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Zoology-Nematology

Effectiveness of Entomopathogenic Nematodes (*Steinernema carpocapsae)* against the *Melolontha hippocastani (Coleoptera: Scarabaeidae)*

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ABSTRACT. The results of application of entomopathogenic nematodes against the pest May beetle (chafer) (*Melolontha hippocastani* Coleoptera: Scarabaeidae), obtained in the laboratory conditions, are presented in the paper. It was established that the concentration of the nematode suspension to be used should be not less than 1000 nematodes/1 ml water. *© 2011 Bull. Georg. Natl. Acad. Sci.*

Kew words: *entomopathogenic nematodes, Melolontha hippocastani - chestnut cockchafer, Infective juveniles (IJs), symbiotic bacteria, septicemia.*

Entomopathogenic nematodes (EPNs) *Steinernematidae* refer to the large group of animals that are in the process of biological progress. The biological connections of insects and pathogenic nematodes are various. For parasite nematodes the insect is the host of nematodes and they feed on its tissues. At the same time tissues of animal's organs are used as inhabitation. There nonfeeding infective juveniles (IJs) locate and invade suitable host [1] insects through natural body openings (i.e., anus, mouth and spiracles). Once inside the host, nematodes invade the haemolymph and release a lethal bacterium of the genus *Xenorhabdus* which is held in the nematodes intestine. The bacteria cause septicemia and rapid death of the host. EPNs can parasitize and kill a wide variety of insects. Death of insects sprayed with nematode suspension is directly dependent on the virulence of symbiotic bacteria [2].

In 2009-2010 we studied the pathogenic effect of nematode species *Steinernema carpocapsae* on the worm of *M. hippocastani*. May beetle (cockchafer) is widely spread in Georgia. It is one of the main pests of fruit and forest plants. Bugs do harm, eating leaves on the trees. Worms do more harm eating roots of young plants. Young plants die and the older ones delay in growth.

Material and Method. Nematodes were cultured in *Galleria mellonella* L. larvae at 25^{0}C following the methods described by Dutky, Thompson & Cantwell 1964 [3]. Infective juveniles were used between 2 and 3 weeks after their emergence from host cadavers and washed 3 times in sterile distilled water. During the interim period, the infective juveniles were held in water in Petri dishes at room temperature.

For laboratory investigations worms of cockhafer were collected from the soil of private estates in the village of Tskhneti in the second half of May, beginning of June. Experiments were carried out under laboratory conditions at temperature 22-24°C according to Abbott [4].

Preliminarily in 10 cuvettes, 25x30 cm in size with soil (1-2 cm) the seeds of wheet were sown. When the shoots appeared 45 worms of May beetle III-IV of age, in control – 15, were placed into each cuvette. Tests were carried out in four variants, out of them three - experimental (for

Table

Results of invasion of *Melolontha hippocastani* by nematode species *Steinernema carpocapsae* under laboratory conditions at 22-24°C

Variant number	Concentration of nematode suspension in 1 ml water	Number of alive larvae in cuvettes	Quantity of dead larvae in cuvettes					Dead larvae (according to the Abbott method)
			4th day	6th day	8th day	10th day	Total	
1	500	45	2	11	12	2	27	60%
2	1000	45	5	17	18	3	43	96%
3	1500	45	6	19	20	-	45	100%
Control	Water	15	-	-	-	-	1	6.6%

each variant 3 replicates) and 1 control. In the first experimental variant insects were sprayed with 15 ml of nematode suspension *S. carpocapsae*, containing 500 nematodes in 1 ml of water, in the second variant 1000 nematodes per 1 ml of water (500, 1000-1500 IJ/ml). In the control the insects were sprayed with water from water-supply.

To evaluate the character of the effect of *Steinernema carpocapsae* on the organism of the insect the microscopic changes of the worm of May beetle, infected by the above pathogenic nematodes, were studied.

Result. After invasions the insects did not die during 1-3 days; however their low activity was observed. On the 4th day 2 insects were found dead in the 1st variant of experimental cuvettes, 5 dead insects were found in the 2nd variant and 6 dead May beetle worms in the 3rd variant. All the rest were weak.

Dissection of the worms showed that several pubescent animals of the indicated species were localizated in their body fat.

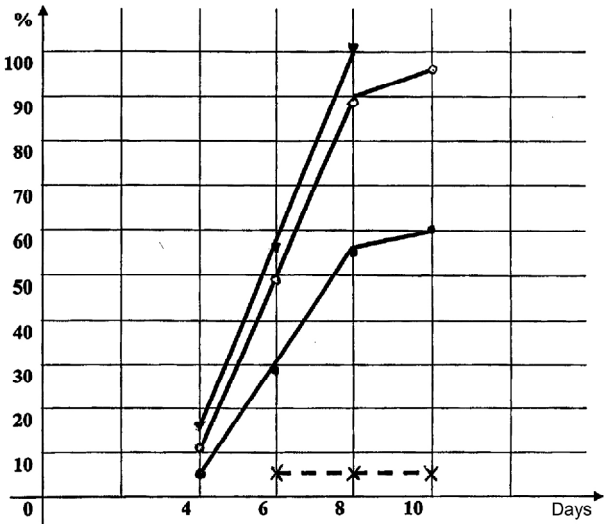

Fig. Intensity of *Melolontha hippocastani* depending on nematode concentration
●–●--●-- 500 IJ/ml water, ○-- ○-- ○-- 1000 IJ/ml water, ◄--◄--◄-- 1500 IJ/ml water, *-*-*-- control- water.

Preparation of the worms of May beetle on the 6th day showed that fatty tissue became yellow, granulated. Haemolymph quantity was a little bit decreasd. In the first variant 11 worms of May beetle were found dead and decomposed, in the 2nd variant 17 and in the 3rd – 19. All the rest were on the verge of death, depressed and low-acting.

On the 8th day microscopic investigations showed that body cavity of the May beetle was filled with *Steinernema carpocapsae* larvae at different stages of their development. In the 1st variant 12, in the 2nd – 18, in the 3rd variant 20 were found dead. Fatty body became pappy. Despite the high level of invasion in the 2nd variant 5 insects did not die. They became less motile, did not eat. Pathogenic effect of nematodes on insects was conditioned mainly by the results of symbiotic bacteria of the genus *Xenorhabdus* action.

On the 10th day a revision of experimental cuvette was carried out (Table). In the 1st variant 2 worms were found dead (totally 27), in the 2nd variant – 3 (totally 43). After dissection of the body of the insect different stages of the larvae development of nematodes *Steinernema carpocapsae* were detected.

In control, the wheat root system appeared to be damaged while in the experiment it was slightly touched. After location of insects into the control cuvette the wheat root system was almost all damaged. Out of the 15 worms of May beetle in control only 1 was dead (Table).

It was shown (Table, Fig.) that in the 2nd variant when the nematode suspension contained 1000 and 1500 nem/ml, the percentage of death of the May beetle worms was almost the same (96-100%). In the 1st variant, when the nematodes suspension was lower – 500 nem/ml, the death level of the worms made up 60%.

The advantage of suspension with concentration 1000 nem/ml was evident. High effectivness of the species *Steinernema carpocapsae* in its application against pests was also established.

ზოოლოგია - ნემატოლოგია

ენტომოპათოგენური ნემატოდის *Steinernema carpocapsae*-ს ეფექტურობა მაისის ღრაჭას *Melolontha hippocastani* (Coleoptera: Scarabaeidae) წინააღმდეგ

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ნაშრომში მოცემულია მავნე მწერის მაისის ღრაჭას (*Melolontha hippocastani* Coleoptera: Scarabaeidae) წინააღმდეგ ენტომოპათოგენური ნემატოდის *Steinernema carpocapsae*-ს გამოყენების შედეგები ლაბორატორიულ პირობებში. ცდებში მავნებლის მატლების წინააღმდეგ გამოყენებული იყო სამი განსხვავებული კონცენტრაციის (500, 1000 და 1500 ნემატოდა 1 მლ წყალში) ნემატოდური სუსპენზია. დავადგინეთ, რომ მეორე და მესამე ვარიანტში, სადაც გამოყენებული იყო მაღალი ნემატოდური სუსპენზიის კონცენტრაცია (1000 და 1500 ნემ. 1 მლ წყალში), დაფიქსირდა მაისის ღრაჭას თითქმის ერთნაირი სიკვდილიანობის პროცენტი 96-100%, ხოლო I ვარიანტში ნემატოდების კონცენტრაცია გაცილებით დაბალი იყო (500 ნემატოდა 1 მლ წყალში) და შესაბამისად მაისის ღრაჭას მატლების სიკვდილიანობამ დაბალი მაჩვენებელი 60% შეადგინა. აქედან გამომდინარე, ლაბორატორიულ პირობებში გვარი *Steinernema*-ს პათოგენური ნემატოდების მაისის ღრაჭას წინააღმდეგ გამოყენების დროს მოქმედი სუსპენზიის კონცენტრაცია უნდა იყოს არანაკლებ 1000 ნემ. 1 მლ წყალში.

მწერის სიკვდილიანობის პროცენტული მაჩვენებლის თანახმად დავადგინეთ, რომ სახეობა *Steinernema carpocapsae* გამოირჩევა მაღალი ეფექტურობით და მისი გამოყენება აღნიშნული მავნე მწერის წინააღმდეგ საკმაოდ გამართლებულია.

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