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Research Article
Efficacy of Selected Local Fungal Isolates in the Management of Root Knot Nematode (*Meloidogyne* spp.) on Tomato (*Solanum lycopersicum*) in Kenya

A.N. Kariuki, J.W. Waceke and M. Mwangi

Department of Agricultural Science and Technology, Kenyatta University, P.O. Box 43844-00100, Nairobi, Kenya

Abstract

Background and Objective: Root knot nematodes (*Meloidogyne* spp.) are a serious threat to tomato production causing 30-100% yield loss in Kenya. Effective management of nematodes primarily depend on the chemical nematicides, which are expensive, a health hazard and can pollute the environment. The main objective of this study was to evaluate the efficacy of the selected fungal isolates in managing root knot nematodes on tomatoes under greenhouse and field conditions. **Materials and Methods:** Field and greenhouse experiments were set up from January-July, 2019 to evaluate the efficacy of local fungal isolates against Root Knot Nematodes (RKN) on tomato (*Solanum lycopersicum* L.). The treatments included local *Trichoderma harzianum*, *T. afroharzianum* and *Purpureocillium lilacinum* isolates, Bionematon® and untreated control. Greenhouse trials arranged in Completely Randomized Design replicated four times while the field experiments were arranged in Randomized Complete Block Design with four replicates. The inoculum was obtained from galled tomato roots using root maceration technique. Data on growth and nematode disease parameters were recorded. The data was subjected to analysis of variance (ANOVA) using SAS software and separation of means done using Fisher's Least Significant Difference (LSD) test at 95% confidence level. **Results:** The results showed consistent significant ($p \leq 0.05$) effects on measured parameters in both greenhouse and field. The treatments had significantly lower mean galling and egg mass indices (>2.5) than the control (>2.7). The treatments had an RF 1 indicating no multiplication compared to >2 in control. The isolates were noted to increase plant height by above 10%, while the dry weight and yield increased by at least 20 and 50%, respectively over the untreated. Overall, the isolates (*T. harzianum*, *T. afroharzianum* and *P. lilacinum*) performed equally as the Bionematon®. **Conclusion:** These results concluded that indigenous fungal isolates are effective bio-control agents for managing RKN on tomato and can therefore be recommended to farmers.

Key words: *Meloidogyne* spp., fungi, *T. harzianum*, *P. lilacinum*, bio-control

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Corresponding Author: A.N. Kariuki, Department of Agricultural Science and Technology, Kenyatta University, P.O. Box 43844-00100, Nairobi, Kenya Tel: +0724739790

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The agriculture sector remains the mainstay of the Kenyan economy contributing 29.3% of the Gross Domestic Product (GDP) and 80% of national employment[1]. The horticulture sector was ranked the third most important sub sector after dairy and tea by the Kenya economy survey 2016. Tomato is one of the most highly consumed vegetable in Kenya[2]. It is grown for fresh market, processing and export market. However, tomato production is hindered by diseases and pests that cause significant yield losses. The major diseases affecting tomatoes are Fusarium wilt (*Fusarium oxysporum*), bacterial wilt (*Ralstonia solanacearum*), early blight (*Alternaria solani*), late blight (*Phytophthora infestans*), leaf spots and tomato mosaic virus. The major pests of economic importance include leaf miner, (*Tuta absoluta*), white flies, (*Bemisia tabaci*), thrips, (*Frankliniella occidentalis*), aphids, (*Aphis gossypii*), mites, (*Tetranychus urticae*) and bollworms, (*Helicoverpab armigera*) and root knot nematodes (*Meloidogyne* spp.)[3-4].

Root knot nematodes have been ranked at the top among the five major plant pathogens and also first among the most important genera of plant parasitic nematodes in the world[5]. The genus *Meloidogyne* is of concern to tomato growers in small-scale and large scale production[6]. Formation of galls on infected roots is a primary symptom to nematode attack which disrupts nutrient and water uptake in plants. Root knot nematodes (*Meloidogyne* spp.) alone cause 30-100% yield loss in tomato crop[7]. Apart from the direct damage caused by root knot nematodes to plants, they have been implicated to form disease complexes with pathogenic fungi and bacteria hence increase the damage that lead to greater yield losses[8].

Initially nematicides proved to be the most effective measure of controlling high levels of *Meloidogyne* spp.[7]. However, high costs of nematicides in the market and their harmful effects on human and animal and also on numerous beneficial microbes found in the soil limit their use[9]. Furthermore, some broad spectrum nematicides including methyl bromide and Aldicard, used against nematodes have been restricted due to their toxicity to human beings and negative effects to the environment.

Effective and sustainable management of RKN is required for profitable tomato production[10]. Biological control methods have been considered as viable alternative to chemical control. Nematophagous fungi including *Aspergillus* sp., *Paecilomyces lilacinus*[11], *Pochonia chlamydosporia* and *Trichoderma* spp.[10] have showed suppressive effects against nematodes including *Meloidogyne* genus. Since most of these fungal antagonists can be found in most agricultural soils, they provide an inexpensive environmentally friendly technique for management of root knot nematodes. The aim of the current study was therefore, to enhance tomato production through management of root knot nematodes using fungal antagonists. Specifically, evaluated the efficacy of the selected fungal isolates in managing root knot nematodes on tomatoes under greenhouse and field conditions.

MATERIALS AND METHODS

Study area: The research was conducted from January to July, 2019 in Kimbimbi area, Mwea, Kirinyaga County. Mwea is a semi-arid region with an altitude of 1100 m above sea level and rainfall ranges from 800-2200 mm annually. It is located at 0°36′8″S 37°21′58″E. Tomatoes in this area are produced throughout the year since farmers use furrow irrigation.

Preparation of nematodes inoculum: Eggs were extracted from galled tomato roots through root maceration method as described by Coyne *et al.*[12]. The roots were gently washed under tap water and blotted dry using a paper towel. The roots were then chopped into one-centimeter-long pieces. Roots were weighed and water added at a ratio of one gram of fresh root to 20 mL water and 1.5% NaOCl into a blender and blended for 15 sec at high speed[13]. The resultant suspension was sieved using 500, 50 and 25 µm aperture sieves into a beaker and eggs enumerated using a dissecting microscope to estimate concentration per two milliliters. The eggs were then incubated for 7 days and freshly hatched J2 collected every 2 days after the third day of incubation. The number of J2 s mL^{-1} of suspension was determined using a counting dish under a compound microscope[14]. The suspension was then adjusted to 500 J2 mL^{-1} and stored at 4°C before being used in the greenhouse experiments.

Isolation and identification of fungal isolates: The fungal isolates were obtained from samples of healthy tomato roots and also from RKN egg masses.

The healthy tomato roots were thoroughly but gently washed under running water. The roots were then sterilized by dipping them in 0.5% sodium hypochlorite (NaOCl) for 3 min after which they were rinsed out twice in sterile water and air dried on sterile blotting paper.

After drying, the roots were cut into 1 cm pieces and placed evenly on the surface of Potato Dextrose Agar (PDA) medium in the Petri plates in which 150 mg L^{-1} streptomycin was added to suppress bacterial growth.

On the other hand, the egg masses were handpicked using forceps from the galled roots under a dissecting microscope and placed aseptically in Petri dishes containing PDA amended with Streptomycin.

The plates were sealed with Para film to avoid desiccation and contamination. The plates were then incubated at 25°C for 7 days to promote fungal growth and sporulation. The fungal cultures were then sub-cultured onto fresh PDA medium to obtain pure cultures and observed after every three days for a period of two weeks. The final pure cultures were preserved on PDA slants at 4°C in the refrigerator until further use.

The various fungal isolates were identified in reference to the morphological features according to identification key[15]. The selected isolates for *in vivo* studies were identified to the species level using molecular techniques.

Mass multiplication of fungal antagonists: The selected local fungal isolates from the *in vitro* studies were mass multiplied using sorghum grains. Sorghum grains were steeped in water (containing 2% dextrose) in a volume ratio of 2:1 (water: sorghum) for 12 hrs, excess water was drained and the grains were placed in polypropylene bag and autoclaved at 121°C at 1 atmosphere pressure for 20 min. The materials were allowed to cool to 25°C and then inoculated with the 5mm cube of the 7 days old fungal colonies[16].

The substrate was incubated for approximately 14 days, air dried, blended and passed through 80 and 50 µm mesh sieves simultaneously to obtain spore powder. The powdered form of sorghum grains containing the fungal isolates was incorporated in to talc in the ratio 1:2 and dried at room temperature then mixed with Carboxyl Methyl Cellulose (CMC) 5 g kg^{-1} the product[17]. The estimation of Colony Forming Units (CFU) of the fungal species in different formulations was done by suspending 1 g of dried product prepared on substrates and serially diluting the powder. The fungal isolates were then used for trials.

Greenhouse experiments: The greenhouse experiments were set up in Kenyatta University in the period of January-April, 2019 and April-June, 2019. Tomato seeds, Kilele F1 variety were sown on sterile soil mixture in nursery for germination in greenhouse conditions. The potting medium was sand and soil mixed in the ratio 2:1 then autoclaved at 121°C for 20 min at 15 psi. Plastic bags of 40 cm in diameter and 20 cm depth were filled with the sterile soil mixture and placed in the greenhouse. Three weeks old tomato seedlings were transplanted one seedling per pot. The treatments were arranged in a Complete Randomized Design (CRD) with 4 replicates. The treatments include, *T. harzianum*, *P. lilacinum*, *T. afroharzianum*, Bionematon® (*Paecilomyces lilacinus* 1.15%) and Untreated control.

A plastic syringe was used to place juveniles into 3 cm deep holes around the root system. The juveniles were applied at the rate of 1000 J2 per pot while the fungal isolates applied at 1.0×10^6 spores mL^{-1} per pot. Proper agronomic practices were observed and the experiment terminated at 90 days after transplanting.

Field experiment: A field with high nematodes infestation (approximately 500-1000 J2/200 cc of soil) was identified and a two-season experiment set up on separate fields. The treatments were arranged in a Randomized Complete Block Design (RCBD) and replicated four times. A plot size of 16 M^2 was used with 1 M left between treatments and replicate. The treatments were, *T. harzianum*, *T. afroharzianum* and *P. lilacinum*, Bionematon® (Positive control) and untreated control. Four weeks old tomato seedlings were transplanted in the respective field and their growth monitored. Application of treatments was done immediately after transplanting as a drench and 2 subsequent applications at 30 days' interval.

Data collection

Plant growth parameters

Plant height (cm): This was measured from the soil line to the shoot apex using a tape measure at 30, 60 and 90 days after transplanting.

Dry shoot and root weights (g): At harvest, plants were gently uprooted and roots separated from the shoots by cutting at the soil line. The roots were rinsed under tap water, surface sterilized using 1.5% NaOCl and then rinsed in distilled water. The roots were then blotted dry using a paper towel. The root and shoots were then oven dried at 60°C until a constant mass was achieved and their dry weight determined as described by Arim *et al.*[18].

Yield: The yield was recorded as weight of marketable and non-marketable fruits.

Nematode disease parameters

J2 population in the soil: The initial J2 population was determined before transplanting then monitored at 30, 60 and 90 days after inoculation. The juveniles were extracted from the soil through modified Bearmann's technique as described by Coyne *et al.*[13].

Reproduction Factor (RF) was obtained from the ratio of final nematode population to initial population in the field experiment.

The galling and egg mass indices were determined by counting the number of galls/egg masses and then scoring according to Quesenberry *et al.*[19] using a scale of 0-5 where: 0 = no gall or egg mass, 1 = 1 or 2, 2 = 3 to 10, 3 = 11 to 30, 4 = 31 to 100 and 5 => 100 galls or egg masses per root system. To facilitate counting of egg masses the roots were stained for 15 min in an aqueous solution of Phloxine B stain (0.15 g L^{-1} water), which stains the gelatinous matrix pink-red increasing egg mass visibility[20].

Data analysis: The data collected on plant height, plant dry weight, juvenile population in the soil, galling index, egg masses index and yield was subjected to Analysis of variance (ANOVA) using SAS software (version 9.2) and separation of means was done using the Fisher's Least Significant Difference (LSD) at 5% level of significance.

RESULTS

Effects of fungal bio-control agents on the growth of tomatoes in greenhouse experiments

Effects of fungal bio-control agents on shoot height (cm): There were highly significant ($p \leq 0.05$) differences in shoot height (cm) of treated plants and the untreated control (Fig. 1, 2).

In both experiments, no significant ($p \geq 0.05$) differences were recorded in shoot heights among the bio-control agents (BCAs) including the commercial BCA (Bionematon®) at 30 DAT (Table 1, 2).

In experiment 1, *T. harzianum* treated plants recorded the highest shoot heights that was significantly ($p \leq 0.05$) different from that recorded in *P. lilacinum* and *T. afroharzianum* treated plants at 90 DAT (Fig. 1).

In the second experiment, *T. harzianum* treated plants still recorded the highest shoot heights at 90 DAT, although this did not differ significantly ($p \geq 0.05$) from Bionematon® treated plants.

Overall, *T. harzianum* performed better than Bionematon® and *P. lilacinum* while Bionematon® treated plants performed significantly ($p \leq 0.05$) better than *P. lilacinum* in terms of shoot heights (Fig. 1, 2). The untreated plants consistently recorded significantly ($p \leq 0.05$) shorter shoot heights than the treated plants.

Effects of fungal bio-control agents on plant dry weights (g): The plant dry weights of treated tomato plants were significantly ($p \leq 0.05$) higher than those recorded in untreated plants (Table 1). In the greenhouse experiment 1, tomato plants treated with Bionematon® had the highest dry shoot weights although they did not differ significantly ($p \leq 0.05$) with those recorded in *T. harzianum* and *P. lilacinum* treated plants (Table 1).

In the greenhouse experiment 2, *T. harzianum* had the highest dry shoot weight although did not differ significantly from Bionematon® and *P. lilacinum* (Table 1). *Trichoderma afroharzianum* on the other hand recorded significantly lower dry shoot weights from *T. harzianum* and Bionematon® treated plants.

The highest dry root weight was recorded in Bionematon® treated plants while the least among the treated plants was noted in *T. afroharzianum* (Table 1).

The untreated plants recorded the lowest dry weights in both roots and shoots during the two greenhouse experiments (Table 1).

Effects of fungal bio-control agents on yield of tomatoes: The results showed significant ($p \leq 0.05$) differences in yield (kg $plant^{-1}$) of tomatoes between the treated and untreated plants (Fig. 3, 4). In both greenhouse experiments, *T. harzianum* treated plants had the highest yield which was not significantly ($p \leq 0.05$) different from Bionematon® treated plants (Fig. 3, 4). *Trichoderma afroharzianum* treated plants on the other hand recorded a lower yield compared to the other treated plants although it was significantly higher than the untreated plants.

The non-marketable yield was not significantly different between the treated and untreated plants although it was slightly higher in the untreated plants than in the treated plants (Fig. 3, 4).

Effects of fungal bio-control agents on nematodes disease parameters

Effects of fungal bio-control agents on Juveniles (J2) populations: The J2 population in the soil was observed to reduce over time in the treated pots while increasing in the untreated control (Table 2, 3).

In the greenhouse experiment 1, *T. harzianum* recorded a lower mean J2 population which did not differ significantly ($p \geq 0.05$) from the mean recorded in Bionematon® and *T. afroharzianum* but differed significantly ($p \leq 0.05$) from *P. lilacinum* at 30 DAI (Table 2).

At 60 DAI, *Trichoderma afroharzianum* treated pots recorded higher mean J2 population which differed significantly from means J2 recorded in *T. harzianum* and Bionematon® treated pots (Table 2).

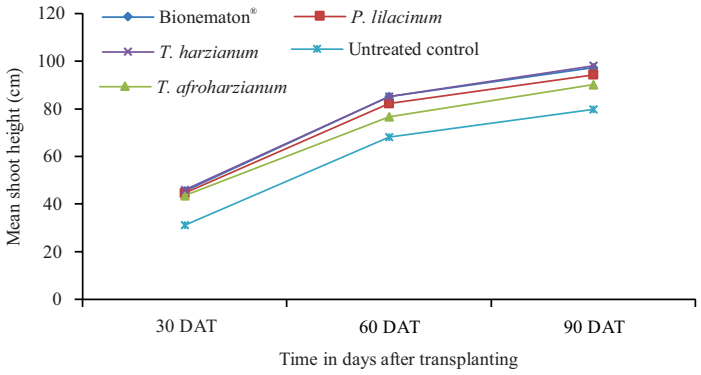

Fig. 1: Mean shoot height (cm) of tomato plants treated with different fungal bio-control agents in the greenhouse experiment 1

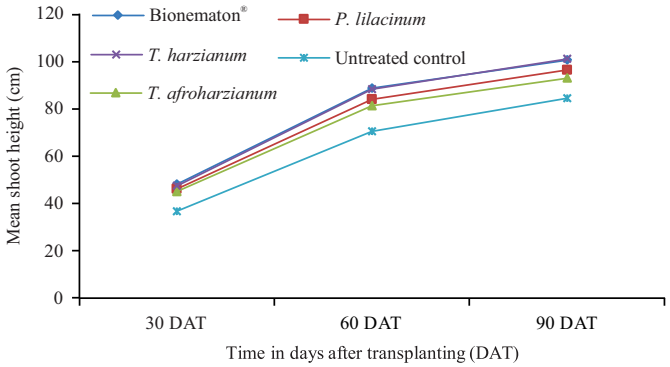

Fig. 2: Mean shoot height (cm) of tomato plants treated with different fungal bio-control agents in the greenhouse experiment 2

Table 1: Mean plant dry weights (g), greenhouse experiments

	Plant dry weight (g)			
	Greenhouse experiment 1		Greenhouse experiment 2	
Treatments	DSW	DRW	DSW	DRW
T. harzianum	7.98±0.12^{a}	4.35±0.25^{a}	8.28±0.13^{a}	4.68±0.20^{ab}
Bionematon®	8.03±0.15^{a}	4.84±0.27^{a}	8.25±0.11^{a}	5.09±0.22^{a}
P. lilacinum	7.83±0.14^{ab}	4.67±0.23^{a}	7.98±0.11^{ab}	4.79±0.21^{ab}
T. afroharzianum	7.41±0.15^{b}	4.36±0.22^{a}	7.58±0.12^{b}	4.48±0.20^{b}
Untreated control	6.21±0.22^{c}	3.32±0.18^{b}	6.33±0.22^{c}	3.45±0.18^{c}
LSD	0.45	0.98	0.41	0.57
p-value	0.0001	0.0001	0.0001	0.0001

Means followed by the same letter (s) within the same column are not significantly ($p \geq 0.05$) different according to Fisher's least significance difference (LSD) test. DSW: Dry shoot weight and DRW: Dry root weight

Table 2: Mean J2 populations per 200 cc of soil, greenhouse experiment 1

	J2/200 cc of soil		
Treatments	30 DAI	60 DAI	90 DAI
T. harzianum	496±39.89^{c}	313±41.13^{c}	133.0±18.66^{c}
Bionematon®	590±28.58^{bc}	335±25.91^{c}	200..0±41.41^{bc}
P. lilacinum	603±28.98^{b}	357±48.18^{bc}	183.0±52.41^{bc}
T. afroharzianum	515±42.07^{bc}	515±86^{b}	319.0±83.45^{b}
Untreated control	816±19.03^{a}	880±37.19^{a}	1005.0±45.55^{a}
LSD	98.87	159.30	158.64
p-value	0.0001	0.0001	0.001

Means followed by the same letter (s) within the same column are not significantly ($p \geq 0.05$) different according to Fisher's least significance difference (LSD) test. DAI: Days after inoculation

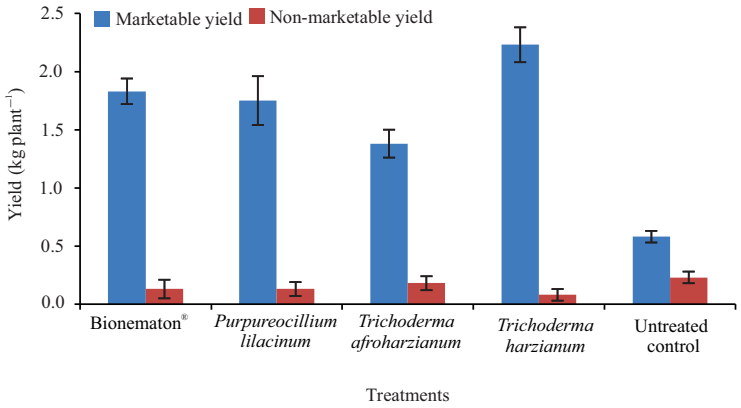

Fig. 3: Mean yield (kg plant^{-1}) of tomatoes in the greenhouse experiment 1

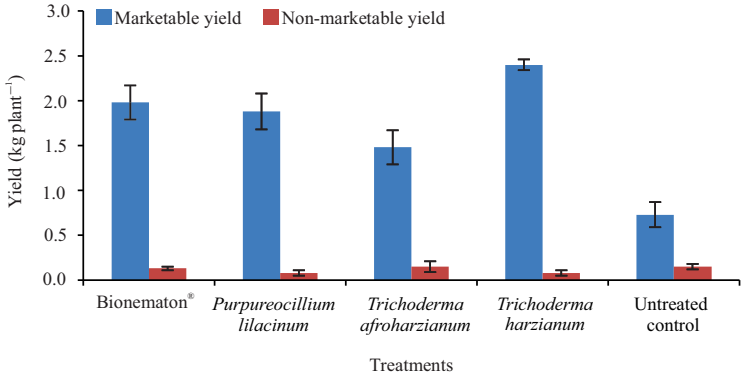

Fig. 4: Mean yield (kg plant^{-1}) of tomatoes in the greenhouse experiment 2

Table 3: Mean J2 populations per 200 cc of soil, greenhouse experiment 2

	Mean J2/200 cc of soil		
Treatments	30 DAI	60 DAI	90 DAI
T. harzianum	466±23.34^{b}	288±36.45^{c}	172±7.67^{c}
Bionematon®	575±36.63^{b}	330±24.06^{c}	175±9.57^{c}
P. lilacinum	577±13.14^{b}	342±53.49^{bc}	220±23.45^{bc}
T. afroharzianum	511±39.08^{b}	490±94.07^{b}	328±56.49^{b}
Untreated control	768±58.56^{a}	860±53.39^{a}	958±54.52^{a}
LSD	103.54	145.01	89.93
p-value	0.0005	0.0001	0.0001

Means followed by the same letter (s) within the same column are not significantly ($p \geq 0.05$) different according to Fisher's least significance difference (LSD) test. DAI: Days after inoculation

In both experiments, the least mean number of J2/200 cc of soil was recorded in *T. harzianum* treated soils which were not significantly ($p \geq 0.05$) different from those recorded in *P. lilacinum* and Bionematon® at 90 DAI. *Trichoderma afroharzianum* was the least in reducing the mean number of J2 in the soil but differed significantly ($p \leq 0.05$) from the untreated control (Table 2, 3).

In the greenhouse experiment 2, no significant ($p \geq 0.05$) difference was noted in the mean number of J2s in the treated soils at 30 DAI (Table 3).

The untreated soils consistently recorded the highest mean number of J2s and were significantly different from the treated pots (Table 2, 3).

Effects of fungal bio-control agents on galling and egg mass indices: There was significant difference ($p \leq 0.05$) in the galling and egg mass indices between the treated and untreated plants during the greenhouse experiments (Table 4). The untreated plants recorded the highest disease severity across the disease parameters (GI and EMI). No

significant ($p \geq 0.05$) difference was observed among the BCAs in line with the different disease parameters in both experiments. A lower GI was noted in Bionematon® treated plants among the BCAs (Table 4).

Effects of fungal bio-control agents on growth and yield of tomatoes in the field

Effects of fungal bio-control agents on shoot height: According to the results on Fig. 5 and 6, the treatments were found to promote the growth of tomato plant as exhibited by increased plant height compared to the untreated plants. *Trichoderma harzianum* treated plants consistently recorded significantly ($p \leq 0.05$) higher shoot heights in both seasons followed by Bionematon® and *P. lilacinum* compared to the untreated plants. The untreated plants were noted to have significantly ($p \leq 0.05$) shorter heights throughout the test period (Fig. 5, 6).

Effects of fungal bio-control agents on dry shoot and root weights (g) of tomato plants: The results on Table 5 depicted a significant positive effect of treatments application on the dry weight of plants in both seasons.

Table 4: Galling and egg mass indices on tomato, greenhouse experiments

	RKN disease parameters on tomato			
	Greenhouse experiment 1		Greenhouse experiment 2	
Treatments	Galling index	Egg mass index	Galling index	Egg mass index
T. harzianum	2.00±0.22^{b}	2.00±0.27^{b}	2.00±0.22^{b}	2.00±0.27^{b}
Bionematon®	1.88±0.27^{b}	1.88±0.27^{b}	1.88±0.27^{b}	1.88±0.27^{b}
P. lilacinum	2.06±0.31^{b}	2.13±0.27^{b}	1.94±0.23^{b}	1.94±0.23^{b}
T. afroharzianum	2.00±0.30^{b}	2.19±0.33^{b}	2.00±0.30^{b}	2.19±0.33^{b}
Untreated control	2.88±0.95^{a}	3.00±0.18^{a}	3.19±0.23^{a}	3.06±0.19^{a}
LSD	0.72	0.76	12.98	9.58
p-value	0.005	0.038	0.002	0.013

Means followed by the same letter (s) within the same column are not significantly different ($p \geq 0.05$) according to Fisher's least significant difference (LSD) test

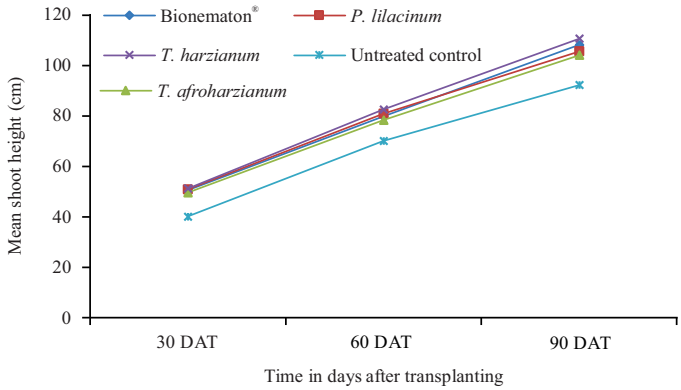

Fig. 5: Mean shoot height (cm) of tomato plants treated with different fungal bio-control agents in the field (season one)

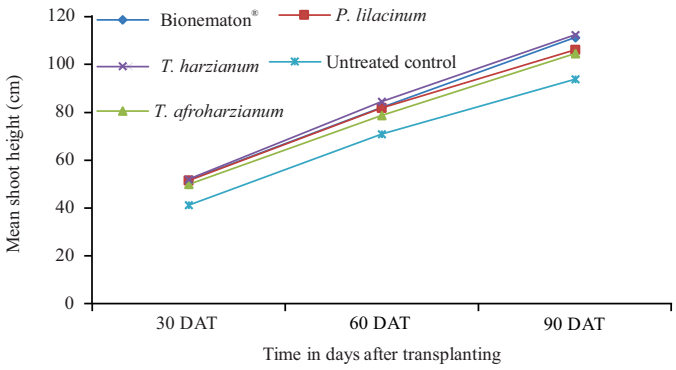

Fig. 6: Mean shoot height (cm) of tomato plants treated with different fungal bio-control agents in the field (season two)

In the first season, Bionematon® treated plants recorded the highest dry weight of shoots and roots followed by *T. harzianum* treated plants and these were not significantly different from *P. lilacinum* and *T. afroharzianum* treated plants (Table 5).

However, in the second season, *T. harzianum* had the highest dry weight of shoots which was significantly higher than those recorded in *P. lilacinum* and *T. afroharzianum* treated plants (Table 5). No significant differences were noted in the dry weight of roots in the treated plants.

The untreated plants had significantly lower dry weights of shoots and roots in both seasons.

Effects of fungal bio-control agents on tomato yield (t ha^{-1}): The soil application of the local fungal bio-control agents had a significant increase in the tomato yield as shown on Fig. 7 and 8. The treated plants had significantly higher yield than the untreated plants in both seasons.

In the first season the highest marketable yield was noted in Bionematon® treated plants followed by *T. harzianum*

Table 5: Mean plant dry weights (g), field experiments

	Plant dry weight (g)			
	Field (season one)		Field (season two)	
Treatments	DSW	DRW	DSW	DRW
T. harzianum	17.71±0.28^{a}	5.00±0.12^{a}	17.96±0.24^{a}	5.27±0.14^{a}
Bionematon®	17.75±0.16^{a}	5.06±0.24^{a}	17.80±0.14^{a}	5.22±0.22^{a}
P. lilacinum	17.12±0.21^{a}	4.79±0.14^{a}	17.17±0.20^{b}	4.90±0.12^{a}
T. afroharzianum	17.16±0.21^{a}	4.90±0.21^{a}	17.22±0.18^{b}	5.01±0.18^{a}
Untreated control	10.12±0.21^{b}	3.73±0.13^{b}	10.34±0.20^{c}	3.88±0.11^{b}
LSD	0.614	0.532	0.547	0.436
p-value	0.0001	0.0001	0.0001	0.0001

Means followed by the same letter (s) within the same column are not significantly ($p \geq 0.05$) different according to Fisher's least significance difference (LSD) test

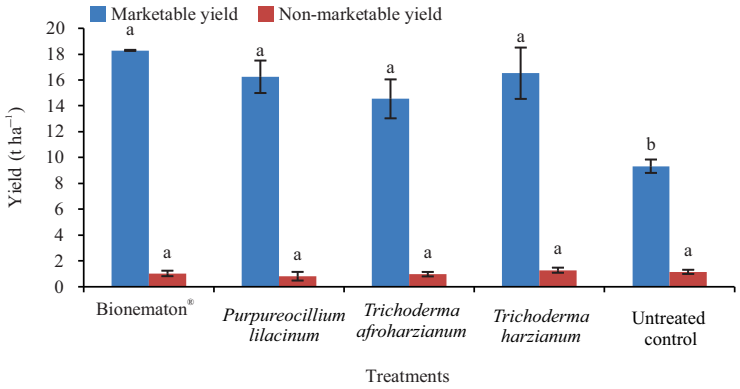

Fig. 7: Mean yield (kg plant^{-1}) of tomatoes in the field (season one)

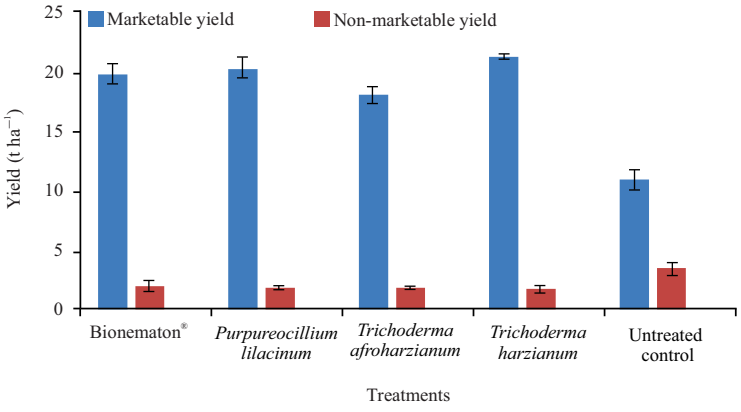

Fig. 8: Mean yield (kg plant^{-1}) of tomatoes in the field (season two)

treated plants, however no significant (p>0.05) differences was established among the treated plants (Fig. 7). No significant (p>0.05) difference was noted in the non-marketable yield between the treated and untreated plants, although the highest non-marketable yield was noted in the untreated plants (Fig. 7).

In the second season, *T. harzianum* treated plants had the highest marketable yield which was significantly (p<0.05) different from that recorded in *T. afroharzianum* treated plants (Fig. 8). The non-marketable yield noted in the untreated plants was significantly (p<0.05) higher than that recorded in the treated plants (Fig. 8).

Effects of fungal bio-control agents on nematode disease parameters in the field

Effects of fungal bio-control agents on nematode soil population: The J2 populations were significantly (p<0.05) reduced by the application of the various fungal bio-control agents. The initial J2 populations in the soil did not differ significantly (p>0.05) in all the treatments in both seasons. Significant (p<0.05) decrease in J2 population was noted at 60 and 90 days after fungal inoculation (Table 7).

The lowest J2 population was noted in Bionematon® treated soils followed by *T. harzianum* although these numbers were not significantly (p>0.05) different from those recorded in *P. lilacinum* and *T. afroharzianum* in both seasons (Table 6, 7).

The J2 population in the untreated control was noted to increase throughout the test period and was significantly (p>0.05) higher than those recorded in the treated soils.

Effects of fungal bio-control agents on nematode disease severity on tomato: The GI and EMI of tomato plants grown on treated soils were significantly (p<0.05) lower than that of the plants on the untreated soils in both seasons. The GI of treated plants did not differ significantly (p>0.05) among the various fungal bio-control agents across the two seasons. *Trichoderma harzianum* treated plants recorded the lowest GI and EMI in both seasons. However, the EMI recorded on *T. afroharzianum* treated plants was higher than that of other treated plants and did not differ significantly (p>0.05) from that recorded in the untreated control in the first season (Table 8).

Effects of fungal bio-control agents on nematode reproduction: The fungal bio-control agents effectively decreased the reproduction and multiplication of the root knot nematodes in the soil as shown by the lower RF (Table 9).

In the first season *P. lilacinum* had the lowest RF which was not significantly (p>0.05) different from that noted in the other treated soils. However, in the second season, *T. harzianum* had the lowest RF, although this was not significantly (p>0.05) lower than that recorded in the other treatments (Table 9).

Table 6: Mean J2 populations per 200 cc of soil in the field (season one)

	Mean J2/200 cc of soil			
Treatments	Initial J2	30 DAI	60 DAI	90 DAI
T. harzianum	500±174^{a}	679±146^{c}	508.0±122^{b}	269.0±61^{b}
Bionematon®	308±74^{a}	427±43^{b}	284.0±25^{b}	213.0±26^{b}
P. lilacinum	508±152^{a}	686±156^{ab}	490.0±100^{b}	287.0±53^{b}
T. afroharzianum	575±107^{a}	857±92^{a}	587.0±69^{b}	459.0±66^{b}
Untreated control	560±118^{a}	760±162^{ab}	1170.0±171^{a}	1692.0±242^{a}
LSD	390.94	381.86	328.28	356.66
p-value	0.616	0.231	0.0001	0.0007

Means followed by the same letter (s) within the same column are not significantly (p≥0.05) different according to Fisher's least significance difference (LSD) test. DAI: Days after inoculation

Table 7: Mean J2 populations per 200 cc of soil in the field (season two)

	Mean J2/200 cc of soil			
Treatments	Initial J2	30 DAI	60 DAI	90 DAI
T. harzianum	500±124^{a}	674±142^{ab}	400.0±79^{b}	219.0±36^{b}
Bionematon®	290±65^{a}	423±42^{b}	280.0±21^{b}	185.0±16^{b}
P. lilacinum	460±125^{a}	619±118^{ab}	473.0±89^{b}	238.0±25^{b}
T. afroharzianum	555±96^{a}	836±83^{a}	568.0±62^{b}	407.0±44^{b}
Untreated control	545±114^{a}	848±146^{a}	1260.0±169^{a}	1646.0±235^{a}
LSD	362.65	341.72	292.23	339.98
p-value	0.5445	0.0977	0.0001	0.0001

Means followed by the same letter (s) within the same column are not significantly (p≥0.05) different according to Fisher's least significance difference (LSD) test. DAI: Days after inoculation

Table 8: Galling and egg mass indices on tomato in the field experiments

	RKN disease parameters on tomato			
	Field (season one)		Field (season two)	
Treatments	Galling index	Egg mass index	Galling index	Egg mass index
T. harzianum	1.90±0.19[b]	1.80±0.25[b]	1.90±0.19[b]	1.65±0.22[b]
Bionematon®	2.00±0.23[b]	1.95±0.22[b]	2.00±0.23[b]	1.90±0.22[b]
P. lilacinum	2.15±0.21[b]	2.15±0.22[b]	2.15±0.21[b]	1.95±0.18[b]
T. afroharzianum	2.20±0.27[b]	2.30±0.27 [ab]	2.20±0.21[b]	2.15±0.28[b]
Untreated control	3.15±0.20[a]	2.90±0.16[a]	3.10±0.18[a]	2.75±0.14[a]
LSD	0.619	0.640	0.609	0.589
p-value	0.0008	0.0107	0.0013	0.0055

Means followed by the same letter (s) within the same column are not significantly ($p \geq 0.05$) different according to Fisher's least significance difference (LSD) test

Table 9: Reproductive factor of RKN on tomato in field experiments

	Reproductive factor (RF)	
Treatments	Field (season one)	Field (season two)
T. harzianum	0.66±0.12[b]	0.59±0.15[b]
Bionematon®	0.83±0.21[b]	0.78±0.22[b]
P. lilacinum	0.65±0.10[b]	0.62±0.12[b]
T. afroharzianum	0.82±0.11[b]	0.78±0.12[b]
Untreated control	3.32±0.55[a]	3.34±0.55[a]
LSD	0.831	0.857
p-value	0.0001	0.0001

Means followed by the same letter (s) within the same column are not significantly ($p \geq 0.05$) different according to Fisher's least significance difference (LSD) test

The nematodes were noted to significantly ($p<0.05$) increase in the untreated control across the two seasons.

DISCUSSION

The results of this study demonstrated that application of *Trichoderma harzianum* and *Purpureocillium lilacinum* isolates enhanced plant growth through increased plant height and plant dry weight compared to the control in the greenhouse and field experiments. Ahmed and Monjil[21], also observed that *Purpureocillium lilacinum* increased plant height, number of leaves, root length and root dry weight of tomato plants in a pot experiment. Mukthar *et al.*[22] demonstrated increased shoot height and length of tomato plants through application of *Trichoderma harzianum* and *T. viride*. However, he observed a decreased root weight of tomato in a dose dependent manner which is in contrast with the current study findings.

Khan *et al.*[23] also recorded an enhancement in the growth and yield of eggplant with bio-control agents, *Pochonia chlamydosporia*, *P. lilacinus* and *T. harzianum* by suppression of galls formation. In another study, inoculation of tomato plants with *T. harzianum* was shown to improve shoot length, root length, dry shoot mass and dry root mass[24]. Lobna *et al.*[25] also reported that soil inoculation with *T. harzianum* resulted in a significantly greater tomato growth increase depicted by enhanced plant fresh shoot weight and plant length. Current findings also agreed with Ering and Simon[26] who reported that there was significant increase in growth and yield of tomato plants treated with *Trichoderma* isolates.

The significant differences observed in plant growth parameters of tomato plants due to application of local fungal isolates (*Trichoderma* spp. and *Purpureocillium* spp.) could be due to one or more mechanisms of the isolates. *Trichoderma* species can lead to increased growth due to improved nutrient intake, improved root growth, control of pathogens or by eliminating growth inhibitors from the soil[27]. *Trichoderma* spp. has proved to assist plants in tolerance to stress condition by enhanced root development[28]. It participates in solubilizing inorganic nutrients hence increased intake by the plants[29]. *Purpureocillium lilacinum* ability to suppress nematodes could have led to the increase in growth and yield of tomato. This hence indicates the yield increase was due to reduction in nematode multiplication.

The lower growth in untreated plants in the current study might be due to the stunting action of root knot nematodes and unavailability of nutrients to the plants. *Meloidogyne* spp. were observed to readily infect tomato, retarded the growth and lead to reduction in plant fresh and dry weights. The galls on the roots could also disturb the important root functions like uptake and transport of water and nutrients hence reduced growth[30]. Kankam and Adomako[31] reported a decrease in plant height and weight due to inoculation of

Meloidogyne J2 at 10 and12 weeks after transplanting. The infective stage second stage juveniles J2 penetrates through the root and migrates to a site near the vascular tissue which disrupts uptake of water and transportation of nutrients as reported by Hussey and Boerma[32].

The results obtained from this study showed that the indigenous isolates of *Trichoderma* species and *Purpure zocillium* species have some suppressive effect as nematode bio control agents. This was explained by reduced *Meloidogyne* spp. population densities in the soil and reduction of nematode damage parameter on tomato (i.e., gall and egg mass indices). Kalele *et al*.[33] reported that *Paecilomyces lilacinus* strain significantly reduced the juveniles' population in both soil and roots of tomato plants when fungal inoculation was done at planting. The fungal strain also reduced galling index and *Meloidogyne* sp. multiplication rate. *Paecilomyces lilacinus* was found effective in suppressing *M. javanica* by reduced root galling and egg mass production of the nematode[34].

The reduction in nematode galls and egg masses in the current study might be due to high rhizosphere competency of fungal isolates as they could easily colonize roots and hence reduce feeding sites for nematodes. The reduction in root gall number may also be due to the failure of most of the juveniles to penetrate the host roots. Bio control agents (*Trichoderma* spp.) have been reported to cause reduction in nematode populations due to secretion of enzymes such as chitinase, cellulose, protease and glucanase involved in pathogen's cell wall degradation[35]. The performance of *P. lilacinum* may be attributed to the main mode of action as egg parasitic[36] that attack nematode eggs in the soil and produce toxin fatal to the juveniles existing and those that may still hatch[37]. Chitinase activity has also been associated with *P. lilacinum* and has been reported to destroy the cuticle and kill the nematodes[38].

Trichoderma harzianum was found to be more effective in improving plant growth parameters and yield than *Purpureocillium lilacinum*.

CONCLUSION

In conclusion, the local isolates of *Trichoderma harzianum* and *Purpureocillium lilacinum* were able to enhance the growth and yield of tomatoes as well as control root knot nematodes. These isolates performed equally as the commercial product, Bionematon®. These isolates, therefore, are potential bio-control agents against root knot nematodes.

SIGNIFICANCE STATEMENTS

Tomato is a valuable vegetable sold in the Kenyan markets. However, tomato production is faced with various constraints along its value chain including pests and diseases. Among them, the nematodes parasitizing plants, *Meloidogyne* spp. that negatively influencing increased tomato production. Chemical nematicides have been used, however, concerns over chemical pesticides in respect of groundwater contamination, residues on food and development of resistance to pests have prompted research for safer management alternatives such as biological control.

Most of the research on the application of biological control methods has been conducted outside Africa. The present study therefore adds significant knowledge to the existing data on potential indigenous species in bio-control of root knot nematodes.

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