Journal of Insect Science, (2018) 18(3): 26; 1–8
doi: 10.1093/jisesa/iey060
Research

Biological Control Potential of Two Steinernematid Species Against the Date Fruit Stalk Borer (*Oryctes elegans* Prell, Coleoptera: Scarabaeidae)

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Subject Editor: Stefan Jaronski

Received 27 December 2017; Editorial decision 18 May 2018

Abstract

The fruit stalk borer (*Oryctes elegans*) is an important pest of date palm (*Phoenix dactylifera*) trees in Saudi Arabia. This study was conducted to determine efficacy of using two species of entomopathogenic nematodes, *Steinernema kushidai* and *Steinernema glaseri*, against *O. elegans* under laboratory and field conditions. Under laboratory conditions, both species of nematodes showed a significant effect on the mortality of *O. elegans* larvae. Significant variations were observed when insects were exposed to nematodes for variable durations under laboratory conditions. They showed no differences in insect larval mortality when tested either in aqueous suspensions or in *Galleria*-infected cadavers. Insects exposed to nematode aqueous suspension for 4 d and those treated with *Galleria*-infected cadavers showed the same rates of mortality, which differed when insects were exposed to nematode-infected cadavers under field conditions. Mean percentages of corrected mortality varied between nematode species and number of infected cadavers. *S. kushidai* caused significantly higher mortality percentages ± SE (72.17 ± 5.57, 95.83 ± 4.17, 94.43 ± 5.57, and 100%) compared with *S. glaseri* when the fruit stalk borer, *O. elegans*, was treated for 6 wk with two, four, six, and eight infected cadavers, respectively.

Key words: *Steinernema kushidai*, *Steinernema glaseri*, *Galleria mellonella*, cadaver, *Oryctes elegans*

Date palm (*Phoenix dactylifera* L.) is an important crop in the Kingdom of Saudi Arabia, from both economical and cultural heritage points of view. The sweet fruit obtained from date palm trees has played a significant role in the culture, traditions, and history of the Arabian Peninsula.

Date palm trees are severely attacked by the larvae of the fruit stalk borer, *Oryctes elegans* Prell (Coleoptera: Scarabaeidae), whereas adult stages of frond palm borer (*Phonapate frontalis* 'Fahraeus', Coleoptera: Bostrichidae) infest date palm orchards (Al-Sayed and Al-Tamiemi 1999). As a result, serious damage to crops of date palm, oil palm, sago palm, coconut palm, and betel nut were reported due to *O. elegans* infestation (Gassouma 2000). Recent studies showed that adults and larvae of *O. elegans* infested different cultivars of date palm with significant economic losses of young date palm orchards in Saudi Arabia (Martin 1972). The larvae damage roots by extensive feeding and also cause rotting of wood, whereas adults feed on plant sap, fruit, and nectar (Bedford 1980). The adults and larvae of *O. elegans* form long tunnels in tissues to feed and attack the apical buds and fruit stalks of growing trees. Subsequently, these tunnels are accessible to other pathogens, which leads to secondary infections and complications.

Controlling populations of *O. elegans* has always been a challenge, mainly because of the unique biology of *O. elegans* larvae and residual effects of chemical insecticides on nontarget organisms. Biological control is a safer and more effective method to manage *O. elegans* population in date palm orchards (Khalaf et al. 2013). Biological control agents integrated with other antagonistic microorganisms, such as green muscardine fungus (*Metarhizium anisopliae*), produce more encouraging results and kill *O. elegans* larvae more effectively (Khalaf et al. 2013).

Entomopathogenic nematodes (EPNs), *Steinernema* (Panagrolaimorpha: Steinernematidae) and *Heterorhabditis* (Rhabditida: Heterorhabditidae), are used to manage insect pest populations in many countries (Grewal and Peters 2005, Grewal et al. 2005, Georgis et al. 2006, Campos-Herrera 2015). The infective juveniles (IJs) of soil-inhabiting nematodes search suitable insect host to penetrate. After penetrating the host *via* natural openings such as spiracles, cuticle, mouth, or anus, juveniles move to the hemocoel and release symbiotic bacteria, e.g., *Xenorhabdus* (Kaya and Gaugler 1993, Lewis and Clarke 2012). *Xenorhabdus* associated with *Steinernema* species produces toxins that kill insect hosts upon entering within 24–48 h. The nematodes then feed on bacteria and decaying host tissues to complete up to three generations within

host cadaver (Burman 1982, Dunphy and Webster 1988, Ehlers et al. 1997, Bowen et al. 1998). EPNs are effective against soil-dwelling insects with least toxicity to mammals and other nontarget organisms (Akhurst and Smith 2002, Ehlers 2005).

Several species and strains of EPNs, effective against insect pests, have been identified (Lewis 2002, Grewal and Peters 2005), and significant improvements were made in their mass production (Friedman 1990, Grewal and Georgis 1998, Ehlers and Shapiro-Ilan 2005, Grewal et al. 2005). Although formulations such as wettable dispersible granules, wettable powders, and infected cadavers have improved the shelf life of EPNs, the short shelf life still remains a major obstacle in their widespread and effective use against insect pests under adverse climatic conditions, except when infected cadavers are used (Grewal 2002, Shapiro-Ilan et al. 2003, Grewal and Peters 2005, Shapiro-Ilan et al. 2010). Susceptibility of EPNs to adverse climatic conditions hinders field applications and reduces chances of nematode survival during and after application.

The EPN formulations, applied as aqueous suspensions using special sprayers, require constant agitation to maintain nematode homogeneity and toxic conditions for nematodes (Wright et al. 2005). However, agitation of suspension stresses out EPNs, by exposing them to increased temperatures within spray tanks, thus killing a portion of them (Fife et al. 2005, Bilgrami and Gaugler 2007). The application of nematodes in aqueous suspension also exposes them to ultraviolet radiation and desiccation on exposed foliage and soil surfaces, resulting in the decrease of field efficacy and persistence of EPNs (Shapiro-Ilan et al. 2015).

In this study, experiments were made to test effects of direct application of EPNs-infected cadavers (*Galleria mellonella*) on the rate of efficacy of two species of steinernematid nematodes, '*Steinernema kushidai* (EIK7c strain) and *Steinernema glaseri* (NJ strain)' in controlling the date fruit stalk borer (*O. elegans*) larvae. Two species of EPNs, *S. kushidai* (EIK7c strain) and *S. glaseri* (NJ strain), were tested as biological control agents against *O. elegans* larvae in date palm orchards. In addition, laboratory and field experiments were also made to test virulence of *G. mellonella*-infected cadavers.

Materials and Methods

O. elegans Collection

The third instar larvae (last instar larvae) of date fruit stalk borer were collected from an infected field from Tomor El-Mamlakha, Al-Qasim region, Kingdom of Saudi Arabia. All collected larvae were washed three times with distilled water to remove the soils and plant fibers before use in experiments.

Rearing of the Greater Wax Moth (*G. mellonella*)

Greater wax moth larvae, *G. mellonella* (L.) (Lepidoptera: Pyralidae), collected from domesticated honey bee hives, were kept in plastic rearing jars, measuring 17 × 17 × 27 cm and containing 250 g of *Galleria* artificial media. To facilitate egg laying by *G. mellonella*, rearing jars were lined with frill paper tissue.

The insects were allowed to lay eggs on frill paper tissue measuring 15 × 15 cm. The eggs were transferred gently to other rearing jars containing 250 g of prepared *Galleria* media. The jars were tightly closed with the double layered muslin cloth to prevent the escape of neonatal larvae and incubated at 28 ± 2°C, photoperiod of 8:16 (L:D) h, and relative humidity of 65 ± 5%. The larvae were separated from other stages upon reaching to the final instar (25 d old), used for mass rearing of the EPNs, and stored at 15°C for 2–4 wk. Insects were reared on artificial diet prepared from wheat bran (260 g), wheat flour (162 g), yeast extract (65 g), glycerol (193 g), and water (158 g; Monastyrskij and Gorbatovskij 1991). The media was freshly prepared, and the stock was frozen at –5°C for 2 wk to prevent secondary contamination.

Nematodes

Two species of EPNs, *S. kushidai* (EIK7c strain) isolated from Egypt by Atwa (2003) and *S. glaseri* (NJ strain) obtained from the laboratory of Professor Randy Gaugler, Rutgers University, New Jersey, were tested. Both species of nematodes were maintained under laboratory conditions using last instar larvae of *G. mellonella* as the host (Woodring and Kaya 1988). The infected cadavers of *G. mellonella* were used to obtain two types of nematode formulations: 1) infected cadavers formulation (capsules) and 2) aqueous suspension of IJs. To obtain an aqueous suspension of nematodes, the infected cadavers were placed on White trap according to White (1927). The IJs were collected from White trap, washed with distilled water three times, and stored at 15°C for 2 wk before use.

Infected Cadaver Formulation Production

To obtain at least 3,000 high-quality infected cadavers, 5,000 last instar larvae of *G. mellonella* were infected by *S. kushidai* and *S. glaseri* and the best chosen for use. Twenty-five healthy *G. mellonella* larvae were exposed to 1,500 *S. glaseri* and *S. kushidai* IJs in each of the 140 Petri dishes measuring 150 × 30 mm, lined with filter paper. Infected cadavers were obtained after 3–5 d post exposure and used for laboratory and field experiments. To prevent sticking or breaking of infected larvae during transportation and application, insect cadavers were coated with baby powder (Johnson's Baby Powder, Johnson & Johnson Consumer Products Company, Skillman, NJ). Infected cadavers of *G. mellonella* were coated with baby powder to prevent cadavers from sticking together.

To rule out any effect of talcum powder, one group of 10 infected *G. mellonella* larvae was treated with talcum powder and the second not, as a control. Upon maturation, the cadavers were placed on White trap (White 1927) to isolate IJs, which were counted and compared to rule out any effect of Johnson's Baby Powder.

Laboratory Experiments

Experiments were made under laboratory conditions in Petri dishes measuring 150 × 30 mm, lined with 200 g of sand moistened with 5 ml of distilled water. *O. elegans* was subjected to infection by both types of nematode formulations, i.e., aqueous suspension under laboratory conditions and infected cadavers under laboratory and field conditions. In laboratory bioassay, sterilized sand was used to test the efficacy of EPNs against *O. elegans*. Small parts of date palm fronds axils were used to feed the larvae. However, sand bioassay methods are easy to set up and closer to field situation and adapted to standard quality control tool for assessing the virulence of EPNs (Grewal 2002). Standard application of EPNs in aqueous suspension have been used to determine the EPNs' virulence against the target insect. While as a delivery of EPNs in their infected host cadavers is a novel method to increased nematode dispersal, survival, infectivity, and efficacy (Shapiro-Ilan et al. 2012) are reported herein.

Nematode Suspension Bioassay

The efficacy of *S. kushidai* and *S. glaseri* was tested against *O. elegans* in separate sets of Petri dishes lined with a layer of sterilized sand. Four concentrations, i.e., 250, 500, 750, and 1,000 IJs in 5 ml of distilled water of each nematode species, were tested separately against five third instar larvae of *O. elegans*. The control treatments

consisted of distilled water only, and each experiment was replicated three times. The mortality of *O. elegans* was recorded 24, 48, 72, and 96 h after inoculation with IJs. The experiments were replicated three times; each replication consisting of three Petri dishes each containing five larvae.

Infected Cadaver Bioassay

The field experiments were conducted to determine efficacy of infected nematode cadavers (capsules) against *O. elegans*. Cadavers were used Larval *O. elegans* were bioassayed with *Galleria* cadavers containing either *S. kushidai* or *S. glaseri* at the rate of one, two, three, and four cadavers per Petri dish. Treatments were made in Petri dishes with cadavers covered with a layer of sterilized sand moistened by 5 ml of distilled water. Five third instar larvae of *O. elegans* were used in each of three replicates. Distilled water was used in the control with the sand moistened as needed. Cumulative mortality was recorded 5, 10, 15, and 20 d after cadaver treatment, and each experiment was replicated three times.

Field Trials

The field experiments were conducted to determine efficacy of infected nematode cadavers (capsules) against *O. elegans*. Cadavers were used to provide protection against destructive biotic and abiotic factors, with the EPNs released having greater energy reserves, greater ability to disperse and infect the target insects, and greater longevity in the soil (Shapiro-Ilan et al. 2003). Each nematode species was applied as two, four, six, or eight infected *Galleria* cadavers per tree to 50 randomly chosen trees in each of three plots. The infected cadavers were applied to the soil close to tree trunks and small shoots, 5–10 cm deep (Fig. 1). Insect mortality was determined after 2, 4, and 6 wk. For this purpose, two trees per treatment were chosen in each of the three replicate plots, for a total of six trees per treatment. Insect mortality was based on a census of live and dead *O. elegans* larvae in the soil close to each tree. Soil samples were collected for testing using *Galleria* for nematode detection in either treated or control area.

Data Analysis

The data were normalized using an arcsine transformation. The significance of differences between the means was determined using analysis of variance (ANOVA). Comparisons were made by using Tukey's multiple range test. The corrected mortality percentage and change rate of control blot mortality in field application were calculated by using Sun-Shepard's formula (Püntener 1981) as follows:

$$\text{Corrected\%} = (\text{mortality \% TP} \pm \text{change \% CPP} / 100 \pm \text{change \% in CPP}) \times 100$$

$$\text{Change \% in CP mortality} = (\text{CPP after T} - \text{CPP before T} / \text{CP before T}) \times 100$$

where TP = treated plot, CPP = control plot population, and T = treatment.

Results

Laboratory Experiments

Efficacy of Nematode Suspension

The efficacy of aqueous suspension of nematodes against fruit stalk borer *O. elegans* larvae is shown in Fig. 2A. Various concentrations of used IJs yielded different rates of larval mortality in *O. elegans* (Fig. 2A). The rates of mortality ± SE from *S. glaseri*

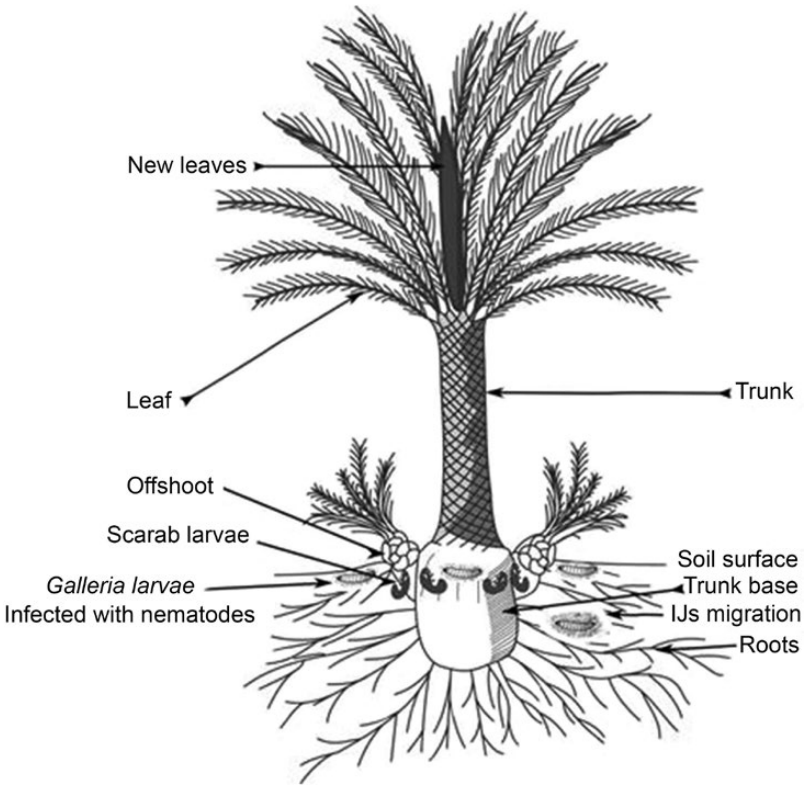

Fig. 1. Field application of infected *Galleria mellonella* cadavers.

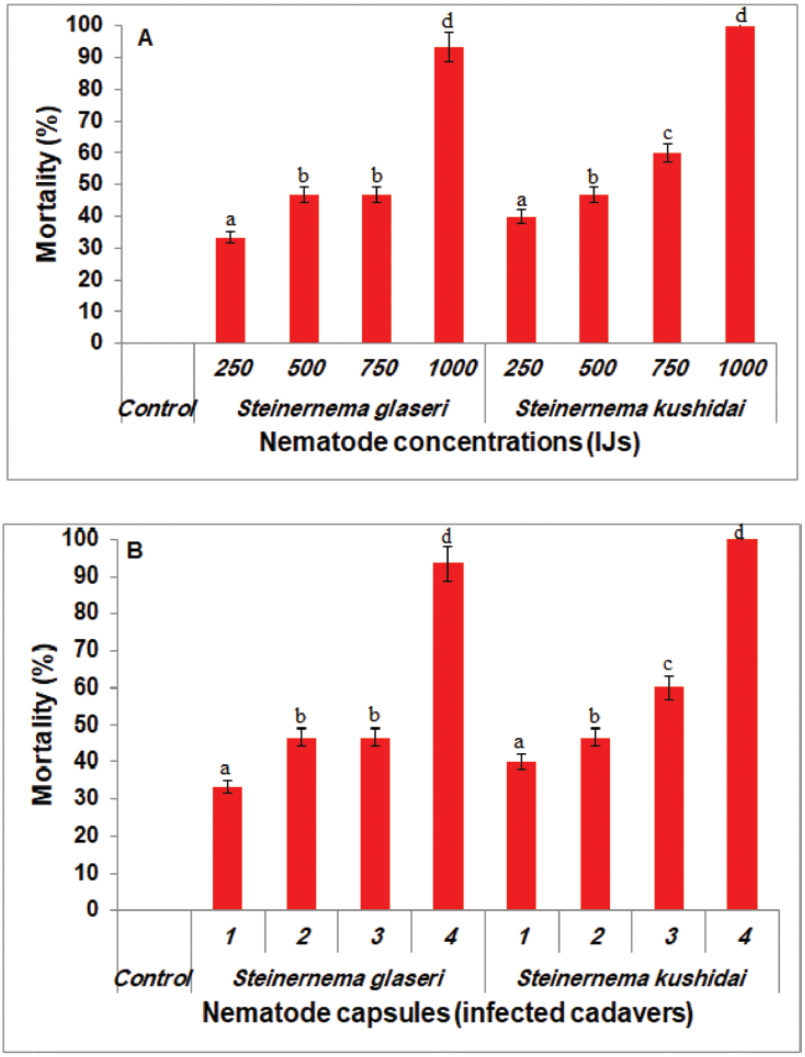

Fig. 2. (A) Rate of mortality of *Oryctes elegans* exposed to various nematode concentrations in aqueous suspension under laboratory conditions. (B) Rate of mortality of *O. elegans* larvae exposed to *Gallaria mellonella*-infected cadavers (NC) under laboratory conditions.

aqueous suspensions were 33.3 ± 12.6, 46.7 ± 13.3, 46.7 ± 13.3, and 93.3 ± 6.7% when *O. elegans* larvae were exposed to 250, 500, 750, and 1,000 IJs, respectively (Fig. 2A). When *O. elegans* was treated with the same concentrations of *S. kushidai*, the rates of insect mortality were 40.0 ± 13.1, 46.7 ± 13.3, 60.0 ± 13.1, and 100.0 ± 0.0% ($P < 0.05$; Fig. 2A). The rates of mortality of *O. elegans* showed significant differences at all concentrations of both nematode species; however, an aqueous suspension containing 1,000 IJs of both nematode species was most effective against *O. elegans* 4 d after treatment (*S. kushidai*: $F = 5.55$, df = 3, $P < 0.01$; *S. glaseri:* $F = 4.96$, df = 3, $P < 0.01$; Fig. 2A). Factorial analysis between species and concentrations of nematode suspension illustrated the differences due to nematode species ($F = 444.0$; df = 2; $P < 0.05$) and nematode concentrations in aqueous suspensions ($F = 88.67$; df = 3; $P < 0.05$). The effects of nematode species and aqueous suspensions on *O. elegans* larvae were also significant ($F = 22.67$; df = 6; $P < 0.05$).

Efficacy of Infected Cadavers

Efficacy of nematode 'capsules' (infected cadavers) against fruit stalk borer *O. elegans* larvae is shown in Fig. 2B. Various numbers of used cadavers yielded different rates of larval mortality in *O. elegans* (Fig. 2B). There was a significant difference in larval mortality by *S. kushidai* and *S. glaseri* when treated with different number of nematodes capsules (infected cadaver). Larval mortality ± SE of *O. elegans* reached 33.3 ± 12.6, 46.7 ± 13.3, 46.7 ± 13.3, and 93.3 ± 6.7% with the application of one, two, three, and four infected cadavers of *S. glaseri* for 20 d, respectively (Fig. 2B). In contrast, larval mortality ± SE of *O. elegans* reached 40.0 ± 13.1, 46.7 ± 13.3, 60.0 ± 13.1, and 100.0 ± 0.0% with one, two, three, and four infected cadavers of *S. kushidai*, respectively, during the same duration of exposure time (Fig. 2B). Significant differences were found between nematode species at different numbers of nematode capsules (*S. kushidai*: $F = 5.55$, df = 3, $P < 0.01$; *S. glaseri:* $F = 4.96$, df = 3, $P < 0.01$). When the data were analyzed as factorial arrangement for nematode species and different numbers of nematode 'capsules' (infected cadavers) in a completely randomized experiment, significant differences were obtained. There was a highly significant variation among total mortalities produced by nematodes species ($F = 1{,}066.33$; df = 2; $P < 0.05$) and different nematode 'capsules' (infected cadavers; $F = 11.00$; df = 3; $P < 0.05$). Means of larval mortality affected by species of EPNs and different numbers of nematode 'capsules' (infected cadavers) were highly significant ($F = 3.67$; df = 6; $P < 0.05$).

Field Trials

The mean percentage of corrected mortality varied significantly between nematode species and among the number of infected cadavers (Table 1). Corrected mortality of *O. elegans* subjected to different numbers of cadavers infected by the two nematode species at different exposure times under field condition is shown in Table 1. The mortality of insects was corrected using Sun-Shepard's formula. The lowest means of corrected mortality ± SE were 0.0 and 5.55 ± 5.55% when two and four infected cadavers of *S. kushidai* were applied, whereas the highest were 94.43 ± 5.57 and 100.0 ± 0.0% when six and eight infected cadavers of *S. kushidai* were applied for the duration of 6 wk. When *O. elegans* was exposed to eight cadavers infected by *S. kushidai* for 8 wk, high variation (100%) was recorded, whereas *S. glaseri* caused 87.5 ± 8.54% mortality under similar conditions (Table 1). Data in Table 1 show that *S. kushidai* caused significant mortalities (72.17 ± 5.57, 95.83 ± 4.17, 94.43 ± 5.57, and 100.0 ± 0.0%) after 6 wk in plots treated with two, four, six, and eight infected capsules, respectively. Plots treated with two, four, six, and eight infected cadavers containing *S. glaseri* yielded 66.63 ± 7.45, 90.27 ± 6.25, 90.27 ± 6.25, and 87.5 ± 8.54% mortality of *O. elegans*, respectively, after 6 wk.

Fig. 3A presents means of corrected mortality as the result of factorial analysis of nematode species and number of infected cadavers applied in a completely randomized experiment (*S. glaseri*: $F = 7.56$, df = 3, $P < 0.01$ and *S. kushidai*: $F = 2.53$, df = 3, $P < 0.01$). The interaction was highly significant with the number of infected cadavers used. The factorial analysis of corrected mortality of *O. elegans* between nematode species and duration of insect exposure was highly significant (*S. glaseri*: $F = 81.4$, df = 2, $P < 0.01$ and *S. kushidai*: $F = 140.16$, df = 2, $P < 0.01$; Fig. 3B).

Table 2 shows factorial analysis of corrected mortality of *O. elegans* at different durations of exposure to nematodes, number of infected cadavers applied, and nematode species. Highly significant variation was recorded among the means of corrected mortality as produced by duration of exposure ($F = 276.97$; df = 2; $P < 0.05$) and different number of infected cadavers ($F = 19.33$; df = 3; $P < 0.05$). The interaction was highly significant ($F = 2.94$; df = 6; $P < 0.05$; Table 2). Variations among the means of corrected mortality between nematode species ($F = 416.39$; df = 2; $P < 0.05$) and duration of insect exposure to nematode species ($F = 90.64$; df = 4; $P < 0.05$), and number of infected cadavers and nematode species ($F = 5.09$; df = 6; $P < 0.05$) were highly significant (Table 2). The interaction between duration of insect exposure, number of infected cadavers, and nematode species was highly significant ($F = 1.44$; df = 12; $P > 0.05$).

Discussion

The two species of EPNs, *S. kushidai* and *S. glaseri*, had significant effects on the population of *O. elegans*. Under laboratory conditions, *S. kushidai* species was more effective than *S. glaseri* against *O. elegans*, when tested in various concentrations of IJs or as infected host cadavers (nematode capsules). Similar results with aqueous nematode suspension and infected cadavers under laboratory conditions suggested that nematode formulations made no differences in the rates of mortality compared with infected cadaver. The relatively delayed effect of nematode cadavers probably resulted due to the emergence of nematode IJs at different times and finding a host on an irregular basis. The differences in the mean mortality of *O. elegans* under laboratory conditions may occur due to several factors (e.g., nematode species, nematode concentrations, and nematode formulations). Individual habits and behavior of nematode species may greatly influence their ability to parasitize white grubs and protect trees (Lewis et al. 2006). The IJs find the host, enter it through its natural apertures (oral cavity, anus, and spiracles) or in some cases through the cuticle (Dowds and Peters 2002). Although the EPNs were able to infect larval stage, there was considerable variability in the number of infected larvae, showing distinct strain dependence, and differences between using infected cadavers or aqueous suspensions of IJs. In general, EPN application within infected cadavers tends to be more efficacious than application in aqueous suspensions, which is why only infected cadavers were used under field conditions.

Use of EPNs to control fruit stalk borer *O. elegans* in palm farms in Kingdome of Saudi Arabia (KSA) is unknown because of the limited data about EPNs' active ingredients or formulations in KSA. Fruit stalk borer *O. elegans* are living as cryptic habitat insect in most life cycle stages with the limited data about behavior, biology, and ecology of fruit stalk borer in KSA, so EPNs are promising for controlling this insect. Choosing the right species of EPNs in a particular formulation against a particular pest in a particular environment is very important for successful biological control (Shapiro-Ilan et al. 2002). For this reason, efficacy of two species of EPNs (*S. kushidai* and *S. glaseri*) against *O. elegans* under laboratory and field

Table 1. Mean percentage of corrected mortality under field conditions of *Oryctes elegans* when exposed to *Galleria mellonella*-infected cadavers 'nematode capsules'

Exposure time	No. of infected cadavers 'capsules'	Mean % of corrected mortality* ± SE	
		Steinernema glaseri	*Steinernema kushidai*
2 wk after treatment	2 nematode capsules	0.0	0.0
	4 nematode capsules	8.33 ± 8.33	5.55 ± 5.55
	6 nematode capsules	12.5 ± 8.54	16.65 ± 7.45
	8 nematode capsules	15.12 ± 8.74	17.47 ± 7.85
4 wk after treatment	2 nematode capsules	38.88 ± 8.24	52.75 ± 5.11
	4 nematode capsules	79.17 ± 10.04	87.5 ± 8.54
	6 nematode capsules	79.4 ± 6.64	83.57 ± 7.35
	8 nematode capsules	78.03 ± 7.04	78.32 ± 7.04
6 wk after treatment	2 nematode capsules	66.63 ± 7.45	72.17 ± 5.57
	4 nematode capsules	90.27 ± 6.25	95.83 ± 4.17
	6 nematode capsules	90.27 ± 6.25	94.43 ± 5.57
	8 nematode capsules	87.5 ± 8.54	100 ± 0.0

*Calculated using Sun-Shepard's formula.

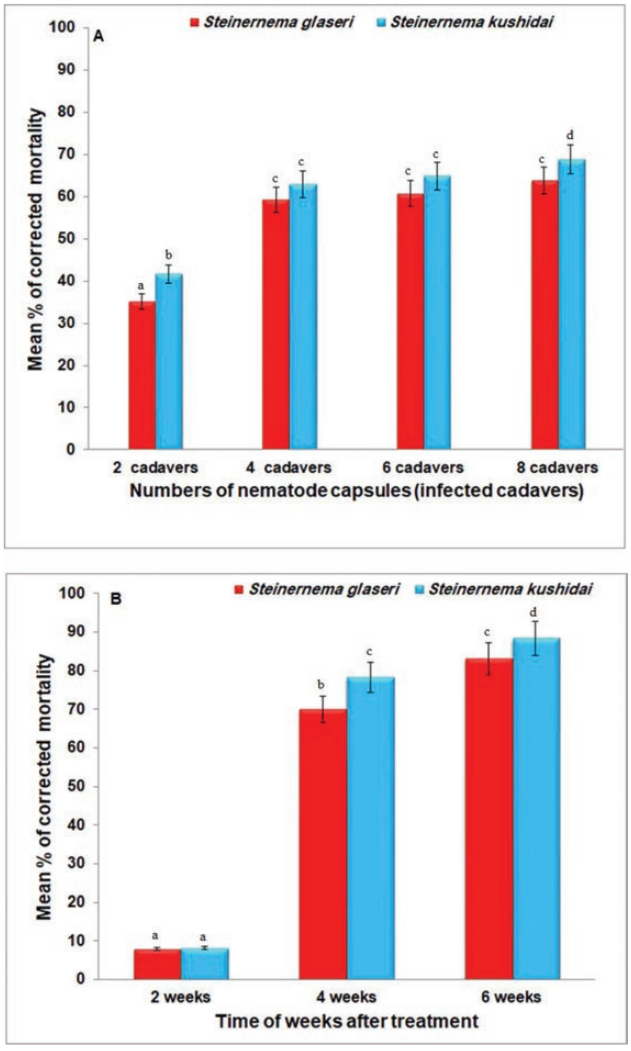

Fig. 3. Cumulative mean percentage of corrected mortality of *Oryctes elegans,* calculated between nematodes species, number of infected capsules, and exposure time under field conditions. (A) Cumulative corrected mortality of *O. elegans* exposed to different numbers of infected cadavers 'capsules' by *Steinernema glaseri* and *Steinernema kushidai.* (B) Cumulative corrected mortality of *O. elegans* calculated between various times of exposure to *S. glaseri*- and *S. kushidai*-infected cadavers 'capsules'.

conditions was evaluated in this article. This is a first report showing EPNs' preference to *O. elegans* and first report to use infected cadavers to control *O. elegans* under field conditions. In the literature, no data on efficacy of EPNs toward the fruit stalk borer *O. elegans* has been reported.

Superior efficacy of EPNs, applied as infected cadavers with application in aqueous suspension, was reported earlier by Shapiro-Ilan et al. 2003. While as Shapiro-Ilan et al. (2003) reported that, compared infected cadavers with application in aqueous suspension, under laboratory studies, greenhouse and field condition have indicated that application of infected cadavers can result in superior nematode dispersal, survival, and infectivity. Correspondingly, several previous reports have suggested that efficacy of the cadaver application approach is approximately equal to application in aqueous suspensions (Shapiro-Ilan et al. 2003), which agrees with the current study, but my experiments focussed on the efficacy of *S. kushidai* and *S. glaseri*, and different of time to get the same results of

Table 2. Effect of time of application, numbers of nematode-infected cadavers 'capsules', and nematode species within field application, and their interactions, on cumulative corrected mortality of *Oryctes elegans*

Source	*F*	df	*P*
Replication	0.93	5	
Time of application	276.97	2	≤0.05
Error		10	
Number of nematode capsules	19.33	3	≤0.05
Times of application by numbers of nematode capsules	2.94	6	≤0.05
Error		45	
Nematode species	416.39	2	≤0.05
Times of application by nematode species	90.64	4	≤0.05
Numbers of nematode capsules by nematode species	5.09	6	≤0.05
Times of application by numbers of nematode capsules by nematode species	1.44	12	≥0.05

insect mortality for infected cadavers (maximum 20 d) compared with aqueous suspension (maximum 4 d).

The current results suggest that the two nematodes species (*S. kushidai* and *S. glaseri*) have very different levels of infectivity against *O. elegans*. Overall, this study indicates that *S. kushidai* and *S. glaseri* have considerable promise as biocontrol agents for *O. elegans*. However, contrasting results were obtained under field conditions, when *O. elegans*-infested date palm trees were treated with *S. kushidai*- or *S. glaseri*-infected cadavers. Such differences might have occurred due to the different durations of nematode exposure, delayed emergence of IJs, stressed conditions (Bilgrami and Gaugler 2007), migration of IJs toward host, etc. Several factors such as nematode species, types of nematode formulation, biotic and abiotic factors, and host-searching abilities greatly influence the efficacy of EPNs (Lewis et al. 2006). Life strategies of *S. kushidai* and *S. glaseri* might also have contributed to the differences in the rates of mortality of *O. elegans*. *S. glaseri* is highly mobile and active in searching its host in the soil (Lewis et al. 2006), whereas the *S. kushidai* acts as an 'ambusher', searching for a host mainly on the soil surface (Campbell and Gaugler 1997).

Cadaver formation in EPNs is a natural way to protect IJs from exposure of hazardous environmental conditions, such as UV light and soil moisture. Ansari et al. (2009) used a kaolin–starch mixture and Del Valle et al. (2009) used unflavored gelatin; both authors demonstrated that the coating provided protection and promoted the conservation of the insect cadavers, herein baby talcum powder is used for the same reason. Coating cadavers with the baby talcum powder (Johnson's Baby Powder) has increased its longevity but restricted IJs to leave cadavers, resulting in reduced percentage of insect mortality. Soil moisture appears to be the key factor in nematode efficacy against insects (Shetlar et al. 1988, Kung et al. 1991, Gaugler et al. 1992, Downing 1994).

Deol et al. (2011) applied *Steinernema carpocapsae*-infected *G. mellonella* cadavers and recorded higher mortality of *Tenebrio molitor* compared with aqueous suspensions. During the present study, *G. mellonella* was used as a host because of its ability to produce more IJs than other insect hosts, such as *T. molitor* (Jansson and Lecrone 1994, Bruck et al. 2005, Deol et al. 2011). Thus, our results support prior studies suggesting that IJs emerging from cadavers possessing high virulence rate than aqueous suspensions, when

tested against insect pest (Shapiro-Ilan and Lewis 1999, Shapiro-Ilan et al. 2003).

The EPNs applied as cadavers have successfully infected larvae of *O. elegans* in the soil, when applied close to small shoots of the tree trunk, exhibiting survival and virulence retention, suggesting the use of host cadavers as the tool to overcome disadvantages of direct application of aqueous suspensions under field conditions.

Insect management using EPNs has been known since 1980s (Georgis 2002); however, their applications to *O. elegans*-infestated date palms in Saudi Arabia have never been done, especially in Saudi Arabia. Therefore, this study was conducted using EPNs as the biological control agent against *O. elegans* in date palm farms of Saudi Arabia. Choosing the right species of pest and parasite and effective modes of application are important parameters to achieve maximum mortality (Shapiro et al. 2002). This study also suggests that the two species of EPNs, i.e., *S. kushidai* and *S. glaseri*, are correct choices to use as biological control agents against *O. elegans,* when applied in the form of infected cadavers under field conditions.

Acknowledgments

This work was funded by the Deanship of Scientific Research (DSR), King Abdulaziz University, Jeddah, under grant no. (305-002-D1434). I, therefore, thank DSR for technical and financial support. I also thank Ms Gehan Abosheashaa for her help in designing the Figure no. (1) in this article using the software Inkscape.

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