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# STEINERNEMATID AND HETERORHABDITID NEMATODES AGAINST *ZABRUS* SPP.

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***Abstract***

*Experiments were conducted to find out the susceptibility of the larvae of Zabrus spp. (Coleoptera: Carabidae), an important insect pest of wheat, against entomopathogenic nematodes (Rhabditida: Steinernematidae and Heterorhabditidae) in the laboratory first time in the World. The entomopathogenic nematodes used in the trials were Steinernema feltiae-Commercial, S. feltiae-Endemic, S. carpocapsae, S. bicornutum, Heterorhabditis bacteriophora, and H. indica. Small plastic pots with a lid (8 cm in height, 6 cm in diameter) containing autoclaved soil have been utilized in trials. In the experiments, rates of 50, 100 and 200 infective juveniles (IJs)/cm$^2$ at 15, 20 and 25°C applied and they were repeated 2 times. Raising rate and temperature expanded the mortalities caused by nematodes. S. carpocapsae produced 75% mortality at the rate of 200 IJs/cm$^2$, which was the highest at 15°C. The lowest mortality with 5% at the rate of 50 IJs/cm$^2$ was caused by S. bicornutum 15°C. Steinernema carpocapsae at the rate of 200 IJs/cm$^2$, and S. feltiae-Endemic and H. indica at the rate of 50 IJs/cm$^2$ provided the highest (85%) and the lowest (20%) mortality, respectively at 20°C. At 25°C, S. carpocapsae at the rate of 200 IJs/cm$^2$ was the nematode caused the highest mortality with 95% while S. feltiae-Endemic at the rate of 50 IJs/cm$^2$ was producing 25% mortality which was the lowest. As a result, S. carpocapsae performed the best efficacy against Zabrus spp. and it was followed by H. bacteriophora and S. bicornutum.*

***Key words***: *Zabrus* spp., entomopathogenic nematods, *Steinernema*, *Heterorhabditis, biological control.*

## INTRODUCTION

Being an important crop in our country and in the world, wheat is an indispensable source of food for human nutrition. Annual wheat production in our country is approximately 18-20 million tons, wheat consumption however is approximately 16-17 million tons (Anonymous, 2008). Despite of an excessive wheat production, the losses in crop quality caused by pests and diseases makes a significant amount of wheat to be imported into our country. In addition to the diseases such as bunt, rust, smut, insect pests; sunn pest, cereal bugs, cereal spike beetles, and cereal ground beetles, *Zabrus* spp. are important and cause losses in wheat yield and quality (Lodos, 1989). Occurrence of high population densities of *Zabrus* spp. (Coleoptera: Carabidae) can cause yield losses of up to 100% in years and areas where none of control methods are applied. In our country, there are only seeds and surface chemical applications as control methods against this important pest.

In terms of plant protection, although there are many different control options against pests, the biological control is highly preferred over other methods, because of being the human, animal and environmental friendly method, maintaining the ecological balance and sustainability. As biological control agents, entomopathogenic nematodes (EPNs) (Rhabditida: Steinernematidae and Heterorhabditidae) attract attention increasingly in research area recently. Their ideal properties such as; the broad host spectrum, to be able to kill their hosts within 24-48 hours, to be producible commercially easily in vivo or in vitro, having ability to search actively their hosts, settling in application areas and staying effective for a long time, having easy applicability, being in compliance with many chemicals and being safe for the environment are important for their preferability. They are soil dwelling, aquatic organism and have motile bacteria living their intestine. The bacteria in Steinernematidae are *Xenorhabdus* spp. and in Heterorhabditidae are *Photorhabdus* spp.

Nematode and bacterium are mutualistic symbionts and obligate, lethal parasites of insects. EPNs can be found everywhere on earth and infect many different insects (Smart, 1995). Nematodes enter insect through natural openings. Once in the insect, the nematode releases the bacteria that are carried in the intestine. Bacterial cells reproduce rapidly and kill the insect within 24-48 hours using many different toxins. They also produce antibacterial and antifungal antibiotics not to allow any other other organisims in the host. The nematodes eat the bacteria and reproduce for 3 or 4 generations depending on the food source. Over 100,000 nematodes exit the insect (Burnell and Stock, 2000).

Entomopathogenic nematodes have been used in controlling insects since the 1930s (Smart 1995) in various climatic regions of the world. They are important biological control agents of soil-inhabiting insects (Gaugler, 1981; Georgis and Poinar, 1984; Klein, 1990) such as Japanese beetles, mole crickets, and root weevils. They have also been used successfully against above-ground insects in cryptic habitats (Bedding and Miller, 1981; Ralph, 1981; Kaya, 1988; Begley, 1990; Kaya, 1990; Vreditelyami, et al., 1992), for example, navel orange worm, the codling moth and the artichoke plume moth, carpenter worms, and clearwing moths.

However, studies on EPN are very limited and some of them have just started in Turkey. Turkey having a diverse ecology shelters nine EPN species but the studies on the investigating of efficacy and usage of these species on pests of cultivated plants are very rare. In this study; we conducted experiments to find out the susceptibility of the larvae of *Zabrus* spp. against entomopathogenic nematodes in the laboratory first time in the World to produce basic data to use in the biological control of the insect.

## MATERIALS AND METHODS

We studied entomopathogenic nematodes as an alternative to chemical control. The entomopathogenic nematodes used in the trials were *Steinernema feltiae-Commercial, S. feltiae*-Endemic, *S. carpocapsae, S. bicornutum*, *Heterorhabditis bacteriophora*, and *H. indica*.

*Zabrus* larvae from wheat fields were collected digging into soil 25-30 cm at the end of March and beginning of April. They brought to lab in ice box. They let stay in plastic containers for 24 hours to differentiate the damaged ones during collecting and transportation. The trials have been conducted in small plastic pots with a lid (8 cm in height, 6 cm in diameter) containing autoclaved soil and repeated 2 times. The nematodes at 3 rates of 50, 100 and 200 infective juveniles (IJs)/cm$^2$ with 4 replicates applied evenly into plastic with pipet. Pots were placed in incubators at dark adjusted 15, 20 and 25$^o$C. They were checked after 7 and 10 days to count dead larvae. Efficacy was evaluated by comparing the treatments with untreated control.

## RESULTS AND DISCUSSIONS

The mortalities caused by nematodes increased by increasing rate and temperature at the 10$^{th}$ day. The highest mortality with 75% at the rate of 200 IJs/cm$^2$ was caused by *S. carpocapsae* followed by *S. carpocapsae* at the dose of 100 and 50 IJs/cm$^2$ with 70% mortality and *H. bacteriophora* at dose of 200 IJs/cm$^2$ with 60% mortality. However they were statistically at the same group. The lowest mortality with 5% mortality at the rate of 50 IJs/cm$^2$ was caused by *S. bicornutum* at 15°C (Table 1).

*S. carpocapsae* at the rate of 200 IJs/cm$^2$, and *S. feltiae*-Endemic and *H. indica* at the rate of 50 IJs/cm$^2$ provided the highest (85%) and the lowest (20%) mortality, respectively at 20°C. *H. bacteriophora* at the rate of 200 IJs/cm$^2$ with 75% mortality and *S. bicornutum* at the rate of 200 IJs/cm$^2$ with 67.5% mortality followed *S. carpocapsae*. They did not differ from each other statistically (Table 2). At 25°C, *S. carpocapsae* at the rate of 200 IJs/cm$^2$ was the one producing the highest mortality with 95% while *S. feltiae*-Endemic at the rate of 50 IJs/cm$^2$ was causing 25% mortality which was the lowest (Table 3). *S. bicornutum* at the rate of 100 and 200 IJs/cm$^2$ with 70% and 85% mortality and *H. indica* at the rate of 200 IJs/cm with 70% mortality followed *S. carpocapsae* at the rate of 200 IJs/cm$^2$, which were at the same group statistically.

Table 1. The efficacy of entomopathogenic nematods against *Zabrus* larvae at 15°C in laboratory

| Nematod | Dose (IJ/ $cm^2$) | Larva used 1st and $2^{nd}$ year | $7^{th}$ day count | | | $10^{th}$ day count | | |
|---|---|---|---|---|---|---|---|---|
| | | | Death (%) | Death (%) | Death average (%) | Death (%) | Death (%) | Death average (%) |
| | | | $1^{st}$ year | $2^{nd}$ year | | $1^{st}$ year | $2^{nd}$ year | |
| *S.feltiae Endemic* | 50 | 20+20 | 30 | 10 | 20bcde | 30 | 30 | 30de |
| *S.feltiae Endemic* | 100 | 20+20 | 35 | 15 | 25cde | 35 | 35 | 35ef |
| *S.feltiae Endemic* | 200 | 20+20 | 45 | 25 | 35def | 50 | 50 | 50f |
| *S.feltiae* Commecial | 50 | 20+20 | 15 | 25 | 20bcdef | 15 | 15 | 15abcd |
| *S.feltiae* Commercial | 100 | 20+20 | 30 | 25 | 27,5cde | 30 | 30 | 30de |
| *S.feltiae* Commercial | 200 | 20+20 | 50 | 30 | 40ef | 55 | 55 | 55gh |
| *H. bacteriophora* | 50 | 20+20 | 15 | 50 | 32,5cde | 40 | 70 | 55fg |
| *H. bacteriophora* | 100 | 20+20 | 25 | 35 | 30cde | 45 | 65 | 55gh |
| *H. bacteriophora* | 200 | 20+20 | 15 | 60 | 37,5ef | 50 | 70 | 60ghi |
| *S. carpocapsae* | 50 | 20+20 | 30 | 50 | 40ef | 55 | 85 | 70hi |
| *S. carpocapsae* | 100 | 20+20 | 40 | 60 | 50f | 60 | 80 | 70hi |
| S. carpocapsae | 200 | 20+20 | 35 | 70 | 52,5f | 65 | 85 | 75i |
| S. bicornutum | 50 | 20+20 | 5 | 0 | 2,5ab | 5 | 5 | 5ab |
| *S. bicornutum* | 100 | 20+20 | 10 | 15 | 12,5abc | 15 | 25 | 20bcde |
| *S. bicornutum* | 200 | 20+20 | 25 | 25 | 25cde | 30 | 30 | 30de |
| *H. indica* | 50 | 20+20 | 5 | 5 | 5ab | 10 | 10 | 10abc |
| *H. indica* | 100 | 20+20 | 10 | 10 | 10abc | 25 | 25 | 25cde |
| *H. indica* | 200 | 20+20 | 15 | 15 | 15ab | 25 | 25 | 25cde |
| Control | 0 | 20+20 | 0 | 0 | 0a | 0 | 0 | 0a |

Table 2. The efficacy of entomopathogenic nematods against *Zabrus* larvae at 20°C in laboratory

| Nematod | Dose (IJ/ $cm^2$) | Larva used 1st and $2^{nd}$ year | $7^{th}$ day count | | | $10^{th}$ day count | | |
|---|---|---|---|---|---|---|---|---|
| | | | Death (%) | Death (%) | Death average (%) | Death (%) | Death (%) | Death average (%) |
| | | | $1^{st}$ year | $2^{nd}$ year | | $1^{st}$ year | $2^{nd}$ year | |
| *S.feltiae Endemic* | 50 | 20+20 | 25 | 15 | 20abc | 25 | 15 | 20b |
| *S.feltiae Endemic* | 100 | 20+20 | 50 | 10 | 30bcde | 50 | 45 | 47,5de |
| *S.feltiae Endemic* | 200 | 20+20 | 50 | 20 | 35bcde | 55 | 50 | 52,5def |
| *S.feltiae* Commercial | 50 | 20+20 | 20 | 25 | 22,5abcd | 25 | 30 | 27,5bc |
| *S.feltiae* Commercial | 100 | 20+20 | 35 | 35 | 35bcde | 40 | 35 | 37,5cd |
| *S.feltiae* Commercial | 200 | 20+20 | 50 | 20 | 35bcde | 55 | 50 | 52,5def |
| *H. bacteriophora* | 50 | 20+20 | 30 | 50 | 40bcde | 55 | 50 | 52,5cde |
| *H. bacteriophora* | 100 | 20+20 | 40 | 35 | 37,5bcde | 50 | 40 | 45cde |
| *H. bacteriophora* | 200 | 20+20 | 60 | 70 | 65g | 70 | 80 | 75gh |
| *S. carpocapsae* | 50 | 20+20 | 35 | 55 | 45defg | 50 | 60 | 55def |
| *S. carpocapsae* | 100 | 20+20 | 55 | 50 | 52,5efg | 55 | 55 | 55def |
| *S. carpocapsae* | 200 | 20+20 | 40 | 80 | 60fg | 80 | 90 | 85h |
| *S. bicornutum* | 50 | 20+20 | 30 | 30 | 30bcde | 45 | 45 | 45cde |
| *S. bicornutum* | 100 | 20+20 | 30 | 30 | 30bcde | 45 | 45 | 45cde |
| *S. bicornutum* | 200 | 20+20 | 45 | 45 | 45defg | 75 | 60 | 67,5fg |
| *H. indica* | 50 | 20+20 | 15 | 15 | 15ab | 20 | 20 | 20b |
| *H. indica* | 100 | 20+20 | 40 | 40 | 40cdef | 55 | 65 | 60efg |
| *H. indica* | 200 | 20+20 | 25 | 35 | 30bcde | 55 | 65 | 60efg |
| Control | 0 | 20+20 | 5 | 0 | 2,5a | 5 | 0 | 2,5a |

Table 3. The efficacy of entomopathogenic nematods against *Zabrus* larvae at 25 °C in laboratory

| Nematod | Dose (IJ/ $cm^2$) | Larva used 1st and 2nd year | 7th day count | | | 10th day count | | |
|---|---|---|---|---|---|---|---|---|
| | | | Death (%) 1st year | Death (%) 2nd year | Death average (%) | Death (%) 1st year | Death (%) 2nd year | Death average (%) |
| *S.feltiae Endemic* | 50 | 20+20 | 25 | 15 | 20abcd | 25 | 25 | 25b |
| *S.feltiae Endemic* | 100 | 20+20 | 15 | 25 | 20abcd | 45 | 55 | 50def |
| *S.feltiae Endemic* | 200 | 20+20 | 15 | 30 | 22,5bcde | 50 | 70 | 60efg |
| *S.feltiae* Commercial | 50 | 20+20 | 20 | 10 | 15abc | 30 | 30 | 30bc |
| *S.feltiae* Commercial | 100 | 20+20 | 15 | 10 | 12,5ab | 35 | 25 | 30bc |
| *S.feltiae* Commercial | 200 | 20+20 | 30 | 40 | 35bcdef | 45 | 45 | 45cde |
| *H. bacteriophora* | 50 | 20+20 | 55 | 65 | 60gh | 60 | 70 | 65fg |
| *H. bacteriophora* | 100 | 20+20 | 15 | 60 | 37,5cdefg | 50 | 80 | 65fg |
| *H. bacteriophora* | 200 | 20+20 | 40 | 60 | 50fg | 50 | 80 | 65fg |
| *S. carpocapsae* | 50 | 20+20 | 45 | 30 | 37,5cdefg | 50 | 50 | 50def |
| *S. carpocapsae* | 100 | 20+20 | 60 | 60 | 60gh | 60 | 60 | 60efg |
| *S. carpocapsae* | 200 | 20+20 | 95 | 60 | 77,5h | 100 | 90 | 95i |
| *S. bicornutum* | 50 | 20+20 | 40 | 40 | 40defg | 60 | 60 | 60efg |
| *S. bicornutum* | 100 | 20+20 | 45 | 45 | 45efg | 70 | 70 | 70gh |
| *S. bicornutum* | 200 | 20+20 | 45 | 20 | 32,5bcdef | 90 | 80 | 85hi |
| *H. indica* | 50 | 20+20 | 45 | 45 | 45efg | 35 | 40 | 37,5bcd |
| *H. indica* | 100 | 20+20 | 45 | 45 | 45efg | 50 | 55 | 52,5defg |
| *H. indica* | 200 | 20+20 | 20 | 45 | 32,5bcdef | 60 | 80 | 70gh |
| Control | 0 | 20+20 | 0 | 0 | 0a | 0 | 0 | 0a |

## CONCLUSIONS

As a result, *S. carpocapsae* showed the best efficacy against *Zabrus* sp. and it was followed by *H. bacteriophora* and *S. bicornutum*. Field trials with these nematodes showed high efficacy against *Zabrus* should be planned for future studies.

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