Colonisation of the Rhizosphere of plants which are poor host to root-knot nematodes by the biological agent *Pochonia chlamydosporia*

N. LUAMBANO[1]*, J.W. KIMENJU[2], R. NARLA[2] & J.W. WACEKE[3]
[1] Sugarcane Research Institute, P. O. Box 30031, Kibaha, Tanzania
[2] University of Nairobi, P. O. Box 29053-00625, Nairobi, Kenya
[3] Kenyatta University, P. O. Box, 43844 Nairobi, Kenya
Corresponding author: nluambano@yahoo.com

Abstract Management of root-knot nematodes (*Meloidogyne* spp.,) using fungi that parasitize eggs of root-knot and cyst nematodes has been gaining popularity. Application of this fungus to plants that are poor host to root-knot nematodes has shown good results. This study was conducted to screen plants that support growth of *Pochonia chlamydosporia* on its rhizosphere. Seedlings of cabbage, sunhemp, maize, velvet bean, African marigold and tomato were planted in pots containing sterilized soil which had *Pochonia chlamydosporia*. Thirty days after planting, the fungal propagules in the soil and roots increased significantly (P<0.05) in all plants with the exception of velvet beans. Ninety days after planting, the counts of the fungal propagules taken from the soil were significant higher (P<0.05) in the rhizosphere where maize was planted than in other plants. This study concluded that maize is a promising rotational in system where *P. chlamydosporia* is used as a biocontrol agent.

Key words: *Chalmydosporia*, nematodes, *Pochonia,* propagules, root-knot

Introduction

Root-knot nematodes (*Meloidogyne* spp.) affects many crops due to its wide host range. Traditionally, root-knot nematodes have been managed using nematicides, the most prominent being methyl bromide. Management of root-knoot nematodes by integrating more than one method has gained popularity in recent years due to the absence of new nematicides and the banning of methyl bromide (Sikora *et al.*, 2005; Anastasiadis *et al.*, 2008). Some of the components in this integrated management package include crop rotation, application of organic amendments and biological control. There are however concerns of effectiveness and sustainability of these methods. *Pochonia chlamydosporia* is a fungus of high potential for biological control of root-knot nematodes. It is a facultative parasite of eggs and females of root-knot and cyst nematodes (Atkins *et al*., 2003).

Sustainable use of *P. chlamydosporia* demands that it survives in the soil even in the absence of the susceptible host crop. Ideally, it should colonise and effectively grow in the rhizosphere of non hosts nematodes (Bourne & Kerry, 1999). It has been noted that addition of *P. chlamydosporia* on crops such as cabbage, bean and kale reduced root-knot nematodes in the soil before planting of the susceptible crop (Sikora & Fernandez, 2005). Crops such as these seem to favour fungal growth in the rhizosphere. Ideally, they should increase the chances of the fungus surviving in the soil when rotated with a susceptible hosts (Leij de & Kerry, 1991). In Cuba, chlamydospores of *P. chlamydosporia* are applied to a poor host crop used in rotation with tomato in a vegetable production system to enhance growth and survival of *P. chlamydosporia* (Sikora & Fernandez, 2005).

Integrated management where *P. chlamydosporia* has been incorporated, have been successfully practised in rotation with a poor host followed by a susceptible crop, either in the following year or after two seasons (Atkins *et al.*, 2003). However, the plant species that are poor hosts to root-knot nematodes differ in their ability to allow fungal growth on their root surface and soil (Bordallo *et al.*, 2002). Selection of a rotational crop where *P. chlamydosporia* will be incorporated in the soil has to consider the capacity of the poor host to fungal growth and reproduction. There is therefore a need for identifying poor hosts of the target nematode that are suitable for colonisation by *P. chlamydosporia*. This study was undertaken with the aim of screening different locally grown and commonly found plants for their support of growth and multiplication of *P. chlamydosporia* in their rhizosphere.

Materials and Methods

This experiment was carried out in a glasshouse using five different plant species known to be poor hosts to root-knot nematodes and which can be rotated with tomato. The plants tested were cabbage (*Brassica oleracea),* maize (*Zea mays)*, African marigold *(Tagetes minuta)*, sunn hemp (*Crotalaria ochroleuca)* and velvet beans *(Mucuna pruriens)*. The experiment had six treatments (five poor host crops and tomato as a control) arranged in a randomised complete block design (RCBD) and was replicated five times. Seeds of the plants were pre-germinated in Petri dishes and transferred into 500g capacity pots containing loam soil mixed with sand at 3:1 w/w. The potted soil was inoculated with *P. chlamydosporia* at a rate of 5000 chlamydospores/g of

soil making a total of 2,500,000 chlamydospores per pot. The plants were kept watered to ensure that normal growth. At 30 and 90 days after application of the fungus into the soil. Soil and root samples were collected and used to estimate the fungal population (CFU) in soil following a method as described by Kerry & Bourne (2002). One gram of soil was suspended in nine millilitres of water agar (0.05% agar) and diluted to 10^{-2} and 10^{-3}. From the dilutions, 0.2ml was cultured on semi-selective media (17g corn meal agar, 17.5 sodium chloride, 75mg rose Bengal, 50mg streptomycin sulphate, 50mg chloramphenical, 50mg chlortetracycline, 37.5mg thiabendazole, 37.5mg carbendazim, 3ml Triton X-100) in Petri dishes and incubated at 25°C for 14 days. After incubation, the colonies of *P. chlamydosporia* were counted and were estimated as number of fungal propagules per gram of soil. Numbers of fungal propagules in the roots were also estimated per gram of ground roots following the same procedures used for soil.

Data analyses. All data were Log_{10} transformed and analysed using one way analysis of variance using GenStat package version 11. Means were compared using least significance differences (LSD).

Results

At 30 days after fungal application, the plant effect on root and soil fungal populations were significant ($P<0.05$), (Table 1). Fungal propagules in soil and roots increased in all treatments with the exception of velvet beans which had low CFU in both roots and soil. Again, at 90 days after infestation, test plant effects were significant ($P<0.001$) for the numbers of *P. chlamydosporia* propagules in the soil and root tissues. The root CFU counts after indicated that cabbage, velvet beans and maize had the higher fungal propagule population while tomato and sunn hemp had no fungal propagules at all (Table 1). In soil, the CFU counts 90 days after infestation were highest in the maize rhizosphere compared to the other plants.

Discussion

This study has demonstrated that different plants differ in their capacity to support growth of *P. chlamydosporia* on their roots and in their rhizosphere. All the crops tested had some ability to support fungal growth on their root surfaces for at least 90 days with the exception of sunn hemp and tomato. The decline in fungal populations associated with sunn hemp and tomato can be attributed to the fact that the root exudates they release may not attract the fungus. According to Kerry (2000) root exudates from tomato infected by root-knot nematodes contain more water soluble and several metal ions which support more colonisation of *P. chlamydosporia* than healthy roots. The decline may have also been occasioned by the degradation of nutrients in the rhizosphere and their subsequent uptake by the growing plants. This study has shown that sunn hemp and tomato do not support external root colonisation of the fungus for an extended period of time. Inability of these plants to support *P. chlamydosporia* for extended periods implies that one time application of the fungus may not sustain high enough fungal propagules to control root-knot nematodes. Addition of *P. chlamydosporia* to crops such as that are poor in supporting fungal growth in their rhizosphere may not be useful (Bordallo *et al.*, 2002).

A similar trend was observed for the fungal populations in soil in the crops tested which increased after 30 days of application and subsequently decreased three months after fungal application in some. These results suggest that, different plant species have different capacities for supporting growth and survival of the fungus in their rhizosphere. Variability in the ability of different plants to support the fungus in the rhizosphere has been reported by several authors (Bourne & Kerry, 1999; Kerry, 2000; Vargas-Ayala *et al.*, 2000). In this experiment maize and cabbage exhibited good support for fungal establishment and growth in their rhizosphere. In addition, maize was able to maintain a high fungal population density in the soil and roots, throughout the growing period.

The differences in capacity of a different plants to support fungal growth could be due to differences in availability of chemical substances such as glucose and nitrogen emanating from the host plant, that might either promote or suppress fungal growth (Kerry, 2000). It has been noted that maize supports a higher degree of fungal growth, even in the presence of high nematode populations compared to tomato (Bourne & Kerry, 1999). From this study, maize has been proved to be for a

Table 1. Colonisation of *Pochonia chlamydosporia* isolate 10 in the soil and in roots of six different crops 30 and 90 days after application.

Crop	CFU /g at 30 days		CFU/g at 90 days	
	Root	Soil	Root	Soil
Cabbage	67500(4.83)	392086(5.58)	13250(4.11)	284(1.38)
Sunn hemp	85000(4.92)	500133(5.70)	0(0.00)	0(0.00)
Maize	110000(5.04)	369507(5.57)	12250(4.04)	2245(3.34)
Velvet bean	10000(2.15)	10478(2.16)	12500(4.09)	307(1.39)
African marigold	20000(4.29)	201323(5.27)	4250(3.62)	283(1.38)
Tomato	182500(5.26)	164889(5.21)	0(0.00)	0(0.00)
LSD	1.55	1.52	0.19	1.36

Values in brackets are Log_{10} transformed means from five replications.

promising crop to be used in rotational cropping systems where *P. chlamydosporia* is used as a biocontrol agent. The use of maize as a rotational crop may provide double effect on root-knot nematodes management; first as a poor host to root-knot nematodes and secondly a better host to this fungus hence suppressing the nematode populations (Bourne *et al.*, 1996). A report by Desaeger & Rao (2000) shows that rotating maize with a root-knot nematode susceptible crop (sesbania) significantly reduced root-knot nematodes infestation. Moreover, in Sub-saharan Africa maize is a readily acceptable crop to smallholder farmers since it improves food security this will make them easily grow in rotation with other nematode susceptible crops.

Acknowledgement

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