Biological Control 119 (2018) 27–32

Contents lists available at ScienceDirect

Biological Control

journal homepage: www.elsevier.com/locate/ybcon

Effect of *Trichoderma* spp. and *Purpureocillium lilacinum* on *Meloidogyne javanica* in commercial pineapple production in Kenya

Agnes W. Kiriga[a,b], Solveig Haukeland[b,*], George M. Kariuki[a], Danny L. Coyne[c], Nikolai V. Beek[d]

[a] Kenyatta University, Department of Agricultural Science and Technology, P.O. Box 43844-00100, Nairobi, Kenya
[b] International Centre of Insect Physiology and Ecology (icipe), Department of Plant Health, P.O. Box 30772-00100, Nairobi, Kenya
[c] International Institute of Tropical Agriculture, IITA East Africa, c/o icipe P.O. Box 30772-00100, Nairobi, Kenya
[d] Kenya Biologics Limited, P.O. Box 4560-01002, Thika, Kenya

ARTICLE INFO

Keywords:
Ananas comosus
Trichoderma
Purpureocillium lilacinum
Host response
Plant parasitic nematodes

ABSTRACT

Plant-parasitic nematodes, in particular *Meloidogyne* species, cause significant yield reduction in commercial pineapple, *Ananas comosus*, worldwide. The efficacy of three *Trichoderma* isolates (*Trichoderma asperellum* M_2RT_4, *T. atroviride* F_5S_{21}, *Trichoderma* sp. MK_4) and two isolates of *Purpureocillium lilacinum* (KLF_2 and MR_2) were evaluated against *Meloidogyne javanica*, using rooted pineapple crowns in a pot experiment under greenhouse conditions. All the three *Trichoderma* isolates successfully colonized pineapple root endophytically. The application of two isolates of *Trichoderma* (*T. asperellum* M_2RT_4 and *Trichoderma* sp. MK_4) and the two isolates of *P. lilacinum* significantly reduced nematode egg and egg mass production reducing root galling damage by between 60.8 and 81.8% and increased the plant root mass growth compared to the untreated control. *T. asperellum* M_2RT_4 most effectively reduced galls, egg mass and eggs, by 81.8, 78.5 and 88.4% respectively. *P. lilacinum* MR_2 most effectively reduced galls, egg mass and eggs, by 71.6, 73.9 and 82.6% respectively. In contrast *Trichoderma atroviride* F_5S_{21} application had no significant effect on nematode multiplication or root damage compared with the control. Inoculation with *T. asperellum* M_2RT_4 increased root fresh weight by 91.5%, *Trichoderma* sp. MK_4 by 63.8%, *T. atroviride* F_5S_{21} by 50.0%, *P. lilacinum* KLF_2 by 43.8% and MR_2 by 32.3%. Results indicate that local isolates *Trichoderma* spp. and *P. lilacinum* directly and indirectly affected nematode reproduction and host response, demonstrating their control potential against *M. javanica* on pineapple. Such alternative options for managing *Meloidogyne* spp. would provide more environmentally sensitive options for combining with other management methods towards more sustainable pineapple production systems.

1. Introduction

Plant parasitic nematodes are a major global limitation to pineapple, *Ananas comosus* (L.) Merr., production (Gianessi et al., 2002; Sipes et al., 2005). The most important of these are root-knot nematodes, *Meloidogyne javanica* (Treub) Chitwood and *M. incognita* (Kofoid & White) Chitwood (Rohrbach and Apt, 1986), causing significant reduction in yields worldwide, alongside the other important species, *Rotylenchulus reniformis* Linford & Oliveira and *Pratylenchus brachyurus* (Godfrey) Filipjev & Schuurmans Stekhoven (Daramola and Afolami, 2014; Sipes et al., 2005; Stirling, 1993). Pineapple is the third most important fruit crop globally, after banana and citrus, contributing to over 23% (24.8 million tons) of global tropical fruit production (FAOSTAT, 2015; Kormelinck and Janssen, 2012; UNCTAD, 2016). Pineapple production is concentrated in the tropical regions of the world, with Brazil, Costa Rica, Philippines and Thailand commanding nearly 50% of the total output (UNCTAD, 2016). Other important producers are India, China, Nigeria, Kenya, Mexico and Indonesia (FAOSTAT, 2015; Kormelinck and Janssen, 2012). In Kenya, pineapple is mostly cultivated on large scale commercial plantations using cv. Smooth Cayenne. However, smallholder farmers are increasingly turning to pineapple production for both home consumption and commercial purposes (HCDA, 2008; Kormelinck and Janssen, 2012; Koech et al., 2014). Kenya is among the main exporters of the 16% of pineapples that Africa produces in the world (FAO, 2012). Under commercial production systems synthetic chemical pesticides (e.g. 1, 3-Dichloropropene-Telone II) are widely used and relied upon to manage root-knot nematode (Daramola and Afolami, 2014; Stirling and Pattison, 2008). However, environmental and human health concerns regarding the use of such nematicides have led to increased interest in identifying alternative strategies that are environmentally sensitive (Singh et al., 2012). Biological control agents (BCAs) have shown

* Corresponding author.
E-mail address: shaukeland@icipe.org (S. Haukeland).

https://doi.org/10.1016/j.biocontrol.2018.01.005
Received 13 April 2017; Received in revised form 10 January 2018; Accepted 11 January 2018
Available online 12 January 2018

promise from both an economic and ecological perspective to reducing pest damage (Singh et al., 2012).

Well-known antagonists of *Meloidogyne* spp. are soil fungi from the genera *Trichoderma* (Hypocreaceae) (Samuels et al., 2012) and *Paecilomyces* (now *Purpureocillium*) (Kiewnick and Sikora, 2006; Kumar et al., 2009). Biocontrol activity of *Trichoderma* species against plant pathogens occurs through various mechanisms: induced resistance in the host plant, antibiosis, competition, direct parasitism and enzymatic hydrolysis (Elad and Freeman, 2002; Harman et al., 2004; Howell, 2003). Moreover, these fungi may additionally promote plant growth in the absence of pest challenge (Sharon et al., 2001; Yedidia et al., 1999). *Trichoderma* spp. have been used to successfully suppress *Meloidogyne* spp. reproduction in tomato roots and other crops (Sharon et al., 2007). They have also been shown to colonize plant roots, both along the root surface as well as endophytically (Harman, 2000, Howell et al., 2000), which has been linked to the successful suppression of root-knot disease severity (Harman et al., 2004; Sharon et al., 2001; Siddiqui and Shaukat, 2003; Yedidia et al., 1999). To enhance maximum root colonization, it has been recommended that *Trichoderma* spp. be applied before transplanting (Dababat and Sikora, 2006; Van Damme et al., 2005). For pineapple, crowns, suckers or slips are used as "seedling" material (Rohrbach and Apt, 1986) but to our knowledge, no studies have reported on the colonization of *Trichoderma* spp. on developing pineapple roots.

The saprophytic fungus *P. lilacinum* (previously called *Paecilomyces lilacinus* (Thom) Samson) (Lopez-Lima et al., 2014) has been widely assessed for the biological control of plant-parasitic nematodes (Atkins et al., 2005). It has shown significant success against *Meloidogyne* spp., parasitizing eggs but also females (Khan et al., 2006a; Mukhtar et al., 2013; Oclarit and Cumagun, 2009; Siddiqui et al., 2000). Diverse mechanisms have been reported for the biological activity of *P. lilacinum*, with direct parasitism of the egg stage (Kiewnick and Sikora, 2006) and females (Holland et al., 1999) after the formation of appressoria, being the main mechanisms of action; the production of proteases and chitinases is also associated with the infection process (Khan et al., 2004; Kiewnick and Sikora, 2006). The enzymes dissolve the vitelline layer of eggshell, facilitating the fungal hyphae to penetrate eggs and destroy the embryonic developmental stages at an early stage (Khan et al., 2006b; Mukhtar et al., 2013). Its high potential for the biological control of nematodes, and its successful use against *M. javanica* and *M. incognita* on tomato, vegetables, banana and other crops has led to its development into commercial products (Goswami and Mittal, 2004; Goswami et al., 2006; Haseeb and Kumar, 2006; Jonathan and Rajendran, 2000; Kumar et al., 2009; Van Damme et al., 2005). To our knowledge, neither of these fungi is used for the control of root-knot nematodes in pineapple.

The objectives of this study were to (i) determine the effect of three selected *Trichoderma* isolates: *T. asperellum* M_2RT_4, *T. atroviride* F_5S_{21}, *Trichoderma* sp. MK_4, and two isolates of *P. lilacinum*, KLF_2 and MR_2, against *Meloidogyne javanica* in pineapple, and (ii) determine the ability of the four *Trichoderma* isolates to colonize pineapple roots.

2. Materials and methods

2.1. Source of nematodes and preparation of nematode inoculum

Meloidogyne spp, were isolated from naturally infected pineapple plants collected from Delmonte, Thika County (01°03′S latitude and 37°05′E longitude) and Kakuzi, Murangá County (0°, 58′S latitude and 37° 16′E longitude) commercial pineapple farms, Kenya. Single egg masses were removed from infected pineapple roots under a dissecting microscope and individually inoculated onto pineapple seedlings (cv. Smooth Cayenne) planted in pots containing autoclaved soil in the greenhouse at *icipe* Duduville Campus, Nairobi, Kenya. *Meloidogyne javanica* was identified from the perineal pattern of mature females (Seinhorst, 1966; Taylon et al., 1956) and confirmed using species specific primers for tropical root knot nematodes (Correa et al., 2014; Tigano et al., 2010; Zijlstra et al., 2000). The specific SCAR primers Fjav/Rjav (*M. javanica*) (Zijlstra et al., 2000) gave consistent results and the products were readily amplified from DNA of individual females.

The pineapple plants (cv. Smooth Cayenne) were uprooted three months after inoculation, and the galled roots gently washed free of soil using clean tap water and then sterilized using 1.5% sodium hypochlorite (NaOCl). The roots were blended for 30 s in 0.6% NaOCl solution (Hussey and Barker, 1973), rinsed with distilled water and eggs collected in a 25 μm sieve. The eggs were incubated at 28 °C to obtain fresh, 1–5 day old infective second stage juveniles (J2) to be used for inoculation.

2.2. Source of the fungal antagonists

Four isolates of *Trichoderma* spp. were sourced locally, all of which originated from within Kenya: three from the *icipe* Arthropod Germplasm Centre, (*Trichoderma asperellum* M_2RT_4; *T. atroviride* F_5S_{21} and *T. harzianum* F_2L_4) and one from Kenya Biologics Limited (KBL) *Trichoderma* sp. (MK_4). Two Kenya isolates of *P. lilacinum* (KLF_2 and MR_2) were also supplied by KBL.

2.3. Mass production of the fungal antagonists

Inoculum for all isolates was multiplied using rice; grains were washed with water, surface dried using a paper towel and 2 kg placed in Milner bags (autoclavable bags), before autoclaving at 121 °C (15 psi) for 50 min. The sterilized rice grains were inoculated with pure cultures of each of the antagonistic fungi. The bags were massaged from the outside to evenly distribute the inoculum over all the rice grains before incubating at 25 ± 1 °C for 21 days. The bags were shaken on alternate days to encourage uniform colonization by the fungus. The bags were then opened to allow the rice and the conidia to dry for seven days, before using to make spore suspensions. A 0.1 g aliquot of conidia for each fungal isolate was placed separately in universal bottles with 10 ml sterile distilled water containing 0.05% Triton X-100 and vortexed for 5 min to produce homogenous conidial suspensions. The spore concentration was estimated using a haemocytometer (Hausser Scientific, Horsham, Pennsylvania, USA) and adjusted to 3×10^6 and 1×10^8 spores per ml for *P. lilacinum* and *Trichoderma* spp., respectively, through dilution. Aliquot 0.75 g and 1.5 g of M_2RT_4 and KLF_2 respectively and 1 g each of F_5S_{21}, MK_4 and MR_2 were used in 30 ml of 0.05% Triton X-100. To assess the viability of the fungus, 100 μL aliquots of conidial suspension for each isolate was inoculated to the surface of two plates (9 cm diameter) of potato dextrose agar (PDA). A sterile microscope cover slip (2 × 2 cm) was placed on top of the agar in each plate before incubation. The inoculated plates were then incubated for 24 h at 25 °C. The percentage conidial germination was assessed by counting the number of germinated conidia per 100 in one randomly selected field. Conidia were considered as germinated when germ tubes exceeded half of the diameter of the conidium. The percent germination of each isolate was over 95% (Parsa et al., 2013).

2.4. Assessment of pineapple root colonization by Trichoderma isolates

The four isolates of *Trichoderma* were tested for colonization of pineapple roots under greenhouse conditions, average ambient temperatures ranged from 23 °C (day) to 13 °C (night) for the experimental period. Pineapple (cv. Smooth Cayenne) crowns were established in 10L pots (24-cm-diameter and 22-cm-height) containing autoclaved soil for three weeks to allow roots to develop. The rooted crowns were then removed from the soil with roots intact, the soil gently rinsed with tap water and the roots immersed in a 250 ml suspension containing 1×10^8 spores per ml *Trichoderma* for 8 h for each isolate; controls were immersed in distilled water for 8 h. Each treatment was replicated four times and arranged in a complete randomized design. The rooted crowns were then planted into pots containing a 10L of 2:1:1 mixture of

autoclaved soil, sand and manure. Two weeks after inoculation the plants were removed from the soil mixture with roots intact and rinsed with tap water to remove the soil. Leaf and root sections were aseptically removed and cut into 1 cm length pieces under a laminar flow hood. These were surface sterilized using 1.5% NaOCl, rinsed first in 70% alcohol and then in distilled water. For each plant, five pieces each of leaves and roots were separately placed 4 cm apart onto PDA plates and incubated at 26 ± 1 °C for 10 days. Mycelial growth was assessed between days 2 to 10. Fungal colonization was recorded for fungal growth according to Koch's postulate for each piece.

2.5. Evaluation of efficacy of the fungal antagonists on Meloidogyne javanica

2.5.1. Application of the fungal antagonists in vivo

The assessment of biocontrol activity of the antagonistic fungi against *M. javanica* was undertaken in the greenhouse at *icipe*. Pineapple crowns were rooted in pots containing autoclaved soil for three weeks as above, before uprooting and rinsing in tap water. Roots of each plant were then totally immersed in 1×10^8 spore per ml (250 ml) suspension for *Trichoderma* isolates, or distilled water (for controls and treatments that required *P. lilacinum*), for 8 h. The rooted crowns were then planted into pots containing 10L of 2:1:1 mixture of autoclaved soil, sand and manure in a greenhouse. After two weeks, three 2 cm deep holes were made around the stem of each plant, and inoculated with 3000 *M. javanica* (infective stage juveniles) J2, using a pipette; the holes were covered with the soil mixture. One day later a small fallow was made around each plant that required the *P. lilacinum* treatment and a 30 ml suspension containing the 3.0×10^6 spores/ml applied. The experiment included seven treatments: a negative control with no nematode-fungus inoculation, a positive control inoculated with nematodes only and a treatment each inoculated with fungal antagonists (three *Trichoderma* and two *P. lilacinum*) and nematodes. *Trichoderma harzianum* F_2L_4 was not used, due to poor colonization results. Treatments were arranged in a completely randomized experimental design replicated six times. The plants were irrigated with tap water as required; the experiment was terminated at 90 days after nematode inoculation. The experiment was repeated once in time following the same procedure with a total of 84 pots in the full experiment.

2.5.2. Disease measurement and data analysis

At harvest, plants were gently uprooted, the roots cut from the plant and the soil gently rinsed under running tap water. The roots were then surface sterilized using 1.5% NaOCl, rinsed first in 70% alcohol and then in distilled water, dabbed dry with a paper towel and root fresh weights recorded.

Galling index was determined on a 1–5 scale: 1 – no galling; 2 – slight; 3 – mild; 4 –moderate and 5 – severe (Coyne et al., 2014). The number of galls on each root system was counted and the nematode density estimated by counting the number of egg masses and eggs under a stereo microscope at ×400 magnification from a representative sample of 5 g chopped up roots from each plant (Holbrook et al., 1983; Shurtleff and Averre, 2000). To facilitate counting of egg masses the roots were first stained with phloxine B, which stains the gelatinous matrix pink-red increasing egg mass visibility (Coyne et al., 2014). Eggs were extracted from galled pineapple roots by cutting into small pieces and blending in 0.6% sodium hypochlorite (NaOCl) for 30 s (Stetina et al., 1997). The contents were then poured onto nested 75 and 25 µm pore sieves. Eggs collected on the 25 µm pore sieve were counted under stereo microscope. The percentage reduction in the number of galls was computed as:

$$\text{Percentage Reduction} = \frac{\text{Number of galls (+ve control)} - \text{Number of galls (Treated)}}{\text{Number of galls (+ve control)}} \times 100$$

The data were subjected to one way analysis of variance (ANOVA) using R software version 3.2.3 (R Core Team, 2015) and the means of treatments found significantly different at $P \leq .05$ separated using Tukey-HSD.

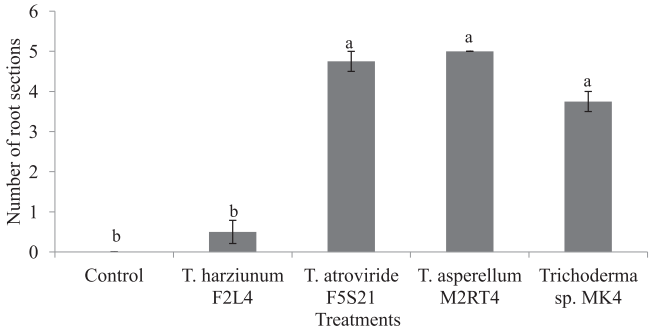

Fig. 1. Mean number of pineapple root sections colonized by four *Trichoderma* isolates, *T. harzianum* (F_2L_4), *T. atroviride* (F_5S_{21}), *T. asperellum* (M_2RT_4) and *Trichoderma* sp. (MK_4), and an untreated control on three weeks old plants. Columns with same letter(s) are not significantly different ($P \leq .05$).

3. Results

3.1. Assessment of pineapple root colonization by Trichoderma isolates

The mean number of root (Fig. 1) and leaf (Fig. 2) sections colonized by *T. asperellum* M_2RT_4, *T. atroviride* F_5S_{21}, and *Trichoderma* sp. MK_4 was similar but significantly greater ($P \leq .05$) than for *T. harzianum* F_2L_4, which had mean scores of 0.5 and 1.25 for roots and leaves, respectively. Hence isolate F_2L_4 (*T. harzianum*) was not further tested as a biocontrol agent.

3.2. Effect of the fungal antagonists on root fresh weight

The two experimental sets were statistically similar ($P \leq .05$) on the effect of the fungal antagonists and so results were combined for analysis. Root fresh weight of the positive control was significantly lower than in all other treatments ($F_{(6,77)} = 14.37$; $P < .001$) (Table 1). Root fresh weights of plants treated with *T. asperellum* M_2RT_4 was similar to that of *Trichoderma* sp. MK_4 but greater ($P \leq .05$) than that of the negative control, *T. atroviride* F_5S_{21} and the two *P. lilacinum* isolates (KLF_2 and MR_2).

3.3. Effect of the fungal antagonists on damage and multiplication of Meloidogyne javanica on pineapple plants

Galling index on plants treated with *T. atroviride* F_5S_{21} and the positive control was moderate and significantly greater ($P < .001$) than on plants treated with *Trichoderma* spp. MK_4 and the *P. lilacinum*

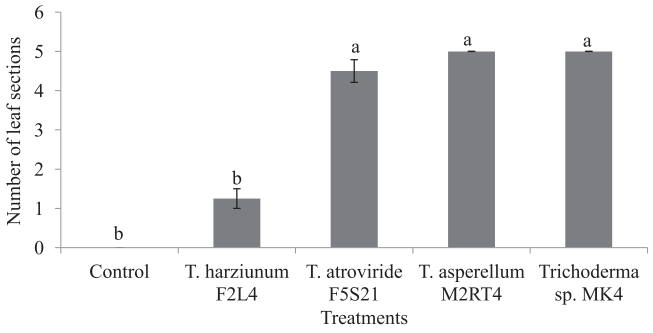

Fig. 2. Mean number of pineapple leaf sections colonized by four local *Trichoderma* isolates, *T. harzianum* (F_2L_4), *T. atroviride* (F_5S_{21}), *T. asperellum* (M_2RT_4) and *Trichoderma* sp. (MK_4), and an untreated control on three weeks old plants. Means followed by same letter (s) are not significantly different ($P \leq .05$).

Table 1
Effect of the fungal antagonists, *Trichoderma* sp. (MK_4), *T. asperellum* (M_2RT_4) and *T. atroviride* (F_5S_{21}) and *Purpureocillium lilacinum* (KLF_2 and MR_2) on fresh root weight of pineapple in pots. Mean values (n = 12) pooled from two experiments repeated in time.

Treatments (T0–T6)	Fresh root weight (grams)*	% Increase
T0 (negative control)**	20.1[b]	54.6
T1 (positive control J2s)***	13.0[c]	–
T2 (*Trichoderma* sp. MK_4 + J2s)	21.2[ab]	63.8
T3 (*T. asperellum* M_2RT_4 + J2s)	24.9[a]	91.5
T4 (*T. atroviride* F_5S_{21} + J2s)	19.5[b]	50.0
T5 (*Purpureocillium lilacinum* KLF_2 + J2s)	18.7[b]	43.8
T6 (*P. lilacinum* MR_2 + J2s)	17.2[b]	32.3

*) Values in the column followed by different letters indicate significant differences among treatments according to Tukey's test (P ≤ .05) per root system. **) Tap water; ***) J2s = 3000 infective stage juveniles of *Meloidogyne javanica*; % Increase (percentage increase of fresh root weight compared to positive control). Each experiment was terminated at 90 days after nematode inoculation.

isolates KLF_2 and MR_2 (Table 2). Galling index on *T. asperellum* M_2RT_4 treated plants was slight and less (P ≤ .001) than for the other *Trichoderma* isolates. There was no galling on negative control plants (Table 2).

All the treatments except *T. atroviride* F_5S_{21} reduced significantly the number of galls compared to the positive control ($F_{(6,77)} = 71.3$; P < .001). The number of galls on plants treated with *T. atroviride* F_5S_{21} (110.0 ± 8.7) was greater (P = .012) than for the positive control. There were also more galls (P < .001) on plants treated with F_5S_{21} and the positive control than for the other fungal treatments. The number of galls for the *T. asperellum* M_2RT_4 treatment was significantly less (P ≤ .002) than for the other fungal treatments. *P. lilacinum* treated plants had a similar number of galls, significantly lower than the positive control (P < .001). There were no galls in the negative control (Table 2).

The number of egg masses on plants treated with *T. atroviride* F_5S_{21} (184.7 ± 14.7) was similar to the positive control (174.7 ± 9.4) but higher than all the other fungal treatments ($F_{(6,77)} = 70.3$; P < .001). The number of egg masses on plants treated with *T. asperellum* M_2RT_4 was similar to *the P. lilacinum* MR_2 but significantly less (P < .001) compared with other fungal antagonist treatments. *Trichoderma* sp. MK_4 and *P. lilacinum* KLF_2 treated plants had a similar number of egg masses, which was less (P < .001) than on the positive control. There were no egg masses in the negative control (Table 2). The number of eggs on plants treated with *T. atroviride* F_5S_{21} (20,920 ± 2016) was higher (P = .03) than for the positive control (15,991 ± 1670); these were also significantly higher than for all the other fungal treatments ($F_{(6,77)} = 72.8$; P < .001). The number of eggs for *T. asperellum* M_2RT_4 plants (1855 ± 169) was less (P ≤ 0.05) than on all other fungal treatments. *Trichoderma* sp. MK_4, *P. lilacinum* KLF_2 and MR_2 had fewer (P < .001) eggs than the positive control. There were no eggs in the negative control (Table 2).

On average, the number of galls was reduced by 81.8% and 60.8% by application of *T. asperellum* M_2RT_4 and *Trichoderma* sp. MK_4 respectively. The number of galls was reduced by 71.6% and 68.7% by the application of *P. lilacinum* MR_2 and KLF_2, respectively. In contrast, application of *T. atroviride* F_5S_{21} led to 30.0% more galls (Table 2).

4. Discussion

Three of the four isolates of the local *Trichoderma* species tested, successfully colonized the pineapple roots endophytically. Various studies have demonstrated the ability of *Trichoderma* spp. to colonize plant roots endophytically (Sharon et al., 2001; Yedidia et al., 1999), which can however differ markedly according to strain (Ahmad and Baker, 1987). The current study confirms that *Trichoderma* spp. will endophytically colonize pineapple roots.

Trichoderma asperellum M_2RT_4 and *Trichoderma* sp. MK_4 effectively suppressed *Meloidogyne javanica* while *T. atroviride* F_5S_{21} did not. Howell (2003) found that strains of *T. koningii,* which were outstanding root colonizers, showed little or no biocontrol activity. Suitability as a biocontrol agent thus needs to consider the broader range of characteristics than the ability to colonize roots. Endophytic *Trichoderma* spp. have previously been shown to suppress the damage caused by nematodes, in part by preventing nematode penetration (Lamovšek et al., 2013). Similarly, the nematicidal activity of *T. asperellum* (T-203) (Sharon et al., 2001) and *T. atroviride* have been confirmed (Sharon et al., 2007), demonstrating that strain, target pest species and host need to be compatible (Al-hazmi and TariqJaveed, 2016), as the *T. atroviride* strain in the current study was not effective against nematodes.

The two isolates of *P. lilacinum* (MR_2 and KLF_2) in our study reduced root galling, egg mass and egg production, and increased host growth, reflecting results by Ganaie and Khan (2010) on tomatoes, when applied 10 days prior to introduction of *M. javanica* inoculum. This fungus is a good rhizosphere competitor (Mukhtar et al., 2013) and its suppressive effect has been variously reported to reduce *M. incognita* and *M. javanica* soil and root populations in tomato (Lara et al., 1996; Siddiqui et al., 2000). Kiewnick and Sikora (2006) demonstrated increased biocontrol efficacy of *P. lilacinus* 251 on *Meloidogyne incognita* in tomato when applied before planting, combined with a seedling drench and second application. According to Rodriguez-Kabana et al. (1984), isolates of *P. lilacinus* differ widely in their biocontrol capacity and ability to establish in soil, emphasising the need to establish compatibility with the local specific circumstances.

This study obtained inoculum from long-term commercially cultivated pineapple fields in Kenya and supported by controlled pot experiments in the greenhouse using cv. Smooth Cayenne, provide strong

Table 2
Effect of the fungal antagonists, *Trichoderma* sp. (MK_4), *T. asperellum* (M_2RT_4) and *T. atroviride* (F_5S_{21}) and *Purpureocillium lilacinum* (KLF_2 and MR_2) on development of *Meloidogyne javanica* on pineapple roots. Mean values (n = 12) pooled from two experiments repeated in time.

Treatments (T0-T6)	Galling index rating[1]	Galls[2] number	% Galls reduction	Egg mass[2] number	% Egg mass reduction	Number of eggs[2]	% Eggs reduction
T0 (negative control)**	1.00[d]	0	–	0	–	0	–
T1 (positive control J2s)***	3.67[a]	84.6[b]	–	174.7[a]	–	15991[b]	–
T2 (*Trichoderma* sp MK_4 + J2s)	2.58[b]	33.2[c]	60.8	70.3[b]	59.8	5531[c]	65.4
T3 (*T. asperellum* M_2RT_4 + J_2s)	2.00[c]	15.4[e]	81.8	37.5[d]	78.5	1855[f]	88.4
T4 (*T. atroviride* F_5S_{21} + J_2s)	3.75[a]	110.0[a]	−30.0	184.7[a]	−5.7	20920[a]	−30.8
T5 (*Purpureocillium lilacinum* KLF_2 + J_2s)	2.25[bc]	26.5[cd]	68.7	64.7[b]	63.0	4408[d]	72.4
T6 (*P. lilacinum* MR_2 + J_2s)	2.25[bc]	24.0[d]	71.6	45.6[c]	73.9	2790[e]	82.6

*) Values in the column followed by different letters indicate significant differences among treatments according to Tukey's test (P ≤ 0.05). [1]per root system, [2]per 5 g root sample.% reduction (Percentage reduction compared to positive control); Galling Index rating (1–5 scale: 1 – no galling; 2 – slight; 3 – mild; 4 – moderate and 5 - severe). The experiment was terminated at 90 days after nematode inoculation. **) Tap water; ***) J2s = 3000 infective stage juveniles of *Meloidogyne javanica*.

support for exploring further the use of fungal biocontrol agents for nematode management on pineapple under field conditions. These results are encouraging, demonstrating significant suppressive effects of the local isolates *Trichoderma asperellum* (M_2RT_2) and the two *Purpureocillium lilacinum* isolates (KLF_2 and MR_2) against *M. javanica* damage on pineapple. The present study is, to our knowledge, the first report on the endophytic colonization of *Trichoderma* spp. on pineapple, and its consequent suppression of root knot nematodes on the crop.

Acknowledgments

The authors are grateful to *icipe* and Kenya Biologics Limited for providing the fungal cultures used in this study. Thanks, are also extended to Dr. Christiaan Kooyman and Chris Beadle for guidance, and to Jane Kimemia, Arthropod Pathology Unit (APU) and laboratory colleagues for technical support. This work was financially supported by Kenya Biologics Limited.

References

Ahmad, J.S., Baker, R., 1987. Rhizosphere competence of *Trichoderma harzianum*. Phytopathology 7, 182–189.
Al-hazmi, A.S., TariqJaveed, M., 2016. Effects of different inoculum densities of *Trichoderma harzianum* and *Trichoderma viride* against *Meloidogyne javanica* on tomato. Saudi J. Biol. Sci. 23, 288–292.
Atkins, S.D., Clark, I.M., Pande, S., Hirsch, P.R., Kerry, B.K., 2005. The use of real-time PCR and species-specific primers for the identification and monitoring of *Paecilomyces lilacinus*. FEMS Microb. Ecol. 51, 257–264.
Correa, V.R., Mattos, V.S., Almeida, M.R.A., Santos, M.F.A., Tigano, M.S., Castagnone-sereno, P., Carneiro, R.M.D.G., 2014. Genetic diversity of the root-knot nematode *Meloidogyne ethiopica* and development of a species-specific SCAR marker for its diagnosis. Plant. Pathol. 63, 476–483.
Coyne, D.L., Nicol, J.M., Claudius-Cole, B., 2014. Practical plant nematology: a field and laboratory guide. In: SP-IPM Secretariat, second edition. International Institute of Tropical Agriculture (IITA), Cotonou, Benin, pp. 1–82.
Dababat, A.A., Sikora, R.A., 2006. Use of *Trichoderma harzianum* and *Trichoderma viride* for the biological control of *Meloidogyne incognita* on tomato. Jordan J. Agric. Sci. 3, 953–961.
Daramola, F., Afolami, S., 2014. Studies on the distribution of plant-parasitic nematodes associated with pineapple in delta, Imo and cross river states of Nigeria. Aust. J. Basic Appl. Sci. 8, 248–256.
Elad, Y., Freeman, S., 2002. Biological control of fungal plant pathogens. In: Esser, K., Benett, J.W. (Eds.), The Mycota A Comprehensive Treatise on Fungi as Experimental Systems for Basic and Applied Research. Springer Press, Germany, pp. 92–109.
FAO, 2012. World production of tropical fruit expected to increase in 2014. accessed 28.03.17. http://www.freshplaza.com/article/101367/World-production-of-tropical-fruit-expected-to-increase-in-2014.
FAOSTAT, 2015. http://faostat3.fao.org/browse/Q/*/E (accessed 28.03.17).
Ganaie, M.A., Khan, T.A., 2010. Biological potential of *Paecilomyces lilacinus* on pathogenesis of *Meloidogyne javanica* infecting tomato plant. Eur. J. Appl. Sci. 2, 80–84.
Gianessi, L.P., Silvers, C.S., Sankula, S., Carpenter, J.E., 2002. Plant biotechnology: Current and potential impact for improving pest management in US agriculture, an analysis of 40 case studies (Online report). Washington, DC: National Center for Food and Agricultural Policy (NCFAP). http://www.ncfap.org/40casestudies.html.
Goswami, B.K., Mittal, A., 2004. Management of root-knot nematode infecting tomato by *Trichoderma viride* and *Paecilomyces lilacinus*. Indian Phytopathol. 57, 235–236.
Goswami, B.K., Pandey, R.K., Rathour, K.S., Bhattacharya, C., Singh, L., 2006. Integrated application of some compatible biocontrol agents along with mustard oil seed cake and furadan on *Meloidogyne incognita* infecting tomato plants. J. Zhejiang Univ. Sci. 7, 873–875.
Harman, G.E., 2000. Myths and dogmas of biocontrol: Changes in perceptions derived from research on *Trichoderma harzianum* T-22. Plant Dis. 84, 377–391.
Harman, G.E., Howell, C.R., Viterbo, A., Chet, I., Lorito, M., 2004. *Trichoderma* species – opportunistic, avirulent plant symbionts. Nat. Rev. Microbiol. 2, 43–56.
Haseeb, A., Kumar, V., 2006. Management of *Meloidogyne incognita-Fusarium solani* disease complex in brinjal by biological control agents and organic additives. Ann. Plant Prot. Sci. 14, 519–521.
HCDA (Horticultural Crops Development Authority), 2008. Horticulture data 2005–2007 validation report. Kenya: HCDA, pp. 1–72. http://www.countrystat.org/country/ken/doc/methodology/horticulturevalidation.pdf (accessed 28.03.17).
Holbrook, C.C., Knauft, D.A., Dickson, D.W., 1983. A technique for screening peanut for resistance to *Meloidogyne arenaria*. Plant Dis. 57, 957–958.
Holland, R.J., Williams, K.L., Khan, A., 1999. Infection of *Meloidogyne javanica* by *Paecilomyces lilacinus*. Nematology 1, 131–139.
Howell, C.R., 2003. Mechanisms employed by *Trichoderma* species in the biological control of plant diseases: the history and evolution of current concepts. Plant Dis. 87, 4–10.
Howell, C.R., Hanson, L.E., Stipanovic, R.D., Puckhaber, L.S., 2000. Induction of terpenoid synthesis in cotton roots and control of *Rhizoctonia solani* by seed treatment with *Trichoderma virens*. Phytopathology 90, 248–252.
Hussey, R.S., Barker, K.R., 1973. A comparison of methods of collecting inocula of *Meloidogyne* spp., including a new technique. Plant Dis. Rep. 57, 1025–1028.
Jonathan, E.I., Rajendran, G., 2000. Biocontrol potential of the parasitic fungus *Paecilomyces lilacinus* against the root-knot nematode *Meloidogyne incognita* in banana. J. Biol. Control 14, 67–69.
Khan, A., Williams, K.L., Nevalainen, H.K.M., 2004. Effects of *Paecilomyces lilacinus* protease and chitinase on the eggshell structures and hatching of *Meloidogyne javanica* juveniles. Biocontrol 31, 346–352.
Khan, A., Williams, K.L., Nevalainen, H.K.M., 2006a. Control of plant-parasitic nematodes by *Paecilomyces lilacinus* and *Monacrosporium lysipagum* in pot trials. Biocontrol 51, 643–658.
Khan, A., Williams, K.L., Nevalainen, H.K.M., 2006b. Infection of plant-parasitic nematodes by *Paecilomyces lilacinus* and *Monacrosporium lysipagum*. Biocontrol 51, 659–678.
Kiewnick, S., Sikora, R.A., 2006. Biological control of the root-knot nematode *Meloidogyne incognita* by *Paecilomyces lilacinus* strain 251. Biol. Control 38, 179–187.
Koech, W., Ithinji, G.K., Kibet, L.K., Ngenoh, E., 2014. Evaluation of allocative efficiency of small scale pineapple (*Ananas comosus*) production in Bureti District, Kenya. Agric. J. 9, 61–67.
Kormelinck, A.G., Janssen, I., 2012. Business case Pineapple West-Kenya-Wageningen UR Centre for Development Innovation, pp 1–15.
Kumar, V., Haseeb, A., Sharma, A., 2009. Integrated management of *Meloidogyne incognita-Fusarium solani* disease complex of brinjal cv Pusa Kranti Ann. Plant Prot. Sci. 17, 192–194.
Lamovšek, J., Urek, G., Trdan, S., 2013. Biological control of root-knot nematodes (*Meloidogyne* spp.): microbes against the pests. Acta Agric. Slovenica 101, 263–275.
Lara, J., Acosta, N., Betancourt, C., Vincente, N., Rodrıguez, R., 1996. Biological control of *Meloidogyne incognita* in tomato in Puerto Rico. Nematropica 26, 143–152.
Lopez-Lima, D., Carrion, G., Núñez-Sánchez, A.E., 2014. Isolation of fungi associated with Criconemoides sp. and their potential use in the biological control of ectoparasitic and semiendoparasitic nematodes in sugar cane. Aust. J. Crop Sci. 8, 389–396.
Mukhtar, T., Hussain, M.A., Kayani, M.Z., 2013. Biocontrol potential of *Pasteuria penetrans*, *Pochonia chlamydosporia*, *Paecilomyces lilacinus* and *Trichoderma harzianum* against *Meloidogyne incognita* in okra. Phytopathologia Mediterr. 52 (1), 66–76.
Oclarit, E.L., Cumagun, C.J.R., 2009. Evaluation of efficacy of *Paecilomyces lilacinus* as biological control agent of *Meloidogyne incognita* attacking tomato. J. Plant Prot. Res. 49, 337–340.
Parsa, S., Ortiz, V., Vega, F.E., 2013. Establishing fungal entomopathogens as endophytes: Towards endophytic biological control. J. Visualized Exp. 74, 1–5.
R Core Team, 2015. R: A language and environment for statistical computing. Vienna, Austria. Available from: http://www.R-project.org (accessed 03.04.17).
Rodriguez-Kabana, R., Morgan-Jones, G., Godoy, G., Gintis, H.O., 1984. Effectiveness of species of *Gliocladium*, *Paecilomyces* and *Verticillium* for control of *Meloidogyne arenaria* in field soils. Nematropica 14, 155–170.
Rohrbach, K.G., Apt, W.J., 1986. Nematode and disease problems of pineapple. Plant Dis. 70, 81–87.
Samuels, G.J., Ismaiel, A., Mulaw, T.B., Szakacs, G., Druzhinina, I.S., Kubicek, C.P., Jaklitsch, W.M., 2012. The Longibrachiatum clade of *Trichoderma*: a revision with new species. Fungal Diversity 55, 77–108.
Seinhorst, W., 1966. Killing nematodes for taxonomic study with hot F.A. 4:1. Nematologica 12, 178–179.
Sharon, E., Bar-Eyal, M., Chet, I., Herra- Estrella, A., Kleifeld, O., Spiegel, Y., 2001. Biological control of the root-knot nematode *Meloidogyne javanica* by *Trichoderma harzianum*. Phytopathology 91, 687–693.
Sharon, E., Chet, I., Viterbo, A., Bar-Eyal, M., Nagan, H., Samuels, G.J., Spiegel, Y., 2007. Parasitism of *Trichoderma* on *Meloidogyne javanica* and role of the gelatinous matrix. Eur. J. Plant Pathol. 118, 247–258.
Shurtleff, M.C., Averre III, C.W., 2000. Diagnosing plant diseases caused by nematodes. In: The American Phytopathological. Society Press, St. paul, USA, pp. 187.
Siddiqui, I.A., Qureshi, S.A., Sultana, V., Ehteshamul-Haque, S., Ghaffar, A., 2000. Biological control of rot-root knot disease complex of tomato. Plant Soil 227, 163–169.
Siddiqui, Z.A., Shaukat, S., 2003. Endophytic bacteria: prospects and opportunities for the biological control of plant-parasitic nematodes. Nematologia Mediterr. 31, 111–120.
Singh, V., Mawar, R., Lodha, S., 2012. Combined effects of biocontrol agents and soil amendments on soil microbial populations, plant growth and incidence of charcoal rot of cowpea and wilt of cumin. Phytopathologia Mediterr. 51, 307–316.
Sipes, B., Caswell-Chen, E., Sarah, J., Apt, W., 2005. Nematode parasites of pineapple. In: Luc, M., Sikora, R., Bridge, J. (Eds.), Plant Parasitic Nematodes in Subtropical and Tropical Agriculture. CABI Publishing, Egham, UK, pp. 709–731.
Stetina, S.R., McGawley, E.C., Russin, J.S., 1997. Extraction of root associated *Meloidogyne incognita* and *Rotylenchulus reniformis*. J. Nematol. 29, 209–215.
Stirling, G.R., 1993. Nematodes. In: Broadley, R.H., Wassman, R.C., Sinclair, E. (Eds.), Pineapple: Pests and Disorders. Queensland Department of Primary Industries, Brisbane, pp. 21–29.
Stirling, G.R., Pattison, A.B., 2008. Beyond chemical dependency for managing plant parasitic nematodes. Examples from banana, pineapple and vegetable industries of tropical and subtropical Australia. Australas. Plant Pathol. 37, 254–267.
Taylon, A.A., Dropkin, H., Marin, G.C., 1956. Perineal patterns of the root-knot nematodes. Phytopathology 46, 26–34.
Tigano, M., De Siqueira, K., Castagnone-sereno, P., Mulet, K., Queiroz, P., Dos Santos, M., Teixeira, C., Almeida, M., Silva, J., Carneiro, R., 2010. Genetic diversity of the root-knot nematode *Meloidogyne enterolobii* and development of a SCAR marker for this guava-damaging species. Plant Pathol. 59, 1054–1061.
UNCTAD, 2016. Pineapple – An INFOCOMM commodity profile unctad.org/en/PublicationsLibrary/INFOCOMM_cp09_Pineapple_en.pdf. (accessed 28.03.17).

Van Damme, V., Hoedekie, A., Viaene, N., 2005. Long-term efficacy of *Pochonia chlamydosporia* for management of *Meloidogyne javanica* in glasshouse crops. Nematology 7, 727–736.
Yedidia, I., Benhamou, N., Chet, I., 1999. Induction of defense response in cucumber plants (*Cucumis sativus* L.) by the biocontrol agent *Trichoderma harzianum*. Appl. Environ. Microbiol. 65, 1061–1070.
Zijlstra, C., Donkers-Venne, D.T.H.M., Fargette, M., 2000. Identification of *Meloidogyne incognita*, *M. javanica* and *M. arenaria* using sequence characterised amplified regions (SCAR) based PCR assays. Nematology 2, 847–853.