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Assessment of potential fungi in the management of potato cyst nematodes *Globodera* spp on potato in Nyandarua, Kenya

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ABSTRACT

Aim: The study was conducted to evaluate the effectiveness of selected indigenous fungi in managing potato cyst nematodes.
Materials and Methods: Experiments were carried out at Kwa Harakain. Treatments comprised of *Trichoderma atroviride*, *T. atrobrunneum*, *T. tomentosum* 1, *T. tomentosum* 2, *Purpureocillium lilacinum*, bionematon (commercial bionematicide) and untreated control. Randomized complete block design with four replicates was used. Treatments were applied as soil drenches at a rate of 6kg/ha at planting, 30 and 60 days after planting. Plant growth was assessed based on plant height, root length and crop biomass while nematode reproductive potential was based on juvenile count and reproduction factor. Data was subjected to one way Analysis of Variance using SAS software and means were separated using Least Significant Difference at P≤0.05.
Results: The fungal isolates significantly enhanced shoot height, root length, dry root and shoot weight and yield compared to the untreated control. The isolates significantly (P ≤ 0.05) reduced PCN juvenile population by 6 to 43% compared to the negative control in which juvenile population increased by 80 to 104%. Significant differences were observed in PCN reproduction factor (RF) with the maximum RF of 1.9 and 2.4 being obtained in the control during first and second season respectively. The least RF (0.42; 0.46) was recorded following application of *T. tomentosum* 2 during first and second season, respectively.
Conclusion: It was concluded that *T. atroviride* and *T. tomentosum* 2 can be adopted to suppress PCN in potato as part of their integrated management.

Keywords: *Globodera* spp, juvenile, reproduction factor, *Trichoderma*.

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Introduction

Horticulture production is very important in Kenya with potatoes being one of the key crops cultivated as an exotic vegetable (HCDA, 2016). In 2017, the area under potato production was 192,341 ha and its production was 1.52 million tons. The crop is ranked the second important food crop in the country after maize (FAOSTAT, 2019). It is a non-cereal crop rich in vitamin B6, potassium, copper, vitamin C, manganese, phosphorus, niacin, dietary fiber, and pantothenic acid (The World's Healthiest Foods, 2019). Potato is ideal as a food security crop because of its short growing period raging between 2-4 months depending on the variety (Kaguongo *et al*., 2014).

Potatoes are mainly sold as fresh produce although due to urbanization and increased demand of fries they are processed into different foodstuffs at the household or industrial level (Kaguongo *et al*., 2014). The production of potatoes in Kenya is constrained by both biotic and abiotic constraints. Potato cyst nematodes, *Globodera* spp. are among the biotic constraints. These nematodes are amongthe second most important plant parasitic nematodes in terms of economic significance after *Meloidogyne* spp. (Jones *et al*., 2013). They were detected in Kenya in 2014 in Nyandarua County(Mwangi *et al*., 2015) and a survey conducted in 2016 revealed that they are present in 71.8% of samples collected (Mburu *et al*., 2020) from potato growing areas thus a major threat to potato production. They are capable of causing severe losses in potato fields because they attack roots and have the ability to survive as cysts in soil for 20 years or more (Turner, 1996).

Management of PCN has been a major concern in many countries due to their ability to survive in form of cysts for a long period, their narrow host range and appearance of new species (Turner, 1996; Handoo *et al.,* 2012; Chitambo *et al*., 2019). Management of plant parasitic nematodes in Kenya has been majorly by use of synthetic nematicides. However, these are associated with risks such as environmental pollution and human health threat(Sarkar *et al*., 2021)and some pesticides such as aldicarb and methyl bromide have been banned for use in Kenya (PCPB, 2019). Thus there is an increasing interest in the development and adoption of environment friendly strategies for managing nematodes all over the world (Schneider *et al*., 2003).A number of PCN management options have been suggested including use of resistant potato varieties, crop rotation, biofumigation, fallowing use of nematicides and use of biological control agents (Trudgill *et al.,* 2014; Belair *et al*., 2016; Chitambo *et al*., 2019).

There has been growing interest in the use of bionematicides as a substitute for synthetic nematicides in managing plant parasitic nematodes. Different fungal species such as *Fusarium* spp., *Verticillium* spp. and *Trichoderma* spp. have demonstrated their potential in managing nematodes (Jacobs *et al*., 2003; Sharon *et al*., 2007; Pau *et al*., 2012). *Paecilomyces lilacinus* Thom and *V. chlamidosporium* Goddard were used successfully against *Globodera* spp. on potato (Jacobs *et al*., 2003). The current study investigated the potential of indigenous fungi from Kenya for the management of potato cyst nematodes on potato under field conditions.

Materials and Methods

Study area

The study was conducted at Kwa Haraka, Nyandarua County, Kenya. The area is located at 0°46'14" S and 36°35'26" E at 2,603 meters above sea level in the Upper Highland 1 agro ecological zone. Rainfall pattern in the area is bimodal with an average of 1542 mm per annum. The soil type in the study area was sandy clay loam with a pH of 4.2. The field was naturally infested with potato cyst nematodes.

Preparation of microorganisms used in the study

The fungal isolates used in this study were isolated from cysts of potato cyst nematodes collected from major potato growing areas in Kenya between March and May 2017. Fungi were isolated by plating cysts directly on Potato dextrose agar (PDA). Fungi were grown on PDA in petri dishes at 25±2°C for 7 days. The isolates were mass multiplied using sterilized sorghum grains for 14 days. The sorghum fungal mixture was air dried, ground and then formulated using talc powder as a carrier in Kenyatta University agricultural laboratory.

Experimental design

The experiment consisted of six treatments namely; *T. atroviride, T. tomentosum* 1Bisset, *T. tomentosum* 2, *T. atrobrunneum* Rocha, Chaverri and Jaklitsch, *P. lilacinum,* Bionematon-(Commercial *P. lilacinus*) as a positive control and an untreated control (negative control). The treatments were arranged in a Randomized Complete Block Design (RCBD) in plots measuring 3 m by 4 m and replicated four times. Experiments were set for two seasons between June and Sept 2018, and Nov 2018 to Feb 2019. Potato tubers (cultivar Shangi) were obtained from Kenya Agricultural and Livestock Research Organization (KALRO) Tigoni and were planted at a spacing of 30 by 75 cm and treatments applied at a rate of 6 Kg/ha at planting, 30 and 60 days after planting. Manual weeding was carried out every month and experiments were terminated after 12 weeks.

Data collection: Data on plant height (cm), root length (cm), dry root and shoot weights (g) were determined 56 days after planting (onset of flowering). Plant height of 15 plants per plot was measured using a tape measure from the soil line to the apex of the longest shoot while root length of 5 plants per plot was measured using a ruler. Five shoots and roots per plot were oven dried at 70°C for 3 days to determine dry weight. Data on nematode population was also collected. Soil sampling was done before planting to determine initial nematode populations and at the end to determine final nematode population. Soils were randomly collected from 5 points in each plot using a soil auger (15 cm depth) and mixed to get a composite sample. Five hundred grams of soil was taken to lab for nematode extraction. Juveniles were extracted from 200 cc of soil using modified Baermann technique (Hooper *et al*. 2005) then counted under a compound microscope using a counting dish. Cysts were extracted from 200 cc of soil using Fenwick can method (Fenwick, 1940) and counted using a dissecting microscope. Reproduction of nematodes was determined by calculating the

reproduction factor (RF) using the following formula:

RF= $\frac{final\ cyst\ population}{initial\ cyst\ population}$(Trifonova *et al*., 2014).

Data analysis

Data on plant height, root length, dry shoot and root weight, potato yield and number of nematodes were subjected to one way Analysis of Variance (ANOVA) using Statistical Analysis Systems (SAS) software Version 9.2. The means obtained were separated using Fisher's Least Significant Difference at 95% confidence interval.

Results and Discussion

Effects of fungal isolates on potato growth and yield:

The fungal isolates significantly (P<0.05) improved potato growth in terms of shoot height, root length, dry shoot and root mass compared to the untreated control. In both seasons, maximum shoot height of 19 and 20.9, root length of 15.6 and 17.1 and dry shoot weight of 18.7 and 21.2 was recorded in plants treated with *T. atroviride* while plants treated with *T. tomentosum* 1 had the heaviest dry roots of 3.3 and 3.6 (Table 1). The control plots recorded the minimum growth parameters. Yield of potato plants was significantly (df = 6, 21; P < 0.05) improved by application of the fungal isolates in comparison to the untreated control in both experiments (Figure 1). The highest yield was obtained in plots applied with *T. tomentosum 2*(6.94t/ha; 8.194 t/ha) followed by *T. atroviride* (6.27 t/ha; 7.52 t/ha) and no significant differences were observed between these isolates. There were no significant (P<0.05) differences observed in yield of plants treated with *T. atrobrunneum*, *P. lilacinum* and bionematon although they varied slightly (Fig 1). In untreated plots, the least potato yield (0.89 t/ha; 2.14 t/ha) were recorded in both seasons, respectively which differed significantly (df = 6, 21; P< 0.05) from yield obtained in plots treated with *T. tomentosum* 2 and *T. atroviride*.

Effect of fungal isolates in suppressing potato cyst nematodes during season one (June to Sept 2018) and season two (Nov 2018 to Feb 2019)

In both the first and the second season, fungal isolates significantly (F= 70.36;df= 6, 27; P ≤ 0.05) reduced juvenile population densities compared to the control while in the control plots juvenile population increased (Fig 2). In the first season ajuvenile population increase of 80 % was recorded in the control plots while *T. tomentosum* 2 and *T. atroviride* reduced J2 populations by 36% and 32%, respectively. There was a significant (P<0.05) difference observed in juvenile reductions by *T. tomentosum* 2 and *T. atroviride* compared with *T. tomentosum* 1, *T. atrobrunneum*, *P. lilacinum* and bionematon. Similar observations were observed in the second season with an increase of 104% in juveniles observed in the control while *T. tomentosum* 2 reduced juvenile population by 43% and *T. atroviride* by 30 %.

Table 1. Mean growth parameters of potato plants treated with different fungal isolates during season 1 (Jun –Sep 2018) and season 2 (Nov 2018- Feb 2019)

	Season 1- Jun to Sept 2018				Season 2- Nov 2018 to Feb 2019			
Treatment	**Shoot height(cm)**	**Root length (cm)**	**Dry shoot weight(g)**	**Dry root weight (g)**	**Shoot height (cm)**	**Root length (cm)**	**Dry shoot weight (g)**	**Dry root weight (g)**
Trichoderma tomentosum 1	16.4±0.8b	13.7±0.5b	17.1±2.2a	3.3±0.6a	20.3±0.7ab	14.9±0.6b	20.1±2.2a	3.6±0.6a
Trichodermato mentosum 2	16.6±0.7b	14.7±0.5ab	16.2±1.2a	3.1±0.2a	20.0±0.7ab	15.9±0.6ab	20.5±1.7a	3.1±0.4a
Trichodermaatr obrunneum	16.5±0.9b	13.4±0.5b	15.3±1.8a	2.7±0.4ab	18.9±0.7b	15.2±0.8b	18.1±1.9a	3.0±0.4a
Trichodermaatr oviride	19.0±0.8a	15.6±0.5a	18.7±2.7a	3.0±0.3a	20.9±0.7a	17.1±0.6a	21.2±2.7a	3.0±0.3a
Purpureocilliu mlilacinum	16.1±0.6b	13.5±0.8b	17.1±3.0a	2.6±0.5ab	19.3±0.7ab	14.4±0.8b	19.7±3.1a	2.6±0.5ab
Untreated	11.7±0.7c	11.4±0.5c	7.5±1.0b	1.8±0.4b	15.4±0.7c	12.6±0.5c	9.6±1.0b	1.8±0.3b
Bionematon-*P. lilacinus*	16.5±0.8b	14.7±0.5ab	16.9±1.8a	3.0±0.2a	19.5±0.6ab	15.9±0.6ab	17.5±1.8a	2.9±0.2a
LSD	2.14	1.53	5.93	1.13	1.90	1.79	6.03	1.13
CV (%)	21.29	17.68	60.98	64.83	27.65	18.91	53.25	63.42
P-Value	<0.0001	<0.0001	0.0083	0.1641	<0.0001	0.0002	0.0038	0.0954

Means followed by the same letters within the same column are not significantly (P>0.05) different according to Fisher's LSD test

Table 2. Mean reproduction factor (RF) of potato cyst nematodes during season 1 (Jun to Sep 2018) and season 2 (Nov 2018 to Feb 2019)

Treatment	RF, Season 1	RF, Season 2
Trichoderma tomentosum 1	0.85±0.02bc	0.83±0.02b
Trichodermatomentosum 2	0.49±0.04d	0.46±0.04c
Trichodermaatrobrunneum	0.91±0.03b	0.94±0.03b
Trichoderma atroviride	0.68±0.01cd	0.70±0.01bc
Purpureocilliumlilacinum	0.82±0.05bc	0.73±0.10bc
Untreated control	1.94±0.17a	2.42±0.21a
Bionematon- *Paecilomyces lilacinus*	0.82±0.02c	0.80±0.01b
LSD	0.21	0.27
CV (%)	15.29	18.51
P- Value	<0.0001	<0.0001

Means followed by the same letters within the same column are not significantly (P>0.05) different according to Fisher's LSD test.

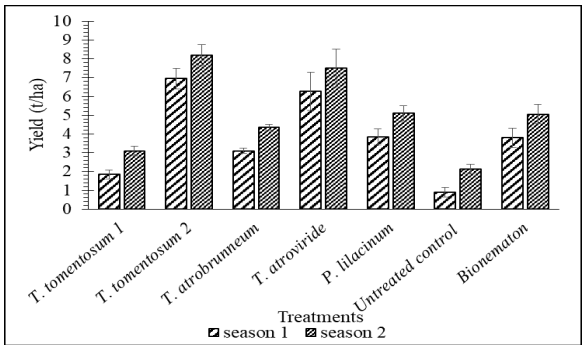

Fig.1 : Yield and treatments of season 1 and 2

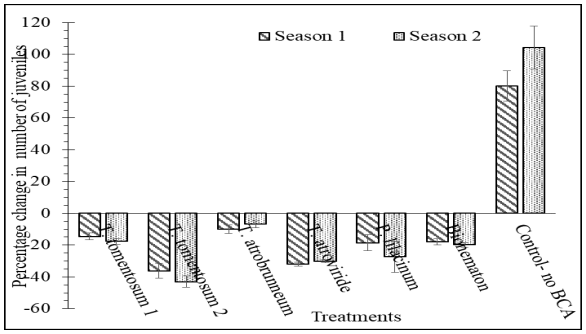

Fig. 2 : Juvenile population densities and treatments

Trichoderma tomentosum 2 recorded the least Reproduction factor (RF) of 0.49 and 0.46 in season 1 and season 2, respectively. These differed significantly (P<0.05) from RF recorded after application of Bionematon, *T. tomentosum* 1, *T atrobrunneum* and the control. The control had the highest RF of 1.94 and 2.42 in season 1 and season 2, respectively (Table 2). The RF in the control were significantly (P<0.05) higher compared to those obtained following application of the fungal agents in both seasons.

The fungal isolates used in the current study enhanced potato growth and yield indicating their growth promoting effects. In addition, they reduced population of second stage juveniles in soils and multiplication of PCN. These were comparable with Bionematon which is a bionematicide for managing PCN. Earlier results have demonstrated the ability of fungi to improve plant growth. In a study by Colla *et al*. (2015), inoculation of tomato with *T. atroviride* MUCL 45632 enhanced dry shoot weight by 68%. Contreras-Cornejo *et al*. (2009), found that inoculating mouse-ear cress *Arabidopsis thaliana*(L.). seedlings with *T. virens* and *T. atroviride* increased root and shoot biomass production through prolific formation of lateral roots. In other studies, *P. lilacinum* reduced *Meloidogyne* spp. populations and improved growth parameters of tomato, cucumber and eggplant (Kalele *et al*., 2010; Ganaie and Khan, 2010; Abbas *et al*., 2016). Application of *P. lilacinus* (2x106 cfu/ gm) @ 50g/m2 in nursery bed + *P. lilacinus* @ 5kg/ha along with 2.5 tons of Farm yard manure (FYM)/ha improved the plant growth and recorded the lowest gall formation of 60.3- 65.4% in tomato caused by *M. incognita* (Wagh and Peamanik, 2014). Our results conform to those of Hajji et al. (2017), who observed that *P. lilacinum* significantly decreased the development of PCN in roots and soil by 76% and 61%. The ability of fungi to promote plant growth is attributed to production of plant hormones such as auxins, cytokinins, and gibberellins (Zhang *et al*., 2006; Aly *et al*., 2011; Redman *et al*., 2011). According to Khalil, 2013, inoculating tomato plants with *P. lilacinum* increased root length by 57 % as well as reducing galling by *M. incognita* by 58.58% and egg masses upto 68.18%.

Earlier reports have indicated the ability of *Trichoderma* species to effectively suppress plant parasitic nematodes (Sahebani and Hadavi, 2008; Yang *et al*., 2010). Khalil *et al*. 2012, evaluated nematicidal activity of *P. lilacinus* against *M. incognita* under greenhouse conditions and observed that it was effective in reducing gall formation and egg mass by 88.23% and 76.94% respectively. It was an agreement with the results of the current study in which the fungi used reduced potato cyst nematode population in soil. Antagonistic fungi suppress nematodes through production of enzymes and secondary metabolites (Al Ajrami, 2016; Abbas *et al*., 2016). *Purpureocillium lilacinum* is associated with production of antibiotics such as lilacin andleucinotoxins and enzymes including

chitinases, and proteases (Park *et al*., 2004). *Trichoderma* spp. secrete trichodermin, dermadin, trichoviridin, and sesquiterpene heptalic acid antibiotics and enzymes such as chitinase, glucanases, and proteases (Askary and Martinneli, 2015). Fungi also attack nematodes through direct parasitism (Hallman *et al*., 2009). These mechanisms could have reduced PCN invasion and consequent infection of the roots hence the reduced juvenile population and PCN multiplication.

Conclusion

It was concluded that the application of *T. atroviride* and *T. tomentosum* 2 enhanced potato growth and yield, and reduced potato cyst nematode population hence have the potential of managing potato cyst nematodes. These can be incorporated in integrated pest management (IPM) strategies of potato cyst nematodes thus increasing potato production, reduce cost of production, pollution and human health.

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