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Entomology

Tolerance of Israel Local Strains of Entomopathogenic Nematodes *Steinernema Feltiae* and *Heterorhabditis Bacteriophora* at the 37°C Temperature

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ABSTRACT. The overall aim was to study the tolerance of some entomopathogenic nematodes. The experiments were conducted at Laboratory of Nematology, Institute of Plant Protection, ARO, Israel. The strains of *Steinernema feltiae* (SFG) - SFG-Besor, SFG-zeelim orange, HP-Besor, HP-Dvora, HP-Grofit and *Heterorhabditis bacteriophora* (HP) were used in experiments. In control tests the SFG was used. The nematodes were transferred to Petri dishes, where the distilled water was poured. Petri dishes with nematode suspensions were placed at the room temperature during 24 hr; after that the alive nematodes were calculated. The tolerance of entomopathogenic nematodes strains *Steinernema feltiae* (SFG-Besor, SFG-Zeelim orange) and *Heterorhabditis bacteriophora* (HP-Besor, HP-Dvora, HP-Grofit) at 37ºC is presented. The higher tolerance of SFG strains - SFG-Besor, SFG-Zeelim orange in comparison with HP (HP-Besor, HP-Dvora, HP-Grofit) is established. The aim of the present study was to isolate native strains of EPNs from varied climatic regions in Israel and to evaluate traits which are important as biological control agents. *© 2015 Bull. Georg. Natl. Acad. Sci.*

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

Entomopathogenic nematodes belong to the genus *Steinernema* and *Heterorhabditis,* which are associated with the bacteria *Xenorhabdus* and *Photorhabdus,* respectively [1]. The life cycle of insecticidal nematodes appears simple: infective juveniles (IJs) search for suitable insect hosts; infective juveniles penetrate into the hemocoel using mechanical and enzymatic means. Shortly after entering the nematode the symbiotic bacterium releases. The nematode and its bacterial partner work together to overcome the immune system of susceptible insects quickly causing their host's death. The nematodes feed upon the rapidly multiplying bacteria, mature, mate and produce two or more generations within the insect cadaver before emerging as infective juveniles in search of fresh hosts. The most limiting factor for nematode activity under laboratory conditions is the heat tolerance. All nematodes are aquatic organisms and need a film of water surrounding their body in order to move in dry conditions adversely

affecting nematode motility and survival [2]. Some free-living stages of several animal and plant-parasitic nematodes can survive exposured to desiccation for long periods [3], which slow the rate of water loss by reducing the area of cuticle exposed to air. On exposed surfaces, *Steinernematids* and *Heterorhabditids* can survive no longer than several hours, depending on species, temperature and relative humidity [4].

In the present study, we used both invasion rate and dose response assays to compare the various isolates. One of the major factors hindering the use of nematodes as biological control agents is their sensitivity to environmental stresses, such as extreme temperatures and low humidity (for a review, see Glazer) [4]. On exposed surfaces, *Steinernematids* and *Heterorhabditids* can survive no longer than several hours, depending on the species, ambient temperature and relative humidity. In dry soil, EPNs can persist for 2-3 weeks [5, 6]. It is assumed that this survival is due to the nematodes' ability to enter a state of anhydrobiosis [7, 3]. Anhydrobiosis is usually reached by the slow loss of water from the body of the nematode [8]. Some nematodes form tight coils when exposed to desiccation [3]. This strategy slows the rate of water loss by reducing the area of cuticle exposed to air. Another environmental factor hampering EPN activities is high temperatures [9]. Nevertheless, it has been reported that EPNs, isolated in a region with a hot climate, can tolerate high temperatures for extended periods [10]. In the present study, the capabilities of the nematodes to withstand desiccation and high temperature were evaluated as part of the overall evaluation of the populations. The aim of the present study was to isolate native strains of EPNs from varied climatic regions in Israel and to evaluate traits which are important as biological control agents.

Entomopathogenic nematodes (EPNs) from *Steinernematidae* and *Heterorabditidae* families are effective biological control agents because of their ease of culture, high lethality against key pests and safety.

Materials and Methods

The experiments were conducted at Laboratory of Nematology, Institute of Plant Protection, ARO, Israel. The strains of *Steinernema feltiae* (SFG) – SFG-Besor, SFG- zeelim orange, HP-*Besor,* HP-Dvora, HP-Grofit and *Heterorhabditis bacteriophora* (HP) were used in experiments. The strains were obtained in the north part of Israel, Golan Mountain, 700 m above sea level. In control tests the SFG was used. The filtration of tested strains (SFG-Besor, SFG-zeelim orange, HP-Besor, HP-Dvora, HP- Grofit) was done in the device Buckner, each filtrated nematode strain *(S.feltiae)* was kept at 22.5ºC and *H.bacteriophora* strains - at 17.3ºC during 24 hr. After that the strains were put in water bath at 37ºC; then 1 ml suspension from each strain was transferred to Petri dishes in every 2 hr during 6 hr, where previously the distilled water was poured. Petri dishes with nematode suspensions were placed at the room temperature during 24 hr; after that the alive nematodes were calculated.

Results

The results were expressed in graphical forms showing the tolerance of nematodes at 37ºC temperature. Survival range for SFG in 120 minutes was 78%, SFG-Besor 65% and SFG - Zeelim orange 61%. Survival range for *SFG* in 240 minutes was 69%, SFG- Besor 53%, SFG- Zeelim orange 48%. Survival range for SFG in 300 minutes was 65%, SFG- Besor 47%, SFG-Zeelim orange 45%. Survival range for SFG in 360 minutes was 58%, SFG-Besor 44%, strain SFG-Zeelim 41%. The survival range of SFG after 2 hr at 37ºC was higher than its other strains (SFG-besor, SFG-zeelim orange). The data are presented in Fig. 1.

Survival range for HP-Besor in 120 minutes was 62%, HP-Dvora 78% and HP- Grofit 58%. Survival range for HP- Besor in 240 minutes was 55 %, HP-Dvora 63%, HP- Grofit 51%. Survival range for HP-Besor in 300 minutes was 48%, HP-Dvora 55%, HP-Grofit was 40 %. Survival range for HP-Besor in 360 minutes was 39%, HP- Dvora 52 %, HP-Grofit 34 %. The survival range of HP-Dvora after 2 hr at 37ºC was

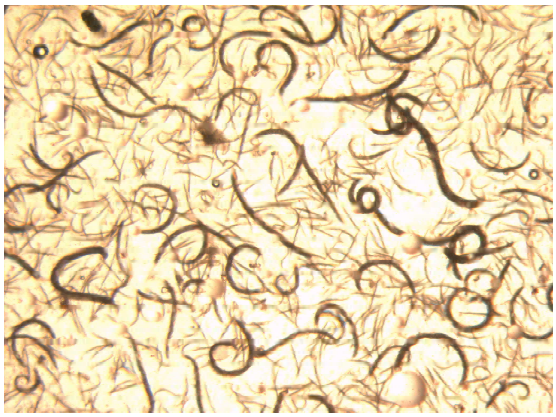

Fig.1. *Steinernema feltiae.*

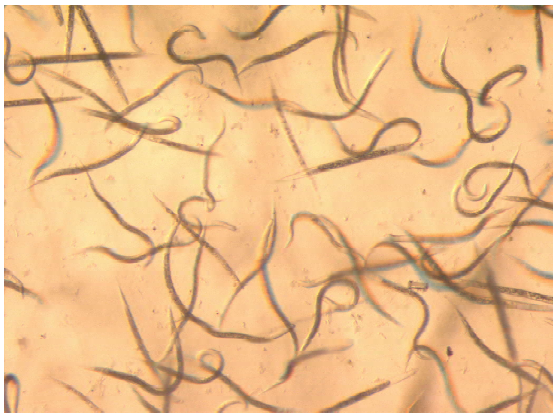

Fig.2. *Heterorhabditis bacteriophora.*

higher than its other strains (HP-besor, HP-grofit). The data are presented in Fig. 2.

Nematode viability was recorded for each population by observing nematode motility and response to probing under a stereomicroscope 24 h later. In each sample 200 individuals were randomly examined for viability. Control treatment consisted of nematode suspension shaken at room temperature. Each treatment consisted of eight replicates. The experiments were repeated three times. The tested strains exhibited different levels of tolerance to a harsh temperature regime (Fig. 3, 4). Several Steinernematid and Heterorhabditid strains were able to survive at levels higher than 78%, whereas the population obtained in Besor and the reference populations (*S. feltiae* SfG and *H. bacteriophora* HP88) did not do well under conditions of excessive heat (>39% survival at 37°C; Fig. 3, 4). Most populations showed moderate levels of heat tolerance (40-78% survival at 37°C).

As a result of investigations the more tolerance of SFG strains (SFG-Besor, SFG-Zelim orange) in compare with HP strains (HP-besor, HP-grophit, HP-Dvora) was established.

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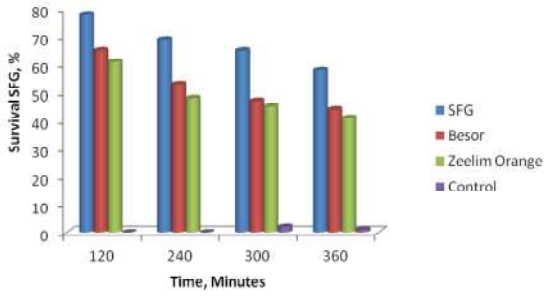

Fig. 3. Survival of infective juveniles SFG after exposure to 37 °C.

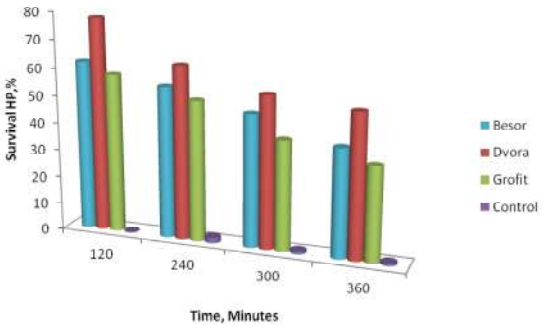

Fig. 4. Survival of infective juveniles HP strains after exposure to 37 °C.

ენტომოლოგია

ისრაელის ენტომოპათოგენური ნემატოდების ადგილობრივი შტამების *Steinernema feltiae* და *Heterorhabditis bacteriophora* მიმღებიანობა 37°C ტემპერატურაზე

ნ. მიქაია

სოხუმის სახელმწიფო უნივერსიტეტი, თბილისი

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ჩვენი მთავარი მიზანი იყო ზოგიერთი ენტომოპათოგენური ნემატოდას ტოლერანტობის შესწავლა 37°C ტემპერატურაზე. ექსპერიმენტები ჩატარებული იყო ისრაელის მცენარეთა დაცვის ინსტიტუტის ნემატოლოგიის ლაბორატორიაში. ექსპერიმენტში გამოყენებული იყო *Steinernema feltiae* (SFG) - SFG-Besor, SFG- zeelim orange, HP-Besor, HP-Dvora, HP- Grofit და *Heterorhabditis bacteriophora* (HP) სახეობები, საკონტროლო ცდაში შესამოწმებლად გამოყენებული იყო SFG თითოეული გაფილტრული ნემატოდას სახეობები. ნემატოდები გადატანილი იყო პეტრის თასებზე, სადაც წინასწარ იყო დასხმული გამოხდილი წყალი. პეტრის თასები ნემატოდური სუსპენზიით მოთავსებული იყო ოთახის ტემპერატურის პირობებში 24 საათის განმავლობაში. შემდეგ ცოცხალი ნემატოდები იყო დათვლილი. ენტომოპათოგენური ნემატოდების *Steinernema feltiae* (SFG - Besor, SFG-Zeelim orange) და *Heterorhabditis bacteriophora* (HP-Besor, HP-Dvora, HP-Grofit ტოლერანტობა 37°C ტემპერატურაზე იყო წარმოდგენილი. მაღალი ტოლერანტობა აღინიშნება SFG სახეობებზე-SFG-Besor, SFG-Zeelim orange, HP (HP-Besor, HP-Dvora, HP-Grofit) შედარებისას. არსებული კვლევის მიზანი იყო ენტომოპათოგენური ნემატოდების ისრაელის ადგილობრივი შტამების იზოლირება განსხვავებული კლიმატური რეგიონებიდან და ნემატოდების ტოლერანტობის შეფასება,რომელიც მნიშვნელოვანია როგორც ბიოლოგიური კონტროლის აგენტები ისრაელისთვის და საქართველოსთვის.

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