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
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 non-target 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 2003, 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 S. kushidai and S. glaseri (NJ strain), when infected cadavers are used (Grewal 2002, Shapiro-Ilan et al. 2003, Grewal and Peters 2003, 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.

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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-Mamlakah, 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 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:

\[
\text{Corrected\%} = \frac{\text{mortality \% TP} \pm \text{change \% in CPP}}{100} \\
\pm \text{change \% in CPP} \times 100
\]

\[
\text{Change\% in CP mortality} = \frac{\text{CPP after T} - \text{CPP before T}}{\text{CPP before T}} \times 100
\]

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*...
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 = 3; *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 capsules 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 Kingdom 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 conditions is important.

### 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 ± 5.37 |
|                 | 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.*
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 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

### 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 | 2.94  | 6  | ≤0.05|
| by nematode species             | 1.44  | 12 | ≥0.05|

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’.

*Journal of Insect Science, 2018, Vol. 18, No. 3*
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*-infested 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-Ilan 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 acknowledged 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 Aboheashaa for her help in designing the Figure no. (1) in this article using the software Inskape.

**References Cited**

Akhurst, R., and K. Smith. 2002. Regulation and safety, pp. 311–332. In R. Gaugler (ed.), Entomopathogenic nematology. CABI Publishing, New Jersey.

Al-Sayed, A. E., and S. S. Al-Tamiemi. 1999. Al-Sayed, A. E., and S. S. Al-Tamiemi. 1999. Evaluation of applica-
tion technologies of entomopathogenic nematodes for control of the black vine weevil, *Phylolobus luminescens*. Science 280: 2129–2132.

Bruck, D. J., D. L. Shapiro-Ilan, and E. L. Lewis. 2005. Evaluation of application technologies of entomopathogenic nematodes for control of the black vine weevil. J. Econ. Entomol. 98: 1884–1889.

Burman, M. 1982. *Neoseiulus cucumeris*: toxin production by axenic insect parasitic nematodes. Nematologica 28: 62–70.

Campbell, J. F., and R. Gaugler. 1997. Inter-specific variation in entomopatho-
genome foraging strategy: dichotomy or variation along a con-
tinuum. Fundamental Appl. Nematol. 20: 393–398.

Campos-Herrera, R. 2015. Nematode pathogenicity of insects and other pests: ecology and applied technologies for sustainable plant and crop protec-
tion, 1st ed. Springer International Publishing, Cham, Switzerland.

Del Valle, E., E. C. Dolinski, E. L. S. Barreto, and R. M. Souza. 2009. Effect of cadaver coatings on emergence and infectivity of the entomopathogenic nematode *Heterorhabditis bacteriophora* LPP7 (Rhabditida: Heterorhabditidae) and the removal of cadavers by ants. Biol. Control 50: 21–24.
Lewis, E. E., and D. J. Clarke. 2012. Nematodes parasites and entomopathogens. In F. E. Vega and H. K. Kaya (eds.), Insect pathology, 2nd ed. Academic Press, Amsterdam, Netherlands.

Lewis, E. E., J. Campbell, C. Griffin, H. K. Kaya, and A. Peters. 2006. Behavioral ecology of entomopathogenic nematodes. Biol. Control 38: 66–79.

Luan, X., S. Zhang, H. Yang, and X. Yang. 1996. Evaluation of entomopathogenic nematodes for control of peanut scarabs, pp. 25–30. In T. A. Jackson and T. R. Glare (eds.), Proceed. 3rd Intl. Workshop on Microb. Control of Soil Dwelling Pests. The Microbial Control Group, Lincoln, New Zealand.

Martin, H. E. 1972. Report to the government of Saudi Arabia on research in plant protection based on the work of H. E. Martin, FAO entomologist. FAO/SAU/TF/63 (AGP: TA/207), pp. 38 (Abstr.: Rev. Appl. Entomol. 61: 1064, 1973.).

Monastyrskij, A. L., and V. V. Gorbatovskij. 1991. Effective rearing of insects for biological plant protection. Agropromizdat (in Russian), Moscow, Russia.

Püntener, W. 1981. Manual for field trials in plant protection, 2nd ed. Agric. Division, Ciba-Geigy Limited, Basle, Switzerland.

Shapiro-Ilan, D. I., and E. E. Lewis. 1999. Comparison of entomopathogenic nematode infectivity from infected hosts versus aqueous suspension. Environ. Entomol. 28: 907–911.

Shapiro-Ilan, D. I., R. Gaugler, W. L. Tedders, I. Brown, and E. E. Lewis. 2002. Optimization of inoculation for in vivo production of entomopathogenic nematodes. J. Nematol. 34: 343–350.

Shapiro-Ilan, D. I., E. E. Lewis, Y. Son, and W. L. Tedders. 2003. Superior efficacy observed in entomopathogenic nematodes applied in infected-host cadavers compared with application in aqueous suspension. J. Invertebr. Pathol. 83: 270–272.

Shapiro-Ilan, D. I., J. A. Morales-Ramos, M. G. Rojas, and W. L. Tedders. 2010. Effects of a novel entomopathogenic nematode-infected host formulation on cadaver integrity, nematode yield, and suppression of Diaprepes abbreviatus and Aethina tumida. J. Invertebr. Pathol. 103: 103–108.

Shapiro-Ilan, D. I., R. Han, and C. Dolinski. 2012. Entomopathogenic nematode production and application technology. J. Nematol. 44: 206–217.

Shapiro-Ilan, D. I., S. Hazir, and L. Lete. 2015. Viability and virulence of entomopathogenic nematodes exposed to ultraviolet radiation. J. Nematol. 47: 184–189.

Shetlar, D. J., P. E. Suleman, and R. Georgis. 1988. Irrigation and use of entomogenous nematodes, Neaplectana spp. and Heterorhabditis beliobidila (Rhabditida: Steinernematidae and Heterorhabditidae), for control of Japanese beetle (Coleoptera: Scarabaeidae) grubs in turf grass. J. Economic Entomol. 81: 1318–1322.

White, C. F. 1927. A method for obtaining infective larvae from culture. Science 66: 302–303.

Woodring, J. L., and H. K. Kaya. 1988. Steinernematid and heterorhabditid nematodes: a handbook of techniques. Southern Cooperative Series Bulletin No. 331, Arkansas Agricultural Experiment Station, Fayetteville, AR, pp. 30.

Wright, D. J., A. Peters, S. Schroer, and J. P. Fife. 2005. Application technology, pp. 91-106. In P. S. Grewal, R.-U. Ehlers, and D. I. Shapiro-Ilan (eds.), Nematodes as biocontrol agents. CABI Publishing, Wallingford, United Kingdom.