Biodiversity and efficacy of fungal isolates associated with Kenyan populations of potato cyst nematode (*Globodera* spp.)

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Abstract

Potato Cyst Nematodes (PCN) (*Globodera rostochiensis* and *G. pallida* Woll.) are quarantine pests of potato (*Solanum tuberosum* L.) worldwide capable of causing significant yield loss and difficult to manage with conventional methods. The study explored the diversity of antagonistic fungi associated with PCN obtained from soil samples collected in Nyandarua and Nakuru Counties in Kenya and their effect on PCN egg viability and hatching was also evaluated. Twelve fungal isolates from five genera were isolated and characterized using morphological and molecular techniques. The twelve isolates were *Trichoderma asperellum* (4), *T. hamatum*, *T. breve*, *T. atrobruneum*, *Amanita basiorubra*, *Setophoma terrestris* (2), *Penicillium chrysogenum* and *Clonostachys rosea*. The most abundant isolate was *Trichoderma* spp. with 58% occurrence. The effect of seven of the isolates on PCN eggs showed that *T. breve* and *P. chrysogenum* reduced egg viability by 41% and 34%, respectively while *T. asperellum* and *T. breve* reduced their hatching by 50% on average. *Trichoderma atrobrunneum*, *T. hamatum*, and *A. basiorubra* also reduced the PCN egg viability by 27% on average. These fungal isolates could provide a potential tool for PCN management in potato production systems for improved yields. However, further studies are warranted to validate these findings under greenhouse and field conditions. A more comprehensive bioprospecting survey for PCN associated antagonistic fungi needs to be extended to other potato growing regions to explore further cyst pathogens.

Keywords:

Solanum tuberosum, Biocontrol, nematode antagonistic fungi, PCN reproductive traits

1. Introduction

Potato (*Solanum tuberosum*) is the second most important staple crop after maize in Kenya and plays a major role in national food and nutritional security (Gitari et al., 2018). Kenya ranks as the 3rd and 1st largest for potato production in Africa and East Africa, respectively (Gildemacher et al 2009; FAOSTAT 2020). Its ability to grow in high altitude areas (1,500-3,000 meters above sea level) under rain-fed conditions where maize does not do well makes it a valuable food crop (Janssens et al., 2013). An estimate of 800,000 farmers cultivate about 161,000 hectares of potato in Kenya per season, with an annual production of about 3 million tonnes in two growing seasons (Muthoni et al., 2017; GIZ 2018; MoALF, 2016).

Kenya earns 50 billion Kenyan shillings (USD 500 million) at farm gate prices annually from potato production (GIZ, 2018; MoALF, 2016). Beyond the farm, about 3.3 million people in Kenya are employed along the potato value chain as input suppliers, traders, transporters, processors, vendors, and exporters (GIZ-PSDA Kenya, 2011; MoALF, 2016). Consequently, potato contributes to poverty alleviation through income generation and unemployment reduction in both urban and rural households.

The most representative potato production regions are concentrated in the former Central, Eastern, and Rift valley provinces. These are areas surrounding Mt. Elgon, Mau escarpment, the Aberdare Range, the edges of the Rift valley, and the slopes of Mt. Kenya (MoA, 2008). Mt. Kenya region mainly comprises of Meru Central and parts of Nyeri and Laikipia counties. Aberdares and Eastern Rift valley mainly include Nyandarua and parts of Nyeri, Kiambu, and Nakuru counties. Mau region comprising Bomet, Kericho, Narok, and parts of Nakuru County. Mt. Elgon, comprising Elgeyo Marakwet, Bungoma, West Pokot, Trans Nzoia, and Uasin Gishu counties. Taita hills in Taita Taveta county is another key highland production zone in the southern border of Kenya and Tanzania (Kaguongo, 2010). Other emerging potato growing areas include Nandi, Baringo, Laikipia, Nyamira, and Kisii counties. Due to increased demand,

potato production has expanded to non-traditional potato growing areas such as Kirinyaga, Naivasha, and Tana River (Figure 1), because of irrigation facilities' availability (MoALF, 2016). The former Central province produces over 37% of the national potato yields, followed by Rift valley province (27%) and Eastern province (19%) (MoA, 2008; FAO, 2013).

Despite the rising acreage under potato production in Kenya (Gitari *et al*., 2018), the yield has remained low at 10 tonnes/ha (FAOSTAT, 2020; Devaux *et al*., 2014). Changes in productivity and suitability of the current potato crop are linked to various factors, including lack of good agricultural practices (GAP), poor seed quality, diseases such as bacterial wilt, and pests such as nematodes (Mburu et al 2020, Mburu et al., 2018; Muthoni et al., 2017; MoALF, 2016; Mwangi et al., 2015, Muthoni & Nyamongo 2009).

Several nematode species have been reported associated with potato. Among these, the potato cyst nematodes (PCN), *Globodera rostochiensis*, and *Globodera pallida* are two of the major species limiting potato yield in various parts of the world, leading to up to 80% and sometimes total crop failure (Mburu et al 2020; Kaczmarek et al., 2019; Mburu et al 2018; Lima et al., 2018; Bohlmann, 2015; Turner & Rowe 2006). These are quarantine pests in about 100 countries worldwide (EPPO 2017). *Globodera* species are sedentary endoparasites of the potato root system, deteriorating the quality and commercial value of tubers and contributing to infections of potatoes by other opportunistic plant pathogens, such as fungi (Lavrova et al., 2017).

Like the potato, PCN species originated from the Andes region in southern Peru and have spread due to anthropogenic activity into many areas of the world (Grenier et al., 2010; Nicol et al., 2011). PCN has been reported throughout Europe, South America, and parts of Asia, North America, Oceania, and Africa, where potatoes are grown (EPPO, 2017). However, it has been reported in only a few countries in Africa. Additionally, Africa's economic estimates on PCN losses have not been reliably established (Mburu et al., 2020; Coyne et al., 2018).

In Kenya, PCN was first reported in significant potato growing areas (Mburu et al., 2020; Ochola et al., 2020; Mburu *et al.,* 2018; Mwangi et al., 2015). However, little is known about the origin and the distribution of this pest in Kenya. Information on pest populations' spatial dynamics is indispensable in integrated pest management (IPM) systems, as it raises considerable interest among plant breeders and plant pathologists for the need to understand better the interactions between pest or pathogen and host, and to estimate the risk of crop damage. Since human activity is the most probable means of spreading PCN, there is a specific interest in evaluating the implemented control measures and their consequences in adopting more effective management practices. Controlling PCN is a difficult task due to its high adaptation to the environment (Eberlein et al., 2016). Solanaceae is the main host plant family, and root exudates from these crops trigger hatchability of the cysts' eggs (Ochola et al., 2020). PCN cysts have prolonged viability in the soil in the form of encysted eggs in the host crop's absence during crop rotation (Deliopoulos et al., 2011). High persistence is due to their lower spontaneous hatchability, and that they hatch over a longer period (Deliopoulos et al., 2011; Fiers et al., 2012; López-Lima et al., 2020) persisting generally for more than 30 years, which reduces crop rotation efficiency. On the other hand, the use of resistant varieties has been a challenge due to a possible selection pressure caused by a shift in nematode virulence (Ebi et al., 2015), leading to partial failure (Schuten & Berniers, 1997; Evans & Haydock, 2000). Besides, most preferred potato varieties by consumers in Kenya are susceptible to PCN (Ochola et al., 2020). Therefore, cultural management strategies like crop rotation and resistant varieties are extremely challenging due to limited land and specialised intensive potato producers (Contina et al., 2017; Eberlein et al., 2016).

Chemical nematicides such as fluopyram and abamectin are available and registered for use in Kenya and might be helpful when used correctly for nematode control, i.e., to bring down high levels of infestation. However, their high cost and PCN devastating damage has made some

farmers give up on potato farming. Nematicides are also toxic to both users and the environment, when used incorrectly. Biological control is an alternative strategy that can be included as a component in integrated nematode management. Fungal biocontrol strategies have been proved effective elsewhere (Collinge et al., 2019; Holgado & Magnusson, 2010; Indarti et al., 2010) in other parts of the world where PCN has been reported but limited information is available for Kenyan conditions (Ochola et al., 2020). There are few fungal-based biocontrol products targeted at plant parasitic nematodes that are available in the Kenyan markets, however, only one product (BIONEMATONE 1.15% WP, ai: *Purpureocillium lilacinum*, Osho Chemical company) is registered for PCN control. Due to this limited number of available fungal based bioproducts and in the prospect of boosting the portfolio of biopesticides for PCN management, bioprospecting is needed to identify locally available fungal species that are associated with PCN and relevant for controlling the pest under Kenyan conditions. The aim of this study was to assess the biodiversity of naturally occurring fungi associated with PCN populations in some selected key potato production areas of Kenya and test their efficacy *in vitro* as a first step for microbial-based biopesticide development against PCN.

2.0 Materials and Methods

2.1 Study sites

The study was carried out in two counties, Nakuru (in Molo, Njoro, and Kuresoi North Sub-counties) and Nyandarua (in Kipipiri and Kinangop Sub-counties) (Figure 2). Nakuru county lies at a latitude of 0^0 16'59.99" N and longitude 36^0 04'0.01" E and the altitude of the farms that were sampled ranged from 1800m to 2200m above sea level. It has an average temperature of 17.5^{o}C and precipitation of 762mm annually. Nyandarua county lies at a latitude of 0^0 32'59.99" N and longitude 36^0 36'59.99" E and the farms from which sampling was done stood

at an altitude of 2200m to 2600m above sea level. It has an average temperature of 12.9^{0}C, average precipitation of 1304mm annually. Potato was the major crop cultivated in the selected farms for at least the last ten years at the time of sampling. The main potato varieties grown were Shangi, Dutch Robijyn, Manitou, Kenya Baraka, and Sherekea, with Shangi being the most preferred variety. Other rotation crops reported included cabbage, peas, snow peas, maize, wheat, tomatoes, oat, and sugar snaps, with the rotation duration ranging from one to two seasons. These counties were selected since they both are among the major potato producing counties (Figure 1).

2.2 Soil sampling

The soil was sampled from small scale potato farmers in both Nyandarua and Nakuru counties between 12th to 16th August 2020. Sample sites were selected using a multistage sampling procedure (Kothari, 2004). Farms that had potato crop at the sampling time were considered a sampling unit and were determined using a simple random sampling technique. Soil sampling per farm was systematically conducted using the 'W' pattern at a depth of 15 - 20 cm using a hand trowel. Thirty sub-samples (scoops) were collected per acre. Samples were combined to make one composite sample of approximately 1.5kg per field. The soil samples were stored in closed paper bags in a cool box for transportation to the laboratory. The samples were analysed soon after arrival at the laboratory.

2.3 Extraction of PCN cysts

The soil samples were air-dried, and PCN cysts extracted using the Fenwick can floatation method (Bellvert *et al*., 2008). The cysts were collected onto a labelled milk filter paper and left to air dry overnight on the shelf at room temperature. The extracted cysts were hand-picked by an entomological forceps and morphologically identified using the EPPO (2017) taxonomic

guide. Cysts that were positively identified as *Globodera* spp were counted under the Leica MZ12.5 dissecting microscope and stored in labelled Eppendorf tubes at room temperature.

2.4 Isolation of fungi from PCN cysts

About 18 cysts of PCN from each farm were placed in tubes with netted bottoms and surface sterilised by first placing them in 5 ml of sodium hypochlorite (1.5%) for 30 seconds, then in 70% Ethanol for 30 seconds followed by three rinses of sterile distilled water. The cysts were blot-dried by placing them on sterile paper towels to remove excess water (Oka *et al*., 1997). Petri dishes (9 cm diameter) containing Potato Dextrose Agar (PDA) amended with streptomycin and chloramphenicol antibiotics were prepared under sterile conditions and kept in the laminar flow. Three surface-sterilised cysts were placed equidistantly on the PDA plates, sealed using parafilm, labelled, and incubated at 22 °C for 7 days to assess fungal growth/ occurrence. The three cysts per Petri dish were replicated six times per farm sample. The last rinse water was also plated out to assess the effectiveness of the surface sterilization procedure (Schultz *et al*., 1998). Fungal occurrence was recorded by counting the number of cysts that showed fungal growth (Petrini and Fisher, 1987). This assessment of fungal occurrence was repeated daily from the first day of incubation until seven-day post-incubation. Isolated fungi were sub-cultured for 4-6 times to obtained pure cultures and preserved at *icipe*'s Arthropod Pathology Unit germplasm. After 14 to 21 days, small cubes (1cm by 1cm) of pure cultures from individual cysts were cut using a sterile surgical blade and transferred to PDA. These fungal samples were incubated at 22 °C for 7-14 days before morphological and molecular identification (Kooliyottil *et al*., 2017). Fungal occurrence rate was expressed as percentage number of cysts exhibiting fungal outgrowth divided by the total number of cysts that were plated out.

2.5 Morphological and molecular identification of the fungi

The isolated fungi were identified morphologically using the glass slide technique as described by Carmichael (1956). Briefly, cellophane squares of about 15-18 mm were arranged in Petri dishes, flooded with water, drained, and autoclaved at 121 °C for 45 minutes. The sterile squares were then picked with forceps and placed on the surface of a solid culture medium. After ensuring that excess surface moisture has disappeared, the fungi to be studied were lifted off the agar (the cellophane squares) and placed colony side up on a drop of lactophenol blue on a glass slide. Each specimen was viewed under a Leica DM2500 microscope. In addition to fungal culture appearance, texture and pigmentation, characteristics such as mycelia type, conidia shape, size, and colour were observed and recorded.

For molecular characterization, fungal mycelia of pure cultures grown on PDA media was harvested into 1.5 ml tubes. The DNA was extracted using Plant/Fungal mini extraction kit as specified by the manufacturer. The quality and quantity of DNA was determined using 1.5 agarose gel electrophoresis and a nanodrop spectrophotometer respectively. Universal oligonucleotide ITS4 and ITS5 were used to amplify the ITS rDNA region (White et al., 1990). The PCR products were purified from the gel using Macrogen purification kit and sent to Macrogen Inc. Europe Laboratory, in the Netherlands for sequencing. The sequences were then cleaned, and blast searched for matching sequences in Genebank database (Nuaima *et al*., 2018; Kooliyottil *et al*., 2016, 2017).

2.6 Germination or viability test of the isolated fungi

The isolated fungal species were cultured on PDA plates and maintained at 25 ± 2 °C in complete darkness. Conidia were harvested by scraping the surface of seven to ten day old sporulated cultures using a sterile spatula. The harvested conidia were then suspended in 10 ml sterile distilled water containing 0.05 % Triton X-100 and vortexed for 5 min at about 700 rpm

to break conidial clumps and ensure a homogenous suspension (Akutse *et al*., 2013). Conidial concentrations were quantified using an improved Neubauer hemocytometer under a light microscope (Goettel and Inglis 1997). The conidial suspension was adjusted to a concentration of 1×10^8 conidia ml^{-1} through serial dilution prior to bioassays. Prior to bioassays, spore viability was determined by plating evenly 0.1 ml of 3×10^6 conidia ml^{-1} onto 9-cm Petri dishes containing PDA. Three sterile microscope cover slips (2 × 2 cm) were placed randomly on the surface of each inoculated plate. Plates were sealed with Parafilm and incubated in complete darkness at 25 ± 2 °C and were examined after 16-20 hours. The percentage germination of conidia was determined from 100 randomly selected conidia on the surface area covered by each cover slip under a light microscope (at x400 magnification) using the method described by Goettel and Inglis (1997). Conidia were considered to have germinated when the length of the germ tube was at least twice the diameter of the conidium (Goettel and Inglis 1997). Four replicates were used for each isolate for the viability assessment.

2.7 Evaluating fungal efficacy on PCN

2.7.1 Effect of fungal isolates on the PCN cysts using sand assay

Ten treatments were set up consisting of seven of the fungal isolates isolated in this study: *Trichoderma asperellum* (oil formulation and pure spores), *T. hamatum*, *T. breve*, *T. atrobruneum*, *Amanita basiorubra*, *Setophoma terrestris* and *Penicillium chrysogenum*, a commercial nematicide Velum® Prime and water as the untreated control.

About 1 kg of river sand was sieved and autoclaved at 121°C for 45 minutes, and approximately 50 grams was dispensed in each Petri dish. About 800 PCN cysts which were originally obtained from a farmer's field in Kipipiri (Nyandarua county) and cultured at *icipe* were placed in plastic tubes with a mesh at the bottom for surface sterilization. The cysts were surface sterilised as described above and blot-dried in a sterile paper towel. Twenty cysts were hand-

picked using an entomological forceps and placed in each Petri dish containing the sterile autoclaved sand.

Spores of pure fungal cultures grown on PDA for two to three weeks were used in evaluating the efficacy effects against the PCN cysts. The plates were flooded with sterile distilled water and harvested in 10 ml triton water to prepare conidial suspensions as described above. The conidial suspension was adjusted to a concentration of 1×10^8 conidia ml^{-1} through serial dilution using a haemocytometer. About 8 ml of the fungal suspension was sprayed on the sterilized sand in the Petri dishes containing the 20 PCN cysts using a precision tower, sealed with Parafilm and labelled. Triton water was used as control treatment without any fungal conidia to treat the cysts. The precision tower was rinsed thoroughly with Ethanol 70% before the commercial nematicide treatment Velum prime was applied. Velum prime was applied at a rate of 1ml/ L of Triton water and sprayed onto the cysts on media using the precision tower. The experiment was laid out in a completely randomised design (CRD) replicated four times and stored at room temperature for two weeks. The treated cysts were air-dried and extracted from the treated sand using the modified bucket method and quantified (Holgado and Magnusson, 2010; Indarti *et al.*, 2010; Kooliyottil *et al*., 2017).

2.7.2 Evaluation of viability and hatching of treated PCN cysts

Ten cysts from each treatment and replicate were selected for the viability test, by placing them in viability chambers and stained with 0.01% Nile blue solution (Ogiga and Ostely, 1974). Five (5) ml of the 0.01% Nile Blue solution was added to each well containing treated cysts and incubated in a dark room at room temperature for 48 hours. The cysts were then triple-rinsed with distilled water and blot-dried using a paper towel. Each of the treated cysts was opened using a sharp needle. The number of stained and unstained eggs observed, were counted under a stereo microscope at a magnification of x40 and recorded. Viable eggs were unstained while

those that stained blue were non-viable (Ebrahimi *et al*., 2014). The number of juveniles was also counted. Egg viability was assessed by counting the number of viable and non-viable eggs and juveniles (J2) per cyst. The number of unstained eggs and J2s (termed as viable) was divided by the total number of eggs and J2s (both stained and unstained) and multiplied by 100 to get the percentage viability of the cysts.

The treated cysts' hatchability was evaluated by soaking 5 treated cysts in tap water for 24 hours then replacing the water with a 3-week-old potato root exudate and incubated at 21 ^{o}C in the dark for five days. The number of hatched juveniles from each treatment was picked using a pipette, placed in a graduated counting dish, counted under a stereo microscope and recorded. The quantified exudate was discarded, and a fresh exudate replaced weekly. The total number of hatched juveniles per week was counted for eight weeks (Ebrahimi *et al*., 2014). The cysts were crushed at the end of 8 weeks, and the number of unhatched eggs and juveniles was counted. Hatching % was calculated as hatched J2/ (hatched + unhatched) *100.

2.8 Statistical analysis

The mean values of fungal species associated with cysts, their diversity and abundance, cyst hatchability, and egg viability were subjected to analysis of variance (ANOVA) using R statistical software version 4.0.2. The means were separated using Tukey's Honestly with a significance level of $p<0.05$.

3.0 Results

3.1 PCN cyst detection

The results of the survey showed that the total PCN prevalence was 61.7% (29 farms with PCN detected out of 47 sampled). PCN cysts were detected in 10 out of the 20 farms sampled in

Nyandarua (50% prevalence) while in Nakuru county, 19 out of the 27 farms sampled had PCN (70%). The PCN were identified to *Globodera* spp., using morphological features of the cysts.

3.2 Fungal species identification, diversity and abundance

Fungi belonging to five (5) genera were isolated from the field collected PCN cysts and a total of twelve (12) fungal isolates were obtained from the cysts collected. These were identified as eight species: *Trichoderma asperellum*, *T. hamatum*, *T. breve*, *T. atrobruneum*, *Amanita basiorubra*, *Setophoma terrestris*, *Clonostachys* spp., and *Penicillium chrysogenum*. The morphological identification of the various fungal species was well confirmed by the molecular identity (Table 1). The most abundant isolate was *Trichoderma* spp. with 58% occurrence rate. Of all the identified fungal isolates obtained, 50% were from Nakuru (altitude 2338 to 2649 metres above sea level) and the other 50% were from Nyandarua (altitude 2484 to 2861 metres above sea level) counties. *Trichoderma* spp. was present in both regions while *Clonostachys* spp., and *Amanita spp.,* were only found in Nakuru county. *Penicilium* spp*.,* and *Setophoma* spp., were present only in Nyandarua county. The fungal occurrence rate was highest in Nyandarua county at 68% while in Nakuru county it was 48% on average. Differences in fungal occurrence in the selected sub counties were also noted in Nakuru county where Mau Narok had 67% occurrence while both Molo and Kuresoi North had 33%. The two selected areas (Kipipiri and Kinangop) in Nyandarua county both had 68.5% fungal occurrence on average.

3.3 Effects of fungal isolates on viability and hatchability of PCN cysts *in vitro*

The viability of PCN cysts used for efficacy was 87% prior to treatment application and the untreated control had 86% at the end of the experiment. The *in vitro* bioassays showed that the fungal isolates significantly reduced PCN egg viability compared to the untreated control. Six fungal isolates (*Trichoderma breve*, *Penicillium chrysogenum*, *T. hamatum*, *T. atrobrunneum*, *Amanita basiorubra*, and *Setophoma terretris*) significantly ($p<0.001$) reduced PCN egg viability by 60% on average (min 51.1% to max 65.7%). *Trichoderma breve* and *Penicillium chrysogenum* reduced the egg viability of *Globodera* spp. by 41% and 34%, respectively, compared with the untreated control (Table 2). Other fungal isolates that were significantly different from the untreated control include *T. hamatum*, *T. atrobrunneum*, *Amanita basiorubra*, and *Setophoma terretris*. These isolates reduced egg viability by 27% on average, and they were not significantly different from the commercial nematicide Velum (Fluopyrum), which had a 19% reduction (Table 2). The two strains of *T. asperellum* (S11 and Al Tememe) were not significantly different from the untreated control as regards to eggs viability.

Velum (Fluopyrum) – Bayer E.A. (the commercial nematicide) outperformed all the tested isolates where only 22.2% eggs hatched compared to 88.5% in the control. *Trichoderma breve* and *T. asperellum* also significantly ($p<0.001$) reduced the hatchability of PCN eggs (>40% in the three fungal isolate treatments) compared to 88.5% in the untreated control on average (Table 2). Hatching was decreased in the rest of the isolates in the range of 30 - 40%, while the commercial nematicide Velum (Fluopyrum) reduced the hatchability by 75% compared to the untreated control. Velum was significantly different from all the tested fungal isolates (Table 2).

4.0 Discussion

Previous studies on PCN incidence in Kenya and its presence across the country in key potato growing areas have been conducted and they showed a 71.8% PCN prevalence with the most prevalent species being *Globodera rostochiensis* (Mwangi et al., 2015; Mburu et al., 2020). The results of our study support these findings with PCN prevalence of 61.7% in Nyandarua and Nakuru counties. From the collected cysts across the two counties, we found eight different fungal species (12 isolates) infecting the cysts. Fungi have also been isolated from PCN by other researchers for instance, Abbasi et al. (2017) isolated 34 fungal isolates belonging to 11 genera, some of which include *Trichoderma, Fusarium, Paecilomyces* and *Cylyndrocarpon* while Kooliyottil et al. (2017) and Ibrahim et al. (2009) isolated *Fusarium, Paecilomyces, purpureocilium* and *Chaetomium* among others. *Malassezia* fungus was also isolated from PCN eggs in potato monoculture fields (Eberlain et al., 2016). Despite the extensive studies being conducted in various areas across the world, information on Kenyan fungal isolates in association with nematodes such as PCN is minimal or completely lacking. Our study therefore serves to provide some primary information on fungal species infecting PCN cysts under Kenyan conditions.

Fungal species occurrence across the two counties was equally distributed with each area having 50% occurrence rate of the isolated fungi. The slight difference in altitude (2338 to 2649 metres above sea level in Nyandarua and 2484 to 2861 metres above sea level in Nakuru) did not affect fungal occurrence. These findings support earlier studies conducted by Li et al., (2020) where they found that altitude did not significantly affect fungal distribution in high altitude areas. Despite the fungal occurrence differences in the subcounties of Nakuru county, altitude did not seem to be the main determining factor. Other factors that may be contributing to the abundance and distribution of fungal isolates in an area include soil organic matter, soil pH clay content, presence of the preferred host among others (Li *et al*., 2020; Kohlmeyer, 1983).

Earlier studies have shown that parasitic fungi capable of infecting PCN cysts and eggs could be exploited as potential biological agents for controlling this pest (Sharma and Rakesh 2009; Siddiqui and Akhtar 2009; Ada et al., 2007; Sun et al., 2006; Larena et al., 2002; Verdejo-Lucas et al., 2002; De Cal and Melgarejo 2001; Hutchinson 1999; Fuchs et al., 1997; Madi et al., 1997). *Trichoderma* spp. has demonstrated great potential as a biocontrol agent against plant parasitic nematodes (Indarti et al., 2021; Martinez-Medina et al., 2017; Bokhari, 2009) including PCN (Indarti et al., 2021). Some species like *Trichoderma harzianum* and *T. longibrachiatum* has showed pathogenicity to cyst nematodes (Contina et al., 2017; Zhang et al., 2014; Bentoumi et al., 2020). In our study, *T. breve* reduced both egg viability and hatchability but *T. asperellum* only reduced egg hatchability. *Trichoderma breve* was previously isolated and described by Chen and Zhuang (2017) from soil samples in Australia and in Africa (Cameroon and Ethiopia) (Carmen et al., 2021) but information on its ability as a biocontrol agent is limited. *Trichoderma breve* is closely related to *T. harzianum* group which is characterized by very high chitinase activity that directly affects egg viability and survival of juveniles (Carmen et al., 2021). This enzymatic activity is hypothesized to be associated with the potency of this isolate. It was not immediately clear within the scope of this study on why *T. asperellum* only affected PCN egg hatchability. The efficacy of *Penicillium chrysogenum* has been studied and it successfully reduced hatching of the root knot nematode *Meloidogyne incognita* and sugar beet cysts (Naz et al., 2021; Sikandar *et al*., 2020; Kassam *et al*., 2021; Sikandar *et al*., 2019; Siddique and Akhtar, 2009). The findings in our study are in line with the above previous results because this fungus successfully reduced egg viability from 86% in the untreated control to 57.2%. We showed that *Setophoma terrestris* also significantly reduced egg viability and hatching of PCN by 20.6% and 28.6% respectively. Interestingly this fungal species is also known to be a serious pathogen of onion (Orio *et al*., 2016). *Setophoma terrestris* has previously been found associated with PCN, beet cyst nematode and cereal cyst

nematode (CCN) (Oro et al., 2021; Nuaima et al., 2020; Dackman 1990). Its pathogenicity was tested on cereal cyst nematode where 60% reduction of CCN eggs hatchability was reported (Chen et al., 1996). *Trichoderma atrobrunneum* is a newly constituted species which is closely related to *T. harzianum* in morphology and functionality and has been isolated from soil or decaying wood in North America, Spain, France, Croatia, Greece and Africa (Algeria) (Haouhach et al., 2020; Jaklitsch et al., 2015; Chaverri et al., 2015). An extensive study on this fungal isolate reveal that *T. atrobrunneum* possesses chemical properties like high level of CAZYmes and high chitinase activity which directly influences the biocontrol activity (Fanelli *et al*., 2018). Chao and Zhuang (2019) reported that *T. atrobrunneum* depicted biocontrol capabilities against *Ralstonia solani* (Wang and Zhuang, 2019). Despite the above findings, there is limited information on its efficacy against PCN. In our study we showed that the isolate significantly reduced PCN egg viability by 30% on average, this may be one of the first reports showing its pathogenicity to PCN. Finally, we showed that *Amanita basiorubra* reduced the viability of PCN eggs by 22%. This fungus has been isolated from soil samples in Australia (Robinson and Haska, 2020; Bougher, 2020); although limited information is available on its biocontrol capabilities, our study has demonstrated that it can reduce PCN egg viability.

Our study confirms and supports previous studies on the potential of using selected fungal species/isolates for managing PCN infestations, such as *Trichoderma* spp. (D'Errico et al., 2021; Trifonova, 2010). However, the interactions of these isolates within the soil environment have not been conducted to ascertain their efficacy, and this warrants further studies before development into biopesticides to sustainably manage the PCN. Some species of *Trichoderma* like *T. asperellum* which is commercially available as MAZAO Sustain (PCPB (CR) 1234) for the control of root knot nematodes (*Meloidogyne* spp.) in French beans from Real IPM Company, Kenya can be evaluated for efficacy against PCN (https://www.pcpb.go.ke/biopesticides-on-crops/).

Biological control, especially the use of fungal isolates, is an attractive potential tool for sustainable nematode management (Collinge et al., 2019; Indarti et al., 2010; Latz et al., 2018; Stirling, 2011). The presence of these natural microbial enemies associated with cyst nematodes could indicate that the pest could be controlled naturally (Holgado & Magnusson, 2010). These nematodes' sedentary lifestyle makes them vulnerable to colonisation by the fungal parasites (López-Lima et al., 2020; Manzanilla-López et al., 2017) which could be exploited as a tool to manage this pest.

Even though some of the fungal species associated with PCN have been reported elsewhere globally, it is vital that local isolates adapted to local conditions be evaluated to ascertain their efficacy in managing this pest (Stirling, 2011). Knowing fungal species associated with PCN is paramount in identifying possible biocontrol agents that could be exploited and developed as fungal-based biopesticide for managing this pest. This study has provided insight into possible fungal microbiomes available in some parts of Kenya and has further demonstrated that they can negatively affect PCN's life-history parameters or reproduction rate. However, further evaluation needs to be undertaken to assess their efficacy *in vivo* and under field conditions. It is also recommendable that a more exhaustive survey be done in crucial potato growing areas to provide a thorough report on the fungal communities (or mycobiota) associated with PCN in Kenya.

The use of more than one control strategy (integrated management) is advised to optimise control efficiency, and the findings of this study prove that these potent fungal species could be integrated in the management of PCN. Information required for proper nematode management, include: (i) proper diagnosis of nematode species and potent/effective fungal isolate; (ii) relationship between population density and yield losses; (iii) nematode biology (life cycle, environmental requirements, and parasitism); (iv) host range; (v) population dynamics; (vi) efficiency of control methods and (vii) economic feasibility of control methods.

5.0 Conclusions

Potato cyst nematode is present in most parts of potato growing regions in Kenya especially in Nakuru and Nyandarua counties. This pest lives in close association with fungal microbes. Twelve fungal isolates belonging to five genera (*Trichoderma, Penicillium, Amanita, Setophoma and Clonostachys*) were isolated from PCN cysts and *Trichoderma* was the most abundant genera. *T. breve* and *T. asperellum* were the best performing isolates in reducing PCN egg viability and hatchability followed by *T. attrobrunneum* and *T. hamatum*. Other fungal isolates that were effective in reducing PCN reproduction potential include: *Penicillium chrysogenum*, *Amanita basiorubra* and *Setophoma terrestris*. The results herein, were obtained *in vitro* under laboratory conditions with success; hence a potential biocontrol tool that could be deployed for PCN management in potato after validating these findings under field conditions. Therefore, there is need for further evaluation under greenhouse and field conditions. A more exhaustive survey for antagonistic fungi needs to be conducted in crucial potato growing regions in the country.

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420

421 **Conflict of Interest**

422 The authors declare that the research was conducted in the absence of any commercial or
423 financial relationships that could be construed as a potential conflict of interest.

424

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County	Sub county	Sample code	Sample	ID from GeneBank	Accession no.	ID %
Nakuru	Mau Narok	NRK-R2	Isolate 1	*Penicillium chrysogenum* strain 2S10	MT421937.1	98.92
Nakuru	Mau Narok	NRK-R7	Isolate 2	*Trichoderma atrobrunneum* isolate Tatro	MT435949.1	99.71
Nakuru	Molo	TURI-R1	Isolate 3	*Setophoma terrestris* isolate Llano grande	MK012554.1	99.35
Nakuru	Molo	TURI-R2	Isolate 4	*Trichoderma asperellum* strain Al-Tememe	MT175982.1	95.62
Nakuru	Molo	TURI-R3	Isolate 5	*Setophoma terrestris* clone 2014	MN522638.1	97.67
Nakuru	Kuresoi North	Sum-R2	Isolate 6	*Trichoderma asperellum* strain S11	MT409888.1	97.89
Nyandarua	Kipipiri	KPR-R1b	Isolate 7	*Trichoderma asperellum* strain S11	MT409888.1	99.69
Nyandarua	Kipipiri	KPR-R1a	Isolate 8	*Trichoderma hamatum* isolate SK19 42F	MT406740.1	99.36
Nyandarua	Kipipiri	KPR-R3	Isolate 9	*Clonostachys* sp. isolate F48	MN611289.1	96.68
Nyandarua	Kinangop	KNG-R1	Isolate 10	*Amanita basiorubra* voucher PERTH7878516 clone 6.31 18S	KF803245.1	96.67
Nyandarua	Kinangop	KNG-R10	Isolate 11	Trichoderma asperellum strain S11	MT409888.1	97.49
Nyandarua	Kinangop	KNG-R5	Isolate 12	*Trichoderma breve* isolate 21	MT441608.1	99.04

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Treatment/ Fungal isolate	Viability (%)	% reduction compared to control	Hatchability (%)	% reduction compared to control
Trichoderma breve	51.1 ± 0.09a	41	44.8 ± 0.21b	49
Penicillium chrysogenum	57.2 ± 0.09ab	34	50.8 ± 0.12bc	43
T. hamatum	61.4 ± 0.11ab	29	50.6 ± 0.13bc	43
Trichoderma atrobrunneum	61.6 ± 0.08ab	29	51.4 ± 0.25bc	42
Amanita basiorubra	64.3 ± 0.04abc	25	59.2 ± 0.08bc	33
Setophoma terrestris	65.7 ± 0.18abcd	24	60 ± 0.17bc	32
Velum (*Fluopyrum*) – Bayer E.A.	70.1 ± 0.05bcd	19	22.2 ± 0.12a	75
T. asperellum S11	76.9 ± 0.06cde	11	43.8 ± 0.16b	51
T. asperellum Al Tememe	79.2 ± 0.04de	8	45.4 ± 0.21b	49
Control	86.3 ± 0.04e		88.5 ± 0.06c	
p values	<0.001		<0.001	

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Table 1. Molecular identification of fungal species isolated from PCN collected from two counties (Nakuru and Nyandarua) in Kenya.

Table 2. Effect of fungal isolates on viability and hatchability of PCN eggs under *in vitro* conditions at $p \leq 0.05$ using Tukey's Honestly Significant difference mean separation.

868 Figure 1. Major potato growing regions in Kenya (MOALF, 2016)

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870 Figure 2. Location of Nakuru and Nyandarua Counties in Kenya. Sampling points are indicated
871 with the red stars.

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