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Research Paper

Occurrence of entomopathogenic nematodes to manage coffee thrips, *Diarthrothrips coffeae* (Thysanoptera: Thripidae) in Kenya

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Abstract. Coffee thrips, *Diarthrothrips coffeae* (Thysanoptera: Thripidae) is a major pest that adversely affects the yield and quality of coffee in Kenya. Its control mainly by application of insecticides has been a challenge. The use of Entomopathogenic nematodes (EPNs) as a component of biological control agents is anticipated to be a more viable option for the management of thrips hence the need to establish the species of EPNs locally available in Kenya. The existing local EPNs were established from the soil samples collected from shaded and un-shaded coffee farming systems from coffee estates managed by Coffee Research Institute. The samples were collected along the coffee drip lines at a depth of 0 to 20 cm in an area of 25 m^2 (5 m × 5 m) by using a garden hoe. The EPNs were extracted from the soil using the baiting technique. The colour of the cadavers was used to distinguish the type of EPNs collected from the soil samples. The soil moisture content for each sampling site was measured using a soil moisture probe meter. The soil samples were also analyzed for pH in the laboratories. Two types of Entomopathogenic nematodes, *Steinernema* spp and *Heterorhabditis* spp were identified and established to occur locally under shaded and un-shaded coffee farming systems. Their distribution diverged with coffee habitats. The two species preferred the un-shaded coffee when compared to shaded ones. Of the two types of EPNs, *Steinernema* spp (62.5%) was the predominant when compared with *Heterorhabditis* spp (37.5%). The soils from un-shaded coffee had significantly ($P < 0.05$) lower moisture content than shaded coffee. This factor was found to contribute more to the EPNs under un-shaded coffee than shaded ones. The study showed that EPNs occurred in both un-shaded and shaded coffee farming systems. Following the establishment and the occurrence of the EPNs that can manage *D. coffeae,* the authors recommend further research in order to determine their pathogenicity.

Keywords: *Diarthrothrips coffeae*, Entomopathogenic nematodes, *Steinernema* spp., *Heterorhabditis* spp.

INTRODUCTION

Coffee thrips, *Diarthrothrips coffeae* (William's) (Thysanoptera: Thripidae) (Figure 1) is the most damaging species of thrips in Kenya during the hot/dry season of the year (Le Pelley, 1968). Coffee thrips adversely affect the production of coffee in Kenya. Mugo *et al.* (2013) stated that *D. coffeae* is more prevalent in un-shaded coffee than in shaded coffee. Its management strategy mainly involves the application of insecticides that unless repeated frequently are not effective. This ineffectiveness is associated with their small size, swift mobility, feeding behavior, protected eggs and pupae stages inhabiting the soil or leaf debris (Pesticide Action Network, 1998). Frequent insecticides spraying has several negative impacts that include residual effects, insecticides resistance, human health and environmental pollution.

Options to manipulate the environment to reduce *D. coffeae* infestations have been attempted without encouraging results. These include the use of shade trees to regulate the hot/dry weather conditions and organic mulch to improve the soil moisture that prevents thrips pupation in the soil (Le Pelley, 1968). Another strategy in managing the thrips is the use of biological control agents

Figure 1. Coffee thrips, *Diarthrothrips coffeae.*

where *Trichogramma* sp. (Hymenoptera: Trichogrammatidae) parasitize eggs, *Flanklinothrips megalops* Trybom (Thysanoptera: Aeolothripidae) predate thrips and *Euesius kenyae* Swirski and Ragusa (Acari: Phytoseiidae) that predate the nymphs and adults (Coffee Research Foundation, 2009; Mugo *et al.*, 2012).

The use of biological control agents is a viable method in pests' management. This follows the increasing awareness of the adverse effects of pesticides use and their impact on human health and the environment (Lacey *et al*., 2001). One of the key options in biological control is the use of Entomopathogenic nematodes (EPNs). This option is safe since EPNs have no effect on human health, and are environmentally friendly and easily isolated, produced in mass and applied using standard spray equipment. There are two known genera; *Steinernema* and *Heterorhabditis,* which belong to entomopathogenic nematodes. The genus *Steinernema* has about 70 species (Nguyen and Buss, 2011; Orozco and Stock, 2013), while *Heterorhabditis* has about 25 species (Plichta *et al.*, 2009; Edgington *et al.*, 2011).

Normally, Entomopathogenic nematodes are parasites of a nematode-bacterium complex. The EPNs are soil-inhabiting and the most effective biological control organisms of soil and above-ground pests (Kaya and Gaugler, 1993; Laznik *et al*., 2010). They control a wide range of insect pests such as cutworms, thrips and beetles among others (Kaya and Gaugler, 1993). Their distribution is primarily limited by the availability of susceptible hosts (Lewis, 2002). Agricultural cropping systems such as monoculture, crop rotation and intercropping affect the occurrence of EPNs (Kawaka *et al*., 2011). Other factors such as soil fertility, management practices, moisture, temperature and pathogenicity for the targeted pest account for the effective use of EPNs as biological control agents (Kawaka *et al*., 2011; Kung *et al*., 1991; Grewal *et al.*, 2005). Hence, the objective of this study was conducted to determine the existence of local Entomopathogenic nematodes under coffee farming systems, suitable to manage *D. coffeae* in Kenya.

MATERIALS AND METHODS

Study sites

The study was conducted at Jacaranda and Azania coffee estates located in the main coffee growing areas in Kiambu County, Kenya (Figure 2). Jacaranda farm lies at co-ordinates 1° 5.599' to 1° 5.900' South and 36° 53.456' to 36° 54.485' East at an elevation of 1608 m above sea level and Azania farm lies at co-ordinates 1° 3.050' to 1° 3.274' South and 36° 58.819' to 36° 59.296' East at an elevation of 1568 m above sea level. A preliminary soil sampling was carried out in December 2016 and a final one in September 2017. The study sites are within the main coffee growing area of Kenya, Upper midland 2 (UM2). Both Jacaranda and Azania estates experience an annual mean temperature of 13.2°C minimum and 24.8°C maximum with a bimodal rainfall of 1465 mm. The rainfall occurs in the months of April-June and October-December for long rains and short rains, respectively. The soils are of friable clays, well-drained and extremely deep.

Determination for the occurrence of Entomopathogenic nematodes

Soil sampling was conducted from shaded and un-shaded coffee habitats (Figure 3) from both Jacaranda and Azania coffee estates. Each estate had two sampling sites that represented shaded and un-shaded coffee giving four (4) sites. The shaded coffee habitat was coffee growing under *Grevillea* sp. and *Cordia africana* while the un-shaded coffee was coffee growing in the open sun. In each site, 20 soil samples were collected. The samples were collected along the coffee drip lines at a depth of 0 to 20 cm using a fork-jembe (Garden hoe) in an area of 25 m^2 (5 m × 5 m). A soil sample composed of five (5) sub-samples was combined and thoroughly mixed to form a composite sample. One (1) kg of the soil sample was placed in a properly labeled plastic bag, then transferred into a cooling

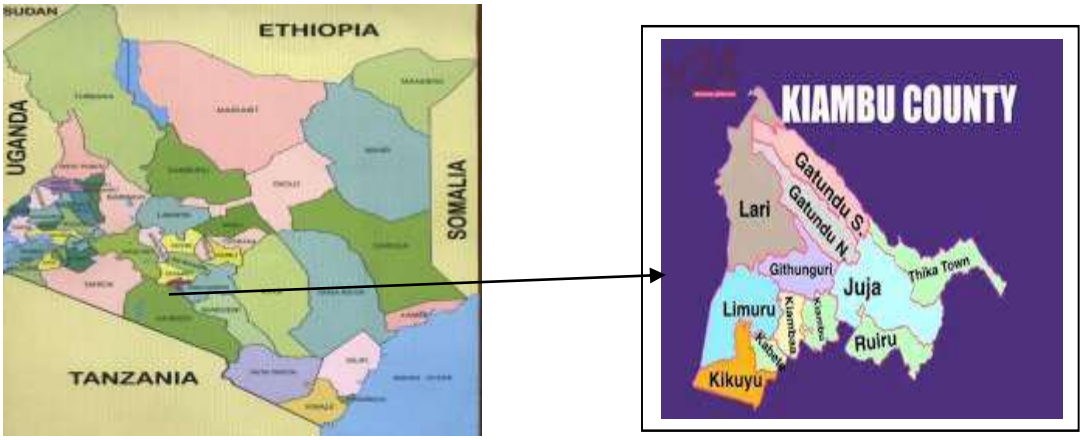


Figure 2. Map of Kenya (0.0236° S, 37.9062° E) showing Kiambu County and its sub-counties (Source: Geographic information services).

a **b**

Figure 3. Shaded (a) and unshaded (b) coffee habitats.

box and later transported to the Coffee Research Institute (CRI) laboratory for EPNs extraction. During soil sampling, soil moisture content was measured using a soil moisture probe meter for each sample area. A portion of each soil sample was analyzed for pH at the CRI laboratories. Information on sampling date, location, altitude, longitude and habitat was recorded for each soil sample.

Extraction of EPNs from the soil

Using the baiting technique, about 200 g of each soil sample was placed in 300 ml sterile plastic bottles. Five (5) last instar (5^{th}) wax moth larvae were added to each bottle. The bottles were placed in an inverted position in the dark. After 5 to 10 days, dead larvae (cadavers) were recovered, recorded and examined for EPNs occurrence. The cadavers were categorized and recorded according to body colour change. Cadavers exhibiting yellow-brown colour were recorded to harbour EPNs of *Steinernema* spp *(*Kaya and Gaugler, 1993). Those exhibiting purple or brick-red colour were recorded to harbour EPNs of *Heterorhabditis* spp *(*Kaya and Gaugler, 1993).

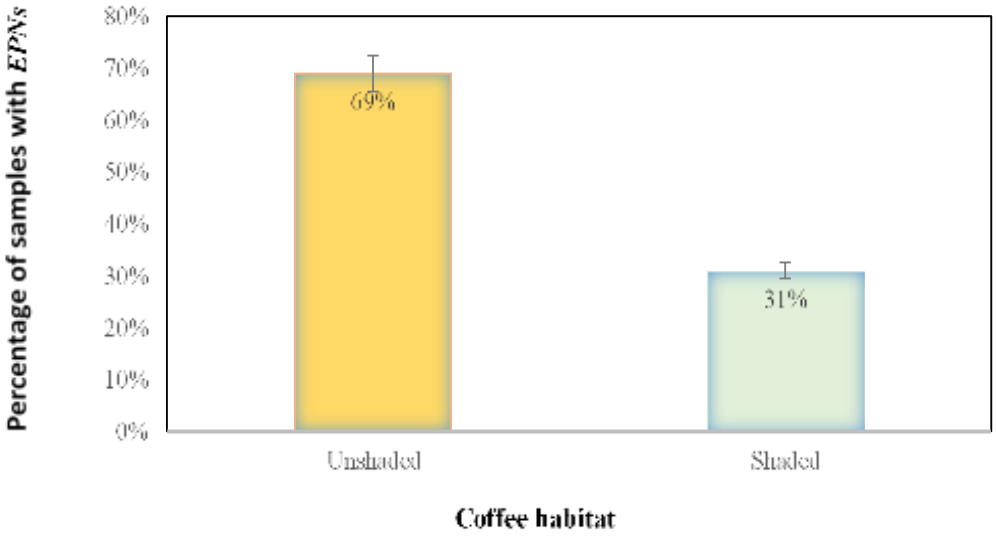
RESULTS AND DISCUSSION

Eighty soil samples were collected (20 per site). Sixteen (16) samples representing 20% of the collected samples, tested positive for the occurrence of EPNs (Table 1). The un-shaded coffee habitat had a significantly ($P < 0.05$) higher number of samples (69%) with EPNs than samples (31%) from shaded habitat (Figure 4).

Two types of Entomopathogenic nematodes, *Steinernema* spp and *Heterorhabditis* spp were identified and established to occur locally in shaded and un-shaded

Table 1. Occurrence of Entomopathogenic nematodes in sampling sites.

Coffee habitat	No. soil samples	EPNs present (%)	EPNs absent (%)
Jacaranda shaded	20	4 (20)	16 (80)
Jacaranda un-shaded	20	6 (30)	14 (70)
Azania shaded	20	1 (5)	19 (95)
Azania un-shaded	20	5 (25)	15 (75)
Total	80	16 (20)	64 (80)



Figures 4. Percentage of samples with EPNs from different coffee habitats.

Table 2. Identified EPNs and their distribution in coffee habitats.

Coffee habitat	Species of EPNs		Total samples with EPNs
	***Steinernema* spp.**	***Heterorhabditis* spp.**	
Jacaranda shaded	2	2	4
Jacaranda un-shaded	3	3	6
Azania shaded	1	0	1
Azania un-shaded	4	1	5
Total	10 (62.5%)	6 (37.5%)	16

coffee habitats in Kenya (Table 2). The distribution of the two species differed with coffee habitats. The two species were most common under un-shaded coffee when compared to shaded (Table 2). This may be due to the availability of susceptible hosts where more thrips according to Mugo *et al.* (2013) occurred in un-shaded coffee as related to shaded coffee. Their availability may also be related to coffee farming systems. According to Kawaka *et al.* (2011), monoculture crop rotation and intercropping affect the occurrence of EPNs. Hence, coffee intercropped with shade trees significantly contributed to the EPNs distribution and their incidences. The *Steinernema* spp (62.5%) was the predominant type of Entomopathogen when compared with *Heterorhabditis* spp (37.5%) from the samples collected and found to have the EPNs (Table 2).

The soil reaction (pH) level for the sites sampled was recorded at the mean range of 4.0 to 4.7 (Table 3). This was within the soil pH, favourable for the survival and propagation of EPNs according to the work of Kung *et al.* (1990). The moisture content ranged from 8.44 to 12.88%. The un-shaded coffee had significantly ($P < 0.05$) lower moisture content than shaded coffee (Figure 5). This factor contributed to more samples with EPNs being under un-shaded coffee than shaded ones. The study showed that EPNs occurred in both un-shaded and shaded coffee farming systems with *Steinernema* spp as the most common species as compared to *Heterorhabditis* spp

Table 3. Soil moisture content and pH from sampling sites.

Coffee habitat	Moisture content (% mc)	Soil reaction (pH)
Jacaranda shaded	12.88	4.7
Jacaranda un-shaded	8.58	4.2
Azania shaded	11.07	4.3
Azania un-shaded	8.44	4.0

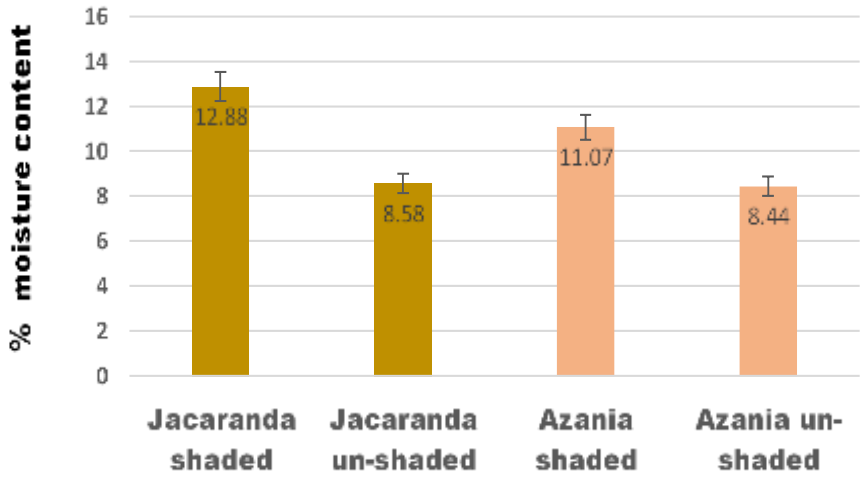


Figure 5. Moisture content from sampled sites.

CONCLUSION

Two types of Entomopathogenic nematodes, *Steinernema* spp and *Heterorhabditis* spp with diverged distribution are locally present in coffee farming systems in Kenya. Of the two, *Steinernema* spp (62.5%) was predominant when compared with *Heterorhabditis* spp (37.5%). Their potential to manage *D. coffeae,* is, therefore, necessary to be determined.

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