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Orijinal araştırma (Original article)

Effect of entomopathogenic nematode species on the corn stalk borer (*Sesamia cretica* Led. Lepidoptera: Noctuidae) at different temperatures[1]

Entomopatojen nematod türlerinin farklı sıcaklıklarda mısır koçan kurdu (*Sesamia cretica* Led. Lepidoptera: Noctuidae) üzerindeki etkinliği

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Summary

Three Turkish strains of the entomopathogenic nematodes, *Steinernema carpocapsae* (Adapazarı), *S. feltiae* (Çanakkale) and *Heterorhabditis bacteriophora* (Kırklareli), were tested in the laboratory for their virulence on the last instar larvae of the corn stalk borer, *Sesamia cretica*. Mortality rates of last instar *S. cretica* larvae were determined for each nematode species under four different temperature treatments. Accordingly, at 15 ºC, mortalities of *S. cretica* exposed to *S. carpocapsae*, *S. feltiae* and *H. bacteriophora* were 48, 56 and 14%, respectively. These mortalities at 20 ºC were 62, 76 and 50%; at 25 ºC, 82, 90 and 90%; and at 30 ºC (the highest application temperature), mortality was 82, 92 and 94%, respectively. These results show that *S. carpocapsae* and *S. feltiae* caused similar mortalities at all applied temperatures and their effectiveness increased as the temperature increased. Although *H. bacteriophora* caused low mortality at low temperatures, it infected and caused very high levels of mortality at relatively high temperatures.

Key words: *Sesamia cretica*, *Steinernema carpocapsae*, *Steinernema feltiae*, *Heterorhabditis bacteriophora*

Özet

Entomopatojen nematodlar; *Steinernema carpocapsae* (Adapazarı), *S. feltiae* (Çanakkale) ve *Heterorhabditis bacteriophora*'nın (Kırklareli) üç Türk izolatının mısır koçan kurdu *Sesamia cretica*'nın son dönem larvalarına karşı etkinlikleri araştırılmıştır. Entomopatojen nematodlar tarafından infekte edilen son dönem *Sesamia cretica* larvalarındaki ölüm oranları nematod türüne ve uygulandığı sıcaklığa bağlı olarak değişmekle birlikte 15 ^{o}C'de, *S. carpocapsae*, *S. feltiae* ve *H. bacteriophora* için sırası ile % 48, 56 ve 14 olarak tespit edilmiştir. Bu oran 20 ^{o}C'de, % 62, 76 ve 50, 25 ^{o}C'de % 82, 90 ve 90, denemede kullanılan en yüksek sıcaklık olan 30 ^{o}C'de ise % 82, 92 ve 94 olarak bulunmuştur. Elde edilen bu sonuçlar *S. carpocapsae* ve *S. feltiae*'nin uygulandığı tüm sıcaklıklarda birbirine yakın sonuçlar verdiğini ve sıcaklığın artması ile etkinliklerinin de arttığını, *H. bacteriophora*'nın ise düşük sıcaklıklarda oldukça düşük olan infekte etme oranının, yüksek sıcaklıklarda çok yüksek seviyelere ulaştığını ve en iyi infeksiyonun bu sıcaklık derecelerinde olduğunu ortaya koymuştur.

Anahtar sözcükler: *Sesamia cretica*, *Steinernema carpocapsae*, *Steinernema feltiae*, *Heterorhabditis bacteriophora*.

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Introduction

Entomopathogenic nematodes have been known to be insect parasites since the 17th century, but until the 1930's their potential importance as biological control agents was poorly explored (Nickle, 1984). However, after the 1970's, after the negative effects of pesticide use against insects were determined, research on entomopathogenic nematodes has been carried out at an ever increasing rate, until it is now a common biological control agent among the alternative methods to chemical control (Smart, 1995). The use of entomopathogenic nematodes against target insect pests now forms the basis for a significant body of research (Nickle, 1984; Kaya, 1985; Klein, 1990; Wouts, 1991; Georgis and Meanweiler, 1994).

Now that the true costs of pesticide use on human health and the environment are becoming better understood, alternative methods to control harmful insects are increasingly in demand. Among these methods, one of the groups especially accentuated is entomopathogenic nematodes, known obligate insect pathogens that are commercially prepared and are effective in controlling some target insect pests (Burnell and Stock, 2000; Liu et al., 2000; Nguyen and Duncan, 2002; Nguyen et al., 2004a, 2004b; Qiu et al., 2004, 2005).

Entomopathogenic nematodes have a wide host range, can effectively suppress their hosts and are naturally available under nearly in all environmental conditions, increasing their potential as successful biological control agents (Gaugler and Kaya, 1990; Koppenhofer, 2000; Nguyen et al., 2004a). Currently, entomopathogenic nematodes are applied with success against many target pests in biological control (Kaya and Gaugler, 1993; Fenton et al., 2000; Koppenhöfer, 2000).

Sesamia cretica may cause severe damage to maize plantations. Larval feeding in the stem leads to tunnelling and quite often, plant breakage. *Sesamia cretica* infests maize throughout its development, from the seedling stage to maturity. The direct feeding on the cob leads to quantitative and qualitative yield losses varying between 20 and 80% (Zeren et al., 1998).

The colour of *S. cretica* adults is usually yellowish-gray with a wingspan of 30-40 mm. The female of the stem borer lays over two hundred eggs in batches. Soon after hatching and feeding at the original point, the borers begin to move into the stem and cob. After 6-7 molting stages, larvae become mature (full grown) and stop feeding. Inside the cocoon found in stem and cob, the mature larva changes into the pupa.

In this study, the effectiveness of three different entomopathogenic nematodes, *Steinernema carpocapsae* Weiser, *S. feltiae* Filipjev and *Heterorhabditis bacteriophora* Poinar, isolated from Turkish soils, were tested on final instar larvae of *Sesamia cretica*, one of the most important pests in corn fields in Turkey. Specifically, the aim of this study was to determine and compare the effectiveness of isolates of the three entomopathogenic nematodes on *Sesamia cretica* at four different temperatures under laboratory conditions.

Material and methods

Isolation and identification of the nematodes

Turkish isolates of the nematodes, *Steinernema carpocapsae*, *S. feltiae* and *Heterorhabditis bacteriophora,* were used in the present study. The nematodes were isolated in different cities (Adapazarı, Çanakkale, Kırklareli) in the western part of Turkey. The species were identified by morphometrics and sequencing of the D2/D3 and ITS regions of rDNA using molecular identification methods (Gözel and Güneş, 2008).

Propagation of nematodes

The nematodes were propagated in the last instar larvae of the greater wax moth, *Galleria mellonella* (Lep.: Pyralidae), as described by Dutky et al. (1964). The emerged infective juveniles (IJs) were stored in Ringer solution (9.0 g NaCl, 0.42 g KCl, 0.37 g $CaCl_2$, 0.2 g $NaHCO_3$ and 1 l distilled water) in culture flasks for 5 days at 4-5 ^{o}C prior to use in the experiment (Solomon, 1999).

Test organisms

Final instar larvae of the corn stalk borer (*Sesamia cretica*, Lep.: Noctuidae) were used as the test insect in this study. Larvae of *S. cretica* were collected from corn fields in Çanakkale in August 2008. Field-collected larvae were mass produced on corn in a climate controlled room at 25±1 ^{o}C and 70±5% moisture.

Bioassays

Infective juveniles (500 individual nematodes) of each entomopathogenic nematode species were put with one corn stalk borer larva in 250 µl distilled water in a 4 cm diameter plastic petri dish lined with Whatman filter paper. Experiments were repeated 50 times for each nematode species at each temperature. Control petri dishes (for all temperatures) consisted of 250 µl of distilled water with a corn stalk borer larva.

Determination of infection

After 5 days, dead *S. cretica* larvae were examined for nematode infection by observing the condition and colour of the cadavers. In addition to this method, all infected *S. cretica* larvae were dissected in Ringer's solution. In this way, the presence of entomopathogenic nematodes, in cadavers, was determined. Thus, all dead larvae encountered in the study were examined in this manner for the existence of entomopathogenic nematodes.

Statistical analysis

Mortality percentage of the corn stalk borer for each variable was determined with the Abbotts's formula initially, and then determined for significance via one-way ANOVA Duncan ($P<0,05$).

Results

We determined the virulence of *Steinernema carpocapsae, S. feltiae* and *Heterorhabditis bacteriophora* to corn stalk borer under laboratory conditions. At four different temperatures and the same application rate, we showed that *Sesamia cretica* infection and mortality due to the three nematode species is correlated with temperature.

While the virulence of each entomopathogenic nematode species varied according to temperature, mortality rates in corn stalk borer larvae were highest at higher temperatures. However, there was significant variation in the mortality rates caused by the three nematode species at the different temperatures.

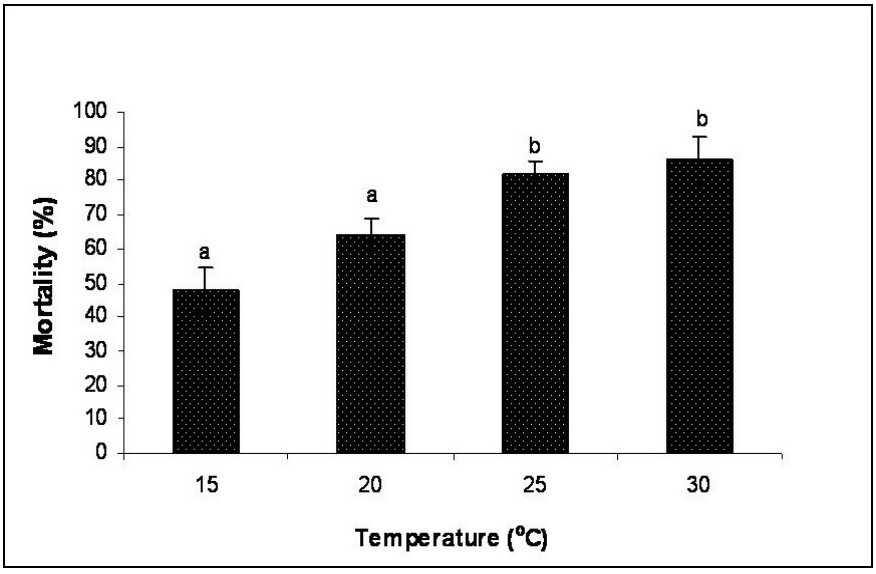

Figure 1. Mortalityof *Steinernema carpocapsae* on *Sesamia cretica* at four different temperatures.

For *S. carpocapsae*, the lowest mortality was 48% at 15 ºC and at 20 ºC it was 62%. The highest mortalities occurred at 25 and 30 ºC (82 and 86% respectively; Figure 1).*Steinernema feltiae* caused mortality of 56% at 15 ºC, 76% at 20 ºC, and 90% at 25 ºC. The highest mortality it caused was 90% at 30 ºC (Figure 2).

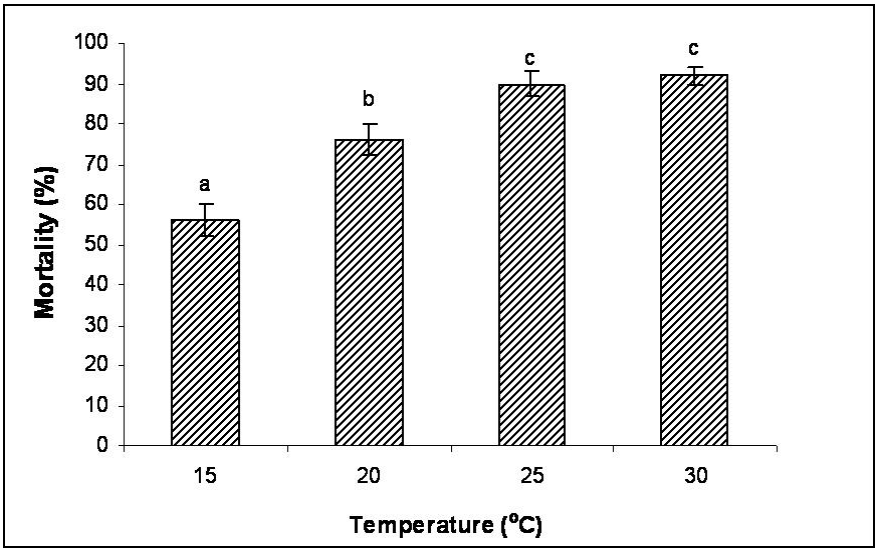

Figure 2. Mortality of *Steinernema feltiae* on *Sesamia cretica* at four different temperatures.

At 15 ºC, *H. bacteriophora* killed 14% of the corn stalk borer larvae, the lowest among the three nematode species tested at this temperature. At 20 ºC, mortality increased to 50%. This trend continued as the temperature increased, resulting in mortality rates of 90% at 25 ºC and 94% at 30 ºC (Figure 3).

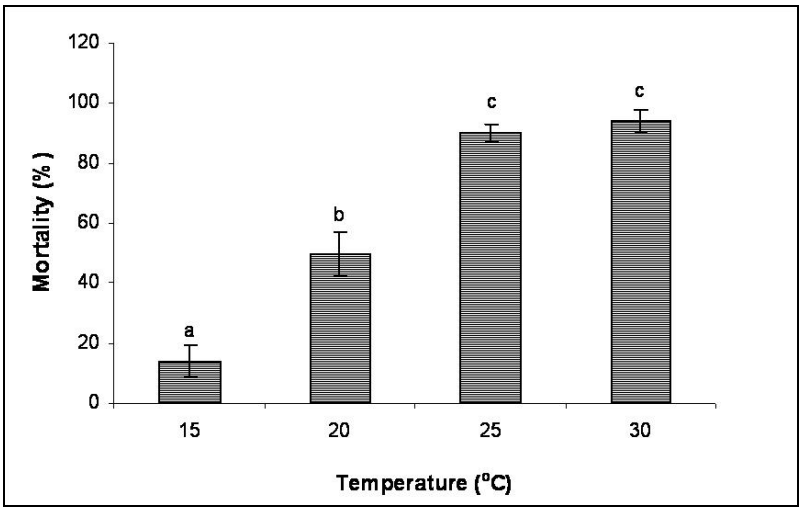

Figure 3. Mortality of *Heterorhabditis bacteriophora* on *Sesamia cretica* at four different temperatures.

For comparative purposes, the mortality caused by the three species is presented in Figure 4. At 15 ºC, the most effective species on corn stalk borer was *S. feltiae* at 56%. *Steinernema carpocapsae* was the second most effective species at this temperature by producing 48% mortality. The least effective nematode species at this temperature was *H. bacteriophora.*

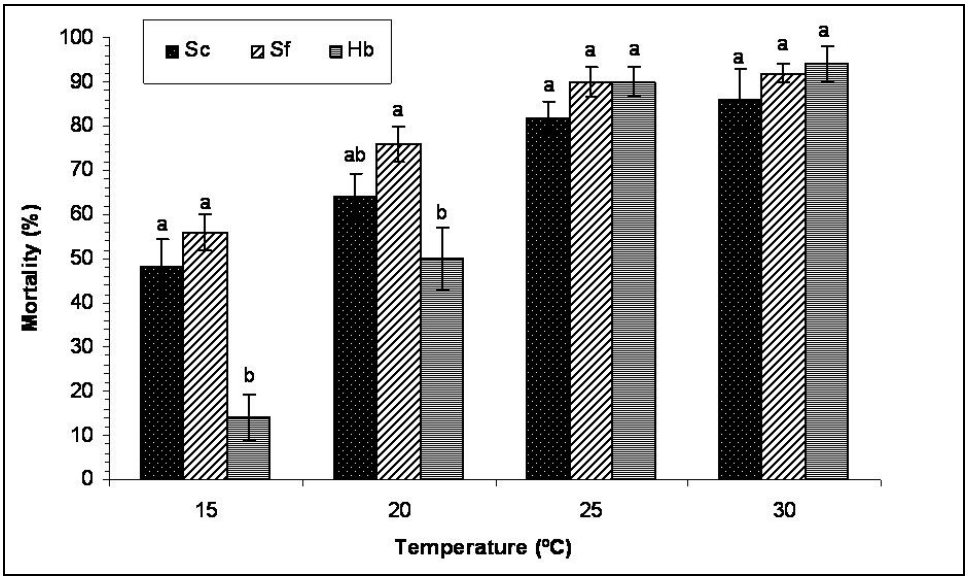

Figure 4. Mortaliity of three entomopathogenic nematode species on *Sesamia cretica* at four different temperatures.

At 20 ºC, there was an increase in mortality on corn stalk borer larvae produced by all nematode species. The most effective species at this temperature was *S. feltiae*, followed by *S. carpocapsae* with a mortality rate of 62%. *Heterorhabditis bacteriophora* was the species that most increased its effectiveness with the increase of temperature of 5 ºC producing a mortality of 50%.

At 25 ºC, the third highest temperature we tested, there were no statistical differences among the efficaciies of all three species; *S. carpocapsae* caused 82% of mortality, and *S. feltiae* and *H. bacteriophora* caused 90% of mortality.

The highest temperature applied (30 ºC) yielded the highest efficiency with a mortality of 94% in *H. bacteriophora,* followed by *S. feltiae* with 92%. The lowest mortality at 86% occurred with *S. carpocapsae*. However, at this temperature the differences in mortality were not statistically significant.

Discussion

This study of virulence of three entomopathogenic nematode species of the corn stalk borer at four different temperatures revealed that mortality rates were a function of temperature. Among the three species, the lowest effectiveness occurred at 15 ºC, whereas the highest effectiveness occurred at 30 ºC, the highest temperature used.

The lowest mortality rate was for *H. bacteriophora* at 15 ºC. However, the same species at 30 ºC produced a 94% mortality rate which was the highest in the experiment. With the increase in temperature, *H. bacteriophora* was the species that increased its effectiveness the most. We showed that at the lowest temperature this species was the least effective, but that it also was the most effective at the highest temperatures. These findings are consistent with those of El-Wakeil and Hussein (2009), who obtained similar results in their study of *S. cretica* exposed to *H. bacteriophora,* by realizing a 97% of mortality one week post infection.

In another study, González-Ramírez et al. (2000) performed laboratory studies to determine the effectiveness of *H. bacteriophora* on *Mocis latipes* (Guenée) (Lep.: Noctuidae). On its larvae, pupae and prepupae, they found mortality that ranged from 22.5 to 100%, depending upon the temperature. The second species used in experiment, *S. sarpocapsae*, was least effective at 15 ºC, but showed the highest effectiveness at 30 ºC, which was again the highest temperature in the experiment, with a mortality of 86%. The effectiveness of *S. carpocapsae* increased with each increase in temperature, although the differences between the 15 and 20 ºC treatments were not statistically significant. However, the increased mortalities at 25 and 30 ºC were significant relative to the lower temperature.

The efficiency of *S. carpocapsae* was less affected by temperature than *H. bacteriophara*. Halawa et al. (2007), for the control of *S. cretica* Led. (Lep.: Noctuidae), carried out a study with *S. carpocapsae* under laboratory and field conditions, and found that the mortality that this nematode caused on *S. cretica* ranged between 60% and 73.3%, depending on the inoculation density of nematodes.

Our study of the effectiveness of *S. feltiae* showed that mortality increases with temperature. At 15 and 30 ºC, the highest effectiveness occurred on corn stalk borer for this species. At the highest temperatures of 25 and 30 ºC, the differences between this and the other species studied were not significant.

Excluding 15 and 20 ºC, *H. bacteriophara*, showed similar effectiveness with *S. carpocapsae* and *S. feltiae* at the two higher temperatures. As revealed by our study, the effectiveness of *H. bacteriophora* on corn stalk borer was low. In the two *Steinernema* species tested, the effect of temperature was less pronounced.

Our results suggest that when considering the effectiveness of different entomopathogenic nematode species, it is important to acknowledge that application temperatures can be important drivers of pest insect infection and mortality.

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