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Full Length Research Paper

The effect of *Glomus mosseae* and *Trichoderma harzianum* on proximate analysis of soybean (*Glycine max* (L.) Merrill.) seed grown in sterilized and unsterilised soil

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Trichoderma harzianum **as well as** ***Glomus mosseae*** **inoculum (separately and combined) were prepared, and soybean plants were treated by these inocula in a greenhouse to compare their proximate analyses in sterilized and unsterilized soils. Soybean plants grown on sterilized soil gave higher percentage of crude fiber (3.66%), carbohydrate (50.00%), ash (4.53%) as well as dry matter (90.41%) than those grown on unsterilized soil. However, soybean grown on unsterilized soil gave higher percentage of crude protein (30.11%), oil (12.93%) and moisture content (9.62%) than those grown on sterilized soil. The inoculated treatment GT and G produced significantly higher (P < 0.05) amount of CP (30.22%) and (90.61%); and MC (9.86%), respectively than the control. Treatment of soil with combined** ***T. harzianum*** **and** ***G. mosseae*** **inoculum or separately in sterilized or unsterilized soil, will act as biofertilizer which can invariably improve yield, seed quality as well as the seed composition of soybean.**

Key words: Moisture content, seed composition, seed quality.

INTRODUCTION

Soybean is an essential component of cropping systems throughout the world, particularly in developing countries such as Nigeria. It plays a unique role due to its high protein content and its ability to fix atmospheric nitrogen. Legumes are important economically and are good sources of proteins, calories, vitamins and minerals in the diet of many individuals in developed and developing countries (El Tinay et al., 1989). Soybean is an important legume plant with a huge potential as a source of inexpensive protein and oil content. Many practices and efforts are directed to improving the seed oil and protein contents (Barbiker et al, 1995). Its newly developed varieties are high yielding, disease and shattering resistant and could be stored relatively better than the older varieties (Danshiell et al., 1987). These improved varieties though specifically released for large-scale production (Oyekan et al., 1986), small-scale farmers take the advantage of them because of their yield and agronomic potential. Diseases and other malading factors are also on the increase thereby reducing soybean grain yield per hectare (Adegbite et al., 2001). Seed oil content is one of the varietal characteristics which is influenced by the environmental and the climatic conditions. It is estimated within a range of 15-22% (Ref?).

This oil norm-ally contains approximately 10% linolenic acid and 55% oleic acid, with up to 50% variation in a specific component. Protein content is also inversely related to oil content, but in some cases high protein content could be associated with lower seed yield. Previously, it was reported that not only are seed compositions not heritable traits but that they also depend to a large extent on environmental conditions (Wilson, 1987; Burton, 1989).

Mycorrhizal fungi influence expression of genetic differences in host plants (Toth et al., 1984), and may

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also affect seed physiology. However, information on the effects of arbuscular mycorrhizal fungi on seed composition is limited, since most of the experiments with these fungi are conducted in pot cultures, where plants are not usually grown up to maturity and where constraints of root-volume is a limiting factor for mycorrhizal fungi effectiveness (Koide, 1991). In recent years, many scientists prefer to apply biofertilizers in place of chemical fertilizers since microbial inoculants such as mycorrhizal fungi enhance plant productivity directly or indirectly (Mahdi, 1993).

Inoculation with mycorrhizal fungi as well as nitrogen fertilizers was found to improve protein contents of soybean (Babiker et al., 1995). Also, it was indicated that fertilization of soybean plants with mycorrhizal fungi increased yield and protein content significantly (Elsheikh and Elzidany, 1997).

There are several studies which have focused on mycoparasitic nature of *Trichoderma* species and its contribution to plant health (Chet, 1987; Harman, 2000). The application of *Trichoderma* to the soil as a biocontrol agent, in screen house or under field conditions, not only resulted in reduced disease severity but also enhanced plant growth (Chang et al., 1986; Inbar et al., 1994; Ousley et al., 1994; Harman and Bjorkman, 1998). Recently, defense responses were demonstrated during the early stages of roots colonization by *Trichoderma* (de Meyer et al., 1998; Yedidia et al., 1999, 2000; Howell et al., 2000).

These observed defense mechanisms, along with biocontrol of major and minor plant pathogens, may somewhat explain growth promotion characteristics following *Trichoderma* application (Kleifeld and Chet, 1992; Harman, 2000).

The objective of the study was to determine the effect of separate and dual inoculation of *Glomus mosseae* and *Trichoderma harzianum* and the control on the seed composition of soybean grown in sterilized and unsterilized soil.

MATERIALS AND METHODS

Sample collection and preparation

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

Greenhouse study

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

Plant growing conditions

Seven and a half kilogram (7.5 kg) of sieved soil was weighed and placed in sixteen plastic pots each for sterilized and unsterilized experiment. The mycorrhizal inoculum (50 g/pot) were added at a depth of 2.5 cm. Fifteen seeds were sown at a depth of 2.5 cm in each pot. 1 ml of *T. harzianum* culture containing 256 spores was used as the inoculum by adding over the seed hole (Elad and Chet, 1983). After germination, plants were thinned to ten plants per pot. The pots were distributed randomly in greenhouse under natural conditions. The pots were watered daily to maintain moisture at field capacity and seeds harvested at maturity after a growth period of 15 weeks.

Proximate analysis

The percentage of dry matter, ash, moisture content, fiber, protein, oil and crude protein in different treated soybean were estimated according to AOAC (1973).

Statistical analysis

Collected data were analyzed statistically by SAS Version 6.08 (SAS Institute, 1990) and means of treatment were grouped using Least Significant Difference (LSD) at $P < 0.05$.

RESULTS

Results showed significant differences among treatments on seed composition of soybean (Tables 1 - 3).The seeds from unsterilized soil gave significantly ($P < 0.05$) higher values for the seed composition, crude protein (CP), oil and moisture contents (MC) while the seeds from sterilized soil gave significantly higher ($P < 0.05$) values of crude fibre (CF), carbohydrate (CHO), ash and dry matter (DM).

The interaction between inoculation and soil treatment showed that in sterilized soil condition, G inoculum produced significantly higher values than control in all except on % oil where control was significantly higher than the inoculated treatments. The unsterilized soil followed the same pattern with the sterilized soil except in % CHO that the control was significantly higher than the inoculated treatment (Table 1).

The *T. harzianum* (T) inoculation (Table 2) produced significantly ($P < 0.05$) higher amount of CF, CHO, Ash and DM on seed from sterilized soil. The interaction between inoculation and soil treatment showed that in sterilized soil condition, T inoculum produced significantly higher values than control in all except on oil where control was significantly higher than the inoculated treatments. The unsterilized soil followed the same pattern with the sterilized soil except in CHO that the control was significantly higher than the inoculated treatment.

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, G inoculum produced significantly higher values than control in all except on % oil where

Table 1. Effect of *G. mosseae* on seed composition of soybeans under sterilized and unsterilized soil condition.

	Treatments	%CP	%CF	%CHO	%Ash	%Oil	%MC	%DM
Soil treatment conditions	Sterilized	27.3800	0.0366	50.0000	0.0453	12.9300	9.6000	90.4100
	Unsterilized	30.1100	0.0355	46.3200	0.0442	13.8400	9.6200	90.3700
	LSD (0.05)	0.0700	0.0003	0.2300	0.0004	0.2000	0.0200	0.0400
Inoculation × soil treatments								
Sterilized	G	27.6300	0.0365	50.3700	0.0440	12.8300	9.8300	90.2600
	C	25.8700	0.0345	49.2600	0.0435	14.1700	9.3800	90.2600
Unsterilized	G	31.1800	0.0352	44.0500	0.0465	14.7900	9.8900	90.4200
	C	27.1900	0.0335	47.8700	0.0425	15.2100	9.4600	90.3600
	LSD (0.05)	0.1000	0.0005	0.3300	0.0006	0.2800	0.0200	0.0600

DM - dry matter, CP - crude protein, CHO - carbohydrate, MC - moisture content, CF - crude fiber, T -*Trichoderma*, G – *G. mosseae* and C - control.

Table 2. Effect of *T. harzianum* on seed composition of soybeans under sterilized and unsterilized soil condition.

	Treatments	%CP	%CF	%CHO	%Ash	%Oil	%MC	%DM
Soil treatment conditions	Sterilized	27.3800	0.0366	50.0000	0.0453	12.9300	9.6000	90.4100
	Unsterilized	30.1100	0.0355	46.3200	0.0442	13.8400	9.6200	90.3700
	LSD (0.05)	0.0700	0.0003	0.2300	0.0004	0.2000	0.0200	0.0400
Inoculation × soil treatments								
Sterilized	T	27.1000	0.0380	49.9300	0.0470	13.0800	9.4600	90.5300
	C	25.8700	0.0345	49.2600	0.0435	14.1700	9.3800	90.2600
Unsterilized	T	30.5800	0.0360	46.5500	0.0430	13.2600	9.5200	90.4700
	C	27.1900	0.0335	47.8700	0.0425	15.2100	9.4600	90.3600
	LSD (0.05)	0.1000	0.0005	0.3300	0.0006	0.2800	0.0200	0.0600

DM - dry matter, CP - crude protein, CHO - carbohydrate, MC - moisture content, CF - crude fiber, T- *Trichoderma*, G – *G. mosseae* and C - control.

Table 3. Effect of dual inoculation of *G. mosseae* and *T. harzianum* on seed composition of soybeans under sterilized and unsterilized soil.

Treatments		%CP	%CF	%CHO	%Ash	%Oil	%MC	%DM
Soil treatment conditions	Sterilized	27.3800	0.0366	50.0000	0.0453	12.9300	9.6000	90.4100
	Unsterilized	30.1100	0.0355	46.3200	0.0442	13.8400	9.6200	90.3700
	LSD (0.05)	0.0700	0.0003	0.2300	0.0004	0.2000	0.0200	0.0400
Inoculation × soil treatments								
Sterilized	GT	28.9200	0.0375	50.0500	0.0470	11.6500	9.7300	90.690
	C	25.8700	0.0345	49.2600	0.0435	14.1700	9.3800	90.2600
Unsterilized	GT	31.5100	0.0370	46.8000	0.0450	12.1200	9.6300	90.5300
	C	27.1900	0.0335	47.8700	0.0425	15.2100	9.4600	90.3600
	LSD (0.05)	0.1000	0.0005	0.3300	0.0006	0.2800	0.0200	0.0600

DM - dry matter, CP - crude protein, CHO - carbohydrate, MC - moisture content, CF - crude fiber, T - *Trichoderma*, G – *G. mosseae* and C - control.

control was significantly higher than the inoculated treatments. The unsterilized soil followed the same pattern with the sterilized soil except in CHO that the control was significantly higher than the inoculated treatment.

DISCUSSION

This study showed the importance of *G. mosseae* and *Trichoderma harzianum* individually and combines inoculation on some soybean index such as percentage of CP, MC and DM. This confirms other reports of favorable legume response to AM fungi inoculation both in sterilized and unsterilized soil (Daft and El-Giahmi, 1976; Manjunath and Bagyaraj, 1984).

It also agrees with the work of Ousley et al (1994) which demonstrated that application of *Trichoderma* to the soil as biocontrol agent in the greenhouse or under field conditions not only resulted in low disease severity but also enhanced plant growth. However, increased growth was shown in a pathogen free environment in experiments carried out in autoclaved soil (Kleifeld and Chet, 1992; Windham et al,, 1986).

The crude protein and dry matter of soyabean significantly increased by all inoculation especially GT treatments. The protein content was significantly increased with mycorrhizal fungi in the presence or absence of N and P treatment. Similar results were found for soybean (Regitano et al., 1995) and faba bean (Elsheikh and Elzidany, 1997). Helen et al (1991) reported that protein content of several legumes was increased by improved plant nutrition, mainly due to normal N-nutritional status.

Dual inoculation with GT and T or T alone did not have effects on fiber, carbohydrate, ash, oil and moisture content of soybean. *G. mosseae* inoculation had effect on all these parameters. Ash content was significantly increased by *G. mosseae* inoculation as well as other inoculants it can represents the inorganic residue remaining after organic matter has been burnt, and ash can be regarded as a general measure of quantity and quality of food. This confirms the work on faba bean grown in Northern Sudan which increased ash content from 2.55% to 3.33% (Salih and El Hardallou, 1986). In Shanbat, the ranges of ash content of chickpea, lentil and soybean are 2.9 to 4.0%, 2.4 - 4.1% and 4.3 -5.0%, respectively (El–Tinay et al, 1989). The crude fiber increased with *G. mosseae* inoculation in unsterilized soil. Oil contents were also significantly increased by *G. mosseae* inoculation.

In all treatments there were significant increases in carbohydrate content of soybean and mycorrhizal fungi had the greatest effects. This contradicts the reports of Elsheikh and Mohammed Zein (1998) who reported that inoculations in the presence or absence of N or P treatments significantly decreased carbohydrate content of groundnut seeds.

Mixture of *T. harzianum* and *G. mosseae* also greatly improved seed composition 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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