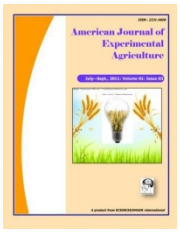

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The Influence of *Glomus mosseae* and *Trichoderma harzianum* on Phytohormone Production in Soybeans (*Glycine max L. Merr*) Planted in Sterilized and Unsterilized Soils

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Authors' contributions

This work was carried out in collaboration with all authors. OEH is the initiator of the study, wrote the proposal, carried out the research, performed the statistical analysis and wrote the first draft of the manuscript. AAS managed the results and the discussion. OAFI and RTF managed literature searches and analysis of the study. The final manuscript was read and approved by all the authors.

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ABSTRACT

This study was carried out to investigate the effect of the mycorrhizae; *Glomus mosseae* and *Trichoderma harzianum;* singly and in combination on the level of phytohormones (auxin, gibberrelin and abscisic acid) of soybean *(Glycine max L. Merr)* planted in sterilized and unsterilized soils. The experimental design adopted was completely randomized with four treatments i.e. *Glomus mosseae (G), Trichoderma harzianum (T)* and combination of *G. mosseae* and *T. harzianum (GT)* and an uninoculated (control (C)). There were four replications harvested at maturity after a growth period of 15 weeks. The experiment was carried out in the Department of Microbiology, University of Agriculture, Abeokuta, Ogun State, Nigeria between March 2009 to April 2010. Phytohormone levels before and after treatments were determined using documented method. The volume of phytohormones obtained were significantly (P>0.05) higher (7.05-70.43mg/100ml) in sterilized than

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unsterilized (6.27-52.73mg/100ml) soil. In all the treatments, auxin volume was highest (63.64mg/100ml) followed by abscisic acid (60.55mg/100ml) and gibberrellin (7.75mg/100ml). Treatment of soil with combined mycorrhizae *G. mosseae* and *T. harzianum* enhanced phytohormone production in soybean compared to soil treatment with single mycorrhiza and trichodermal inoculations.

Keywords: Glomus mosseae; trichoderma harzianum; randomized design; soybean; phytohormone.

1. INTRODUCTION

The legume, Soybean *(Glycine max (L.) Merr)* is an important crop particularly in the developing countries such as Nigeria where it is a part of human diet and an inexpensive source of protein (IITA, 1989). Soybean is an essential component of cropping systems throughout the world. Soybean is tolerant to a wide range of soil conditions, with the highest yield obtained on well-drained fertile loams.

Mycorrhizae fungi are natural inhabitants of tropical soils (Muller, 1979). They engage in a symbiotic relationship with the roots of many plants. Direct inoculation of plants with these fungi have been found to enhance plant nutrient uptake particularly phosphorus (Kang et al., 1980; Harley and Smith, 1983; Hayman, 1986; Sieverding, 1991; Atayese et al., 1993). One major factor that determines successful plant-fungi relationship is the fungal ability to compete with the other microbes in the soil including other arbuscular mycorrhiza fungi (Sieverding, 1991).

Trichoderma sp. is common inhabitants of the rhizosphere and has been recognized as biocontrol agent of soil borne pathogens (Chet, 1987; Harman and Lumsden, 1990; Chet et al*.,* 1997). However, several years of trials carried out with *Trichoderma* gave results that were not always convincing mostly due to the type of the *Trichoderma* used. The *Trichoderma* stocks TRI 002/003 were tested in many trials and have been shown to improve root development with a positive effect on the balance of microorganisms in the soil. Application of the correct strain of Trichoderma in a large scale field trial and greenhouse have given good results of better root system with more hair roots, hence better developed plants.

Several mechanisms by which Trichoderma influences plant development have been suggested, such as production of plant growth hormones (Windham et al*.,* 1986) and increased uptake of less-available minerals (Inbar et al*.,* 1994). Uptake of minerals, such as phosphorus and nitrogen is of key importance considering their role in plant growth (Johansen, 1999).

The rate of growth of a plant is influenced by several factors such as the availability of nutrients, water, humidity, temperature, light, pH of the environment and certain chemical substance called plant hormones (Sarajini et al*.,* 1979). Plant hormones are chemicals produced in very minute amounts within the plant body. They play a very important part in the regulation of growth and development of plants. Plant have five classes of hormones (Purves et aI., 2010). The categories of plant hormones associated with seed physiology are

the gibberellins e.g. (GA_3), Abscisic acid (ABA), Cytokinins, auxins, ethylene and compounds with hormone like properties such as thiourea and phenols (Jones, 1973; Agboola and Adedire, 1998).

Auxins have different effects on plants, including cell division, root stem elongation, induced root growth cuttings, inhibition of branching (apical dominance) and inducing fruit set. The naturally occurring auxin (found in plant tissues) is indole acetic acid (IAA). Gibberellins are known to be more effective than auxin in causing stem elongation in plants. In some cases, gibberellins can change the morphology of treated plants. For example, it can cause dwarf pea plants to grow very tall. Cytokinins, unlike auxins and gibberellins, generally promote growth by cell elongation and modify plant growth by altering cell divisions. Cytokines like *kinetin* promote growth cultured organs and tissues of plants. Ethylene is a gas that functions as a plant hormone. Its primary effect is in enhancing ripening of fruits. Placing unripe fruit in a paper bag makes the fruit ripen much faster than if it had sat on the counter top. This is because ethylene accumulates in the bag, which induces ripening in the fruit. Abscisic acid (ABA) is considered to be a growth more inhibitor. The fall of leaves in deciduous trees at the beginning of the autum season is generally attributed to the production of ABA in the leaves and buds. Buds remain dormant in the winter because they contain ABA (Jones, 1973; Agboola and Adedire, 1998).

2. MATERIALS AND METHODS

Seeds of the soybean TGx-1448 and the test organisms *(T. harzianum* and *G. mosseae)* used in this study were collected from The International Institute of Tropical Agriculture (IITA), Ibadan. The *T. harzianum* was cultured and maintained at 4°C on Potato Dextrose Agar (PDA) plate.

The soil used (top soil) in this study was collected from University of Agriculture, Abeokuta, Ogun State, Nigeria; farmland using soil auger at a depth of 10cm. After collection, the soil was sieved (4mm) to remove extraneous particles before sterilization. Some of the soil samples were sterilized at 180°C for 2 hours and some were left unsterilized.

G. mosseae spore density was determined according to Daniel and Skipper (1992).

2.1 Determination of *G. mosseae* Spore Density

One hundred grams of the mycorrhiza spore inoculums containing the spores as well as root fragments and sand were thoroughly mixed after which they were suspended in water inside a 150ml beaker two-thirds full. The soil-water mixture was thoroughly stirred after which the suspension was allowed to stand for 20 seconds to allow sedimentation of coarse sand. The suspension was decanted over a series of soil sieves arranged in descending order of mesh sizes i.e. 212, 106, 53µm mesh sieve sizes. The *G. mosseae* spores were collected by the 53µm mesh along with fine soil particle. The spores were further cleaned (i.e. separated from dust particles) by the sucrose gradient centrifugation method of (Daniel and Skipper, 1982). Twenty per cent (20%) and 6% sucrose/water wt/vol. sucrose solutions were gently introduced into the current. The sucrose was topped gently with the suspension of spores and the content centrifuged at 3,000 revolutions per minutes for 4 minutes. The soil particles settled at the bottom and the spores floated at the interface of the sucrose solutions. The cleaned spores were extracted by means of syringe placed in a clean 53µm mesh sieve and washed several times with water before being transferred into water in a clean Petri dish.

The spores were counted in 1cm grid line nematode dish using a dissecting microscope at 15-45X. The *G. mosseae* spore density was expressed as a number of spores/100g of dry soil.

2.2 Preparation of *T. harzianum* Spores

One milliliter of 10^{-8} (containing 256 spores as counted by hemocytometer) of 7 days old *T. harzianum* cultured on Potato Dextrose Agar was used as inoculum in a 250ml flask containing 100ml of synthetic medium. The flasks were shaken on a rotary shaker at 150 rpm for 24 hour at 30°C to allow spore germination. After 24 hour, the mycelia inoculum were separated from the growth medium by centrifugation at 10,000 rpm, 4°C and washed twice in 200ml of sterile distilled water.

2.3 Screenhouse Experiment

Study on the interaction between *T. harzianum* and *G. mosseae* on soybean was carried out in a greenhouse experiment using a complete randomized design. There were four treatments with four replicates each for sterile and unsterile soil conditions as follows: Control (uninoculated): -C; Inoculated with *T. harzianum*: -T; Inoculated with *G. mosseae:* -G; Inoculated with both *T. harzianumand and G. mosseae:* GT.

2.4 Planting and Inoculation

Soybeans were grown as the test crop. Seven point five kilogram (7.5kg) of sieved soil was weighed into each of the sixteen plastic pots. The *mycorrhiza* inoculum (50g/pot) were added at a depth of 2.5cm. Fifteen seeds were planted in each pot at a depth of 2.5cm. Uniform inoculation of *T. harzianum* was done introducing 1ml of culture containing 256 spores (Elad and Chet, 1983) over the seed hole. Two weeks after planting, the plants were thinned to ten plants per pot. The 16 pots were distributed at random in a screen house. The pots were watered daily to maintain moisture at field capacity and harvested at maturity after a growth period of 15 weeks.

2.5 Plant Growth Hormonal Determination

2.5.1. Preparation of growth regulators stock solution

Ten milligram (10mg) of a growth regulator was weighed and placed in a 100ml beaker. A few drops of 0.5N HCl and 25ml of 96% ethanol were added. This was mixed till the regulator was completely dissolved. Double Distilled Water (DDW) was added, the content was poured into a 100ml volumetric flask and the solution was made up to100ml. The flask was covered, sealed with paraffin wax and mixed well. The flask was thereafter labeled appropriately. This stock solution would give 0.1ml of growth regulator for each ml that was pipette.

2.5.2. Determination of phytohormones

The phytohormones; auxin (IAA), abscisic acid (ABA) and gibberelin (GA_3) were determined using the methods of Horgan (1987) and Horgan and Smith (1991). Briefly, for auxin, chloroform (10mL) and methanol (10mL) were added to sample (1g). After 1 h, the mixture was filtered and filtrate was separated in a separating funnel. 10ml of glacial acetic acid and

20ml warm distilled water were added, the mixture was thoroughly shaken and the organic layer was separated. The concentration of auxin was determined with a spectrophotometer with absorbance reading at 510 nm. For absicisic acid, Propanol (50%; 20ml) was added to sample (1g) and the mixture was stirred. Double distilled water (20ml) was added and allowed to stand for 1h. The extract was filtered (Whatman No 1) into a 100mL volumetric flask using 50% propanol to rinse. One milliliter (1ml) of sample extract was pipetted into a 30ml centrifuge tube. Methanol (5ml), NaOH (0.5 M; 5ml) and butanol (5%) were added and thoroughly mixed. The mixture was allowed to stand for 10 minutes to develop colour and the concentration of abscisic acid was determined with a spectrophotometer at a wavelength of 500nm. For gibberellins, methanol (10mL) was added to sample (1g), HCl (8%; 20ml) in glacial acetic acid were added, and allowed to stand for 1 hour. The extract was filtered (Whatman No. 2) into a 100mL beaker and 20mL of Propanol was added to totally remove the organic extract. Twenty milliliters (20ml) of warm distilled water was added to remove any aqueous contaminant. The organic layer was siphoned out into a 30ml centrifuge tube and the concentration of gibberellins, was determined with a spectrophotometer at a wavelength of 470nm.

2.6 Statistical Analysis

The data collected were subjected to statistical analyses (ANOVA) using Statistical Analysis System (SAS) Version 6.08 (SAS Institute 1990). The treatment means were separated using Least Significant Differences (LSD) at $P < 0.05$.

3. RESULTS AND DISCUSSION

There were significant differences in root phytohormone levels among the different soil treatments. The root of the plants in the sterilized soil contained significantly higher level of all phytohormones than those in the unsterilized soil. Auxins (IAA) value was highest, followed by abscisic acids (ABA) and Gibberellins (GA_3) was the least produced in sterilized soil while in unsterilized soil, ABA value was highest followed by IAA and GA_3 the least.

The interaction between inoculation and soil treatment showed that in sterilized soil condition, *G. mosseae* inoculation produced significantly higher phytohormones than control in all except on ABA where control was significantly higher than the inoculated treatments. The unsterilized soil followed the same pattern with the sterilized soil (Table 1). *T. harzianum* (T) inoculation produced significantly ($P > 0.05$) higher amounts of both IAA and GA_3 in root of soybeans grown in sterilized soil whereas a significantly higher amount of ABA was produced in the root of soybean grown in unsterilized soil (Table 2). The interaction between inoculation and soil treatment showed that in sterilized soil condition, *T. harzianum* inoculums produced significantly higher values than control in all except on ABA where control was significantly higher than the inoculated treatments. The unsterilized soil followed the same pattern as the sterilized soil. The effect of dual inoculations of *G. mosseae* and *T. harzianum* (GT) is presented in Table 3. The interaction between inoculation and soil treatment showed that in sterilized soil condition, Combined *G. mosseae* and *T. harzianum* inoculum produced significantly higher values than control in all except on ABA where control was significantly higher than the inoculated treatments. The unsterilized soil followed the same pattern as the sterilized soil.

Results of this investigation showed that the combined application of G and T enhanced the volume of phytohormones of soybean in both sterilized and unsterilized soil.

Table 1. The effects of *Glomus mosseae* on the production of phytohormones in the root of soybean

Treatments	IAA (mg/100ml)	GA3 (mg/100ml)	ABA (mg/100ml
Sterilized soil only	70.43	7.05	55.23
Unsterilized soil only	28.52	6.27	52.73
LSD (0.05)	0.42	0.37	0.61
Inoculations G	55.68	7.01	36.06
C	37.73	6.18	76.51
LSD (0.05)	0.59	0.56	0.87
Sterilized soil with G	68.18	7.76	30.60
Control	63.64	5.45	87.44
Unsterilized soil with G	43.18	6.05	41.53
Control	11.82	4.36	65.58
LSD (0.05)	0.59	0.56	0.87

Control (uninoculated): -C; Inoculated with G. mosseae:-G;
IAA- Auxin, GA_3-gibberelin, ABA- abscisic Acid

Table 2. The effects of *T. harzianum* on the production of phytohormones in the root of soybean

Treatments	IAA (mg/100ml)	GA3 (mg/100ml)	ABA (mg/100ml)
Sterilized soil only	70.43	7.05	55.23
Unsterilized soil only	28.52	6.27	52.73
LSD (0.05)	0.42	0.37	0.61
Inoculations T	40.86	6.18	37.78
C	37.73	5.20	76.51
LSD (0.05)	0.59	0.56	0.87
Sterilized soil with T	67.09	6.04	26.38
Control	63.64	5.45	87.44
Unsterilized soil with T	13.64	6.99	49.19
Control	11.82	4.36	65.58
LSD (0.05)	0.59	0.56	0.87

Control (uninoculated): -C; inoculated with Trichodermal harzianum: -T;
IAA- auxin, GA_3-gibberelin, ABA- abscisic acid

Table 3. The effects of combined inoculation of *G. mosseae* and *T. harzianum* on the production of phytohormones in the root of soybean

Treatments	IAA (mg/100ml)	GA_3 (mg/100ml)	ABA (mg/100ml)
Sterilized soil only	70.43	7.05	55.23
Unsterilized soil only	28.52	6.27	52.73
LSD (0.05)	0.42	0.37	0.61
Inoculations GT	63.64	7.75	65.58
C	37.73	6.18	76.51
LSD (0.05)	0.59	0.56	0.87
Sterilized soil with GT	81.82	8.96	76.51
Control	63.64	5.45	87.44
Unsterilized soil with GT	45.46	7.72	54.65
Control	11.82	4.36	65.58
LSD (0.05)	0.59	0.56	0.87

Control (uninoculated): -C; Inoculated with both T. harzianuum and G. mosseae:GT. IAA- Auxin, GA_3-gibberelin, ABA- abscisic acid

Trichoderma, a fungus that is useful to control plant diseases caused by soil-borne plant pathogens is also known to stimulate plant growth. Its application has been previously demonstrated in lettuce, radish bean, cucumber, pepper, corn and periwinkle under green house and field conditions (Kleifeld and Chet, 1992; Inbar et al., 1994; Ousley et al., 1994; Bailey and Lumsden, 1998; Harman, 2000). The studies on agricultural crops treated with Trichoderma commonly demonstrate a significant enhancement of plant growth during vegetative and reproductive growth stages. There are some mycorrhizal fungi in the soil that form symbiotic association with most economically important crop plants, including legumes. Their application enhances plant growth and this is thought to be the result of improved mineral nutrition of the host plant (Krishna and Bagyarag, 1984; Pacovsky and Fuller, 1986) and increase hormonal activity (Hedge, 2003).

Growth analysis of soybean plant showed that combined inoculation of mycorrhiza fungi and *Trichoderma spp* increased the hormone level of auxins and gibberellins in the root of the plant. The higher value of IAA, GA_3 and ABA in sterilized soil than unsterilized soil may be because the treatments did not have effect on abscisic acid. This might be due to the fact that the soil microorganisms in unsterilized soil had metabolized the IAA and GA_3 (Strzelczgk et al., 1973). The IAA and GA_3 levels were higher in inoculated treatments than unionculated treatments (control) but ABA were higher in control than inoculated treatments. The reason could be that IAA and GA_3 are growth stimulators while ABA is a growth inhibitor. IAA and GA_3 were higher in combined *G. mosseae and T. harzianum (GT)* than single application of either *G. mosseae* or *T. harzianum*. Also, IAA and GA_3 were higher in *G. mosseae* alone than *T. harzianum*. Mycorrhizal fungi increased root auxin levels at comparable values. The simplest explanation is that improved P nutrition creates a sink for photosynthate in the root and this increases auxin delivery from the shoot with the photosynthate (Marschner, 1995). There are changes in concentration of growth regulating compounds such as IAA, GA_3 and Cytokinin in mycorrhizal plant parasitic rates increases and the partitioning of photosynthate to shoots and roots changes (Linderman, 1992). Harman (2000) also suggested that *Trichoderma sp* are opportunistic plant colonizers that affect plant growth by promoting abundant and healthy plant roots, possibly via production or control of plant hormones (Baker, 1989; Kleifeld and Chet, 1992).

4. CONCLUSION

Mixture of *T. harzianum* and *G. mosseae* greatly improved phytohormones level particularly auxin and gibberellin in soybean. Proper fertilization program with special focus on biofertilizers should be implemented to improve productivity of food legumes. This would increase total food production and improve the supply of good quality proteins in people diet most of who largely depend on food legume crops.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

Agboola, D.A., Adedire M.O. (1998). Responses of treated dormant seeds of three tropical tree species to germination promoters. Nigerian Journal of Botany, 11, 103-110.

Atayese, M.O., et al. (1993). Composition of the influence of vesicular arbuscular mycorrhizal on productivity of hedgerow woody legumes and cassava on top and base of a slopping land in alley cropping system. Biology Fertilizer of Soil, 16, 198-204.

Bailey, B.A., Lumsden, R.D. (1998). Direct effects of *Trichoderma* and *Gliocladium* on plant growth and resistance to pathogens. In *Trichoderma* and *Gliocladium*. Vol II editions. G.E. Harman and C.P. Kubicek, 185-204. Taylor and Francis, London.

Baker, R. (1989). Improved *Trichoderma spp*. for promoting crop productivity. Trends Biotechnology, 7, 34 -38.

Daniel, B.A., Skipper, H.O. (1982). Methods of recovery and quantitative estimation of propagates form soils. In: Scherk, N. C (Ed). Method and Principles of *Mycorrhizal* Research, 29-35. The American Psychopathological Society, St. Pant, Minnesota.

Elad, Y., Chet, I. (1983). Improved selective media for isolation of *Trichoderma* or *Fusarium spp*. Journal of Phytoparasitica, 11, 55-58.

Harley, J.P., Smith, S.E. (1983). Mycorrhizal symbiosis. Academic Press, New York, 12-15

Harman, G.E. (2000). Myth and dogmas of biocontrol changes in perceptions derived from research on *Trichoderma harzianum* T-22. Journal of Plant Diseases, 84, 377-393

Hayman, D.S. (1986). *Mycorrhizal* of nitrogen-fixing legumes. MIRCEN Journal of Applied Microbiology Biotechnology, 2, 121-145.

Hedges, S.V. (2003). Microbial biotechnology in agriculture. Department of Agricultural Microbiology, University of Agricultural Sciences, Bangalore, 560-565.

Horgan, R., Scott, I.M. (1991). Methods in Plant Biochemistry. L.J. Rogers Edition, 5, 91-120, London Academic Press.

Horgan, R. (1987). Instrumental method of plant hormone analysis. In Plant Hormone and their Role in Plant Growth and Development. P. J Davies edition, 222-239. Darkdrecht Nijhaff.

IITA. (1989). How to grow soybeans in Nigeria, international institute of tropical agriculture, Ibadan, Nigeria. IITA (PUB), 10.

Inbar, J., et al. (1994). Plant growth enhancement and disease control by *Trichoderma harzianum* in vegetable seedlings under commercial conditions. European Journal of Plant Pathology, 100, 337-346.

Johansen, A. (1999). Depletion of Soil Mineral Nitrogen by roots of *Cucumis sativus L*. colonized or not by *Arbuscular mycorrhizal* fungi. Plant Soil, 209, 119-127.

Jones, R.L. (1973). Gibberellins: Their physiological role. Annual Review of Plant Physiology, 24, 571-578.

Kang, B.T., et al. (1980). Effect of phosphate fertilization and inoculation with VAM mycorrlizal fungi on performance of cassava (Manihot Essential Craritz) grown on an alfisol. Field Crop Research, 3, 83-94.

Kleifeld, O., Chet, I. (1992). *Trichoderma* plant interaction and its effect on increased growth response. Plant and Soil, 144, 267-272.

Krishna, K.R., Bagyaraj, D.J. (1984). Growth and nutrient uptake of peanut inoculated with the mycorrhizal fungus *Glomus fasciculatum* compared with non-inoculated ones. Plant and soil, 77, 405-408.

Linderman, R.G. (1992). Vesicular Arbuscular Mycorrhizae and Soil Microbial Interaction.

Marschner, H. (1995). Mineral nutrition of higher plants. Academic Press, London, UK, 889.

Muller, R.M. (1979). Some occurrences of VAM in natural and disturbed ecosystem of the red desert. Canadian Journal of Botany, 57, 619-623.

Ousley, M.A., et al. (1994). Potential of *Trichodermal* as consistent plant growth stimulation. Biology Fertilizer Soils, 17, 85-90.

Pacovsky, R.S., Fuller, G. (1986). Development of two endomycorrhizal symbioses on soybean and comparison with phosphorus fertilization. Plant Soil, 95, 361-377.

Purves, et al. (2010). Plant hoemones, nutrition and transport. In: Life- The Science of Biology. 4th Edition by Sinauer Associates.

Sarajini, T.R., et al. (1979). Modern Biology for schools. FEP INTER-PRIVATE LIMITED, 290, 292, 318, 323, 346-56.

SAS institute. (1990). SAS user guide 6.08 edition. SAS Institute Incorporation; SAS circle, BOX 8000, Cary, NC 27515-8000, USA.

Sieverding, E. (1991). Vesicular-arbuscular *Mycorrlizal* management in tropical ecosystems. deusche gessellschaft fur techishe zusamenarbeit (GTZ) Eschborn, Germany.

Strzelczyk, E., et al. (1973). Production and decomposition of indole acetic acid (IAA) by microorganisms isolated from the root of a crop. Plants Microbiology, 5, 71.

Windham, M.T., et al. (1986). A mechanism for increased plant growth induced by *Trichoderma spp.* Phytopathology, 76, 518-521.