

PARASITOLOGY AND HELMINTHOLOGY

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**Efficacy of the Nematodes *Steinernema disparsica* (Steunernematidae) and
Heterorhabditis bacteriophora (Heterorhabditae) Against Worms of *Culex pipiens*
pipiens
in Laboratory Conditions**

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ABSTRACT. Entomopathogenic nematodes *Steinernema disparsica* and *Heterorhabditis bacteriophora* have been tested against worms of mosquito *Culex pipiens pipiens* in water reservoirs of different depths (6, 20, and 60 mm). The 300 nematode samples were used against each mosquito worm. The maximum mortality rate of mosquito worms was achieved at low depths of water (from 6 to 20 mm), 100-52.3 % respectively. The nematodes turned out to be less effective in deeper reservoirs water. As a result of invasion at average 3.5 specimens of immature nematodes were found in each worm of the mosquito. During the experiments water temperature was 20 °C. Efficacy of nematodes in water reservoirs turned out to be dependent on direct contact with mosquito worms, quantity of nematodes and abiotic factors.

Key words: entomopathogenic nematodes, *Steinernematidae*, *Heterorhabditae*, invasion, ectoparasite, polyphage, chemical method of control, biological method of control, *Galleria mellonella*, blood sucking insect, *Culex pipiens pipiens*.

Of non malaria mosquitoes in Georgia the genus *Culex*, comprising two forms *Cx. pipiens pipiens* and *Cx. pipiens molestus*, is especially distinguished as a numerous one. Both species are active blood-sucking thermophilic ectoparasites, polyphages. The species cause grate damage to human health both as ectoparasites and carriers of different pathogenic microorganisms. It has been scientifically proved, that application of chemical method of control against blood-sucking insects threatens human health and the environment. At present it is extremely urgent search for ecologically safe methods of control of blood-sucking arthropods and their implementation in medical practice.

Of insect parasites the nematodes are distinguished by great pathogenicity, which makes them the most prospective agents of biological control.

Entomopathogenic nematodes of *Steinernema* and *Heterohabditis* genera are widely used against parasitic insects [1,2]. Effect of the nematodes against pests of agriculture is well studied [3]. Comparatively less numerous are the data concerning the nematodes application against insects of medical importance [4].

The aim of our research was to test the efficacy of *S. disparsica* and *H. bacteriophora* against worms of mosquito *Cx.p. pipiens* in water reservoirs of different depths.

Material and methods. Mosquito worms for the experiments were obtained from micro reservoirs of 50-70 cm depth located on the right riverside of the riv. Mtkvari in Tbilisi. surroundings The worms were gathered using a special net. The collected material was identified till the species. More than 1500 samples of II- IV instars worms of *Cx.p. pipiens* were used in tests. The nematodes *S. disparsica* and *H. bacteriophora* were cultivated on larvae of honeycomb moth *Galleria mellonella* according to methods elaborated by Datky [5] and White [6]. Invasion of mosquitoes by nematodes was performed by researchers of the Laboratory of Entomonematology of the Institute of Zoology (Georgian Academy of Sciences) and those working at the Department of Transmission and Medical Entomology of the S. Virsaladze Scientific-Research Institute of Medical Parasitology and Tropical Medicine. Air temperature during experiments 24-26°C and relative humidity fluctuated between 81-83%. At invasion of worms water temperature in vessels was 20°C. According to the technique by Veremchuk [7] in 1 ml of water suspension of nematodes was determined quantity of nematodes *S. disparsica* and *H. bacteriophora*. Nematode suspensions of both low (1:100) and high (1:300) concentrations were tested against mosquito worms. The concentration 1:300 turned out to be the most effective.

Invasion of *Cx.p. pipiens* worms with nematodes *S. disparsica* and *H. bacteriophora* has been performed in vessels with different levels of water (6, 20 and 60 mm) in two variants. In experiments of the first variant on the bottom of 6 mm depth water vessels the 3 mm layer of river sand and 30 samples of mosquito worms were placed. The 9000 samples of the nematode *S. disparsica* have been used for invasion.

The same experiments were repeated in vessels with 20 and 60 mm level of water mixed with sand. Proportion between warms and nematodes *S. disparsica* was the same. The experiments performed in sand-containing vessels with all depths of water were repeated 4-times together with control experiments.

Similar tests were carried out using the second nematode species *H. bacteriophora*. In this case thickness of sand, water depth and proportion between mosquito warms and the nematodes(1:300) were the same as described above.

In the second variant the analogous experiments were performed on mosquito worms using the same nematode species with only difference- water vessels did not contained sand.

Efficacy of nematodes and the number of nematodes in each mosquito worm were evaluated using methods entomohelminthology [8, 9].

Results. In experiments of the first variant nematodes did not caused death of mosquito worms in sand-containing vessels with either water level. This phenomenon can be explained by the fact, that the nematodes find themselves on the sandy bottom and the contact between mosquito warms and nematodes was not achieved. Consequently the nematodes failed to infect the worms.

In experiments of the second variant, where the water vessels did not contained sand, mortality rate of warms attained 100% in the vessels with 6 mm level of water and 52.3% in vessels with 20 mm level of water. In vessels with 60 mm level of water mortality of worms was not observed

Dissection of *Cx.p. pipiens* worms has revealed that number of nematodes in dead individuals varied within the range of 1-9 samples. An average quantity of nematodes per worm was 3.5 samples. Mature phase of the nematode has not been revealed in mosquito worms. Out of registered nematodes 29% were alive and 71% were dead.

The experiments performed *Cx.p. pipiens* worms have shown that the entomopathogenic nematodes *S. disparsica* and *H. bacteriophora* are able to penetrate the worm organism and cause its death only at low level of water (from 2 up to 20 mm).

Nematode efficacy has been found to be dependent on their direct contact with mosquito worms, quantity of nematodes and their activity.

It has been established, that application of entomopathogenic nematodes of the genera *Steinernema* and *Heterorhabditis* against malaria and non malaria worms is effective in spring and autumn in open, shallow, not covered with vegetation water reservoirs, where the populations of first generations of winter passed mosquitoes develop. The above mentioned nematodes can also be used against worms of *Cx. p. molestus*, which inhabit stagnant puddle water in basements. This insect develops all year round, without diapause.

The results obtained in laboratory conditions allow us to conclude, that the studied entomopathogenic nematodes can be used against malaria and non malaria mosquitoes in natural conditions.

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