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Occurrence of Entomopathogenic Nematodes and their Potential in the Management of Diamondback Moth in Kale

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Abstract: This study was aimed at determining the occurrence of entomopathogenic nematodes (EPNs) in different agroecosystems and their potential as biocontrol agents in the management of DBM. Soil samples were taken from a planted forest, pasture, a coffee field and a vegetable garden. EPNs were isolated from the soil using *Galleria mellonella* as the bait insect. Laboratory bioassays were conducted to determine the lethal time fifty (LT_{50}), which is time till 50% lethality, of the EPN isolates to DBM larvae using the leaf disc bioassay method. Five isolates of EPNs namely *Heterorhabditis indica*, *Steinernema karii*, *Steinernema wesieri*, *Steinernema* sp. and *Heterorhabditis* sp. were used. The frequency of occurrence of EPNs was lowest, 27%, in the soil from vegetable garden, followed by forest soil, 33%. EPNs were present in 50 and 77% of the soil samples from pasture and coffee ecosystems, respectively. The LT_{50} of *S. karii*, *H. indica* and *S. wesieri* was 38.10, 20.27 and 23.80 h, respectively. *Heterorhabditis indica*, *S. karii*, *S. wesieri*, *Steinernema* sp. and *Heterorhabditis* sp. caused 96.0, 93.3, 92.0, 88.0 and 86.7% mortality in the DBM larvae within 72 h, respectively. This study has demonstrated that the frequency of occurrence of EPNs is different in various agroecosystems. The study has also showed that EPNs have a great potential that may be exploited along with other suitable strategies in integrated management of DBM.

Key words: Biocontrol agent, *Heterorhabditis* spp., lethal time fifty, *Plutella xylostella*, *Steinernema* spp.

INTRODUCTION

Entomopathogenic nematodes (EPNs) in the genera *Steinernema* and *Heterorhabditis* are effective biocontrol agents against several groups of pest insects, such as sciarid flies, weevils, scarab grubs thrips and mole crickets. These nematodes are also known to infect a number of lepidopterous species where they are able to recycle in the infected host and hence persist in the environment for longer periods of time (Tomalak, 2003). The diamondback moth (*Plutella xylostella* L.) is a major pest of cabbage, particularly in tropical regions. Excessive use of chemical insecticides against this pest has promoted the development of insecticide resistance (Schroer *et al*., 2005).

Entomopathogenic nematodes (EPNs) are aggregated rather than randomly distributed in the soil as demonstrated by Spiridonov and Voronov (1995). The prevalence (percentage of samples that are positive) and intensity (number of individuals in a sample) of EPNs can vary with time, crop grown and soil type (Hummel *et al*., 2002). There are dominant species and comparatively rare ones. Factors influencing the motility and survival of infective juveniles are soil moisture, temperature, soil texture, soil pH and biotic factors (Glazer, 2002). EPNs require adequate soil moisture for survival and movement, but too much moisture may cause oxygen deprivation and restrict movement (Glazer, 2002). Studies have shown that EPN survival is poor once water has been lost from the substrate (Solomon *et al*., 1999). Soil temperature can have a great effect on nematode activity (Shapiro and McCoy, 2000). Optimum temperatures for infection and reproduction vary among nematode species and strains (Hazir *et al*., 2001). Soil pH in most agroecosystems, having a range of 4-8, is not likely to have any significant effect on EPNs, but a pH of 10 or higher is likely to be detrimental (Kung *et al*., 1990a). Soil texture affects nematode movement and survival (Glazer, 2002). Generally, compared with lighter soils, soils with higher clay content restrict nematode movement and have potential for reduced aeration, which can result in reduced nematode survival and efficacy. Survival decline most rapidly in clay soil followed by clay loam and sand or sandy loam (Kung *et al*., 1990b).

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Various agricultural land use systems are characterized by a varying degree in the use of farm inputs. When applied at recommended rates, most fertilizers have little impact on EPN efficacy (Shapiro *et al*., 1996). However, fresh manure or high rates of chemical fertilizers (e.g., urea) can have detrimental effects to EPNs survival and efficacy (Mannion *et al*., 2000). Lack of physical disturbance and favourable soil conditions, as characterized by stable land use systems, favours the success of control attempts using EPNs (Shapiro-Ilan *et al*., 2002). Under a conventional tillage regime, the soil surface tends to have greater fluctuations in temperature and moisture than under no-tillage or reduced tillage management and EPNs are often more frequently detected in reduced tillage regimes (Millar and Barbercheck, 2002). *Steinernema carpocapsae* Weiser has been used in leaf bioassays and was able to cause mortality in the DBM larvae (Schroer and Ehlers, 2005). Much of the research has been done to determine the occurrence of EPNs in different geographical regions. There is little information on the influence of agroecosystems on the occurrence of EPNs. The aim of this study was to determine the occurrence of EPNs in different agroecosystems and their potential as biocontrol agents in the management of diamondback moth.

MATERIALS AND METHODS

Soil samples were taken from a coffee field, vegetable garden, pasture and a planted forest in the month of May, 2006 to June, 2007 at the University of Nairobi, Kabete, Kenya. The vegetable garden was taken to represent a highly disturbed habitat in terms of tillage, short-term (four months) crop rotation regimes and where fresh manure and chemical fertilizers and pesticides are regularly used. The coffee field represented moderate disturbance in terms of tillage and an area where few chemical fertilizers and pesticides are used. Pasture and planted forest habitats were taken to represent areas where no chemical fertilizers and pesticides are used and where minimal local disturbance has occurred. The sampling areas were subdivided into three blocks. Three sampling points along each block at 10 m intervals were identified using a measuring tape. Soil samples were taken at a depth of 5-30 cm (where nematodes are abundant) from three points making a triangular grid in each sampling point. Soils from the three points were mixed together to make a composite sample. The composite sample was subdivided into two sub-samples and EPNs were isolated from the soil using the baiting technique. The soil samples were put in shallow dishes and then six final instar of greater wax moth, *Galleria mellonella*, were placed into each soil sample. The soil was incubated at room temperature for five days. The soils were kept moist during incubation. Consecutive exposures of *Galleria mellonella* to the soil samples was done to increase recovery of EPNs considering that not all the nematodes infect bait insects at the same time.

The dead *G. mellonella* larvae were recovered from the soil after five days, washed with distilled water and then examined for nematode infestation before being transferred to the white's trap. The cadavers were transferred into white's traps for seven days to allow the Infective Juveniles (IJs) to move out of the cadaver into the surrounding water. The nematode suspension was harvested and stored in shallow dishes. The EPNs collected were exposed to fresh *G. mellonella* larvae to confirm infectivity. Aliquots of the nematode suspension were pipetted and transferred into nematode counting dishes for observation under a dissecting microscope. Soil from where the EPNs were isolated was tested for pH, organic matter content and texture.

Insect mortality bioassays were conducted with five different isolates of EPNs; *Heterorhabditis indica*; *Heterorhabditis* sp.; *Steinernema wesieri*; *Steinernema karii* and *Steinernema* sp. as treatments. Ten 3rd instar DBM larvae were placed in 9 cm filter paper-padded-petri dishes and then 2 mL of nematode suspension containing 200 IJs mL^{-1} of each isolate was applied onto the filter papers as described by Rosa *et al*. (2002). Kale leaves were washed with distilled water and cut into 9 cm leaf discs and allowed to air-dry for 45 min before being used as the nutrient medium in the petri dishes. The petri dishes were incubated at room temperature for 72 h from the start of the experiment in a completely randomized design with five replications. A control where 0.5 mL of distilled water was used to wet the filter paper before placing the DBM larvae and kale leaf disc was included. Data on mortality was collected after 24, 48 and 72 h from the start of the experiment.

RESULTS

Soils from the forest and land under vegetables had the least number of positive samples for nematodes compared to the soils from the coffee field and pasture (Table 1). The dominant species in all the soils from coffee, forest and pasture was *Steinernema* spp. except in vegetable garden, which had *Heterorhabditis* spp. as the dominant species. The soil from the coffee field had the highest number of EPNs compared to the soil from other agroecosystems.

Table 1: Occurrence of entomopathogenic nematodes in different agroecosystems at Kabete, Nairobi, Kenya (2007)

Nematode species found	No. of positive soil samples (%)	No. of samples	Textural class	Carbon	Sand	Silt	Clay	pH 0.01 M $CaCl_2$	Source of soil sample
				---------- (%) ----------					
Steinernema spp. (Major) *Heterorhabditis* spp. (Minor)	6 (33%)	18	Clay	3.78	14	32	54	5.37	Forest
Steinernema spp. (Major) *Heterorhabditis* spp. (Minor)	9 (50%)	18	Silty clay	2.98	14	42	44	5.29	Pasture
Steinernema spp. (Major) *Heterorhabditis* spp. (Minor)	14 (77%)	18	Clay	2.92	12	38	50	4.52	Coffee
Steinernema spp. (Minor) *Heterorhabditis* spp. (Major)	5 (27%)	18	Silty clay loam	2.88	14	48	38	5.66	Vegetable garden

Galleria traps were used in extraction of the entomopathogenic nematodes from soil

Table 2: Lethal time fifty (LT_{50}) levels of the EPNs on 3rd instar larvae of diamondback moth

Nematode species	Estimated LT_{50}±SE	Fiducial limits	
		Lower 95%	Upper 95%
S. wesieri	23.83±5.41	10.89	31.44a
H. indica	20.27±5.00	8.27	27.36a
Heterorhabditis sp.	31.61±4.14	22.35	37.96ab
Steinernema sp.	26.93±5.18	14.73	34.32ab
S. karii	38.12±2.30	33.45	42.24b

*S = *Steinernema* and H = *Heterorhabditis*; Values followed by the same letter(s) within a column are not significantly different

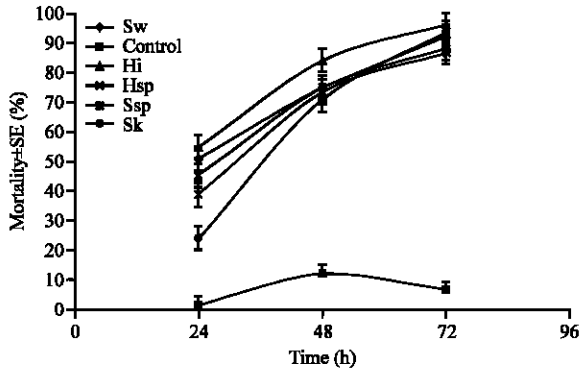

Fig. 1: Percentage mortality of 3rd instar larvae of diamondback moth caused by exposure to entomopathogenic nematodes (*Steinernema wesieri*: Sw; *Heterorhabditis indica*: Hi; *Heterorhabditis* sp.: Hsp; *Steinernema* sp.: Ssp and *Steinernema karii*: Sk) for varying durations

All the EPN isolates tested caused mortality to the DBM larvae (Fig. 1). *H. indica*, *S. karii*, *S. wesieri*, *Steinernema* sp. and *Heterorhabditis* sp. caused up to 96.0, 93.3, 92.0, 88.0 and 86.7% mortality, respectively, within 72 h. The time of exposure of the various EPN isolates tested had significantly variable ($p<0.05$) effects on mortality of the 3rd DBM instars. The proportion of DBM larvae mortality increased with increased exposure time to all the EPNs.

The time taken for half the number of (LT_{50}) *G. mellonella* to die on exposure to the EPN isolates was significantly variable (Table 2). *Steinernema karii* recorded the highest LT_{50} (38.1 h) compared to *H. indica*, which recorded the lowest LT_{50} (20.3 h). *Steinernema wesieri*, *Steinernema* sp. and *Heterorhabditis* sp. had intermediate LT_{50} values of 23.8, 26.9 and 31.6 h, respectively.

DISCUSSION

Frequency of occurrence of EPNs was lowest in the intensively cultivated vegetable garden compared to the pasture, coffee and planted forest agroecosystems. This indicates that EPNs might be less adapted to highly disturbed soils characterized by frequent tillage, high agrochemical input use and frequent fluctuations in environmental conditions. Regular cultivation in the vegetable garden exposes nematodes to desiccation and lethal radiations of the sun. In addition, organic amendments, chemical fertilizers and pesticides are commonly used in vegetable production and these may have had detrimental effects on EPNs. Shapiro *et al.* (1999) have reported that fresh manure or high rates of chemical fertilizers such as urea can be detrimental to survival and efficacy of EPN. Several studies have been conducted on the effects of agrochemicals including acaricides, fungicides, herbicides and insecticides on different species of entomopathogenic nematodes. While some chemical pesticides such as dodine, methomyl and parathion have proven to be toxic to EPNs.

Frequency of occurrence of EPNs was moderate in forest soil. This may be due to the fact that the forest soil had a high proportion of clay, which is prone to poor aeration and high water retention, a situation that limits their chances of survival. Millar and Barbercheck (2002) have shown that soil texture affects nematode movement and survival. Other researchers have reported that clay soils restrict nematode movement and are poorly aerated, which results in reduced nematode survival and efficacy when used as biocontrol agents (Shapiro-Ilan *et al.*, 2002). As expected, forest soil also had the highest amount of organic matter, which helps in proliferation of microbes some of which may be antagonistic to EPNs. Kaya (2002) has demonstrated that EPNs are subject to infection or predation by certain phages, bacteria, protozoans, nematophagous fungi, predacious mites and nematodes.

Surprisingly, despite the high clay content, the frequency of occurrence of EPNs was highest in soils from coffee ecosystem. This may be attributed to the moderate disturbance in terms of tillage and low amounts of inorganic fertilizers and pesticides that are applied. Given the dense canopy in coffee fields, there is little fluctuation in soil temperature and moisture, which favours nematode survival. In addition, exceptions have been recorded on the effect of clay content on the population of EPNs whereby survival and efficacy of the EPNs increased in clay soils (Shapiro *et al*., 2000). Soil pH in most agroecosystems, having a range of 4-8, is not likely to have any significant effect on EPNs, but a pH of 10 or higher is likely to be detrimental (Kung *et al*., 1990a). In this study, the pH range of the soils was 4.5-5.7 which was well within the range that is considered tolerable by the EPNs.

The dominant nematode species in all the soils was *Steinernema* spp. This indicates that *Steinernema* spp. are probably more versatile compared to *Heterorhabditis* spp. In addition, this implies that *Steinernema* spp. are more persistent and have a high recycling ability (ability to initiate a new life cycle). The high recycling ability of *Steinernema* spp. can be explained by their mode of reproduction: *Steinernema* spp. are amphimictic whereas *Heterorhabditis* spp. are hermaphrodites. Research has revealed that nematode species competitively exclude one another, depending on the host species present, with the steinernematids being intrinsically superior competitors to the heterorhabditids (Kaya, 1990). Spiridonov and Voronov (1995) have also reported that there are dominant species and comparatively rare ones.

All the five isolates of EPNs tested caused mortality in the DBM larvae. This implies that the isolates were favourable hosts of DBM larvae and that the symbiotic bacteria associated with the nematodes are lethal to the larvae. These findings are in agreement with those of other workers who have also reported the efficacy of different *Steinernema* spp. and *Heterorhabditis* spp. against DBM (Belair *et al*., 2003; Mahar *et al*., 2004; Somvanshi *et al*., 2006). A linear increase in the percentage mortality of DBM with increase in exposure time was observed. At 24 h, the percentage mortality of the DBM larvae was lowest for all the EPN isolates and highest at 72 h for all the EPNs. This is because bacterial infection starts after the penetration of the nematodes into the body cavity of the insects. Therefore, infective nematode juveniles must locate the insect host and gain entry into the haemocoel. However, Dowds and Peters (2002) have shown that nematode juveniles encounter various behavioural and mechanical forms of resistance during entry into the insect hosts. These behaviours include rubbing with an abrasive raster situated on the ventral end of the abdomen, brushing with prolegs or mouthparts and scraping and chewing motions with their mandibles (Koppenhöfer *et al*., 2000).

This study has shown that disturbance of soil in terms of tillage and soil organic matter content have an influence on the occurrence of EPNs. The study has also demonstrated that frequency of occurrence of EPNs is different for various agroecosystems. This study is also a clear demonstration that EPNs have a potential that may be exploited in the management of DBM.

CONCLUSION

A series of field trials would be appropriate to ascertain the results. More work also needs to be done on the economics of using EPNs in the management of DBM. This is because two basic elements are necessary for EPNs to be successfully used: a suitable nematode for the target pest and favourable economics.

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