Available online: www.notulaebiologicae.ro
Print ISSN 2067-3205; Electronic 2067-3264
Not Sci Biol, 2018, 10(4):503-507. DOI: 10.15835/nsb10410320
Original Article

Efficacy of Entomopathogenic Nematode *Steinernema feltiae* (Filipjev) as a Biological Control Agent of Lentil Weevil, *Bruchus lentis*, Under Laboratory Conditions

Karim SAEIDI[1]*, Hossein PEZHMAN[1], Hadi KARIMIPOUR-FARD[2]

[1] *Fars Agricultural and Natural Resources Research and Education Center, Department of Plant Protection Research, AREEO, Shiraz, Iran;*
[2] *Kohgiluyeh and Boyerahmad Agricultural and Natural Resources Research and Education Center, Department of Plant Protection Research, AREEO, Yasouj, Iran; saeidi391@yahoo.com, hossein.pezhman@yahoo.com karimipourfard@yahoo.com*

Abstract

Stored-product pests in the family Bruchidae of Coleoptera represent important pests affecting legume seeds. The lentil weevil, *Bruchus lentis* Froelich (Coleoptera: Chrysomelidae: Bruchinae) is one of the major lentil pests in Iran and in the world. The economic losses caused by this pest on lentil grow up to 40%. Synthetic pesticides are currently the chosen method to protect stored grain from insect damage. However, their widespread use has led to the development of pest strains resistant to insecticides and pest resurgence. In recent years, nonchemical methods, including biological agents are considered safe methods to control the stored grain pests. Positive characteristics of entomopathogenic nematodes as biological control factors of arthropod pests, introduce them as an appropriate option for controlling the integrated pest management of lentil weevil. In the present study, an isolate of entomopathogenic nematode, *Steinernema feltiae*, isolated from soils around Yasouj and based on morphological traits and morphometric data were identified. Adult insects of lentil weevil were exposed to concentrations of 0; 500; 1,000; 2,000 and 3,000 infective juvenile/ml of distilled water at different temperatures (20, 23, 26 and 29 °C). Insects were placed on filter paper impregnated with 1 ml of nematode suspension in Petri dishes for three days. Mortality of the insects was recorded every 24 hours. The highest mortality was recorded after 72 hours, which represented 79.40%, at the concentration of 3,000 infective juvenile at 26 °C.

Keywords: *Bruchus lentis*; entomopathogenic nematode; lentil weevil; *Steinernema feltiae*

Introduction

Lentil is one of the most important plants in the legumes family, rich in protein and starch and it is very important in human diet (Deeba *et al.*, 2006; Cokkizgin and Shtaya, 2013). Lentil in Iran is often cultivated in marginal lands and in soils that are not very fertile and in rainfed form. In this case, its performance is often insignificant due to lack of rainfall and plant pests and diseases (Saeidi and Shahab-Ghayoor, 2015). The total area under cultivation, production and yield of lentil in Iran is 225 thousand hectares, 115 thousand tons per hectare. Mean grain yield of lentils in Iran is only 511 kg / ha (Saeidi and Shahab-Ghayoor, 2015).

In the cultivation and production of lentil it is possible to apply and improve management practices which can lead to an increased product performance. One of the most important strategies for achieving maximum yields is the change in planting date, selection of suitable cultivars, management of water and fertilizer use, control of pests, diseases and weeds (Sarwar, 2012).

Lentil weevil is one of the most popular pests, which is found in all parts of Iran (Saeidi and Shahab-Ghayoor, 2015). Lentil weevil is more harmful to lentil and mushrooms.

Duke and Yadav (2000) reported 30-60% loss in seed weight and 40-65% loss in protein content due to its damage and pulse seeds become out of shape for human consumption, as well as for planting (Rehman *et al.*, 1992; Polanco and Rennie, 2004; Saeidi and Shahab-Ghayoor, 2015).

Larvae feed lentils from legumes, causing heavy damage to crop products (Hominic *et al.*, 1996). The larvae of lentil weevil cannot feed dry seeds. Therefore, on farms, it feeds only juicy grains. All activities of lentil weevil larvae occur in field, but the insects go overwintering in the diapauses mode in the stores and in the infested grains of the legumes (Saeidi and Shahab-Ghayoor, 2015).

Received: 06 Jun 2018. Received in revised form: 03 Dec 2018. Accepted: 21 Dec 2018. Published online: 21 Dec 2018.

Adult beetles fly to the lentil farm in the spring and after a bit of feeding from nectar and pollen, the flowers begin to spawn, the eggs are clear yellow and the females lay their eggs on young pods lentils leave. Young larvae go to the pod when it leaves the egg and grows slowly, which lasts about six weeks. Before digging the larvae to the outside of the grains, they dig a corridor to reach the surface of the skin (White, 2001). The insect has a life span of 45 days and lasts for 10 days. Lentil weevil is a generation per year. The first sign on the lentil seed is the presence of a very small dark circle of spheres or if there is a hole on the grain during harvesting. If we open the grain, there is a larva in it. Later a window appears on the seed and the larva feeds on the seed (Ward, 1999).

Bruchus lentis Frölich is one of the most important pests causing serious damages to lentil in Iran and around the world (Sarwar, 2012). They appear in high population in fields and storages in central and western Europe (Shahina and Salma, 2009), Mediterranean coasts (White, 2001) and Iran (in the provinces of east- Azerbaijan, west- Azerbaijan, Hamedan, Ghazvin, Tehran, Isfahan and Fars) (Shahhosseini and Kmali, 1989).

In Gachsaran, with geographical position (51° 35' 21" N 57° 25' 21" E) the warm regions of Kohgiluyeh va Boyerahmad, *B. lentis* have been reported by for the first time in 1998 (Saeidi, 1999).

Lentil weevils are difficult to control with chemicals due to their cryptic lifestyles of hiding in crevices, where they are protected from insecticidal sprays (Saeidi, 1999). Entomopathogenic nematodes belonging to the family of Steinernematidae and Heterorhabditidae are the most effective and useful biological control agents for insects (Georgis *et al*., 1991; Gaugler *et al*., 1997; Gaugler, 2002; Stuart *et al*., 2006). Entomopathogenic nematodes are safe for the environment while controlling insects (Jess and Bingham, 2004). They can also be manipulated through genetic engineering, and their pathogenesis and stability are altered.

In the present study, the main objective was to establish the potential of *S. feltiae* to control adult *B. lentis* under laboratory conditions.

Materials and Methods

Insects rearing of B. lentis

B. lentis were obtained from a laboratory colony established in 2015 at the Department of Entomology, Faculty of Agriculture, Urmia University. The colony of *B. lentis* originated from field collected individuals from crops that included lentil in Yasouj, Iran.

Collect soil samples

Soil samples were collected from oak forests, grasslands and alfalfa fields in mid-southern regions of Kohgiluyeh va Boyerahmad province, Iran including: Sisakht, Dashtroom, Yasouj, Amir Abad and Sargachineh with geographical position (51° 36' 22" N 51° 37' 23" E). Elevation of sampling locations was between 1,670 and 2,450 meters above sea level. The samples were taken during March and April 2015 at depth of 0-20 cm from an area of 100-500 m^2, each sample consisted of 5-10 subsamples of 50 g soil. Totally, 75 soil samples were collected.

Isolation of Entomopathogenic nematode from soil

Entomopathogenic nematode, *Steinernema feltiae*, was isolated from soil samples by using Galleria bait technique (Bedding and Akhurst, 1975). *Galleria mellonella* (wax moth larva) was reared on an artificial diet suggested by Hala *et al*. (2012) at room conditions. Dead *Galleria* larvae were moved to a white trap for obtaining IJs (Infective Juvenile larvae) from the cadavers, while a few cadavers were directly dissected to obtain adult nematodes of the first and second generations.

Pathogenicity test

Factorial experiment with three factors, including time (24, 48 and 72 hours), concentration (0; 500; 1,000; 2,000 and 3,000 IJs in 1 ml distilled water) and temperature (20, 23, 26 and 29 °C), within a completely randomized design with four replications was done. To obtain the effective concentrations of nematode, a sequential dilution method was used. For each concentration and each repetition, petri dishes with a diameter of 9 cm were considered. The floor of each petri dish was covered with two layers of filter paper and 10 adult insects in each petri dish were placed. Petri dishes were kept for three days at a temperature of 20, 23, 26 and 29 °C in the incubator. Mortality rate of insects was recorded on a daily basis.

Statistical analysis

Data were evaluated through analysis of variance by using SPSS software (version 16, SPSS Institute, Cary, NC, USA) and mean comparison of treatments were done with Tukey's test ($\alpha \leq 0.01$).

Results and Discussion

A species of insect pathogenic nematode, *Steinernema feltiae*, using morphological and morphometric characteristics and using a Lucskai key (1999) was identified and on greater wax moth, *G. mellonella* multiplied. Using the Koch method, a pathogenicity test was performed to confirm the pathogenicity of the nematode. Results of the statistical analysis of data, about the effect of *S. feltiae* on the adult insect lentil weevil in laboratory conditions are shown in Table 1. According to the analysis of variance table, all treatments showed a significant difference compared to control.

Also, the effect of treatments (temperature, time and different concentrations of the pathogen nematode), as well as the interaction of temperature at nematode concentration and time in nematode concentration were statistically significant at 1% level. Considering the significance of the differences, the comparison of the averages of the tested treatments was carried out and the results of the comparison of the mean are given in Table 2.

The results of this section are consistent with the results obtained by Aydin and Susurluk (2005). The results of probit analysis for determination of LC_{50} are given in Table 3. According to this table, it is observed that the best result in this experiment was obtained by treatment of 26 °C after 72 hours (LC_{50} = 698).

Table 1. Analysis of variance of the effect of *Steinernema feltiae* on adult of *Bruchus lentis*

Source of variance	df	Mean square
Temperature	3	9.123**
Time interval	2	181.345**
IJ*S	4	69.125**
Temperature × IJ*S	12	3.451**
Time interval × IJ*S	8	14.211**
Time interval × temperature × IJ*S	24	0.615 ns
Error	180	0.666

*Infective juvenile
ns and ** respectively indicate Non-significant and significant at the probability level of one percent

Table 2. Percentage mortality of adult *B. lentis* due to effect of *S. feltiae* in different temperatures and times

Time interval (hours)	No. of IJs	Mean mortality of *B. lentis*			
		20 °C	23 °C	26 °C	29 °C
24	3,000	19.10(7.23)	9.21(3.12)	9.31(3.10)	7.02(6.22)
	2,000	17.11(4.44)	7.02(3.23)	7.02(3.23)	12.01(5.02)
	1,000	9.45(5.11)	9.22(3.24)	4.51(3.25)	4.42(3.22)
	500	4.23(3.21)	4.33(3.23)	0.00(0.00)	4.52(3.22)
	control	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)
48	3,000	42.10(8.12)	27.01(3.56)	47.00(7.22)	22.01(5.04)
	2,000	27.10(6.11)	17.02(3.56)	29.30(5.65)	17.03(3.25)
	1,000	22.10(6.33)	17.01(3.56)	19.20(5.65)	14.25(3.10)
	500	17.10(3.75)	9.30(3.23)	7.00(3.23)	14.34(3.20)
	Control	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)
72	3,000	64.43(11.25)	59.44(5.12)	79.40(7.12)	54.30(7.41)
	2,000	52.12(11.11)	39.32(9.32)	54.33(5.26)	32.02(5.06)
	1,000	44.42(3.10)	39.21(5.11)	42.02(5.05)	24.40(3.23)
	500	32.11(5.03)	37.04(7.23)	24.45(9.22)	29.32(5.12)
	control	4.61(3.21)	0.00(0.00)	0.00(0.00)	7.01(3.26)
LSD5%			3.6569		
LSD1%			4.5364		

The number of repetitions of four, the mean outside the parentheses, and the standard error inside the parentheses are given.

Table 3. Values of LC_{50} for *S.feltiae* on adults of *B. lentis* at different temperatures and times

Time interval (hours)	LC_{50}			
	20 °C	23 °C	26 °C	29 °C
24	13,878	13,122,263	28,221	1,341,132
48	5,433	16,353	3,121	521,312
72	857	1,511	698	3,123

In a study by Jess and Bingham (2004) on the decay of nematodes Heterorhabditidae and Steinernematidae on the ages of helio this larvae of high ages were shown to be more susceptible to nematode concentrations than larvae of the same ages. They also got similar results. So that 79 to 100% of larvae and 66% of adult flour beetles were controlled by nematode *S. feltiae*. Yee and Lacey sensitivity of larval, pupal and adult stages, cherry fruit fly to three species of *Steinernema* genus nematode were investigated and concluded that mortality in the laboratory was 10 to 62 percent.

Shahina and Salma (2009) tested the effect of *Steinernema* on rice weevil and concluded that 55 to 370 nematodes per adult were the best concentrations and could be used to control the nematode in rice weevil control. Csontos (2002), studying the effect of nematode on adult rice weevil at four different temperatures, obtained similar results to this study. The experiment of the effect of the nematode *S. feltiae* on the last instar larvae of *Tenebrio molitor* at 12, 18 and 25 °C showed that 97% of death occurred at 25 °C, which are similar to the results of this research.

The results of the experiment on the effect of nematode *S. feltiae* on adult weevil rice showed that the concentration of 2,000 IJs per milliliter, at 30 °C and after 72 hours, caused the highest mortality and therefore had the highest impact. On the other hand, not only the concentration of nematode influences the control of this pest, but the ambient temperature and the time after inoculation of the nematode of the patient have a significant role in increasing the efficiency of the nematode. So that the efficacy's nematode increases as the temperature rises to 30 °C.

It seems that an increase in the amount of mobility, nutrition, body volume and consequently the increase in the size and dimensions of the natural pores (mouth, denominator, and respiratory holes), the age of the insect, and most likely the sensitivity of the developmental stage of the pest to the sympathetic bacterium (X) with the nematode, the patient has been the main cause of the temperature effect at this stage of insect growth.

It can be said that the possible reason for observing the different effects of different temperatures on the efficacy of nematodes in the patient is that the metabolism of the nematode and the bacteria symbiotic with it, along with the increase in temperature to 30 °C, increases.

Therefore, not only the percentage of the penetration of nematodes increases, but also the production of poison and the growth of the symbiotic bacteria are very high. As a general conclusion, it can be noted that the *S. feltiae* isolated from soils in the province of Kohgiluyeh va Boyerahmad at 29 °C had the greatest effect on adult weevil lentil. Time also had a significant effect on mortality, so that the highest mortality was observed after 72 hours.

References

Aydin H, Susurluk A (2005). Competitive abilities of entomopathogenic nematodes, *Steinernema feltiae* and *Heterorhabditis bacteriophora* in the same host at different temperatures. Turkish Journal Biology 29(1):35-39.

Bahl FS, Averbeck D, Averbeck B, Idaomar M (2008). Biological effects of essential oils a review. Food and Chemistry Toxicology 46(2):446-475.

Bakr MA (1994). Plant protection of lentil in Bangladesh (*Lens culinaris*). In: Erskine W, Saxena MC (Eds). Lentil in South Asia, Proceedings of the seminar on Lentil in South Asia, Indian Council of Agricultural Research, New Delhi, India: International center for Agricultural Research in the dry areas (ICARDA), Aleppo (Syria) pp 177-186.

Bedding A, Akhurst RJ (1975). A simple technique for the detection of insect parasitic rhabditid nematodeisn soil. Nematologica 21(1):109-110.

Cokkizgin A, Shtaya MJY (2013). Lentil: origin, cultivation techniques, utilization and advances in transformation. Agricultural Science 1(1):55-2.

Crespi M (2007). Analysis of the response to abiotic stress in legumes. Retrieved 5.01.2008 from www.grainlegumes.com.

Csontos AS (2002). Lateral movement of the entomopathogenic nematodes *Steinernema glaseri* and *Heterorhabditis bacteriophora* in sand at different temperatures in response to host seeking. Biocontrol Science and Technology 12(1):137-139.

Deeba F, Sarwar M, Khuhro RD (2006). Varietal susceptibility of mungbean genotypes to pulse beetle, *Callosobruchus analis* (Fabricius) (Coleoptera: Bruchidae). Pakistan Journal of Zoology 38(4):265-268.

Duke GT, Yadav TD (2000). Feeding of *Callosobruchus maculatus* (Fab.) and *Callosobruchus chinensis* L. in green gram. Indian Journal of Entomology 40:108-112.

Ehlers RU (2011). Current and future use of nematodes in biocontrol: Practice and commercial aspects in regard to regulatory policies. Biocontrol Science and Technology 6(3):303-316.

Forst S, Nealson K (1996). Molecular biology of the symbiotic pathogenic bacteria *Xenorhabdus* and *Photorhabdus* spp. Microbiological Reviews 60(1):21-43.

Gaugler R (2002). Entomopathogenic nematology. CABI Publishing, Wallingford, UK pp 400.

Gaugler R, Lewis E, Stuart RJ (1997). Ecology in the service of biological control: the case of entomopathogenic nematodes. Oecologia 109(4):483-489.

Georgis R, Kaya HK, Gaugler R (1991). Effect of steinernematid and heterorhabditid nematodes (Rhabditida: Steinernematidae and Heterorhabditidae) on nontarget arthropods. Environmental Entomology 20(3):815-822.

Gouge DH, Hague NGM (1993). Effects of *Steinernema feltiae* against sciarids infesting conifers in a propagation house. Tests of Agrochemical and Cultivars.

Groot ID (2004). Protection of stored grains and pulses. Fifth edition, Digigrafi, Wageningen, the Netherlands. Agromisa Foundation 18 pp 78.

Hala MS, Metwally Gehan A, Hafez Mona A, Hussein MA, Hussein HA, Salem MME (2012). Low cost artificial diet for rearing the greater wax moth, *Galleria mellonella* L. (Lepidoptera: Pyralidae) as a host for entomopathogenic nematodes. Egyptian Journal of Biological Pest Control 22(1):15-17.

Hominick WM, Reid AP, Bohan DA, Briscoe BR (1996). Entomopathogenic nematodes: Biodiversity, geographical distribution and the convention on biological diversity. Biocontrol Science Technology 6(3):317-331.

Jess S, Bingham JFW (2004). Biological control of sciarid and phorid pests of mushroom with predatory mites from the genus *Hypoaspis* (Acari: Hypoaspidae) and the entomopathogenic nematode *Steinernema feltiae*. Bulletin of Entomological Research 94(2):159-167.

Khan MZ, Ali MR, Bhuiyan MSI, Hossain MA (2015). Eco-friendly management of pulse beetle, *Callosobruchus chinensis* Linn. using fumigants on stored mungbean. International Journal of Scientific and Research Publications 5(5):1-6.

Kossou DK, Gbehounou G, Ahanchede A, Ahohuendo B, Bouraima Y, Huis AV (2001). Indigenous cowpea production and protection practices in Benin. International Journal of Tropical Insect Science 21(2):123-132.

Lucskai A (1999). Identification key to entomopathogenic nematdoe species. Acta Phytopathologica et Entomologica Hungarica 34(4):317-325.

Majka CG, Langor D (2011). The bean weevils (Coleoptera: Chrysomelidae: Bruchinae) of Atlantic Canada. Journal of the Acadian Entomological Society 7(11):75-82.

Polanco I, Rennie M (2004). Evaluation of certain spices for the control of *Callosobruchus maculatus* (Fabricius) (Coleoptera: Bruchidae) in cowpea seeds. African Journal of Entomology 3(1):87-89.

Rehman M, Mohammad F, Ahmed A, Skari M (1992). Screening of tropical plants for the presence of bioactive compounds. Pertanika 15:131-135.

Saeidi K (1999). Investigation on the timing of chemical control of lentil weevil, *Bruchus lentis* (Coleoptera:Bruchidae) in lentil fields in Gachsaran region. Final Report, Agricultural and Natural Research Centre of Yasouj. Yasouj, Iran pp 45.

Saeidi K, Shahab-Ghayoor H (2015). Evaluation of botanical insecticides against lentil weevil, *Bruchus lentis* Frolich (Coleoptera: Chrysomelidae: Bruchinae) under laboratory conditions. Agricultural Communications 3(4):23-27.

Sarwar M (2010). Some possibilities on the effectiveness of plant powders as grain protectants against cowpea weevil, *Callosobruchus maculatus* (Fabricius) Walp (Coleoptera: Bruchidae) infestation in chickpea.

International Journal of Agronomy and Plant Production 1(2):45-50.

Sarwar M (2012). Assessment of resistance to the attack of lentil beetle *Bruchus lentis* (F.) in lentil genotypes on the basis of various parameters during storage. Songklanakarin Journal of Science and Technology 34(3):287-291.

Shahina F, Salma J (2009). Laboratory evaluation of seven Pakistani strains of entomopathogenic nematodes against a stored grain insect pest, pulse beetle *Callosobruchus chinensis* (L.). Journal of Nematology 41(4):255-260.

Stuart RJ, Barbercheck ME, Parwinder SG, Robin AJ, Taylor CW (2006). Population biology of entomopathogenic nematodes: Concepts, issues, and models. Biological Control 38(1):80-102.

Taghizadeh-Saroukolai A, Meshkatalsadat MS (2010). Insecticidal properties of *Thymus parsecs* essential oil against *Tribolium castaneum* and *Sitophilus oryzae*. Journal of Pest Science 83(1):3-8.

Ward RL (1999). Monitoring of the bean seed beetle (*Bruchus rufimanus*) in field beans (*Vicia faba*). Aspects of Applied Biology 56:145-150.

White NDG (2001). Protection of farm-stored grains, oilseeds and pulses from insects, mites and moulds. Agriculture and Agri-Food Canada Publication pp 53.