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Entomology

Efficacy of Entomopathogenic Nematodes *Steinernema feltiae* and *Heterorhabditis bacteriophora* against the Melon Aphid (*Aphis gossypii* Glow., *Hemiptera, Aphididae*)

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(Presented by Academy Member Irakli Eliava)

ABSTRACT. This study was aimed to determine the efficiency of biological control of entomopathogenic nematodes *Steinernema feltiae* and *Heterorhabditis bacteriophora* against the melon aphid (*Aphis gossypii*) in the 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.feltiae* 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 formula. The trials were conducted on 10 cm Petri dishes. The obtained results have shown that the nematode *S.feltiae* is more effective against *A.gossypii* than *H.bacteriophora* and 500 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 infective juveniles/ml of *S.feltiae* in the given trial reveal 20, 58 and 78% mortality rate whereas *H.bacteriophora* 15, 28, and 46% respectively. The obtained results show that under laboratory conditions the efficiency of *S.feltiae* and *H.bacteriophora* against *A.gossypii* can be controlled by *S. feltiae* rather than *H.bacteriophora* and therefore, future study is to be conducted in greenhouse and field conditions. *© 2017 Bull. Georg. Natl. Acad. Sci.*

Key words: *Steinernema feltiae, Heterorhabditis bacteriophora, Xenorhabdus, Photorhabdus*

Introduction

The damage caused by pests reaches the 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, as well as social insects: cockroaches,

Figs. 1,2. Colonies of *A.gossypii* on cucumber leaves.

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

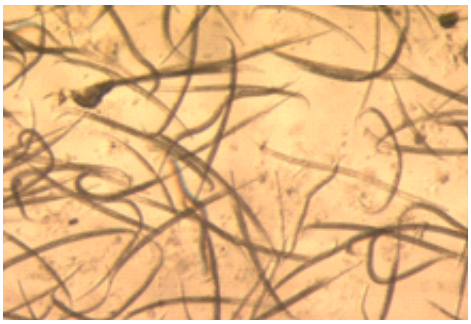
The purpose of our study was to determine the efficiency of using entomopathogenic nematodes *Steinernema feltiae* and *Heterorhabditis bacteriophora* in relation to melon aphid or *Aphis gossypii* and their use for biological pest control. Today *Aphis gossypii* is widespread in almost all regions of Georgia. Melon aphid, *Aphis gossypii* Glov. , lives on the lower part of the leaves of cultural and wild, melons plants - melon, cucumber, pumpkin and sucking sap from the plants due to which leaf veins are damaged, cramped and distorted.

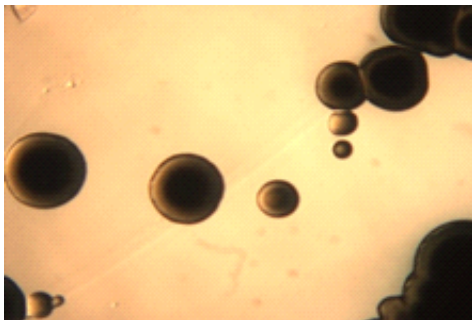
Besides leaves, aphids do damage to shoots and flower stems and fruit fails to develop. At the beginning, when the plant is tender and contains a large amount of sap, aphids feed avidly and leave the large amount of excrement. These excrements represent the best substrate for saprophytic fungi of the genus *Capnodium*. Such plants, as a rule, strongly attract ants, flies, wasps and other insects.

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 melon aphid, which must be carried on the basis of the State Catalogue of pesticides permitted for use in Georgia and consulting with appropriate service [1, 2]. New safe biological means or suspensions of entomopathogenic nematodes give the best result in melon aphid control. These pathogens were introduced from Israel and Germany [3,4].

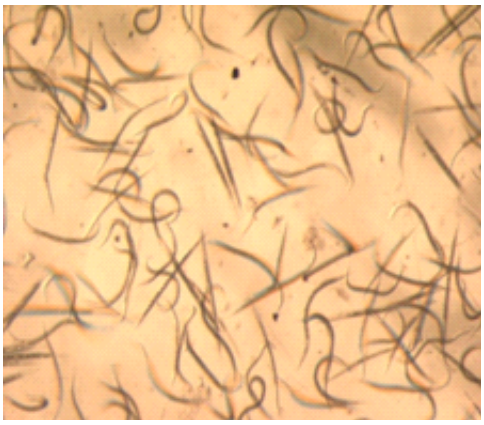
As is known, entomopathogenic nematode belongs to *S.feltiae* genus of Steinernema and is associated with bacteria *Xenorhabdus*, while *Heterorhabditis* bacteriophora belongs to the genus *Heterorhabditis* and is associated with bacteria *Photorhabdus*. Joint action of bacterium and nematodes leads 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 for the development of *S.feltiae*: 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 into 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.

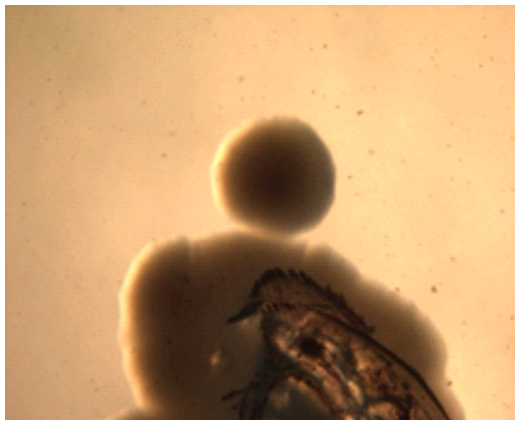
The life cycle of H. bacteriophora consists of an egg, four juvenile stages and the adult. Only third-stage juveniles attack and infect host insects. This





Figs. 3, 4. *S. feltiae* and symbiotic bacteria *Xenorhabdus*





Figs. 5, 6. *H. bacteriophora* and symbiotic bacteria *Photorhabdus.*

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.

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 [3,4].

Materials and Methods

Prior to the use of entomopathogenic nematodes in the experiment, their cultivation occurred in an incubator at temperature 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 a temperature of 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.feltiae* 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 formula**.** (Abbot, 1925) [5,7]. Our study aimed to determine the efficacy of entomopathogenic nematodes *Steinernema feltiae* and *Heterorhabditis bacteriophora*- biological control of melon aphid (*Aphis gossypii*) in laboratory conditions. Experiments were conducted on a 10 cm Petri dishes. One infected cucumber plant leaf contained approximately 120-150 of *A.gossypii*, which were placed on each Petri dish. The trial used *S.feltiae*- and *H.bacteriophora* of 500, 1000, 1500 infective juveniles/ ml. Insect mortality was examined on 3 5, 7 days after treatment [6,8].

Results

The results have shown that the high virulence of *S. feltiae* against *A.gossypii* insect mortality than *H.bacteriophora* depended on the time, type and concentration of the nematodes. On the 7th day after treatment with a nematode suspension 500, 1000, 1500 infective juveniles/ml of *S.feltiae* in the given trial reveals 20, 58 and 78% mortality rate whereas *H.bacteriophora* 15, 28, and 46% respectively. The obtained results show that under laboratory conditions the efficiency of *S.feltiae* and *H.bacteriophora* against *A.gossypii* can be controlled by *S. feltiae* rather than *H.bacteriophora* and therefore, future study is to be conducted in greenhouse and field conditions.

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ენტომოლოგია

ენტომოპათოგენური ნემატოდების *Steinernema feltiae* და *Heterorhabditis bacteriophora*-ს ეფექტურობა ნესვის ბუგრის (*Aphis gossypii Glow, Hemiptera Aphididae*) 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 feltiae* და *Heterorhabditis bacteriophora*-ს ბიოლოგიური კონტროლის ეფექტურობა ნესვის ბუგრის (*Aphis gossypii*) მიმართ ლაბორატორიულ პირობებში. ენტომოპათოგენური ნემატოდების ექსპერიმენტში გამოყენებამდე, მათი კულტივირება ხდებოდა თერმოსტატში 24-25^{0}C ტემპერატურაზე ცვილის დიდი ჩრჩილის (*Galleria mellonella*) ბოლო ხნოვანების მატლებზე (Kaya, Stock 1997) სათანადო მეთოდით. კულტივირების შედეგად მიღებული სუსპენზიები ინახებოდა მაცივარში 4-6^{0}C ტემპერატურაზე. ნემატოდების აკლიმატიზირება მიმდინარეობდა ოთახის 24-25^{0}C ტემპერატურის პირობებში. მიღებული ბიომასის გამოყენება შესაძლებელი იყო 6-10 სთ. შემდეგ. *S.feltiae* და *H.bacteriophora* ეფექტურობის დასადგენად ოთახის 24-25^{0}C ტემპერატურისა და 75% ტენიანობის პირობებში საცდელად გამოყენებული იყო მავნებლის ზრდასრული ფორმა-იმაგო. ინდივიდების სიკვდილიანობის პროცენტი განისაზღვრებოდა აბოტის ფორმულით (Abbot, 1925). ექსპერიმენტები ჩატარებული იყო ფილტრგადაკრულ 10 სმ პეტრის თასზე. ერთი ინფიცირებული კიტრის მცენარის ფოთოლი მიახლოებით შეიცავდა 120-150 *A.gossypii*, რომელიც მოთავსებული იყო თითოეულ პეტრის თასზე. ექსპერიმენტში გამოყენებული იყო *S.feltiae*-ს და *H.bacteriophora*-ს 500, 1000, 1500 ინფექციური იუვენილები/მლ. მწერების სიკვდილიანობა იყო შემოწმებული დამუშავების მერე 3, 5, 7 დღის შემდეგ. შედეგებიდან ნაჩვენებია, რომ ნემატოდა *S. feltiae* იყო მაღალი ვირულენტობის *A.gossypii*-ის წინააღმდეგ, ვიდრე *H.bacteriophora* და მწერის სიკვდილიანობა დამოკიდებული იყო დროზე, ნემატოდების სახეობასა და კონცენტრაციაზე. მე-7 დღეს ნემატოდა *S.feltiae*-ს 500, 1000, 1500 ინფექციური იუვენილები/მლ სუსპენზიით დამუშავების შემდეგ მოცემული ექსპერიმენტი აჩვენებს *S.feltiae*-ს 20, 58, და 78% სიკვდილიანობას, ვიდრე *H.bacteriophora* 15,28, და 46% შესაბამისად. როგორც შედეგიდან ჩანს, ლაბორატორიულ პირობებში განსაზღვრული იყო *S.feltiae* და *H.bacteriophora* ეფექტურობა *A.gossypii*-ის მიმართ და ის შეიძლება იყოს კონტროლირებული *S. feltiae*-თი, ვიდრე *H.bacteriophora*-თი, ამიტომ სასურველია მომავალი კვლევა ჩატარდეს სათბურსა და მინდვრის პირობებში.

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