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Entomology

EPNs *Steinernema carpocapsae* and *Heterorhabditis bacteriophora* for Control of the Brown Marmorated Stink Bug (BMSB) *Halyomorpha halys* (*Hemiptera, Pentatomidae*)

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ABSTRACT. The efficiency of entomopathogenic nematodes *Steinernema carpocapsae* and *Heterorhabditis bacteriophora* for biological control of the brown marmorated stink bug (BMSB) *Halyomorpha halys* was determined under laboratory conditions. Prior to conducting the trial on entomopathogenic nematodes, their cultivation occurred in an incubator at 24-25^{0}C on last-instar large wax moth (*Galleria mellonella*) larvae according to a procedure described by Kaya and Stock (1997). The suspensions obtained as a result of cultivation were kept in a refrigerator at 4-6^{0}C. Acclimation of nematodes proceeded at room temperature 24-25^{0}C. The application of the obtained biomass was possible after 6-10 hours. For determination of the efficiency of *S. carpocapsae* and *H.bacteriophora* under room temperature at 24-25^{0}C and 75% humidity, last instar–imago of the pest was used for trial. Mortality rate of individuals was determined by Abbott's formula. The trials were conducted on 10 cm Petri dishes. The obtained results have shown that the nematode *S. carpocapsae* is more effective against *H. halys*, than *H.bacteriophora* and 500, 1000, 1500, 2000, nematode/ml suspensions mortality depended on time, nematode variety and concentration. Pest mortality was tested for treatment after 3, 5, 7 days. On the 7th day after treatment with a nematode suspension 500, 1000, 1500, 2000 infective juveniles/ml of S. carpocapsae in the given trial reveal 18, 32, 43 and 68% mortality rate where as *H.bacteriophora* 12, 24, 26 and 48% respectively. The obtained results show that under laboratory conditions the efficiency of *S. carpocapsae* and *H.bacteriophora* against *H. halys* can be controlled by *S. carpocapsae* rather than *H.bacteriophora* and therefore, future study is to be conducted in private houses, field and greenhouse conditions. © *2018 Bull. Georg. Natl. Acad. Sci.*

Key words: *Steinernema carpocapsae, Heterorhabditis bacteriophora, Xenorhabdus, Photorhabdus, Halyomorpha halys*

Damage caused by brown marmorated stink bug has reached a large scale in Georgian agriculture. This requires protection of agricultural crops, garden-melons, fruit trees, vines, housing, etc. In this respect particularly dangerous pests are distinguished: American white butterfly, colorado beetle, calla, gardens aphids, small mulberry Allure, marmorated stink bug (BMSB) as well as

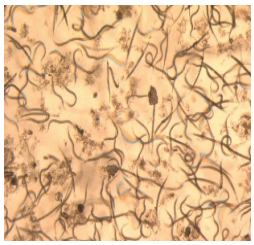
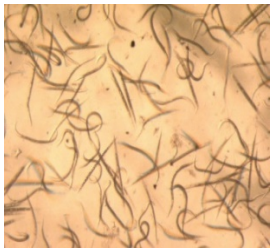


Figs.1,2,3. *Halyomorpha halys* (Stink bug), colonies at home

social insects: cockroaches, ants, which were applied with entomopathogenic nematodes: *Steinernema carpocapsae, Steinernema feltiae and Heterorhabditis bacteriophora*.

The purpose of our study was to determine the efficiency of using entomopathogenic nematodes *Steinernema carpocapsae* and *Heterorhabditis bacteriophora* against brown marmorated stink bug (BMSB) or *Halyomorpha halys* and their use for biological pest control. Today *Halyomorpha halys* as dangerous pest insect is widespread almost throughout all regions of Georgia.

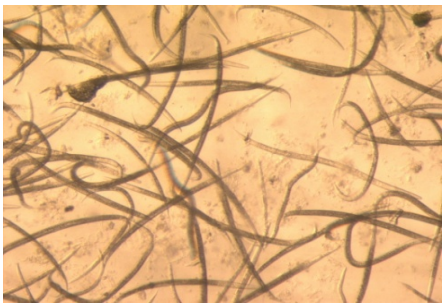
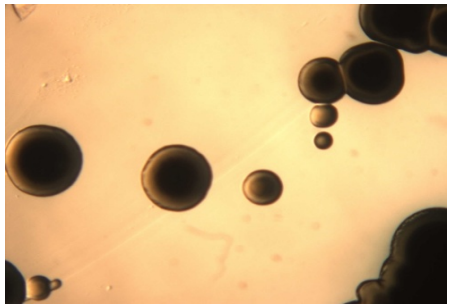
The brown marmorated stink bug is an invasive agricultural pest that has caused great damage to the crops across the west and eastern Georgia. It feeds on a wide array of plants including apples, apricots, Asian pears, cherries, corn, grapes, lima beans, peaches, peppers, tomatoes, and soybeans. This makes them extremely versatile as they do not require a specific plant to feed on. The adults are approximately 1.7 cm long. They are various shades of brown on both the top and undersides, with gray, off-white, black, copper, and bluish markings. The abundance and activity of brown marmorated stink bugs, *Halyomorpha halys* Stål, over-wintering inside a home in west Georgia were documented. The brown marmorated stink bug is more likely to invade homes in the fall. The bug survives the winter as an adult by entering houses and structures when autumn evenings become colder. Adults can live from several months to a year. They will enter under siding, into soffits, around window and door frames, chimneys, or any space which has openings big enough to fit through. Once inside the house, they will go into a state of hibernation. They wait for winter to pass, but often the warmth inside the house causes them to become active, and they may fly clumsily around light fixtures. During the 14-day study period 200 adult brown marmorated stink bugs were collected inside the house. Control measures to block exit from walls into living space reduced collection rate, but failed to halt it. This heavy infestation in a single home demonstrates the potential nuisance to lots of homes across the west part of country Georgia if the range and population of the brown marmorated stink bug continues to expand.

Figs. 4,5. Application of *S.carpocapsae* and *H. bacteriophora* against the *Halyomorpha halys*.

Among agro-technical measures, the fighting with unwanted weed plants, moderate irrigation, extra nutrition of the plant, etc. are used. The application of chemical methods also occurs in fighting against stink bug *(Halyomorpha halys)*, which must be carried on the basis of the State Catalogue of pesticides permitted for use in Georgia and consulting with appropriate service [1-3]. New safe biological means or suspensions of entomopathogenic nematodes give the best result in stink bug *(Halyomorpha halys*) control. These pathogens were introduced from Germany [4,5].

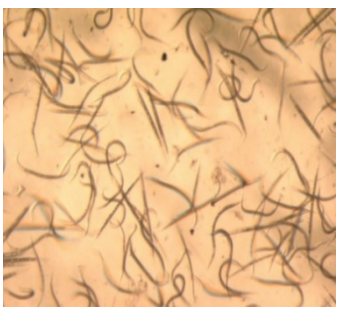
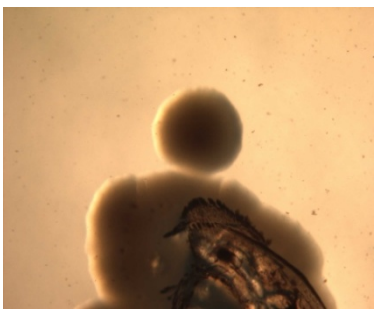
As is known, entomopathogenic nematode belongs to *S.carpocapsae* genus of *Steinernema*

Figs.6,7. *S.carpocapsae* and symbiotic bacteria *Xenorhabdus*.

and is associated with bacteria *Xenorhabdus*, while *Heterorhabditis bacteriophora* belongs to the genus *Heterorhabditis* and it is associated with bacteria *Photorhabdus*. Joint action of bacterium and nematodes leads to insect mortality which plays an important role in the regulation of the number of harmful insects. These species of nematodes are distinguished by safety to humans and the environment and they are effective biological agents for biological control of pests. The following cycle is characteristic of the development of *S.carpocapsae*: egg, four juvenile stages and the adult form. After covering with cuticle - protective film of the second stage juveniles, the nematodes stop feeding, leave the dead host and carry with them reproductive bacterium for infestation of a new host. Nematodes penetrate the hemolymph of a living host, inject into it symbiotic bacteria which causes insect mortality in approximately 24-72 h. Nematodes produce amphimictic population (nematodes of male and female genus) in the host intestinal.

The life cycle of *H. bacteriophora* consists of an egg, four juvenile stages and the adult. Only third-stage juveniles attack and infect host insects. This stage is the only free-living stage in the life cycle of the nematode, and is adapted to remain in the environment without feeding for a prolonged time. All other stages exist only inside the host. The infective juveniles move through soil in search of hosts. Once a host is encountered, the nematodes enter though natural openings or use their dorsal tooth or hook to break the outer cuticle of small, fragile insects to allow the juvenile to enter. Once the infective juveniles are in the host insect, they begin development. Their alimentary canal becomes functional and they release symbiotic bacteria to multiply in the insect. These bacteria are consumed and digested by the developing nematodes. The symbiotic bacterium *Photorhabdus luminescens* is only pathogenic to insects when introduced into the insect body, not if it is ingested. The bacterium is unable to survive in soil or water, so the nematode provides protection for the bacterium outside the insect host and a means of transmission to new hosts. The nematode is unable to reproduce without the nutrients the bacterium provides.

Figs.8,9. *H. bacteriophora* and symbiotic bacteria *Photorhabdus*

The bacteria kill the host, usually within 24-48 hours. Nematodes feed on the bacteria and host remains, and each infective juvenile develops into a hermaphroditic female. These females then produce eggs which will develop into both males and females. Only a portion of the eggs are laid outside the female; the remainder hatch inside the female and the juveniles destroy their mother as they develop. These nematodes mature, mate and produce infective juveniles that emerge from the cadaver 12-14 days after infection [4,5].

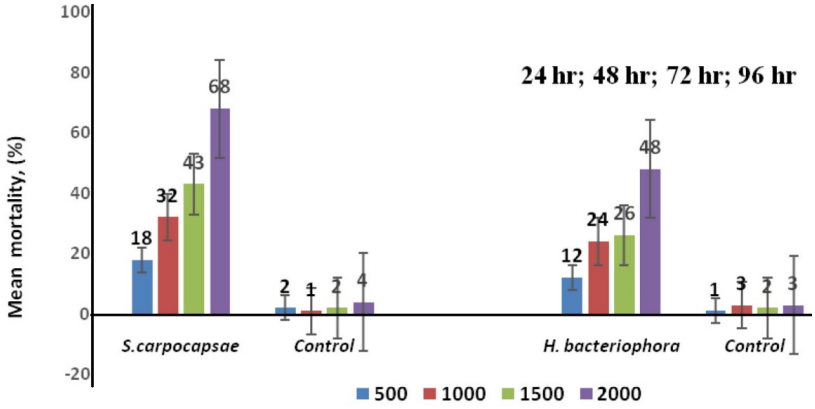


Fig. 10.Virulence of *S.carpocapsae* and *H.baceteriophora* against *Halyomorpha halys*(stink bug)

Materials and Methods

Prior to the use of entomopathogenic nematodes in the experiment, their cultivation occurred in an incubator at 24-25^{0}C on last-instar larvae of large wax moth (*Galleria mellonella*) using the appropriate method (Kaya, Stock 1997). Suspensions obtained as a result of cultivation were kept in a refrigerator at 4-6^{0}C. Acclimatization of nematodes proceeded under the conditions of room temperature 24-25^{0}C. The use of the obtained biomass was possible 6-10 h later. To determine the efficiency of *S.carpocapsae* and *H.bacteriophora* in conditions of room temperature 24-25^{0}C and 75% humidity for trial were used pest-grown form of imago. Mortality rate of individuals was determined by Abbott's formula (Abbot, 1925) [6,8].Our study aimed to determine the efficacy of entomopathogenic nematodes *Steinernema carpocapsae* and *Heterorhabditis bacteriophora* for biological control of stink bug*(Halyomorpha halys)*, in laboratory conditions. Experiments were conducted in a 10 cm Petri dishes. During the 14-day study period 200 adult brown marmorated stink bugs *(Halyomorpha halys)* were collected inside the home. They were placed on each Petri dish. The trial used *S. carpocapsae* and *H.bacteriophora* of 500, 1000, 1500, 2000 infective juveniles/ml. Insect mortality was examined on 3 5, 7 days after treatment [7,9].

Results

The results have shown that the virulence of *S. carpocapsae* against *H. halys* insect mortality is higher than that of *H.bacteriophora* which depends on the time, type and concentration of the nematodes. On the 7th day after treatment with a nematode suspension 500, 1000, 1500 and 2000 infective juveniles/ml of *S. carpocapsae* in the given trial reveals 18, 32,43,52 and 68% mortality rate whereas *H.bacteriophora* 12, 24, 26 and 48% respectively. The obtained results show that under laboratory conditions the efficiency of *S. carpocapsae* and *H.bacteriophora* against *H. halys* can be better controlled by *S. carpocapsae* rather than *H.bacteriophora* and therefore, future study is to be conducted in private houses, field and greenhouse conditions.

I would like to thank the students participating in the experiments from the Faculty of Natural Sciences and Health Care of the Sokhumi State University for their technical assistance

ენტომოლოგია

ენტომოპათოგენური ნემატოდების *Steinernema carpocapsae* და *Heterorhabditis bacteriophora*-ს კონტროლი აზიური ფაროსანას **Stink bug,** ***Halyomorpha halys (Hemiptera, Pentatomidae)*** მიმართ

ნ. მიქაია

სოხუმის სახელმწიფო უნივერსიტეტი,საბუნებისმეტყველო მეცნიერებათა და ჯანდაცვის ფაკულტეტი, თბილისი, საქართველო

(წარმოდგენილია აკადემიის წევრის ი.ელიავას მიერ)

ჩვენი კვლევის მიზანი იყო განგვესაზღვრა ენტომოპათოგენური ნემატოდების – ***Steinernema carpocapsae***-სა და ***Heterorhabditis bacteriophora***-ს ბიოლოგიური კონტროლი აზიური ფაროსანას, ***Halyomorpha halys*** მიმართ ლაბორატორიულ პირობებში. ენტომოპათოგენური ნემატოდების ექსპერიმენტში გამოყენებამდე, მათი კულტივირება ხდებოდა თერმოსტატში 24-25°C ტემპერატურაზე ცვილის დიდი ჩრჩილის ***(Galleria mellonella)*** ბოლო ხნოვანების მატლებზე (**Kaya, Stock 1997**) სათანადო მეთოდით. კულტივირების შედეგად მიღებული სუსპენზიები ინახებოდა მაცივარში 4-6°C ტემპერატურაზე. ნემატოდების აკლიმატიზება მიმდინარეობდა ოთახის 24-25°C ტემპერატურის პირობებში. მიღებული ბიომასის გამოყენება შესაძლებელი იყო 6-10 სთ შემდეგ. ***S. carpocapsae*** და ***H.bacteriophora*** ეფექტურობის დასადგენად ოთახის 24-25°C ტემპერატურისა და 75% ტენიანობის პირობებში საცდელად გამოყენებული იყო მავნებლის ზრდასრული ფორმა-იმაგო. ინდივიდების სიკვდილიანობის პროცენტი განისაზღვრებოდა აბოტის ფორმულით (**Abbot, 1925**). დასავლეთ საქართველოს, მარტვილის რაიონის კერძო სახლში შეგროვებული იყო აზიური ფაროსანას***(H.Halys)*** 200 ეგზემპლარი. ექსპერიმენტები ჩატარდა 10 ფილტრგადაკრულ 10 სმ პეტრის თასზე, თითოეულ პეტრის თასზე მოთავსებული იყო აზიური ფაროსანას ***(H.Halys)*** 20 ეგზემპლარი. ექსპერიმენტში გამოყენებულ იქნა ***S. carpocapsae*** და ***H.bacteriophora***-ს 500, 1000, 1500 და 2000 ინფექციური იუვენილი/მლ. მწერების სიკვდილიანობა შემოწმდა დამუშავების 3, 5, 7 დღის შემდეგ. შედეგებიდან ნაჩვენებია, რომ ნემატოდა ***S. carpocapsae*** მაღალი ვირულენტობისაა ***H. halys***–ის წინააღმდეგ, ვიდრე ***H.bacteriophora*** და მწერის სიკვდილიანობა დამოკიდებული იყო დროზე, ნემატოდების სახეობაზე და კონცენტრაციაზე. მე-7 დღეს ნემატოდა ***S. carpocapsae***-ს 500, 1000, 1500 და 2000 ინფექციური იუვენილები/მლ სუსპენზიით დამუშავების შემდეგ მოცემული ექსპერიმენტი აჩვენებს ***S. carpocapsae***-ს 18, 32, 43 და 68% სიკვდილიანობას, ვიდრე ***H.bacteriophora*** 12, 24, 26 და 48% შესაბამისად. როგორც შედეგიდან ჩანს, ლაბორატორიულ პირობებში განსაზღვრულია ***S. carpocapsae*** და ***H.bacteriophora*** ეფექტურობა ***H. halys***-ის მიმართ და ის შეიძლება იყოს კონტროლირებული ***S.carpocapsae***-თი, ვიდრე ***H.bacteriophora***-თი, ამიტომ სასურველია მომავალი კვლევა ჩატარდეს დასავლეთ საქართველოს კერძო სახლებში, მინდვრის პირობებსა და სათბურში.

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