

Original article (Orijinal araştırma)

Implementing local entomopathogenic nematodes to control Mediterranean fruit fly *Ceratitis capitata* (Wiedemann, 1824) (Diptera: Tephritidae)¹

Akdeniz meyve sineği *Ceratitis capitata* (Wiedemann, 1824) (Diptera: Tephritidae)'yı kontrol etmek için yerel entomopatojen nematodların uygulanması

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Abstract

The Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann, 1824) (Diptera: Tephritidae), is one of the world's most destructive fruit pests. *Ceratitis capitata* pupates in the soil, making it a target of many soilborne pathogens like entomopathogenic nematodes (EPNs). Entomopathogenic nematodes are highly lethal to many important pests, safe to non-target organisms and they might be good alternatives for control of *C. capitata*. In this study, the efficacy of four local EPN species; *Steinernema affine* Bovien, 1937, *Steinernema carpocapsae* Weiser, 1955, *Steinernema feltiae* Filipjev, 1934 (Rhabditida: Steinernematidae) and *Heterorhabditis bacteriophora* Poinar, 1976 (Rhabditida: Heterorhabditidae) against the third instar larvae and pupae of *C. capitata* were evaluated. The study was conducted in 2019-2020 both in the laboratory (in plastic cups) and in a climate room (in wooden cages with plastic pots) at doses of 100 and 200 IJs/larva-pupa and 7,650 and 15,300 IJs/pot, respectively. Larvae of *C. capitata* were found more susceptible to EPNs than pupae in the study. *Steinernema feltiae* isolate 113 and *H. bacteriophora* isolate 12 showed the highest efficacy while *S. affine* isolate 47 showed the least efficacy against the pest larvae and pupae. Suppression of *C. capitata* population by EPNs indicates that these EPNs can be considered as a biological control agent potentially useful for the control of this pest. After further support by field studies, these two local EPN isolates could be used as promising eco-friendly biological agents against *C. capitata*.

Keywords: Biological control, *Ceratitis capitata*, efficacy, entomopathogenic nematodes, local isolates

Öz

Akdeniz meyve sineği, *Ceratitis capitata* (Wiedemann, 1824) (Diptera: Tephritidae), dünyanın en tahripkar meyve zararlılarından biridir. *Ceratitis capitata* toprakta pupa olur ve bu durum onu entomopatojen nematodlar (EPN) gibi toprak kökenli birçok patojenin hedefi haline getirir. Entomopatojen nematodlar birçok önemli zararlı için oldukça öldürücü, hedef dışı organizmalar içinse güvenlidir ve *C. capitata*'yı kontrol etmek için iyi bir alternatif olabilirler. Bu çalışmada, dört yerel EPN türü; *Steinernema affine* Bovien, 1937, *Steinernema carpocapsae* Weiser, 1955, *Steinernema feltiae* Filipjev, 1934 (Rhabditida: Steinernematidae) ve *Heterorhabditis bacteriophora* Poinar, 1976 (Rhabditida: Heterorhabditidae)'nın *C. capitata*'nın üçüncü dönem larvalarına ve pupalarına karşı etkinlikleri değerlendirilmiştir. Çalışma 2019-2020 yıllarında hem laboratuvarında (plastik kaplarda) hem de iklim odasında (plastik saksılarda ahşap kafeslerde) sırası ile 100 ve 200 IJs/larva-pupa ile 7.650 ve 15.300 IJs/saksı dozunda yürütülmüştür. Çalışmada EPN'lere karşı *C. capitata*'nın larvalarının pupalarından daha duyarlı olduğu bulunmuştur. Zararlı larva ve pupalara karşı en yüksek etkinliği *S. feltiae* 113 ve *H. bacteriophora* 12 izolatları gösterirken, *S. affine* 47 izolatu en düşük etkinliği göstermiştir. Entomopatojen nematodlar tarafından *C. capitata* popülasyonunun baskılanması, bu EPN'lerin zararlıların kontrolü için potansiyel olarak faydalı biyolojik mücadele etmenleri olarak kabul edilebileceğini göstermektedir. İleride yapılacak arazi çalışmaları ile desteklendikten sonra bu iki yerel EPN izolatu, *C. capitata*'ya karşı ümit var çevre dostu biyolojik etmenler olarak kullanılabilir.

Anahtar sözcükler: Biyolojik kontrol, *Ceratitis capitata*, etkinlik, entomopatojen nematodlar, yerel izolatlar

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Introduction

Mediterranean fruit fly, *Ceratitis capitata* Wiedemann, 1824 (Diptera: Tephritidae), is a devastating fruit fly with a broad global distribution. It is a cosmopolitan quarantine pest that causes damage to more than 360 different hosts ranging from citrus to soft and stone fruits and vegetables (Liquido et al., 1991; Papadopoulos et al., 1998; Satar et al., 2016). It is able to tolerate climatic conditions better than most other fruit flies and the introduction of *C. capitata* to the almost all parts of the world have negative impacts on fruit production.

Turkey has suitable ecological conditions for many fruit species because of its geographical location and *C. capitata* is one of the zero-tolerance species on the quarantine list of Turkey (Anonymous, 2013). It significantly affects the export of fresh fruit; therefore, the control of the pest is crucial but controlling *C. capitata* remains problematic due to the degree and frequency of damage, and the complications of applying control methods (Harbi et al., 2018).

Control strategies of this pest are mainly based on an integrated pest management (IPM) approach using different methods. Chemical control (Magaña et al., 2007), mass trapping (Navarro-Llopis et al., 2008), sterile insect technique (Katsoyannos et al., 1999; Hendrichs et al., 2002) and biological control (Montoya et al., 2005) are the most commonly used methods. However, due to the problems and the failures occurring in these methods scientists have been focused on different studies on alternative biological control agents like entomopathogenic nematodes (EPNs) against *C. capitata* under laboratory and field conditions (Lindengren, 1990; Laborda et al., 2003; Kepenekçi & Susurluk, 2006; Karagöz et al., 2009; Malan & Manrakhan, 2009; Rohde et al., 2010, 2012; Mokrini et al., 2020).

Entomopathogenic nematodes of the genus *Steinernema* and *Heterorhabditis* (Nematoda: Rhabditida) find their hosts in cryptic habitats, sometimes in soil and kill them within 2-3 days by their mutualistic bacteria in the genera *Xenorhabdus* and *Photorhabdus*, respectively (Dillman et al., 2012; Lacey et al., 2015). Nematode and bacteria both deal with the host by producing specific compounds. The bacteria kill host larvae and start reproduce inside the hemocoel and it also create better environmental conditions for nematode development of inside the hemocoel (Boemare, 2002; Bode, 2009; Lu et al., 2017). These nematodes are non-polluting and safe, can be applied by agronomic equipment, and EPNs are also adaptable with many pesticides (Forschler et al., 1990; Georgis, 1990; Rovesti & Deseo, 1991). The host range of a species/strain is generally quite limited so they do not produce untargeted deaths (Smart, 1995). These safe agents are successful in controlling many agricultural pests belonging to different orders/families (Belair et al., 2003; Head et al., 2004; Lacey et al., 2010; Shapiro-Ilan et al., 2010; Gözel & Kasap, 2015; Gözel & Gözel, 2019).

This study aimed to evaluate the control potential of local EPNs on the third instar larvae and pupae of *C. capitata*. The efficacy of *Steinernema affine* (Bovien, 1937) isolate 47 (İstanbul), *Steinernema feltiae* (Filipjev, 1934) isolate 113 (Balıkesir), *Steinernema carpocapsae* (Weiser, 1955) isolate 1133 (Sakarya) (Rhabditida: Steinernematidae) and *Heterorhabditis bacteriophora* (Poinar, 1976) isolate 12 (Çanakkale) (Rhabditida: Heterorhabditidae) obtained from different locations in Turkey was investigated both in laboratory and climate room conditions.

Materials and Methods

Entomopathogenic nematodes

The study was conducted between 2019 and 2020 under laboratory and climate room conditions at Faculty of Agriculture. Four local EPN isolates from different provinces of Turkey were reared at 25±1°C and 65±5% RH on the final instar larvae of *Galleria mellonella* L. (Lepidoptera: Pyralidae) (Kaya & Stock, 1997). Freshly emerged infective juveniles (IJs) were harvested and used in the bioassays.

Mediterranean fruit fly, *Ceratitis capitata*

Ceratitis capitata colony was previously established on its natural hosts (Genc & Yücel, 2017) and then adapted to the artificial diet in Insect Molecular Biology Laboratory (Tsitsipis & Kontos, 1983; Tzanakakis, 1989; Genc, 2008). Daily collected eggs from the adult cages were transferred to the artificial diet and reared until the third instar in the laboratory at $25\pm 1^\circ\text{C}$ and $60\pm 5\%$ RH. Mature larvae or pupae were collected from the artificial diet with 2 mm diameter sieve for the bioassays.

Bioassays

Laboratory bioassay

The bioassay was conducted at 10% moisture in sterile sand in 60 ml plastic cups with 20 individuals (third instars or pupae). Two EPN doses of 100 and 200 IJs per larva or pupa were used in this study. Cups were capped by a lid, then punctured with a needle for aeration and kept at room temperature ($23\text{-}24^\circ\text{C}$). Mortality was recorded 7 days after EPN inoculation, to approve the infection the dead larvae and pupae that shown typical infection signs were placed to White traps (White, 1927).

Emerged adults were counted, and mortality calculated by subtracting the emerged adults from the initial number of larvae or pupae. Mortality of larvae and pupae and the efficiency of EPNs were also determined according to the EPN harvested from cadavers. In control groups, only distilled water was given to *C. capitata* larvae and pupae. Four replicates for each nematode isolate were used and the bioassay was performed twice.

Climate room bioassay

The bioassay in a climate room was conducted in plastic pots, with a depth of 13 cm, a diameter of 14 cm and a surface area of 153 cm^2 . Pots were filled with autoclaved sand at 10% moisture, by 50 individuals (third instars or pupae) for each application. Two EPN doses used for application were 7,650 and 15,300 IJs per pot. Pots were covered by tulle, placed in wooden cages and kept at climate room ($23\text{-}24^\circ\text{C}$). All other procedures were similar as the laboratory bioassay. Mortality was recorded 21 days after EPN inoculation. Three replicates for each nematode isolate were used and the bioassay was performed twice.

Statistical analysis

The experiment was conducted by a completely randomized design. The mortality resulted from the effect of EPNs was calculated and corrected according to Abbott's formula (Abbott, 1925) and ANOVA analysis was performed on Minitab 17 Statistical Software. Significant means were compared by Tukey's comparison test ($p \leq 0.05$).

Results

The mortality of third instars and pupae of *C. capitata* (Figure 1) caused by EPNs in the laboratory bioassays are shown in Figure 2 (upper panels). It was determined that the third-order interaction of EPN isolate, *C. capitata* stage and EPN dose was significant, which means that mortality of *C. capitata* changed with biological stages of the *C. capitata* and the EPN dose in each EPN isolate. Significant differences were determined between doses. Among the EPNs doses, 200 IJs caused the highest mortality both on mature larvae and pupae of *C. capitata*.

In the larval stage at dose of 100 IJs, the mortality was recorded as the highest by *H. bacteriophora* 12 (79%) and *S. feltiae* 113 (83%). The similar trend was also observed in 200 IJs and the highest mortality was reached 91 and 96% for the same isolates, respectively. The lowest mortality was reported by *S. affine* 47 with 49 and 77% at dose of 100 and 200 IJs, respectively.

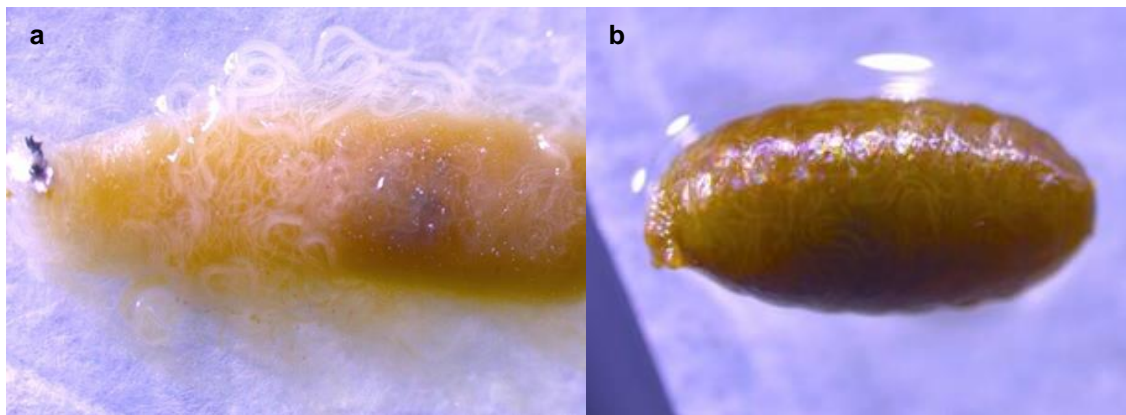


Figure 1. Entomopathogenic nematode-infested a) larva; b) pupa.

The mortality was lower in pupae of *C. capitata*, the dose of 100 IJs the mortality was recorded as the highest by *H. bacteriophora* 12 (21%) and *S. feltiae* 113 (23%). Similar rise occurred with 200 IJs and the highest mortality reached 34% with these two isolates. The lowest mortality was obtained by *S. affine* 47 with 8 and 15% with 100 and 200 IJs, respectively.

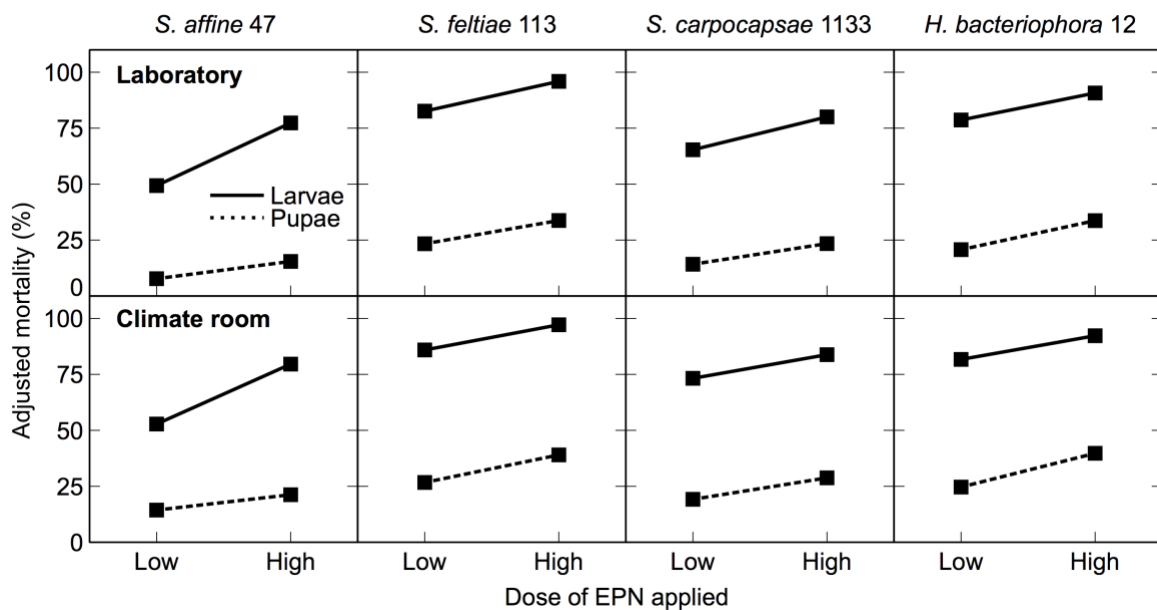


Figure 2. Mean adjusted mortality *Ceratitis capitata* larvae and pupae exposed to entomopathogenic nematodes in the laboratory (low, 100 IJs/larva-pupa, and high 200 IJs/larva-pupa) and climate room (low, 7,650 IJs/pot; and high, 15,300 IJs/pot).

The mortality of third instar larva and pupa of *C. capitata* caused by EPNs occurred in a climate room bioassay are shown in Figure 2 (lower panels). The third-order interaction of EPN isolate, *C. capitata* stage and EPN dose was significant for mortality as in the laboratory bioassay. In the larval stage with 7,650 IJs, the mortality was recorded as the highest by *H. bacteriophora* 12 (82%) and *S. feltiae* 113 (86%). The similar tendency was also observed with 15,300 IJs and the highest mortality was reached 92 and 97% by the same isolates, respectively. The lowest mortality was reported by *S. affine* 47 with 53 and 80% with 7,650 and 15,300 IJs, respectively.

Mortality was lower in pupae of the pest, and with 7,650 IJs the mortality was recorded as the highest by *H. bacteriophora* 12 (25%) and *S. feltiae* 113 (27%). With 15,300 IJs, the highest mortality was 40% by *H. bacteriophora* 12 and 39% by *S. feltiae* 113. The lowest mortality was 14 and 21% by *S. affine* 47 with 7,650 and 15,300 IJs, respectively.

Discussion

A member of Tephritidae family, Mediterranean fruit fly is considered one of the most important and cosmopolitan pests of the fruits throughout the world (Zucchi, 2001) and it is also a key pest of citrus and many other fruit species in Turkey. Entomopathogenic nematodes are beneficial biological control agents that adapted to soil and can be safely used against numerous pests (Kaya & Gaugler, 1993; Koppenhöfer, 2007).

This study showed the potential of EPNs as biopesticides against *C. capitata*. All tested EPN isolates caused mortality, however, the third instar larvae were more susceptible to infection than pupae under both laboratory and climate room conditions. This result is similar with the studies of Gazit et al. (2000), Karagöz et al. (2009), Rohde et al. (2012), Nouh & Hussein (2014) and Minas et al. (2016).

It was emphasized by Yee & Lacey (2003) that the higher susceptibility of larvae to EPNs may be related with the higher release of CO₂ at that stage, attracting the nematodes. Also, large natural openings and the poorly sclerotized integument of the larva enable EPNs infect more easily. In contrast, the lower susceptibility of pupae could be due to the small spiracle opening size for nematode penetration (Toledo et al., 2005). The closure of all-natural openings owing largely to sclerotization and thickening of the cuticle into puparial cells is a main reason of pupal resistance (Grewal et al., 2005). It was also confirmed by Chergui et al. (2019), who used a Turkish *S. feltiae* isolate and observed that the final instar larvae and newly formed pupae of *C. capitata* were more susceptible to EPNs than old pupae under laboratory conditions.

Steinernema feltiae and *H. bacteriophora* species gave better performance than *S. carpocapsae* and *S. affine* in the present study and this was similar to the findings of Glazer (1992) that *S. carpocapsae* isolate All was less effective than *H. bacteriophora* isolated HP88 against different lepidopteran pests. Karagöz et al. (2009) found that mortality was higher with *S. feltiae* (78%) compared to *S. carpocapsae* (56%) on the last instar larvae of *C. capitata*. Rohde et al. (2012) observed that *Heterorhabditis* sp. isolate PI, *Heterorhabditis* sp. isolated JPM4, *H. bacteriophora* isolate HP88 and *S. feltiae* were the best against pupal stage of *C. capitata* (ranging from 35 to 44% mortality). Mokri et al. (2020) found high larval mortality (80%) by *S. feltiae* isolate SF-MOR9 under the laboratory conditions.

Based on our findings, *H. bacteriophora* was able to cause higher pupal mortality than *S. carpocapsae* and *S. affine*. This can be explained by dorsal tooth of *Heterorhabditis* species used to penetrate the host cuticle more easily (Griffin et al., 2005). Mortality of larvae and pupae caused by all nematode isolates increased as the dose increased. Studies conducted by Nouh & Hussein (2014) and Minas et al. (2016) gave similar results with higher mortality with higher IJs doses. Kepenekçi & Susurluk (2006) used two Turkish isolates against *C. capitata* pupae and obtained higher mortality with 100 IJs/insect compared to 50 IJs/insect.

Similar trends in the efficacy of the EPN isolates were observed in the bioassays performed under different conditions. The findings of the present study demonstrated that EPNs, specifically *S. feltiae* isolate 113 and *H. bacteriophora* isolate 12, can effectively control *C. capitata*. In conclusion, implementing these biopesticides as part of an IPM program of *C. capitata* might successfully reduce pest damage to acceptable levels. The findings of this study need to be further evaluated by testing the most effective isolates under field conditions.

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