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EFFICACY OF LOCAL ENTOMOPATHOGENIC NEMATODES AGAINST PIERIS BRASSICAE (L., 1758) (LEPIDOPTERA: PIERIDAE)

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ABSTRACT

Pieris brassicae (L., 1758) (Lepidoptera: Pieridae) is considered one of the main pests of cabbage crops; The article describes the use of local entomopathogenic nematodes (EPNs) (*S.thesami, S.tbilisiense, S. borjomiense, S. gurgistana* and *Heterorhabditis* sp.) against *P. brassicae* in laboratory conditions. These nematodes were first used for cabbage pests. The tests were carried out with different doses of a suspension of each species of nematode (100, 50 and 25 nematodes against one insect). As a result of the experiments, it was found that all the above-mentioned nematodes have a rather high efficacy against *P. brassicae* not only high (100), but also using medium (50) doses. When using 4 local species of Steinernema EPN (*S. thesami, S. tbilisiense, S. borjomiense,* and *S. gurgistana*) against the *P. brassicae* nematodes *S. thesami* showed the highest results (96.6%); the remaining three species showed the same effect against insect (93.3%); among the local EPNs used against *P. brassicae, Heterorhabditis* sp. was the most effective. Pest mortality caused by its nematodes was 97.5%.

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INTRODUCTION

Larvae of butterflies of the genus Pieris (Lepidoptera: Pieridae) may substantially decrease yields andmarket value of plants of the family Brassicaceae (Mazurkiewicz *et al*., 2017). Active feeding of the caterpillars of the cabbagebutterfly leads to leaves skeletonizing (Figure 1 B) while single caterpillars of the small white butterfly gnaw holes and enter the loose heads of cabbage, and cauliflower and flower buds ofbroccoli (Kochman and Wegorek, 1997; Boczek and Lewandowski, 2016). Of cabbage pests like *Pieris brassicae* (L., 1758) (Lepidoptera: Pieridae) is an economically important pest of the Brassicaceae (Cartea *et al*., 2009; Metaspalu *et al*., 2009). This pest has five larval stages, of which the last two can cause significant economic damage to the crucifers (Karowe and Schoonhoven, 1992). Currently, the most reliable pest control is provided by chemical insecticides (Cartea *et al*., 2009). These factors highlight the need to develop and use biological control agents as an alternative to chemical insecticides. Currently, biocontrol is such a tool. Entomopathogenic nematodes (EPNs) of the genera Steinernema and Heterorhabditis, which are obligatory and lethal insect parasites, associated at least in one of the growth stages, with (Adams *et al*., 2006). In Georgia, on cabbage plantings there are many types of harmful insects that cause significant damage to the crop (Kalandadze, Jashi, 1956; Kelendjeridze, 1975). *P. brassicae, P. rapae, Mamestra brasicae, Plutella maculipennis* are most harmful. Caterpillars of the second, third and fourth generations *P. brassicae* are most harmful for the cabbage culture in Georgia. In the valleys of Georgia, *P. brassicae* has four generations, in the mountainous regions, three (Aleksidze, 1952). Laboratory studies provide the information necessary to assess the specificity of species/strains of EPN for taking into account crop pests (Toth, 2006). The study was conducted to assess the effectiveness of local strains of EPNs (*S. thesami, S. tbilisiense, S. borjomiense, S. gurgistana* and *Heterorhabditis sp.*) in the control of *P. brassicae* larvae. Experiments with these nematodes against *P. brassicae* have not yet been carried out.

MATERIALS AND METHODS

In 2018, a cabbage pest laboratory experiments were conducted at the Institute of Zoology at the Ilia State University (Georgia). Research materials for experiments were collected from cabbage cultures of the private sector from the

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Mtskheta district. Only *P. brassicae* (III-IV ages) was chosen for the experiments because it is considered one of the main pests of cabbage. We collected worms from cabbage leaves with special soft tweezers. The collected material (worms and cabbage leaves) was placed in a special mesh container to prevent damage to their transport. We sorted the collected material and prepared them for our experience. Tests were conducted in five variants. Against *P. brassicae*, we used local forms of entomopathogenic nematodes of the genus Steinernema *S. thesami* (Gorgadze *et al*., 2016), *S. tbilisiense* (Gorgadze *et al*., 2015), *S. borjomiense* (Gorgadze *et al*., 2018), *S. gurgistana* (Gorgadze and Lortkipanidze, 2006) and the not yet identified form of *Heterorhabditis sp.,* In the experiments, we used the Petri dish with a diameter of 10 cm, on which we placed filter paper, pieces of cabbage leaves and 10-15 pests; Each Petri dish was developed by nematode suspensions. The titer of the suspension on the cup of Peter was determined in accordance with the method of Veremchuk (Veremchuk, 1986). We used different doses of nematode suspensions (100, 50, and 25 nematodes against one insect) against pests. We watched every 24 hours. 48 hours after infection, the dead worms of the pest were cut under a stereomicroscope. As a result of the autopsy, we determined the number of nematodes in the insect. As a result of the experiments, the minimum, maximum and average number of nematodes found in dead insects was determined. Accounting for mortality of insects occurred 120 hours after the start of the experiment. In the control Petri dishes, cabbage leaves and worm pests were treated with distilled water. For experiment nematodes (*S.thesami, S. tbilisiense, S. borjomiense, S. gurgistana* and *Heterorhabditis sp*.) were cultured as on *Galleria mellonella* (Lepidoptera: Pyralidae) in accordance with the method of Dutky (Dutky, 1964), and Bombyx mori (Lepidoptera: Bombycidae) (Kakuliya *et al*., 1983). Insect mortality accounting in experiments was carried out by the Abbot formula (Abbot, 1925). Each variant of the experiment was carried out in triplicate under similar conditions (at a temperature of 21-23 ° and a relative humidity of 61-74%). The obtained results were processed by the method of mathematical statistics of Dospekhov (Dospekhov, 1979).

RESULTS

In laboratory experiments against *P. brassicae*, in each variant, different species of local entomopathogenic nematodes were used (S.*thesami,S. tbilisiense, S. borjomiense, S. gurgistana* and *Heterorhabditis sp*.); the experiments were conducted simultaneously three replications. The nematode suspension of each species was used in three different concentrations (100, 50, and 25 nematodes against one insect). Accounting for mortality of insects was carried out after 24, 48, 72, 96 and 120 hours. When using *S. thesami* nematode suspension (dose -100 it against 1 insect), the maximum mortality rate of the pest (96.6%) was obtained after 72 hours (see Table 1); Dose - 50 him against 1 insect - after 120 hours (73.5%); when using 25 nematodes against 1 insect, the maximum mortality of the pest was obtained after 72 hours - 39.2%. The maximum mortality rate of pests caused by 100 nematode doses of *S. tbilisiense* suspension was observed after 120 hours (93.3%); When using 50 nematode doses - after 96 hours (56.3%); And when using 25 nematode doses of the suspension - after 72 hours (27.9%). The maximum mortality rate of pests (93.3%) was noted 120 hours after using *S. borjomiense* suspension with a dose of 100 nematode against 1 insect. The maximum mortality rate of the pest was 96 hours after using the suspension in doses of 50 and 25 nematodes (69.2%, 38.5%). The maximum mortality rate of harmful insects was obtained 72 hours after using all three doses (100, 50, and 25 nematodes against 1 insect) of the nematode suspension of *S. gurgistana*, respectively: 93.3%; 72.4% and 36.7% (Table 2); As for the suspension of *Heterorhabditis sp*. against a pest, the maximum result of mortality with a dose of 100 nematodes was obtained after 48 hours (97.5%); 50 nematode dose - after 96 hours (93.3%); 25 nematode dose - after 72 hours (55.3%). A small percentage of mortality (1.5%) of harmful insects was observed in the control Petri dishes; This was taken into account when determining the mortality rate of *P. brassicae*. After 36-48 hours, infected dead insects were cut under a binocular microscope (Figure 1 A). We calculated the number of nematodes that penetrated the insect. During the study, we examined the dose of infection of the worms. Nematodes of various quantities were found in various dead insects. Their number ranged from 2 to 97. The average value was 26.5. The study showed that in insects infected with a high dose of suspension, more nematodes were exposed to those infected with a low dose.

In those Petri dishes, where *P.brassicae* worms were infected with high doses of nematode suspensions, the mortality rate of the pests was much faster. Based on the test results, it was found that a high dose of nematode suspension, the optimum temperature (24°) and humidity gives a high lethal effect of harmful insects. However, it is important to note that using the average dose of nematode suspension (50 nematode against 1 insect), the lowest mortality rate of harmful insects was 56.3%, and the maximum - 93.3% (Table 1). Of the 4 local EPNs species (*S. thesami, S. tbilisisiense, S. borjomiense and S. gurgistana*) used against *P. brassicae,* the highest results were shown by the nematode *S. thesami* (96.6%); the remaining three species showed the same effect (93.3%) against the harmful insect. Among local EPNs used in experiments against *P. brassicae, Heterorhabditis sp*., proved to be the most effective; the mortality rate of the pest caused by its nematodes was 97.5%. As a result of laboratory experiments, it was revealed that local species of EPN are effective against *P. brassicae*; they can be successfully applied against harmful insects as environmentally friendly agents in biocontrol.

A

B

Figure 1. A: Infected with nematodes Pieris brassicae ; B: Skeletonized cabbage sheet

Table 1. Pathogenicity of the 3th-4th larval stage of *Pieris brassicae* with three different doses of entomopathogenic nematode species

Treatment	% Mortality of after different time periods				
	24 h	48 h	72 h	96 h	120 h
S. thesami - 100 IJs	0	64.5	96.6	0	0
- 50 IJs	0	27.3	58.4	69.7	73.5
- 25 IJs	0	28.6	39.2	0	0
S. tbilisiense - 100 IJs	0	53.7	68.0	88.5	93.3
- 50 IJs	0	22.8	45.5	56.3	0
- 25 IJs	0	14.0	27.9	0	0
S. borjomiense -100 IJs	1.7	59.4	70.3	92.7	93.3
- 50 IJs	0	28.3	63.4	69.2	0
- 25 IJs	0	24.6	36.2	38.5	0
S.gurgistana -100 IJs	0	91.0	93.3	0	0
- 50 IJs	0	37.2	72.4	0	0
- 25 IJs	0	29.4	36.7	0	0
Heterorhabditis sp.					
-100 IJs	3.4	97.5	0	0	0
- 50 IJs	0	68.5	90.3	93.3	0
- 25 IJs	0	52.0	55.3	0	0
Control	0	00	1.5	1.0	1.0

Sx%≤ 0.33

Table 2. The effectiveness of entomopathogenic nematodes against larvae *Pieris brassicae*

Concentracion (IJs / larvae)	Mortality larvae (%)			The death of larvae on average in (%)
	Repetition			
	1	2	3	
S. thesami -100	100	100	96.6	97.0
- 50	65.5	78.5	77.3	72.2
- 25	44.3	36.4	39.0	39.0
S. tbilisiense -100	96.6	96.6	100	95.5
- 50	58.5	60.0	52.5	56.3
- 25	24.5	29,0	26.4	26.0
S. borjomiense -100	100	100	100	98.5
- 50	75.0	70.5	76.5	73.5
- 25	38.5	37.0	43.4	39.6
S. gurgistana -100	100	100	93.3	97.0
- 50	71.0	69.6	72.5	70.5
- 25	45.5	39.5	48.7	45.0
Heterorhabditis sp.-100	100	100	100	97.0
- 50	96.6	96.6	100	96.6
- 25	56.3	52.0	49.5	50.5
Control	1.0	2.0	1.5	1.5

Sx%≤ 0.33

DISCUSSION

Laboratory data show that five indigenous species EPNs (*Steinernema thesami, S. tbilisiense, S. borjomiense, S. gurgistana* and *Heterorhabditis sp*.) against *Pieris brassicae* are characterized by high pathogenicity. The intensity and extensiveness of infectivity of *P. brassicae* worms mainly depend on the doses of nematode suspension. When using the *S. thesami* suspension (dose 100 / IJs against a single insect), the maximum mortality rate of *P. brassicae* was observed after 72 hours (96.6%), and with a dose of 50 / IJs - 120 hours (73.5%). Similar results were obtained when using nematodes of the Steinnernema genus (Table 1). High results were observed (100% mortality) within 48 hours (dose 100 / IJs) while using *Heterorhabditis sp*., Using average doses (50 / IJs), 100% mortality was taken within 72 hours. As for the low dose (25 / IJs) of EPN, the maximum result of the death of insects (55.3%) was observed after 120 hours. According to Ramliana and Yadava (2009) less percent of the death of harmful insects (45-35%) were observed 48 hours after using a nematode suspension (*S. thermophilidium* and *S. glaseri*) with a dose (50 / IJs) against *P. brassicae*. According to the above authors 80% (using *S. glaseri*) and 85% (using *S. thermophilidium*) mortality of the pest were obtained after 72 hours. 100% mortality was observed after 120 hours. Sanderman and Pezowicz (1983), using the nematode *S. carpocapsae* against *P. brassicae* larvae, resulted in 100% death after 96 hours. The intensity of mortality in the experiments was 48 hours. Similar results were found in the study by Mazurkiewicz *et al*., (2017). Using nematode suspensions against *P. brassicae* larvae, experiments under laboratory conditions were carried out in many countries at different times. For example, using the nematode *S. feltiae* against *P. brassicae*, 75-97.5% of the mortality of worms was obtained (Wu and Chow, 1989); With the use of nematodes - *Heterorhabditis indica, H. bacteriophora, S. carpocapsae* - 100% of the pest deaths are observed in Mahara et al., (2005) in a study; *S. thermophilidium* 12.5-100%, *S. glaseri* 37.5-100% of mortality is mentioned by Lalramliana and Yadav (2010). The high pathogenicity of local EPNs used against *P. brassicae* in our laboratory experiments allows the use of nematodes in the field.

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