Research Article

iMedPub Journals
www.imedpub.com

Journal of Plant Sciences and Agricultural Research

2018
Vol.3 No.1:17

In-vitro and *In-vivo* Evaluation of *Entomopathogenic nematodes*, *Steinernema yirgalemense* and *Heterorhabditis bacteriophora* for control of Red Teff Worm, *Mentaxya ignicollis*

Tesfaye Hailu*, Belay Habtegebriel and Teshal Daba

Ethiopian Institute of Agricultural Research, Ambo Plant Protection Research Center, P.O.Box-37, Ambo, Ethiopia

Corresponding author: Tesfaye Hailu

yonatentes@yahoo.com

Ethiopian Institute of Agricultural Research, Ambo Plant Protection Research Center, P.O.Box-37, Ambo, Ethiopia.

Citation: Hailu T, Habtegebriel B, Daba T (2018) *In-vitro* and *In-vivo* Evaluation of *Entomopathogenic nematodes*, *Steinernema yirgalemense* and *Heterorhabditis bacteriophora* for control of Red Teff Worm, *Mentaxya ignicollis*. J Plant Sci Agri Res. Vol.3 No.1:17

Abstract

Red Teff Worm (RTW), *Mentaxya ignicollis* (Lepidoptera: Noctuidae), is one of the most serious pests of teff causing high economic losses in the growing areas in Ethiopia. Control of RTW heavily relies on chemical pesticides which pose serious safety concerns on the environment and human and animal health. An integrated approach of management of RTW should include biological control as a component. Five Entomopathogenic nematodes (EPN) (three *Steinernema yirgalemense* (HI, Z9, Aw3) and two *Heterorhabditis bacteriophora* (AEH and AAM8B) strains were evaluated under laboratory and glass house conditions for efficacy against 3rd instar larvae of the RTW. In the laboratory bioassays a 1ml suspension containing 400 Infective juveniles (IJ)/ ml of each of the EPN strains was applied to 10 RTW larvae in a Petri-dish. Two selected strains (AEH and HI) were then tested in glass house pot experiments at a rate of 3 ml/pot and a concentration of 400 IJ/ml. One and three ml of sterile water was applied to negative control treatments in the laboratory and pot experiments respectively. Karate 5% EC served as a positive control in the pot experiments. A completely randomized design and a randomized complete block design with three replications were used for the laboratory and glass house pot experiments respectively. Each of the experiments was conducted twice. The bioassay results showed that each of AEH and HI strains caused 91% mortality on RTW larvae within ten days of application significantly differing (P=0.0001) from the rest of the treatments which caused 33.75% (AW3), 43.75% (AAM8B), and 46.25% (HH) while mortality in the control was 10%. In the first glass house pot experiments, AEH and HI caused 74% and 67% mortality of RTW larvae significantly differing (P=0.0001) from the positive control (91%). Similarly, in the second glass house pot experiments, it was observed that, AEH and HI caused 76% and 77% larval mortality respectively ten days after treatment application and the result was not significantly different from the positive control but when compared with untreated check all treatments were significantly different (P=0.0002). It is concluded that the two EPN strains are promising candidate biological control agents against the RTW. Further experiments are recommended to test the efficacy of the strains under field conditions.

Keywords: RTW; teff; *Steinernema yirgalemense; Heterorhabditis bacteriophora;* bioassay; biological control

Abbrevation: RTW: Red Teff Worm; EPN: Entomopathogenic Nematode; CRD: Completely Randomized Design; RCBD: Randomized Complete Block Design; IJ: Infective Juveniles

Received: October 23, 2018; **Accepted:** November 06, 2018; **Published:** November 17, 2018

Introduction

Teff, *Eragrostis tef* and Trotter is a staple food crop of Ethiopia where it originated and diversified. It is the most preferred national diet and accounts for about two thirds of the daily protein in the diet of the population. Over 3.02 million hectares of land was covered with teff in the main season of 2016/2017 year with a total production of over 50.2 million quintals and its mean productivity at national level predicted at 16.64 q/ha [1]. Insect pests, diseases and weeds are among the biotic factors that cause substantial reduction and loss of teff yield in Ethiopia where the crop is widely cultivated. The major insect pests recorded on the crop in the country are *Mentaxya ignicollis. Acrotyus* spp*. Aolopus longicornis, Carbula recarva, Eriangerits iger, and Odontoteres* spp. [2].

Red teff worm (RTW), *Mentaxya ignicollis* (Lepidoptera: Noctuidae), is a serious pest of teff grown on black or heavy deeply cracking clay soils. The reddish light green larvae are observed on plants in the early morning and in the evening feeding on leaves and developing grains in their milky stage [3]. In the hotter hours of the day they hide in cracks in the soil and under shade. The status of the red teff worm, *M. ignicollis* as a major pest of teff was reported in Shewa, Kefa, Gojam and in some places of Tigray and Wollega [4] and could cause up to 24% loss in yield [5].

Control measures of RTW, include cultural, chemical and biological methods which have been attempted to some extent. Tadesse reported that application of the biological agent of *Bacilius tharingrensis* to RTW at rate of 700 mg (WP) reduced larval density and increased grain yield of teff compared to untreated checks. Several studies were conducted to determine the most effective control methods without using insecticides and insect growth regulators. One of the latest discovered methods is the use of Microbial Biocontrol Agents (MBA) such as bacteria, virus nematodes and fungi [6,7] which are natural enemies of the insects. Biopesticide formulations based on *Beauveria bassiana* and *Metarhizium anisopliae*, a wide host range insect pathogenic fungi, are being marketed and used in insect pest management [8,9]. These entomopathognic hyphomycete fungi have great potential as biological control agents against insects and are an important component within integrated pest management systems. They are being developed worldwide for the control of many pests of agricultural importance [10,11]. Fortunately enough lepidopterans are amongst the choicest hosts of this fungus, including several species of agriculturally important insect pests [12-14].

EPN are beneficial roundworms that can prey on harmful insects [15] The two families to which the EPN belong are Steinernematidae and Heterorhabditidae and are known to have potential in biological control of many insect pest of crops [16,17]. Describing the mechanism of these nematodes, Boemare and Adams state that the nematodes that belong to *Steinernema* and *Heterorhabditis* harbor symbiotic bacteria in the family Enterobacteriaceae, such as *Xenorhabdus* and *Photorhabdus* in their gut [18,19]. These bacteria are then carried in to the insect body and released in the hemocoel, multiply and cause septicemia that ultimately kills the host insect in 24-48hrs [18]. Infective juveniles are then produced *en masse* after the nematodes develop and multiply in the insect body completing up to 3 generation before emerging to infect another host [20].

Although there are over a hundred companies in the USA involved in the mass production, formulating and marketing of EPN as biological control agents against pests of crops such as fruits and turf grasses, their use in pests such as the RTW has not been explored substantially.

The use of these environmentally safe EPN against lepidopteran insect pests such as the RTW, *M. ignicollis,* has not been explored substantially. To develop integrated pest management package for the insect pest, information about safe alternative control methods of the pest is necessary. Since RTW passes the harsh dry season in the soil as pupae and the larvae hide in the soil during the day, biological control agents that inhabit the soil easily are of considerable importance for substantial control targeting the larvae and pupae. Therefore the objective of this study was to determine the efficacy of entomopathogenic nematode species, *Steinernema yirgalemense* and *Heterorhabditis bacteriophora,* against RTW (*M. ignicollis*) under laboratory and greenhouse conditions and to generate information for the development of integrated pest management package against the pest.

Materials and Methods

Nematode culturing

Five strains of the two species of entomopathogenic nematodes (AEH, HI, Aw3, AAM8B and HH) were tested in the bioassays. Culturing and preparation of EPN was performed by using standard methods described by Keya and Stock [21]. Fifth instar larvae of the wax moth larvae *G. mellonella* was used to culture the entomopathogenic nematodes *in-vivo* from which Infective juveniles were harvested on a daily basis for 7 days. The culture of the strains used in the experiments had been maintained for over 8 years. Harvested nematodes were kept at 14˚C in 250 ml flasks and used within a week of culture preparation.

Rearing of RTW

For mass rearing of RTW, larvae were collected from infested teff fields of Dendi, Ambo, Becho Dawo and Seden Sodo woredas of west and South west shawa zones of Oromiya region and mass reared at Ambo Plant Protection Research Center biocontrol laboratory using teff plants. The larvae were maintained in the laboratory under 27˚C. The larvae were fed with teff plants until changed to pupae. The pupae were transferred to soil filled container for pupation purpose. When the pupae changed to adults, they were collected and kept in cages in which tissue paper was provided for oviposition. The adults were provided with sugar solution for feeding. The newly laid eggs were collected and kept in an incubator at 25˚C and 70 ± 5% of relative humidity which is favorable for hatching of the eggs [22]. The newly emerged larvae were used for the experiments.

Laboratory bioassays

Bio-assays were conducted in Petri dishes to select the most virulent strains of EPN using 3rd instar larvae of RTW according to the procedure. Five strains of EPN were used including three *Steinernema yirgalemense* (HI, Z9, Aw3) and two *Heterorhabditis bacteriophora* (AEH and AAM8B) strains. Ten 3rd instar RTW larvae were added to each Petri-dish and 1ml of aqueous nematode suspension of infective juveniles was applied into each of the Petri-dishes at a dose of 400 IJs per ml [23,24]. The control

group was treated with 1 ml of sterile water. All Petri-dishes were incubated at 25 ± 2°C. The experiments were conducted in Completely Randomized Design (CRD) with four replications. Mortality of the RTW larvae was recorded at 24, 48, and 72h after initial inoculation. The experiment was repeated after a week.

Mortality data for bioassay experiments were corrected for the corresponding control mortality by the formula:

%CM=((T-C) ÷ (100-C)) × 100

Where CM is corrected mortality, T is mortality in treated insects and C is mortality in untreated insects [25].

Dose response experiments

Two potential nematode strains, namely AEH and HI were used for the experiments. The strains were selected based on the observed potential high virulence in the laboratory bio-assays. For each strain, aqueous nematode suspension of 200, 400 and 600 IJs/ml were prepared and used for inoculation. Sterilized distilled water was used for the control group. Ten 3rd instar larvae of RTW were transferred to a Petri-dish containing young chopped teff seedlings. Each Petri-dish was sprayed with 1/2 ml of suspension of isolates having a concentration of 200, 400 and 600 IJs/ml. Each treatment was replicated four times. After spraying, all larvae on Petri-dishes were incubated at 27°C, 70 ± 5% RH, photoperiod of 12:12h day and night; and examined daily. Mortality data were collected, starting from 24h after inoculation.

Glass house experiments

The experiments were conducted in the glass-house at Ambo Plant Protection Research Center (PPRC), on teff plants (variety Kuncho) under pot culture. The pots were filled with soil composed of vertisol: compost: sandy soil at 2:1:1 ratio. Teff seeds were then sown on the soil filled pots at the recommended rate per hectare and watered at three days interval until the crop started to flower. The experiments were conducted in a randomized complete block design (RCBD) with four replications and ten 3rd to 4th instar larvae of the RTW were introduced to the teff plants at the flowering stage of the crop. The two EPN strains, *Steinernema yirgalemense* strain HI and *Heterorhabditis bacteriophora* strain AEH which were selected from the laboratory bio-assays were used for these experiments. A three ml suspension of each of the nematode strains containing 400 IJs/ml was applied to the teff plants in each pot containing the RTW larvae by hand held sprayer early in the morning. Sterile water was applied to control treatments. Daily temperature and relative humidity of the green house was recorded throughout the experiment. The mean temperature and relative humidity of the glass house during the first experiment were 22.25°C and 58.5% respectively and inoculation was conducted on 19/4/2015. Similarly the temperature and relative humidity during the second experiment were 19.75°C and 63.5% respectively and inoculation was conducted on 25/10/2015. Percentage of efficacy of the isolates was determined by means of the formula:

%Efficacy=((Cd–Td)×10 ÷ Cd) × 100

Where Cd=Number of live individuals in the control plots after the treatment, Td=Number of live individuals in the treated plots after the treatment.

Statistical analysis

Mortality data were corrected for control mortality and subjected to the ANOVA procedure of SAS software version 9.2. Means were separated with LSD test. The median lethal concentration (LC_{50}) and median lethal time (LT_{50}) were determined using SPSS statistical software version 20.

Results

Laboratory bioassays

Cumulative mortality of the larval population of red teff worm over the ten days reached 90.81% for AEH and HI strains of the entomopathogenic nematodes **(Table 1).** Among the EPN strains AW3, AAM8B and HH incurred lowest mortality in ten days after treatment application as compared to the other strains. One strain of *Steinernema yirgalemense* (HI) and one strain of *Heterorhabditis bacteriophora* (AEH) which showed better performance in terms of mortality of the RTW were selected for the pot experiments.

Dose-response

Determination of medium lethal concentration of *Steinernema yirgalemense* strain HI and *Heterorhabditis bacterophora* strain AEH against 3rd instar larvae of RTW under laboratory conditions showed that there were significant differences among the two tested strains**.** The relative potency and the estimated LC_{50} and LC_{90} values based on the mortality trends across dosage are presented in **Table 2**. The comparison of the virulence of the nematodes *Steinernema yirgalemense* strain HI and *Heterorhabditis bacterophora* strain AEH showed that *S. yirgalemense* strain HI exhibited highest virulence with the lowest LC_{50} (191.55 IJ/ml) and LC_{90} (605.46 IJ/ml). The *Heterorhabditis bacterophora* (AEH) showed the lowest virulence with the highest LC_{50} (231.81 IJ/ml) and LC_{90} (1072 IJ/ml). The results showed that mortality of RTW was dose related as observed between the two nematode strains **(Table 2).**

Table 1. Percentage mortality of the Red teff worm larvae treated with different EPN isolates of *Steinernema* and *Heterorhabditis spp*. at the rate of 400 IJ/ml.

Isolates	Mortality 10DTA ± SE	F-value	P-value
AEH	90.81 ± 1.01a	21.06	0.0001
HI	90.81 ± 1.01a		
Aw3	33.75 ± 0.54b		
AAM8B	43.75 ± 0.87b		
HH	46.25 ± 0.97b		
Control	10 ± 12.65c		
CV	17.16		
LSD	14.75		

Table 2. LC_{50} and LC_{90} of *Steinernema spp.* (HI) and *Heterorhabditis spp.* (AEH) Isolates (IJ/ml) to 3rd Instar Larvae of RTW at 72hrs after application.

Treatments	LC_{50} (95% CI)a	LC_{90} (95% CI)	Relative potency[b]	
			LC_{50}	LC_{90}
HI	191.55 (125.89-246.37)	605.46 (387.03-4224)	1	1
AEH	231.81 (0.25-604)	1072 (477.16-1.58)	1.21	1.77

The relative potency values indicated that the nematode isolate *Steinernema yirgalemense* (HI) was more effective than *Heterorhabditis bacterophora* (AEH) with 1.21 times more potency at the LC_{50} and 1.77 times more potency at the LC_{90} level, respectively.

Determination of medium lethal time of *Steinernema yirgalemense* strain HI and against 3rd instar larvae of RTW under laboratory conditions also showed that there were significant differences among the two tested strains**.** The lowest LT50 was observed from *H. bacteriophora* strain AEH (3.1 days) followed by *S. yirgalemense* strain HI (5.2 days) while it took nearly 19 days for 50% of the RTW larvae to die in the controls **(Table 3).**

The statistical analysis of percentage mortality at 24, 48 and 72 hrs after application of nematodes at 200, 400 and 600 IJ/ml also showed significant differences among the two strains of the selected nematode species **(Table 4)**. The highest RTW mortality was induced by HI strain of *S. yirgalemense* (72.6%) 72hrs after application at 600 IJ/ml indicating that mortality of was related both to dose and duration. The lowest RTW mortality other than the control was observed in AEH strain of *H. Bacteriophora* (34.34%) at a dose of 200 IJ/ml. All of the EPN strains showed increased efficacy as the concentrations of the strains increased.

Pot experiment

In first glass house pot experiment, AEH and HI caused 74% and 67% mortality of RTW larvae significantly differing (p=0.0001) from the positive control (91%). Similarly, in the second glass house pot experiment, it was observed that AEH and HI caused 76% and 77% larval mortality respectively ten days after treatment application and the result was not significantly different with the positive control (Karate) but when compared with Untreated check all treatments (Karate, HI and AEH) were significantly different (P=0.0002) **(Table 5).**

Table 3: LT_{50} of Red teff worm treated with 2 EPN isolates at the rate of 400IJ/ml.

Isolates	LT_{50} days ± SE	95% Confidence limits		Slope
		Lower	Upper ± S.E	
AEH	3.1 b	1.94	4.22	4.68
HI	5.2 b	4.24	5.95	4.12
Control	19.11a	3.39	4.54	2.35
CV	18.09			
LSD	3.74			

Table 4. Mean Present Mortality of 3rd Instar Larvae of RTW treated different concentration on HI and AEH Isolates under laboratory Conditions.

Treatment	Doses (IJ/ml)	Mean 3rd Instars larvae of DBM Mortality +SD		
SS		24hrs	48hrs	72hrs
HI	200	12+0b	24.07+5.25b	37.77+3.85c
	400	16.33+5.77b	52.2+8.1a	70.61+5a
	600	30+0a	54.81+8.98a	72.6+4.5a
AEH	200	10+0b	20.74+1.28c	34.44+5.1c
	400	22+0a	42.7+9.91a	69.41+3.01a
	600	23.7+0a	44.44+9.62a	71.52+2.57a
Control		0+0c	3.33+5.77d	3.33+5.77d
CV		8.15	16.96	6.86

Table 5: Mortality of RTW larvae treated with 2 EPN isolates at the rate of 400 IJ/ml and Karate 5% EC in pot planted teff seedlings.

Treatment	1st expt Mort. 10DAT ± SE	2nd expt Mort. 10DAT ± SE
Karate	90.81a	94.58a
HI	67.49b	77.49a
AEH	73.74b	75.99a
Untreated	0c	0b
CV	27.15	29.54
LSD	22.96	24.24
F-value	26.3	21.03
P-value	0.0001	0.0002

Discussion

Although there are few studies on the use of EPN against RTW larvae, there are several examples of their use in similar lepidopterous larvae. For instance, the findings of the current study are in agreement with Anbesse [24], who evaluating the same isolates of nematodes against the barley chafer grub, *Coptognathus curtipennis,* and reported that there were significant differences among isolates, species and concentrations of entomopathogenic nematodes. They also reported that mortality increased with increased concentration of IJ/ml with highest mortality (88.7%) observed at 1000 IJ/ml for *H. bacteriophora*. Using *S. carpocapsae* and *H. indica,* Hussaini *et al.* (2003) also reported 96 to 98% mortality of the diamond back moth larvae 72 hrs post infection indicating the high potential of EPN in the control of larval pest of crops. Differences in virulence of entomopathogenic nematode species and isolates have been reported by several authors including those reported by Ricci, Shapiro and Shapiro and McCoy [26-28].

In this study however, the percent mortality of the red teff worm larvae treated with different EPN strains of *S. yirgalemense* and *H. bacteriophora* applied at the rate of 400 IJ/ml showed both similarities and differences in virulence of the strains. The *S. yirgalemense* strain HI and the *H. Bacteriophor* strain AEH which caused highest mortality on the larvae of the RTW did not significantly differ from each other. On the other hand as shown in **Table 1**, these strains showed significant differences with other stains of the same species. This may be attributed to different environmental condition under which the experiments are conducted. Similar discrepancies have been reported by Anbesse [24] indicating that inconsistencies are not uncommon. Entomopathogenic nematodes are also known to differ in their parasitism strategies, foraging behaviours and to have specific host ranges and environmental requirements [29].

Base on the experiments of the current study, it is concluded that the two EPN strains are promising candidate biological control agents against the RTW for an eco-friendly and sustainable management of the pest. The strains can also be used as an alternative to the synthetic pesticides. Further experiments are recommended to test the efficacy of the strains under field conditions.

References

1. CSA (2017) Central statistics agency. Agricultural sample survey. Area and production of major crops of private Peasant holdings in Meher season. Addis Ababa Ethiopia. Statistical Bulletin 584.
2. HARC (1998) Holleta Agricultural Research Center, Progress Report for 1998.
3. Crowe TJ, Gebremedhin (1977) Crop pest handbook. Institute of Agricultural Research, Addis Ababa, pp: 55.
4. Tadesse E (1987) Tef (Eragostis tef) cultivars: morphology and classification part II. Experiment station Bulletin No.66. College of Agriculture, Addis Abeba University, Dire Dawa, Ethiopia pp: 73.
5. IAR (1986) Department of crop protection progress report 1984/85. IAR, Addis Ababa, Ethiopa.
6. Ainsworth GC (1956) Agostino Bassi, 1773-1856. Nature 177: 255-257.
7. Begley CG , Waggoner P (1992) Soft contact lens contamination by Beauveria bassiana. Int contact lens clinic 19: 247-251.
8. Drouhet E, Dupont B (1980) Chronic mucocutaneous candidosis and other superficial and systemic mycoses successfully treated with ketoconazole. Reviews of infectious diseases 2: 606-19.
9. Kenis M, Hurley BP, Hajek AE, Cock MJW (2017) Classical biological control of insect pests of trees: Facts and figures. Biol Invasions 19: 3401-3417.
10. Freour P, Lahourcade M, Chomy P (1966) A new mycosis: clinical and mycological study of a pulmonary localization of "Beauveria". Bulletins and memoirs of the Medical Society of Paris Hospitals 117: 197-206.
11. Freour P, Lafourcade M, Chomy P (1966) On a new pulmonary mycosis due to "Beauveria". J Medicine Bordeaux and South-West 143: 823-35.
12. Henke MO, De Hoog GS, Gross U, Zimmermann G, Kraemer D et al. (2002) Human deep tissue infection with an entomopathogenic Beauveria species. J Clin Microbiol 40: 2698-2702.
13. Gonzalez CJ, Espejo SJ, Barcena AM (1995) Mycotic pulmonary disease by Beauveria bassiana in a captive tortoise. Mycoses 38: 167-9.
14. Li Z, Alves SB, Roberts DW, Fan M, Delalbera I Jr, et al. (2010) Biological control of insects in Brazil and China: history, current programs and reasons for their successes using entomopathogenic fungi. Biocontrol Sci Technol 20: 117-136.
15. Mwaniki SW (2008) Roundworms useful as biological control agents in the central rift valley, Kenya. Research Brief Norman Borlaug LEAP.
16. Gaugler R (2002) Entomopathogenic Nematology. CABI Publishing,Wallingford, UK, pp: 388.
17. Kaya HK, Aguillera MM, Alumai A, Choo HY, de la Torre M, et al. (2006) Status of entomopathogenic nematodes and their symbiotic bacteria from selected countries or regions of the world. Biological Control 38: 134-155.
18. Boemare N (2002) Biology, taxonomy and systematics of Photorhabdus and Xenorhabdus. Entomopathogenic Nematology (Gaugler R., ed.). CABI Publishing pp: 35-56.
19. Adams BJ, Fodor A, Koppenhöfer HS, Stackebrandt E, Stock SP et al. (2006) Biodiversity and systematic of nematode-bacterium entomopathogens. Biological Control 38: 4-21.
20. Ganguly S, Somvanshi VS (2007) Efficacy of foliar applications of entomopathogenic nematodes against the crucifer diamondback moth, plutella xylostella-a review. Nematol Medit 35: 5-14.
21. Keya Hk, Stock SP (1997) Techniques in insect nematology. In: Lacey, S.A. (Ed.), Manual of Techniques in Insect Pathology. Academic Press, London, pp: 281-324.
22. Tariku T (2012) Efficacy of entomopathogenic fungi and insect growth regulators against red tef worm, Mentaxya ignicollis. Lambert Academic Publication.
23. Wang CL, Powell JE, Nguyen K (2002) Laboratory evaluations of four entomopathogenic nematodes for control of subterranean termites (Isoptera: Rhinotermitidae). Environmental Entomology 31: 381-387.
24. Anbesse S, Bekele J, ADGE, Wondirad M (2008) Laboratory screening for virulent entomopathogenic nematodes (Heterorhabditis bacteriophora and Steinernema yirgalemense) and fungi (Metarhizium anisopliae and Beauveria bassiana) and assessment of possible synergistic effects of combined use against grubs of the barley chafer Coptognathus curtipennis. Nematology 10: 701-709.
25. Abbott WS (1925) A method of computing the effectiveness of an insecticide. J Econ Entomol 18: 265-267.
26. Ricci M, Glazer I, Campbell JF, Gaugler R (1996) Comparison bioassay to measure virulence of different entomopathogenic nematodes. Biocontrol Sci Technol 6: 235-245.
27. Shapiro DI, Cate JR, Pena J, Hunsburger A, MCcoy CW (1999) Effect of temperature and hostage on suppression of Diaprepes abbreviatus (Coleoptera: Curculionidae) by entomopathogenic nematodes. J Econ Entomol 92: 1086-1092.
28. Shapiro D, MCCoy CW (2000) Susceptibility of Diaprepes abbreviatus (Coleoptera: Curculionidae) larvae to different rates of entomopathogenic nematodes in laboratory and green house. J Econ Entomol 93: 1090-1095.
29. Lewis EE, Campbell J, Griffin C, Kaya H, Peters A (2006) Behavioral ecology of entomopathogenic nematodes. Biol Control 38: 66-79.