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RESEARCH ARTICLE

THE EFFICACY OF ENTOMOPATHOGENIC AGENTS AGAINST HALYOMORPHA HALYS (HEMIPTERA: PENTATOMIDAE)

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ABSTRACT

With the aim ofestablishing the efficacy of entomopathogenic agents experiments were carried outunder laboratory conditions against the third instar nymphae and imagoes. The following entomopathogenic nematodes (EPN) were used as agents in the experiments: *Steinernema tbilisiensis*, *S. thesami*, *S. gurgistana*, *S. carpocapsae*, *Heterorhabditis bacteriophora* and the local strain of the fungus *Isaria fumosorosea* (Wize). The experiments were carried out in two stages: at the first stage 100 nematodes were used against each insect, while at the second stage – 200 nematodes were applied. In parallel to this the efficacy of two concentrations of the fungus *I. fumosorosea*- 1.10^8 conidia/ml and 3.10^8 conidia/ml- was testedagainst the pest insect. On the second stage of experiment EPN and the fungus were jointly applied against the *H. haly* as a combination at high doses (200 nematodes + fungus *I. fumosorosea* - 3.10^8). The results of experiments have shown that while applying separately EPN and the fungus against nymphae and imagos, *S. carpocapsae* and the fungus *H. bacteriophora* turned out tobecomparatively effective: the effect of low dose of *S. carpocapsae* against nymphae was 77.2% and against imagoesit was equal to 62.2%. The effect of high dose was 82.4% and 65.3% respectively. As a result of application of low doses of *H. bacteriophora* 79.6% mortality of nymphae and 69.0% mortality of imagoes was achieved. When using high doses of this agent, the 83.3% of nymphae and 73.7% of imagoes were killed. EPN and the fungus were used in experiments in combination as well: the joint effect of *S. carpocapsae* and the fungus *H. bacteriophora* was 94.2% mortality of nymphae and 88.5% mortality of imagoes of *H. halys*. Combination of *H. bacteriophora* and *I. fumosorosea* yielded 95.6% and 90.5% mortality rate of nymphae and imagoes respectively. At all stages of the experiment emtomopathogenic agents revealed higher effect against nymphae as compared with imagoes. Our experiments have demonstrated that the combined applicationofEPN and fungus was more effective, that application of the same agents separately. The nematodes *S. tbilisiensis*and*S. thesami*, which turned out to be less effective at the first stage of experiment have revealed higher effect against the pest when applied in combination with the fungus.

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INTRODUCTION

Halyomorpha halys (Stål, 1855) (Hemiptera: Pentatomidae), known as Brown Marmorated Stink Bug, (BMSB), is an agricultural pest, native to China, Japan, Koreas and Taiwan (Hoebeke and Carter 2003; Smith and Whitman, 2007).This is the polyphage pest, causing heavy damage to vegetables, fruits, ornamental plants and other crops. Its importance increased since it has been identifiedthat it feeds on more than 300 different plants, which are economically important to agriculture (Parker *et al*., 2015). In addition, they are also a nuisance pests by entering homes in the fall to overwinter.

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The pest was first registered in Georgia in 2015. It has significantlydamaged orchards and vegetable crops. During the season of 2016 the pest caused significant loss of the hazelnut harvest in the West Georgia (Samegrelo, Guria). According to the data of the National Food Agency of Georgiathe damage caused by *H. halys*made approximately 70% of the total cost of hazelnut harvest. To date only chemical insecticides are used for the control of this pest. Excess use of chemical pesticides in agriculture brings serious harm to the soil, air, water, flora, fauna and humans(Georgis, 1992; Vos *et al*., 2000). That's why it is so urgent to develop ecologically safe alternative methods of pest control such as entomopathogenic agents (bacteria, nematodes, fungi, etc.). Entomopathgenic nematodes belonging to families Steinernematidae and Heterorhabditidae are such agents. EPN - obligate parasites of insects have demonstrated high potential against the agricultural pests (Poinar, 1990; Georgis и др., 2006; Lacey & Shapiro-Ilan, 2008; Сан-Blas, 2013; Tofangsazi *et al*., 2014; Půža, 2015; Martínez *et al*., 2017).The role of entomopathogenic fungi for the pest controlshould also be outlined. For example, *Isaria fumosorosea* Wize is a well-known entomopathogenic fungus with a world wide distribution and a relatively wide host range, which makes it an interesting agent for the developmentof biocontrol methods (Zimmermann 2008; Hunter *et al*., 2011).

MATERIALS AND METHODS

To carry out different laboratory trials it was necessary to ensurecontinuous supply of nymphae (larvae)and imagoesof *H. halys*. Material was periodically collected in the West Georgia in summer of 2017, in hazelnut plantations, located in region Guria and Samegrelo. For the infestation of *H. halys* the local entomopathogenic nematodes *Steinernema gurgistana* (Gorgadze & Lortkipanidze, 2006), *S. tbilisiensis* (Gorgadze *et al*., 2015) and *S. thesami* (Gorgadze *et al*., 2016) as well as the introduced species*S. Carpocapsae* (Weiser, 1955) and *Heterorhabditis bacteriophora* (Poinar, 1976) were used. Only one species of the local entomopathogenic fungus *Isaria fumosorosea* strain (ARSEF access no. 10244) was used in the bioassays together with nematodes. It was isolated from pupae of *Hyphantria cunea* in Georgia (Kereselidze *et al*., 2014).

Filter paper, hazelnut leaves and 10-15 individuals of the pest were placedon experimental glass vessels - Petri dishes of 90mmdiameter. Laboratory experiment was carried out in two stages: at the first stage in the first variant of the experiment 100 nematodes were applied against each pest insect, placed on Petri dishes, while in the second variant 200 nematodes were placed. In both variants the nymphae (III instar) as well as imagoes were subject to experiments. In parallel to this, the two concentrations (1.10^{8} conidia/ml and 3.10^{8} conidia/ml) of the fungus *I. fumosorosea*were tested. Conidial suspension of the appropriate concentration was sprayed individually on the pests, placed on each of Petri dishes. At the second stage of the experiment entomopathogenic nematodes and the fungus were applied against the pest together, as a combination, at high doses (200 nematode+the fungus*I. fumosorosea*at high concentration 3.10^{8} conidia/ml). Each variant of experiment was carried out in 3-4 repetitions. Hazelnut leaves and pests, placed on control Petri disheswere treated by distilled water. Mortality of insects in experiments was monitored every 24 hours. Mortality percent of insects was recorded in the experiment on the 3rd, 5th and 7th days using the Abbott's formula (Abbot, 1925). The obtained results were statistically processed using the computer program and the known method by Dospekhov (Dospekhov, 1979). Each experiment in laboratory conditions was carried out at almost the same temperature (20-22ºC) and in conditions of 55-65% relative humidity ofair.

Table 1. Result of the use entomopathogenic nematodes and fungus against *Halyomorphahalys* in laboratory conduction

Name and dose of drugs	Death of yalyomorphahalys nymph and imago by days (%)					
	3		5		7	
	Hymph	Imago	Nymph	Imago	Nymph	Imago
Sr. No *I stage*						
1. *S. tbilisiensis* 100 IJs	28.5*	15.3	52.4	33.3	60.3	35.3
2.*S.tbilisiensis*200 Ijs	37.2	22.0	65.7	42.0	68.6	44.8
3. *S. thesami*100 IJs	31.0	20.6	50.5	40.8	53.2	42.0
4.*S. thesami* 200 IJs	40.6	17.4	52.0	39.2	58.5	39.2
5.*S. gurgistana* 100 IJs	38.0	29.5	59.6	48.0	68.3	50.0
6.*S. gurgistana*200 IJS	36.5	28.3	63.4	52.6	72.6	59.5
7.*S. carpogapcae*100 IJS	44.5	29.0	68.2	57.5	77.2	62.2
8.*S.carpocapsae* 200 IJs	52.4	53.5	73.3	59.0	82.4	65.3
9.*H. bacteriophora* 100 IJs	48.5	53.8	71.7	69.3	79.6	69.0
10.*H. bacreriopyora* 200 Ijs	46.3	66.5	76.2	72.5	83.3	73.7
11.*I. fumosorosea*(1.10^{8}conidia/ml)	16.5	10.0	28.3	20.0	39.3	30.0
12.*I. fumosorosea* (3.10^{8}conidia/ml)	29.3	22.0	40.0	37.4	43.3	38.4
II stage						
13. *S. tbilisiensis*200 Ijs+ *I. fumosorosea*(3.10^{8}conidia/ml)	42.6	34.5	77.0	62.0	78.1	63.2
14.*S. thesami* 200 Ijs+ *I. fumosorosea* (3.10^{8}conidia/ml)	50.0	37.2	72.3	69.7	80.5	72.4
15.*S. carpocapsae* 200 Ijs+ *I. fumosorosea*(3.10^{8}conidia/ml)	56.5	65.0	90.4	85.8	94.2	88.5
16. *H. bacteriophora* 200 Ijs+ *I. fumosorosea*(3.10^{8}conidia/m)	71.2	73.3	92.3	87.0	95.6	90.5
17. Untreated control (distilled water)	0.0	0.0	0.0	0.0	0.0	0.0

Sx%≤ 0. 33

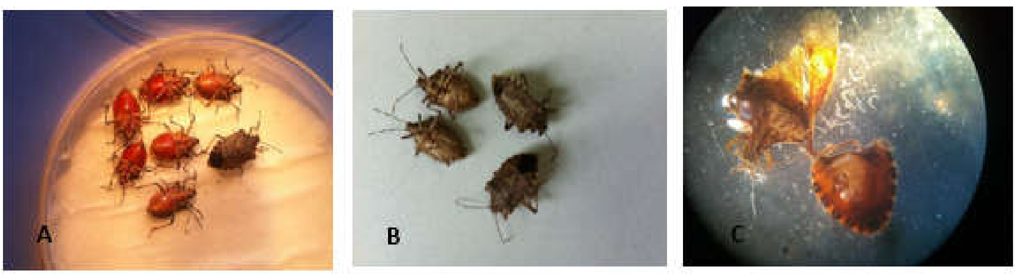

Figure 1. Mortality *of Haliomorpha halis* after usage of entomopathogenis nematodes. A: Mortality caused by *Heterorhabditis bacteriophora;* B: Mortality caused by of *Steinernema tbilisiensis;* C: Invade of nematodes into the insect body under stereomicroscope

RESULTS AND DISCUSSION

At the first stage of the experiment as a result of application of low doses (100 nematodes against each pest) of entomopathogenic nematodes (*S. tbilisiensis, S. thesami, S. gurgistana, S. carpocapsae* and*H.bacteriophora*) against nymphae and imagoes of *H. halys* at average 67±11(53.2-79.6)% mortality of nymphae and 51.7±13 (35.3-69.0)% mortality of imagoes was stated (Table 1. Figure 1). In the second variant as a result of application of the increased dose (200 nematodes against each pest insect) 73.0±9 (58.5-83.3)% of nymphae and 56.5±8 (39.2-73.7) %of imagoes were killed.

Two concentrations of the fungus *Isaria fumosorosea* (1.10^8conidia/ml and 3.10^8conidia/ml) were testedas wellagainst the nymphae and imagoes of *H. halys*. Low concentration of the fungus (1.10^8conidia/ml) killed 39.3% of nymphae and 30.0% of imagoes, while increased concentration of *I. Fumosorosea* (3.10^8conidia/ml) killed 43.3% of nymphae and 38.4%of imagoes.The results of experiments have shown that application of nematode suspension as well as of the fungus at the higher doses had a better effect than the low doses of the same agents.

At the second stage of the experiment entomopathogenic nematodes and the fungus *I. fumosorosea* were used in combination, at high doses (200 nematodes + fungus *I. fumosorosea* - 3.10^8conidia/ml). Joint application of *S. tbilisiensis* and the fungus caused respectively 78.1% and 63.2% mortality of nymphae and imagoes of the pest insect. When using together *S. thesami*and the fungus*I. fumosorosea*respectively80.5% and 72.4% mortality of nymphae and imagoes was registered. Combination of *S. carpocapsae*and the fungus*I. fumosorosea*killed 94.2% of nymphae and 88.5% of imagoes of *H. halys.H.bacteriophora* and the fungus together yielded 95.6% mortality of nymphae and 90.5% mortality of imagoes.The zero mortality was observedin the control vessel.The results of testing separately of EPN and the fungus against the nymphae and imagos of *H. halys* show higher efficacy of *S. carpocapsae* and *H. bacteriophora* in comparison with other entomopathogenes. It has also been established that the mortality of the pest insect was higher at the combined application of nematodes and the fungus, than in case when they were applied separately. At every stage of experiments, carried out against *H. halys* the mortality rate, caused by the entomopathogenic agents was higher in nymphae than in imagoes. Thus, our experiments have proven the high effectiveness of entomopathogenic organisms against *Halyomorphahalys*. These are the first results of studying the influence of pathogens on this harmful insect in the conditions of Georgia.

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