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RESEARCH ARTICLE

EFFECTIVENESS OF ENTOMOPATHOGENIC NEMATODES *STEINERNEMA TBILISIENSE* AND *S. THESAMI* AGAINST PEST OAK - *LYMANTRIA DISPAR* (L.) AND *TORTRIX VIRIDANA* (L.)

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ABSTRACT

Steinernematids and Heterorhabditids are lethal insect pathogens with biocontrol potential for managing insect pests of agricultural importance.The efficacy of entomopathogenic nematodes (EPNs) of *Steinernema tbilisiense* and *S. thesami* was evaluated by oak pests, such as *Lymantria dispar* L. (Lepidoptera: Liparidae) and *Tortrix viridana*L. (Lepidoptera: Tortricidae). To determine the efficacy of nematodes under laboratory conditions, suspensions of the following concentrations were tested: 700 nematode, 350 nematode, and 200 nematode in 1 ml of water. The experimental objects were mainly *L. dispar* and *T. viridana*. In those experiments, where a suspension of *S. tbilisiense* 700, 350 and 200 nematodes in 1 ml of water was used against *L. dispar,* 96.5, 62.0 and 55.0% insect mortality were respectively recorded. When applying *S. thesami* - 98.0; 71.0 and 57.5% deaths.During the action of *S. tbilisiense* on the caterpillars of *T.viridana,* 95.5, 58.0 and 53.0% died. When using *S. thesami,* respectively, 90.5., 66.0 and 56.0%.The most effective concentration was 700 nem. in 1 ml of water, in which a maximum biological efficiency (99-100%) was achieved in a number of replicates.When using combined suspensions of two species of *S. tbilisiense* and *S. thesami* nematodes (350 N each per 1 ml of water) caused the death of 98.0% of *L. dispar* caterpillars, while the effectiveness of *S. tbilisiense* alone was 62.0% and *S. thesami* - 71.0%.The same high efficacy was observed when using this suspension against *T.viridana* - 92.0%.Thus, in the joint application of two species of nematodes, higher results were obtained than when each nematode was used separately. From this, we can conclude that in this case there is a phenomenon of synergism.Field surveys were conducted in Saguramo, Lelubani, Tezami (Mtskheta district).Oak trees were treated with suspensions of *S. tbilisiense* and *S. thesami* (700 nematode in 1ml of water). The best results were obtained in the fight against caterpillars of III and IV stages (mortality 62.5 - 65.2%).It was found that when using *S. tbilisiense* at 19-22^0 and relative humidity of 75 to 81%, the mortality of caterpillars of III and IV ages was 59%. In case of *S. thesami*, the mortality of caterpillars reached 64.5%.With an increase in the dose of nematodes in a suspension of up to 1000 nematodes in 1 ml of water, the effectiveness of the preparation also increased accordingly.The mortality of caterpillars in the case of *S. tbilisiense* reached 74.2%; when using *S. thesami* - 76.5%.On the basis of the conducted studies, it was established that the nematodes *S. tbilisiense* and *S. thesami* used in our experiments are equally effective against leaf-eating pests of oak. The effectiveness of nematode preparations varies with the temperature and relative humidity of the air. The main condition for obtaining high effects is high air humidity (above 80%) and an increase in the concentration of nematode suspension. It is proved the mortality of insect increases with an increase in the concentration of up to 1000 nematode 1 ml of water.

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INTRODUCTION

The need to protect agricultural and forestry crops from pests that destroy a significant share of the crop and reduce the productivity of forests is obvious. Biological methods of plant protection are of particular importance at present (Poinar, 1990; Georgis*et al.,*2006;Сан-Blas,2013;Tofangsazi*et al.,*2014; Půža, 2015). Among the biological agents, an important role belongs to entomopathogenic nematodes. Entomopathogenic nematodes of the families Steinernematidae and Heterorhabditidae are naturally occurring pathogens of insects (Kaya and Gaugler, 1993; Hominick *et al.,* 1996) which are environmental friendly alternative for insectpest management (Kaya and Gaugler, 1993; Kaya, 1990).Most bio control agents require days or weeks to kill their hosts, yet nematodes, working with their symbiotic bacteria, kill insects within 24 to 48 hours (Anes and Ganguly, 2015). Key to the success of EPN as a control strategy is an understanding, based on both laboratory and field observation. Proceeding from this, the task was set to determine the biological effectiveness of the use of local nematodes *Steinernema tbilisiense* (Gorgadze *et al.,* 2015) and*S. thesami* (Gorgadze *et al.,* 2016) against the caterpillars of

Table 1. The effectiveness of entomopathogenic nematodes against caterpillars of the III-IV stage of the pest of oak

Species of insects	Concentration of nematodes (in 1 ml of water)	Mortality of caterpillars in % Repetitions					The death of caterpillars in view of their death in control on average in %
		1	2	3	4	5	
	S. tbilisiense						
Lymantria lispar	700	98,0	95.5	100	96.5	99,0	96.5
"--------------------"	350	62.5	68,0	57.5	68,0	66,0	62,0
"--------------------"	200	56.5	52.5	60,0	54,0	61,0	55,0
Control (water)		2,0	1,0	3,0	0	0	2,0
Tortrix viridana	700	100	94.5	98.5	95	97.5	95.5
"--------------------"	350	60.7	62	59.5	54,0	0	58,0
"--------------------"	200	55,0	57.5	51,0	48.5	59,0	53,0
Control (water)		1,0	—	2,0	2,0	1,0	1,0
		Sx%	0.68				
	S. thesami						
Lymantria lispar	700	100	98.5	97,0	100	0	98,0
"--------------------"	350	73.5	68,0	65.5	78.5	77,0	71,0
"--------------------"	200	60.5	54,0	58,0	56.5	65,0	57.5
Control (water)		2,0	3,0	1,0	1,0	—	1.8
Tortrix viridana	700	95,0	97,0	88.5	90,0	90.5	90.5
"--------------------"	350	68.5	73,0	70.5	62,0	68,0	66,0
"--------------------"	200	77,0	52.5	63,0	59.5	64.5	56,0
Control (water)		2,0	1,0	3,0	—	1,0	1.8
		Sx% 0.71					

oak pests - *Lymantria dispar* and *Tortrix viridana*, establish the methods, norms, and timing of applying *S. tbilisiense* and *S. thesami* separately and jointly and determine the effectiveness of the action. *L. dispar* in Georgia is found mainly in the mountainous regions of Kartli. It should be noted that *L. dispar* is one of the dangerous pests of early blossoming forms of fruit and deciduous trees, especially oak forests (Kanchaveli and Supatashvili, 1968). *T. viridana* represents a polyphage. Prefers oak, hornbeam, and fruit; In Georgia, they are found both in the Eastern and Western regions (Shavlyashvili *et al.,* 1980). Caterpillars of *T. viridana* appear in late April - early May. The appearance of caterpillars of pests coincides with the opening of the buds of the early form of oak. They penetrate into the kidneys and eat them, then eat out a part of the leaves, and in the older ages, they are eaten whole.

MATERIALS AND METHODS

Experiments were conducted in 2016-2017. In the laboratory of the Institute of Zoology of the Ili University and in the territory of Eastern Georgia in the Mtskheta region. For laboratory experiments, oak pests were collected at different locations in the Mtskheta district. The collected material was transported in special gauze pouches and vessels. The insects delivered to the site were sorted by phases and species. In the experiment on infection of insects, nematodes *S.tbilisiense* and *S. thesami* were used. Petri dishes and 0.5 l cans were used as dishes. In the Petri dishes, filter paper was placed, the leaves of oak and caterpillars in an amount of 20 to 30. In the cans of water, branches with leaves were placed. On leaves, the number of insects was 30-40 copies for each can. Their treatment was conducted in the same way as in Petri dishes. In laboratory experiments, the following doses of the nematode suspension were used: 200. 350, and 700 units of nematodes in 1 ml of water. The leaves were treated with an entomonematods suspension using a hand pulvelizer. Observations were conducted every 24 hours. The percentage of insect death was calculated on days 3, 5, and 7 according to the method of Ebot (Abbot, 1925). In control, the branches and leaves were treated with water. Experiments for each variant were carried out in 3-5 fold replicates. Laboratory experiments were carried out at a temperature of 20-21^0 and a relative humidity of 65-75%. In the field conditions, low oak trees (3-4.5 m) were chosen for treatment with nematode suspensions. The intensity of occupancy of trees by leaf-eating pests was established according to Vorontsov's method (Vorontsov, 1982): 1. The cut of the branches. 2. The recalculation of the leaves. To conduct field experiments, nematode preparations *S. tbilisiense* and *S. thesami* were used. The treatment of plants against caterpillars of 3-4 ages was carried out with a manual sprayer (OVH-14), in the evenings with cloudy weather. Норм expenditure of the working solution during spraying was 700-900 l / ha. In the field experiments, suspensions with a titer from 700 to 1000 nematode in 1 ml of water were used. The calculation of the biological effectiveness of the drugs used was carried out according to the formula of Franca (Franca, 1968). The effectiveness of the action of nematode preparations was established by methods of mathematical statistics (Dospehov, 1979).

RESULTS AND DISCUSSION

In the laboratory, in experiments where a suspension of the *S. tbilisiense* 700 nematode, 350 and 200 nematodes in 1 ml of water was used against *L. dispar,* 96.5, 62.0 and 55.0% insect mortality (Table 1), respectively, was observed, with *S. thesami* - 98.0; 71.0 and 57.5% deaths.During the action of *S. tbilisiense* on the caterpillars of *T. viridana*, 95.5, 58.0 and 53.0% died (Table 1). When using *S. thesami*, respectively, 90.5., 66.0 and 56.0%. In all cases in the control, the mortality of caterpillars varied from 1.5 to 3.0%.As can be seen, the most effective concentration was 700 nem. in 1 ml of water, in which a maximum biological efficiency (99-100%) was achieved in a number of replicates.Even better results were when using a mixture of suspensions of two species of nematodes. Thus, a mixture of nematode suspensions from *S. tbilisiense* and *S. thesami* (350 Nm per 1 ml of water) caused the death of 98.0% of the *L. dispar* caterpillars (Table 2), while the effectiveness of *S. tbilisiense* alone was 62.0% and *S. thesami* - 71.0% (Table 1).The same high efficacy was observed when using this suspension against *T. viridana* - 92.0% (Table 2).Thus, in the joint application of two species of nematodes, higher results were obtained than when each nematode was used separately. From this, we can conclude that in this case there is a phenomenon of synergism.Field experiments on the use of nematodes of the genus Steinernema

Table 2. The effectiveness of the joint action of *Steinernema tbilisiense* and S. *thesami* (350 nematode in 1 ml of water) against caterpillars of the third age of some pests of oak

Species of insects	Mortality of caterpillars in % Repetitions				The death of caterpillars in view of their death in control on average in %
	I	II	III	IV	
Lymantria lispar	100	96.5	97,0	100	98,0
Tortrix viridana	92.5	97,0	98,0	89.5	92,0
Control (water)	2.5	0	0	5.2	3.8

Table 3. Biological effectiveness of Steinernema against the different ages of caterpillars *Lymantria dispar* and *Tortrix viridana* on oak trees (at a temperature of 16 to 230 and relative humidity of 75 to 87%)

Preparations	Number of caterpillars on a branch 1 m long (average)			
	Age caterpillars	Before processing	On day 10 after processing	% Mortality
S. tbilisiense 700 nem. in 1 ml of water	II - III	46.0±1.33	10.0±0.66	57.5
S. thesami 700 nem. in 1 ml of water	II - III	42.0±1.00	20.0±1.00	53.2
Control (water)	II - III	40.0±1.00	38.5±2.00	0
S. tbilisiense 700 nem. in 1 ml of water	III-IV	21.1±2.00	19.0±1.00	62.5
S. thesami 700 nem. in 1 ml of water	III-IV	50.5±2.33	18.0±2.00	65.2
Control (water)	III-IV	50.0±3.00	49.0±2.33	0
S. tbilisiense 700 nem. in 1 ml of water	IV-V	48.5±2.33	24.0±1.00	48,0
S. thesami 700 nem. in 1 ml of water	IV-V	39.0±1.00	19.5±1.00	46,0
Control (water)	IV-V	47.5±1.33	44.5±2.33	0
Sx%≤ 3.0				

Table 4. Biological efficacy of the III-IV ages of *Lymantria dispar* and *Tortrix viridana* at 19 - 22° C and relative humidity 78 - 81%

Name and concentration of preparations	Number of caterpillars on a branch 1 m long (average)					
	L. dispar			*T. viridana*		
	Before processing	After processing	% Mortality	Before processing	After processing	% Mortality
S. tbilisiense 700 nem. in 1 ml of water	40.5±2.33	16.5±1.00	58.0	18.0±1.33	7.5±0.33	59.0
S. thesami 700 nem. in 1 ml of water	35.0±2.66	13.0±0.66	60.5	23.5 ± 2.00	2.5 ± 0.33	64.5
S. tbilisiense 1000 nem. in 1 ml of water	34.5±1.33	9.5±0.66	69.5	19.0 ± 1.33	5.0 ± 0.33	74.2
S. thesami 1000 nem. in 1 ml of water	37.0±2.33	10.5±0.33	71.5	15.5 ± 1.00	3.5 ± 0.33	76.5
Control (water)	36.0±2.66	35.0±2.33	0	18.5 ± 1.66	17.5 ± 1.33	0
Sx%≤ 4.7						

(*S. tbilisiense* and *S. thesami*) in the fight against insect pests of oak were conducted in the Mtskheta region.As a result of our studies, it was established that the number of damaged leaves in the Mtskheta forestry on oak, hornbeam, and walnut was 50 - 75%. In this case, on the branch of an oak with a length of 1 m, there was an average of 52 to 64, on a hornbeam from 44 to 51, in a walnut 55 to 62 caterpillars of *L.dispar*. In the forest area of the pests, as a rule, more than in the forest was observed (50 and 27%, respectively).*T.viridana* also causes great damage to forests; it is often distributed along with other leaf-eating insects. One of the tasks was to establish the age of caterpillar pests, against which the use of nematode suspension in natural conditions was bi-most effective. For this, the trees were treated with suspensions of *S. tbilisiense* and *S. thesami* (700 nematode in 1ml of water) at different times (Table 3). The best results were obtained in the fight against caterpillars of III and IV ages (mortality 62.5 - 65.2%).It is well known that the efficiency of nematode use can vary with the temperature and humidity of the air. It was found that when using *S. tbilisiense*at 19 - 22^0 and relative humidity of air 75 - 81%, the mortality of caterpillars of III and IV ages was 59. 0%., In the case of *S. thesami*, the mortality of caterpillars reached 64.5% (Table 4).When the dose of nematodes in the suspension (up to 1000 nematode in 1 ml of water) was increased, the effectiveness of the drug was also increased accordingly. The mortaliti of caterpillars in the case of *S. tbilisiense* reached 74.2%, with *S. thesami*76.5% (Table 3.4).On the basis of the conducted studies, it was established that the nematodes *S. tbilisiense* and *S. thesami* used by us in the experiments are equally effective against harmful insects of oak.

Once again it is proved that the effectiveness of nematode preparations varies with temperature and relative air humidity. The main condition for obtaining a high effect is high air humidity (above 80%) and an increase in the concentration of nematode suspension. It is proved that the percentage of insect death significantly increases with increasing the concentration to 1000 nematode 1 ml of water.

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