Turkish Journal of Biology

Volume 27 | Number 3 Article 5

1-1-2003

Evaluation of the Reproductive Potential and Competition between Two Entomopathogenic Nematodes, Steinernema feltiae Filipjev, 1934 (Rhabditida: Steinernematidae) and Heterorhabditis bacteriophora, Poinar 1976 (Rhabditida: Heterorhabditidae)

IŞIK OĞUZOĞLU ÜNLÜ

NURDAN ÖZER

Turk J Biol
27 (2003) 149-155

Evaluation of the Reproductive Potential and Competition between Two Entomopathogenic Nematodes, *Steinernema feltiae* Filipjev, 1934 (Rhabditida: Steinernematidae) and *Heterorhabditis bacteriophora*, Poinar 1976 (Rhabditida: Heterorhabditidae)

Işık OĞUZOĞLU ÜNLÜ*, Nurdan ÖZER
Department of Biology, Faculty of Science, Hacettepe University, 06532 Beytepe, Ankara - TURKEY

Received: 20.09.2002

Abstract: The emergence in *Galleria mellonella* (L.) larva, which was infected by *Steinernema feltiae* and *Heterorhabditis bacteriophora*, began on the ninth day after exposure to the former nematode and the sixth day of exposure to the latter nematode (25 ± 0.5 ºC). The average number of nematodes developing in each host was as 13,829, and the range was from 4365 to 27,510 for S. feltiae infection. The average number of nematodes per host was 141,562, and the range was 50,905 - 271,593 for *H. bacteriophora* infection. When the 2 different species were inoculated simultaneously into the same host, *H. bacteriophora* caused higher mortality rates than S. feltiae. As a result of the application of 2 nematode species to the same host at different times, the first nematode species to be inoculated was responsible for the highest mortality rate in all cases.

Key Words: Entomopathogenic nematodes, *Steinernema feltiae*, *Heterorhabditis bacteriophora*, Reproductive potential, Competition

Steinernema feltiae Filipjev, 1934 (Rhabditida: Steinernematidae) ve *Heterorhabditis bacteriophora*, Poinar 1976 (Rhabditida: Heterorhabditidae) Arasındaki Üreme Potansiyeli ve Rekabetin Değerlendirilmesi

Özet: *Steinernema feltiae* ve *Heterorhabditis bacteriophora* ile enfekte olan *Galleria mellonella* larvalarından enfektif juvenil çıkışı, S. feltiae'de karşı karşıya kaldıktan 9 gün sonra, *H. bacteriophora*'da ise 6 gün sonra başladı. Her bir konakta gelişen nematodların ortalama sayısı 13.829 olup S. feltiae enfeksiyonu için bu sayı 4365 ile 27.510 arasında değişti. Konak başına düşen ortalama nematod sayısı 141.562 olup *H. bacteriophora* enfeksiyonu için bu sayı 50.905 ile 271.593 arasındadır. İki farklı tür aynı anda aynı konağa inoküle edildiğinde, *H. bacteriophora* S. feltiae'den daha fazla ölüme neden olmuştur.Aynı konağa farklı zamanlarda iki farklı nematod türünün uygulanması sonucunda gözlenen yüksek ölüm oranlarından, her durumda ilk olarak ortalama verilen nematod türü sorumludur.

Anahtar Sözcükler: entomopatojen nematodlar, *Steinernema feltiae*, *Heterorhabditis bacteriophora*, üreme potansiyeli, rekabet

Introduction

Nematodes of the families Steinernematidae and Heterorhabditidae have been the subject of interest lately because of their great potential as biological agents. These nematodes, mutualistically associated with bacteria in the genera Xenorhabdus and Photorhabdus, are similar in their actions. The bacterial cells voided from the nematode's intestine into the hemolymph propagate and kill the insect host by septicemia within 48 h. The nematodes feed on bacterial cells and host tissue, produce 2 or 3 generations and emerge from the host as infective juveniles in search of new hosts. Infective juveniles are the only free living stage of the nematodes in nature, and after entering susceptible hosts they and their bacterial partners kill their insect hosts so quickly that they do not form the intimate, highly adapted host-parasite relationships characteristic of other insect-nematode infections (1-7). They possess many key attributes for biological control, including a broad host range, high virulence, great ability for host seeking, ease of mass production and safety (8). Furthermore, researchers recently realized that both living and dead infective juveniles of EPN (*Steinernema carpocapsae*) cause more than a 50% reduction in total populations of plant parasitic nematodes (9).

* This is part of I. (Oğuzoğlu) Ünlü's MS thesis, under the supervision of Assoc. Prof. Nurdan Özer.

The host ranges of steinernematids and heterorhabditids are very broad, including several hundred insect species from at least 10 orders (10). Despite this impressive spectrum of activity, considerable variation has been found in the virulence of different nematode strains to specific insects (11-18). In the present study, 2 entomopathogenic nematode species, *Steinernema feltiae* (All type) and *Heterorhabditis bacteriophora* TUR-H2, were used in a host-parasite model and the differences between their reproduction capacity and competition properties were investigated. We think that studies of this kind will provide fundamental knowledge useful in increasing our ability to use these 2 species effectively in biological control (19). We know very little about the population biology of entomopathogenic nematodes, yet such information is fundamental to understand their persistence, reproductive potential and effect on insect populations, and to develop predictive models for control programs (3,7,18).

Materials and Methods

The *S. feltiae* (All type) used in the experiments was first isolated from Turkish soil samples taken from the Black Sea coast (20). The other species, *H. bacteriophora* TUR-H2, was obtained from the study field of the Agricultural Research Institute in Ankara (21). Nematode identifications were confirmed by Imperial College and Christian Albrechts University, Kiel (20,21). This species has been harvested from last instar larvae of *Galleria mellonella* (L.) in the laboratory at 25 ºC and 86.77% humidity since that time. The infective juveniles were stored in deionized water and stored at 15 ºC and used within 10 days of harvest.

Reproductive potential of *S. feltiae* and *H. bacteriophora*

Our experiments were conducted with larvae of a suitable host, *G. mellonella*, at 25 ºC. One hundred infective juvenile nematodes (IJs) of *S. feltiae* and 100 *H. bacteriophora* in 1.4 ml of deionized water were allowed to each invade last instar *G. mellonella* larva, which weigh 200 mg, in filter paper-substrate petri dishes (3.5 x 1.2 cm). Density-dependent factors within a host can have an important influence on the population dynamics of parasites. According to Selvan et al., *S. carpocapsae* and *H. bacteriophora* have optimum survival at a density of approximately 100 nematodes per host. In addition, *H. bacteriophora* is better able to tolerate higher densities (22). Depending on this data, we also decided to utilize 100 infective juveniles per host. Afterwards, 10 closed petri dishes, each containing 1 *G. mellonella* larva, were enclosed in plastic bags to maintain moisture and kept at 25 ± 0.5 ºC. White traps were used to collect IJs emerging from *G. mellonella* cadavers (23) and the experiments were replicated 10 times. White traps for *S. feltiae* and *H. bacteriophora* were arranged using 2 x 1.2 cm plastic petri dish lids lined with a single Whatman No. 1 filter paper; 3.5 cm in diameter moistened with deionized this lid was floated on sterilized deionized water in a 10 x 1.2 cm glass petri dish for collecting IJs. Dead larvae were observed until the last infective juveniles emerged. The number of nematodes yielded was determined daily for each host by counting 10 subsamples per replicate in 0.2 ml (24,25).

Competition Trials

Application of 2 nematode species to the same host simultaneously

In the first test, 40 IJs of each nematode species in 0.60 ml of deionized water (*S. feltiae* and *H. bacteriophora*) were simultaneously applied to the same host synchronically in petri dishes (10 x 1.2 cm) padded with filter paper (Whatman No. 1) containing 12 *G. mellonella* last instar larvae. In the second test we used a concentration of 80 IJs in 0.60 ml of deionized water. In this manner we observed the competition between *S. feltiae* and *H. bacteriophora* in *G. mellonella* (26).

For this procedure we used different kinds of control groups. One group was exposed to 40 IJs (and 80 IJs for the other procedure) of either *S. feltiae* or *H. bacteriophora* only, and the other group that was free of nematodes. Three dishes were used for each nematode/host combination. The experiments were replicated 3 times. After 72 h the cadavers were examined for changes in coloration. The larvae infected by *H. bacteriophora* turned from red to dark brown (26). An ocher color was observed in *S. feltiae* infected larvae. Following the period of nematode emergence (6-7 days) the cadavers were dissected and examined to verify species. At the end of the trials when the nematodes were present, slides containing juvenile heterorhabditis and steinernematids were readily separated by several morphological characters (26). In this study, we

distinguished the nematodes from each other by IJ body length, tail shape and the different color of the infected larvae. Twenty nematodes were used to determine which species was present in the cadaver (26).

Application of 2 nematode species to the same host at different times

The infective juveniles of 1 species were inoculated into filter paper-substrate petri dishes containing *G. mellonella* last instar larvae 5 h earlier than the application of the other species (50 *S. feltiae* and 50 *H. bacteriophora*). Mortality was recorded after 72 h. The cadavers were dissected to ascertain nematode invasion and to determine which nematode species had entered the host. Observations of dead hosts were also made on the color of the larvae to determine whether they had a reddish look, a characteristic of infection by the symbiotic bacteria associated with heterorhabditids (26).

Results

The emergence of infective nematode stages from the nematode-killed wax moth larvae infected by *S. feltiae* began on day 9 after the hosts' death and continued for approximately 10 days (8-12 days). The mean number of nematodes emerging from each host larva was 13,829, ranging from 4365 to 27,510 (Fig. 1).

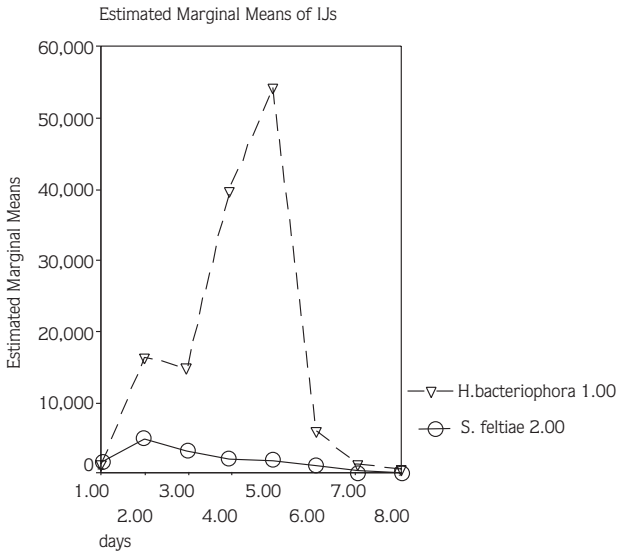

Figure 1. The mean number of nematodes (*H. bacteriophora* and *S. feltiae*) recovered from hosts between 1 and 8 days after the beginning of emergence.

Emergence first occurred on the day 7 after the hosts' death when infected with *H. bacteriophora* and continued for approximately 12 days (11-14 days). The same treatments were repeated and similar results were obtained in the proceeding experiments. The mean number of nematodes was 141,562, from a minimum of 50,905 to a maximum of 271,593 (Fig. 1).

The highest number of emerged infective juveniles from *G. mellonella* larvae was observed on days 4 and 5 after the beginning of the emergence period for *H. bacteriophora,* and on days 2 and 3 for *S. feltiae* (Fig. 1).

A two-way ANOVA of the data (Table 1) shows that the difference between the species in the number of nematodes released is significant (two-way ANOVA as performed by log-transformed data). The specific time at which nematodes were released is also significant within and between species. Overall results strongly suggest that the 2 species have distinct host features with substantial dependence on releasing time.

In competition bioassays, the mortality rate of the *G. mellonella* infected by *S. feltiae* only was 62.9% (40 IJs per petri dish) The mortality rate was 67.5% with *H. bacteriophora* (40 IJs per petri dish). When 80 IJs were inoculated per petri dish we found the same rate of mortalities for both *S. feltiae* and *H. bacteriophora* (75.8%). However, when both nematode species were inoculated into the same host simultaneously, the mortality rate was 99% (20 *S. feltiae* and 20 *H. bacteriophora*). When 40 infective juveniles from each species were given simultaneously, the mortality rate was 98%. Those rates were much higher than that of each species inoculated respectively (Table 2).

In another procedure, the 2 nematode species were applied at definite time intervals rather than being applied synchronically. *G. mellonella* larvae were exposed to *S. feltiae* infective juveniles and after 5 h *H. bacteriophora* infective juveniles were added to the medium. At the end of 72 h when *S. feltiae* was first applied a 96.2% infection rate was observed and 21.2% of the mortality rate was due to *H. bacteriophora,* while 75% was due to *S. feltiae*, according to dissections and color changes (Table 3). When *H. bacteriophora* was applied first, the infection rate was 94.4% after 72 h and 72.2% of the mortality was due to *H. bacteriophora*, while 22.2% of the mortality rate was due to *S. feltiae* (Table 3). The mortality rate of *G. mellonella* larvae exposed to only one entomopathogenic species was less than the rate after the

Table 1. ANOVA of the nematodes released by the two species.

Dependent Variable A					
Source	Type III Sum of Squares	df	Mean square	F	Sign.
Species	22.717	1	22.717	97.589	.000
Day	57.717	7	8.257	35.469	.000
Species Day	7.308	7	1.044	4.485	.000
Error	33.521	144	0.233		

a. R Squared = 0.724 (Adjusted R Squared = 0.695)

Table 2. Infection rate of *G. mellonella* by *H. bacteriophora* and *S. feltiae*, following simultaneous inoculation of the species.

Number of IJs	Time	Rate of infection (%)		
		Sf [a] (%)	Hb [b] (%)	Sf and Hb(%)
40	48	50.9	53.7	87.9
	72	12.03	13.8	11.1
	Total	62.93	67.5	99
80	48	66.6	71.2	95.3
	72	9.2	4.6	2.7
	Total	75.8	75.8	98

[a] *S. feltiae*
[b] *H. bacteriophora*

Table 3. Infection rates of *G. mellonella* by *H. bacteriophora* and *S. feltiae* following consecutive inoculations.

Nematodes	Number of IJs	Rate of infection (%)		
		Sf [a] (%)	Hb [b] (%)	Sf ve Hb(%)
S. feltiae[c]	50	75	21.2	96.2
H. bacteriophora[d]		22.2	72.2	94.4

[a] *S. feltiae*
[b] *H. bacteriophora*
[c] *S. feltiae* first inoculated
[d] *H. bacteriophora* first inoculated

inoculation of the 2 species. In reciprocal tests, the species inoculated first had the competitive advantage. According to statistical analyses, the differences between these results ($X^2_{0.001(5)} = 20.517$) were significant.

Discussion

It is difficult to know how many IJs have entered a host body for such a procedure, because we have to trace the dauer larvae that leave the host body (25). The

experiments showed that the reproductive potential of *H. bacteriophora* was higher than that of *S. feltiae* considering the number of infective juveniles emerging from the cadavers and the duration of emergence. Due to *H. bacteriophora*'s hermaphroditic life cycle, this result is not suprising (22,23).

According to Poinar, entomopathogenic nematodes can be reared by in vivo methods, with yields of 100,000-200,000 infective stage juveniles per *G. mellonella* larva (10). According to Woodring and Kaya (1988), up to 350,000 *H. bacteriophora* infective juveniles have been harvested from one last-instar *G. mellonella* larva (23). Average production is much less, in the order of 30,000 to 50,000 infectives per insect. In our study we obtained similar numbers of IJs for *H. bacteriophora*; however, we could not attain such great numbers for *S. feltiae* IJs. The body size of the host definitely affects the total number of IJs developing inside the cadaver. We used 200 mg of *G. mellonella* for our experiments and this apparently affected the result.

Differences between the reproduction potential of entomopathogenic nematodes may be related to the isolates, species, host susceptibility, number of bacteria per infective stage, invasion rate, temperature and humidity. It is possible that differences in virulence between species and isolates might be greater for a less susceptible host. Prior to biocontrol applications, bioassays should be performed against the target pest insect (27). Supporting the ideas above, according to Mari et al., 30,400 IJs were yielded from *G. mellonella* (100 IJs per larvae) when they were infected with *H. megidis* NLH-E87.3, although this result is not as high as is normally expected from the genus Heterorhabditis (28).

Under in vivo conditions, *H. bacteriophora* IJs entering a host insect encounter a food signal that immediately induces the recovery of IJs. The hermaphrodite that develops from recovered IJs first lays eggs until the egg production rate decreases. At that moment, the first stage juveniles hatch within the uterus. Owing to the low food concentration in the uterus, the development of infective juveniles is induced. The juveniles develop and feed on the body of the hermaphrodite until all tissues are digested (endotokia matricida). After the hermaphrodite has died, the IJs emerge from the empty carcass. These IJs either recover and develop into a second generation of hermaphrodites or are arrested in the infective juvenile stage, depending on the food signal concentration in the surrounding medium. Thus the bacterial food signal indicates whether food is abundant for another propagative life cycle or whether the resources have been used up (29). If the nutrients are consumed, the IJs leave the insect cadaver in search of other hosts (30). An overview of *H. bacteriophora*'s life cycle supports our findings about the early emergence of *H. bacteriophora*. Time of emergence also strongly depends on the host, especially on its size. It is known from *S. feltiae* that time of emergence can vary dramatically with the size of the host. In the small sciarid flies nematodes will emerge within 6-7 days whereas in *G. mellonella* it can take 2-3 weeks (31). Probably, the use of 200 mg *G. mellonella* larvae is responsible for such surprising results in our study.

According to Rosas and Kaya (26), in competition trials between *S. carpocapsae* and *H. bacteriophora*, *S. carpocapsae* was the most successful species in every competitive condition they tested. In our applications, the nematode species inoculated first has an advantage in parasitizing the host. According to these researchers, *S. carpocapsae* infected more *G. mellonella* larvae than *H. bacteriophora* when *H. bacteriophora* was inoculated first. We speculate that *S. feltiae* does not seem to have as much competitive advantage as *S. carpocapsae*. In their research they also obtained a high mortality rate when they used 2 species together, and our results resemble their findings in this respect.

In biological-control campaigns, it is vital to know whether releasing one natural enemy against a pest is likely to be more effective than the release of many, especially where competition between enemies might reduce their overall effectiveness (22). As Gaugler and Kaya (8) reported, the combination of 2 nematode species with different search strategies to control 1 or 2 susceptible insect pest species in a soil habitat appears feasible. Indeed, combinations of different nematode species and other biological control agents may increase their overall efficacy against an insect pest (27).

Acknowledgments

We are grateful to Dr. W.B. Hominick and Dr. Bernie Briscoe, who gave us the fundamental information on enthomopathogenic nematodes and encouraged us to study them. We are also grateful to our friend Dr. İlker

Kepenekçi, who supplied the nematode isolate *H. bacteriophora,* and to Ergi D. Özsoy and Hakan Gür for their help with statistical analyses. We thank Dr.M. Nihat Şişli for sharing his knowledge with us. We also thank Z. İpek Ekmen for her help.

Corresponding author:
Işık OĞUZOĞLU ÜNLÜ
Hacettepe University,
Department of Biology Faculty of Science
06532 Beytepe, Ankara - Turkey

References

1. Kondo E. Studies on the Infectivity and Propagation of Entomogenous Nematodes, Steinernema spp. (Rhabditidae: Steinernematidae), in the Common Cutworm, *Spodoptera litura* (Lepidoptera: Noctuidae). Reprinted from Bulletin of the Faculty of Agriculture Saga University, 67, 88 pp., 1989
2. Kaya HK. Entomopathogenic Nematodes in Biological Control of Insects, New Directions in Biological Control: Alternatives for Suppressing Agricultural Pests and Diseases, Proceeding of a VCLA Colloquium held in Frisco, Colorado 20-27 Jan 1989. alan R. Liss Publ., New York; 189-198, 1990.
3. Hominick WM. Entomopathogenic Rhabditid Nematodes and Pest Control. Parasitology Today, 6: 148-152, 1990.
4. Hominick WM, Briscoe BR. Occurrence of Entomopathogenic Nematodes (Rhabditida: Steinernematidae and Heterorhabditidae) in British Soils, Parasitology 100: 295-302, 1990.
5. Epsky ND, Capinera JL. Quantification of invasion of two strains of *Steinernema carpocapsae* (Weiser) into three Lepidopteran Larvae. Journal of Nematology 25: 173-180, 1993.
6. Lacey AL. Techniques in insect nematology, Manual of Techniques in Insect Pathology (Biological Techniques), Academic Press Limited, London, 409 pp., 1997.
7. Pino FG, Palomo A. Natural occurrence of entomopathogenic nematodes (Rhabditida: Heterorhabditidae and Steinernematidae) in Spanish soils. Journal of Invertebrate Pathology 68: 84 -90, 1996.
8. Gaugler R, Kaya H K. Entomopathogenic Nematodes in Biological Control, CRC Press Inc., Boca Raton 349 pp., 1990.
9. Jagdale GB, Somasekhar N, Grewal PS, et al. Suppression of plant parasitic nematodes by application of live and dead infective juveniles of an entomopathogenic nematode, Steinernema carpocapsae on boxwood (Buxus spp.) Biological Control 24: 42-49, 2002.
10. Poinar GO Jr., Nematodes for Biological Control of Insects, Boca Raton CRC Press Inc., 94-201, 1979.
11. Barbercheck ME, Kaya HK. Competitive interactions between entomopathogenic nematodes and Beauveria bassina (Deuteromycotina: Hyphomycetes) in soilborne larvae of Spodoptera exigua (Lepidoptera: Noctuidae), Environ. Entomol. 20: 707-712, 1991.
12. Capinera JL, Pelissier D, Menout GS, et al. Control of Black Cutworm Agrotis ipsilon (Lepidoptera: Noctuidae), with Entomogeneous Nematodes (Nematoda: Steinernematidae, Heterorhabditidae). Journal of Invertebrate Pathology 52: 427-435, 1988.
13. Ebssa L, Borgemeister C, Berndt O, et al. Efficacy of Entomopathogenic Nematodes against Soil Dwelling Life Stages of Western Flower Thrips, Frankliniella occidentalis (Tysanoptera: Thripidae). Journal of Invertebrate Pathology 78: 119-127, 2001.
14. Koppenhöfer AM, Brown IM, Gaugler, et al. Synergism of entomopathogenic nematodes and Imidacloprid against white-grubs: greenhouse and field evaluation. Biological Control 19: 245-251, 2000.
15. Mc Coy CW, Shapiro DI, Duncan LW, et al. Entomopathogenic nematodes and other natural enemies as mortality factors for larvae of Diaprepes abbreviatus (Coleoptera: Curculionidae). Biological Control 19: 182-190, 2000.
16. Medeiros JS, Rosa JS, Tavares J, et al. Susceptibility of Pseudaletia unipuncta (Lepidoptera: Noctuidae) to entomopathogenic nematodes (Rhabditida: Heterorhabditidae and Steinernematidae) isolated in the Azores: effect of nematode strain and host age. Journal of Economic Entomology 93: 5, 2000.
17. Stark JEP, Lacey LA. Susceptibility of western cherry fruit fly (Diptera: Tephritidae) to five species of entomopathogenic nematodes in laboratory studies. Journal of Invertebrate Pathology 74: 206-208, 1999.
18. Ricci M, Glazer I, Campbell JF, et al. Comparison of Bioassays to Measure Virulence of Different Entomopathogenic Nematodes, Biocontrol Science and Technology 6: 235- 245, 1996.
19. Glazer I. Invasion Rates as a Measure of Infectivity of Steinernematid and Heterorhabditid Nematodes to Insects. Journal of Invertebrate Pathology 59: 90-94, 1992.
20. Özer N, Keskin N, Kırba? Z, et al. Occurrence of Entomopathogenic Nematodes (Steinernematidae: Heterorhabditidae) in Turkey, Nematologica 41: 639- 640, 1995.
21. Susurluk A, Dix I, Stackebrandt E, et al. Identification and ecological characterisation of three entomopathogenic nematode-bacterium complexes from Turkey, Nematology 3: 833- 841, 2001.

22. Selvan S, Campbell JF, Gaugler R, et al. Density-dependent effects on entomopathogenic nematodes (Heterorhabditidae and Steinernematidae) within an insect host, Journal of Invertebrate Pathology 62: 278-284, 1993.

23. Woodring JL, Kaya HK. Steinernematid and Heterorhabditid Nematodes: A Handbook of Techniques, Southern Coop. Ser. Bull. 331, Arkansas Agri. Exp. St. Fayatteville, AZ. 29 pp., 1988

24. Gaugler R, Georgis R. Culture Method and Efficiacy of Entomopathogenic Nematodes (Rhabditida: Steinernematidae and Heterorhabditidae). Biological Control 1: 269-274, 1991.

25. Koppenhöfer AM, Baur ME, Kaya H, et al. Competition between two steinernematid nematode species for an insect host at different soil depths. J. Parasitol. 82: 34- 40, 1996.

26. Rosas RA, Kaya H K. Interaction between two entomopathogenic nematode species in the same host. Journal of Invertebrate Pathology 57: 1-6, 1991.

27. Stiling PD, Introductory Ecology, Prentice Hall Engewood Cliffs, NJ. 1992.

28. Mari IC, Boff Gerrie L, Wiegers Lonne JM, et al. Development of the Entomopathogenic Nematode *Heterorhabditis megidis* Strain NLH-E87.3 in *Galleria mellonella*. Nematology 2: 303-308, 2000.

29. Strauch O, Ehlers RU. Food signal production of *Photorhabdus luminescens* inducing the recovery of entomopathogenic nematodes Heterorhabditis spp. in liquid culture. Appl. Microbiol. Biotechnonol 50: 369-374, 1998.

30. Ehlers RU. Mass production of entomopathogenic nematodes for plant production. Appl. Microbiol. Biotechnonol 56: 623-633, 2001.

31. Gouge DH, Hague NGM. Development of *Steinernema feltiae* (Steinernematidae: Nematoda) in *Bradysia paupera* (Sciaridae: Diptera). Bulletin IOBC SROP/WPRS 17: 132-135, 1994.