Bio-control of Root-knot Nematode (*Meloidogyne incognita*) infecting tomato (*Lycopersicon esculentum*) using *Trichoderma harzianum*

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ABSTRACT

Field experiment was conducted at the Teaching and Research farm, University of Ilorin, Ilorin, Nigeria, to investigate the effectiveness of a bio-control agent, *Trichoderma harzianum* in the management of plant parasitic nematodes infecting two varieties of tomato (UC8LB and ROMA-VF). The experimental plot was divided into treated and untreated (control) plots and each plot was further sub-divided into two sub-plots. Thus the experiment was a 2x2 factorial fitted into a Randomised Complete Block Design. Three weeks old seedlings were transplanted and the *T. harzianum* treatment was applied. The experimental field was inoculated with pure culture of *Meloidogyne incognita* obtained from root knot heavily infested *Celosia argentea* at 2 weeks after transplanting. Data were collected plant height, number of leaves, number of branches, number of flowers, fruit weight and initial and final soil nematode population. All data were subjected to analysis of variance and significantly different means were separated using the Least significant difference at 5% level of probability. Though there was no significant difference in plant height and number of leaves, the main effects of *Trichoderma harzianum* treatment on number of flowers and yield of tomato were significantly higher in treated plants than in control. There was no varietal difference. Equally soil nematode populations were also significantly reduced in the treated soil compared with the control. This study has elucidated the efficacy of *T. harzianum* in the management of root-knot nematode infecting two tomato varieties, UC8LB and ROMA-VF.

Keywords: Root-knot nematode, *Trichoderma harzianum*, Bio-control agent, Variety

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INTRODUCTION

Tomato (*Lycopersicon esculentum*) belonging to the family Solanaceae, is a major crop of world commerce and one of the widely grown vegetables. Vegetables are eaten either raw or cooked for their nutritional value. Tomato is a good source of vitamins A and C, potassium and fiber. It is rich in lycopene (Di Mascio *et al*., 1989) which, is used to fight against cancer, especially prostrate cancer (Giovannuci *et al*.,1995). Tomatoes are attacked by many pests and diseases one of which is root-knot nematode. Root-knot nematodes are common pathogens that parasitize vegetables and other crops and cause significant yield reduction globally. Umar and Jada (2000) reported that root-knot nematodes particularly, *Meloidogyne* spp. make it difficult and sometimes impossible to grow tomato in the tropics and semi-tropics. These pests and diseases are largely controlled with synthetic pesticides to increase crop productivity, improve quality and profits. However, pesticides residues can be detected in raw and processed tomato fruits. Thus misuse of and persistence use of pesticides can present risks to human health. To minimize or avoid the deleterious effects of chemicals on human and environment, using acceptable alternative control methods become inevitable (Izuogu *et al*., 2015; Sahebani and Hadavi, 2008).

Biological means of control is proving to be a favourable alternative for the management of root-knot nematodes as it is economical, sustainable and environmental friendly. Biological control is the reduction of inoculum density or disease producing activity of a pathogen or parasite in its active or dominant state by one or more organisms accomplished naturally or through manipulation of the environment, host or antagonist (Baker and Cook, 1974). A large number of biocontrol agents have been tested so far to control root-knot nematodes with encouraging results. These include bacteria such as *Burkholder acepacia, Pasteuria penetrans, Pseudomonas fluorescens, Paecilomyces lilanus,* e.t.c (Siddiqui and Shaukat, 2004; Walia and Dalal, 1994; Rohana *et al.* 1987).

However, the activities of several other micro-organisms with potential as biocontrol agents need to be investigated. This study was therefore conducted (i) to determine the efficacy of a bio-agent, *T. harzianum* in the

management of root-knot nematode (*Meloidogyne incognita*) infection in two varieties of tomatoes on the field and consequent effects on the growth and yield of the tomato plants.

MATERIALS AND METHODS

Experimental Site

The experiment was carried out at the Teaching and Research farm University of Ilorin, Ilorin Kwara State, Nigeria. The piece of land measuring 70m x 6m (420m^2) was ploughed, harrowed and ridged. The experimental field was divided into two plots: (1) the control plot which was not treated with *Trichoderma harzianum*, and (2) the plot that was treated with *Trichoderma harzianum*. Each plot was divided into five (5) blocks while each block was divided into two (2) sub plots. Thus, the experiment was a 2 x 2 factorial fitted into a Randomized Complete Block Design.

Soil test

Initial soil samples were randomly collected from the whole plot before incorporation of galled roots and were taken to the laboratory to determine the population of nematodes. Using the Whitehead and Hemming (1965) modified extraction tray method, the extraction of second stage juveniles was carried out. Hundred grams (100 mls) of each sample of soil was measured with a beaker for the set-up. The extraction tray was set on a flat table and a plastic sieve lined with facial serviette placed on the tray while the soil sample was thinly spread on the serviette. Thereafter clean water was gently applied into the trays until the soil was moist. The set-up was left for 24 hours undisturbed. After 24 hours all the nematodes were expected to have migrated into the extraction tray. The plastic sieve was removed gently with its contents, and the nematode-water extract was carefully poured into a beaker and left for some time to allow the extracted nematode to settle down, it was decanted a little at a time. The remaining nematode suspension poured into the counting dish which was placed under the stereoscopic microscope for counting. The number of the second stage juveniles (j2) present in each sample was counted. The procedure was repeated after

harvest, to determine the population of nematodes present in the soil after inoculation with *Trichoderma*, as against the population prior to inoculation.

Source for root-knot nematode

One hundred kilograms of heavily galled roots, *Meloidogyne incognita* infected, *Celosia argentea* plants, were collected from a vegetable garden in Ilorin, Kwara State, Nigeria. The roots were then chopped into smaller pieces.

Inoculation ***procedure***

Fifty kilograms of galled roots were each incorporated into the treated plot as well as the control plot, three weeks before transplanting.

Source of tomato

Two varieties of tomato were collected from Institute of Agricultural Research and Training, Ibadan, Nigeria. The varieties collected were UC82B and ROMA-VF.

Nursery preparation

The nursery beds used for raising the tomato seedlings prior to transplanting to the field were prepared for the two tomato varieties. The seedbeds measuring 3m x3m and 25cm high were sown to tomato seeds of varieties UC82B and ROMA-VF separately by broadcast, after which they were covered with fine sand and labelled accordingly. The beds were also mulched using straws to conserve the soil moisture and to protect the emerging seedlings from intense sunlight.

Transplanting

The tomato seedlings were propagated for 3 weeks in the nursery, before being transplanted on the field and spaced 45cm apart on the ridges. They were watered within 10minutes of transplant to prevent transplanting shock. The two varieties were randomized on the plots following the layout designed on both the control plot and the treated plot.

Antagonistic incorporation

Sixty ml of *Trichoderma harzianum* filtrate obtained Crop and Environmental Protection, Ladoke Akintola University of Technology, Ogbomoso, Nigeria, was serially diluted and then sprayed only on the experimental plot, using the knapsack sprayer.

Cultural practices

The plants were weeded at three-week interval to enhance their growth as well as remove weeds that might serve as secondary hosts to pests and disease causal agents.

Parameters collected weeks after transplanting

The following parameters were collected: Plant height, number of leaves, number of branches, length, Numbers of flowers and fruit weight at harvest. Data were collected at weekly intervals for a period of seven weeks after transplanting. Soil initial and final nematode population were determined.

Statistical analysis

Data collected were subjected to analysis of variance and significantly different means were separated using the least significant difference at 5% level of probability.

RESULTS

Tables 1 shows the main effect of *Trichoderma harzianum* and varietal treatments on the plant height of two varieties of Tomato from 4-10 weeks after planting. There was no significant difference in the plant height between the plants treated with *Trichoderma harzianum* and those not treated with *Trichoderma harzianum*. There was also no significant difference in varietal response to the treatment. Tables 2 and 3 show the main effects of *Trichoderma harzianum* and varietal treatments on the number of branches and leaves of tomato from 4-10 weeks after planting. There was significant difference in the number of branches between the plants treated with

Trichoderma harzianum and those not treated with *T. harzianum* from 4-10 weeks after planting. The plants that were treated with *Trichoderma* had significantly higher number of branches and than those that were not treated. However, variety did not result in any significant differences among the treatment.

Table 1: Main effects of *Trichoderma harzianum* and variety on the plant height (cm) of Tomato

TREATMENT	4WAP		5WAP	6WAP		7WAP	8WAP	9WAP	10WAP
Trichoderma	9.1		14.8	22.3		29.7	36.6	43.8	44.5
No Trichoderma	8.1		12.2	16.8		23.3	28.2	35.1	38.2
S.E.D	1.22		2.04	3.21		4.05	4.88	6.06	6.63
LSD	NS		NS	NS		NS	NS	NS	NS
UC82B	*9.0*	13.9	20.3	25.8	32.3	40.3	47.5		
ROMA-VF	*8.2*	13.0	18.8	27.2	32.5	38.6	43.5		
S.E.D	*1.22*	2.04	3.21	4.05	4.88	6.06	6.63		
LSD	*NS*	NS	NS	NS	NS	NS	NS		

Means in the same column followed by different letters are significantly different.
L.S.D= Least significant difference
WAP= Weeks after planting

Table 2: Main effects of *Trichoderma harzianum* and variety on the number of branches of Tomato

TREATMENT	4WAP	5WAP	6WAP	7WAP	8WAP	9WAP	10WAP
Trichoderma	5.90a	6.60a	8.10a	10.70a	12.90a	15.60a	18.30a
No Trichoderma	4.20b	5.30b	6.40b	7.90b	8.70b	10.00b	11.10b
S.E.D	0.574	0.632	0.652	0.791	1.093	1.283	1.373
LSD	1.218	1.341	1.382	1.676	2.317	2.719	2.911
UC82B	5.0	6.0	7.0	9.0	11.0	13.0	14.0
ROMA-VF	5.0	6.0	7.0	9.0	11.0	10.0	15.0
S.E.D	0.574	0.632	0.652	0.791	1.093	1.283	1.373
LSD	NS	NS	NS	NS	NS	NS	NS

Means in the same column followed by different letters are significantly different.

L.S.D= Least significant difference

WAP= Weeks after planting

Table 3: Main effects of *Trichoderma harzianum* and variety on the number of leaves of tomato

TREATMENT	4WAP	5WAP	6WAP	7WAP	8WAP	9WAP	10WAP
Trichoderma	15	21	32	42	52a	60a	64a
No Trichoderma	11	18	26	28	32b	35b	39b
S.E.D	3.04	3.65	5.88	7.33	6.80	6.62	6.36
LSD	NS	NS	NS	NS	14.42	14.04	13.49
UC82B	14	20	33	37	44	49	53
ROMA-VF	12	19	25	33	40	46	49
S.E.D	3.04	3.65	5.88	7.33	6.80	6.62	6.36
LSD	NS	NS	NS	NS	NS	NS	NS

Means in the same column followed by different letters are significantly different.

L.S.D= Least significant difference

WAP= Weeks after planting

Table 4: Main effects of *Trichoderma harzianum* and variety on the number of flowers and yield of tomato.

Treatment	Number of flowers	Yield (kg)
Trichoderma	22.0a	0.480a
No Trichoderma	9.0b	0.175b
S.E.D	2.92	0.0877
LSD	6.20	0.1859
Variety one	15.0	0.345
Variety two	17.0	0.310
S.E.D	2.92	0.0877
LSD	NS	NS

Means in the same column followed by different letters are significantly different.

L.S.D= Least significant difference

WAP= Weeks after planting

Table 5: Main effect of *Trichoderma harzianum* and variety on the number of Initial and final nematode population of tomato

Initial nematode population		Treatment	Final nematode population	
			Trichoderma	No Trichoderma
Sample 1	232	V1	9	401
Sample 2	300	V1	5	453
Sample 3	256	V2	9	507
Sample 4	270	V2	6	480
Means	264.50		7.25	460.25

Table 4 shows the main effect of *Trichoderma harzianum* and varietal treatments on the number of flowers and yield of tomato. Number of flowers and yield were significantly higher in the plants treated with *Trichoderma harzianum* than in those not treated with *Trichoderma harzianum.* There was no significant difference in the number of flower and in the yield between variety one and variety two. Table 5 shows the initial population of nematodes present in the soil and final soil nematode population after treating with *Trichoderma harzianum*. The population of nematodes in the untreated plot were much higher as compared to those present in the treated plot.

DISCUSSION

Root-knot nematodes (*Meloidogyne* sp.) are sedentary endoparasites and are among the most destructive pests of agricultural crops. They are worldwide in distribution having a very wide host range. *Trichoderma* isolates have been used successfully to control the damage caused by soil-borne pathogens in greenhouses and under opened-field conditions

(Papavizas, 1985). *Trichoderma* species also have been shown to have activity toward root-knot nematodes (Windham *et al*., 1989; Sharon *et al*., 2001).

This experiment has therefore shown that *Trichoderma harzianum* can reduce the number of *Meloidogyne incognita* juveniles' counts, as the counts were much lower in the treated plots than the control plots. There were more *M. incognita* juveniles in the control plots that were not treated with *T. harzianum* after harvest. The fungus provided gave some level of nematode suppression. Similar results were obtained in other researches with other biocontrol agents. Rohana *et al*.(1987) reported that Paecilomyces lilanus inhibited population increase of *Meloidogyne* sp. on tomato.

In the current study, inoculating the seedling with *Trichoderma* did not have a consistent positive effect on plant height. The plant height treated with *Trichoderma harzianum* was not significantly different from those not treated with *Trichoderma harzianum*. The results are similar to those of Sankaranarayanan et al. (2002) who showed that maximum plant height was reached in the non-inoculated control plants followed by those treated with the biocontrol agent.

Tomato plants that were treated with *T. harzianum* were less attacked by the root-knot nematodes. The untreated plants had significantly lower number of flowers, leaves, and fruit weight, conversely, significantly higher nematode population was recovered in the untreated control plots for the two varieties.

Significant increase in growth and yield of tomato plants treated with *T. harzianum* compared to those not treated with *T. harzianum*. Is in agreement with findings of Sasser, (1980) who reported that root-knot nematodes *(Meloidogyne* sp) are capable of causing reduced growth rate and poor yield. This observation also agrees with those of earlier researchers (Papavizas, 1985; Windham *et al*., 1989; Sharon *et al*., 2001) who reported the importance of *Trichoderma* isolates in enhancing plant growth, increasing crop yield and reducing root-knot nematode population build up in the soil as well as their damage.

RECOMMENDATION

Considering the large fraction of scientific efforts being channeled towards the control of plant parasitic nematodes and the practical significance of bio control agent used in this study as possible alternative control in tomato production, we therefore recommend that *T. harzianum* isolates with enhanced activity towards root-knot nematodes be cultured and synthesized into forms that would be durable and available to farmers for use at subsistence and commercial levels.

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