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Parasitology

Fauna of Nematodes of Georgian Hazelnut Soils and its Entomopathogenic Forms

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Hazelnuts occupy one of the most important places in agriculture in Georgia. It accounts for 6.4% of total exports. In recent years, hazelnut crops have declined sharply due to exposure to pests. In the fight against plant pests, it is important to use environmentally friendly biological preparations that are harmless to humans. These are the natural enemies of pests – entomopathogenic nematodes (EPNs). Therefore, the aim of our study was to study nematodofauna in soils with hazelnuts in the regions of Guria, Samegrelo and Kakheti, detect and identify nematodes EPN, and select highly virulent, effective forms against hazelnut pests from identified nematodes. Nematodes identified as a result of investigation of material taken from hazelnut soils belong to 6 orders (Dorilamida, Mononchida, Areolaimida, Enoplida, Tilenchida, Rhabditida), 17 families and 25 genera. Of the 30 identified forms, 11 were identified to species. By way of life, saprobiotic, free-living, parasitic, and entomopathogenic nematodes were identified. Experiments on test insects (Galleria mellonella and Tenebrio molitor) in the laboratory showed that of the order Rhabditida: genus Steinernema sp., Parasitorhabditis sp. and Phasmarabditis sp. nematodes, are pathogenic. We used these EPNs against hazelnut pests – Halyomorpha halys, Myzocallis coryli and Lymantria dispar. According to the results of experiments, the most effective of the pathogenic forms we identified against pests was Steinernema sp., His action resulted in mortality of H. halys – 94.6% ± 2.33, M. coryli – 72.2% ±1.00 and L. dispar – 98.4% ± 2.00. *© 2020 Bull. Georg. Natl. Acad. Sci.*

Nematodofauna, larvae, entomopathogenic nematodes, Steinernema sp., Parasitorhabditis sp., Phasmarhabditis sp

Hazelnuts occupy one of the main places in the agricultural export of Georgia; It accounts for 6.4% of total exports. After Turkey and Italy, Georgia ranked third in the world for the production of hazelnuts in 2014-2015 [1]. Unfortunately, over the past two years, hazelnut yields have declined sharply. To increase hazelnut yield and fruit quality, various measures must be taken against harmful insects in orchards. Among them, it is important to seek, identify and use biological means that are safe for the environment. Entomopathogenic nematodes are one of the natural enemies of harmful insects; They are used in biocontrol against harmful insects of forest and

agricultural plants [2]. The aim of our study was to study the soil nematode fauna in orchards with hazelnuts, selected in the regions of Guria, Samegrelo and Kakheti; Selection of highly virulent, effective and active entomopathogenic forms from identified nematodes.

Materials and Methods

In 2018-2019, the nematodofauna of the soil of hazelnuts was studied in private sector gardens in the region of Guria (Anasseuli, Naruja, Chakvi, Chochkhati), Samegrelo (Akhalsopeli, Senaki, Tkviri, Shamgona) and Kakheti. The research material was taken at different depths of the soil (up to 20 cm). In each area of 50 m^2, we used a special metal drill to obtain 5-5 samples of the same weight (300 g) in each area of 50 m^2 using the Metlitsky method [3]. We examined all five of these materials in one sample. In the laboratory, we extracted entomopathogenic nematodes from the soil using the Hominic, method [4]; Free-living, proparasitic and phytoparasitic nematodes were isolated from the soil according to the Berman method [5]. In laboratory studies in material taken from hazelnut soil, we used *Galleria mellonela* larvae as bait for nematodes. Each container (5 containers in total) contained 250 grams of soil and 15 larva *G. mellonella*; We placed the containers in a thermostat at a temperature of 24°C; After 3-5 days, the worms killed in the container were transferred to the White bait [6] to obtain invasive nematode larvae. For identification (before fixation), we killed the separated nematodes in a hot water bath at 60ºC. We placed dead nematodes according to the Poinar method in the fixator TAF [7]. We prepared temporary and permanent preparations with nematodes removed from the fixative; Measurements of nematodes were carried out using a light microscope – Motic-DMB1 with 10x, 20x, 40x and 100-fold magnification according to the De Mann formula [8]. We used both local [9] and foreign identifiers [10] to identify nematodes.

Permanent preparations of the studied nematodes of holotypes and paratypes were placed in the collectible Fund of the Institute of Zoology of Ili State University of Georgia.

The pathogenicity of the separated nematodes was tested on test insects, *G. mellonella* and *Tenebrio molitor* larvae. Laboratory tests were carried out at 20-21º temperatures and 65-67% relative humidity. The tests were carried out in parallel with both insects: we placed 10–10 worms of different ages on the target insects in 10x10x10 containers with preloaded filter paper.

In the tests, suspensions were used in different doses – 200, 100 and 50 nematodes against 1 insect. The tests were repeated 3-3 times. Experimental nematodes were cultured according to the Datka method [11]. Mortality Insect was recorded on days 3, 5, and 7 according to the Abbott method [12]. The pathogenic nematodes (Steinernema sp., Phasmarabditis sp. and Parasitorhabditis sp.) Identified in the tests, we used against harmful hazelnut insects: *Halyomorpha halys*, *Myzocallis coryli* and *Lymantria dispar*. To compare pathogenicity, trials with *Steinernema thesami* and *Steinernema borjomiense* were also conducted. The tests used equally high doses (200 nematodes versus 1 insect). The obtained data were processed by the method of mathematical statistics [13].

Results

The study revealed 30 forms of nematodes in the hazelnut soils of the regions of Guria, Samegrelo and Kakheti (Table 1). Of these, 11 were identified to species. Registered nematodes belong to 6 orders, 17 families and 25 genera. The study showed that the hazelnut soils of the three regions studied by us were characterized by almost the same variety of nematode fauna (20 forms of nematodes were identified in Guria, 23 in Samegrelo and 21 in Kakheti). Of the registered nematodes, the most diverse are the order Dorilaimida (including 6 families and 9 genera (Table 1).

Table 1. Taxonomic groups of nematodes isolated from hazelnut soils; nematode detection site

Order	Family	Genus	Species	Region		
				Guria	Samegrelo	Kakheti
Dorilaimida	Dorilaimidae	Dorilaimus	*D. carteri*	+	+	+
		Mesodorilaimus	*M. bastiani*	+	+	-
			Mesodorilaimus sp.	+	+	+
		Sicadutur	Sicadutur sp.	-	-	+
		Thornenema	Thornenema sp.	+	+	-
	Qudsianematidae	Eudorilaimus	*E. acutus*	+	+	+
			E. meridionals	-	+	-
			Eudorilaimus sp.	+	+	+
	Aporcelaimidae	Aporcelaimus	Aporcelaimus sp.	+	+	+
	Nygolaimidae	Nugolaimus	*N. paravulvus*	+	-	-
	Tylencholaimide	Tilencholaimus	Tilencholaimus sp.	+	+	-
	Belondoridae	Belondira	*B. aptica*	+	-	+
Mononchia	Mylonchulidae	Mylonchulus	Mylonchulus sp.	+	+	+
	Iotonchidae	Iotonchus	Iotonchus sp.	-	+	-
	Mononchidae	Mononchus	Mononchus sp.	+	+	+
		Prionchulus	*P. punctatus*	+	+	-
Areolaimida	Plectidae	Plectus	Plectus sp.	+	+	+
Enoplida	Tripylidae	Tripula	Tripula sp.	-	-	+
Tylenchida	Hoplolaimidae	Heliocotylenchus	Heliocotylenchus sp.	+	-	+
	Heteroderidae	Meloidogyne	*M. hapla*	+	+	+
			M. arenaria	-	+	+
			M. incognita	-	+	+
	Criconematidae	Pratylenchus	Pratylenchus sp.	+	+	+
		Criconema	Criconema sp.	-	-	+
Rhabditida	Rhabditidae	Rhabditis	*R. strongiloides*	+	+	-
		Parasitorhabditis	**Parasitorhabditis sp.**	+	+	+
		Mesorhabditis	Mesorhabditis sp.	-	-	+
		Phasmarhabditis	**Phasmarabditis sp.**	+	+	-
	Steinernematidae	Steinernema	**Steinernema sp.**	-	+	+
	Cephalobidae	Cephalobus	Cephalobus sp.	-	-	+

The second in this respect is Tylenchida (contains 3 families and 4 genera), followed by Rhabditida (represented by 3 families and 6 genera) and Mononchida (including 3 families and 5 genera). Nematodes Areolaimida and Enoplida differ in a small number of nematode forms. Both are represented by the same family and one surname. Representatives of the genus Parasitorhabditis, Phasmarhabditis, and Steinernema from the Rhabditidae family, identified in the study area, are entomopathogenic nematodes (EPNs).

To study the pathogenicity of the latter, laboratory studies were first conducted on *Galleria mellonella* and *Tenebrio molitor,* then on harmful hazelnut insects. In opitis was used Steinernema sp., Parasitorhabditis sp. and Phasmarhabditis sp. – Three different doses: 200, 100 and 50 nematodes against 1 insect. To determine the degree of effectiveness of nematodes, the same doses were used on test insects with local EPN (*Steinernema thesami* and *Steinernema borjomiense*) as a standard. As a result of the high concentration of Steinernema sp. (200 nematodes per 1 insect) killed 97.2%±3 *G. mellonella* (Fig. 1,2) and 95.8%±4 *T. molitor*. At the same dose of *S. thesami*, we got 98.1%±2 mortality of *G. mellonella* and 94.6%±2 *T. molitor*. The same high results were observed when using *S. borjomiense*, killing 99.0%±2 and 98.7%±1, respectively. Based on test results, the effectiveness of Steinernema sp. on insects tested is as high as *S. thesami* and *S. borjomiense.*

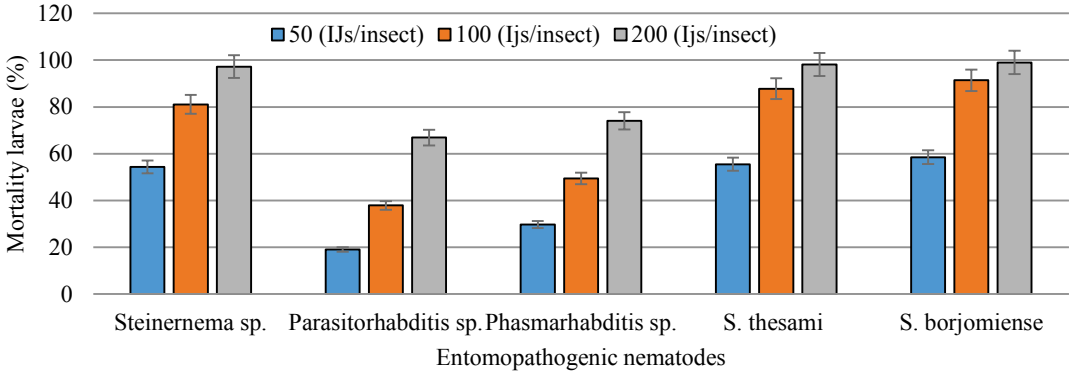

Fig. 1. The Mean percentage infection of different concentrations (50, 100 and 200 IJs/insect) of nematodes found in hazelnut soils on *Galleria mellonella.*

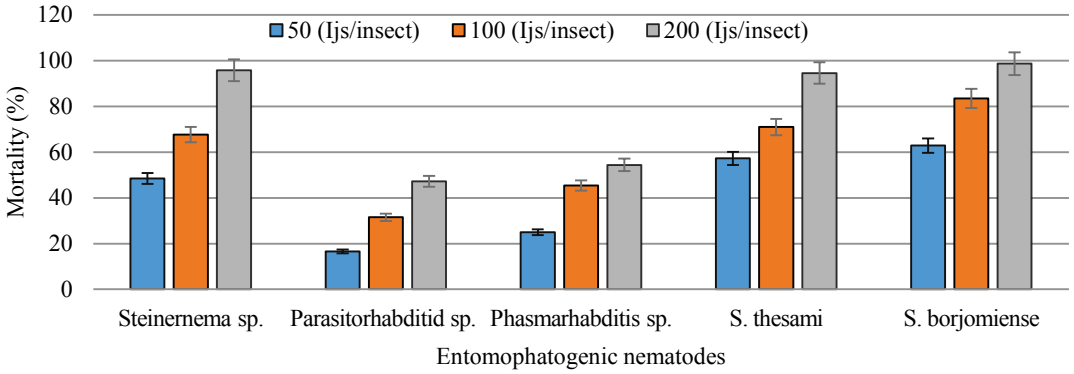

Fig. 2. The Mean percentage infection of different concentrations (50, 100 and 200 IJs/insect) of nematodes found in hazelnut soils on *Tenebrio molitor.*

Regarding the degree of pathogenicity of Parasitorhabditis sp. and Phasmarabditis sp., In opits carried out at the same high doses (200 nematodes against 1 insect) by the action of Parasitorhabditis sp. 66.9%±4 of *G. mellonela* and 47.2%±4 of *T. molitor* were killed; When using Phasmarabditis sp. – 74.1%±3 and 54.4%±5 respectively. Was found, of the three forms of nematodes (Steinernema sp., Parasitorhabditis sp. and Phasmarabditis sp.) used in opites, Steinernema sp. it has a high pathogenicity on tested insects. Parasitorhabditis sp. and Phasmarabditis sp. Compared to Steinernema sp. gives a lower mortality rate.

After carrying out tests on test insects (*G. Me-llonella, T. molitor*), pathogenicity of Steinernema sp., Phasmarabditis sp. and Parasitorhabditis were tested for hazelnut pests: *H. halys*, *M. coryli* and *L. dispar*. To compare pathogenicity, trials with *S. thesami* and *S. borjomiense* were also conducted. The tests used equally high doses (200 nematodes versus 1 insect). When using Steinernema sp. 94.6%±2.33 of *H. halys* were killed (Fig. 3), *M. coryli* 72.0%±1.00 and *L. dispar* 98.4%±2.00; As a result of the action of Phasmarabditis sp. were killed, respectively – 25.9%±1.00, 49.5%±2.33 and 66.2%±1.33, while using Parasitorhabditis sp. we received mortality, respectively: 16.5%±0.66, 39.7%±1.33 and 60.4%±0.66.

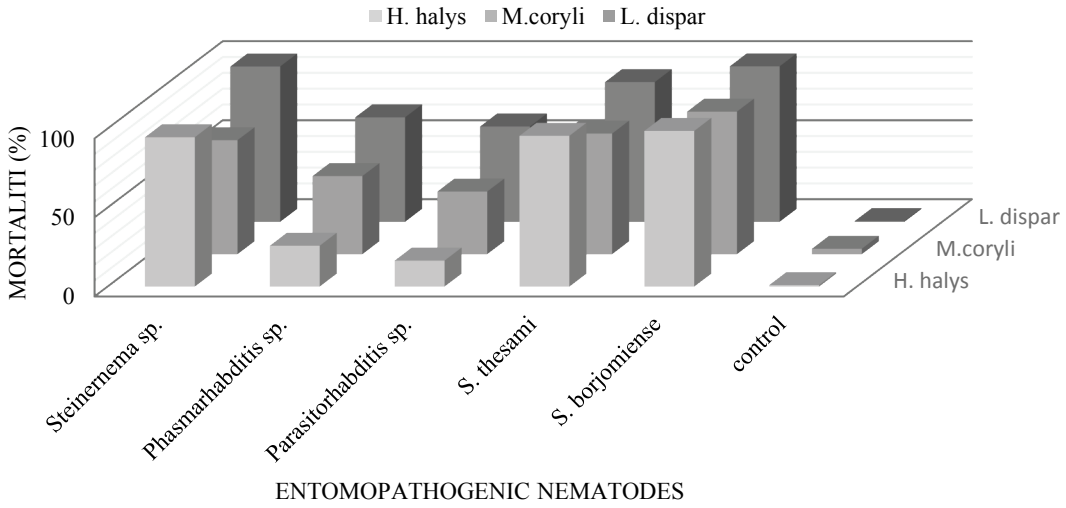

Fig. 3. Efficacy of detected nematodes using 200 EPN (IJs / Insect) concentrations against harmful insects hazelnut.

Using *S. thesami*, the mortality rate of harmful insects was 95.5%±2.00, 76.4%±1.00, 88.5%±1.00. Under the action of *S. borjomiense*, respectively – 98.6%±2.33, 90.3%±2.00 and 98.5%±0.66 died. As in the case of experiments conducted on test insects Steinernema sp. also showed high pathogenicity against hazelnut insects. It is as effective against harmful hazelnut insects as the local entomopathogenic species – *S. thesami* and *S. borjomiense*.

Regarding Phasmarhabditis sp. and Parasitorhabditis sp. they showed lower efficiency compared to other pathogenic nematodes used in opitis against hazelnut pests. Using Phasmarhabditis sp. as a result 25.9%±1.00 of *H. halys*, 49.5%±2.33 of *M. coryli* and 66.2%±1.33 of *L. dispar* died. Under the action of Phasmarhabditis sp. 16.5%±0.66, 39.7%±1.33 and 60.4%±0.66 mortality were recorded. Based on the results, nematodes Steinernema sp. can be used in biocontrol against hazelnut pests.

Conclusion. The nematode fauna of hazel soils of the regions of Guria, Samegrelo and Kakheti was studied. During the study, free-living, parasitic, and entomopathogenic nematodes of the orders Dorilamida, Mononchida, Areolaimida, Enoplida, Tilenchida, Rhabditida were identified. Among them in Rhabditida were identified, entomopathogenic forms: Steinernema sp., Parasitorhabditis sp. and Phasmarabditis sp. Their pathogenicity was studied for *H. halys*, *M. coryli* and *L. dispar*. The highest pathogenicity is characterized by nematodes of the genus Steinernema sp.

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პარაზიტოლოგია

საქართველოს თხილნარის ნიადაგების ნემატოდოფაუნა და მისი ენტომოპათოგენური ფორმები

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**სოფლის მეურნეობის სამეცნიერო კვლევითი ცენტრი, თბილისი, საქართველო

(წარმოდგენილია აკადემიის წევრის ი. ელიავას მიერ)

ნაშრომში შესწავლილია გურიის, სამეგრელოსა და კახეთის თხილნარის ნიადაგების ნემატოდოფაუნა. დადგენილია გამოვლენილი ნემატოდების ტაქსონომიური ჯგუფები. საკვლევ ტერიტორიაზე რეგისტრირებული ნემატოდები განეკუთვნება 6 რიგს (Dorilaimida, Mononchida, Areolaimida, Enoplida, Tylenchida, Rhabditida), 17 ოჯახსა და 25 გვარს. გამოვლენილი 30 ფორმიდან სახეობამდე გარკვეულია 11. ფორმათა მრავალფეროვნებით გამოირჩევა რიგი Dorilaimida, რომელიც აერთიანებს 9 გვარს. ლაბორატორიული კვლევის შედეგად დადგინდა, რომ Rhabditida-ს რიგის წარმომადგენლებიდან გვარ Steinernema sp., Parasitorhabditis sp. და Phasmarabditis sp.-ის ნემატოდები პათოგენურობით ხასიათდება. ცდებში გამოყენებული ამ სამი ფორმიდან Steinernema sp.-მა ტესტ-მწერების მიმართ საკმაოდ მაღალი პათოგენურობა გამოავლინა: დაიხოცა *Galleria mellonella*-ს 97,2%±3, *Tenebrio molitor*-ის 95.8%±4; შედარებით დაბალი პროცენტი იქნა მიღებული Parasitorhabditis sp.-სა (66,9%±4, 47,2%±4) და Phasmarabditis sp.-ის გამოყენების დროს (74,1%±3, 54,4%±5). გამოვლენილი ენტომოპათოგენური ნემატოდებით ექსპერიმენტები ჩატარდა ასევე თხილის მავნე მწერებზე – აზიურ ფაროსანაზე (*Halyomorpha halys*), თხილის ბუგრსა (*Myzocallis coryli*) და არაფარდი პარკხვევიაზე (*Lymantria dispar*). შედეგებმა აჩვენა, რომ ცდებში გამოყენებული ზემოთ აღნიშნული სამი ნემატოდური ფორმიდან მავნე მწერების მიმართ ყველაზე მაღალი პათოგენურობით Steinernema sp. ხასიათდება (Steinernema sp. შესაბამისად: 94,6%±2,33, 98,4%±2,00, 72,2%±1,00; Parasitorhabditis sp. – 16,5%±0,66, 39,7%±1,33, 60,4%±0,66; Phasmarabditis sp. – 25,9%±1,00, 49,5%±2,33, 66,2%±1,33).

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