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Zoology and Nematology

Isolation of Entomopathogenic Nematodes from the Soil

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ABSTRACT. Two approaches are used for isolation of insect parasitic nematodes from their natural environment: 1) from soil by a physical separation technique, 2) from insects naturally infected in the field; they are most commonly recovered from soil by baiting with susceptible insects. The wax moth, *Galleria mellonella (L.),* larvae are most commonly used as a bait. A number of infective stage juveniles (IJ) were isolated from the soil by insect bait, *G. mellonella* and cylinder biological trap. *© 2010 Bull. Georg. Natl. Acad. Sci.*

***Key words**: entomopathogenic nematodes, larvae, biological trap, bait, soil.*

Insect- pathogenic (entomopathogenic) nematodes (EPNs) occur naturally in almost all soils and reproduce only in insect hosts which they have killed. Entomopathogenic nematodes have good potential as a biological control agent of insects, especially in the environment. They infect many different types of soil insects, including the larval forms of butterflies, moths, beetles [1]. EPNs of the genera *Steinernema* and *Heterorhabditis* (*Nematoda: Rhabditida*) are ubiquitous in distribution and have been recovered from soils throughout the world [2] .

Thirty *Galleria* baits were laid in three plots at the bordering forest zone in a humid cool area of the town of Tbilisi: the National park (NP), the Vake park (VP), and the territory of the Turtle lake (TL), in June and July 2009, which were located at a distance about 4 km. The discovery of strain was performed by Berman method: using 3-5 late instar dismember *G. mellonella*. For the first series *Galleria mellonella* bait (GM) were laid at different depths of the soil layer. The depth of the first layers was about 0-1 cm, the depth of the second layers was 1-3 cm, and the third layers was about 5-10 cm. After two days we checked all the baits. Further work was held in laboratory conditions. *Galleria* baits were divided into two batches. One was dissected to obtain adult nematodes of the second generation, while the second batch was used for culturing on a water trap to obtain infective juveniles, for which fresh *Galleria* larvae were used. In the second series further work goes on in accordance with the unified method – after 48 hours dead *G. mellonella* larvae situated on Petri dishes which (13.5 cm diameter, 1.7 cm depth) were turned upside down and laid by filtered paper. Then 30 Petri dishes are placed on water traps to obtain IJs, all water traps were numbered. Every day for two weeks samples were taken from the bottom of the water trap, where new IJs get from their hosts and accumulated of nematode suspension was examined and checked up under the microscope. At the end of the experiment all IJs were moving actively in the liquid and number was determined. Species identification was mostly done at light microscopical magnification using morphological characters of the infective-stage juveniles. The nematode suspension was poured into the retorts and kept in the refrigerator at the temperature $+4^0$-5^0C.

In the given paper we also offer a new convenient method, which can be applied for catching nematodes in the soil during the whole year. For this purpose we

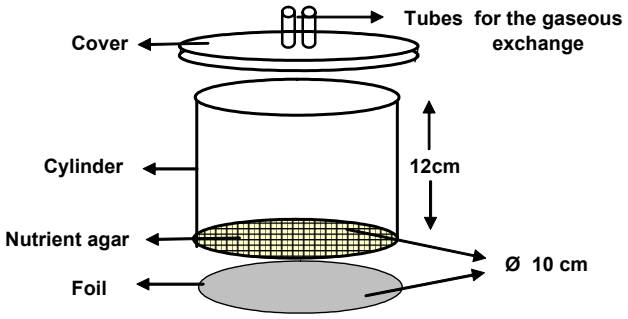

Fig. Special CBT for catching nematodes in the soil.

constructed a special cylinder - biological trap (CBT), which consisted of an aluminum sieve (diameter 10 cm, height 12 cm) covered with a lid containing two tubes to facilitate gaseous exchange (Fig.).

The nutrient agar (3 g beef extract, 5 g peptone, 8 g NaCl, 1 g agar) 2-3 mm was poured on the surface of the trap and 7-8 larvae of *G. mellonella,* 3-5 late instars were placed on the surface. To keep the broth on the trap, the lower side of the sieve should be covered with foil, when nutrient agar becomes solid; foil is discarded from the surface to open a way for nematodes to get into the cylinder. Naturally 15 prepared cylinders are placed on the soil in a zigzag line 10-15 m apart from one another. The depth of the soil was 1-2 cm. About 4-5 days, the infected larvae and pieces of broth are removed from the cylinder and examined under the microscope.

The results of discovery of *Steinernema* infective-stage juveniles by the *Galleria mellonella* baiting (GM)

Table

GM baiting technique and CBT for discovery of *Steinernema* infective stage juveniles

Plot	Samp. No	*Steinernema gurgistana*		*Steinernema feltiae*		*Steinernema* sp.	
		GM	CBT	GM	CBT	GM	CBT
NP I	1	+	+	+	-	-	-
	2	-	+	-	+	-	+
	3	+	+	-	+	+	-
	4	-	-	+	-	-	-
	5	+	-	-	-	-	-
	6	-		+		-	
	7	+		-		-	
	8	-		-		-	
	9	-		-		-	
	10	-		-		-	
VP II	1	-	+	-	-	-	-
	2	+	-	-	+	-	-
	3	-	+	+	-	-	+
	4	+	+	-	-	-	-
	5	+	-	-	-	-	-
	6	-		+		+	
	7	+		-		-	
	8	-		-		-	
	9	-		-		-	
	10	-		-		-	
TL III	1	-	-	-	-	-	-
	2	-	+	-	+	-	-
	3	+	-	-	-	-	-
	4	-	+	+	+	-	-
	5	+	-	-	-	+	-
	6	-		-		-	
	7	+		+		-	
	8	-		-		-	
	9	-		-		-	
	10	-		-		-	

technique and cylinder - biological trap (CBT) are presented in Table.

In June, when the average temperature of air was 22.5^{0}C and air humidity equaled 72.5%, the largest number of nematodes was revealed in the first layer of the soil- 19. 5 per larva. In bait of the second layer the number of nematodes was approximately- 5.5 per larva. Nematodes were not revealed at all in the third layer. But in July, when average temperature was 25.5^{0}C and air humidity equaled ≥66.5%, the position of nematodes in the soil changed. Number of nematodes became larger in the second layer – 15.6, than in the first layer – 6.8. Nematodes were not revealed at all in the third layer of soil. Three *Steinernema* species: *S. feltiae,* local entomopathogenic nematodes - *S. gurgistana* [3] and *Steinernema* sp., which at present are in the process of studying, were revealed all three plots: the National park, the Vake park and the Turtle Lake.

Total number of GM baits was 30. Among 30 larvae 21 were positive for steinernematids. Local EPNs - *S. gurgistana* were found in 11 baits, *S. feltiae* in 7, but *Steinernema* sp. was rarely or not at all found. The number of local EPNs *S. gurgistana* was more common and higher than *S. feltiae,* and *Steinernema* sp. Though most GM baits were invaded in the National park, *Steinernema* sp. were fewer from the revealed IJs. The maximum number of *Steinernema* specimens isolated from one larva of *G. mellonella* was 400.

Among 15 CBT 12 contained EPNs. Local nematodes *S. gurgistana* were found in 8 traps, *S. feltiae* in 5 traps and *Steinernema* sp. only in two cases. Nematodes were not revealed at all in the third traps from Turtle Lake. It is explained by the climate changes. The experiment was carried out in natural conditions in July and the temperature increases from 24-25^{0}C to 34^{0}C. The place chosen was in comparatively warm and dry environment, which became important risk factor for nematodes. The number of infected *G. mellonella* larvae in the traps varied: the percentage of infected larvae may depend on the number of infective larvae in the soil, but not all infective stage juveniles present in a soil sample can invade the bait and raise infections at any time [4-5]. All three species were recovered with both methods, but the GM bait was less effective than CBT, which provided a large amount of nematodes. From the results obtained in the present study it is evident that CBT preference is: first, has a high volume, second, it is possible to use both in natural and laboratory conditions in winter months. Taken in nature soil samples are transferred and distributed on the cuvette, so as the depth of the soil sample is 3-4 cm. The cylinder is placed on the soil and as the temperature in laboratory conditions is desirable, nematodes become active and get into the trap. 24-48 hr exposure must be enough for nematodes to get into insect larvae or broth. The method makes possible the discovery of EPNs all the year round.

ზოოლოგია-ნემატოლოგია

ენტომოპათოგენური ნემატოდების მოპოვება ნიადაგიდან

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(წარმოდგენილია აკადემიის წევრის ი. ელიავას მიერ)

ნაშრომში განხილულია ნიადაგში მობინადრე მწერების პათოგენური ნემატოდების მოპოვების ორი მეთოდი. პირველი მეთოდის თანახმად, ნიადაგიდან ნემატოდების მოსაპოვებლად ბიოლოგიური საჭერის სახით გამოიყენება მწერი *Galleria mellonella (L.)*. საჭერები ნიადაგში თავსდება სხვადასხვა სიღრმეზე (1-

10 სმ) ზიგზაგისებურად. ნემატოდები, რომლებიც იკვებებიან ცოცხალი ორგანიზმებით, იოლად პოულობენ საჭერებს და იჭრებიან მწერის სხეულში. მეორე მეთოდი შედარებით გაუმჯობესებულია და წარმოადგენს ხელსაწყოს – ბიოლოგიურ ცილინდრს (CBT), რომლის ფსკერზე ისხმება აგარი ნემატოდების მოსაზიდად. ბუნებაში ორივე ბიოლოგიური საჭერის გამოყენების შედეგად გამოვლენილია შტეინერნემა გვარის სახეობები **S. *feltiae*, S. *gurgistana*** და ***Steinernema* sp.** და სხვ. აღმოჩნდა, რომ ნემატოდების რაოდენობა ჭარბობდა ბიოლოგიურ ცილინდრში, რაც აიხსნება ცილინდრის დახვეწილი კონსტრუქციითა და მოცულობით. ბიოლოგიური ცილინდრის უპირატესობა ასევე მდგომარეობს იმაში, რომ მისი გამოყენება შესაძლებელია მთელი წლის განმავლობაში, ლაბორატორიულ და საველე პირობებში.

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