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Biocontrol of Root-Knot Nematode (Meloidogyne incognita) using Trichoderma harzianum on Tomato (Lycopersicon esculentum L. MILL)

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Abstract
A field experiment was conducted at the Teaching and Research farm of the University of Ilorin, Ilorin to investigate the effectiveness of a biocontrol antagonist (*Trichoderma harzianum*) in the control of plant parasitic nematodes on two varieties (UC 82B and ROMA-VF) of tomato. *Trichoderma* treatment resulted in significant higher plant height, number of leaves, length and breadth of leaves, number of flowers and fruit weight as compared with the untreated control. The treatment significantly reduced the nematode population at harvest when compared with the population in no – Trichoderma treated plot. Thus, the treatment of the soil with the antagonistic fungus improved nematode control as the isolate significantly reduced the nematode populations. The fungus enhanced plant growth and yield in all the treated plots.
Keywords: root-knot nematodes, *Trichoderma harzianum*, biocontrol, variety

Introduction

"Tomato (*Lycopersicon esculentum* L. Mill) is a major crop of world commerce and one of the most widely grown vegetables. It belongs to the family of Solanaceae, which contains about 85 genera and 2,300 species. Tomato supplies essential nutrients in human diet; it is a good source of vitamins A and C, potassium, and fiber. Tomato is rich in lycopene"[1], "which is used in the fight against cancer, especially the prostate cancer[2]".

"Plant-parasitic nematodes cause great economic losses to agricultural crops worldwide by causing severe damage to a wide range of important crops"[3]. "In order to reduce these losses, an estimated amount of US$ 500 million is spent on nematode control globally"[4]. "Unlike other pathogens, nematodes give more problems because nematodes live in the soil and cannot be easily seen by the farmers. They are only noticed when the population is widespread and yield reduction is high"[5].

"They can severely damage growing plants, including tomato and other vegetables. The galls are of various sizes. Each gall may contain thousands of fertilized nematode eggs, resistant to environmental hazards. The eggs contain second stage juveniles. The infected plants often show symptoms of chlorosis, rotting, wilting, stunted growth, premature shedding of leaves and are usually non-productive"[6]." At present, more than 65 species of *Meloidogyne* have been reported from different parts of the world. Four of these are economically important including *M. incognita, M. javanica, M. arenaria* and *M hapla*"[7], "responsible for 95% of the infestations in cultivated lands"[8].

In order to reduce the population of the nematodes, several control measures are being employed including cultural practices, chemical and biological control methods. "Unfortunately there are several problems associated with the use of chemicals such as their poor penetration into the nematode egg, rapid leaching and degradation, high cost, environmental pollution including ground water contamination, food safety and worker's protection. Therefore, chemical control of root-knot nematodes may no longer be a good option" [9]. "Biological means of control has emerged recently as a promising alternative to the use of synthetic pesticide"[10]. Biological control is a favourable alternative for the management of root-knot nematodes, as it is economical, sustainable and environment friendly. Such a strategy is considered to be a major tactic in integrated pest management systems.

"A large number of biocontrol agents have been tested so far to control root-knot nematodes with encouraging results. These include bacteria such as *Burkholderacepacia*, *Pasteuriapenetrans, Pseudomonas fluorescens*

[1] Di Mascio *et al*., (1989)
[2] Giovannucci *et al*., (1995)
[3] Sasser and Freckman, 1987
[4] Keren-Zur *et al.,* 2000
[5] Mai, 1977
[6] Agrios, 2004
[7] Eisenback and Triantaphylou, 1991
[8] Sasse and Carter, 1982
[9] Ploeg, 2002
[10]Wilson *et al*., 1993
[11] Walia *et al.,* 1994

"Some species of *Trichoderma* have been used widely as biocontrol agents against soil-borne plant diseases"[1]. "*Trichoderma* isolates have been used successfully to control the damage caused by soil-borne pathogens in greenhouses and under opened-field conditions"[2]. "*Trichoderma* species also have been shown to have activity towards root-knot nematode. Some *Trichoderma* isolates were reported to both enhance plant growth and reduce root-knot nematode damage" [3].

Rohana *et al*. (1987) reported that *Paecilomyces lilacinus* inhibited the population increase of *Trichoderma* spp on tomato at 0.5 and 1.0g inoculum/750g soil. Leij *et al*. (1991) investigated the potential of three *Verticullium chlamydosporium* isolates as biological control agents against *M. arenaria* on tomato plants under glass house conditions. All three isolates survived well in the soil but showed marked differences in their ability to colonize un infected roots, nematode galls and nematode eggs. Significant population reductions of more than 80% after the first nematode generation were achieved with one isolate, which resulted in significant damage control, but no population control in subsequent generations.

Khan and Khan (1992) tested 15 different fungal filterates for their nematicidal properties against *M. incognita*. The percentages mortality and the inhibition of hatching of nematodes were directly proportional to the concentration of the culture filterates. *Nigrospora sphaerica* and *Paecilomyces lilacinus* had the most nematicidal activity and *Theilaviaterricola* the least.

However, the activities of several other micro organisms with potentials as biocontrol agents need to be investigated. This study was therefore conducted to determine the efficacy of an antagonist, *Trichoderma harzianum* in controlling root-knot nematode (*Meloidogyne* spp.) infection in 2 varieties of tomato on the field and consequent effects on the growth and yield of the tomato plants.

MATERIAL AND METHODS

Experimental site

The experiment was carried out at the Teaching and Research farm, University of Ilorin, Ilorin Kwara State. The piece of land used for the project was ploughed and ridged. Thereafter, It was marked out using the pegs. The experimental plot was divided into 2 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 by 2 factorial fitted into a Randomized Complete Block Design.

Soil test

Samples of soil from both the treated and control plots were taken to the laboratory to determine the population of nematodes they contain after inoculation with galled roots. White head and Hemming (1965) modified extractions tray method was used for the extraction of second stage juveniles. 100g of each sample of soils was measured and poured into the beaker. The extraction tray was set on the flat table by placing the plastic sieve on the extraction tray; facial tissue paper was carefully laid on the plastic sieve while the soil sample was thinly spread on it. Thereafter clean water was gently applied into the trays until the soil was moist. The experiment was left for 24 hours.

After 24 hours all the nematodes are expected to have migrated into the extraction tray. The plastic sieve was removed gently with its content, and the nematode-water suspension was carefully poured into a beaker and left for some time to allow the extracted nematodes settle down, it was decanted a little at a time. The remaining nematode suspension was poured into the counting dish which was placed under the stereoscopic microscope and the tally counter was used to count the number of the second stage juveniles nematodes (j2) present in it.

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

Sufficient galled roots from *Celosia argentia* plant infected with root-knot nematodes were collected from a vegetable garden in Lao area, Ilorin, Kwara State. The roots were then chopped into smaller pieces.

Inoculation procedure

The galled roots were incorporated into the experimental 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 tending the tomato seedlings prior to transplanting to the field were thoroughly

[1] Harman *et al.,* 2001
[2] Papavizas, 1985
[3] Windham *et al.,* 1989

prepared. Two beds were prepared, to be used for the 2 varieties of tomato each. The seedbed was 60cm wide, 6cm long and 25cm high. Clods and stubbles were removed from the seedbed. Tomato seeds of varieties UC82B and ROMA-VF were sown on separate beds by broadcasting, after which they were covered with fine sand and labelled carefully. 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 3weeks 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 experimental plot.

Antagonist incorporation

Sixty ml of *Trichoderma harzianum* culture filtrate obtained from Italy was diluted with 30L of water and then sprayed only on the experimental plot using the knapsack sprayer. The antagonist is a cosmopolitan fungus that was being cultured; it was blackish in colour due to the material used in culturing it.

Cultural practices

The plants were weeded every three weeks to enhance their growth as well as remove weeds that might serve to habour pests and disease causal agents.

Parameters collected weeks after transplanting

The following parameters were collected:

Growth parameters (cm);Plant height, number of leaves, number of branches, length and breadth of leaves. All being collected at weekly intervals for a period of seven weeks after transplanting.

Yield parameters; Number of flowers and fruit weight at harvest

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

Table 1: Main effects of *Trichoderma harzianum* and variety on the plant height of Tomato (cm) WAP
Means in the same column followed by different letter are significantly different.
L.S.D= Least significant difference
WAP= weeks after planting

PLANT HEIGHT (cm)

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

Table 1 shows the main effect of *Trichoderma harzianum* and varietal treatments on the 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 no significant difference in varietal response to the treatment.

Table 2: Main effects of *Trichoderma harzianum* and variety on the breadth of leaves of Tomato (cm) WAP

BREADTH OF LEAVES

TREATMENT	4WAP	5WAP	6WAP	7WAP	8WAP	9WAP	10WAP
Trichoderma	2.45	3.20	3.40	3.55	3.75	4.10	4.30
No Trichoderma	2.89	2.98	3.20	3.60	3.76	3.95	4.10
S.E.D	0.143	0.194	0.235	0.194	0.260	0.318	0.296
LSD	NS	NS	NS	NS	NS	NS	NS
UC82B	2.02	3.08	3.25	3.60	3.81	4.05	4.30
ROMA-VF	2.31	3.10	3.35	3.55	3.70	4.00	4.10
S.E.D	0.413	0.194	0.235	0.194	0.260	0.318	0.296
LSD	NS	NS	NS	NS	NS	NS	NS

Table 2 shows the main effect of *Trichoderma harzianum* and varietal treatments on the breadth of leaves of Tomato from 4-10 weeks after planting. *Trichoderma harzianum* treatment did not result in any significant difference among plants. Similarly, the varietal treatment did not result in any significant difference among

plants throughout the period of data collection.

Table 3: Main effects of *Trichoderma harzianum* and variety on the number of branches of Tomato (cm) WAP

NUMBER OF BRANCHES

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

Table 3 shows the main effect of *Trichoderma harzianum* and varietal treatments on the number of branches 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 *Trichoderma harzianum* from 4-10 weeks after planting. The plants that were treated with *Trichoderma* had significantly higher number of branches than those that were not treated. However, variety did not result in any significant differences among the treatment.

Table 4: Main effects of *Trichoderma harzianum* and variety on the length of leaves of Tomato (cm) WAP

TREATMENT	4WAP	5WAP	6WAP	7WAP	8WAP	9WAP	10WAP
Trichoderma	4.45	5.70	7.40a	8.35a	8.90a	9.15a	9.60a
No Trichoderma	3.54	4.48	5.30b	6.05b	6.25b	6.60b	6.85b
S.E.D	0.595	0.667	0.860	0.911	0.980	0.984	0.893
LSD	NS	NS	1.825	1.931	2.078	2.087	1.893
UC82B	3.87	5.05	6.45	7.65	8.05	8.50	8.70
ROMA-VF	4.12	5.13	6.25	6.75	7.10	7.25	7.75
S.E.D	0.595	0.667	0.861	0.911	0.980	0.984	0.893
LSD	NS	NS	NS	NS	NS	NS	NS

Table 4 shows the main effect of *Trichoderma harzianum* and varietal treatments on the length of leaves of Tomato from 4-10 weeks after planting. There was significant difference in the length of leaves between the plants treated with *Trichoderma harzianum* and those not treated with *Trichoderma harzianum* from 6-10 weeks after planting. Length of leaves was significantly higher in the *Trichoderma* treated plants than the untreated ones. The varietal treatment did not result in any significant difference among plants throughout the period of data collection.

Table 5: Main effects of *Trichoderma harzianum* and variety on the number of leaves of tomato (cm) WAP

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

Table 5 shows the main effect of *Trichoderma harzianum* and varietal treatments on the number of leaves of Tomato from 4-10 weeks after planting. There was significant difference in the number of leaves between the plants treated with *Trichoderma harzianum* and those not treated with *Trichoderma harzianum* from 8-10 weeks after planting, as treated plants had significantly higher number of leaves than the untreated plants. There was no significant differences in the number of leaves between variety one and variety two.

Table 6: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		

Table 6 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 7

	At planting (both plots)	At harvest Trichoderma	No Trichoderma
Sample 1	232	9	401
Sample 2	300	5	453
Sample 3	256	9	507
Sample 4	270	6	480
Means	264.50	7.25	460.25

Table 7 shows the population of nematodes present in the soil before and 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, as the population continue to increase ad no treatment was given to it. Whereas, the population present in the treated plot reduced drastically when compared with those present before treating with *Trichoderma.*

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. Average crop losses due to these nematodes in the tropical and sub-tropical countries are 15% annually. However, in vegetable crops, these loses may reach up to 50-80% (Walker, 1983). *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 as much as synthetic nematicides. 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. Similar results also were obtained by Meyer *et al.* (2001) with *Trichoderma*.

Tomato plants that were treated with T. harzianum were less attacked by the root-knot nematodes. The untreated plants had smaller number of flowers, branches and leaves, reduced length of leaves and fruit weight (kg) and high nematodes population/100g soil.

There was significant increase in growth and yield of tomato plants treated with *T. harzianum* compared to those not treated with *T. harzianum*. This is in agreement with Alam, (1973). Sasser, (1980) reported that root-knot nematodes *(Meloidogyne* sp) are capable of causing reduced growth rate. This observation also agrees with those of earlier researchers; Papavizas, 1985; Windham et al., 1989; Sharon et al., 2001, as well as Meyer 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

The level of root-knot nematode reduction recorded with the use of *T. harzianum* in this study can prove effective for further development of the antagonistic fungi as a commercial product.

REFERENCES

Agrios, G.N. 2004. Plant pathology. Fourth edition. Academic press, San Diego, California, USA. Pp.635.

Di Mascio, P.S., Kaiser,H., Sies. 1989. Lycopene as the most efficient biological carotenoid singlet oxygen quencher. Arch Biochem Biophys. 274:532-8.

Eisenback,J.D. and Triantaphyllou, H.H. 1991. Root-knot nematodes: *Meloidogyne* spp. and races. Pages. 191-274. In: Manual of Agricultural Nematology. W.R. Nickle Marsel Dekker, Inc. New York, USA.

fruits and vegetables. Sci. Hortic., 53: 183–189

Giovannucci, E., Ascherio, A., Rimm, E.B., Stampfer, M.J., Colitz, G.A., Willett, W.C.,1995. Intake of carotenoids and retinol in relation to risk of prostate cancer. J. Natl. Cancer Inst. 87:1767-76.

Harman, G.E., Tronsmo, G.E., Rabeendran, R.M., and Whipps, 2001. Chitinolytic enzymes of *Trichoderma harzianum*, Purification of chitobiosidase and endochitinase. Phytopathol. 83: 313-318. *harzianum.* Soil Biol. Biochem., 40;2016-2020.

Kerry, B.R. and De-leij, F.A.A.M.,1992. Key factors in the development of fungal agents for the control of cyst and root-knot nematodes. Biological control of plant disease: progress and challenges for the future: Proc. NATO Adv. Res. Workshop.139-144.

Khan, S.T. and Khan, T.A. 1992. Effect of culture filtrates of soil fungi on the hatching and mortality of root knot nematode (*Meloidogyne incognita*). Current Nemato. 3:53-60.

Mai, W.F. 1977. World-wide distribution of potato-cyst nematodes and their importance in crop production. J. Nematol.9:30-34.

Papavizas, G.C. 1985. Evaluation of new biotypes of *Trichoderma harzianum* for tolerance to benomyl and enhance biocontrol capabilities. Phytopathol. 72: 126-132.

Ploeg, A.T.2002. Effect of selected Marigold varieties on root-knot nematodes of tomato and melon yields. Plants Dis. 86:505-508.

Rohana, D., Marwoto, B. and Djatnika, I. 1987. The effects of application of P. lilacinus and population levels of root knot nematodes (Meloidogyne spp.) and tomato plant growth. Buletin Penelitian Hortikultura. 15: 109-111.

Sankaranarayanan C., Hussaini S.S., Sreerama Kumara P. and Rangeswaran R. 2002. Parasitism of *M. incognita* Eggs by *F. oxysporum* and other fungi, Indian J. Nematol., 32(1): 33-36.

Sasser JN. Root knot nematodes: A global menace to crop production. Plant Disease. 1980;64:36–41

Sasser, J.N. and Carter, C.C. 1982. Overview of the International Meloidogyne project-rationale, goals, implementation and progress to date. Proc. 3rd Res. Plan. Conf. On root-knot nematodes *Meloidogyne* spp. Panama 1-7.

Sasser, J.N., and Frekman, D.W.,1987. A world perspective on Nematology. The role of society. Pages 7-14 in Vista on Nematology, Veech, J.A., and Dickson, D.W.,eds. SON.Inc., Hyattsville, MD, USA.

Walia, R.K. and Dalal, M.R. 1994. Efficacy of bacterial parasite *Pasteuria penetrans* application as nursery soil treatment incontrolling root-knot nematode *M. Javanica* on tomato. Pest Manag. and. Economic. Zool. 2:19-21.

Walker, J.C. 1983. Diseases of vegetable crops. (1st Ed.). McGraw Hill Book Co. Inc. New York. Pp. 431-515.

Wilson CL, Wisniewski ME, Droby S, Chalutz E (1993). A selection strategy for microbial antagonists to control postharvest diseases of

Windham, G.L., Windham, M.T. and Meyer, W.P. 2001. Effects of *Trichoderma* spp. on maize growth and *Meloidogyne arenaria* reproduction. Plant Dis. 73: 493-495.

Windham, M.T., Elad, Y. and Sharon, A.R., 2001. A mechanism for increased plant growth induced by *Trichoderma* spp. phytopathol 76: 518-521.