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RESEARCH

Efficacy of Native Iranian Entomopathogenic Nematodes Against Mediterranean Fruit Fly, *Ceratitis capitata* Wiedemann (Diptera: Tephritidae)

Nazila Samadaei[1] · Mehrdad Rahimpour[1,2] · Shokoofeh Kamali[1,3] · Javad Karimi[1] · Albrecht M. Koppenhöfer[4]

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Abstract

The Mediterranean fruit fly, *Ceratitis capitata*, is a severe pest of orchards around the world and has recently invaded orchards in northern Iran. The soil-dwelling larvae of this pest are amenable to control by entomopathogenic nematodes (EPNs) but have been studied only in tropical orchards. To assess the biocontrol potential of Iranian strains of two EPN species, *Heterorhabditis bacteriophora* (boj) and *Steinernema carpocapsae* (z1), for control of *C. capitata*, we conducted a series of laboratory and a field experiment. the highly virulent nature of *S. carpocapsae* towards *C. capitata* larvae was apparent, demonstrating near 80% efficacy at a concentration of 25 infective juveniles (IJs) per cm^2 in loam soil, surpassing the 40% control exerted by *H. bacteriophora*. When tested at temperatures of 19, 25, and 30 °C, *Steinernema carpocapsae* caused the highest mortality at 25 °C (85%) and the lowest at 30 °C (58%), whereas control by *H. bacteriophora* was not affected by temperature (44–50%). Both EPN strains caused higher mortality in loam and clay loam soils than in sandy loam. *Heterorhabditis bacteriophora* produced significantly more IJ progeny from infected larvae than *S. carpocapsae.* Both *S. carpocapsae* and *H. bacteriophora* caused only moderate mortality of *C. capitata* pupae with 38 and 27%, respectively, at a rate of 50 IJs/cm^2. Under field conditions, both species caused significant mortality when sprayed at a concentration of 25 IJs/cm^2 on soil and tangerine fruits infested with *C. capitata* larvae, with 45 and 69% mortality for *H. bacteriophora* and *S. carpocapsae*, respectively. Our findings suggest that *S. carpocapsae* (z1) could be implemented as a biological agent in a *C. capitata* management program in temperate climate orchards.

Keywords Insect pathology · Biocontrol · Infective juveniles · Pest management · Soil pest

Introduction

The Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), is one of the most important pests of tropical, subtropical, and temperate fruit crops in many parts of the world (Elqdhy et al. 2024). It is highly polyphagous, known to attack 362 species of wild and cultivated plants within 161 genera and 63 families (Liquido et al. 2024). The significant losses caused by this fly result from damage caused by the egg-laying activity and the feeding of larvae within the fruits' pulp (Dias et al. 2022; James et al. 2018; Mokrini et al. 2020). Moreover, the presence of the pest also restricts the export of fruit to uninfested areas, further adding to financial losses of producers (Ovruski and Schliserman 2012). Many of the control methods used for this pest focus on controlling the adults, using insecticides, baiting methods (Flores et al. 2011; Mokrini et al. 2020; Urbaneja et al. 2009) and, in some places, the release

The Authors Nazila Samadaei and Mehrdad Rahimpour contributed equally to the manuscript.

✉ Javad Karimi
jkb@um.ac.ir

[1] Department of Plant Protection, School of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran

[2] Department of Plant Protection, College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran

[3] Department of Entomology and Nematology, Citrus Research and Education Center, University of Florida, Lake Alfred, FL, USA

[4] Department of Entomology, Rutgers University, New Brunswick, NJ, USA

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of sterile males (James et al. 2018; Toledo et al. 2023). Much research on this pest has been dedicated to finding environmentally friendly control techniques and production methods to avoid negative impacts on non-target organisms, environmental pollution, and the development of insecticide resistance, all resulting from excessive insecticide use (Desneux et al. 2007; Dolinski 2016).

The fly spends a significant part of its life cycle in the soil (Elqdhy et al. 2024). The third-instar larvae drop from the fruit to the ground to bury to a depth of 5–15 cm where they pupate and eventually emerge as adults. This tephritid can go through 3–7 generations per year (Papadopoulos 2008). Egg hatch duration is influenced by seasonal temperature which ranges from 10–15 d during autumn and spring, shortened to 2–3 d during summer, and extended during winter contingent upon ambient temperature conditions. The larval period lasts 10–14 d during summer to 2–3 weeks in spring and autumn. The duration of each stage also can be affected by the specific type and quality of the fruit that serves as the host. The pupal stage may last 6–13 d at 24.4–26.1 °C to a minimum of 19 d at 20.6–21.7 °C (Abd-Elgawad 2021).

A broad spectrum of soil-dwelling biocontrol agents such as entomopathogenic nematodes (EPNs) (Rhabditida: Steinernematidae and Heterorhabditidae) may target multiple life stages of the target pest. Specifically, the subterranean developmental phases, encompassing the larval, prepupal, and pupal stages, as well as the vulnerable period immediately following adult emergence, represent potential opportunities for biocontrol intervention. EPNs are obligate parasites of insects with an infective juvenile (IJ) stage that seeks out a host and penetrates it through natural body openings to ultimately reach the host's hemolymph, where it releases symbiotically associated bacteria (*Xenorhabdus* and *Photorhabdus* species) from its gut (Kaya and Gaugler 1993). These bacteria, producing different antibiotics and secondary metabolites, kill the host within 48–72 h and then break down the host tissues, thus providing the conditions and nutrients for the nematodes to feed on and develop. The nematodes go through 1–3 generations until the host cadaver is depleted at which point a new cohort of IJs emerges from the cadaver and seeks a new host.

EPNs in the genera *Steinernema* and *Heterorhabditis* possess great potential to control a broad range of soil-borne insect pests (Koppenhöfer et al. 2020) including several species of tephritid fruit flies in orchards of tropical and temperate climates (Maniania et al. 2017; Shapiro-Ilan et al. 2017; Elqdhy et al. 2024). A number of studies have examined the potential of EPNs for control of *C. capitata* in laboratory and greenhouse studies (Elqdhy et al. 2024) but field studies have been very limited and restricted to tropical orchards (mango, papaya, guava) and to the EPN species *Steinernema feltiae, S. riobrave*, and *Heterorhabditis baujardi* (Maniania et al. 2017).

Given that *C. capitata* has in recent years invaded orchards, particularly citrus orchards, in the northern regions of Iran along the southern shore of the Caspian Sea (Mazandaran Province), which have a warm temperate climate, it appeared timely to study the potential of EPNs for control of *C. capitata* under these different conditions. We were particularly interested in testing indigenous EPN strains which may be better adapted to the regional climate conditions. Identifying native isolates and assessing their performance against the susceptible life stages of the fruit fly under field conditions to find the most efficient isolate may enhance pest management programs for this pest while avoiding exotic biological agents and the risk of their adverse effects (Mokrini et al. 2020). Our investigation aimed to explore the potential of two Iranian entomopathogenic nematode (EPN) strains, *S. carpocapsae* (z1 strain) and *H. bacteriophora* (boj strain), in controlling *C. capitata* larvae and pupae.

Material and Methods

Insect and Nematode Rearing

A *C. capitata* colony was started from adults obtained from infested tangerine fruits from citrus orchards in Sari, Mazandaran Province in northern Iran (36°30′38.5″N, 53°00′00.1″E) and was kept in plastic containers (40 × 30 × 15 cm) in a growth chamber (25 ± 1 °C, 65 ± 10% RH, 13 h dark:11 h light). Healthy last instar larvae (7 d old) were selected and used for all tests. Another cohort of healthy larvae were maintained for an additional 24 h to pupate. Newly formed pupae were utilized immediately for subsequent experiments. *Heterorhabditis bacteriophora* (boj) and *S. carpocapsae* (z1) were obtained from the Insect Pathology and Biocontrol Laboratory of Ferdowsi University (Mashhad, Khorasan Razavi, Iran) and reared in late-instar larvae of the greater wax moth, *Galleria mellonella* (Lepidoptera: Pyralidae) (Kaya and Stock 1997). Before use in experiments, the IJs were acclimatized at 25 ± 1 °C for 1 h and their viability checked by observing their motility under a stereo microscope.

Laboratory Bioassays with *C. capitata* Larvae

In all below experiments in the laboratory, the same experimental arena was used. It consisted of a cylindrical plastic container (4 cm diam × 4 cm ht) that was filled to depth of 2.5 cm with 25 g of dry soil (soil surface area 12.6 cm^2). The soil had been sieved (5 mm), then autoclaved (121 °C for 120 min) and finally air dried. If not mentioned otherwise,

the soil used was a loam (36% sand, 43% silt, 21% clay, pH 7.34). IJ EPNs were evenly pipetted over the soil surface in 2.5 ml water resulting in a soil moisture of 10% (w/w). Untreated controls received 2.5 ml water only. If not otherwise mentioned, 10 third-instar *C. capitata* larvae were added and allowed to bury into the soil. Then the containers were closed with a lid and kept in a growth chamber at 35 ± 10% RH and, if not otherwise mentioned, 25 ± 1 °C (13 h dark:11 h light). For evaluation, the soil in each container was searched for larvae and pupae, mortality recorded, and EPN infection confirmed by dissection under a stereomicroscope.

Dose Response to EPNs *Heterorhabditis bacteriophora* (boj) and *S. carpocapsae* (z1) were applied at seven concentrations (1.63, 3.13, 6.25, 12.5, 25, 50, and 100 IJs/cm^2). Mortality was evaluated after 72 h. Each treatment had four replicates, and the whole experiment was conducted twice.

Reproductive Potential Medfly in Larvae *Heterorhabditis bacteriophora* (boj) and *S. carpocapsae* (z1) were applied at 10, 25, 50, 100, and 150 IJs/cm^2. After 5 d of exposure, the *C. capitata* cadavers were collected, rinsed with distilled water, and placed individually on emergence traps. The traps were kept at room temperature (25 ± 1 °C) for 28 d to allow for the complete emergence of any produced IJs. To calculate the reproduction potential, the total number of emerged IJs was determined under a stereo microscope. Each rate of each EPN species had four replicates. For each strain, an additional two containers were treated with 50 IJs/cm^2 to observed EPN development. After 4 days of exposure, some of the cadavers infected with *H. bacteriophora* were rinsed and then dissected under a stereomicroscope to ensure the presence of hermaphrodite individuals. The remaining cadavers infected for both EPN species were dissected under a stereomicroscope after 6–8 d of exposure to differentiate between male and female specimens. Microscopic slides were prepared for any individuals that needed to be further identified under a light microscope.

Effect of Temperature On EPN Virulence The virulence of *Heterorhabditis bacteriophora* (boj) and *S. carpocapsae* (z1) applied at 25 IJs/cm^2 was compared at 19, 25, and 30 °C. Larval mortality was determined after 72 h of exposure. Each treatment had four replicates, and the experiment was conducted twice.

Effect of Soil Type On EPN Virulence The virulence of *H. bacteriophora* (boj) and *S. carpocapsae* (z1) applied at 25 IJs/cm^2 was compared in three soil types: a sandy loam (63% sand, 24% silt, 13% clay, pH 8.56), a loam (36% sand, 43% silt, 21% clay, pH 7.34) and a clay loam (36% sand, 30% silt, 34% clay, pH 7.67). Larval mortality was determined after 72 h of exposure. Each treatment had four replicates, and the experiment was conducted twice.

Virulence Against Pupae in the Laboratory

The virulence of *H. bacteriophora* (boj) and *S. carpocapsae* (z1) to *C. capitata* pupae was tested at the concentration of 50 IJs/cm^2 in the same arenas as used in the above experiments with larvae. Pupae (1 d old) were placed individually in a 2 cm deep hole in the soil and then covered with soil. Ten pupae were placed per container. The containers were covered with lids and transferred to a growth chamber (35 ± 10% RH, 25 ± 1 °C, 13 h dark: 11 h light). After 2 weeks, the soil in each container was searched for pupae and mortality recorded. Each treatment had three replicates and the experiment was conducted twice.

EPNs Efficacy Against Larvae Within Fruits On Soil in Field Condition

The virulence of *H. bacteriophora* (boj) and *S. carpocapsae* (z1) against larvae within infested fruits lying on the soil surface was evaluated in a tangerine orchard located in Sari, Mazandaran province, North Iran (36°30′38.5″N, 53°00′00.1″E). Experimental units were plastic containers (23 cm length × 16 cm width × 10 cm height) containing 25 g of the same loam soil as in previous tests. Six tangerine fruits infested by fruit flies were placed on the soil surface of each container. The EPNs were prepared in 10 ml distilled water and sprayed onto the soil and fruits at a rate of 25 IJs/cm^2 by using a hand sprayer (BEHCO, BP-2087, BEHCO Trading Co, Hemei, Taiwan). Untreated controls received 10 ml of distilled water only. The containers were covered with a screen mesh and placed under tangerine trees. Each treatment had four replicates, and the experiment was carried out twice. Mortality was determined 3 d after treatment by searching the soil and the fruits in the containers and recording the number of live and dead larvae and pupae. Recovered cadavers were dissected under a stereomicroscope to confirm EPN infection.

Statistical Analysis

Abbott's formula was used to adjust insect mortality (Abbott 1925). Data were normalized by arcsine transformation if necessary. Data in the experiments on dose response, reproductions, temperature, and soil type were subjected to two-way ANOVA and means separated with LSD test ($P < 0.05$). Data on virulence against pupae were analyzed with student t-test ($P < 0.05$). The mortality data for EPNs efficacy against larvae within fruits on soil were analyzed by one-way analysis of covariance (ANCOVA), where the EPN species was considered as the main factor and the total

number of larvae as a covariate followed up by Tukey HSD test to distinguish the differences among means whether the covariate was significant ($P > 0.05$). All analyses were performed using SAS software, version 9.4. Means are shown with the standard error of the mean (SEM).

Results

Dose Response to EPNs

No mortality was observed in the untreated control group. Conversely, all recovered dead individuals whether it was larvae or pupae from treated arenas exhibited infection by the applied EPN species. Most larvae succeeded to enter the pupal stage before their death. IJ concentration ($F_{6,70} = 125.09$, $P = 0.0001$) and EPN species ($F_{1,70} = 425.09$, $P < 0.0001$) had a significant effect on *C. capitata* mortality, but EPN species and IJ concentration significantly interacted ($F_{6,70} = 10.51$, $P = 0.0001$). For both species, mortality significantly increased with IJ concentration (Fig. 1) and both species caused >90% mortality at 100 IJs/cm^2. However, *S. carpocapsae* caused >50% mortality at all but the lowest rate of 1.63 IJs/cm^2 whereas *H. bacteriophora* caused >50% mortality only at ≥ 50 IJs/cm^2. Mortality increased in a linear fashion with IJ rate for *H. bacteriophora* [$y = 0.0185X + 27.27$; $R^2 = 0.8$, $F_{1,40} = 168.86$, $P < 0.001$] and *S. carpocapsae* [$y = 0.0103X + 65.94$; $R^2 = 0.43$, $F_{1,40} = 24.22$, $P < 0.001$].

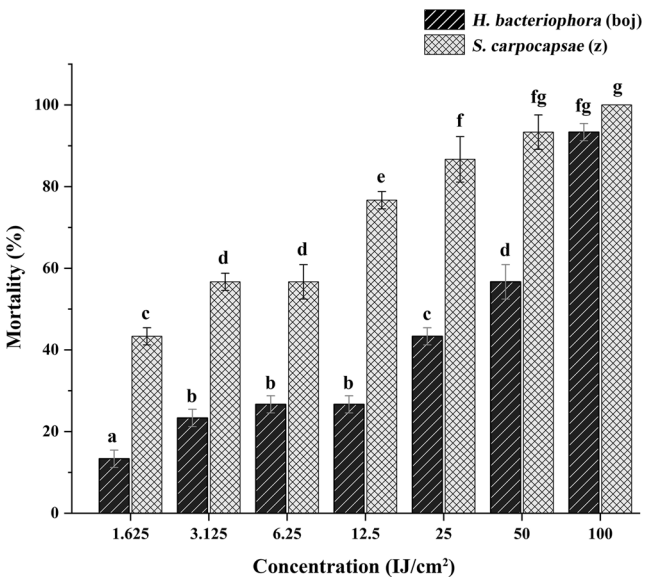

Fig. 1 Mortality (%; mean ± SEM) of last instar *Ceratitis capitata* larvae exposed to different infective juvenile (IJ) concentrations (1.63, 3.13, 6.25, 12.5, 25, 50, and 100 IJs/cm^2) of the entomopathogenic nematodes *Steinernema carpocapsae* and *Heterorhabditis bacteriophora* in the laboratory. Means with the same letter do not differ significantly (two-way ANOVA and Fisher's protected LSD, $P < 0.05$)

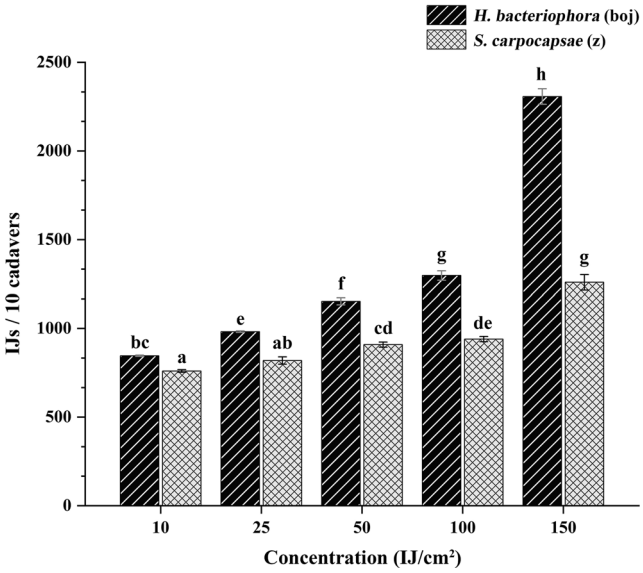

Fig. 2 Number of infective juveniles (IJs) (mean ± SEM) of the entomopathogenic nematodes *Steinernema carpocapsae* and *Heterorhabditis bacteriophora* emerged per 10 nematode-infected last instar *Ceratitis capitata* larvae. The larvae had been exposed to 10, 25, 50, 100, and 150 IJs/cm^2 in the laboratory. Means with the same letter do not differ significantly (two-way ANOVA and Fisher's protected LSD, $P < 0.05$)

EPN Reproduction Potential in Larvae

Both EPN species successfully reproduced in *C. capitata* larvae. EPN species ($F_{1,30} = 610.28$, $P < 0.0001$) and IJ concentration applied ($F_{4,30} = 505.26$, $P < 0.0001$) significantly affected IJ production, but EPN species and IJ concentration interacted significantly ($F_{4,30} = 127.18$, $P = 0.0001$). *Heterorhabditis bacteriophora* produced significantly more offspring than *S. carpocapsae* at all concentrations applied (Fig. 2). IJ production increased with IJ concentration applied for both species, but the effect was stronger for *H. bacteriophora*, particularly at the highest IJ rate applied. All nematode life stages (IJ, male, female, and hermaphrodite (for *H. bacteriophora*)) were observed within the cadaver.

Effect of Temperature On EPN Virulence

Mortality in the untreated control was 10%. In the treated arenas all stages recovered dead were infected by the applied EPN species. At a rate of 25 IJs/cm^2, *S. carpocapsae* caused significantly higher mortality than *H. bacteriophora* ($F_{1,12} = 13.09$, $P = 0.003$) but temperature had no significant effect on larval mortality ($F_{2,12} = 1.3$, $P = 0.3$). There was no significant interaction between EPN species and temperature ($F_{2,12} = 1.7$, $P = 0.22$). While mortality caused by *H. bacteriophora* varied very little among the three temperatures (44–50%), *S. carpocapsae* caused greater mortality at 25 °C (87%) than at 30 °C (57%) with mortality at 19 °C

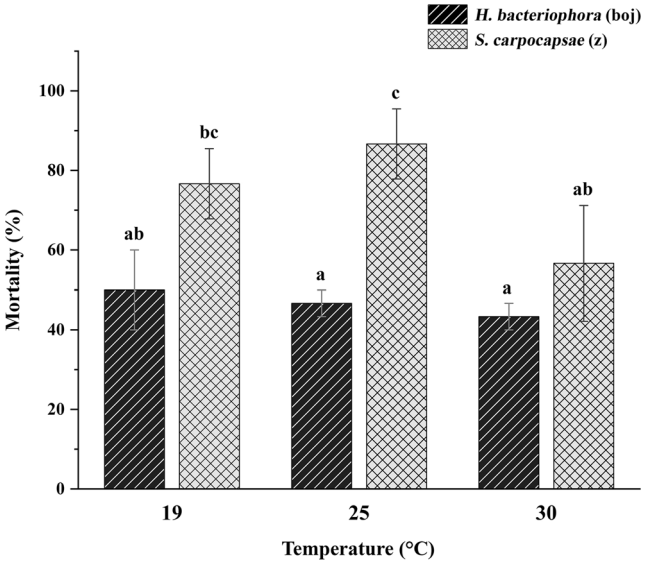

Fig. 3 Mortality (%; mean ± SEM) of last instar *Ceratitis capitata* larvae exposed to 25 IJs/cm^2 of the entomopathogenic nematodes *Steinernema carpocapsae* and *Heterorhabditis bacteriophora* at different temperatures (19, 25, and 30 °C). Means with the same letter do not differ significantly (two-way ANOVA and Fisher's protected LSD, $P < 0.05$)

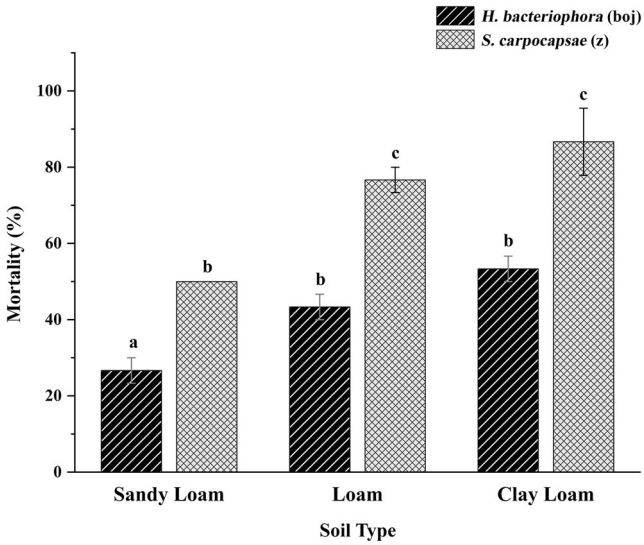

Fig. 4 Mortality (%; mean ± SEM) of last instar *Ceratitis capitata* larvae exposed to 25 IJs/cm^2 of the entomopathogenic nematodes *Steinernema carpocapsae* and *Heterorhabditis bacteriophora* in different soil types in the laboratory. Means with the same letter do not differ significantly (two-way ANOVA and Fisher's protected LSD, $P < 0.05$)

not significantly different from that at the other temperatures (Fig. 3).

Effect of Soil Type On EPN Virulence

Mortality in the untreated control was 5%. In the treated arenas all stages recovered dead were infected by the applied EPN species. At a rate of 25 IJs/cm^2, *S. carpocapsae* caused significantly higher mortality than *H. bacteriophora* ($F_{1,12} = 66.27$, $P < 0.001$) and both EPN species caused higher mortality in loam and clay loam than in sandy loam ($F_{2,12} = 25.73$, $P < 0.001$). EPN species and soil type did not interact ($F_{2,12} = 0.82$, $P = 0.46$). *S. carpocapsae* caused significantly higher mortality than *H. bacteriophora* (by 24–44%) in every soil type with 77 and 87% mortality in loam and clay loam, respectively, but only 50% in sandy loam (Fig. 4).

Virulence Against Pupae

No mortality was observed in the untreated control group. All recovered dead pupae from treated arenas was infected by the applied EPN species. Both EPN species caused moderate mortality of pupae at 50 IJs/cm^2 without a significant difference ($t_{1,10} = 0.98$, $P = 0.35$) between *H. bacteriophora* (27 ± 9%) and *S. carpocapsae* (38 ± 7%).

EPNs Efficacy Against Larvae Within Fruits On Soil in Field Condition

Mortality in the untreated control was 5%. In the treated arenas all stages recovered dead were infected by the applied EPN species. Both EPN species caused significant mortality when sprayed with a concentration of 25 IJs/cm^2 on soil and tangerine fruits infested with the fruit fly. The mean mortality of larvae was significantly influenced by the total number of larvae (*i.e.,* covariate) ($F_{1,13} = 23.9$, $P = 0.003$). The adjusted percent mortality did not differ significantly between *S. carpocapsae* (69 ± 6%) and *H. bacteriophora* (45 ± 11%) ($F_{1,13} = 3.58$, $P = 0.08$). In the *H. bacteriophora*-treated containers, out of the total of 47 ± 9 (range 20–100) larvae recovered per container 25 ± 10 (range 1–87) were infected with *H. bacteriophora*. In the *S. carpocapsae*-treated containers, out of the total of 49 ± 6 (range 30–72) larvae recovered per container 35 ± 6 (range 16–68) were infected with *H. bacteriophora*.

Discussion

Ceratitis capitata larvae were susceptible to two Iranian EPN strains in laboratory and field tests. *Steinernema carpocapsae* was more virulent than *H. bacteriophora* and caused significant mortality even at very low concentrations (e.g., 43% at 1.63 IJs/cm^2). This is in line with previous studies with *C. capitata* (Karagoz et al. 2009), *Dacus cilica-*

tus (Diptera: Tephritidae) (Kamali et al. 2013), *Rhagoletis indifferens* (Diptera: Tephritidae) (Yee and Lacey 2003), and *Anastrepha ludens* (Diptera: Tephritidae) (Lezama-Gutiérrez et al. 2006). In our laboratory trials, observations indicated a high mortality rate among larvae as pre-pupae and pupae showing that the IJs infected the larvae before entering the pupal stage which is similar to results of Rohde et al. (2010) with *C. capitata*. The observed differential efficacy between *S. carpocapsae* and *H. bacteriophora* might be linked to the former specie's capacity for horizontal movement and persistence near the soil surface within the containers which increased the probability of infecting *C. capitata* larvae during their initial soil penetration phase within the first 24 h post-treatment, prior to pupation. At the highest concentration tested, *H. bacteriophora* induced mortality levels equivalent to those achieved by *S. carpocapsae* while further increases in the concentration of *S. carpocapsae* did not result in elevated mortality as it already approached 100% at 50 IJs/cm^2. In contrast to our observations, Shaurub et al. (2021) found the increased susceptibility of *C. capitata* to *H. bacteriophora* than *S. carpocapsae* All and *S. riobrave* ML29 which may be attributed to the smaller IJ size, enabling penetration through natural host openings and possessing a dorsal tooth facilitating cuticular penetration. Their results were similar to those of Gözel and Genç (2021) and Jean-Baptiste et al. (2021). Medfly larvae exhibited a jumping behavior, potentially reducing *H. bacteriophora* IJ infection due to their active search strategy. In contrast, *S. carpocapsae* demonstrated higher virulence at lower concentrations, suggesting efficient attachment to mobile hosts. In addition to ambusher as well as nictation foraging behavior of *S. carpocapsae*, the observed differences in efficacy may be attributed to isolate-specific characteristics, as *S. carpocapsae* performance can vary across diverse environmental conditions such as soil texture (Gümüşsoy et al. 2022).

According to our observation, both EPN strains demonstrated substantial progeny production within host cadavers. However, *H. bacteriophora* exhibited significantly higher reproductive potential compared to *S. carpocapsae* across all tested concentrations. This difference may be attributed to the smaller size of *H. bacteriophora* nematodes, the rapid proliferation and high density of their associated bacterial symbiont, and the relatively smaller size of medfly larvae, which could provide sufficient nutrients to support a larger *H. bacteriophora* progeny compared to *S. carpocapsae* (Godjo et al. 2018). Previous studies examining other tephritid species have also reported a superior reproductive potential for *Heterorhabditis* spp. than *S. carpocapsae* spp. (Malan and Manrakhan 2009; Godjo et al. 2018; Aryal et al. 2022).

Temperature is a key abiotic factor affecting the infection of insects by EPNs (Hazir et al. 2001). Both EPN strains testing in the present study have high potential to control the *C. capitata* larvae at 25 °C due to the existence of similar temperatures in northern, northeastern, and southern Iran as well as Africa and Middle-East. Although the mean mortality of larvae was a little bit higher at 19 °C for *H. bacteriophora*, which might be due to the superior adaptation of the nematode strain at cooler temperatures near 20 °C. High temperature, 32 °C and above (Maushe et al. 2023), is detrimental to the ability of EPNs to infect the host as well as their reproduction and survival, especially in term of Steienrnematids (Kung et al. 1991). Our observation is in line with other studies on *C. capitata* (Shaurub et al. 2015) and other fruit fly species (Sirjani et al. 2009; Langford et al. 2014; Kepenekci et al. 2015; Mandour et al. 2021). Conversely, Rohde et al. (2010) found the highest and the lowest mortality of *C. capitata* produced by EPNs at 31 and 19 °C, respectively. Aatif et al. (2020) stated the highest infection of *B. dorsalis* (Diptera:Tephritidae) when exposed to *H. bacteriophora* and *S. carpocapsae* at 35 °C. These variations in survival and virulence of EPNs across different temperatures is likely attributable to their geographic origin. Heat tolerant strains originating from warmer climates may exhibit higher thermotolerance but reduced virulence and longevity (Ulu and Susurluk 2014). Host species may influence the optimal temperature range for EPN infection, suggesting that host-derived cues vary in their composition or intensity under different thermal conditions, thereby affecting EPN attraction (Chen et al. 2003).

In our study, both EPN strains followed the same trend for infectivity and produced higher mortality in finer textured soils (e.g., clay loam and loam). This finding contradicts the widely accepted notion that nematodes exhibit superior performance in sandy soils compared to finer soils. The impact of soil characteristics on EPNs cannot be generalized due to their varying effects, as noted by Koppenhöfer and Fuzy (2006). In contrast to our observations, various studies indicate that sandy soil is superior to both silt and clay soils in providing a more favorable microhabitat for nematode-caused infection of late third instar larvae of *C. capitata* (Shaurub et al. 2015). Shapiro-Ilan et al. (2000) reported the elevated mortality of *Anastrepha obliqua* (Diptera: Tephritidae) larvae by *S. carpocapsae* in sand clay mixture compared to sand and loamy sand soil (Toledo et al. 2009). In a recent study, Elhadidy et al. (2021) found the optimal virulence for *S. carpocapsae* (EGAZ 9) in sandy loam and sandy soils at 10% moisture while a greenhouse bioassay conducted by Mokrini et al. (2020) demonstrated enhanced efficacy of *H. bacteriophora* (HB-MOR7) and *S. feltiae* (SF-MOR9, SF-MOR10) against Medfly larvae and pupae in sandy clay loam and loamy sand soils with low moisture content (10 and 15%) compared to clay soil. This difference might be related to discrepancies in exclusive properties of the soils (Barbercheck 1992). The survival

of IJs can also be affected by other abiotic factors like soil pH and moisture level as highlighted by Glazer (1996). Unlike at greater soil depths or in water-saturated soil, oxygen level below 10% is not commonplace in the top 30 cm of soil (Shapiro-Ilan et al. 2000; Dzięgielewska and Skwiercz 2018; Glazer 2022); Thus, this factor is not considered as the most crucial limiting factor affecting the efficacy of EPNs in our experiment. Given that we found the maximum virulence in the finest soil type in our experiment, dispersal was not a limiting constraint. Furthermore, the nematodes did not need to travel long distances within the experiment containers, and thus the fine soil texture (e.g., clay loam) was not an obstacle. Given the significant role of soil moisture level in EPN persistence and efficacy (Koppenhöfer and Fuzy 2006), we suggest that the soil moisture content may have contributed to the observed trend. A similar trend was observed with *S. riobrave* and *H. bacteriophora* against *Diaprepes abbreviatus* (Coleoptera: Curculionidae) (Shapiro-Ilan et al. 2000) and *H. bacteriophora* against *P. japonica* larvae (Georgis and Gaugler 1991). The pH tolerance of indigenous EPNs may differ among different EPN species or strains (Grewal et al. 1994, 1996) or based on the region where they were isolated (Khathwayo et al. 2021). In the current study, the EPN strains might be better adapted to soils with pH < 8 as the infectivity was the lowest in sandy loam soil (pH 8.56).

The susceptibility of pupae to both EPN strains was lower than that of larvae, and both EPN strains cause similar mortality. Other studies also found lower efficacy of EPNs against the pupae than larvae in *C. capitata* and other fruit fly species (Toledo et al. 2023). Karagoz et al. (2009) even observed no susceptibility of pupae of *C. capitata* to different *Steinernema* and *Heterorhabditis* nematodes. This may be due to several factors, such as cues related to host-seeking by EPNs (Kamali et al. 2013), higher mobility of the larvae causing the emission of a large amount of the EPN attractant CO_2 (Lewis et al. 1993; Shapiro-Ilan et al. 2017), poorly sclerotized integument of larvae as well as the small size of the spiracle opening in pupae which hinders IJ penetration (Gözel and Genç 2021; Griffin et al. 2005). Nonetheless, some studies reported the high susceptibility of *C. capitata* pupae to *H. bacteriophora* and *S. riobrave* (Barbosa-Negrisoli et al. 2009; Gözel and Genç 2021; Shaurub et al. 2021) likely because of the IJs getting into the body before the final sclerotization of the integument. This fact explains our results of pupal mortality. Yağcı et al. (2021) found a similar result with different isolates of *S. carpocapsae* and *H. bacteriophora* against *C. capitata.*

When the nematodes were sprayed over the infested fruits in field conditions, they showed high infectivity to larvae pointing at the high potential of these nematodes to manage the overwintering population of *C. capitata* on tangerine fruits. As the temperature decreases in autumn, the final instar larvae within the fruits enter the soil to commence the pupal stage (Sirjani et al. 2009) giving the nematodes an opportunity to infect them. Our observation indicates that IJs successfully infected host larvae within fruits, particularly in case of *H. bacteriophora*, known for its cruiser foraging behavior, or on the soil prior to pupation, when host resistance is typically elevated. Similarly, *S. feltiae* produced higher mortality than *H. bacteriophora* of *C. capitata* larvae in apricot-infested fruits on soil (Mokrini et al. 2020). Heve et al. (2017) reported the high capacity of *H. bacteriophora* to control *Anastrepha suspensa* (Diptera: Tephritidae) at 100 IJs/cm^2 in the field. The differences between the performance of EPNs in such tests may be related to environmental factors such as temperature, humidity, UV radiation that have a direct effect on their mobility, persistence, development, reproduction and pathogenicity. The optimum level of these factors differs among isolates and species (Rohde et al. 2020). In a similar study, Shapiro-Ilan et al. (2015) stated that *S. carpocapsae* was more resistant to UV and desiccation than *H. bacteriophora* which confirms our findings in this test.

Our study shows that *S. carpocapsae* could be used as a biological agent in the management program of the *C. capitata*. Application of these nematodes, especially as a soil drench, could be an effective measure to control this pest in citrus orchards with longer harvesting periods in which many infested fruits fall onto the wet soil. Nonetheless, additional attempts should be made to identify more virulent native EPN isolates in field conditions and to study their ecological characteristics such as optimal soil moisture, soil type, temperature, etc. against this pest. Furthermore, the efficacy and biology of the EPNs should be studied in more detail in combination with other biotic and abiotic factors under field conditions.

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Declarations

Conflict of interest N. Samadaei, M. Rahimpour, S. Kamali, J. Karimi and A.M. Koppenhöfer declare that they have no competing interests.

Ethical standards *Informed consent*: All authors consent to publication. *Research involving human and animals' participants*: The present article does not involve any research conducted on human participants or animals (including vertebrates) by any of its authors.

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