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Original article (Orijinal araştırma)

Orientation of some *Heterorhabditis bacteriophora* (Poinar, 1976) (Rhabditida: Heterorhabditidae) strains to *Lolium perenne* L. (Poales: Poaceae) and *Galleria mellonella* (L., 1758) (Lepidoptera: Pyralidae)

Bazı *Heterorhabditis bacteriophora* (Poinar, 1976) (Rhabditida: Heterorhabditidae) ırklarının *Lolium perenne* L. (Poales: Poaceae) ve *Galleria mellonella* (L., 1758) (Lepidoptera: Pyralidae)'ya yönelimi

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Abstract

Entomopathogenic nematodes (EPNs) have different host-finding (foraging) behavior that varies from species to species. Besides their foraging behavior, the orientation of some EPNs can vary depending on plant roots. In the present study, the orientation of some *Heterorhabditis bacteriophora* (Poinar, 1976) (Rhabditida: Heterorhabditidae) strains to perennial ryegrass *Lolium perenne* L. (Poales: Poaceae) roots and greater wax moth *Galleria mellonella* (L., 1758) (Lepidoptera: Pyralidae) larvae were investigated. For this purpose, three different strains of *H. bacteriophora* were used. One of them was a hybrid strain (HBH) and the two were parents (HB1138 and HB4) of the hybrid strain. Three experimental combinations were conducted in the study conducted in 2019 under laboratory conditions in Bursa, Turkey: (A) plant roots, larvae and control; (B) plant roots, control I and control II; and (C) mechanically damaged plant roots, control-I and control-II in Y-tube olfactometers filled with moist sand. The results indicated that for combination A, most orientation to plants and larvae was seen in HB1138; for combination B, most orientation to plant roots was was seen in HBH; and for combination C, strain HB4 showed the most orientation to mechanically damaged plant roots. According to the results, each strain of the same EPN species may have a different response to plant roots and host insects.

Keywords: Entomopathogenic nematodes, perennial ryegrass, orientation

Öz

Entomopatojen nematodlar (EPN'ler) türlere bağlı olarak, birbirinden farklı konukçu bulma davranışlarına sahiptirler. Konukçu arama davranışlarının yanı sıra, bazı EPN'lerin topraktaki yönelimleri bitki köklerine bağlı olarak değişebilir. Bursa'da laboratuvar koşullarında 2019 yılında yapılan bu çalışmada bazı *Heterorhabditis bacteriophora* (Poinar, 1976) (Rhabditida: Heterorhabditidae) ırklarının İngiliz çimi *Lolium perenne* L. (Poales: Poaceae) köklerine ve Petek Güvesi *Galleria mellonella* (L., 1758) (Lepidoptera: Pyralidae) larvalarına yönelimleri araştırılmıştır. Bu amaçla, üç farklı *H. bacteriophora* ırkı kullanılmıştır. Bunlardan birisi hibrit tür (HBH) diğer ikisi ise hibrid türün ebeveynidir (HB1138, HB4). Çalışma, üç farklı deneysel kombinasyon ile laboratuvar koşullarında yapılmıştır. Y-Olfaktometre düzeneğinde A kombinasyonu bitki kökleri, larvalar ve kontrol; B kombinasyonu bitki kökleri, kontrol-I ve kontrol-II; C kombinasyonu ise mekanik olarak yaralanmış bitki kökleri, kontrol-I ve kontrol-II bölümlerini içermektedir. Elde edilen sonuçlar; Kombinasyon A'da bitki köklerine ve böcek larvalarına karşı en yüksek yönelimi HB1138 göstermiştir. Kombinasyon B'de bitki köklerine en yüksek yönelimi HBH ve kombinasyon C'de mekanik olarak yaralı bitki köklerine en yüksek yönelimi HB4 sergilemiştir. Bu çalışmada, aynı türe ait farklı EPN ırklarının bitki köklerine ve konukçu böceklere yönelimi değişebileceği tespit edilmiştir.

Anahtar sözcükler: Entomopatojen nematodlar, İngiliz çimi, yönelim

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Introduction

Entomopathogenic nematodes (EPNs) belonging to the families Heterorhabditidae and Steinernematidae are used commercially in place of chemical pesticides in biological control against insect pests, especially of soil inhabitants (Ehlers, 2001; Susurluk, 2011; Lacey & Georgis, 2012). Heterorhabditidae and Steinernematidae are associated symbiotically with *Photorhabdus* and *Xenorhabdus* bacteria, respectively (Boemare et al., 1993). In the soil, only the third-stage larvae (infective juveniles, IJs) of EPNs actively move to find their hosts. The IJs release the bacteria when they reach to the host insect hemocoel, so the bacteria cause the insect to die from septicemia within 36-48 h (Kaya & Gaugler, 1993). EPNs have different host-finding strategies (foraging behavior), known as ambusher, cruiser and intermediate (Lewis et al., 1993; Grewal et al., 1994; Susurluk et al., 2004). Cruiser IJs move continuously to find their hosts and they are mostly more effective against sedentary insect pests (Lewis et al., 1992). *Steinernema glaseri* (Steiner, 1929) (Rhabditida: Steinernematidae) and *Heterorhabditis bacteriophora* (Poinar, 1976) (Rhabditida: Heterorhabditidae) have cruiser behavior (Susurluk et al., 2004; Bal & Grewal, 2015). However, the IJs that have ambusher behavior are more effective at infecting mobile insect hosts and tend to stay in the same place waiting for their hosts (Campbell & Gaugler, 1993; Kaya & Gaugler, 1993). *Steinernema carpocapsae* (Weiser, 1955) (Rhabditida: Steinernematidae) shows ambusher host finding strategy (Bal & Grewal, 2015). The intermediate IJs have behavior between the two foraging strategies, which are cruiser and ambusher. This foraging behavior is shown by *Steinernema feltiae* (Filipjev, 1934) (Rhabditida: Steinernematidae) (Susurluk, 2009). The difference in host-finding strategies depends on the species of EPNs (Grewal et al., 1994).

EPNs tend to respond to the chemical cues of their hosts, such as carbon dioxide, feces and plant roots (Boff et al., 2002; Webster & Cutler, 2003; Bal & Grewal, 2015). Plant roots have chemical and physical defense mechanisms against harmful insects and can secrete volatile chemicals as a sign to allure natural enemies of herbivores (Turlings & Wäckers, 2004; Rasmann et al., 2005; Tonelli et al., 2016). For example, carbon dioxide is an important signal for EPNs secreted by both plant roots and host insects (Susurluk, 2009; Turlings et al., 2012). However, the plant roots also can produce a repellent effect against EPNs (Boff et al., 2002; Bal & Grewal, 2015).

The aim of the study was to determine the orientation of three *H. bacteriophora* strains (one a hybrid strain *H. bacteriophora* HBH and two parents of the hybrid strain *H. bacteriophora* HB1138 and *H. bacteriophora* HB4) to the perennial ryegrass roots, *Lolium perenne* L. (Poales: Poaceae) and insect host larvae, *Galleria mellonella* (L., 1758) (Lepidoptera: Pyralidae). The experiment was performed in three combinations: A) plant roots, larvae and control; B) plant roots, control and control II; and C) mechanically damaged plant roots, control I and control II in moist sand filled Y-tube olfactometers.

Materials and Methods

Culture of the EPN strains

In this study, the hybrid strain *H. bacteriophora* HBH and their parents that isolated from Antalya (*H. bacteriophora* HB1138) and Şanlıurfa (*H. bacteriophora* HB4) in Turkey were used. The last-instar larvae of the *G. mellonella* were selected as host for the production of infective juveniles (IJs). IJs were extracted using the White trap method (White, 1927) and stored in standard Ringer's solution (distilled water + KCl + $CaCl_2 \cdot 2H_2O$ + NaCl + $NaHCO_3$) at 4°C. The experiments were conducted with 1-week-old IJs.

Culture of *Galleria mellonella*

The greater wax moth, *G. mellonella,* larvae have been used as a host since it can be grown easily and have a suitable size for the production of EPNs. Sufficient number of EPNs can be obtained by in vivo production using the last-instar larvae of the *G. mellonella* (Kaya & Stock, 1997). Honey (200 g), glycerin

(200 g), yeast (50 g), milk powder (100 g), soybean flour (100 g), cornstarch (150 g) and bran (200 g) were used in the mass production of the larvae (Wiesner, 1993). The insect larvae with the food were kept in glass jars at 30ºC.

Culture of *Lolium perenne*

Lolium perenne plants with about 10 cm long roots were taken from the Department of Field Crops (Bursa Uludağ University, Faculty of Agriculture). Two kinds of roots were used as healthy roots and damaged roots of the grass, which were cut 1 cm from end by scissors.

Experimental design and methodology

The Y-tube olfactometer (Boff et al., 2001; Susurluk, 2011) was used to test the orientation of the strains to perennial ryegrass root, insect hosts *G. mellonella* and control arms. Olfactometer has three separate arms (diameter 2.5 cm by 12.5 cm long), which were filled with sterilized quartz sand (particle size: 300-400 µm) 10% moistened by sterilized tap water. Three separate experiments were established simultaneously with the different combinations (Figure 1). This study was performed in nematology laboratory in Department of Plant Protection in Faculty of Agriculture in University of Bursa Uludağ in 2019.

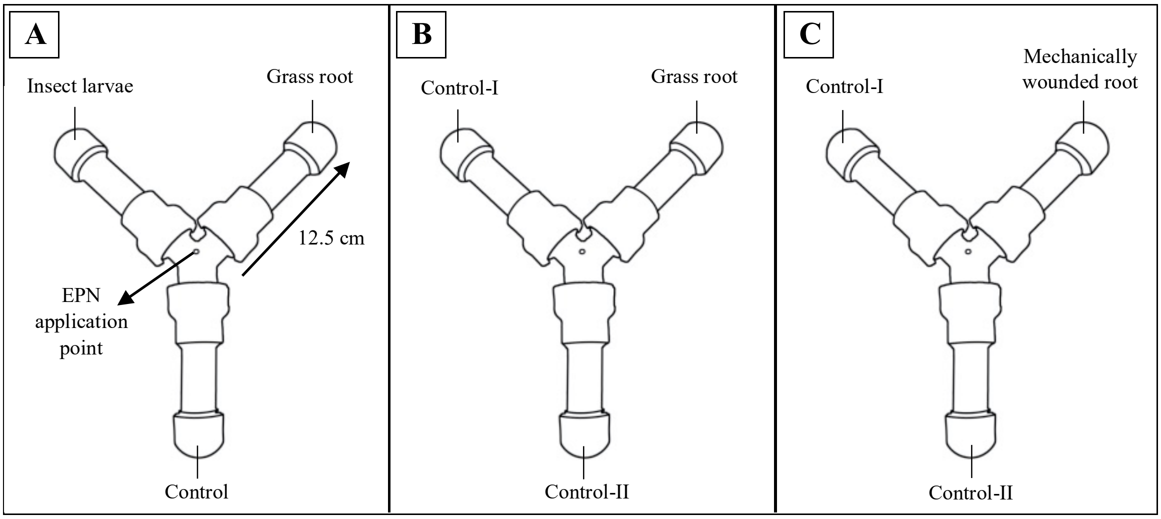

Figure 1. Schematic drawing of used Y-tube olfactometer in the study. A, B and C show the experimental combinations.

In the experimental combination A, the insect host larvae and the plant roots were placed at the end of the first two olfactometer arms. The other arm was kept as a control without any treatment. In the experimental combination B, only the plant roots were transferred at the end of one arm. The other arms, control-I and control-II were without any treatment. The experimental combination C was constructed in a similar manner to the B. However, the plant roots used in this experiment were mechanically damaged (Figure 1).

Before adding the strains in the center of the olfactometers, the different combinations were incubated horizontally at 25ºC for 24 h to for chemical gradient into the olfactometer. Then, about 1000 IJs were applied to the center of each olfactometer. After inoculation of the IJs, incubation was continued for a further 96 h (4 d). Four d after the inoculation, the IJs were recovered from the quartz sand using the Cobb’s (1918) decanting and sieving method (Klein et al., 1994). Afterwards, the number of the IJs showing orientation towards different arms was determined and each combination was repeated three times.

Statistical analysis

Statistical differences were detected in the orientations of the strains by using one-way ANOVA in JMP®7.0 Software. LSD test ($P < 0.05$) was used to settle the difference between means.

Results and Discussion

The orientations of the strains towards *Lolium perenne* roots and *Galleria mellonella* larvae (combination A)

Among the strains, HB1138 showed statistically higher orientation to *L. perenne* roots and *G. mellonella* larvae than HB4 and HBH. The statistically highest orientation towards control was shown by HB4, while the lowest by HB1138, HBH and HB4 strains showed more tendency towards the control arm. Also, the orientations of the HBH, HB4 and HB1138, to *G. mellonella* larvae and *L. perenne* roots were not statistically different (F = 32.6, df = 8,18, P < 0.0001) (Figure 2).

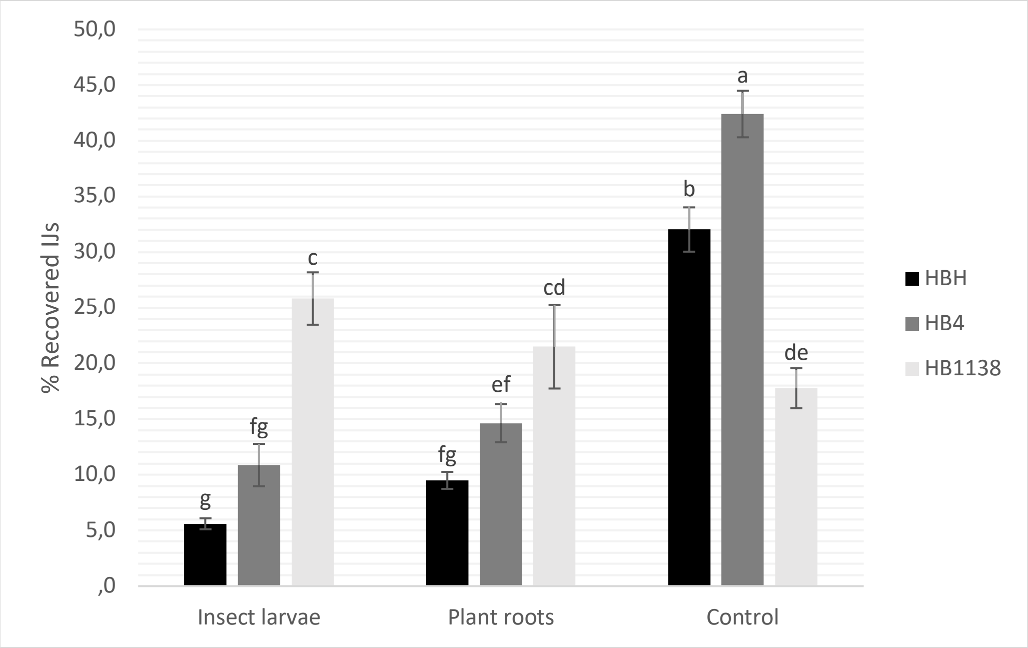

Figure 2. *Heterorhabditis bacteriophora* strain (HBH, HB4 and HB1138) orientations towards perennial ryegrass roots and wax moth.

The orientations of the strains towards the healthy roots of *Lolium perenne* (combination B)

Among the strains, HBH showed significantly highest orientation to the grass roots. The orientations of HB1138 and HB4 to plant roots were not statistically different. HBH and HB1138 strains showed more tendency towards the control-II, but the orientations of the three strains were not statistically different towards control-I. (F = 22.0, df = 8,18, P < 0.0001) (Figure 3).

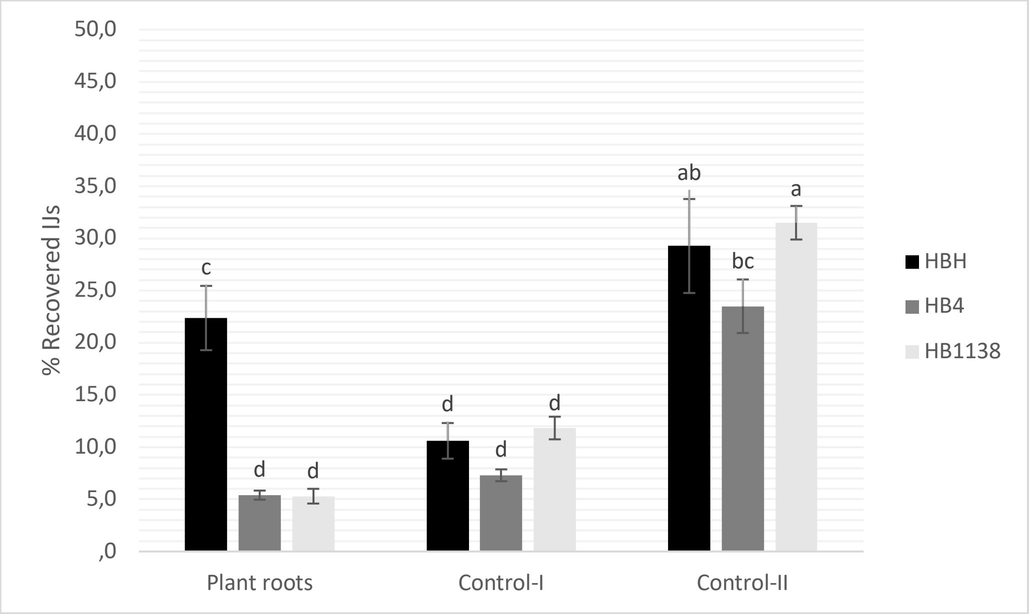

Figure 3. *Heterorhabditis bacteriophora* strains (HBH, HB4, HB1138) orientations towards perennial ryegrass roots.

The orientations of the strains towards the damaged roots of *Lolium perenne* (combination C)

Among the strains, HB4 had the significantly highest orientation to the damaged plant roots. The orientations of HB1138 and HBH to the damaged plant roots were not statistically different. Also, HB4 showed the statistically highest orientation towards control-II (F = 91.8, df = 8,18, P < 0.0001) (Figure 4).

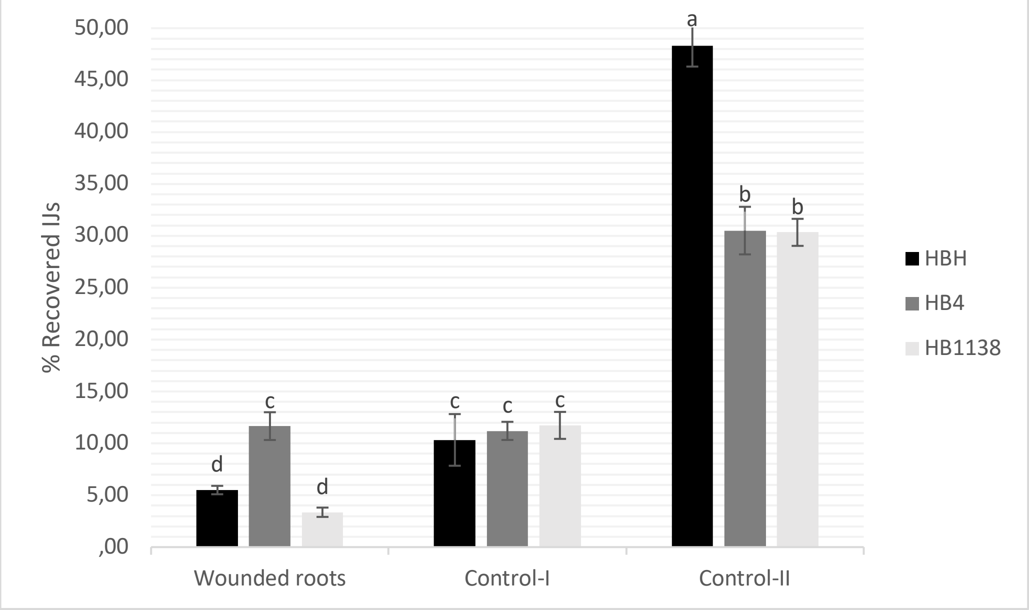

Figure 4. *Heterorhabditis bacteriophora* strains (HBH, HB4, HB1138) orientations towards perennial ryegrass roots that were damaged.

In biological control, the effectiveness of entomopathogenic nematodes (EPNs) is mainly determined by host-finding (foraging) strategy, insect species and soil conditions (Shapiro-Ilan et al., 2006). It is known that the foraging strategies (ambusher, cruiser and intermediate) depend on the EPN species (Grewal et al., 1994). Campbell & Gaugler (1993) described *H. bacteriophora* as a cruiser. All the strains (HBH, HB4 and HB1138) used in this trial belong to *H. bacteriophora* species. The plant roots of *L. perenne* and *G. mellonella* larvae were selected to determine the orientations between the *H. bacteriophora* strains. The effectiveness and dispersal of *H. bacteriophora* are better for sedentary insect hosts as evidenced in the research of Bal & Grewal (2015). Investigations have proved that EPNs use chemical signals secreted by plants or insects to find their hosts (Boff et al., 2001; Rasmann et al., 2005; Rasmann & Turlings, 2008; Turlings et al., 2012; Lortkipanidze et al., 2016; Tonelli et al., 2016). EPNs use volatile chemicals released from the plant roots and insect, such as carbon dioxide, to find their hosts (Susurluk, 2009; Turlings et al., 2012; Bal & Grewal, 2015; Tonelli et al., 2016). However, there should be some EPN strains that do not show any inclination to elevated amounts of the plant volatiles (Anbesse & Ehlers, 2013; Laznik & Trdan, 2013).

In the present study, both mechanically damaged and healthy plant roots of *L. perenne* were tested separately. In the experimental combination A, which contained both the insect larvae and plant roots in their separate arms, had the statically highest orientation to *G. mellonella* and *L. perenne* roots for HB1138. The orientation of HBH, HB4 and HB1138 individually to the insect larvae and the plant roots was not statistically different. Similarly, Rasmann & Turlings (2008) investigated the tritrophic interactions containing maize plant roots, herbivore insects and EPNs. They found that the orientation of *H. bacteriophora* towards healthy plant roots and insect larvae was not statically different, consistent with the results of the present study. However, the nematode *Heterorhabditis megidis* Poinar, Jackson & Klein, 1987 (Rhabditida: Heterorhabditidae) showed more orientation to plant roots than to insect larvae (Rasmann & Turlings, 1987). Susurluk (2011) studied the larvae of *Delia radicum* L. (1758) (Diptera: Anthomyiidae) (Diptera: Anthomyiidae), oilseed rape roots and in a combination of both using Y-tube olfactometer at 8 and 15ºC. In contrast, in that study the maximum orientation of the *S. feltiae* was to the insect larvae of *D. radicum* at both temperature values. Different result might be obtained when using other *S. feltiae* strains. Although the response of HB1138 to the insect was statically more significant than other strains (HBH and HB4) and the control. The orientation of HBH and HB4 towards control was higher compared to the plant roots and the insect larvae. However, Rasmann & Turlings (2008) indicated that using maize plant roots, *H. bacteriophora* did not have a statistically significant response towards healthy plant roots and insect larvae only *Diabrotica virgifera virgifera* LeConte, 1868 (Coleoptera: Chrysomelidae) or the control. However, *H. megidis* migrated significantly towards undamaged plant roots than control (Rasmann et al., 2005). The nematode, *H. megidis*, had different behavior from strains of *H. bacteriophora* in the present study. Tonelli et al. (2016) found, when the roots of sugarcane were used, that bothof *Heterorhabditis indica* (Poinar, 1992) (Rhabditida: Heterorhabditidae) and *S. carpocapsae* responded equally to the plant roots and the control of moistened sand only.

In combination B; among the strains, HBH had the highest orientation to the undamaged *L. perenne* roots. Rasmann & Turlings (2008) found that the orientation of *H. megidis* to maize and cotton plant roots was significantly higher than to damaged cowpea roots, which is consistent with the findings of the present study. In combination C; in all the strains, HB4 had the highest orientation to the mechanically damaged *L. perenne* roots. However, Rasmann et al. (2005), using a six-arm olfactometer, found the orientation of *H. megidis* towards mechanically damaged and undamaged maize plants roots were not statically different. Boff et al. (2001) found that *H. megidis* had more orientation towards mechanically damaged strawberry roots than to roots damaged by *Otiorhynchus sulcatus* (Fabricius, 1775) (Coleoptera: Curculionidae) larvae, which is again consistent with the results of the current study. Whereas, *Thuja occidentalis* L. (Pinales: Cupressaceae) roots damaged by *O. sulcatus* larvae were more attractive to *H. megidis* than to mechanically damaged roots (Boff et al., 2002), which differs from the findings of the present study, because there might be a difference between roots damaged with scissors and those damaged by insect. Additionally, Ali et al. (2010) reported that when citrus roots were damaged by insect larvae, *Diaprepes abbreviates* (L., 1758) (Coleoptera: Curculionidae), more EPNs were attracted than to mechanically damaged roots. According to those results, roots with an insect bite can be more attractive to EPNs. To date, all studies on the orientation of EPNs have been conducted with different EPN species. However, in the present study different strains of the same *H. bacteriophora* were used.

The results of the present study indicate that the orientations between the strains of *H. bacteriophora* towards *G. mellonella* larvae and undamaged or mechanically damaged *L. perenne* roots may be significantly different. The response of the EPN species and strains on plant roots or insect hosts can significantly affect the biological control potential of the EPNs. However, further studies should be conducted in the field to confirm these findings. This would allow the proper application of EPNs and enhance biological control.

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