Türk. entomol. derg., 2022, 46 (4): 399-405
DOI: http://dx.doi.org/10.16970/entoted.1162125

ISSN 1010-6960
E-ISSN 2536-491X

Original article (Orijinal araştırma)

Biocontrol potential of *Heterorhabditis bacteriophora* Poinar, 1976 (Rhabditida: Heterorhabditidae) HBH hybrid strain against the beet webworm, *Loxostege sticticalis* L., 1761 (Lepidoptera: Pyralidae)[1]

Heterorhabditis bacteriophora Poinar, 1976 (Rhabditida: Heterorhabditidae) hibrit HBH ırkının ayçiçeği çayır tırtılı, *Loxostege sticticalis* L., 1761 (Lepidoptera: Pyralidae)'e karşı biyolojik mücadele potansiyeli

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Abstract

With limits on the use of pesticides, biological control has become increasingly important. Consequently, entomopathogenic nematodes (EPN) are now used widely in biological control. EPNs can potentially be used against beet webworm, *Loxostege sticticalis* L., 1761 (Lepidoptera: Pyralidae), which established in sunflower-growing areas in Türkiye in 2022. Therefore, the hybrid EPN strain, *Heterorhabditis bacteriophora* Poinar, 1976 (Rhabditida: Heterorhabditidae) HBH, was assessed for this purpose. The study was conducted in Bursa Uludağ University, Faculty of Agriculture, Plant Protection Department, Nematology Laboratory in 2022. Four nematode doses (2, 5, 10 and 20 IJs) were applied to the last instars of *L. sticticalis* at three temperatures (20, 25 and 30°C). The highest mortality was 97% with 20 IJs dose nematodes at 30°C. LD_{50} and LD_{90} of the nematode were determined at all tested temperatures. The lowest LD_{50} and LD_{90} were at 30°C; 4.37 and 11.0 IJs, respectively. These results indicated that the HBN strain has potential for control of *L. sticticalis*.

Keywords: Biological control, *Heterorhabditis bacteriophora, Loxostege sticticalis,* sunflower

Öz

Pestisit kullanımının sınırlandırılması ile birlikte buna alternatif olan biyolojik mücadele giderek daha önemli hale gelmiştir. Bu nedenle, 2022 yılında Türkiye'de ayçiçeği tarlalarında ayçiçeği yetiştirilen tarım alanlarında istilaya neden olan çayır tırtılı, *Loxostege sticticalis* L., 1761 (Lepidoptera: Pyralidae) zararlısına karşı EPN'lerin potansiyel olarak kullanılabileceği düşünülmektedir. Bu çalışmada *Heterorhabditis bacteriophora* Poinar, 1976 (Rhabditida: Heterorhabditidae) hibrit ırkı HBH kullanılmıştır. Bu çalışma 2022 yılında Bursa Uludağ Üniversitesi, Ziraat Fakültesi, Bitki Koruma Bölümü, Nematoloji Laboratuvarı'nda yürütülmüştür. Bu çalışmada 4 farklı nematod dozu (2, 5, 10 ve 20 IJs), böceğin son dönem larvası üzerine üç farklı sıcaklıkta (20, 25 ve 30°C) uygulanmıştır. Sonuçlara göre, en yüksek ölüm oranı %97 olarak 20 IJs doz nematod yoğunluğunda 30°C'de elde edilmiştir. Ayrıca, HBH hibrit ırkının LD_{50} ve LD_{90} değerleri uygulamada kullanılan tüm sıcaklık değerlerinde belirlenmiştir. En etkili LD_{50} ve LD_{90} değeri sırasıyla 4.37 ve 11.0 IJs olarak 30°C'de gözlenmiştir. Sonuçlar, bu HBH ırkın *L. sticticalis*'ye karşı potansiyel bir ajan olabileceğini göstermiştir.

Anahtar sözcükler: Biyolojik mücadele, *Heterorhabditis bacteriophora, Loxostege sticticalis,* ayçiçeği

[1] This study was supported by the TUBITAK (The Scientific and Technological Research Council of Türkiye), Grant Project No: 219O370.
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Received (Alınış): 15.08.2022 Accepted (Kabul ediliş): 10.10.2022 Published Online (Çevrimiçi Yayın Tarihi): 17.10.2022

Introduction

Insect pests cause considerable yield and economic losses in agricultural production worldwide. Although the decrease in agricultural productivity can be attributed to various reasons, insects and diseases are the most important cause worldwide. Losses from plant pests range from 20 to 40% (Savary et al., 2012).

Chemical control methods have been used for many years in control of insect pests that cause economic losses to crop plants (Gaugler, 2002). Chemical insecticides can have adverse effects on non-target organisms such as humans, animals and natural enemies of pests (van der Blom et al., 2009). With the regulations adopted by the European Union in 2009 (Marchand & Robin, 2019), it was recommended that the use of pesticides should now be reduced (Barzman et al., 2015).

One of the best alternatives to chemical control is biological control. One of biological agents that can be used effectively is entomopathogenic nematodes (EPNs) (Gaugler, 2002) with no negative effects on beneficial and non-target organisms (Ehlers, 1996; Lacey et al., 2015).

The EPNs, belonging to the order Rhabditida in the families Heterorhabditidae and Steinernematidae, live underground and seek insects in which to complete their life cycle. Infective juveniles (IJs) of EPNs can search for a host in the soil for long periods without feeding. The IJs are third stage juveniles (Glazer, 2002).

Heterorhabditis bacteriophora Poinar, 1976 (Rhabditida: Heterorhabditidae), in particular, is effective in for control of pests in a range of crop plants and remains alive in the soil for about 2 years (Susurluk & Ehlers, 2008). Nematodes in the Heterorhabditidae and Steinernematidae have a symbiotic relationship with bacteria, *Photorhabdus* spp. and *Xenorhabdus* spp., respectively. These bacteria belonging to the family Enterobacteriaceae and are present in the digestive system of third stage juvenile and pass to the host following nematode infection of the host, causing death of the host (Boemare et al., 1996).

Heterorhabditis bacteriophora HBH strain created by hybridization and patented was used in the study. This HBH hybrid strain has superior life characteristics and has adapted to the conditions of Türkiye.

The beet webworm, *Loxostege sticticalis* L., 1761 (Lepidoptera: Pyralidae), is commonly seen in eastern and western Europe (Pepper, 1938; Lizhi et al., 2009; Kong et al., 2010). *Loxostege sticticalis* is a highly invasive species that damage to crop plants such as sunflowers and maize. High populations of this pest are found on adult migration routes (Yue & Yuan, 1983; Luo, 2004). When population explosion occurs in a particular area, this stimulates migration behavior (Tammaru et al., 2000; Kong et al., 2010). *Loxostege sticticalis* is also capable of traveling particularly long distances as a result of the morphology and physiology of their adults (Yajie & Ruilu, 1995). Despite there being many studies on this insect, there are very few studies on control methods and especially biological control (Malysh et al., 2021).

The objective of this study was to assess the effectiveness of HBH hybrid EPN strain against the beet webworm in laboratory bioassays. Three temperatures and four nematode doses were tested. The efficacy of EPNs against *L. sticticalis* has not been assessed before and therefore this study is of primary importance for determining potential options for control of *L. sticticalis*.

Materials and Methods

Beet webworm and entomopathogenic nematode

HBH, a hybrid strain of *H. bacteriophora*, which has features such as high efficiency and longevity, was used based on the results of earlier hybridization studies. This hybrid strain was patented in 2018 (Patent No: TR 2013 06141 B). HBH is a superior breed adapted to the climatic conditions of Türkiye and due to these characteristics (Ulu & Susurluk, 2014). *In vivo* production of the strain was done in the last instar of *Galleria mellonella* L., 1758 (Lepidoptera: Pyralidae) larvae in the study. 2-3-day-old IJs formed using *in vivo* methods were used (Kaya & Stock, 1997; Şahin & Susurluk, 2020).

The last instars larvae of *L. sticticalis* were collected from infested sunflower fields of Bursa Uludağ University, Agricultural Research and Application Center. Simultaneously with the collection of these pests, the experiment phase was started after the larvae were brought to laboratory.

Experimental design

The experiment was conducted in 24-well tissue culture plates (each well; 1.5 cm diameter x 3 cm deep). One larva was placed in each well, and then filled with soil at 10% moisture. Four doses (2, 5, 10 and 20 IJs) of HBH were then applied to the top of the soil (under a binocular microscope at low doses) and the plates sealed with paraffin. Each HBH does rate was incubated at 20, 25 and 30°C for 3 days. Water only controls were also included.

After 3 days, all plates were open and the mortality of larvae determined. All dead larvae were dissected and examined for EPN juveniles in order to determine if their death was a result of dead by EPN infestation. Three plates were used for each dose and temperature with 20 larvae used in each plate (n = 20) and all assessment were repeated three times.

Statistical analyses

Mortality data were analyzed by analysis of variance using JMP®7.0 software. In addition, the least significant difference test ($p < 0.05$) was also performed. Probit analysis was performed with XLSTAT® software to calculate LD values. Data were fit to a response model using a non-linear regression.

Results

With increasing dose, mortality also increased at all temperatures except for 10 and 20 IJs at 30°C. At 20°C, the mortality ranged between 8 and 73%. The differences between the mortalities in all doses were statistically significant ($F = 75.6$; df = 3,8; $P < 0.0001$). At 25°C, the mortality ranged between 10 and 95% and were all statistically significant ($F = 188$; df = 3,8; $P < 0.0001$). At 30°C, the mortalities ranged between 15 and 97%, with 2 and 5 IJs the mortality was statistically different ($F = 265$; df = 3,8; $P < 0.0001$) whereas between the 10 and 20 IJs there was no significant difference. No larvae used in control treatments died. HBH application gave substantive mortality even at the low dose 5 IJs with over 70% mortality at the 30°C (Figure 1).

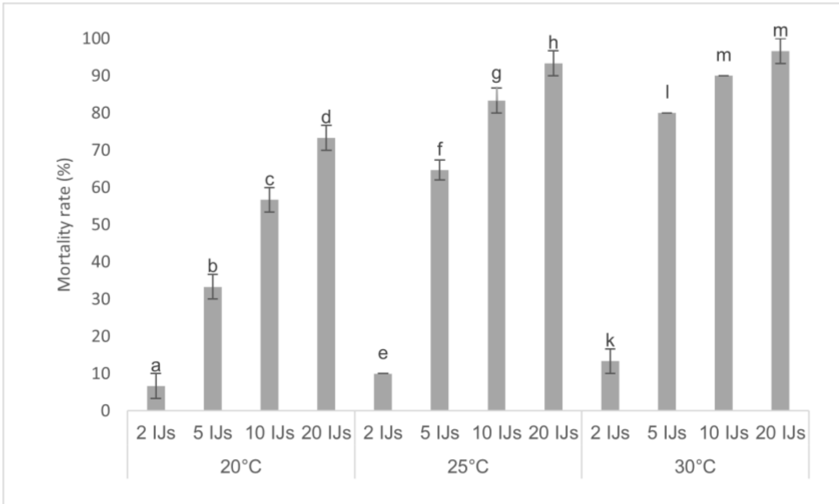

Figure 1. Mortality of *Loxostege sticticalis* was analyzed separately and statistically at each temperature value. Means in columns followed by the same letters are not significant different.

Across all temperatures, mortalities of 2, 5, 10 and 20 IJs doses ranged between 8 and 15%, 35 and 80%, 60 and 90%, 75 and 97%, respectively (Figure 1).

LD_{50} and LD_{90} values deceased with increasing temperatures as mortality increased with temperature. The LD_{50} (4.38) and LD_{90} (11.0) values at 30°C were lower than those at the other temperatures. The greatest effect of the HBH strain was at 30°C. The LD_{50} and LD_{90} values at 25 and 30°C were closer than at 20°C (Table 1). Regression analyses of the mortality values over the dose range for each temperature are presented graphically in Figure 2.

Table 1. LD_{50} and LD_{90} values of the HBH strain on *Loxostege sticticalis*

Temperature (°C)		Dose (IJs)	Std. Error	Lower	Upper
20	LD_{50}	11.2	1.26	8.97	14.3
	LD_{90}	24.6	3.21	19.8	34.2
25	LD_{50}	4.69	0.79	2.84	6.18
	LD_{90}	13.6	1.61	11.2	18.3
30	LD_{50}	4.38	0.75	2.64	5.82
	LD_{90}	11.0	1.28	9.06	14.7

Discussion

A decision was made by the European Union in 2009 to limit the use of pesticides. This has stimulated the use of biological control, which can be a highly effective method of combating pests (Marchand and Robin, 2019)).

Loxostege sticticalis is a species that can damage a wide range of field crop species. Although this varies between regions, it can can complete five life cycles per year. In addition, many larvae can invade plants at the same time, give the large number of egg-laying females (Kong et al., 2010; Frolov, 2015). *Loxostege sticticalis* has recently established in sunflower fields in Türkiye and has caused considerable damage. Therefore, this study was conducted to assess an EPN as alternative to pesticides. Entering soil to pupae after larval stage, this insect is a vulnerable host for EPNs. Therefore, there is potential to use EPNs in its control but this has not previously been assessed.

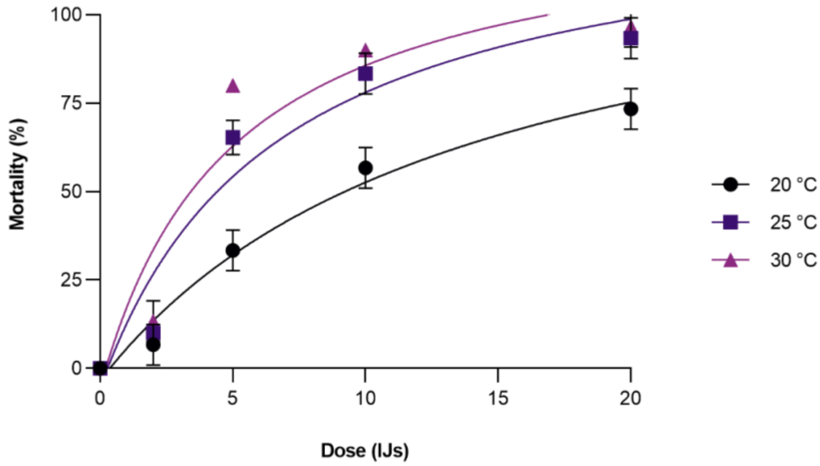

Figure 2. Regression analysis of mortality values at 20, 25 and 30°C against applied EPN doses (2, 5, 10 and 20 IJs).

Heterorhabditis bacteriophora has been widely and effectively used in the agricultural areas against many insect pests (Ehlers, 1996; Susurluk & Ehlers, 2008; Şahin et al., 2018). There are many commercial products based on this species. These are mostly used in temperate climatic regions; since they are not effective below 12°C (Boemare et al., 1996; Gaugler, 2002; Glazer, 2002). The fact that *H. bacteriophora* is cruiser is one of the reasons it is a preferred EPN species. Given that the last instar larvae of *L. sticticalis* move into soil for pupation, it is likely that EPNs could be used for its control in Türkiye. The patented hybrid strain (*H. bacteriophora* HBH) was assessed for this purpose because of its known suitability for the conditions of Türkiye and this choice was supported by the high mortalities founding in the present study.

In general, it has been found that mortality increases as the temperature increases in most EPNs efficacy trials (Fitters et al., 2001; Susurluk, 2008; Ulu & Susurluk, 2014). Consistent with previous studies, the same was found in the present study. Doses used in previous studies have generally been high, 50, 100 or 200 IJs/larva and even more. However, the usefulness of a species or strain is increase if it is effective at low doses (Ulu et al., 2015). Therefore, in this study, doses of *H. bacteriophora* HBH hybrid strain were used as low as possible, that is, 2 and 5 IJs/larva, with mortality ranging from 8 and 80%. This indicates the high the susceptibility of *L. sticticalis* and the efficacy of this EPN strain (Frolov, 2015; Cheng et al., 2016). Efficacy at low doses is critical for achieving economically acceptable commercial outcomes for EPNs in field applications.

The temperatures used in the study were those at which EPNs are most active. Many EPNs efficacy studies have been conducted in this temperature range (Susurluk, 2008; Mukuka et al., 2010). In this respect, the present study is consistent with many studies. Also, the beet webworm completes its life cycle during the summer months within the soil temperatures used in this study (Kong et al., 2010; Frolov, 2015), so the test temperatures were appropriate.

Ulu et al. (2015) found that LD_{50} and LD_{90} values for *H. bacteriophora* against the larvae of the yellow saw fly, *Hoplocampa flava* (L., 1761) (Hymenoptera: Tenthredinidae), were 6.5 and 15.5 at 25°C, respectively. These findings are consistent with the results of present study at 25°C.

There are few studies on biological control of *L. sticticalis*. One of these used a microsporidium, *Nosema pyrausta* (A.Paillot) J. Weiser, 1961 (Protozoa: Nosematidae), against the insect and it was determined to be a promising biocontrol agent for *L. sticticalis* (Malysh et al., 2018, 2021). However, this microsporidium is unlikely to be commercially viable for large scale field application. Lizhi et al. (2016) found that the parasitoid wasp, *Orgilus ischnus* Marshall, 1898 (Hymenoptera: Braconidae), is also an a potentially effective biocontrol agent but only for early stages of larval *L. sticticalis*. Consistently, Luo et al. (2018) found that some hymenopteran wasps attack beet webworm, but their effects indicated limited potential for control at a field scale.

According to results of the present study, it is clear that the test EPN was quite effective for the control of *L. sticticalis* in a laboratory context. Therefore, this demonstrates that is a promising choice for evaluation of field control of this invasive species. If this proved successful, it would help in limiting the use of pesticides by the adoption of alternative control methods against agricultural pests.

Acknowledgements

This study was financially supported by the TUBITAK (The Scientific and Technological Research Council of Türkiye), Project number: 219O370. Dr. Tufan Can Ulu is thanked for statistical support.

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