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Efficacy of Turkish isolate of *Steinernema feltiae* (Rhabditida: Steinernematidae) in controlling the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), under laboratory conditions

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Abstract: The Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), is one of the most destructive pests in fruit growings. It pupates in the soil. The pupae are target of many organisms sheltering the soil such as the entomopathogenic nematodes (EPN). Pathogenicity of the Turkish strain of the EPN, *Steinernema feltiae*, was evaluated against late instar larvae, pupae, and adults of *C. capitata* under laboratory conditions. Suspensions of the nematode were applied at four increasing concentrations of (0 (for control) 50, 100, and 200 IJs/ml) in 1 ml of distilled water. The infectivity of *S. feltiae* against soil stages of *C. capitata* under different soil moisture levels of 100, 75, and 50% of field capacity was evaluated. Mortality rates were recorded after 5 days of treatment. In order to confirm the nematode infection, the dead larvae and pupae were collected and incubated until the appearance of the infectious juvenile (IJs) or dissected under a stereomicroscope to check for nematodes. The last instar larvae and newly formed pupae were more susceptible to EPN infection than old pupae. The infectivity was directly proportional to the increase of soil moisture. The highest mortality (75%) was obtained. *S. feltiae* was able to infect adults easily because of the multiple ways of entrance for the nematodes (mouth, anus, and spiracles) than the larvae and/or pupae. Therefore, the Turkish isolate of *S. feltiae* could be useful for an integrated pest management program of *C. capitata*.

Keywords: *Ceratitis capitata*, *Steinernema feltiae*, Nematode density, Soil moisture

Background

The Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), is one of the most dangerous pests of fruit crops in the Mediterranean region. The use of insecticides as the sole way of combating this pest has caused environmental pollution and represents a risk for humans and animals in addition to the resistance that has appeared in the insect. The difficulty of controlling this pest's larvae, especially by beneficial insects, comes from the hiding of the larvae inside the infested fruits far from the parasitoids and/or the predators. However, after going through two molts, these larvae leave the fruit by a characteristic jump, sink a few centimeters deep into the soil, and pupate to become the target of entomopathogenic nematodes (EPN). After locating a host, infectious juvenile stages (IJs) penetrate into the host through natural orifices or the cuticle of the insect (Peters and Ehlers 1994) and release their symbiotic bacteria into the hemocoel. The host is quickly killed by sepsis.

EPNs of the genera *Steinernema* and *Heterorhabditis* are widely studied, so far about 90 species of Steinernematidae and 20 species of Heterorhabditidae have been described (Labaude and Griffin 2018); however, only few species are commercially produced for use in biological control (Lacey et al. 2015) mainly *Steinernema carpocapsae*, *S. feltiae*, and *Heterorhabditis bacteriophora*. These EPNs are widely used to control insect pests with life

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stages in the soil (Grewal et al. 2005). Several entomopathogens have shown great efficacy in controlling the populations of the first and second larval instars than the third instar of different scarab grub species. The combination of EPN with entomopathogenic fungi optimizes their efficacy against these stages (Laznik and Trdan 2015).

Susceptibility varies with EPN species and the stage of the host development. Indeed, insect larvae are often more susceptible to EPN infection than adults (Odendaal et al. 2016). Trdan et al. (2009) indicated that both the temperature and the developmental stage of the pest have an important influence on the efficacy of EPN as pest-control agent. The mortality of *Bactrocera dorsalis* induced by *Heterorhabditis taysearae* ranged from 51.2 to 96.1% depending on isolates, despite the fact that all isolates were from Benin (Godjo et al. 2018).

Laboratory bioassays are important and allow selecting the most virulent species and isolates of EPN. *Steinernema feltiae* was tested against several pests of different orders including Coleoptera, Diptera, and Hemiptera. Previous research on the biology and the ecology of EPNs made better forecasts of their performance in the field. Indeed, environmental parameters such as temperature, moisture, vegetation types, and soil properties can affect the survival and virulence of nematodes, while infection depends on interactions between IJs, their symbiotic bacteria and the host (Labaude and Griffin 2018). In this regard, several researches are carried out to increase the efficiency thanks to the selection of the strains and the improvement of production methods (Testa and Shields 2017), formulation (Kim et al. 2015) and application (Bai et al. 2016).

It is in this context, the present study aimed at evaluate the pathogenicity of the Turkish isolate of *S. feltiae* against immature stages of *C. capitata* under laboratory conditions.

Materials and methods

Target pest

C. capitata larvae and pupae were obtained from the mass-rearing unit of the Plant Protection Department, Suleyman Demirel University, Turkey. The colony was maintained under controlled conditions at 25 ± 1 °C, 65% RH, under 14:10 (L:D) photoperiod. Larvae were reared in sterile Petri dishes containing artificial diet of: water (56 ml), sugar (12 g), Hcl (0.3ml), wheat germ (4 g), yeast (3 g), Benzoic acid (0.3 g), and bran (23 g). Adults were provided by water and a solid diet consisting of sucrose and yeast.

Entomopathogenic nematode

Bioassays were carried out with Turkish commercial strain of *S. feltiae* (Nematac 10 million) obtained from BioGlobal Campany (Antalya). Aqueous suspensions of nematodes were prepared at different concentrations of 0, 50, 100, or 200 IJs/ml.

Susceptibility of *Ceratitis capitata* soil stages to entomopathogenic nematodes

The bioassays took place in a controlled environment room at 25 ± 1 °C, 62 ± 5% RH and 16:8 (L:D) photoperiod. Pupae and third instar larvae used for these experiments were collected from the artificial rearing. Effectiveness of the Turkish strain of *S. feltiae* in controlling soil stages of *C. capitata* was evaluated by exposing individuals to different concentrations of nematode suspensions (0 (C0), 50 (C1), 100 (C2), or 200 (C3) IJs/ml). These concentrations were chosen starting from the recommended commercial application of EPNs ($2.5 - 5 \times 10^9$ IJs/ha = 25–50 IJs/cm^2) (Georgis and Hague 1991). Four replicates with 25 individuals were tested for each treatment. In the control treatment, 1 ml of distilled water without nematodes (D0) was applied. The dead individuals were dissected under a stereomicroscope to determine if the nematodes were present.

Contact method

Appropriate amounts of nematode concentrations (0, 50, 100, or 200 IJs/ml) were counted under a stereomicroscope and added to a filter paper with 1 ml of distilled water in a 9-cm Petri dish (Mahmoud 2007). All concentrations were tested on filter paper against the third instar *C. capitata* larvae and pupae in four replicates. Twenty-five individuals of each were exposed for 24 h to each concentration. Controls were treated with 1 ml of distilled water without nematodes. Mortality percentages were recorded for larvae at 24 h after treatment and after 15 days post-treatment for pupae to record emergence rate and pupal mortality.

Soil method

Infection took place in plastic cups (9 cm diameter and 5 cm deep) containing 50 g of natural sieved soil at 10% of moisture. EPNs were applied on the soil surface at the concentrations of 0, 50, 100, and 200 IJs in 1 ml of distilled water. Controls were sprayed by 1 ml of distilled water without nematodes. One hour after treatment, 25 newly formed pupae (0.0–24 h old) and 25 6-day-old ones of *C. capitata* were placed on the treated soil surface, where 25 third instar medfly larvae were placed on the soil surface in each cup and were left to move into the soil. These cups were covered by a lid and placed in a closed plastic container. Pupation of full-grown larvae took place within 6–10 h; 48 h after nematode treatment, the soil in each cup was sieved to obtain *C. capitata* larvae and pupae. Pupae were monitored daily for a period of 15 days for the emergence of adults. Dead pupae and larvae were dissected under a stereomicroscope to confirm the presence of EPN inside.

Susceptibility of *C. capitata* adults to entompathogenic nematodes

One millimeter suspension of *S. feltiae* at different concentrations of 50, 100, or 200 IJs was mixed with 1 ml of 10% sugar solution for adults provided with a piece of cotton. Flies consumed the processed diet within 2–3 days of treatment. At the same time, a nematode-free diet was provided to adults used as controls. The adults were placed in (10 × 20 × 15 cm) cages under rearing conditions. Each treatment was repeated four times. Adult mortality was recorded 5 days after treatment.

Influence of soil moisture on *Steinernema feltiae* infectivity to *C. capitata* soil stages

The efficiency of *S. feltiae* against *C. capitata* larvae and pupae was investigated under three levels of relative soil moisture (100, 75, and 50% of soil field capacity). First, a soil sample was sent to the Laboratory of Soil Sciences, Suleyman Demriel University, in order to determine the soil field capacity and moisture. The field capacity in the soil samples used in the experiment was determined as 28.96% soil moisture. Therefore, in the treatment at 100% of field capacity, soil moisture was standardized at 28.96% in the 75% treatment, moisture was standardized at 21.72%; and in the 50% treatment, moisture was standardized at 14.48%. The bioassay was carried out according to the experimental procedure and was maintained in an incubator at 25 ± 1 °C, 70 ± 10% RH, and a 12-h photophase. A completely randomized experimental design was used with four replicates.

Influence of soil moisture on *Steinernema feltiae* infectivity to *C. capitata* larvae and pupae

The efficiency of *S. feltiae* against larvae and pupae of *C. capitata* was studied under three different soil moistures in a completely randomized design with four replicates each. Ten larvae and pupae of *C. capitata* were transferred to plastic pots (12 cm × 6 cm) containing 100 g of soil treated with an aqueous suspension of 100 IJs/ml. The control treatment received 2 ml of distilled water without nematodes. The plastic pots were covered and maintained in incubators at 25 ± 1 °C, 70 ± 10% RH, and a 12-h photophase. Mortality rates were recorded after 5 days of treatment.

Statistical analysis

Mortality rates were corrected according to Abbott's formula (Abbott 1925). One-way ANONA was used to compare the mortality of *C. capitata*. Means were compared at the P = 0.05 level, and Tukey's test was used to separate means (Prism 7).

Results and discussion

Pathogenicity of the Turkish strain of the EPN, *S. feltiae* against last instar larvae, pupae, and adults of *C. capitata* at 4 different concentrations of 0, 50, 100, or 200 IJs/ml was evaluated under laboratory conditions.

Susceptibility of *C. capitata* larvae

Results indicated that in both treatments, soil application and contact method, all concentrations caused higher cumulative mortality than the control treated with C0, where no infection was observed (F = 32.53, DF = 2, P < 0.0001). However, the Tukey test revealed no difference between the two treatment methods (Fig. 1).

Infection of *C. capitata* larvae by *S. feltiae* occurred in a very short time. Eighty-two percent of mortality in 24 h post-nematode treatment in larvae treated with the highest concentration C3 for the treatment in the soil against 69% of mortality recorded in larvae treated at the same concentration in contact method.

Results in Fig. 2 show that mortality rates increased with the increase of nematode concentration with a shock effect obtained in 24 h post treatment with C2 and C3 causing respectively 54 and 82% mortality in the treatment carried out in the soil and 56 and 69% mortality in the contact treatment carried out on a filter paper. In fact, the first signs of nematode infection appeared in the first hour after treatment, when a color change in the larvae was noticed. The first dead individuals were recorded 6 hrs post treatment. Dead larvae were dissected under a stereomicroscope in order to confirm the infection by the EPN (Fig. 3).

Larvae escaped from infection developed to pupae but some of them died as pupae. The monitoring of these pupae until emergence revealed a very significant difference in the emergence rate in treated larvae than the control (F = 43.91, DF = 2, P < 0.0001). Obtained observation is consistent with other reports that most EPN-infected larvae of *C. capitata* and other tephritids died after forming puparia (Sirjani et al. 2009). In

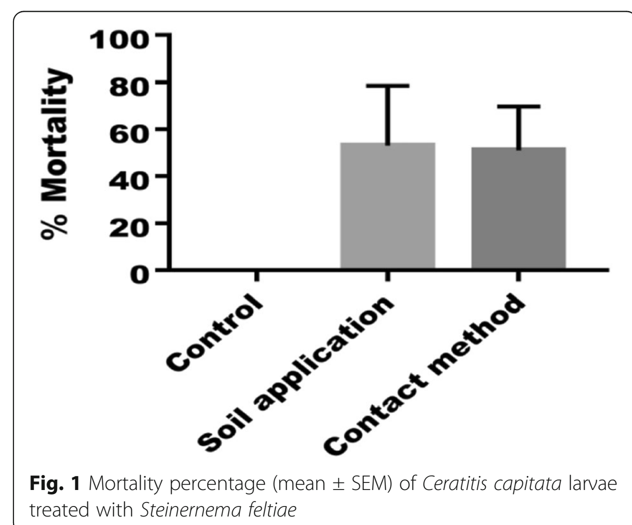

Fig. 1 Mortality percentage (mean ± SEM) of *Ceratitis capitata* larvae treated with *Steinernema feltiae*

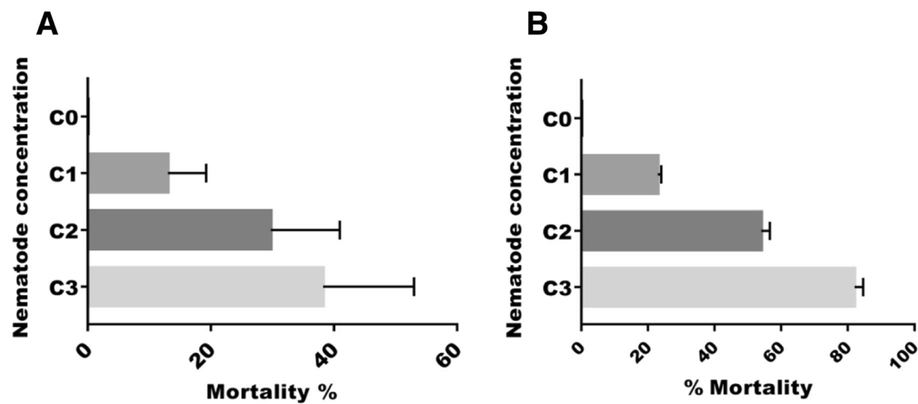

Fig. 2 Mortality percentage (mean ± SEM) of *Ceratitis capitata* larvae treated with different concentration of *Steinernema feltiae*. **a** Soil application. **b** Filter paper application

another study, the pathogenicity of a Turkish strain of *S. feltiae* (09–31) showed that the majority of medfly larvae were killed before they could form puparia. These data suggested that *S. feltiae* (09–31) Aydin isolate was highly virulent to medfly larvae (Karagoz et al. 2009) and may support the conclusion that this species is adapted to dipterous larvae (Lewis et al. 2006). In an additional treatment, *S. feltiae* had an effect on the emergenced flies of *C. capitata*. We recorded 11.76 and 11.23% of adult mortality from larvae treated in soil and filter paper, respectively.

Susceptibility of *C. capitata* pupae

Treatment with EPNs led to the mortality of pupae of *C. capitata* and resulted in a decrease in pupal emergence rate than the untreated ones (F = 21.17, DF = 2, P < 0.0001). However, older pupae and newly formed ones did not respond in the same way to treatment. Indeed, the statistical analysis revealed significant differences between the mortality rate for young and older pupae (F = 94.11, DF = 2, P < 0.0001). The Tukey test showed that the sensitivity of young pupae to nematodes was greater than that of older ones. In fact, mortality was high in newly formed pupae than older pupae and with a response concentration-related mortality increased as inoculation rate of nematodes increased (F = 187.2, FD = 3, P < 0.0001). According to Mahmoud and Osman (2007), the pathogenicity of *S. feltiae* against *Bactrocera zonata* (Diptera: Tephritidae) caused a high mortality reaching a rate of 32% for pupae of 4 days old and 20% for pupae of 6 days old. In a study on *Ragholetis indifferens* pupae, Yee and Lacey (2003) justified that the EPNs had probably penetrated intersegmental membranes before the last sclerotization of the integuments.

Despite low percentages of mortality occurred in old pupae of *C. capitata* (6 days); mortality in emerged flies from survived pupae however was high. A mortality rate of 10.19% in adults from young treated pupae for nematode suspensions and 37.04% mortality in adults from older pupae. These adults are probably infected from the soil during the emergence from the pupae. Uncompleted emergences and dead adults showed wings that were not fully spread and juvenile nematodes in the entire body.

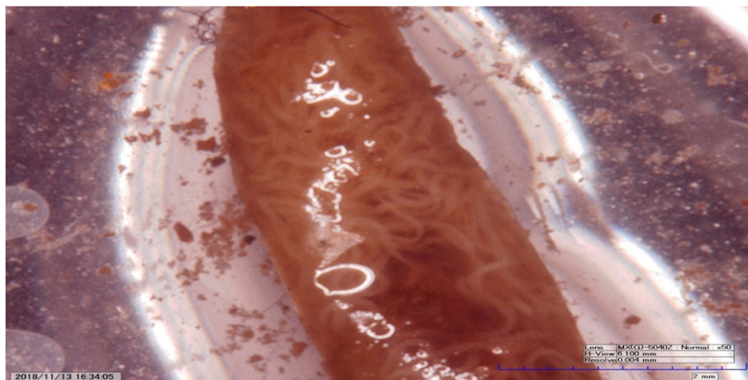

Fig. 3 Last instar larvae of *Ceratitis capitata* parasitized by *Steinernema feltiae* seen under a stereomicroscope

Williams et al. (2015) stated that *Heterorhabditis downesi* or *S. carpocapsae* infected pine weevils (*Hylobius abietis*), the insects died from emergence into adulthood, suggesting that nematode juveniles can infect pupae and survive metamorphosis of their host and adults.

Susceptibility of *C. capitata* adults

S. feltiae was effective and very virulent on *C. capitata* adults (Fig. 4). The application of nematode suspension caused higher cumulative mortality than the control treated with concentration C0, where no infection was observed (F = 219.8, DF = 3, P < 0.0001). The treatment with nematode suspension at concentration C2 and C3 caused (54 and 69%), respectively. The mean mortality percentage increased in a parallel manner with the increase in EPN concentrations.

The results revealed the great pathogenicity of the Turkish strain of *S. feltiae* against *C. capitata*. However, susceptibility of different stages of *C. capitata* was different; larvae and newly formed pupae were more susceptible to nematode infection than old pupae (> 48 h). Obtained results are consistent with several studies conducted on Tephritid flies and indicated that larvae are the most susceptible stage to EPNs infection (Yee and Lacey 2003**;** Kamali et al. 2013; Nouh and Hussein 2014 and Shaurub et al. 2015). Kamali et al. (2013) explained reasons for high incidence of infection of larvae compared to pupae and adults are due to their developmental duration, activity in soil, output of cues related to host finding by EPNs and larger natural openings. For example, the low susceptibility of pupae, which is highly observed in other studies (Hübner et al. 2017) can be attributed to lack of natural pathway entry for nematodes, as well as a more tough cuticle (Garriga et al. 2018). In fact, the large natural openings of the body of the larvae and the weakly sclerotized larval integument (relative to the nymphal integument) facilitate infection by the nematode. Labaude and Griffin (2018) justified these differences in susceptibility by various mechanisms, such as differences in behavior, especially high activity levels and avoidance behaviors in adults, as well as more potent immune system or physical barriers to penetration of nematodes. Thus, the highest susceptibility of larvae to EPNs may be related to a greater locomotion at this stage, with greater release of CO_2, a chemical compound that plays a role in the attraction of the EPNs (Yee and Lacey 2003).

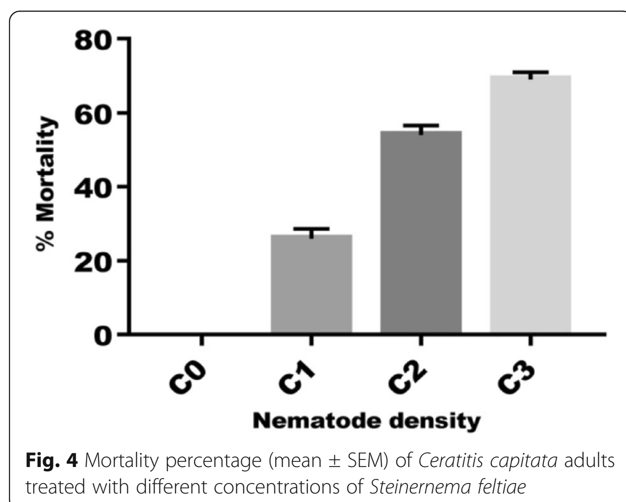

Fig. 4 Mortality percentage (mean ± SEM) of *Ceratitis capitata* adults treated with different concentrations of *Steinernema feltiae*

Influence of soil moisture on *S. feltiae* infectivity to *C. capitata* soil stages

The infectivity of *S. feltiae* to larvae and pupae of *C. capitata* was determined, under laboratory conditions for different soil moisture levels of 100, 75, and 50% of field capacity. According to the results illustrated in Fig. 5, the soil moisture level had an effect on the infectivity of *S. feltiae* to *C. capitata* soil stages. A highly significant difference was recorded between the control and the batch of larvae treated at different moisture levels (F = 41.32, DF = 3, P < 0.0001) and (F = 19.1, DF = 3, P < 0.0001) for pupae.

The efficiency of nematodes was similar at 100 and 50% of field capacity, but at 75% of field capacity, nematodes were more effective against the two soil stages of *C. capitata.* In fact, *S. feltiae* induced a great host mortality when soil moisture was at 75% of field capacity, causing respectivement (82 and 38%) of mortality in larvae and pupae, with low efficiency in the other treatments (100 and 50%). No mortality was observed in the control.

Environmental parameters such as temperature, humidity, vegetation types, and soil properties can affect the survival and virulence of nematodes. Shaurub et al. (2015) indicated that nematode infectivity decreased with increase in exposure time to UV light, whereas it increased with increase in temperature. Infectivity increased in sandy soil, whereas it decreased in silt and clay soils. Soil moisture plays a key role in the mobility of nematode infective juveniles and thus their ability to search for and infect a host. Several studies indicated that soil moisture influence infectivity of EPNs, demonstrating, in general, a decrease in infectivity as soil moisture decreases (Grant and Villani 2003 and Alekseev et al. 2006) and many studies reported low nematode infectivity in extreme, low, and high soil moistures (near the saturation point) (Koppenhöfer et al. 1995).

Glazer (2002) demonstrated that the low infectivity at the highest moisture can be explained by the fact that soil saturation with water reduces oxygen concentration and restricts nematode mobility, which is required to infect the host; however, the low infectivity of nematodes at the lowest moisture content is probably related to the lack of water between the pores, which is also limiting for nematode locomotion. Another possibility for the

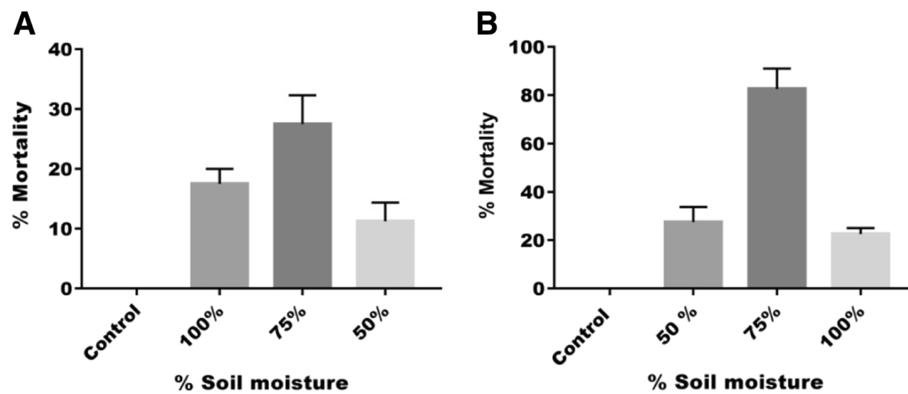

Fig. 5 Mean mortality (±SEM) of *Ceratitis capitata* pupae (**a**) and larvae (**b**) inoculated with *Steinernema feltiae* at a rate of 100 IJs/ml and maintained under different levels of soil moisture

lowest infectivity at the lowest moisture content is that these nematodes have developed physiological and behavioral adaptations that allow them to reduce their metabolism, in case of dehydration entering a state of anhydrobiosis (Glazer 2002). Anhydrobiosis can be reversed by wetting the soil, causing a recovery of nematode infectivity and virulence. Studies have demonstrated that some species of the *Steinernema* have the ability to enter a state of anhydrobiosis, when exposed to low moisture contents (Koppenhöfer et al. 1995), but nothing is clear on this issue regarding *Heterorhabditis* spp. Since adequate humidity is essential for the survival and movement of this nematode species (Baimey et al. 2015 and Filgueiras et al. 2016).

Conclusion

Future work should focus on selecting more EPN species/strains to select the most virulent for field trials. In addition, their biology (persistence, reproduction, survival) post-application of EPNs should be studied, as well as their performance in combination with other biological agents and agrochemicals. Although it is often difficult to control moisture in the field, one solution is the addition of adjuvants or the use of a surfactant in the suspension of nematodes that are used as a biocontrol agent. The Turkish isolate of *S. feltiae* may be an early solution for an integrated pest management program for certain dipteran including *C. capitata.*

Abbreviations

C.capitata: *Ceratitis capitata*; EPN: Entomopathogenic nematodes; *S.feltiae*: *Steinernema feltiae*

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Authors' contributions

SC carried out the experiment and was a major contributor in writing the manuscript. AB conceived and planned the experiments. KB carried out the experiment. HB corrected and revised the manuscript, corrected language mistakes and translation, and corrected references. IK supervised the work. All authors read and approved the final manuscript

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