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Pathogenicity of entomopathogenic nematodes to *Eurygaster maura* L. (Hemiptera: Pentatomidae)

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Summary. Pathogenicity of the entomopathogenic nematodes *Steinernema carpocapsae* (Anamur strain) (Rhabditida: Steinernematidae), *Heterorhabditis bacteriophora* (Tur-H1), *H. bacteriophora* (Tur-H2) (Rhabditida: Heterorhabditidae) was tested against the cereal insect pest, *Eurygaster maura* (Hemiptera: Scutelleridae). The cup assay was used to assess nematode pathogenicity in single dose and dose mortality bioassays. The single-dose assay showed that all three species caused significant host mortality. Nematode-induced mortality increased as the exposure time increased from 72 to 144 h. The mortality induced by the nematodes *S. carpocapsae*, Tur-H1 and Tur-H2, were 55%, 69% and 95% respectively. In single-dose exposures, the Tur-H2 strain of *H. bacteriophora* was the most pathogenic among the three nematodes tested. LC_{50} values of the nematodes varied from 8 to 62. These results indicate that entomopathogenic nematodes may be useful for control of *E. maura*.

Key words: Biological control, entomopathogenic nematodes, *Eurygaster maura*, *Heterorhabditis bacteriophora*, *Steinernema carpocapsae,* sunn pest.

Sunn pests are serious pest of cereal throughout the Balkans, the Middle East, and the former USSR (Critchley, 1998). The migratory hemipteran *Eurygaster maura* L. (Scutelleridae) is the most important and common species in Turkey (Kınacı *et al.*, 1998). This pest spends two to three months in the early summer feeding on cereals. Thereafter, from July to October, the insect rests within or beneath bushes at higher altitudes (2000-2500 m) near cereals. In November, *E. maura* migrates back to lower altitudes (1700-1900 m) where it overwinters beneath oak and pine trees, 20-40 mm deep in the soil. Overwintering densities range from 10 to 60 bugs/m^2 (Kınacı *et al.*, 1998). *E. maura* causes damage to leaves and stems, but direct damage is caused by feeding on grains. Injection of saliva enzymes results in destruction of gluten and subsequent unfavourable baking properties in flour. Control methods for sunn pests are varied but chemical control is the main method and usually involves the application of broad spectrum organophosphates and pyrethroids. In 2001, 1.1 million ha were treated with chemical insecticides in Turkey at cost of US$ 5 million (Anonymous, 2001). The overuse of chemicals over the past decade and the shortcomings of pesticide usage in sunn pests control have become evident.

In this study, the pathogenicity of two entomopathogenic nematodes species and strains, *Steinernema carpocapsae*, isolated from the soil of forest areas of Anamur (İçel) (Kepenekci, 2002), *H. bacteriophora* (Tur-H1) and *H. bacteriophora* (Tur-H2), isolated from Turkish soil samples taken from Agricultural Faculty of Ankara, Turkey (Susurluk *et al.*, 2001; Kepenekci & Susurluk, 2003) was studied on *E. maura* to evaluate their promise for control of the sunn pest.

MATERIAL AND METHODS

Entomopathogenic nematode species and strains (*S. carpocapsae,* Tur-H1 and Tur-H2) were obtained from stock cultures maintained at the Plant Protection Central Research Institute of Ankara (PPRI of Ankara), Turkey. All nematodes were reared on last instar greater wax moth, *Galleria mellonella* (L.), at 25°C as described by Woodring & Kaya (1988). The wax moth larvae were obtained from the Plant Protection Central Research Institute. After harvesting, the nematodes were stored at 5±1°C for 2 weeks. All experiments were conducted at 25°C.

Table 1. LC_{50} and fiducial limits of *Heterorhabditis bacteriophora* (Tur-H1), *H. bacteriophora* (Tur-H2) and *Steinernema carpocapsae* (Anamur) against *Eurygaster maura* adults.

Nematode species	Slope±SE	LC50 (IJs/0.2ml)	Fiducial Limits (IJs/0.2ml)
Heterorhabditis bacteriophora (Tur-H2)	1.20±0.25a	8a	4-13
H. bacteriophora (Tur-H1)	0.96±0.23ab	25ab	14-43
Steinernema carpocapsae (Anamur)	0.78±0.23b	62b	32-237

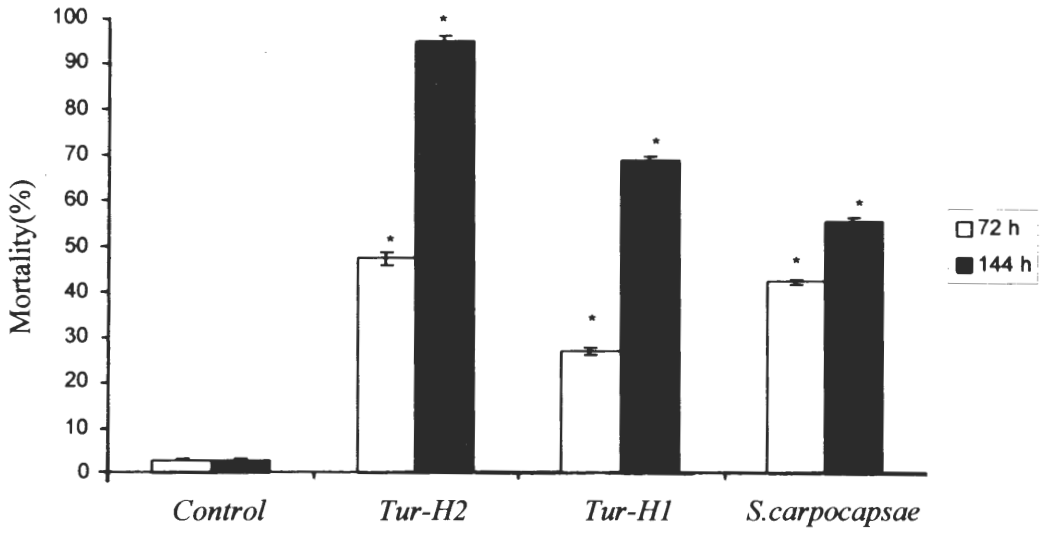

Fig. 1. Pathogenicity of *Heterorhabditis bacteriophora* (Tur-H1), *H. bacteriophora* (Tur-H2) and *Steinernema carpocapsae* (Anamur) against *Eurygaster maura* after 72 and 144 h. Bars showing mortality data with * are significantly different from the control. Each bar is the mean of three replicates.

Eurygaster maura adults were collected from overwintering areas in Haymana, (Ankara, Turkey). The soil was clay loam (sand: silt: clay =27%: 44%: 29%) with a pH =of 7.7, and organic matter of 1.22% by weight. Soil for the study was taken where the insects were collected, autoclaved, and dried at room temperature.

Experiments were carried out in plastic cups, 2.2 x 2 cm, according to the procedure described by Shapiro *et al.* (1999). One *E. maura* adult was placed at the bottom of each cup and the cup was filled with 7-8 cm^3 of soil.

In single dose assays, 50 infective juveniles of each nematode species and strain suspended in 0.2 ml sterile distilled water was applied to each cup. Insect mortality was recorded after 72 and 144 h. Controls received 0.2 ml sterile distilled water. There were three replicates of twelve wells per treatment; each replicate was treated on a separate date. Before all the tests, 0.5 ml sterile distilled water was applied to the soil surface.

In dose-mortality assays, four nematode concentrations (5, 25, 50 and 100 nematodes per 0.2 ml) were tested using the cup assay method. Each well contained 7-8 cm^3 of soil. *E. maura* adults were buried individually to a soil depth of 2 mm in each well. Each nematode species and strain was tested against twelve *E. maura* adults per nematode concentration. In controls, 0.2 ml sterile distilled water was applied. The plates were labeled and incubated for 96 h. Two replicate bioassays at separate dates were tested as described above.

The data from single dose assays was normalized using arcsine transformation (Zar, 1999). Analysis of variance was applied to test the significance of nematode mortality and Tukey's multiple comparison test for comparing the means using minitab computer program (Minitab, 1993). Dose-mortality data were analyzed using probit analysis. Regression lines, LC_{50}, and the equality

and parallelism of the regression lines between replicates for each nematode strain were determined using the POLO-PC probit procedure (LeOra software, 1994) according to Finney (1971).

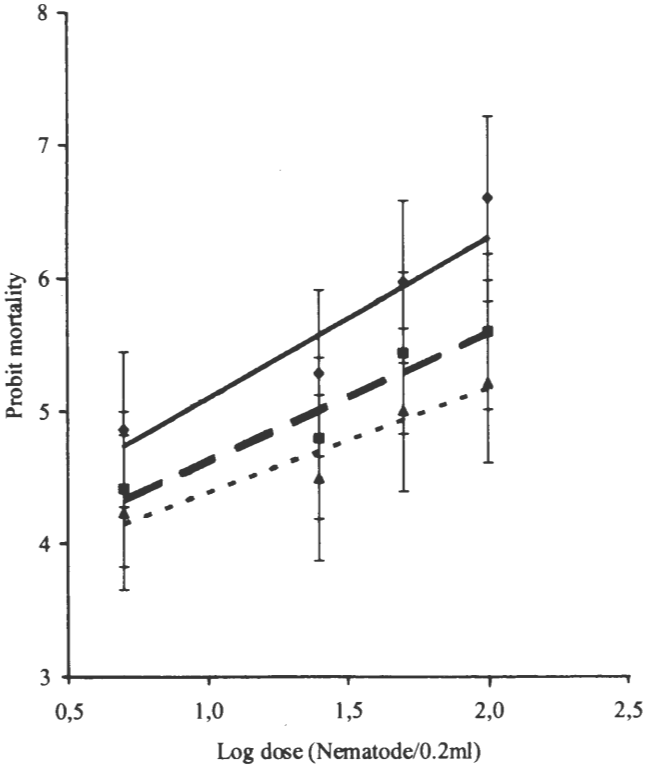

Fig. 2. Probit mortality regression lines for *Eurygaster maura* 96 h post-exposure to the nematodes *Heterorhabditis bacteriophora* (Tur-H1) (■), *H. bacteriophora* (Tur-H2) (♦) and *Steinernema carpocapsae* (Anamur) (▲). Each data point (mean ± SEM) represent the mean of three estimates.

RESULTS

Single dose assays showed that all entomopathogenic nematodes tested were able to kill *E. maura* adults (Fig. 1). All nematodes increased in their effectiveness as the exposure period increased. The largest increase, between 72 h and 144 h, was noted with Tur-H1 and Tur-H2 here effectiveness increased nearly two-fold over this period. Mortality among control insects was 4%.

After 72 h, all nematode species caused significantly greater mortality than in the control ($F=28.85$, $df=3.8$, $P<0.10$). There was no significant difference between strains in nematode pathogenicity after 72 h, although Tur-H2 caused the greatest mortality (47.3%).

All nematode strains caused significant mortality of adult *E. maura* relative to the controls after 144 h ($F=108.51$, $df=3.8$, $P<0.10$). Mortality ranged from 55% for *S. carpocapsae* to 95% for Tur-H2, which was significantly more pathogenic than the other two strains. All three nematode strains showed a high degree of uniformity in that minor variation was found between replicates (Fig. 1).

The dose-mortality bioassay provided further evidence that the nematode species were not equally pathogenic to *E. maura* ($F=2.21$, $df=2.6$, $P<0.20$). The Tur-H2 strain of *H. bacteriophora* was the most pathogenic nematode, showing the steepest slopes and narrowest fiducial limits (Fig. 2). The Tur-H1 strain and *S. carpocapsae* had similar pathogenicity since their fiducial limits overlapped considerably (Table 1). LC_{50} values of the nematodes varied from 8 for Tur-H2 to 62 for *S. carpocapsae*.

DISCUSSION

The single-dose assay demonstrated variation between nematode strains in their laboratory effectiveness. The Tur-H2 strain of *H. bacteriophora* was highly pathogenic against *E. maura* whereas the other two nematodes showed moderate pathogenicity.

The results from single-dose and dose-mortality bioassays indicated that the Tur-H2 strain of *H. bacteriophora* shows the greatest promise for control of *E. maura* based on this strain's high laboratory pathogenicity to the pest. Further research is called for on different aspects of this nematode strain to accelerate its development as a biological control agent.

These dose-mortality results for *E. maura* exposed to three indigenous entomopathogenic nematode strains provide the first data against this key Turkish pest. Treatment of *E. maura* showed that there was a linear relationship between dose and mortality. The maximum-likelihood test revealed that each nematode species had a different slope and there were significant differences between LC_{50} values. The LC_{50} represents a single estimate on a regression line and is a convenient index of pathogenicity determination. However, different mortality agents having the same values for LC_{50} could exhibit dramatic differences when the corresponding mortality regression lines are not equal such that they are unsuitable for comparison of effectiveness of agents (Finney, 1971; Robertson & Preisler, 1992).

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Kepenekci İ. Патогенность энтомопатогенных нематод для *Eurygaster maura* L. (Hemiptera: Pentatomidae).

Резюме. Определена патогенность энтомопатогенных нематод *Steinernema carpocapsae* (изолят Anamur) (Rhabditida: Steinernematidae), *Heterorhabditis bacteriophora* (изолят Tur-H1) и *H. bacteriophora* (изолят Tur-H2) (Rhabditida: Heterorhabditidae) для вредной черепашки *Eurygaster maura* (Hemiptera: Scutelleridae) при фиксированных дозах заражения, а также зависимость смертности от дозы заражения. Все три вида нематод вызывают значительную смертность этого хозяина, составляющую 55%, 69% и 95% соответственно, от *S. carpocapsae,* Tur-H1 и Tur-H2. Смертность вредных черепашек возрастала при увеличении времени обработки с 72 до 144 часов. При заражении одинаковыми дозами изолят Tur-H2 *H. bacteriophora* оказался наиболее патогенным. Значения LC_{50} варьировали в пределах 8 - 62. Результаты указывают на перспективность использования энтомопатогенных нематод для контроля *E. maura.*