Efficacy of *Trichoderma harzianum* T_{22} as a biocontrol agent against root-knot nematode (*Meloidogyne incognita*) on some soybean varieties

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Summary

In 2012 and 2013, a two-year field study was conducted at the University of Ilorin Teaching and Research Farm, Ilorin, the Southern Guinea Savannah Zone, Nigeria, with the aim to investigate the effect of *Trichoderma harzianum* T_{22} as a bio-control agent against a root-knot nematode *(Meloidogyne incognita)* on some soybean varieties. The experimental field, which naturally has been known for the presence of some nematodes such as *Pratylenchus*, *Helicitylenchus*, *Radopholus*, *Meloidogyne*, *Rotylenchulus*, *Xyphinema*, was divided into two blocks, each block consisting of three plots with alleys between blocks and plots measuring 5 m and 1.5 m respectively. All treatments were replicated five times by means of a Randomized Complete Block Design. The initial soil nematode population was increased by chopping six kilograms of *Meloidogyne incognita* galled roots of *Celosia agentea* from a pure culture into all the plots. One block was treated with bio-control agent *Trichoderma harzianum* T_{22} while the second block served as a control unit. The results show that in terms of plant height, the number of branches, yield and reduction of the soil nematode population and root galls, the plants on the *Trichoderma* treated plots performed significantly better (P=0.05) than those in the control unit did. This therefore implies that root-knot nematodes represent a major constraint in the production of soybean while *Trichoderma harzianum* T_{22} improves the yield growth and the yield of soybean as well as better controls soil nematode populations with respect to the control trials.

Keywords: Trichoderma harzianum T_{22}, *Glycine max* L., *Meloidogyne incognita*, resistance, susceptible

Introduction

Soybean (*Glycine max* (L.) Merr.) is a leguminous vegetable of the pea family widely grown in tropical, sub-tropical and temperate regions. It is a cheap source of protein in human diets in many developing countries and in the animal feed industry worldwide (Oyedunmade, 2003). Many leguminous crops also provide some proteins, but soybean is the only available crop that provides an inexpensive and high quality source of proteins similar to those obtained from meat and eggs (IITA, 2009). The plant is classified as an oil seed rather than a pulse by the Food and Agricultural Organization (FAO). Soybean products are also used as substitutes for meat and other animal proteins.

In spite of numerous applications of this valuable crop, it is attacked by a wide array of pests and pathogens which pose a great threat to its profitable production. Plant parasitic nematodes are considered insidious, yield reducing and silent enemies of most food, vegetables, horticultural and fibre crops. Root knot nematode *Meloidogyne incognita* belongs to the most damaging agricultural pests attacking a wide range of crops (Katooli et al., 2010). Nematode management is complicated and difficult and at present, keeping their populations within economic thresholds requires chemical control as far as many crops are concerned (Eapen et al., 2005). Although synthetic nematicides are very effective in this view, there is an increasing need for reappraisal of more acceptable alternatives due to the high costs, environmental hazards and effect of these chemical pesticides on non-target organisms. Regarding the above issue, the potential of bio-control agent use is deemed as safer and more nature-friendly within the context of overcoming problems associated with synthetic chemicals. Some microorganisms, such as bacteria, have been successfully used as bio-control agents against root knot nematodes. Meyer et al. (2001) reported the effectiveness of some fungal agents in reducing a range of plant parasitic nematodes.

Okada and Kadota (2003) reported the host status of some fungal isolates for two nematode species. Some *Arthrobotys* spp., trapping fungi have been reported to give mixed results in nematode control (Viaene et al., 2006).

Trichoderma species are fungi residing in nearly all agricultural soils and reportedly possessing some beneficial abilities. The strains of *T. virens* have been used to protect cotton seedlings from *Pythium ultimum* (Howell, 1998). El-Kafrawy et al. (2002) used Trichoderma and *Glirocladium* spp. to control *Fusarium oxysporum*. The metabolites released from some *Trichoderma* species have fungistic effects on the growth of *Ceratocystis paradoxa* (Eziashi et al.,

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2006). Though *Trichoderma* spp. have shown a high level of efficacy against myriads of commercially important pathogens, there is a dearth of information on their potential as bio-control agents in the management of root-knot nematodes.
The objectives of this study include investigation of the efficacy of *Trichoderma harzianum*T_{22} as a bio-control agent against a root-knot nematode infecting three soybean varieties, evaluation of the resistance and susceptibility of selected soybean varieties to root-knot nematodes and determination of the effects of the treatments on the growth and yield of root-knot nematodes infesting soybean varieties.

Materials and methods

Site description

The two-year study was conducted at the University of Ilorin Teaching and Research Farm (8 29N, 4 35E), the Southern Guinea Savannah Zone, Nigeria between August and November in 2012 and 2013.

The source of soybean seeds

Three different varieties; TGX1448-2F (V1), TGX1984-37F (V2) and TGX1987-62F (V3) were collected from the International Institute for Tropical Agriculture, (IITA) Ibadan, Nigeria.

Experimental design

The experiment was designed as a 2×3 factorial fitted into a Randomized Complete Block Design (RCBD) and replicated five times.

Field layout and planting of soybean seeds

The experimental field, which is well-drained coarse sandy-loam, was ploughed, harrowed and divided into two blocks, each plot measuring 70 m × 4 m (280 m^2). There was a 5 m space between the plots to avoid bio-pesticide interference. While one block was treated by the agent, the second one was a control block. Each block was divided into 3 plots with a 5 m alley between the blocks and a 1m alley between the plots. The blocks were replicated four times. Each block was further sub-divided into 5 sub-plots with a 0.5 m alley between them. The soil samples were collected randomly from all the plots to assess the initial population using modified Baerman's method as described by Whitehead and Hemming (1965).
Five kilograms of roots of *Celosia argentea* infested with *Meloidogyne incognita* were collected from an established pure culture and were chopped into small pieces in our screen house and then incorporated in the plots to increase the initial soil nematode population. The seeds were planted at the rate of 3 seeds per hole at the depth of 4-5 cm and separated at the distance of 40 cm. Three weeks after the planting, the seedlings were thinned to a two -plant stand before application of the treatment.

Source of Trichoderma harzianumT_{22} and application

The cultured filtrates of *Trichoderma harzianum*T_{22} were collected from the Ladoke Akintola University of Technology (LAUTECH), Ogbomosho, Oyo State, Nigeria. Thirty mills (30 ml) of *Trichoderma harzianum*T_{22} mixed in 15 litres of water were applied only to soybean varieties in plot 1 by spraying them with a knapsack sprayer at the base of the plants two weeks after the planting. This exercise was repeated after one month. After the harvest, the roots were rated for galling using the method proposed by Taylor and Sasser (1978).
Along with counting the soil nematode population, five grams of roots from all the replicates of the three varieties from the two (block treated by the agent and the control block) blocks were macerated and extracted in separate trays while the population of different stages of *M. incognita* were counted using the method described by Whitehead and Hemming (1965).

Data collection

The data collected include: plant height, the number of branches, the number of pods, grain yield per plot, mean root gall and soil nematode and root nematode (egg, J2 and an adult female population).

Data analysis

All the collected data were subject to an analysis of variance and separated using Duncan's Multiple Range Test (DMRT) at P=0.05.

Results and discussion

The results from the two-year trials generally followed a similar trend. Significant differences between the *Trichoderma* treated plants and the untreated ones were observed (Tables 1-3). The untreated plants were characterized by better performance than the control (untreated) ones in terms of plant height, the number of branches and plant yield. There were also significant differences in the varietal response to nematode infection with respect to the growth and yield of soybean. Variety three (V3) performed significantly better than the other varieties did.

Table 1. Effects of *Trichoderma harzianum* on the plant height of selected soybean varieties inoculated with a root-knot nematode (in cm)

	4WAP		7WAP		10	
Treatment	1st trial	2nd trial	1st trial	2nd trial	1st trial	2nd trial
Trichoderma	21.3	23.9	26.8	29.2	31.6	34.3
No Trichoderma	19.3	21.2	22.6	24.1	25.5	27.0
SED	0.96	0.92	0.94	0.92	0.86	0.96
Level of significance	S	S	S	S	S	S
TGX1448-27F(V_1C)	18.3^b	20.7^b	22.5^b	24.6^b	26.1^b	28.6^b
TGX1984-37C(V_2C)	19.9^b	21.9^b	24.2^b	25.8^b	27.8^b	29.7^b
TGX1987-62F(V_3C)	22.7^a	25.1^a	27.4^a	29.6^a	31.7^a	33.7^a
SED	1.16	1.13	2.37	1.14	1.05	1.17

Each value is a mean of three replicates. The figures with the same letter in the same column are not significantly different using Duncan's Multiple Range Test (DMRT) at P=0.05

Table 2. Effects of *Trichoderma harzianum* on the number of branches of selected soybean varieties inoculated with a root-knot nematode

	4WAP		7WAP		10	
Treatment	1st trial	2nd trial	1st trial	2nd trial	1st trial	2nd trial
Trichoderma	4.5	5.6	6.9	8.2	10.3	10.3
No Trichoderma	4.3	5.2	6.1	6.9	8.3	8.3
SED	0.31	0.31	0.33	0.31	0.37	0.37
Level of significance	NS	NS	S	S	S	S
TGX1448-27F(V_1C)	3.5^b	4.5^b	5.5^b	6.4^b	8.1^b	8.1^b
TGX1984-37C(V_2C)	4.0^b	5.0^b	6.1^b	7.1^b	8.8^b	8.6^b
TGX1987-62F(V_3C)	5.7^a	6.7^a	7.9^a	9.1^a	10.9^a	11.1^a
SED	0.38	0.38	0.41	0.38	0.45	0.93

Each value is a mean of three replicates. The figures with the same letter in the same column are not significantly different using Duncan's Multiple Range Test (DMRT) at P=0.05

Table 3. Effects of *Trichoderma harzianum* on the pod number and grain yield of selected soybean varieties inoculated with a root-knot nematode in field conditions

	Number of pods per plant		Grain yield per plot (tons ha^{-1})	
Treatment	1st trial	2nd trial	1st trial	2nd trial
Trichoderma	16.4	24.3	2.32	2.65
No Trichoderma	8.3	14.2	0.75	0.82
SED	1.11	1.01	0.38	0.36
Level of significance	S	S	S	S
TGX1448-27F(V_1T)	11.6^b	16.8^b	1.27^b	1.29^b
TGX1984-37C(V_2T)	12.4^b	17.6^b	1.29^b	1.29^b
TGX1987-62F(V_3)	16.1^a	24.2^a	2.24^a	2.68^a
SED	2.28	2.55	0.22	0.29

Each value is a mean of three replicates. The figures with the same letter in the same column are not significantly different using Duncan's Multiple Range Test (DMRT)

Studies have shown that among a wide range of commercially important plant pathogens, *Trichoderma*T_{22} species are highly efficient in biological control (Whipps and Lumsden, 2001). The result of our trials suggest that *Trichoderma harzianum*T_{22} in the treated plots has remarkable positive impact of inhibiting the development and parasitic effects of root knot nematode on the three soybean varieties while increasing the growth and yield of the plant. This can be attributed to the neutralization of organic compounds in soil at a faster rate by *Trichoderma*, increasing the nutrient intake and growth rate of the plants which developed higher ability to resist nematode problems. Our observations to the present investigation reveal that increased growth of plants by *Trichoderma* spp. is an added advantage and this conclusion is in accordance with the reports made by

Sharon et al. (2001). This might also result from the control of minor pathogens and/or production of growth regulatory factors by *Trichoderma harzianum*T_{22}. It has been demonstrated that bio-agents produce different metabolites and antibiotics which directly or indirectly stimulate plant growth (Kloepper et al., 1991). *Trichoderma* spp. is utilized in the production of a number of antibiotics, such as trichoderin, trichodermol A and harzianolide, due to the nematicidal effect of the treatment which resulted in general plant growth and yield of soybean. This is also in line with the findings of Nawar (2007) who reported the inhibition of most fungal phytopathogens by compounds produced by *Trichoderma* spp. Control of other fungal pathogens in soil will additionally provide a more conducive, enabling environment for a plant to develop more sturdily without being overwhelmed by an attack of other microorganisms that would otherwise adversely affect it or weaken it to succumb to secondary infections caused by plant parasitic nematodes.

According to these studies, *Trichoderma harzianum*T_{22} shows a remarkable level of antagonistic characteristic against root-knot nematodes attacking soybean. Because a very high premium is placed on this crop due to its nutritional, oil yielding and other numerous valuable uses, care must be taken to protect it from attacks by pests and pathogens which pose a great threat to its profitable production. Soybean farmers are therefore encouraged to apply *Trichoderma harzianum*T_{22} as an effective biological agent, which represents a safer, more nature- and environmental-friendly approach to overcome the problems caused by standard synthetic nematicides. A varietal and treatment combination plays a significant role in this view. Variety TGX1987-62F (V3) has turned out to be more resistant to nematode infections and has given the highest yield despite the prevailing environmental factors. A combination of TGX1987-62F with *Trichoderma*T_{22} in a nematode infested field will give a significantly higher yield. Use of resistant varieties is considered the cheapest and probably one of the best methods for treating plant parasitic nematodes.

Table 4. Effects of *Trichoderma* treatments and soybean varieties on mean final egg, J_2 and adult females in a 5 gm root of planted soybean infected with a *Melodogyne incognita* infected field

	Egg Pop.1		J2 Pop.		Adult female Pop.	
Treatment	1^{st} trial	2^{nd} trial	1^{st} trial	2^{nd} trial	1^{st} trial	2^{nd} trial
V_1T	$388.9^{\mathbf{b}}$	336.5^b	49.6^b	39.0^{ab}	18.33^c	10.00^c
V_1C	532.8^d	663.5^d	132^d	124.67	23.30^e	22.63^e
V_2T	355^b	326^b	36.7^{ab}	40.00^{ab}	7.33^b	5.4^{ab}
V_2C	522.2^d	611.3^d	88.00^c	87.3^c	$21.7d^e$	20.8^{de}
V_3T	236.6^a	222.4^a	24^a	27.3^a	3.3^a	4.3^a
V_3C	428.8^c	405.6^c	60.64^{bc}	62.3^b	16.4^d	15.2^d
S.E	36.7	39.3	37.96	6.98	1.8	1.09

Each value is a mean of three replicates. The figures with the same letter in the same column are not significantly different using Duncan's Multiple Range Test (DMRT) at P=0.05 (SAS 1997 Statistical package)

Table 5. Effect of *Trichoderma harzianum* on mean root galling of selected soybean varieties inoculated with a root-knot nematode

	1^{st} trial	2^{nd} trial
Treatment	2012	2013
V_1 Treated	1.8^b	2.0^b
V_1 Control	4.2^c	3.9^c
V_2 treated	1.7^b	2.0^b
V_2 Control	3.9^c	3.8^c
V_3 treated	0.8^a	0.8^a
V_3 control	2.6^b	2.6^b

Each value is a mean of three replicates. The figures with the same letter in the same column are not significantly different using Duncan's Multiple Range Test (DMRT) at P=0.05

Conclusions

Enhancement of the naturally existing *Trichoderma harzianum*T_{22} species in soil is expected to be highly beneficial to man and his crops.

Trichoderma harzianumT_{22} should be isolated and cultured by plant pathologists and made easily accessible to farmers for inoculation into soil before the planting as this could boost the naturally existing initial population.

Improved soybean varieties such as TGX1987-62F should be made available particularly to farmers in nematode endemic zones.

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