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Comparison of Some Biological Characterizations of the Entomopathogenic Nematodes, *Steinernema weiseri* and *S. feltiae* (Rhabditida: Steinernematidae), Isolated in Turkey

Alper SUSURLUK[1]

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Abstract: The entomopathogenic nematodes, *Steinernema weiseri*, was firstly isolated in Beytepe Campus of Hacettepe University, in Ankara, Turkey and *S. feltiae* (Rhabditida: Steinernematidae), which was found in Ankara University Campus. In the present study, reproduction capacities and effectiveness of the both entomopathogenic nematodes were compared in variable conditions. The reproduction capacities were examined at the following temperatures; 10, 15 and 20 ºC and the doses of 10, 50 and 100 infective juveniles-dauer juveniles (DJs) in the last instar larvae of greater wax moth, *Galleria mellonella* L. (Lep: Pyralidae). New generation DJs of each species, which produce within the host larvae and emerging from cadaver were counted and the results showed that *S. weiseri* was more productive than *S. feltiae* in all experimental conditions. Effectiveness of the both species on the last instar *G. mellonella* larvae as LD_{50} was calculated 48 h after penetration. According to the results, *S. weiseri* is more effective than *S. feltiae*. The present results indicated that *S. weiseri* can be more useful for biological control.

Key Words: Effectiveness, reproduction capacity, entomopathogenic nematodes, *Steinernema weiseri, S. feltiae*, Turkey

Türkiye'den İzole Edilen Entomopatojen Nematodlar, *Steinernema weiseri* ve *S. feltiae* (Rhabditida: Steinernematidae)'nin Bazı Biyolojik Özelliklerinin Karşılaştırılması

Öz: Türkiye'de ilk kez entomopatojen nematod *Steinernema weiseri* Ankara, Hacettepe Üniversitesi Beytepe Kampüsünden ve *S. feltiae* (Rhabditida: Steinernematidae) Ankara Üniversitesi kampüsünden izole edilmiştir. Bu çalışmada her iki entomopatojen nematodun değişik koşullarda etkinlik ve üreme kapasiteleri karşılaştırılmıştır. Üreme kapasiteleri Balmumu Güvesi, *Galleria mellonella* L. (Lep: Pyralidae) 'nın son dönem larvaları üzerine 10, 15 ve 20 ºC sıcaklıklarda 10, 50 ve 100 adet infektif larva nematodun uygulanması ile belirlenmiştir. Konukçu larva içerisinde üreyerek kadavradan dışarı çıkan her iki nematoda ait yeni generasyon jüvenilleri sayılmıştır. Tüm deneme koşullarında *S. weiseri*i'nin *S. feltiae*'ye göre daha fazla üreme kapasitesine sahip olduğu belirlenmiştir. *G. mellonella*'nın son dönem larvaları üzerinde her iki nematodun etkinliği penetrasyondan 48 saat sonra LD_{50} olarak belirlenmiştir. Bu sonuçlara göre *S. weiseri*'nin, *S. feltiae*'ye göre daha etkili olduğu ve biyolojik mücadelede kullanımının daha yararlı olabileceği bulunmuştur.

Anahtar Kelimeler: Etkinlik, üreme kapasitesi, entomopatojen nematodlar, *Steinernema weiseri, S. feltiae*, Türkiye

Introduction

Entomopathogenic nematodes, steinernematids and heterorhabditids, are safe biocontrol agents that are used to manage soil-borne insect pests as recorded in several studies (Gaugler and Kaya 1990, Gaugler 2002). When the dauer juveniles (DJs) of the entomopathogenic nematodes enter the host hoemocoel, they exist from this stage, release their symbiotic bacteria, which multiply and kill the host within only few days. The nematodes feed on the cells of their symbiotic bacteria in the host body. After approximately 2 weeks, the DJs emerge from the insect cadaver and search for new host insects (Gaugler and Kaya 1990, Poinar 1990, Kaya and Gaugler 1993, Gaugler 2002). This unique mutualistic relationship with the bacteria *Xenorhabdus* and *Photorhabdus* has been the subject of host-parasite interactions, evolution of mutualism and etc. (Fenton and Hudson 2002,

[1]Institute for Phytopathology, Department of Biotechnology and Biological Control, Christian-Albrechts-University Kiel, Raisdorf 24223-Germany.

Fenton and Rands 2004). The broad host range and high virulence of entomopathogenic nematodes make them amenable for inundative insect pest control (Gaugler 1988, Hui and Webster 2000). They have a high control potential and can be mass produced in liquid culture economically reasonable costs (Ehlers 1996). Entomopathogenic nematode isolates have been recovered from soil samples and insect bodies. Hundreds of different laboratories explore *Steinernema* and *Heterorhabditis* nematodes and their bacterial symbionts in more than 60 countries (Gaugler 2002). In Turkey, *Steinernema feltiae* (Filipjev 1934) (Rhabditida: Steinernematidae) has been recorded for the first time from the soil samples of Black-sea coasts (Özer, et al. 1995) and then the same species was also isolated in Ankara and identified by PCR-RFLP (Susurluk et al. 2001). Then, *Heterorhabditis bacteriophora* (Poinar 1976) (Kepenekçi et al. 1999, Susurluk et al. 2001) and *Steinernema anatoliense* (Hazır et al. 2003) have been isolated in Turkey. The species *S. weiseri* is the last record for Turkey. This species was identified by morphological characterizations, cross-breeding experiment and molecular technique PCR-RFLP (Unlu et al. unpublished). In the present study, reproduction abilities and activity of *S. weiseri* and *S. S. feltiae* on *Galleria mellonella* L. larvae were evaluated, in order to determine differences between the both species.

Materials and Methods

Nematodes: *Steinernema feltiae* and *S. weiseri* species were reared at 25°C by using of last instar *G. mellonella* larvae. Dauer Juveniles (DJs) harvested from White traps were stored in deionized water at 4–8 °C till using.

Insect: The greater wax moth, *Galleria mellonella* L., was reared on a mixture of 900 g of liquid honey, 900 g of glycerin, 200 g of bees wax, 400 g of yeast flakes and 1300 g of whole meal at 25 °C. The insect culture was reared in 1,500 ml volume glass containers (11 cm diameter and 15 cm height) at 30-32 °C on an artificial medium according to Wiesner (1993).

Determination of the infectivity: Single last instar of *G. mellonella* larva of an average weight of 0.20–0.25 g was placed in multiwell plates covered with a lid during the experiment. *G. mellonella* larvae were trapped individually in sand and exposed to different nematode concentrations of 10, 25, 50, 100, 150, 300 DJs. One hundred *G. mellonella* larvae were used for each concentration. The wells were kept in the dark at 25ºC. Insect mortality caused by each nematode species was recorded after 48 h. All experiments were repeated three times and the LD_{50} values were also calculated.

Reproductive potential at different temperatures and doses: In order to evaluate the reproductive potential of *S. weiseri* and *S. feltiae*, the number of nematode offspring per insect was evaluated. Experiments were carried out as described in the infectivity bioassays using the concentrations of 10, 50 and 100 DJs per *G. mellonella* larva at the following temperatures; 10, 15 and 20 °C. Each concentration of DJs was tested on 10 numbers *G. mellonella* larvae. This experiment was repeated three times. After 48 h incubation the infected cadavers recognized by their yellowish color, were removed from the sand, rinsed, transferred to water traps and incubated in the dark at 25°C. All emerged DJs from a single host insect were recovered over a period of 10 days and stored in a 50 ml flask (Boff et al., 2000). The content of each flask (nematode suspension from individual cadavers) was mixed thoroughly using air bubbles. Eight samples of 10 µl from each suspension were examined under a stereomicroscope and the total number of DJs per cadaver was calculated.

Statistical analysis : Reproductive capability of *S. feltiae* and *S. weiseri* at the following doses and temperatures; 10, 50 and 100 DJs; 10, 15 and 20 °C was analyzed by analysis of variance (F-test) ANOVA (breakdown one way Anova) and followed by a Least Significant Difference (LSD) test as post-hoc comparisons of the reproduction means. The minimum level of significance was taken as $p<0.05$ (Statistica, 1991). LD_{50} for each nematode was estimated by Probit analysis according to Finney (1971).

Results

The results indicated that *S. weiseri* showed higher infectivity than *S. feltiae* for densities used in the study, especially at 10 DJs dose, although no infection caused by *S. feltiae* was detected, 22.5 % mortality by *S. weiseri* was recorded (Table 1). LD_{50} values of *S. weiseri* and *S. feltiae* were calculated as 25.68 and 80.70 DJs, respectively (Table 2). The reproductive experiments showed that the species of *S. weiseri* more productive than *S. feltiae* at the temperature and doses used in the study. It was found that the differences of reproduction rates between the both species were statistically significant at all temperatures and doses used in the study (Fig. 1. A, B and C).

Table 1. Mortality rates of *Galleria mellonella* larvae by *Steinernema feltiae* and *S. weiseri.*

Incubation Time	Number of DJs	Mortality Rates (%)	
		S. feltiae	*S. weiseri*
48 h	10	0	22.5
	25	19	30
	50	26	67.5
	100	67.7	100
	150	70.3	100
	300	86	100

Table 2. LD_{50} values of *Steinernema feltiae* and *S. weiseri* calculated for *Galleria mellonella* larvae

Species	Incubation Time (h)	LD_{50}
S. feltiae	48	80.70
S. weiseri	48	25.68

Especially, the differences at 10 ºC were detected statistically different at doses used (Fig. 1.A; F=768.63; df=5, 174; p=0.00). But the differences of reproduced juveniles at a dose of 50 DJs for *S. weiseri* and at a dose of 100 DJs for *S. feltiae* were not significant at the temperatures; 15 and 20 ºC (Fig. 1. B and C; F=348.87; df=5, 174; p=0.000 and F=571.54; df=5, 174; p=0.000, respectively). This result showed that *S. feltiae* could reach the statistically same mortality level at the half of the dose for *S. weiseri* used. It was also expectedly observed that the rate of reproduction also increased, when the temperatures increased. Therefore, max. DJs were obtained as approximately the number of 35000 per larva at 20 ºC (Fig. 1. C).

Discussion

The biogeography of entomopathogenic nematodes was comprehensively assessed by Hominick et al. 1996. In the broader sense, they are widespread (Griffin et al. 1990). A large number of unknown species also exist in laboratories around the world (Hominick 2002). The first record coming from Turkey was *S. feltiae*, in terms of entomopathogenic nematodes. Then, *H. bacteriophora* (Kepenekçi et al. 1999) and then, *S. feltiae* and *H. bacteriophora* isolated in Ankara-Turkey were identified by cross-breeding and molecular technique, PCR-RFLP (Susurluk et al. 2001). *S. anatoliense* (Hazir et al. 2003) have been isolated during the surveys in Turkey. Hazir et al. (2003) reported *S. feltiae* has been isolated as most common species from 10 sites in 6 regions in the most extensive survey study carried out in different regions of Turkey. The last record in Turkey, *S. weiseri* firstly reported for Europe by Mracek et al. 2003 from a road side with apple trees near Ceske Budejovice, Czech Republic (Mracek et al. 2003). *Steinernema weiseri* was seen as the most similar to *S. feltiae* that is the most prevalent species in the world. Present experiment is the first study on biological properties of *S. weiseri* Turkish isolate.

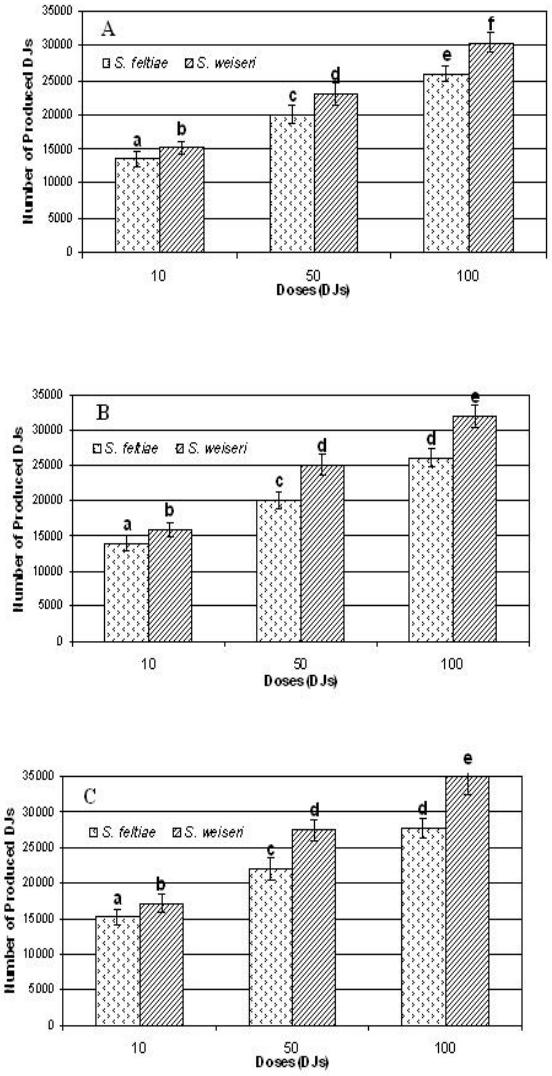

Figure 1. Mean±SE of the reproduced number of *S. feltiae* and *S. weiseri* per *G. mellonella* larva at the following concentrations: 10, 50, and 100 DJs and at temperatures: 10 (A), 15 (B) and 20 ºC (C). Bars followed by the same letter are not significantly different from each other. (P<0.05).

S. weiseri and *S. feltiae* seem similar to each other as recorded in Mracek et al. (2003) study. For this reason, the both nematode species were chosen in this experiment. Infectivity and temperature relations have been the subject in many studies. Temperature affects morphology, behavior and many biological processes in ecology. Biological control agents generally act more active at the temperature that is similar to where they were isolated from. Boff et al. (2000) studied on a reproduction examination of *H. megidis* (strain NLH-E87.3) at different doses (10, 30, 100, 300, 1000 and 3000 DJs) and indicated that total production of the species nearly 28000 and 30000 DJ per larva at the doses of 10 and 100 DJs, respectively. But the present study, the both species *S. feltiae* and *S. weiseri* were less productive than *H. megidis* at a dose of 10 DJs and at the temperatures used, however, the production rate was similar at a dose of 100 DJs at the temperatures, although they might be statistically significant. However, Molyneux et al. (1983) stated that DJs of *Heterorhabditis* spp. were only able to reproduce in *Lucilia cuprina* larvae subjected to low dosages of DJs, whereas *Steinernema* spp. were not able to reproduce at any dosage. We found that *S. feltiae* and *S. weiseri* were able to reproduce on *G. mellonella* at all doses and temperatures. With the same agreement, Boff et al. (2000) indicated that *H. megidis* produced well at all doses. In addition to the differences of reproduction rate between two different species in the study, Susurluk (2005) reported that it can be even observed reproduction differences between *in vivo* and *in vitro* culture of the same species. The number of DJs emerged from the host cadaver does not always show the success of their biological control effort, establishment and persistence in applied area. But high numbers of DJs can create more chances to catch the potential hosts (Susurluk 2005). There are some LD_{50} studies of entomopathogenic nematodes, but the results vary very much. It was found that LD_{50}= 80.70 and 25.68 DJs for *S. feltiae* and *S. weiseri* respectively, even the both species belong to same genus. But, for *S. weiseri*, 10, 25, 50 and 100 DJs of doses were accepted to Probit analyse. Since the doses of 150 and 300 had the same effect on mortality. Aguillera (1992) stated that LD_{50} of *S. scapterisci* to the pest *Scapteriscus* spp. was 4000 DJs, whereas the LD_{50} to a field cricket, *Gryllus rubens*, and the lubber grasshopper, *Romalea guttata*, was 1000 DJs. Akhurst (1982) found LD_{50} for *S. kraussei* as 16.5 DJs on *G. mellonella*. Bhatnagar et al. (2004) reported that lower doses of *H. bacteriophora* were necessary to kill the host *Maladera insanabilis* (LD_{50}= 14 DJs; after 72 hours). In addition to insect studies, infectivity of some entomopathogenic nematodes on tick, *Boophilus annulatus* (Arachnida: Ixodidae) also was examined by Samish and Glazer (1992). They used two strains of *S. carpocapsae* and the results varied each other. LD_{50} and LD_{90} of *S. carpocapsae* "DT" were 15 and 165 DJs, respectively. However, for *S. carpocapsae* "All strain", LD_{50}= 372 and LD_{90}= 9251 DJs. These results were agreement with the present study in this paper. LD value can vary largely even in same species. According to the LD_{50} values in present results, using of *S. weiseri* in biocontrol program might be more effective than *S. feltiae* isolated in Turkey.

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İletişim Adresi:
Alper SUSURLUK
Turkish Ministry of Agriculture and Rural Affairs, Plant Quarantine Service, Department of Nematology, Liman Str. No: 11, 35230 Alsancak/İzmir-Turkey
Fax: + 90 232 463 0994
E- posta: alpersusurluk@yahoo.com