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Integrated management of root-knot nematodes in a tomato-maize crop system using the biocontrol fungus *Pochonia clamydosporia*

Nessie D. Luambano [a, *], Rama D. Narla [a], Waceke J. Wanjohi [b], John W. Kimenju [a], Brian R. Kerry [c]

[a] *University of Nairobi, P.O. Box 29053-00625, Nairobi, Kenya*
[b] *Kenyatta University, P.O. Box 43844, Nairobi, Kenya*
[c] *Nematode Interactions Unit, Rothamsted Research, Harpenden, Herts AL5 2JQ, UK*

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ABSTRACT

Integrated approaches are fast gaining popularity in the management of root-knot nematodes (RKN). This study was undertaken to assess the potential of integrating a biological control agent (BCA, *Pochonia chlamydosporia* isolate 10), crop rotation (CR, maize) and organic amendment (OA, maize stover) in the management of RKN in tomato production. One glasshouse and two field experiments were conducted with tomato as the investigated crop. The treatments were applied either singly (OA, BCA and CR) or in combination (BCA + OA, CR + OA, CR + BCA and CR + BCA + OA) and a control where tomato was grown without treatment. Application of CR consistently led to a reduction in the numbers of second-stage juvenile of RKN on roots during the first season, though to be effective in the second season, inclusion of the BCA was necessary. Galling index was also generally reduced by CR. Fruit count in the field experiments increased as a result of combining CR with BCA and combining CR + BCA + OA. In the first field experiment, yield increase was proportional to increase in the number of fruits as a result of CR + BCA and CR + BCA + OA. Application of *P. chlamydosporia* in the field where maize was planted increased the yield of tomato by up to 63% in the first season compared to plots where no nematode management measure was done. This study has demonstrated that rotating maize with tomato can be effective alone and for some benefits, in combination with a biological control agent and organic amendment.

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1. Introduction

Root-knot nematodes (RKN), *Meloidogyne* spp. Goeldi 1892, which cause galls on affected roots leading to reduced efficiency of water and nutrient uptake, are a major problem affecting vegetable crops in sub-tropical and tropical countries (Nono-Womdim et al., 2002). In East Africa, high value crops like tomato (*Solanum lycopersicum* L.), okra (*Abelmoschus esculentus* Moench) and lettuce (*Lactuca sativa* L.) are severely damaged by RKN (Sikora and Fernandez, 2005). Small-scale farmers are particularly affected because their dependence on nematicides has proved to be unreliable, and methyl bromide which was one of the most effective in controlling RKN, has been banned (Abawi and Widmer, 2000). Also, in developing countries where many farmers are resource-poor, nematicides are unaffordable and not always readily available (Akhtar and Malik, 2000). These dilemmas have led to the demand for a more sustainable approach to the management of nematodes.

Crop rotations (CRs) that incorporate the use of non-host crops have been used in many countries to reduce the build-up of RKN (Widmer et al., 2002). However, due to the wide host range of RKN and variation in host susceptibility, these crops have to be selected with care (Kiewnick and Sikora, 2006). Crops like cabbage (*Brassica oleracea* L.), green amaranth (*Amaranthus viridus* L.) and onion (*Allium cepa* L.) are considered poor hosts to RKN (Sikora et al., 2005). Cultivars of maize (*Zea mays* L.) including sweetcorn have also been found to have a low susceptibility to *Meloidogyne incognita* and associated with reduced nematode populations when used in rotation with tomato and chilli (*Capsicum annuum* L.) (Sikora and Fernandez, 2005).

Addition of organic amendments (OA) to the soil has been found

* Corresponding author. Sugarcane Research Institute, P. O. Box 30031, Kibaha, Coast, Tanzania.
E-mail address: nluambano@yahoo.com (N.D. Luambano).

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to be beneficial in management of RKN (Akhtar, 1997). One of the modes of action of OA is to suppress nematode populations through the enhancement of fungal antagonists (Arim et al., 2006). For example, application of a small quantity of dried alfalfa leaves as an OA to soil increased populations of the nematode-trapping fungus *Dactylellina haptotyla* in the rhizosphere and this was associated with a significant reduction ($P < 0.05$) in the population of plant parasitic nematodes (Jaffee, 2004). Cattle manure mixed with the fungus *Pochonia chlamydosporia* applied as an OA to less RKN-susceptible crops (beans [*Phaseolus vulgaris* L.] and cabbage) greatly increased fungal populations prior to the growing of a more RKN-susceptible crop (tomato) in the following season (Atkins et al., 2003). Less RKN-susceptible crops have smaller galls which are more easily infected by the fungal antagonist. Also, during decomposition, some OA release toxic compounds like phenols and fatty acids which have nematicidal properties (Lazarovits, 2001). In addition, OA generally improve soil structure and fertility thus encouraging plant growth (Sikora and Fernandez, 2005) which may promote greater resistance to RKN.

Use of biological control agents has also been considered as one of the options in integrated management of RKN. *P. chlamydosporia* is a biological control agent (BCA) which colonises the egg masses of RKN and has been tested commercially in vegetable production systems (Atkins et al., 2003). When the hyphae get in contact with the egg surfaces, appressoria that penetrate and destroy the eggs can be formed (Lopez-Llorca et al., 2002; Morton et al., 2004). *P. chlamydosporia* is more effective if first incorporated with crops that are less susceptible to RKN but which favour its growth in their rhizosphere (Leij de and Kerry, 1991; Sikora and Fernandez, 2005). Tomato which is susceptible to RKN favours the growth of *P. chlamydosporia* within its rhizosphere; however in some crops which are less susceptible such as kale (*B. oleracea* L.) and bean, fungal development tends to be poor (Bourne and Kerry, 1999). An exception is maize (Bourne et al., 1996) which appears to support high fungal growth in spite of being a poor host to RKN. . As *P. chlamydosporia* is saprophytic, it can grow and live without a host nematode (Dallemole-Giaretta et al., 2012), thus enabling it to increase its population if applied together with OA before the susceptible crop is planted. However, it has been noted that application of *P. chlamydosporia* alone as one of the method in management of RKN did not have positive effects as compared to the use of chemical nematicides alone (Tzortzakakis and Petsas, 2003).

Integrated control that simultaneously applies more than one of the above methods has been proven to be more effective in managing RKN than one method in isolation (Atkins et al., 2003). For example, combining neem oil cake as an OA together with the BCA *Paecilomyces lilacinus* and a chemical nematicide reduced populations of *Meloidogyne javanica* and *M. incognita* more than if each was used alone (Akhtar, 1997). If *P. chlamydosporia* was integrated into a rotational cropping system where the less-RKN susceptible bean was planted and followed by kale and then tomato, populations of RKN were reduced by up to 85% in the next two subsequent bean crops than in untreated micro-plots (Kerry and Bourne, 1996).

The objective of this study was to optimise the use *P. chlamydosporia* for RKN control when integrated with maize as a rotational crop and maize stover as an OA in the context of tomato production. Maize stover, locally available after harvesting of maize, is a convenient material for use as an OA. We tested the hypothesis that integrating *P. chlamydosporia*, crop rotation and organic amendments decreases populations of root-knot nematodes in tomato-maize cropping systems and is associated with higher tomato yields.

2. Materials and methods

2.1. Integrated management of root-knot nematodes

Pot and field experiments were conducted at the College of Agriculture and Veterinary Sciences of the University of Nairobi (Kenya). In both there were eight treatment combinations and two phases for both pot and field experiments (Table 1). In phase I, treatments 1–4 were planted with tomato and treatments 5–8 with maize (CR); chlamydospores of *P. chlamydosporia* isolate 10 (BCA) originally from Brazil and sourced from the Rothamsted culture collection (Isolate Deposit Number IMI133157) were incorporated into treatments 3, 4, 7 and 8 at planting. In phase II, all treatments were planted with tomato and maize stover (OA) was incorporated into the soil in treatments 2, 4, 6 and 8 (Table 1).

The physical and chemical properties of the soil used in this study were analysed in the Soil Science Department, Kabete, Nairobi following the procedure and techniques outlined by Okalebo et al. (2002). The soil was predominantly clay: pH was 6.3, total organic carbon was 2.32%, total nitrogen was 0.24%, extractable phosphorus was 31.7 ppm and exchangeable potassium was 1.80 cmol/kg.

2.1.1. Glasshouse experiment

The pot experiment was conducted in glasshouse conditions where temperature and light were not controlled. For phase I, pots were filled before planting with 1 kg soil taken from the site where the field experiments were conducted and which had a history of build-up of RKN following planting of susceptible crops, including tomato. The soil was evenly mixed before being transferred to the pots. For the BCA treatments, chlamydospores of *P. chlamydosporia* isolate 10 had been previously mixed with the soil at the rate of 5000 chlamydospores/g soil. One pre-germinated tomato (*S. lycopersicum*) var. Roma VF or maize (*Z. mays*) (sweetcorn var. Pacific Hybrix 5) seed was transferred into the appropriate pots (Table 1). This phase of the experiment was terminated after three months where all plant materials were discarded and the soil retained.

For phase II, maize stover at a rate of 5% of the total soil mass (50 g in 1 kg soil) was evenly incorporated into the soil used in the OA treatments; the soil was then returned to the allocated pot. One pre-germinated tomato seed was then transferred to all pots. In both phases, the treatments (Table 1) were arranged in a

Table 1
Distribution of treatments used in Phase 1 and Phase 2 of the glasshouse and field experiments to assess the potential of integrated root-knot nematode strategies in tomato.

Treatment number	Phase 1	Phase 2	Treatment[a]
1	Tomato	Tomato	Control (C)
2	Tomato	Tomato + maize stover	Organic amendment alone (OA)
3	Tomato + Pc 10	Tomato	Biological control agent alone (BCA)
4	Tomato + Pc 10	Tomato + maize stover	Biological control agent and organic amendment (BCA + OA)
5	Maize	Tomato	Crop rotation alone (CR)
6	Maize	Tomato + maize stover	Crop rotation and organic amendment (CR + OA)
7	Maize + Pc 10	Tomato	Crop rotation and biological agent (CR + BCA)
8	Maize + Pc 10	Tomato + maize stover	Crop rotation, biological agent and organic amendment (CR + BCA + OA)

Pc.10 = *Pochonia clamydosporia* isolate 10 used as biological control agent.
[a] OA = organic amendment; BCA = biological control agent; CR = crop rotation.

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