38a |
| C | 38.43a | 20.02a | 3.48b | 5.60a | 6.51b | 0.20b | 0.64a | 0.67b | 0.17b | 0.29b |
| Main effect | | | | | | | | | | |
| R | * | * | * | * | * | * | * | * | * | * |
| M | * | * | * | * | * | * | * | * | * | * |
| MR | * | * | * | * | * | * | * | * | * | * |
| Intractions | | | | | | | | | | |
| M x R | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns |
| M x MR | ns | ns | ns | ns | ns | ns | ns | ns | ns | * |
| R x MR | ns | ns | ns | ns | ns | ns | ns | ns | ns | * |
| Tmtx I x $R_o$ | * | ns | ns | ns | ns | ns | ns | ns | ns | ns |
| I x $R_o$ | * | * | * | * | ns | * | ns | ns | * | ns |

The values are means of three replicates. Means with the same letter (within the same column) are not significantly different at P = 0.05 according to Duncan Multiple Range Test (DMRT).

I = Intercropping

R = Rotation

**Table 4.21: Proximate and Nutrient contents of soyabean as affected by system of farming**

| Treatment | % Crude protein | % Ether extract | % Crude fibre | % Ash | % Moisture content | N | P | K | Ca |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | (ppm) | | | |
| Intcr Soy | 36.60a | 19.64a | 3.18b | 5.04b | 6.35a | 0.17b | 0.64a | 0.78a | 0.21a |
| Rot. Soy | 38.57a | 20.34a | 3.95a | 6.00a | 6.31a | 0.22a | 0.66a | 0.81a | 0.21a |

The values are means of three replicates. Means with the same letter (within the same column) are not significantly different at P = 0.05 according to Duncan Multiple Range Test (DMRT).

Intcr = Intercropped

Rot. = Rotated

Soy = Soyabean

### 4.23 Effects of mycorrrhizal inoculation on proximate contents of maize

There was effect of mycorrhizal inoculation on proximate and nutrient contents of both intercropped and rotated maize (Table 4.22). Except for crude fibre, ash and moisture contents, the proximate and nutrient contents of mycorrhizal inoculated intercropped maize were significantly higher than those of uninoculated counterparts. The crude fibre and moisture contents of uninoculated intercropped maize were greater than their inoculated counterparts. There was no significant difference in ash content between inoculated and uninoculated maize plants (Table 4.22). In the rotated maize, however, except for ash and content, the proximate and nutrient contents of the mycorrhizal inoculated maize were significantly greater than their uninoculated counterparts. The moisture content in the uninoculated maize plant was significantly greater than the inoculated counterparts (Table 4.22).

### 4.24 Effects of systems of farming on proximate and nutrient contents of maize

Although there were no significant differences in proximate and nutrient contents between the intercropped and rotated maize at the end of experimental period, however, the contents of the rotated maize were slightly higher than that of the intercropped maize (Table 4.23).

### 4.25 Effects of rhizobium and mycorrhizal inoculation on yield of soyabean

There were significant effects of inoculations with mycorrhizal and rhizobium on the yield of both intercropped and rotated soyabean. The treatments inoculated with mycorrhiza, rhizobium and the two microsymbiont combined were 23, 24 and 27% respectively higher than their uninoculated counterparts in intercropped farmland. In rotated soyabean similar higher percentages were obtained in inoculated soyabeans yield than their uninoculated counterparts (Table 4.24).

### 4.26 Effects of systems of farming on the yield of soyabean

The result showed that there was significant difference between the yields of intercropped and rotated soyabean. The yield (kgha$^{-1}$) of the rotated soyabean was about 11% higher than what was obtained in the intercropped soyabean (Table 4.25).

**Table 4.22: Proximate and Nutrient contents of maize plant as affected by mycorrhizal inoculation**

| Tmt | % Crude protein | % Ether extract | % Crude fibre | % Ash | % Moisture content | N | P | K | Ca |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | (ppm) | | | |
| **Intercropped Maize** | | | | | | | | | |
| M+ | 19.21±0.56 | 17.98±0.02 | 18.80±0.40 | 6.26±0.16 | 8.49±0.15 | 0.57±0.04 | 0.62±0.02 | 0.48±0.02 | 0.36±0.13 |
| M- | 17.92±0.72 | 16.30±0.48 | 20.31±0.40 | 6.16±0.19 | 9.35±0.12 | 0.46±0.02 | 0.57±0.03 | 0.45±0.09 | 0.33±0.13 |
| **Rotated maize** | | | | | | | | | |
| M+ | 19.70±0.75 | 18.20±0.20 | 18.92±0.47 | 5.97±0.18 | 8.17±0.29 | 0.57±0.04 | 0.62±0.01 | 0.48±0.00 | 0.36±0.01 |
| M- | 17.88±0.95 | 17.54±0.44 | 17.32±0.41 | 5.90±0.22 | 9.59±0.06 | 0.46±0.01 | 0.57±0.03 | 0.45±0.00 | 0.33±0.01 |
| | ns | ns | ns | ns | ns | ns | ns | ns | ns |

* = Significantly different at P = 0.05 level of significance

ns = Not significant

± = Standard error

M+ = Mycorrhizal inoculated treatment

M- = Mycorrhizal uninoculated treatment

**Table 4.23: Proximate and nutrient contents of maize as affected by systems of farming**

| Treatment | % Crude protein | % Ether extract | % Crude fibre | % Ash | % Moisture content | N | P | K | Ca |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | (ppm) | | |
| Intcr | 18.57±0.63 | 17.65±0.25 | 30.06±0.40 | 6.21±0.18 | 8.92±0.14 | 0.52±0.03 | 0.60±0.03 | 0.47±0.06 | 0.35±0.13 |
| Rot | 18.79±0.85 | 17.87±0.32 | 30.11±0.44 | 5.94±0.20 | 8.88±0.18 | 0.52±0.03 | 0.60±0.02 | 0.47±0.00 | 0.35±0.07 |
| | Ns | ns | ns | ns | Ns | ns | Ns | ns | ns |

Intcr = intercropped maize

Rot = rotated maize

**Table 4.24: Grain yield (kgha$^{-1}$) of soyabean as affected by mycorrhizal and rhizobium inoculation**

| Treatment | Intercropped soyabean | Rotated soyabean |
|---|---|---|
| M | 648.13a | 715.03a |
| MR | 668.69a | 738.70a |
| R | 651.28a | 721.44a |
| C | 524.63b | 584.89b |

The values are means of three replicates. Means with the same letter (within the same column) are not significantly different at P = 0.05 according to Duncan Multiple Range Test (DMRT).

M = Mycorrhizal inoculated
R = Rhizobium inoculated
MR = Mycorrhizal and rhizobium inoculated
C = Uninoculated treatment

**Table 4.25: Effects of system of farming on grain yield (kgha$^{-1}$) of soyabean**

| Treatment | Yield/hectare |
|---|---|
| Intercropped soyabean | 617.68b |
| Rotated soyabean | 684.89a |

### 4.27 Effects of mycorrhizal inoculation on maize grain yield

There was significant effect of mycorrhizal inoculation on the yield of both intercropped and rotated maize (Table 4.26). The maize grain yield in mycorrhizal inoculated treatment was 18% higher than yield obtained in uninoculated treatment in intercropped maize while in the rotated maize the yield obtained for the inoculated treatment was 23% higher than the uninoculated treatment (Table 4.26).

### 4.28 Effect of systems of farming on maize grain yield

Comparing the two systems of farming statistically, that there was no significant difference between the intercropped and rotated maize. However, the grain yield of rotated maize was 6% higher than that of the intercropped maize (Table 4.27).

### 4.29 Yield of soyabean as affected by systems of farming

Systems of farming affected the yield of soyabean with the highest yield obtained in the rotated soyabean followed by sole soyabean and the least in intercropped soyabean (Table 4.28). Although, there was no significant difference between the yield of the sole and intercropped soyabean, the yield of rotated soyabean showed significant different from either the sole or intercropped soyabeans (Table 4.28).

There was no significant difference in the yield between intercropped and rotated maize, but higher grain yield was obtained in the rotated maize followed by intercropped maize with the least grain yield from the sole maize (Table 4.29). The yields of both rotated and intercropped maize were 14 and 5% respectively higher than that of sole maize (Table 4.29).

**Table 4.26: Grain yield (kgha$^{-1}$) of maize as affected by mycorrhizal inoculation**

| Treatment | Intercropped maize | Rotated maize |
|---|---|---|
| M$^{+}$ | 596.63 ± 13.05 | 646.44 ± 13.05 |
| M$^{-}$ | 504.14 ± 13.05 | 524.84 ± 13.05 |
| | * | * |

**Table 4.27: Effect of farming systems on grain yield (kgha$^{-1}$) of maize**

| Treatment | Yield | % difference |
|---|---|---|
| Intercropped maize | 550.41 ± 21.22 | 6.40 |
| Rotated maize | 585.64 ± 29.29 | |
| | ns | |

ns = Not significant

**Table 4.28: Comparison of soyabean grain yield (kgha$^{-1}$) in different systems of farming**

| Treatment | Yield/hectare |
|---|---|
| Sole soyabean | 624.07b |
| Intercropped soyabean | 605.14b |
| Rotated soyabean | 684.89a |

**Table 4.29: Comparison of maize grain yield (kgha$^{-1}$) in different systems of farming**

| Treatment | Yield | % difference |
|---|---|---|
| Sole maize | 512.96 ± 14.39 | |
| | | 6.40 |
| Intercropped maize | 550.41 ± 21.22 | |
| | | 14.17 |
| Rotated maize | 585.64 ± 29.29 | |

# CHAPTER FIVE

## 5.0 DISCUSSION

The results indicated that soyabean treatments inoculated with either mycorrhiza, rhizobium or both microsymbionts performed better than their un-inoculated counterparts. This showed that inoculation with either or both microsymbionts had effects on the growth of soyabean. It means that there was positive and synergistic rhzosphere interactions between the two microorganisms used in the experiment. This positive interaction brought about mobilization of nutrients especially phosphorus and nitrogen which are the main essence of inoculating the two microsymbionts. The positive interaction and nutrient mobilization was eventually converted to tissue development leading to increased growth and higher biomass production of the inoculated soyabean. This result was in line with finding of Amusat (1998) which reported that soyabean performed best when inoculated with efficient rhizobium and mycorrhiza. The higher significant effect of rhizobium inoculation obtained in leaf number as well as height of plant in both sole and intercropped soyabean showed the effectiveness of rhizobium strain used in the experiment.

Growth parameters of maize were not so influenced by AM fungus inoculation, although higher values of number of leaves and plant height were obtained in the plots inoculated with mycorrhiza. The insignificant difference may be due to different edaphic factors operating in the soil which influenced the behaviour and the effectiveness of symbiotic organisms. Among these factors were high extractable phosphorus and presence of indigenous mycorrhiza in the experimental plots which can render the inoculated strain ineffective. It has been reported that introduction of AM endophytes seems likely to be successful where indigenous ones are sparse or ineffective (Mosse, 1977b; Sanders and Hayman, 1977).

The higher dry matter weight obtained in the intercropped maize could be due to the fact that soyabean and maize are different in their nutritional requirements, which are drawn from different soil level. Another reason that could be adduced for this is the combination of greater photosynthetic active radiation interception of the two crops as suggested by Ennin (2002). Increase in the dry matter productivity of intercrops had been reported to occur when light interception of the intercrop is higher than that on monocrop (Keating and Carberry. 1993).

The fact that dual inoculation with both microsymbionts produced significant differences on number of leaves, dry matter production, number of pods, and shoot dry weight of soyabean in both sole and intercropped soyabean confirms the effectiveness of the two micro-organisms especially rhizobium. Furthermore, since there was significant difference between the soyabean inoculated with either of the organisms or both and their uninoculated counterparts show that the two organisms were effective and have synergistic effect on each other. This may be as a result of beneficial rhizoshpere interaction between the two microsybionts as suggested by (Arias *et al*., 1991; Requena *et al*., 1997; Galleguillos *et al*., 2000; Tsimilli-Michael *et al*., 2000; Biro *et al*., 2000) that AMF interact with beneficial rhizosphere microorganisms including free living N- fixing bacteria and general plant growth promoting rhizobacteria (PGPR). In the same manner, increased nodulation observed in treatments inoculated with both or either of the two microsymbionts suggests positive interaction between the strains of microbes and soyabean. This may be as a result of the fact that the organisms found a suitable environment in the root of soyabean which eventually boost their activities and converted into modified form called bacteroid and arbuscules which convert $N_2$ into the form that was utilized by plant and facilitate nutrients and uptake respectively. The resultant effects of this were the increase in growth, higher dry matter production and yield observed in the inoculated treatments. The increased nodulation may also be attributed to enhancement of nitrogen fixation of soyabean due to AM fungus facilating the mobilization of certain mineral nutrients especially phosphorus and other nutrients that are involved in the synthesis on nitrogenase and leghamoglobin. It is clear that inoculation with rhizobium and AM fungi as bioferlizer is effective for promoting growth of legumes and reflect synergistic relationship (Abd –Allah *et al*., 2014). This finding was also corroborated by Scheublin *et al.* (2004), who suggested that legume–rhizobium symbiosis is strongly influenced by AMF and there is some evidence to suggest that legume nodules contain AMF communities quite distinct from those found in the roots of legumes. Yanni *et al.* (1997) reported that inoculation with strains of rhizobium significantly increased grain yield, and harvest index.

The negative effect of intercropping was manifested on the intercropped soyabean as reflected from the values obtained in all the parameters monitored. Low dry matter production was obtained in the intercropped soyabean compared with sole soyabean. This suggests that maize had a higher competitive ability than soyabean probably due to shading effects of maize on the intercropped soyabean. Boehner *et al.* (1991), Egle and Yu, (1991) and Oyetunji, (2009) attributed lower soyabean yields and seed numbers to shading or competition for moisture and nutrients.

It was evident that total dry matter production of the intercropped maize was significantly higher than that of the sole maize. The higher value may be due to two reasons, the spacing between maize plants which involved soyabean sandwitching. It might also be that the maize had been able to benefit from the nitrogen fixed by rhizobium which lives symbiotically in the root nodules of soyabean previoiuly planted in the intercropped farmland. The other reason may be attributed to weaker interspecific competition between the two component crops as a result of the fact that the crops i.e maize and soyabean have different resource requirements as well as different growth pattern which may be of higher competitive advantage for maize over soyabean. Fujita *et al.* (1990) reported total dry matter production by a soyabean/sorghum intercrop to be greater than sole crop due to increasing sorghum growth. Ennin *et al.* (2002) in their study observed increased total dry matter in intercropped maize with closer proximity of maize and soyabean rows.

The higher biomass production obtained in the intercropped maize may be as a result of benefit derived from soyabean intercropped component. This result is in line with the findings of Osiru (1972) and Gray and Charles (1999) which showed that the biomass production of intercropped maize was higher than that of monoculture. The finding was also in line with that of Long li *et al.* (2007) which reported that there was a stronger and more consistent positive effect of intercropping on maize than on faba bean and that maize yield significantly increased when intercropped with legume. Long li *et al.* (2007), however, attributed yield increase of maize dry matter weight to below ground interaction between faba bean and maize. The higher nitrogen content of the intercropped maize further confirms that intercropping cereals with grain legumes can increase the cereal grain N concentration (Hauggaard-Nielson, 2006). The reason for this may be due to poor competition of legumes for soil nitrogen due to their symbiotic ability for N fixation.

The higher yield of soyabean obtained in mycorrhizal and rhizobium inoculated treatments showed that the microsymbionts used in the experiment were effective and confirmed their complementary efforts of mobilizing nutrients especially phosphorus and nitrogen. These effectiveness and nutrients mobilization contributed significantly to increase in soyabean grain yield production. This finding is in line with that of (Galleguillos *et al*., 2000) which observed large increase in grain yield of soyabean over un-inoculated counterparts. Galleguillos *et al*. (2000), further opined that there is rhizosphere interaction between AMF and beneficial rhizosphere microorganisms including free living N fixing bacteria and general plant growth promoting rhizobacteria (PGPR).

The significant differences obtained between mycorrhizal inoculated and uninoculated maize further confirmed the efficacy of the AM fungus – *Glomus etunicatum* used for the experiment and the result is in line with the finding of the previous researchers that maize overshoot when inoculated with appropriate AMF (Simanungkalit, 2002, Heggo and Barakah, 2003, Muralanda *et al*., 2003).

The results also showed that there was siginificant difference in cob dry weight between the intercropped and sole maize but this did not eventually lead to significant different in their grain yields. This meant there was more investment of nutrients obtained by intercropped maize on cob production than in the grain. That there was no significant difference in the grain yield of sole and intercropped maize means that intercropping does not significantly influences maize grain yield as it was for soyabean despite that the two crops were intercropped on the same piece of land. This may be due to the fact that soyabean and maize have their nutrient supply from different soil level and as such their underground competition for nutrient is hereby minimized.

The higher values of nitrogen contents obtained in the inoculated plant may be as a result of mutual interactions between the two microsymbionts and the host plant – soyabean. Since the important benefit confers by AM fungi on host plant is nutrient mobilization, uptake and use efficiency, (Thompson, 1987; Smith and Read, 1997; Graham, 2000; Gosling *et al*., 2006). Phosphorus use efficiency on the other hand has positive influence on $N_2$ fixation ability in the host plant which subsequently caused increased nitrogen content in the plant. This may be associated to increased growth, biomass production and yield obtained in the inoculated treatments (Ibibijen *et al*., 1996; Koide *et al*., 2000).

Higher proximate contents of the inoculated over uninoculated treatments confirm futher the mutualistic tripartite interactaction between the two microsymbionts and soyabean and implicates that the two organisms not only have quantitative but also qualitative impacts on the products obtained from the plant.

In both experiment one and two, there was significant effects of inoculation with both or either of the two microsymbionts on growth and biomass production of soyabean. In experiment one; highest plant total dry weights were obtained in the rhizobium inoculated treatments, while in the experiment two, highest dry matter weights were obtained in the combined inoculated treatments. Similarly, the values of the growth parameters of the experiment one were slightly higher than those in the experiment two.

The number of nodules and pods in the rotated soyabean were slightly higher than that of the corresponding sole soyabean in the experiment one while there was no clear cut difference in the number of nodules and pods between the intercropped and rotated soyabean in both experiments.

Similar trend of event was obtained in the dry matter weights of maize in experiments one and two. Higher dry matter weights was obtained in the rotated maize in experiment two compared with its corresponding sole maize in experiment one. The higher value may be associated benefit of rotation due residual effect of nitrogen initially fixed by the roots of soyabean which was initially planted on the soil.

The results of the experiment two showed that inoculation with either mycorrhizal, rhizobium or both combined together have positive impact on growth, biomass and grain yield of soyabean.

The significant differences in growth parameters between the inoculated and uninoculated soyabean showed the synergistic influence of the two microsymbionts i.e rhizobium and mycorrhizal on soyabean. Many researchers have reported that when the plant is inoculated by effective strains of the microorganisms the plant's growth is influenced and biomass production is equally boosted. Reports (Manjunath and Bagyaraj, 1984; Purcino *et al.,* 1986; Benthlefalvy *et al.,* 1981) indicated that AM would increase P uptake, which would itself increase plant growth especially when phosphorus is the limiting nutrient. Improved P nutrition in turn would favour the nitrogen fixation process by rhizobium. The combined effects of the two microsymbionts resulted in further growth enhancement. (Subba Rao and Tilak, 1986; Azcon-Aguilar and Barea, 1981).

The results showed that soyabean biomass was significantly affected by rhizobium and mycorrhizal inoculation. These results confirmed the effectiveness of the strain of rhizobium and mycorrhiza used in the experiment and they confirmed the findings of Amusat *et al*. (2008) which submitted that soyabean performed best when inoculated with efficient rhizobium and mycorrhiza. The results of the investigation showed that soyabean benefited from inoculation with both microorganisms used. In addition, significant differences in the biomass production of inoculated treatments may be attributed to production by rhizobium of growth enzyme – indoacetic acid (IAA), solubilize phosphate and fix nitrogen which could be used for soyabean growth (Sobral *et al*., 2004).

Zarrin *et al.* (2006) suggested that root life span is important for sustained P uptake during reproduction, nutrient translocation in to the developing seeds and for whole plant carbon budget. This may explain the importance of mycorrhiza and why higher biomass was obtained in the mycorrhizal inoculated soyabean and maize. Other workers have shown that mycorrhizal inoculation bring about elongation of plant roots and mobilizes nutrients especially phosphorus which may be otherwise in the absence of mycorrhiza roots be difficult for plants to obtain from the soil. This may account for the reason why higher biomass of maize was obtained in the mycorrhizal inoculated treatments in both the intercropped and rotated maize.

Higher significant differences in growth soyabean and mycorrhizal root colonization in the dual inoculated treatments compared with uninoculated treatments might have produced the synergistic interactions between mycorrhiza and rhizobium used in the experiment. These suggestions have been proposed by some workers (Daft and ElG-Ghiami, 1974; Cluett and Boucher, 1983; Kawai and Yamamoto, 1986; Pacovsky *et al* 1986; Chaturveli and Singh, 1989) who combined inoculation of mychorrhiza with rhizobia and attributed the enhanced plant growth and soyabean yield to the dual inoculation rather than inoculation with either microsymbiont alone. The higher mycorrhizal root colonization obtained in soybean could be due to some substances exuded by the host plant roots and taken up by AM fungus, since some of these exudates stimulate fungal metabolism and branching (Parniske, 2008). Consequent upon this was the increased growth and higher dry matter production obtained in the inoculated soybean.

Greater number of nodules due to inoculation with either of the two or both microsymbionts suggested that there was better combining and symbiotic relationship between the strain of rhizobium used and soyabean. The results is in line with previous findings (Revellin *et al*., 2000; Abbasi *et al*., 2008; Majid *et al*., 2009) showed higher number of nodules per plant in inoculated treatments compared to uninoculated. This study showed that combination of rhizobium with mycorrhiza resulted in higher number of nodules than the uninoculated controls. In the plots inoculated with mycorrhizal alone but without rhizobium, the number of nodules was almost equal and not statistically different to those inoculated with rhizobium. This suggested that indigenous rhizobium/rhizobia can be equally effective as the introduced one under the conditions enhanced by mycorrhiza. This also suggested synergistic interactions between the two microsymbionts and increased nodulation had been reported (Tahir *et al*., 2009) to result in more nitrogen fixation that leads to incrased yield components.

The increased nodulation obtained may be as a result of below the ground phosphorus mobilization activities of the inoculated mycorrhiza. Previous findings have shown that P in coincidence with the plant demand for N controls the nodule growth and nodulates the symbiotic processes of the legume and rhizobium (Wall *et al.* 2000; Hellsten and Huss-Danell, 2000 and Majid, *et al*., 2009).

Increase in number of pods per plant, dry matter yield, nodules and seed yield obtained in the soyabean due to inoculation with either rhizobium, mycorrhiza or both could possibly be as a result of the symbiotic interaction between the two organisms – *Rhizobium japonicum* and *Glomus etunicatum* and soyabean. It is well documented worldwide that increase in number of pods, seeds and dry matter yield of soyabean is brought about by rhizobium inoculation (Tien *et al*., 2002; Egamberidiyeva *et al*., 2004; Okereke *et al*., 2004 and Tahir *et al*., 2009).

The highest grain yield obtained in the rotated maize may be attributed to enhanced N availability in the soil due the previously grown soyabean. This makes the study to be in line with the reports of Sanginga *et al,* (2002) that the residual N benefits of promiscuous soyabean to the subsequent cereal crop increase the crops yield better than sole corn. The higher yield obtained in both rotated soyabean and maize may be attributed to rotational effects of the crops

Higher values of yield obtained in the rotated soyabean compared with the intercropped showed that rotation favoured biomass production and yield of soyabean than intercropping and further confirmed what was obtained in the first experiment that intercropping has negative impact on yield of soyabean though combination of soyabean with maize serves as a viable strategy for higher crop yield, reduce cost of production and increase monetary return to the farmers. The result also agreed with the findings of Lesoing and Francis (1999) and Mbah *et al.* (2007) that the sole crop components yielded higher than the corresponding intercropping situation and that of Tesfa *et al*., 2001 that intercropping significantly reduced the biomass yield of the intercropped legumes. This result contradicts what had been reported by Joe *et al*. 1997 who reported a negative yield effects of rotation on soyabean compared with sole cropping and attributed the higher continuous soyabean yields to better environmental conditions which as he suggested, eventually reduced the advantages of crop rotation.

Different farming systems generally gave higher total maize grain yield and confer general advantages to the farmers than sole cropping. This may be as a result of reduced competition and increased complimentary effects between the components crops used in the study. Similar results had been reported by Martin *et al*. 1991, Mudita *et al*. 2008 and Kutu and Asiwe 2010.

Significant increase in number of maize leaves, plant height, total dry weight, number of cobs and cob dry weight between mycorrhizal and non-mycorrhizal inoculated treatments was due to positive effects of inoculation on maize. It has been reported (Marschner 1998) that one of the four major methods or mechanisms been adopted by plants to increase access to native or applied soil P is better symbiosis with soil microbes such as arbuscular mycorrhizal fungi. The increase in yield components of maize further support synergistic interactions between maize and inoculated mycorrhizal fungus i.e. *Glomus etunicatum* and other rhizosphere organisms referred to as plant growth promoting organisms. Researchers (Simanungkalit, 2002, Heggo and Barakah, 2003, Muralanda *et al*., 2003) reported that mycorrhiza has synergistic effects with most of other microorganisms that have increasing effect on yield and yield components of most crops.

Significant biomass production and grain yield obtained in sole maize due to rotation may be attributed to the fact that maize had benefitted significantly from rotation due to transfer of residual nitrogen which had been fixed by the root of the soyabean previously planted on the plots. This in addition shows the effectiveness of the mycorrhizal strain used in the experiment. This result is in line with the previous findings of Sanginga *et al*. (2002) that indicated that maize grain yields generally are higher when the crop is planted following soyabean than in monocropping. The reason was however hypothesized to be due to enhanced N availability and the so-called 'rotational effect'.

The fact that the rotated soyabean had biomass and dry matter production which was greater than what was obtained in the intercropped soyabean may be as a result of the shadding effects of maize on soyabean intercrop which was grown on the same land with soyabean

Increase in N contents in soyabean shoot and total N uptake due to *Rhizobium* inoculation was mainly due to significant increased in nodulation, resulted in higher accumulation of N due to atmospheric $N_2$-fixation. Increase in N contents in shoot and total N uptake due to *Rhizobium and mycorrhizal* inoculation was mainly due to significant increased in nodulation, resulted in higher accumulation of N due to atmospheric $N_2$-fixation which has been reported to be sensitive to phosphorus deficiency. Significant increase in shoot N of soyabean inoculated with *B. japonicum* was previously reported by Seneviratne *et al*. (2000), Sarr *et al*. (2005), Zhang *et al*. (2002) and Majid *et al.* (2009).

The insignificant differences in proximate contents especially protein content between intercropped and rotated maize is an indication that maize had benefited from soyabean grown in either intercrop or rotation with them because the protein contents of both were significantly higher than that of sole maize. The result was in line with finding of Odense, (2010) who observed the heightening of the protein content in the cereal grains when intercropped with grain legume.

Higher yield component obtained in sole maize in rotation compared with intercropping is a further indication of the fact that maize crop in sole plots had enough nutrients with reduced or no competition unlike in the intercropped plots where there was competition no matter how small or insignificant. The result was in line with finding of Mbah *et al*. (2007) who suggested that the nutrient requirements of soyabean and maize in intercropping system were higher than the nutrient need of either crop in sole cropping. The high yield of intercropping and rotated maize may also be attributed to better environmental condition such as high but not excessive rainfall, temperature, solar radiation during the growing season and improved soil fertility as suggested by Joe *et al.* (1997).

The result confirmed that intercropping increases the cereals grain nitrogen concentration. Increased nitrogen concentration may be due to the fact that legume competes poorly with maize for nitrogen below ground due to its natural N- fixation ability but competes for light above ground. Thus giving maize the opportunity to make use of the available soil nitrogen and eventually converted it to tissue formation.

Maize yield improvement as a result of the preceding legumes can be attributed to soil fertility improvement from the sufficient nitrogen fixed by soyabean. Although the effects of intercropping were significantly different from effect of rotating soyabean on maize yields

Intercropping significantly reduced the biomass yield of the intercropped soyabean. In contrast, maize biomass yield was significantly increased. This showed that intercropping favoured the dry matter production and yield of maize probably due to its higher competitive ability than soyabean. It may also be due to transfer and mobilization of some of the nutrients especially nitrogen fixed by soyabean grown in intercropping with maize.

Increase in soil mineral nutrients of the experimental soil after planting and rotation with legumes and maize may be attributed to multifunctional usefulness of mycorrhiza which has potential of improving physical, chemical and biological qualities of the soil through the soil food web (Cardoso and Kuyper, 2006). It may also be attributed to symbiotic nitrogen fixation between the rhizobium strain and soyabean used for the trial which led to increase in the concentration of mineral nutrients especially the nitrogen in the soil.

Increase in organic matter of the soil and biomass production of maize may be attributed to sustainable cropping systems which favoured beneficial microbial interaction between rhizobium and mycorrhizal strain used for the experiment. Sustainable cropping system had been reported (Seguy and Bouzinac, 1998; Seguy *et al*., 1998) to preserve organic matter and increase microbial biomass especially those affecting plant nutrition and protection like those used in the experiment. This can also be attributed to incorporation of legumes into maize farming systems. It has been documented that when crop rotation is properly planned with appropriate crop sequence in well designed short-term rotation like maize after soybean, soil texture could be improved for the succeeding crop. This short-term rotation is used to create optimal growing conditions for subsequent crops. (CSA, 2000). In addition, it may also be as a result of the effectiveness of the inoculated microorganisms used in the experiment which has positive impact on the soil. The use of non- pathogenic soil organisms in organic farming as biofertilizer had been reported to have positive impact on the soil physical properties. This may therefore be attributed to the role of mycorrhizal on soil structure formation (Rilling, 2004).

The increase in the soil nutrient contents obtained at the end of the second experiment may also be attributed to sustainable cropping systems coupling with beneficial rhizosphere interactions between the two microsymbionts used. The result of the study is in line and consistent with the findings of previous researchers like Galleguillos *et al*., 2000; Xavier and Germida 2002; and El-Ghandor *et al.,* 2005 that the synergistic interactions between AM fungus and rhizobium has positive impact on legumes biomass production. Rhizobium inoculation has a consequent effect upon nitrogen accumulation and in effect increase yield production of maize.

# CHAPTER SIX

## 6.0 SUMMARY, CONCLUSION AND RECOMMENDATION

### 6.1 Summary

The experiment was conducted to investigate and evaluate the effects of rhizobium and mycorrhizal interactions in sustainable maize and soyabean based farming systems. The systems included sole, intercropping and crop rotation. The experiment site was divided into three farmlands. On the first farmland in the first planting season, was sole soyabean, on the second farmland was sole maize and on the third farmland were soyabean intercropped with maize. In the second season, maize and soyabean were rotated such that maize was grown after soyabean and soyabean after maize while the intercropped farmland was maintained. The experimental design was a randomized complete block design in split plot with three replications. These comprised of four treatments; mycorrhizal, rhizobium and combined inoculated treatments with their appropriate controls. Maize was inoculated with mycorrhiza while soyabean was appropriately inoculated with both mycorrhiza and rhizobium. The plant growth was monitored till maturity but growth parameters were measured for eight (8) weeks at two week interval. The results obtained revealed significant differences in growth and biomass productions between treated plants inoculated with rhizobium, mycorrhiza or both microsymbionts and their uninoculated counterparts in the sole, intercropped and rotated soyabean. Higher biomass was obtained in the mycorrhiza plots compared with non-inoculated counterparts in the sole intercropped and rotated maize. Significant effect of intercropping was observed with higher significant values in the sole soyabean. There was also significant effect of intercropping on maize but in contrary, higher significant values were obtained in sole maize.

Higher dry matter production, number of nodules, number of pods and AM fungus root colonization were obtained in rotated soyabean compared with intercropped soyabean. There was no significant difference in dry matter production, number of cobs and cob dry weights between intercropped and rotated maize, yet higher of the values of these parameters were obtained in the rotated maize.

The nutrient contents and the yield of soyabean also revealed significant effects of inoculation with either of the two microsymbionts or combined inoculation in all the systems farming. Similarly, the proximate and nutrient contents and yield of maize revealed positive impact of inoculation with the arbuscular mycorrhizal fungus.

Corn-soyabean rotation resulted in higher maize and soyabean grain yields than the respective monocultures. Higher significant yield was obtained in the rotated maize compared with either sole or intercropped maize, indicating the rotational benefit of soyabean on maize due to residual impact of nitrogen fixation.

## 6.2 Conclusion

The results of the research revealed that inoculation with *Rhizobium* or mycorrhiza solely or in combination has significant effects on growth and biomass production of soyabean.

P and N availability due to activities of the two microsymbionts significantly increased nodulation of soyabean. The tripartite interactions between rhizobium, mycorrhizal and soyabean brought about increase in growth and biomass production and yields of soyabean and mycorrhizal inoculation had positive effects on the growth of maize in both sole and intercropping condition.

Higher significant biomass and grain yield of maize reconfirmed that legumes preceding maize provided sufficient N requirement to the succeeding maize crop. Therefore, soyabean had residual effects and had contributed no matter how little to the growth, biomass and yield of maize.

The fact that there was significant differences in growth, biomass production and yields between the inoculated soyabean and maize and their corresponding uninoculated counterparts suggested alternative means of boosting production by resources poor farmers through procurement of less expensive plant growth booster such as rhizobium and mycorrhizal inoculants which has no negative ecological impact on ecosystem.

The result showed that soyabean-maize intercropping system had a higher net benefit than their sole system in term of land use efficiency, cost and profit advantages. Despite higher grain yield obtained in the rotated maize and soyabean, the result indicated that intercropping is still beneficial in term of efficient land use when combined yields of the two component crops is considered. There was no significant difference in the grain yield between intercropped and rotated maize, an indication that maize yield was not so much affected but in addition to the maize is the yield of the intercropped soyabean which may serve as additional source of income to the farmer. Apart from the below ground nitrogen transfer from soyabean to maize intercrop, there is likelihood that if any other non leguminous crop is grown subsequently on the land it may benefit from the residual nitrogen fixed by the roots systems of soyabean grown in intercropping with maize. In effect, the intercropping then provides both economic and environmental benefits to the farmers by increasing monetary gain in the current year and improving the soil fertility against subsequent season.

Moreover, since most of the nutrient obtained by intercropped maize is invested on cob production, it is pertinent that plant breeder should look into the trade or enterprise that can make a better utility of of carbohydrates in the grain than in the cob production.

However, rotation on the other hand is beneficial to farmer by improving soil fertility and reduces cost of fertilizer application of the subsequent maize due to residual nitrogen fixed by soyabean which eventually leads to increase in maize yield.

Government at all level should educate our local farmers on the importance of biological fertilizers and give training in obtaining necessary skills on the appropriated methods of inoculation of these beneficial soil microorganisms through extension services.

Research institutes should be adequately funded and charged with the responsibilies of making available commercially these benefial inoculants to local farmers at affordable price.

## 6.3 Recommendations

The use of non-pathogenic soil microorganisms such as rhizobium and mycorrhiza should be encouraged in both small and large scale farming for the following reasons:

The organisms are environment friendly unlike that of nitrogen fertilizer which had been observed to cause disruption to the ecological system which affects the balance of the global nitrogen cycle, soil and ground water pollution chemical spills and increase atmospheric nitrous oxide.

The source of nitrogen fertilizer is natural gas while energy requirement of soil microorganism especially the symbiotic ones like rhizobium and mycorrhiza are met by renewable sources such as plant-synthesized carbohydrates.

For long-term sustainability which is desirable in other to cater for ever increasing population growth, the use of symbiotic organisms are economically and ecological attractive means of reducing external input, serve as aid to resources poor farmers and improving the quality and quantity of internal resources.

Researchers should also note the period of application and inoculation of the microorganisms because their survival are affected by a number of factors such as physical factor – land ploughing, crop rotation, soil acidity, pattern intercropping and rotation e.t.c. chemical factor - concentration of nitrogen in the soil, application of pesticides, fungicides and insecticides and climatic factors - emperature, moisture among others. These factors must be put into consideration during soil inoculation because they can affect AM hyphae or propagules from survival. For instance, if the inoculum is to be applied to the soil when the soil temperature is high, the inoculum should be placed into the deeper layer to enhance the organisms' survival (Van Kdessel and Hartley 2000).

In addition crop matching for intercropping and rotation should be well considered. Odense (2010) suggested that a wrong choice of cereals and grain legumes causes a great risk of loss of yield and reduction in grain quality. Even if the crops match, an extra uncertainty is introduced in intercropping compared to sole cropping. So intercropping cereals with grain legumes does not automatically give an improvement of yield reliability. Therefore, proper matching of crops with similar growth pattern or with less competition for nutrients should be considered to reap maximum benefits of intercropping.

The present challenge is to sustain soil fertility in cropping systems operating at high productivity levels so as to be able to cater for ever increasing population growth. One of the ways by which soil fertility can be sustained for sustainable food production is incorporation of legumes into maize farming systems with the use of farmer's friendly non pathogenic organisms such as arbuscular mycorrhiza fungus and rhizobium as biofertilizer. The organisms when appropriately inoculated into food crops such as soyabean and maize combined with appropriate systems of farming will boost crop production and improve farmers' monetary returns.

In addition the combination of these microorganisms with farming systems will help improving soil fertility especially when cereal such as maize is grown in intercropping and rotation to succeed soyabean. Report has it that the nitrogen contribution of legumes to the growth of *Zea mays* in intercropping systems is equivalent to the application of 96 kg of fertilizer-N ha$^{1}$ at a ratio of plant population densities of one maize plant to four groundnut plants.

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## Appendix 1: The experimental layout of soyabean farmland

| | BLOCK 1 | BLOCK 2 | BLOCK 3 |
|---|---|---|---|
| PLOT 1 | M | R | C |
| PLOT 2 | R | M | M R |
| PLOT 3 | C | M R | M |
| PLOT 4 | M R | C | R |

M = Mycorrhiza uninoculated

R = Rhizobium inoculated for soyabean plot.

M R = Inoculated with both Mycorrhiza and Rhizobium

C = Control experiment (without inoculation)

**Appendix 2: The experimental layout of the maize farmland**

| | BLOCK 1 | BLOCK 2 | BLOCK 3 |
|---|---|---|---|
| PLOT 1 | $M^+$ | $M^-$ | $M^+$ |
| PLOT 2 | $M^-$ | $M^+$ | $M^-$ |
| PLOT 3 | $M^+$ | $M^-$ | $M^+$ |
| PLOT 4 | $M^-$ | $M^+$ | $M^-$ |

$M^{+=}$ Mycorrhiza inoculated

$M^-$ = Mycorrhiza uninoculated