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Effectiveness of Entomopathogenic Nematodes against Sweet Potato Weevil (*Cylas puncticollis* Boheman (Coleoptera: Apionidae)] Under Semi-Field Conditions in Kenya

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Abstract: Sweet potato (*Ipomoea batatas* (L.) Lam.) ranks second in production and value after cassava among the root crops in Kenya. Its production has been declining mainly due to the damaging effects of *Cylas puncticolis* Boheman (Coleoptera: Apionidae), its primary pest. Therefore, this study was done to determine effectiveness of two Entomopathogenic nematodes (EPNs) against *C. puncticolis*. The experiment was conducted under semi-field conditions using potted plants at Kibwezi, Eastern Kenya in two consecutive growing seasons in 2002 and 2003. Two species of EPNs were used and their efficacy compared: *Steinernema karii* Waturu, Reid and Hunt (Rhabditida: Steinernematidea) and *Heterorhabditis indica* Poinar, Karunakar and David (Rhabditida: Heterorhabitidea). Both EPNs significantly suppressed emergence of adult weevil from the tubers. The EPNs were also very effective on larvae and reduced the number of pupae significantly. The effect of the pest on the tuber quality was significantly high compared with its effect on tuber quantity. Among the EPNs, *H. indica* was more efficient in reducing the pest population and damage. In addition, the EPNs persisted in the soil for more than three 3 after their release. It is suggested that these EPNs have field potential in controlling the *Cylas* weevil and may provide the local solution to this pest problem in Kenya.

Key words: EPNs persistence, *Heterohabditis indica*, *Ipomoea batatas*, potato quality, *Steinernema karii*

INTRODUCTION

Sweet potato (*Ipomoea batatas* L. Lam: Convolvulaceae) production in Kenya is by small-scale farmers mainly for home consumption and as source of income. However, production is highly impaired by presence of pests, sweet potato weevils (*Cylas puncticollis* Boheman and *Cylas brunneus* (Fabricius), Coleoptera: Apionidae) being the major pest known to contribute 60-70% yield loss in East Africa (Kabi *et al*., 2001). Sometimes, the damage to tubers can reach up to 90% (Ekanayake *et al*., 2001). Minor damage can render infested tubers unmarketable due to presence of feeding marks and oviposition holes. While main damage is on the tubers, yield losses also occur due to adults and larvae feeding on vines (Ekanayake *et al*., 2001). Tubers can also be rendered unfit for human consumption as a result of terpenoid production that is induced by the feeding larvae, causing bitter taste. The subterranean habitats of these *Cylas* weevil make them less accessible to insecticides, their predators and parasitoids. However, such habitats increase the chances and impact of entomopathogens because they are protected and the cool and humid environments enhance their

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survival and reproduction (Janssons *et al*., 1991). Apart from the conventional insecticides, sex pheromones have been explored but with less success in Africa (Kabi *et al*., 2001). There is not yet an effective resistant variety to date against the weevils. Local farmers use cultural means, e.g., earthling, to control the weevil, with less effects (Smit and Matengo, 1995). One of the reasons is because of their harvesting management. Most farmers practice the so called in-ground storage and peace-meal harvesting (Kabi *et al*., 2001). This enables presence of sweet potatoes in the field throughout the year, providing suitable environment for the weevil population growth. The weevils are especially of concern in areas with long dry seasons (Kabi *et al*., 2001). Entomopathogenic nematodes (EPNs) are reportedly effective against many crop pests, particularly those found in soil inter-phase and cryptic habitats (Smart, 1995; McGraw and Koppenhöfer, 2008). Among the EPNs, it is steinernematids and heterorhabditids that have received more attention as potential biological control agents for insect pests (Smart, 1995). Results from laboratory experiments in Kenya have confirmed the susceptibility of the sweet potato weevil larvae to both *Steinernema karii* Waturu, Reid and Hunt (Rhabditida: Steinernematidea) and *Heterorhabditis indica* Poinar, Karunakar and David (Rhabditida: Heterorhabitidea) (Waturu, 1998). These EPNs are also reported as effective against weevil pests of other crops, e.g., banana weevil (*Cosmopolites sordidus* Germar) in Kenya (Waturu, ibid) and carrot weevil (*Listronotus oregonensis*) in USA (Micklasiewicz *et al*., 2002). The ability of EPNs to control *Cylas* pests outside laboratory conditions has not been determined in Kenya. Therefore, the aim of this study was to assess the efficacy of these EPNs in reduction of the *C. puncticollis* populations on sweet potatoes under semi-field conditions, and also to determine their persistence on the soil after application under same conditions. The findings were expected to offer solutions to the perennial problems of *Cylas* pests in Kenya, which would thus improve the crop productivity.

MATERIALS AND METHODS

Crop and Experiment Establishment

This experiment was carried out at Kibwezi, which is a major growing area of sweet potato in Eastern Kenya. The crop was established in two consecutive growing seasons, first in 20 November 2002, then a repeat on 4 June 2003. Two sweet potato vines each measuring 30 cm were planted in a 25 L capacity plastic pot with a mixture of sterilized soil, ballast and sand (6:2:2, respectively). Vines were first disinfected with Furadan 5G solution (Carbofuran 0.5 kg a.i ha^{-1}) for 15 min before planting. The pots were perforated at the base with holes 1 cm wide to avoid water stagnation. After establishment, weaker vines were thinned out, leaving one vine per pot. Two months after sprouting of vines, they were caged with cloth netting held into a square frame around each pot by sticks 1 m tall. The netting material was placed in a way to ensure a height of 30 cm above the sweet potato was maintained. Ten pairs of adult weevils aged four to 7 days were artificially introduced on the caged plants and the netting material held properly at the base of vines to prevent weevils from escaping and also to block other insects from infesting the vines. Three treatments were used: application of *S. karii* and *H. indica* (each 500000 dauer juveniles) on potted plants infested with artificially introduced *C. puncticollis* and control plots infested with the *C. puncticollis* but no EPNs applied. Each treatment was replicated 3 times in a Complete Randomized Design (CRD). The EPNs used were reared and mass produced *in vivo* in larvae of *Galleria mellonella* L. (Lepidoptera: Pyralidae) at the National Fibre Research Center, Mwea-Tabere, Kenya. Their suspension was drenched over the crown of sweet potato using a watering can. The plants were watered before and after application of EPNs to provide moist conditions.

Sampling

Plants were uprooted and tubers measured on day 2, 7, 14 and 21 after treatment application. Three plants were used per treatment on each sampling period. From each plant, the density and life

stages of weevils on the crown region, inside tubers and in 13 cm vine-section immediately above the tubers were recorded. The damaged tuber parts were chopped off using a knife and their weight measured.

To determine the persistence of the EPNs in the soil, two 200 mL soil samples were collected around the crown of three plants on 2, 7, 14 and 21 days post EPNs treatment and placed on 250 mL containers in the laboratory. Four late instar larvae of *G. mellonella* were buried in each of these soil samples. The containers were then incubated in room temperature and larval mortality recorded. Cadavers were washed in distilled water to drain out all EPNs on their body surface. To determine EPNs penetration, the cadavers were carefully dissected in Ringer solution [Nad 6.75, KCl 0.09, $CaCl_2$ 0.115 and $NaHCO_3(2H_2O)$ 0.215 g in 1 L of distilled water (Taylor and Baker, 1978) under a dissecting microscope. The dissected insect cadavers were allowed to stand on the bench for at least 30 min for the EPNs to drain into the Ringers solution. Finally, EPNs were counted in a 9 cm diameter petri dish with grids engraved on the bottom using a tally counter.

Data were subjected to one-way analysis of variance (ANOVA) and where it showed skewness, it was transformed by square root formula. Genstat statistical software vers. 7.1 (VSN International, 2007) was used to aid in the analysis. The Standard Error (SE) was used as a post ANOVA test.

RESULTS AND DISCUSSION

The findings from the two seasons were not significantly different ($p>0.05$) hence data were pooled for analysis. There were no dead weevils, adults or other stages, recorded on the control plants. However, observations from the crown showed that EPNs were effective against the adult weevils up to seven days post their application, when the weevil mortality peaked (Fig. 1). Afterwards, there was no recorded adult mortality, both males and females. The effect of *S. karii* on female *C. puncticollis* adults was higher (9.52%) than the males (8.37%) but in both instances the nematodes caused lower mortality compared with mortality due to *H. indica* infection (15.86 and 18.99% female and male weevil, respectively). In contrast to *S. karii*, *H. indica* caused high mortality of male than female

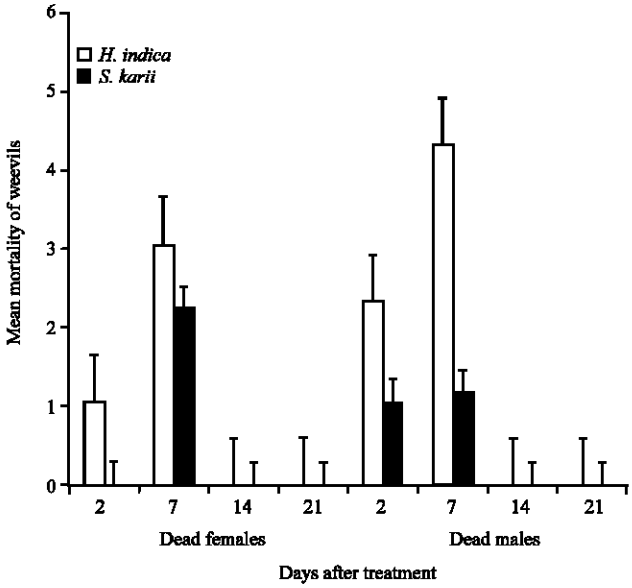

Fig. 1: Mean number (±SE) of adult weevil mortality cause by EPNs around the crown of potted sweet potatoes grown under semi-field conditions at Kibwezi, Eastern Kenya in 2002 and 2003

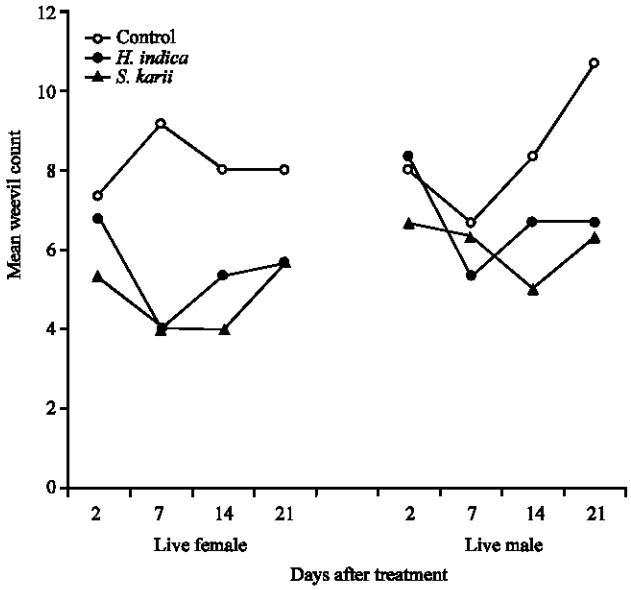

Fig. 2: Mean number of live weevils on and around sweet potato plants subjected to different treatments under semi-field conditions at Kibwezi, Eastern Kenya in 2002 and 2003

weevils. This is also reflected in Fig. 2 that shows the number of live weevils counted on the crown and vines reduced during the sampling period.

The effects of treatments on larval mortality in vines was highly significant ($p<0.001$). *Heterorhabditis indica* was more effective, causing 23.33%. The mortality peaked 14 days after application of the EPNs, *H. indica* causing higher mortality than *S. karii* although not significantly different ($p>0.05$; Fig. 3).

On tubers, the larval mortality was significantly ($p<.05$) different among the treatments (Fig. 4). The *H. indica* caused higher mortality than *S. karii*. The mortality peaked at day 7 after treatment application. While larval mortality caused by *S. karii* continued up to 21 days post treatment in the tubers, the larval mortality in plants treated with *H. indica* was recorded up to 14 days after treatment.

The effect of *S. karii* and *H. indica* on pupae in the vines was recorded till 14 days after treatment application (Fig. 5). Higher mortality was recorded in the *H. indica* treated plants compared with other treatments. There was no mortality in the control plants, as expected a priori. The control plants also recorded the highest number of live pupae as expected, followed by *S. karii* treated plants.

Pupal mortality in the tubers was recorded even 21 days post-treatment application (Fig. 6). Pupae mortality peaked 7 days post-treatment and higher mortality was recorded in plants treated with *H. indica* compared with those treated with *S. karii*.

The total weight of tubers from plants subjected to different treatments was not significantly different ($p>0.05$) and averaged 1.5 kg per plant. However, the weight of the damaged portions within a tuber was significantly different ($p<0.05$, Fig. 7). Plants infested with *C. puncticollis* but not treated recorded the highest weight of the damaged tuber parts. The weight of the damaged parts among the EPNs treated plants was not significantly different though higher weight was recorded on *S. karii* treated plots.

The number of *G. mellonella* larvae killed by EPNs that were collected from infested soils around the potted plants reduced as the time since EPN application increased (Fig. 8). More than 90% of the

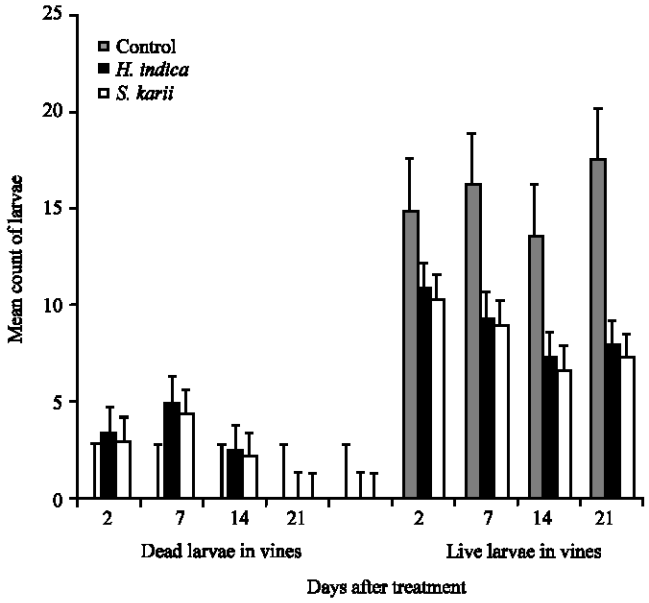

Fig. 3: Mean number (±SE) of *Cylas puncticollis* larvae on sweet potato vines under different treatments in semi-filed conditions at Kibwezi, Eastern Kenya in 2002 and 2003

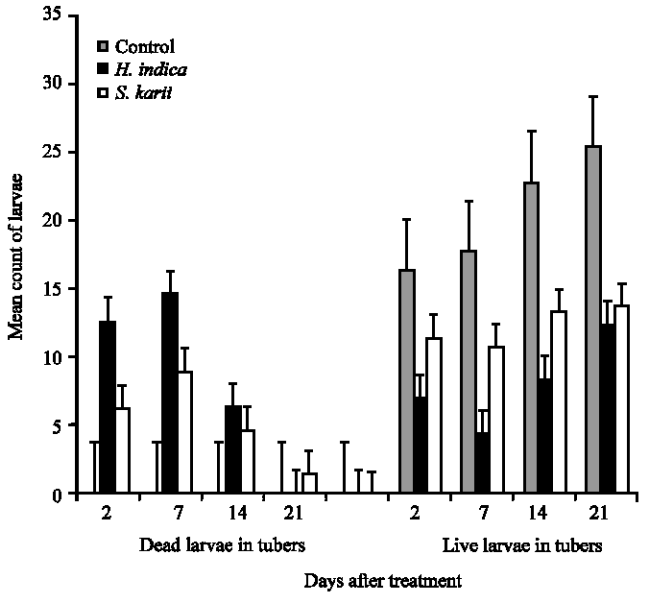

Fig. 4: Mean number (±SE) of *Cylas puncticollis* larvae on sweet potato tubers under different treatments in semi-filed conditions at Kibwezi, Eastern Kenya in 2002 and 2003

larvae died when exposed to soil sample that was collected two days after EPNs application. The soil samples collected from plants after 21 days of EPN application did not show larval mortality except those treated with *H. indica*.

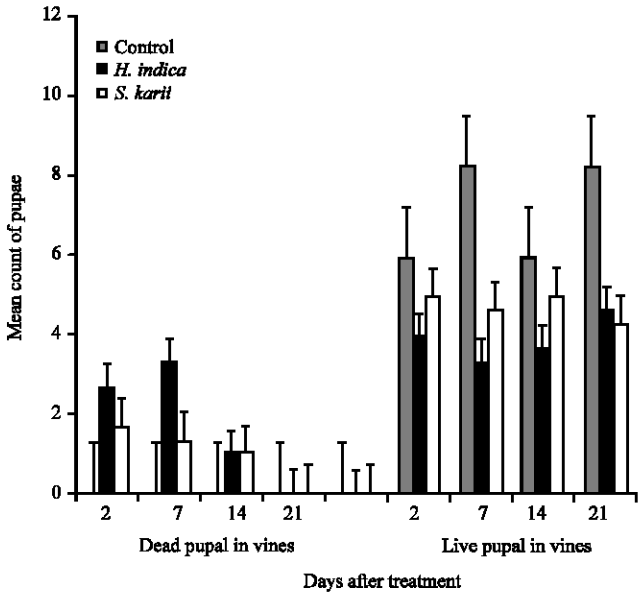

Fig. 5: Mean number (±SE) of *Cylas puncticollis* pupae on sweet potato vines under different treatments in semi-filed conditions at Kibwezi, Eastern Kenya in 2002 and 2003

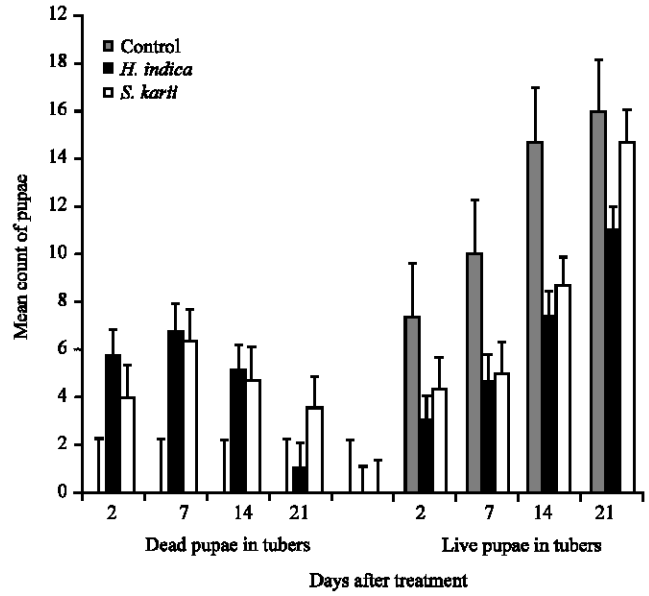

Fig. 6: Mean number (±SE) of *Cylas puncticollis* pupae on sweet potato tubers under different treatments in semi-filed conditions at Kibwezi, Eastern Kenya in 2002 and 2003

The number of EPNs recovered from the cadavers of *G. mellonella* larvae was high from larvae exposed to the infested soils collected two days after EPNs application (Fig. 9). There were more *S. karii* recovered on the cadavers than *H. indica*. It was only *S. karii* that was recovered from cadavers obtained from larvae exposed to soils collected 21 days after EPNs application.

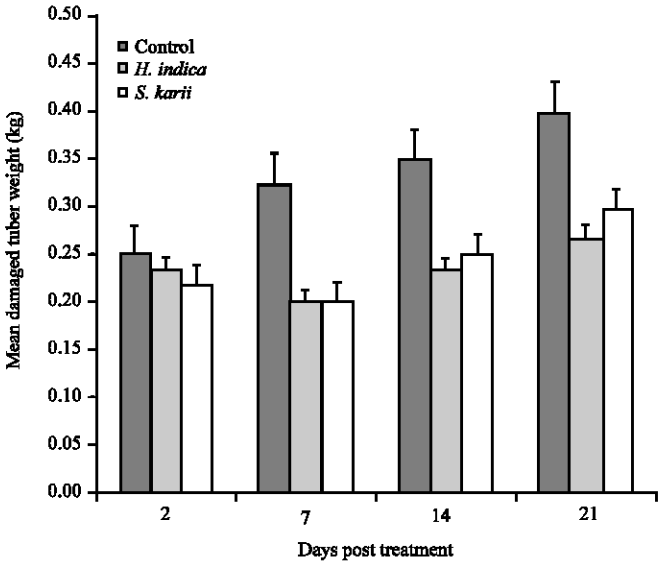

Fig. 7: Mean weight (±SE) of tuber parts damaged by the sweet potato weevil infesting sweet potatoes under different treatments in semi-field conditions at Kibwezi, Eastern Kenya, 2002-2003

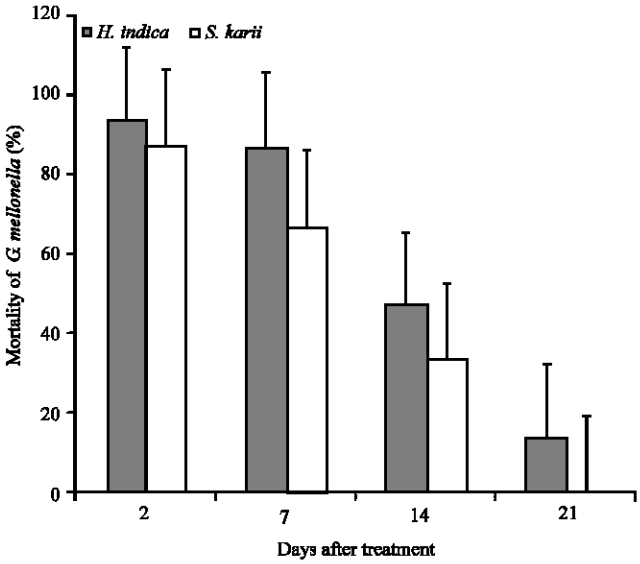

Fig. 8: Mean (±SE) percent mortality of *G. mellonella* larvae after exposure to EPNs-treated soils at Kibwezi, Eastern Kenya in 2002-2003

The results showed that *H. indica* was more effective against *C. puncticollis* populations than *S. karii*. Most earlier studies also suggest that heterorhabditids are more effective than steinernematids in controlling root weevils (Bedding *et al*., 1983). Usually, the ability of any pest control approach to cause adequate mortality of target pest within a short period is the best measure for its success in reducing damage on crops. The results obtained showed that both *S. karii*

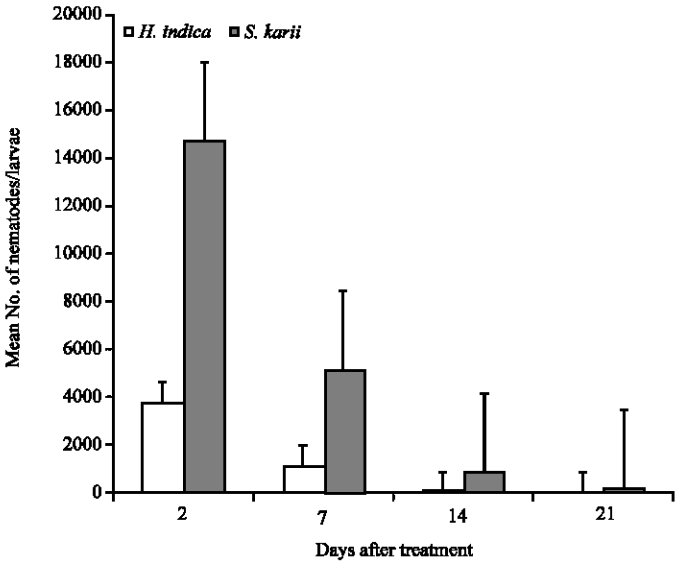

Fig. 9: Mean number (±SE) of EPNs recovered from cadavers of *Galleria mellonella* larvae when exposed to soils collected after different days of EPNs application at Kibwezi, Eastern Kenya in 2002 and 2003

and *H. indica* significantly suppressed adult weevil emergence from the tubers. The fact that various stages of sweet potato weevil are found within roots at the same time favored effectiveness of the EPNs in checking the weevil abundance, by virtue of their attraction and mobility towards the insect host. The larval stage was found to be the most susceptible probably due to the fact that they have soft cuticle and their period lasts two to three weeks, giving EPNs enough action time, which explains the high mortality of the larvae. This is in addition to nematodes invasion of their host through the natural openings. Ekanayake *et al*. (2001) also reports a high mortality of *Cylas* sp. larvae due to heterorhabditid and steinernematid. Ekanayake *et al*. (2001) found the heterorhabditid to be more effective. Considering that pupation lasts one week, the pupae would have short exposure period, in addition to their covering, which may restrict EPNs penetration. These two characteristics could have aided in reducing pupal mortality compared with the larvae. In contrast to infestation of young stages, EPNs penetration of the adult weevil may be impended by the hard outer elytra and limited inter-segmental soft areas, leading to lower mortality of the adults. Low effectiveness of the EPNs on other adult weevil pests with similar habits as the *Cylas* sp. compared with their larvae has also been reported by McGraw and Koppenhöfer (2008). However, effectiveness of the EPNs on the larvae is more crucial in reducing the weevil damage, since the next generation of the weevils is highly diminished. Considering that target farmers rarely use chemical insecticides, the EPNs can be applied effectively against the sweet potato weevil. That the EPNs are able to seek for their hosts and hence overcome the limitations of the chemical or other control means, makes them the best candidates for the weevil control. The findings from this present study confirm earlier laboratory experiments that the sweet potato weevil larvae are susceptible to the EPNs in Kenya (Waturu, 1998). Males usually frequent the crown and leaves of sweet potato than females and this may be the reason why higher mortality was recorded on those regions compared with female mortality (Sathula *et al*., 1997). It was observed in this study that male weevils usually moved to the upper leaves and waited for females seeking mates. This is probably why there have been efforts to use sex pheromones to trap weevils, as a management option (Downham *et al*., 2001; Smit *et al*., 2001). Although, *H. indica* was more

effective than *S. karii* in reducing numbers of the weevil, *S. karii* was more persisted. However, heterorhabditids are known to strongly seek their host in the soil (Georgis *et al*., 2006), explaining why *H. indica* was more effective. That there was no significant difference in the weight of tubers obtained from the EPNs treated and control pots confirmed the general understanding that weevil feeding usually affects the quality of the infested tubers, rendering them unmarketable. This is because the weevil feeding induces terpenoid production by tubers, making them unpalatable. The high number of *S. karii* recovered from the cadavers confirms earlier studies that showed steinernematids have higher invasion efficiency of their hosts than heterorhabditids (Epsky and Capinera, 1993; Caroli *et al*., 1996). Since, this study showed that the EPNs reduced populations of the sweet potato weevil under semi field conditions, it is suggested that field trials should provide evidence of their utility before recommending these biocontrol agents to farmers.

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