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Research Application Summary

Indigenous fungi with antagonistic potential against potato cyst nematodes in Kenya

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Abstract

Potato (*Solanum tuberosum*), which is the second most important food crop in Kenya, has various production constraints including potato cyst nematodes (PCN). This nematode which was first reported in 2014 is now ranked highest. The main option for managing nematode pests is use of synthetic nematicides but their usage is being phased out due to environmental risks, human health concerns, availability and cost. Thus, the need to develop safe, affordable and effective management strategies is very critical. Management of cyst nematodes using biological control agents is a viable alternative. The objective of the study was therefore to test the efficacy of indigenous nematophagous fungi against potato cyst nematodes in Kenya. Potato cyst nematodes were extracted by floatation from soil samples collected from major potato growing areas. Fungi were isolated from cysts by plating them on Potato Dextrose Agar (PDA). The effects of fungal suspensions on juvenile mortality was then determined. Completely Randomized Design (CRD) was used for the in vitro tests. Data were subjected to Analysis of Variance (ANOVA) using SAS version 9.1. Means were separated using Fisher's protected Least Significance Difference (LSD) test at Pd"0.05. Fungi in nine genera were found associated with PCN; *Trichoderma sp, Aspergillus sp, Fusarium sp, Penicillium sp, Paecilomyces sp, Acremonium sp, Chaetomium sp, Verticillium sp* and *Rhizopus sp*. There was a significant isolate effects ($P<0.05$) on juvenile mortality. Juveniles treated with *Paecilomyces* isolate 1, *Trichoderma* isolates 1, 2 and 3 resulted in the highest mortality rates of 98-100%. This demonstrated that *Trichoderma sp*, and *Paecilomyces sp* are potential biocontrol agents of PCN.

Key words: Biological control agents, Cyst nematodes, juvenile mortality, *Solanum tuberosum*

Résumé

La pomme de terre (*Solanum tuberosum*), qui est la deuxième culture vivrière la plus importante au Kenya, a diverses contraintes de production, y compris les nématodes à kystes de la pomme de terre (NPC). Ce nématode qui a été signalé pour la première fois en 2014 est maintenant le mieux classé. La principale option pour lutter contre les nématodes ravageurs est l'utilisation de nématicides synthétiques, mais leur utilisation est progressivement supprimée en raison des risques environnementaux, des problèmes de santé humaine, de la disponibilité et du coût. Ainsi, la nécessité de développer des stratégies de gestion sûres, abordables et efficaces est très critique. La gestion des nématodes à kystes à l'aide d'agents de lutte biologique est une alternative viable. L'objectif de l'étude était donc de tester l'efficacité des champignons nématophages indigènes contre les nématodes à kystes de la pomme de terre au Kenya. Les nématodes à kystes de la pomme de terre ont été extraits par flottation d'échantillons de sol prélevés dans les principales zones de culture de la pomme de terre. Les champignons ont été isolés des kystes en les plaçant sur de la gélose au dextrose de pomme de terre (PDA). Les effets des suspensions fongiques sur la mortalité juvénile ont ensuite été déterminés. Une conception entièrement aléatoire (CRD) a été utilisée pour les tests in vitro. Les données ont été soumises à une analyse de variance (ANOVA) en utilisant SAS version 9.1. Les moyennes ont été séparées en utilisant le test protégé de la différence de moindre signification (LSD) de Fisher à Pd « 0,05. Les champignons de neuf genres ont été trouvés associés au NPC ; *Trichoderma sp, Aspergillus sp, Fusarium sp, Penicillium sp, Paecilomyces sp, Acremonium sp, Chaetomium sp, Verticillium sp* et *Rhizopus sp*. Il y avait un effet isolat significatif (P <0,05) sur la mortalité juvénile. Les juvéniles traités avec l'isolat 1 de Paecilomyces, les isolés 1, 2 et 3 de *Trichoderma* ont donné les taux de mortalité les plus élevés de 98 à 100%. Cela a démontré que *Trichoderma sp* et *Paecilomyces sp* sont des agents potentiels de lutte biologique contre le PCN.

Mots clés : agents de lutte biologique, nématodes à kystes, mortalité juvénile, *Solanum tuberosum*

Introduction

In Kenya, potato (*Solanum tuberosum*) is the second important food crop after maize and is grown in high altitude areas where maize production is not preferred (Janssens *et al*., 2013). The potato industry in Kenya contributes to food security, poverty eradication and economic development. Potatoes are grown by approximately 800,000 small scale farmers in Kenya employing 2.7 million people and contributing about 50 billion shillings annually to the economy. Its production is however limited by abiotic and biotic constraints which include lack of certified seeds, pests and diseases (Riungu, 2011). Thus, Kenya produces less than 10 tonnes per hectare compared to a potential production of 40 tons per hectare (NPCK, 2017). Among the production constraints, diseases are the most important. According to Kaguongo *et al*. (2010) bacterial wilt is common in most areas of Kenya affecting 77% of potato farmers. Other important diseases are late blight (67%) and the virus complex (12%). According to Khurana and Garg (2003), commercially sold tubers are frequently infected by potato virus X and potato leaf roll virus.

The major nematodes attacking potato are potato cyst nematodes (PCN) (Globodera sp.). This nematode is among the recently reported pests in Kenya (Mwangi *et al*., 2015) and has rapidly spread to most of the potato growing areas. A survey done in 20 counties in 2016 showed that the pest was present in all potato growing areas with prevalence ranging from 53% to 100% and an average of 82% (Solveig Haukeland , unpublished work). There are two common species of PCN, namely, *Globodera rostochiensis* and *G. pallida* which are highly regulated in the world due to their economic significance and are readily spread in infested tubers or soil on potato tubers (Dandurand, 2017). They limit root functioning by decreasing soil volume that can be explored by plants for water and nutrient absorption leading to decreased size and number of tubers (Aires *et al*., 2009).

Repeated growing of potato in fields infested with the nematodes can cause up to 80% yield loss (Globodera Alliance Newsletter, 2015). The common approach for managing nematodes in Kenya is the use of synthetic nematicides despite their adverse effect. Therefore, it is important to develop management strategies that are safe, affordable and effective against the pest. Among the alternatives is the use of biological control agents which include nematophagous fungi for managing plant parasitic nematodes. The biological control potential of fungi and bacteria against PPNs has been well demonstrated (Cumagun and Moosavi, 2015). Nematophagous fungi include more than 200 species of taxonomically different fungi (Pandit and Kunjandia, 2014). It is also important to develop control measures for managing PCN in Kenya because it has just been reported hence scanty information is known.

Material and methods

Soil samples were randomly collected using a soil auger in March and April 2017 from potato farms in Nyandarua, Nakuru, Nyeri, Nandi, Kericho, Kiambu and Meru Counties which are major potato growing areas in Kenya. Cysts of potato cyst nematodes were extracted from the soils based on floatation technique (OEPP/EPPO,2013). Soil samples were air dried in trays for 24 hours at 23^0C. Then 200g of the dry soil was added to one litre of water in a beaker and stirred. The mixture was allowed to settle till the water became clearer. The suspension contained the floating organic debris and cysts and was decanted into a funnel with a Whatman No. 4 filter paper. Cysts adhered on the filter paper and were picked using a mounting pin and stored in 1.5ml Eppendorf tubes at ambient room temperatures of 23 ± 2^oC.

Fungi were isolated from cysts by plating cysts on potato dextrose agar, incubating for seven days and identifying fungi showing clear zones of inhibition against others. Such fungi were sub cultured on potato dextrose agar (PDA) to obtain pure cultures. Slant cultures were prepared for preserving these isolates. These were then identified based on morphological characteristics (Watanabe, 2010).

To determine the bioactivity of the isolates on PCN juveniles, 20 second stage juveniles (J2S) were placed in 10 ml fungal spore suspension in three replicates. Juveniles were obtained by placing cysts on potato root diffusate for hatching. The diffusate was prepared by placing five three weeks old potato roots in 200 ml of sterile distilled water for five hours. Suspensions of 14 days old fungal isolates were prepared by flooding the surface of fungal cultures with sterile distilled water and scraping the aerial mycelium with a sterile glass slide. Experiments were arranged in completely randomized design and mortality determined after seven days. Dead juveniles were confirmed by probing with a mounting pin and actively moving juveniles were considered alive. Fungal isolates with highest mortality were regarded potential candidates for managing potato cyst nematodes.

Results and discussion

Eighty fungi were isolated from cysts extracted from soil samples collected from the major potato growing areas in Kenya. The isolates belonged to the following genera *Trichoderma sp. Aspergillus sp., Fusarium sp.,Penicillium sp., Paecilomyces sp.,Acremonium sp.,Chaetomium sp.,Verticillium sp.* and *Rhizopus sp*. The most abundant genera was *Trichoderma* with a frequency of 24 followed by *Fusarium* (16) and the least abundant were *Acremonium, Verticilium* and *Rhizopus* with a frequency of 2. The highest number of *Trichoderma* species observed could be due to the diverse number of existing members of the general and their competitive ability. According to Devrajan *et al*. (2011) similar genera were found to be associated with cysts from Nilgiris district. *Trichoderma sp., Paecilomyces sp., Vericillium sp., Fusarium sp.* and *Aspergillus sp.* were identified as PCN cyst parasites. In addition, seven fungi, *C. globosum, F. oxysporum, F. solani, F. tricinctum, M. bolleyi, P. lilacinum,* and *P. cucumerina* were isolated from *G. pallida* cysts collected from Shelley Idaho, USA (Kooliyottil *et al*., 2017).

The effect of the various isolates on juvenile mortality differed significantly as shown in Table 1. Means were separated using Fischer's protected Least Significance Difference (LSD) test at 95% level of significance. Juvenile mortality above 50 % was observed in most *Trichoderma, Penicillium* and *Paecilomyces* species indicating their potentiality in maintaining potato cyst nematode populations below the economic thresholds. Hussain *et al*. (2017) reported that *Trichoderma harzianum* and *T. hamatum* caused juvenile mortality of root knot nematodes hence could be used for their management.

Therefore, the isolates from this study associated with juvenile mortality can be used for managing potato cyst nematodes. In particular *P. lilacinus* causes substantial egg deformation in *M. incognita* and these deformed eggs never mature or hatch. There was overall significant isolate effect on juvenile mortality. This difference can be associated with the varying genera and species of the isolates which can influence their mechanisms of action. Fungal modes of action include antibiosis, nutrient competition, direct contact and production of cell wall degrading enzymes. For instance *Trichoderma* species is known to release toxic compounds and lytic enzymes mainly chitinases, glucanases and proteases.

Conclusions

Potato cyst nematodes are associated with a diverse group of fungi which can be exploited in their management. Most of the fungi led to juvenile mortality hence could reduce nematode populations in farmers' fields. Therefore when incorporated as biocontrol agents for managing PCN can lead to reduced usage of synthetic nematicides and increased potato yield.

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Table 1. Juvenile mortality of fungal Cyst Nematodes caused by fungal isolates from different Counties in Kenya

Isolate	County	Genus	Mean mortality	± SE
NY9C	Nyandarua	*Paecilomyces*	100	±0.00a
NYA3C	Nyandarua	*Trichoderma*	98.33	± 1.67a
KE S2	Kericho	*Trichoderma*	98.33	± 1.67a
KE S1	Kericho	*Aspergillus*	95.00	± 5.00ab
NYES	Nyeri	*Trichoderma*	98.33	± 1.67a
ME S2	Meru	*Trichoderma*	100	±0.00a
KB S2	Kiambu	*Fusarium*	73.33	± 26.667abcd
NK S2	Nakuru	*Trichoderma*	85.00	± 5.77abc
NYAC	Nyandarua	*Penicillium*	80.00	± 8.66abcd
BG S1A	Bungoma	*Trichoderma*	85.00	± 2.89abc
NYES1	Nyeri	*Trichoderma*	73.33	± 12.02abcd
BGS1	Bungoma	*Trichoderma*	80.00	± 2.89abcd
ND S2	Nandi	*Penicillium*	68.33	± 13.02abcd
NY9C4	Nyandarua	*Fusarium*	73.33	± 6.67abcd
NY93C	Nyandarua	*Paecilomyces*	65.00	± 8.66cdef
NYC3	Nyandarua	*Penicillium*	61.67	± 10.14cdefg
BGS1B	Bungoma	*Trichoderma*	60.00	± 7.64cdefgh
NYES1B	Nyeri	*Trichoderma*	55.00	± 2.88defghi
Control (water)			0.00	± 0.00n

Means attached with different letters differ significantly ($P<0.05$)

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