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Parasitology

The Effectivenes of Entomopathogenic Microorganizsms (Nematodes and Fungi) Against *Curculio nucum* (Coleoptera: Curculionidae)

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The nut weevil (NW), *Curculio nucum*, is one of the main pests of hazelnuts which damages nut buds, leaves and especially fruits. Sometimes it destroys 80-90 percent of the crop. The study presents the results of using entomopathogenic microorganisms (nematodes and fungi) against NW. Local entomopathogenic nematodes (EPN) *Steinernema borjomiense*, *S. thesami*, Heterorhabditis sp. and Boverin's fungi were used against this pest. The tests were carried out in laboratory and field conditions. Nematodes of this species were first used against NW. The tests were carried out separately and together with different doses of nematode concentracions (100, 50 and 25 IJs/insect) and Boverin – (2 g/L and 4 g/L). Among the nematodes used separately against insect pests, *S. borjomiense* and Heterorhabditis sp. (in a dose of 100 IJs/insect) showed the greatest effect (100%). As for the separate application of Boverin, it turned out to be less effective (42.5% of adults and 43.2% of larvae died). On insect pests, a much greater effect was observed when using combined preparations of nematodes and fungi in laboratory and field trials. In the laboratory, after the use of combined preparations, 100% death of harmful insects is recorded. Field experiments using *S. borjomiense*+Bowerin resulted in 85.9% mortality in adults and 72.6% in larvae. When using Heterorhabditis sp.+Bowerin, adult mortality was 87.5%, larvae 80.5%. According to the results of experiments, a combination of EPNs and fungi can be used in biocontrol against *Curculio nucum*.

Biological control, hazelnuts, Curculio nucum, entomopathogenic nematodes, Steinernema, fungi, mortality

Nut weevil (NW), *Curculio nucum,* is one of the main pests of hazelnuts. It can destroy 80-90 percent of hazelnuts [1, 2]. These pests (worms and beetles) damage the buds of all varieties of hazelnuts, leaves and especially the fruit. Hazelnut production in Georgia's agriculture is one of the most important places. Hazelnut plantations are widespread in Western (Samegrelo, Guria, Adjara) and Eastern Georgia (Lagodekhi region). Hazelnuts are one of the most important exports. As of 2014, Georgia ranked third in the production and export of hazelnuts in the world; total production of 40,000 tons and 11% of world exports [3]. However, according to statistics from International Nut and Dried for 2017/2018 Fruit Council [4]: Hazelnut production decreased to 15,000 tonnes and total exports to 4% as of 2018. Therefore, it is very important to protect hazelnuts from harmful insects, especially NW. Currently chemical preparations are used against hazelnut pests. These factors highlight the need to develop and use biological control agents as an alternative to chemical insecticides. One of them is now considered as biocontrol. Entomopathogenic nematodes (EPNs) of the genera Steinernema and Heterorhabditis cause the death of harmful insects due to their pathogenicity [4, 5]. The study was carried out (in the Lagodekhi region) to assess the effectiveness of local EPN strains: *S. borjomiense* [6], *S. thesami* [7], Heterorhabditis sp. and entomopathogenic fungi (EPF) Boverin in NW control. Experiments with local nematodes against NW have not yet been carried out.

Materials and Methods

Experimental insects. Materials for experiments of imago (adult) and larvae of nut weevil (NW), were collected in 2018–2019 at the gardens with hazelnuts in the private sector of the Lagodekhi region (East Georgia); The imago forms of NW were collected into an umbrella of leaves and hazelnut branches; It was placed in special containers and transferred to a laboratory for research purposes. As for the larvae of NW, we collected them in from damaged hazelnut fruits, as well as from soil from a surface 5 cm deep under a hazelnut bush. The larvae with soft tweezers were placed in special bags and transferred to the laboratory for research purposes.

Production of EPN and EPF. Local EPN (*S. borjomiense, S. thesami* and Heterorhabditis sp.) were cultivated on larvae *Bombyx mori* (L) (Lepidoptera: Bombycidae) [8], as well as on the larvae of *Galleria mellonella* (L) (Lepidoptera: Pyralidae) [9]. Infectious juveniles collected from white traps [10] were washed with distilled water and stored at 7°C in a cold box [11]. IJs were used in experiments for two weeks after emergence from dead insects. EPF Boverin is a mycopesticide, a liquid biologic; It is produced in Georgia based on the strain of the entomopathogenic fungus *Beauveria bassiana*.

Bioassay. Laboratory experiments were conducted at the Institute of Zoology, Ilya State University (Georgia). The tests were carried out in two stages. At the first stage, local EPNs (*S. borjomiense, S. thesami,* Heterorhabditis sp.) with three different doses – 100, 50, and 25 IJs /insect were used separately against imago and larvae nut weevil. Bowerin was also used separately in two different doses – 2 gram/Liter (g/L) and 4 g/L. After establishing the effective form (at the second stage), experiments were conducted on the combined form of Heterorhabditis sp. 100 IJs/insect+Bowerin – 4 g/L and *S. borjomiense* – 100 IJs/insect+Bowerin – 2 g/L: In the experiments, Petri dish with a diameter of 10 cm were used. Filter paper was placed in 3-3 doughs and one control Petri dish. 15 insects of this species were placed in each cup and were treated with various doses of nematode suspensions. The titer of the suspension was set on Petri dishes according to the method of Veremtschuk [12]. Control Petri dishes were treated with distilled water. Experiments were checked every 24 hours. 48 hours after infection, the detected dead pest worms were cut out under a stereomicroscope. Each test variant was carried out in triplicate under similar conditions (21-23°C and relative humidity 61-74%).

Field tests of nut weevil were carried out in the hazelnut orchards of the Lagodekhi region in two stages: in May on the imago and in late June on the larva. Three trial and one control zones (each of 20 m^2) were allocated. Before testing, the number of harmful insects in the test and control zones was calculated. In the walnut forest gardens under the hazelnut trees of nut weevil (on 1 square leafy branches and in the soil), 2.5±1.33 individuals were found imago on average, and 1.6±1.00 instances of its larvae. For field trials, we prepared a suspension of *S. borjomiense* and Heterorhabditis sp. in a high dose (2000 IJs/in ml water), as well as the mushroom Bowerin in a dose of 4 g/L. In the experimental areas, hazelnut and soil were processed using a manual spray apparatus. Tests were carried out 3 options; In the first variant, only nematodes with a high concentration (2000 IJs/in ml water) *S. borjomiense* and Heterorhabditis sp. were used against adults and larvae of *C. nucum.* In the second variant, only Boverin (4 g/L), and in the third, *S. borjomiense* and Heterorhabditis sp. with Boverin in combination. The experiments were carried out in cloudy weather to avoid evaporation of the nematode suspension.The tests were carried out under conditions at a temperature of 22-26°C and 85-88% relative humidity. On the 7th day after spraying, the pests were counted on both experimental and control plots.

Statistical analysis. The final recording of the death of insects from nematodes and fungi occurred on the 7th day (after 120 hours) by the method of Abbott [13]. Accounting for mortality was conducted according to the method of Franz [14]. Each test variant was carried out in triplicate replicates per treatment conditions (21-23°C and relative humidity 61-74%). The results obtained were processed in accordance with the mathematical statistics (Sx%≤) of Dospekhov [15].

Results

Laboratory experiments against *C. nucum* were carried out in 2 stages: at the first stage against imago and larvae *C. nucum,* nematode suspension with a dose of -100 nematodes against 1 insect was the most effective (The data on mortality of imago and larvae are shown in Table 1). As for the three different types of nematodes (*S. borjomiense, S. thesami* and Heterorhabditis sp.), Used in the experiments, *S. borjomiense* and Heterorhabditis sp. showed high efficiency against a harmful insect; When these nematodes were used within 96 hours, 100% mortality of harmful insects, both adults and

Table 1. Mean percente mortality of *Curculio nucum* from different doses of local entomopathogenic nematodes and fungi at different hours after inoculation

Treatments	Mortality (%) Imago					Mortality (%) Larva				
	24h	48h	72h	96h	120h	24h	48h	72h	96h	120h
S.borjomiense -100 IJs/insect	*1.6	76.5	91.4	**100**	0	2.5	78.6	93.0	**100**	0
- 50 IJs/ins	0	37.4	62.5	68.50	72.4	0	44.3	66.7	70.8	74.0
- 25 IJs/ins	0	26.8	38.2		0	0	28.4	42.5	0	0
S. thesami -100 IJs/ins	0	60.8	77.5	91.7	95.3	0	65.4	81.8	95.6	98.5
- 50 IJs/ins	0	30.4	62.3	68.5	0	0	36.6	64.5	70.3	73.0
- 25 IJs/ins	0	22.6	36.2	38.5	0	0	23.5	39.3	0	0
Heterorhabditis sp.-100 IJs/ins	1.5	84.5	94.6	**100**	0	2.5	85.3	98.5	**100**	0
- 50 IJs/ins	0	44.7	68.3	71.6	75.0	0	46.6	70.2	73.4	76.6
- 25 IJs/ins	0	28.2	40.4	47.3	0	0	32.4	44.5	49.0	0
** *Bowerin* - 2 g/L	0	0	0	24.6	29.5	0	0	0	28.6	32.5
Bowerin - 4 g/L	0	0	2.5	33.7	42.5	0	0	0	35.5	43.2
H. sp. -100 IJs/ins+*Bow.* 4 g/L	3.5	95.8	**100**	0	0	4.8	95.6	**100**	0	0
S. b.-100 IJs/ins+*Bow*-4 g/L	2.6	93.4	**100**	0	0	3.5	94.8	**100**	0	0
Control	0	0	0	1.5	2.0	0	0	1.2	0	1.6

*Mortality percentages were corrected according to Abbot's [13] formula;
**Boverin (Made in Georgia based on strain *Beauweria bassiana*).

larvae, was achieved (Table 1). *S. thesami* caused the maximum death of insects (98.5%) after 120 hours. When using two different doses (2 g/L and 4 g/L) of Boverin against imago and larvae *C. nucum,* low mortality of harmful insects were shown. When using a low dose of Boverin, the death of the pest began only after 96 hours; After 120 hours, adults died 29.5% and larvae 32.5%; As a result of high doses – after 120 hours, 42.5% and 43.2% respectively, died. At the second stage, the experiments were carried out in combination with two variants against imago and larvae *C. nucum*: in the first variant, Bowerin (dose - 4 g/L) was used in combination with Heterorhabditis sp. (dose of 100 IJs/insect); In the second variant – Bowerin (dose- 4 g/L) + *S. borjomiense* (100 IJs/insect). In the first variant of the experiment, the combined suspension began to act within 24 hours, and after 72 hours, 100% mortality of the pest (larvae and imago) was observed (Table 1). A similar result was obtained in the second version. The number of infected nematodes in each dead insect on the third day after the test was at a minimum of 2, an average of -16.5 and a maximum of 38 instances.

Field experiments with imago and larvae of *C. nucum* were carried out in two varianta in gardens with hazelnuts. In the first variant, nematodes on day 7 demonstrated a lonely effect against a harmful insect; *S. borjomiense* in titer (2000 IJs/ml of water) caused the adult to die 76.5±1.33% and 65.5±2.66% of larvae (Table 2); Heterorhabditis sp. the same dose itself - 81.2± 1.66% of imago and larvae 68.7±0.33%. 21.7±2.33 % of the larvae and 37.8±3.0% of the adults were killed using high doses of Bowerin (dose - 4 g/L). In the second variant, combined tests of nematodes and fungi were used, in which high doses of microorganisms were used (nematode - 2000 Ijs/ml + Bowerin), *S. borjomiense* with Boverin caused adult mortality of 85.9±1.33% and 72.6±1.66% of larval *C. nucum.* Nematodes Heterorhabditis sp. in combination with Bowerin, caused the death of 87.5±2.33% of imago and 80.5±0.33% of larvae *C. nucum.*

As field tests showed, when using nematodes, high mortality pests was observed. Especially good results were obtained with the combined use of nematodes with Bowerin. A small percentage of mortality was obtained in control trials; This was taken into account when determining the results of trial tests.

Table 2. Mortality *Curculio nucum* from various doses of local entomopathogenic nematodes and fungi in the field

Treatments	Mortality (%)	
	Imago	Larvae
I variant		
S. borjomiense-2000 Ijs/ml of water	76.5±1.33	65.5±2.66
Heterorhabditis sp.-2000 IJs/ml of water	81.2± 1.66	68.7±0.33
Bowerin-4 g/L	21.7±2.33	37.8±3.0
II variant		
S. borj.-2000 IJs/ml of water+*Bow.*-4 g/L	85.9±1.33	72.6±1.66
Het. sp.-2000 IJs/ml of water+*Bow.*-4 g/L	87.5 ±2.33	80.5±0.33

Statistical processing of research results to Dospekhov [15] Sx%≤ 4.7.

პარაზიტოლოგია

ენტომოპათოგენური მიკროორგანიზმების (ნემატოდებისა და სოკოების) ეფექტურობა თხილის ცხვირგრძელას *Curculio nucum*-ის (Coleoptera: Curculionidae) წინააღმდეგ

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თხილის ცხვირგრძელა (*Curculio nucum* L.) თხილის ერთ-ერთი ძირითადი მავნებელია; აზიანებს კვირტებს, ფოთლებსა და განსაკუთრებით ნაყოფს. მას შეუძლია გაანადგუროს მოსავლის 80-90%. ნაშრომში მოცემულია თხილის ცხვირგრძელას წინააღმდეგ ენტომოპათოგენური მიკროორგანიზმების (ნემატოდებისა და სოკოების) გამოყენების შედეგები. აღნიშნული მავნებლის წინააღმდეგ გამოყენებულ იქნა ადგილობრივი ენტომოპათოგენური ნემატოდები (ეპნ) *Steinernema borjomiense, S. thesami,* Heterorhabditis sp. და სოკო ბოვერინი. ცდები ჩატარდა ლაბორატორიულ და საველე პირობებში. ეს ნემატოდები პირველად იქნა გამოყენებული თხილის ცხვირგრძელას წინააღმდეგ. ცდები ტარდებოდა ცალკე და კომბინირებული სახით ნემატოდური სუსპენზიების განსხვავებული დოზებით (100, 50 და 25 ინვაზიური ლარვა ერთი მწერის წინააღმდეგ) და ბოვერინი დოზით: 2 გრ/ლ და 4 გრ/ლ. ცდებში მავნებლის წინააღმდეგ (100 ნემატოდიანი დოზით) ცალკე გამოყენებული ნემატოდებიდან მაღალი ეფექტი აჩვენეს *S. borjomiense*-მ და Heterorhabditis sp.-მა (100%). რაც შეეხება ბოვერინის ცალკე გამოყენებას, იგი ნაკლებ ეფექტური აღმოჩნდა (დაიხოცა ცხვირგრძელას მატლების 43,2% და ზრდასრული ფორმების 42,5%). გაცილებით უკეთესი შედეგი დაფიქსირდა ნემატოდებისა და სოკოს კომბინირებული პრეპარატების გამოყენების დროს როგორც ლაბორატორიულ, ისე საველე ცდებში. ლაბორატორიულ ცდებში კომბინირებული პრეპარატების გამოყენების შედეგად დაიხოცა მავნე მწერების 100%. საველე ცდებში, სადაც გამოყენებულ იქნა *S. borjomiense*+Bowerin-ი, დაიხოცა ცხვირგრძელას ზრდასრული ფორმების 85,9% და მატლების 72,6%; Heterorhabditis sp.+Bowerin-ის პრეპარატის გამოყენების შედეგად მიღებულ იქნა ზრდასრული ფორმების 87,5% და მატლების 80,5% სიკვდილიანობა. ცდების შედეგებიდან გამომდინარე, ენტომოპათოგენური ნემატოდებისა და სოკოს კომბინირებული პრეპარატი შეიძლება გამოყენებულ იქნეს თხილის ცხვირგრძელას წინააღმდეგ ბიოკონტროლში.

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