Bioactivity of *Glomus mosseae* (arbuscular mycorrhiza) on Soybean Infected with *Sclerotium rolfsii* and *Meloidogyne incognita*.

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Accepted on June 30, 2012

Abstract

This study investigated the bioactivity of Glomus mosseae (arbuscular mycorrhiza) and Meloidogyne incognita on two soybean cultivars and also assessed their interactive effects on soybean. Two soybean cultivars (TGX1903-7F and TGX1449) known to be susceptible to the test pathogens were sown in sterilized soil in a greenhouse. Pots were arranged in a Randomized Complete Block Design. Inoculations with arbuscular mycorrhiza and the pathogens near the root zones of soybean plants were carried out two weeks after planting. The parameters measured are fresh shoot weight, dry shoot weight, number of pods, pod weight and grain weight. Data were taken at two weeks interval after inoculation till maturity. Also, field experiments were conducted during the late cropping seasons of 2008 and 2009 at Obafemi Awolowo University Teaching and Research Farm, Ile-Ife. Treatments, inoculation and data collection were carried out in the greenhouse. Data collected were analysed using Analysis of Variance (ANOVA) and Duncan's Multiple Range Test (DMRT) at P = 0.05. Inoculation of soybean plants with G. mosseae alone gave the highest fresh shoot, dry shoot weight, nodule number and weight, and grain weight. The least fresh shoot weight, dry shoot weight, nodule number and grain weight were obtained from soybean cultivars treated with pathogens alone. Numbers of pods in soybean plants treated with G.mosseae alone were significantly higher compared with other treatments on the field. The application of mycorrhiza reduced disease severity, improved growth and grain weight of soybean.

***Keywords**: Bioactivity, arbuscular mycorrhiza, legumes, sustainable agriculture, environmental safety.*

Introduction

Glycine max (L. Merrill) is the cheapest source of protein in the developing nations of the world where malnutrition is predominant (Anonymous, 2008). It is one of the world's most important grain legumes in terms of production and international trade (Weiss, 1993). Soybean is described as the "golden bean" or "miracle bean" due to its high nutritional value. It contains 48% high quality protein, 20% edible vegetable oil and a good balance of amino acids (Nworgu, 1993). They are primary sources of vegetable oil and protein. The oil is used in cooking and could be processed as margarine and salad oils. The soy meal or cake obtained after extracting the oil is used for animal feeds and as meat extender (Anonymous, 2002). Soybean is used in the formulation of new low-cost nutritionally balanced high protein foods and beverages for human consumptions. Soybean products such as soy cheese and soymilk are good source of vitamins B_1, B_2

and B_6 and an excellent food for proper growth and development of children (Ogundipe and Osho, 1990). Various industrial uses of soybean also include the production of biodiesel fuel, paints, plastics, insecticides and adhesives (James, 1998). It is an important crop in the farming system most especially in the tropics.

Soybean diseases including those caused by *Sclerotium rolfsii* (Sacc.) *and Meloidogyne incognita* (Kofoid and White) Chitwood which have increased in proportion with the intensification and expansion of the crop in south western Nigeria (Akinsanmi and Adekunle, 2003). *Sclerotium rolfsii* is a soil-borne fungus which primarily attacks host stems though it may infect all portions of the plant causing wilting and death. However, control of *S. rolfsii* by soil fumigation and use of fungicides can be expensive and not completely effective because of the clumped distribution of inoculum and resistance nature of sclerotia (Stephen and Rebecca, 1992). *Meloidogyne incognita* on the other hand has been identified as a major obstacle to the production of sufficient food and fibre crops in Nigeria (Adekunle and Fawole, 2003). The disease caused by this group of nematodes is root knot disease characterized by gall formation which disrupts flow of water and mineral salts leading to chlorosis and wilting. Wounds created by feeding actions of nematodes serve as entry points for other pathogens such as fungi and bacteria. The control of *M. incognita* has equally been difficult because of their ubiquitous nature and very extensive host range. Soybean diseases reduce yields and monetary returns by retarding desirable plant development and seed quality. The yield potential for soybean field was reduced to 20% by soil-borne diseases and in the overall, was found to cause more yield depression than leaf, pod and upper stem diseases such as downy mildew in Florida (Tom, 2005). Due to hazards posed by the use of synthetic chemical to people and their environment, bio-control of plant pathogens is currently recognized worldwide as an efficient environmentally friendly control option in order to establish Maximum Residue Limit (MRL) in Integrated Pest Management (IPM).

Mycorrhiza is a symbiotic relationship between arbuscular mycorrhiza fungi (AMF) and the root of majority of vascular plants. Through extensive specialized structures, the fungi improve the capture or relatively immobile nutrients such as Phosphorus (Sourchie *et al.*, 2006) as well as improving plant water absorption (Auge, 2004). Arbuscular mycorrhiza fungi are increasingly been considered as a natural plant health insurance (Giovenneti and Gianinazi-Pearson,1994)and examples of their positive impact on plant develoment and health, land reclamation and phytoremediation are continually increasing (Leyval *et al.*, 2002). Recently there was great awareness of biodiversity issues including those concerning soil microbial communities and acceptance of these natural technologies as alternative to agro-chemicals (Barea, 2000). Therefore, the society is demanding more sustainable means of production with a consequent feedback to farmers and land conservationists. Studies have shown great advantage of inoculation of soybean with mycorrhiza. This is very important in the tropics where the soils are deficient in Phosphorus (Sharma and Johri, 2002). Soybean has become so important especially in the developing country to alleviate malnutrition and also to improve soil fertility naturally. The protection of this "golden crop" with minimum use of synthetic pesticide against the increasing root knot and stem rot to obtain optimum yield becomes essential. It is therefore against this background that this study was conducted to investigate the bioactivity of *G.mosseae* on soybean infected with *S.rolfsii* and *M. incognita*.

Materials and Methods

Seeds of two susceptible soybean cultivars TGX 1903-7F and TGX 1449 were collected from the Institute of Agricultural Research and Training (IAR&T), Moor Plantation, Ibadan, Oyo State. Isolation and subsequent purification of the fungal pathogen (*S.rolfsii*) was carried out in the Department of Crop Production, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria. Crude inoculum cultures of arbuscular mycorrhiza (*G.mosseae*) containing spores and root fragments were obtained from Crop Production laboratory of the same University. This was propagated using the bucket method on (*Zea mays* L.) grown for four months. Cultures of *M.incognita* raised on *Celosia argentea* were obtained from nematode culture plot from the same Department. This study was conducted under both greenhouse and field conditions. In the greenhouse, surface sterilized of soybean were sown in 15 cm diameter plastic pots containing steam-sterilized soil. Pots were arranged in a Completely Randomized Block Design. Seedlings were thinned to four plants per pot six days after emergence. The seedlings were inoculated near the root zones two weeks after planting. The treatments consisted of: 30 g of *G. mosseae* alone, 16 ml of *S.rolfsii* alone, 1,500 eggs *M.incognita* alone, 30 g of *G.mosseae* + 16 ml of *S.rolfsii*, 30 g of *G.mosseae + M. incognita*, 30g of *G.mosseae* + 1,500 eggs of *M.incognita* +16 ml of *S.rolfsii* and non-inoculated control. Each treatment was replicated four times. Data collection commenced from two weeks after inoculation till maturity. Data were collected on fresh and dry shoot weight, number and weight nodule, number and weight of pod. The soil belongs to Iwo series derived from coarse-grained granite gneiss parent rock and classified as ultisol low base status forest soils, it is well drained grayish brown to brownish red with predominantly high low acidity clay-kaolinite (Harpstead, 1973). The soil sample was analyzed for pH, available nitrogen, available phosphorus and exchangeable potassium before planting and at harvest. Two separate studies were conducted at the Teaching and Research Farm of Obafemi Awolowo University Ile-Ife, located at $7^{o}28$ 'N, $4^{0}33$ 'E at 224 m a.s.l., above the sea level. A plot of 10.2 m x 27.5 m was cleared, ploughed and harrowed. Soybean seeds with about 70% viability were sown in August at the onset of late raining season of 2008 and 2009. The experiment was laid out in a Randomized Complete Block Design. The field consisted of four blocks of 1.8 m x 27.5 m. Each block was subdivided into fourteen plots each of 1.8 m x 1.5 m with an alley of 0.5 m between plots and 1metre between blocks. Seeds were drilled at the spacing of 0.36 cm x 5 cm with five rows in each plot. The three middle rows were inoculated and samples were taken from the innermost row. Inoculation time, method and data collection was similar to that of greenhouse experiment with slight modification in nematode treated plots. The modification was that eggs of *M. incognita* were used in the greenhouse study while equal quantity of chopped galled Celosia roots infected with *M. incognita* was used for the field inoculation. The field was hand weeded and kept clean throughout the growing period. Bulk soil sample before planting and soil from each plot at harvest were analyzed. Data collected were subjected to Analysis of Variance (ANOVA) using the Statistical Analysis System (SAS).

Results

The results showed observable differences in various treatments in relation to the growth parameters and above ground symptoms. Figures 1-4 show the bioactivity of inoculation of *Glomus mosseae* , *Sclerotium rolfsii* and *Meloidogyne incognita* either singly or simultaneously on mean fresh and dry shoot weight of soybean cultivars under both greenhouse and field conditions respectively. Consistently, both greenhouse and field study showed that soybean plants treated with *G.mosseae* alone gave the highest biomass.

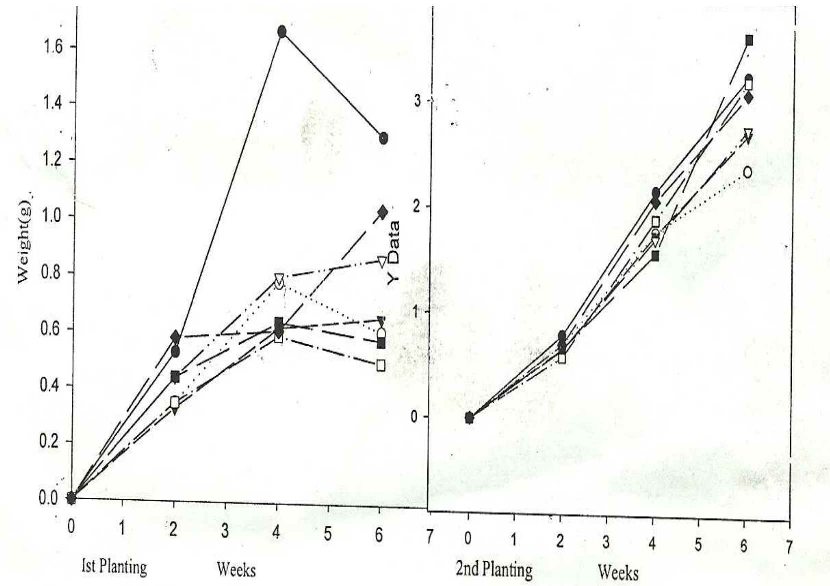

Figure1. Effects of inoculation of *Glomus mosseae*, *Sclerotium rolfsii* and *Meloidogyne incognita* either singly or simultaneously on the mean fresh shoot weight of two soybean cultivars under greenhouse conditions

Gm Alone, Mi Alone, Sr Alone, Control, Gm+Sr, Gm+Sr+Mi, Gm+Mi

Mi Alone, Gm Alone, Sr Alone, Control, Gm+Mi, Gm +Sr, Gm+Sr+Mi

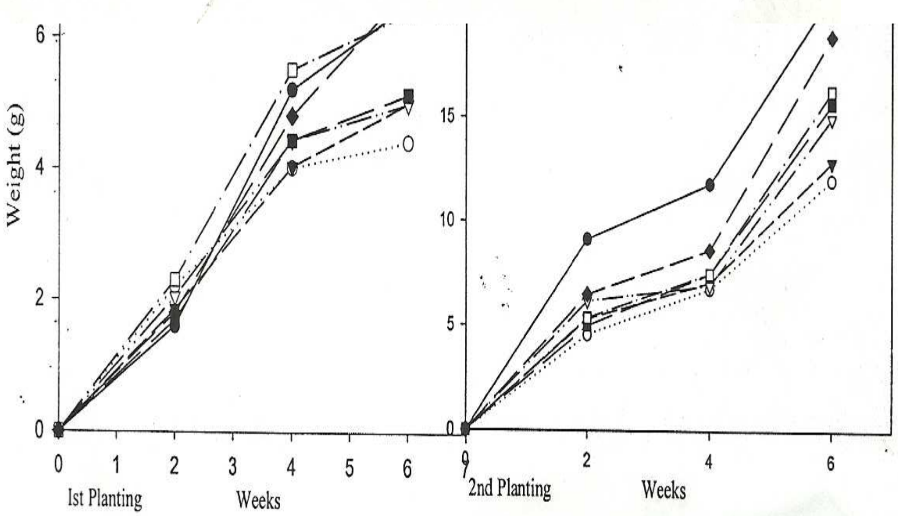

Figure 2: Effect of inoculation of soybeans with *G. mosseae*, *S. rolfsii* and *M. incognita* either singly or simultaneously on FSW under field conditions

Gm alone, Sr Alone, Gm Alone, Gm+Mi, Gm+Sr, Gm+Sr+Mi, Control

Gm Alone, Sr Alone, Mi Alone, Gm+Mi, GM +Sr, Gm+Sr+Mi, Control

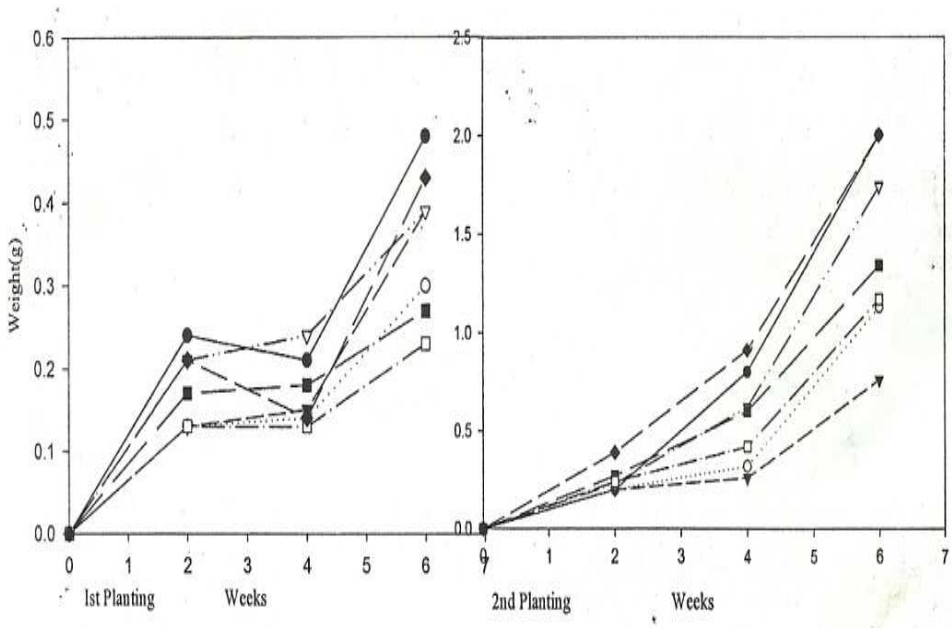

Figure 3: The effect of inoculation of soybean with *G. mosseae, S. rolfsii* and *M. incognita* on mean dry shoot weight (g) DSW under ambient condition

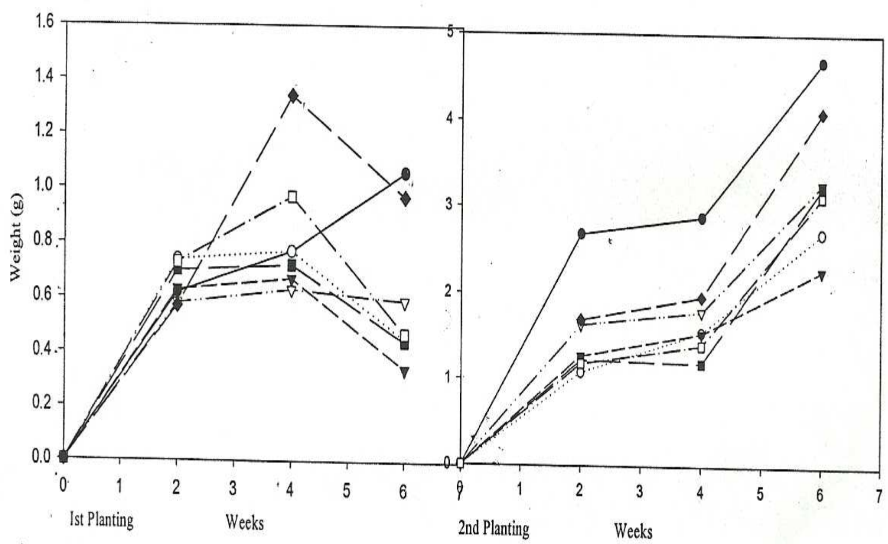

Figure 4: Effects of inoculating soybean with *G. mosseae, S. rolfsii* and *M. incognita* on mean DSW on field conditions

The effect of mycorrhiza on nodulation of soybean infected with *S.rolfsii* and *M.incognita* presented in Table 1 indicated that the number of nodules in mycorrhizal plants though not significantly different from that of control was significantly better than other treatments.

Table 1: Effects of inoculating *Glomus mosseae, Sclerotium rolfsii* and *Meloidogyne incognita* (either singly or simultaneously) on soybean nodulation (field overall)

	Nodule Number (g)	Nodule weight (g)
Gm alone	5.09a	0.44a
Sr alone	3.82cb	0.22d
Mi alone	3.51c	0.24dc
Gm+ Sr	4.13c	0.28c
Gm + Mi	4.31b	0.25dc
Gm+Sr +Mi	4.11cb	0.25dc
CONTROL	4.97a	0.36b

Each value is mean of four replicates. Figures with the same letters in the same column are not significantly different (P = 0.05) by DMRT

Gm = *Glomus mosseae*
Sr = *Sclerotium rolfsii*
Mi = *Meloidogyne incognita*

However, the weights of fresh nodules in mycorrhizal infected plants were significantly higher than other treatments. The plants inoculated with *M.incognita* alone had the least number of nodules while those treated with *S.rolfsii* alone had the least nodule weight. There was no significant difference in the number of pod across the treatments but pod weight of *G.mosseae* treated soybean plants was significantly higher than those of other treatments. The least pod weight was obtained in soybean plants inoculated with *M.incognita* alone (Table 2).

Table 2: Effects of inoculating *Glomus mosseae, Sclerotium rolfsii* and *Meloidogyne incognita* singly or simultaneously on the number of pod and pod weight under field conditions

Treatments	August 2008		August 2009	
	No of Pod	Pod Weight	No of Pod	Pod Weight
Gm alone	20.00a	6.06a	51.13ba	13.5ba
Sr alone	13.75cb	4.20d	40.25c	11.25c
Mi alone	10.63d	4.56cbd	40.38c	11.36c
Gm + Sr	11.75cd	4.96b	48.0bac	12.58bc
Gm + Mi	11.75cd	4.45cd	40.38c	10.73c
Gm+ Sr + Mi	13.38cb	4.65cb	47.75bc	12.20bc
CONTROL	14.63b	4.96b	54.50a	14.49a

Each value is mean of four replicates. Figures with the same letters in the same column are not significantly different (P = 0.05) by DMRT

Gm = *Glomus mosseae*
Sr = *Sclerotium rolfsii*
Mi = *Meloidogyne incognita*

Under the field condition, number and weight of pod of mycorrhizal soybean plants was highest only in the first set of planting. At the second set of planting, control plant had significant higher pod numbers than the treatments. The least pod number and weight were recorded in soybean plants inoculated with the pathogens alone (Table 3).

Table 3: The effects of inoculating soybeans with *Glomus mosseae, Sclerotium rolfsii* and *Meloidogyne incognita* either singly and simultaneously under greenhouse conditions on number of pod and pod weight

Treatments	No of pod	Pod weight
Gm alone	7.38a	1.84a
Sr alone	6.63a	1.38cd
Mi alone	7.00a	1.27c
Gm+ Sr	7.25a	1.54cd
Gm + Mi	7.50a	1.60bc
Gm+ Sr+ Mi	6.63a	1.58cd
CONTROL	7.25a	1.78ba

Each value is mean of four replicates. Figures with the same letters in the same column are not significantly different (P = 0.05) by DMRT

Gm = *Glomus mosseae*

Sr = *Sclerotium rolfsii*

Mi = *Meloidogyne incognita*

The highest grain weight per plants under both greenhouse and field condition were obtained from soybean plants inoculated with *G.mosseae* (Table 4). There was no significant cultivar effect in almost all the observed parameters.

Table 4: Effects of inoculating *Glomus mosseae, Sclerotium rolfsii* and *Meloidogyne incognita* on mean grain weight of soybean mean grain weight per plant (g)

Treatments	April 2009	August 2008	August 2009
Gm alone	1.14a	6.05a	12.24a
Sr alone	0.70b	4.20d	10.03b
Mi alone	0.65b	4.56cbd	10.07b
Gm+ Sr	0.76b	4.45d	10.57b
Gm + Mi	0.84b	4.65cb	9.52b
Gm+ Sr + Mi	0.80b	4.65b	10.46b
CONTROL	2.00a	4.96a	12.42a

Each value is mean of four replicates. Figures with the same letters in the same column are not significantly different (P = 0.05) by DMRT

Gm = *Glomus mosseae*

Sr = *Sclerotium rolfsii*

Mi = *Meloidogyne incognita*

Discussion

The results of this study show that the inoculation of *G.mosseae* both under greenhouse and field conditions increased fresh and dry shoot weight of soybean plants. This is in line with the results of various researchers. Gupta *et al.* (2002) reported that the inoculation of *Glomus fasciculatum* improved plants height, dry shoot weight as well as of yield of mint. Vansantha Krishna *et al.* (1994) observed that dual inoculation of Frankia (an actinorrhizal fungus) with arbusular mycorrhiza (AM) fungi increased the total dry weight of shoots and roots numbers of nodules, weight of nodular tissues as well as levels of Nitrogen and Phosphorus in casuarinas. The ability of mycorrhizae to increase plant growth can in most cases be explained by an increased phosphorus uptake which is greatly influenced by the hyphal spread of AM fungi (Gupta *et al.*, 2002). There are many reports of inter and intraspecific efficiency of AM fungi in terms of plant growth and protection (Ruiz-Luzano and Azcon, 1995). The possible role of hydrolytic activities of external hyphae of AM fungi has also been identified as a factor affecting fungal ability to colonize the root and influence plant growth. Legumes have been repeatedly shown to require high levels of phosphate for effective nodulation and growth which can adequately be mediated by mycorrhiza fungi. The AM fungi associated with legumes are an essential link for adequate phosphorus nutrition leading to enhanced nitrogenase activity that in turn promotes root and mycorrhizal growth (Sharma and Johri, 2002). *Glomus mosseae* treated soybean plants had higher number of nodules with corresponding higher weights than other treatments. Ibijbjen *et al.* (1996) observed that nitrogen fixation was enhanced in AM inoculated plants when compared to non AM inoculated plants. They also reported that symbiotic efficiency was found to be dependent on specific combination of Rhizobium strain of *Glomus* species indicating selective integration between the micro-symbionts. Also, Claudia *et al.* (2009) found that inoculation of pepper with mycorrhizal fungi decreased transplanting stress thus, accelerating the maturation stage of the plants and resulted into higher and better yield quality. Oyetunji, (2001) also found on that VAM fungi improved nutrient uptake and yield of cassava. The efficacy of *G. mosseae* as useful biological control agent against soil borne pathogenic organisms including *S. rolfsii* and *M. incognita* was demonstrated in this study. Both greenhouse and field study showed that the plants inoculated with pathogens alone expressed severe disease symptom together with high incidence. Earlier workers with similar result include (Todd, 2001; Tom, 2005; Salami, 2008).

Mycorrhizal infection of plants though not the only factor responsible for the growth and survival of the plants but have been observed to have a great potential a promoting plants growth and adaptation to abiotic factors. The outcome of AM symbiosis also depends on environmental factors, AM fungal characteristic and plants variables.

Conclusively, this study has shown the beneficial effects of *G.mosseae* (mycorrhiza) association against infection by *S.rolfsii* and *M.incognita* in both ditrophic and tripartite interaction. *G.mosseae* acted as a bioprotective agent been able to suppress the incidence and severity of *S.rolfsii* and *M.incognita*. It also enhanced the growth parameters of soybean plants.

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