Effectiveness of the Entomopathogenic Nematodes *Heterorhabditis bacteriophora* and *Steinernema feltiae* against *Tenebrio molitor* (Yellow Mealworm) Larvae in Different Soil Types at Different Temperatures

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**Abstract:** The efficiency of the entomopathogenic nematodes *Steinernema feltiae* Tur-S3 and *Heterorhabditis bacteriophora* Tur-H2, isolated in Turkey, against larvae of *Tenebrio molitor* L. was investigated in different soil type and temperature conditions. Sterilized and non-sterilized silver sand, clay-loam soil, and compost soil were tested, each at 12, 18, and 24 °C. Temperature had the greatest effect on the mortality of *T. molitor* larvae caused by both nematode species. The efficiency of the 2 nematodes was greater in sterile than in nonsterile conditions, and was greater in sandy soils than in clay soils. The results showed that *Steinernema feltiae* was more efficient than *H. bacteriophora* at all temperatures tested, especially at 12 °C.

**Key Words:** Biological control, Entomopathogenic nematodes, *Tenebrio molitor*, Soil type, Temperature

**Introduction**

Entomopathogenic nematodes belonging to the families Steinernematidae and Heterorhabditidae are parasites that are lethal to soil-dwelling insects (1,2). The specialized third stage juvenile, called the infective juvenile, is the only stage that survives outside of a host and searches for a susceptible insect host. Steinernematids and hetrohabditids are mutualistically associated with entomopathogenic bacteria of the genera *Xenorhabdus* and *Photorhabdus*, respectively (3). The nematode-associated bacterium complex has been used successfully as a biological control agent against a number of soil insect pests (4). The free-living infective juveniles enter their hosts through natural openings (anus, spiracles, mouth), penetrate the hemocoel, and release the symbiotic bacteria that multiply and kill the hosts within 48 h by septicemia (5). Entomopathogenic nematodes survive naturally in the soil and are effective as inundative biological control agents of many soil insects (6,7); however, in some cases, the efficiency of these nematodes against some insect species has varied from poor to excellent (8). Many factors influence the...
successful use of nematodes as biological insecticides, the most critical factors being soil moisture, air and soil temperatures, soil texture, application time, and crop variability and features (2,7). Therefore, this investigation was undertaken to determine the effect of different soil types and temperatures on the effectiveness of 2 entomopathogenic nematode species, *Heterorhabditis bacteriophora* Poinar, 1976 (Rhabditida: Heterorhabditidae) and *Steinernema feltiae* Filipjev, 1934 (Rhabditida: Steinernematidae), on the mortality of *Tenebrio molitor* L. (Coleoptera: Tenebrionidae).

**Materials and Methods**

**Nematode and insect cultures**

The nematode strains *H. bacteriophora* Tur-H2 and *S. feltiae* Tur-S3 were identified by PCR-RFLP (9), and were cultured in wax moth larvae under laboratory conditions (10). Dead larvae of wax moths were placed into White traps and the infective juveniles of the entomopathogenic nematodes were harvested daily and stored (11). Infective juveniles were used within 10 days after harvesting. The yellow meal worm larvae of *T. molitor* were used in the study as this insect is less sensitive than *Galleria mellonella*, which is used in most experiments of entomopathogenic nematodes. Moreover, the infectivity of the nematodes can be more accurately determined using the meal worm (12). The host insects, *T. molitor* larvae, were obtained from the Morio Zoobedarf Company in Röttingen, Germany. The weight of the larvae was 70.28 – 8.65 mg.

The effect of different combinations of soil type and temperature on the mortality of *T. molitor*:

Six experimental plastic boxes (13 × 13 × 5 cm) were prepared for the infectivity tests. The boxes were filled with 330 ± 32 g of sterilized or non-sterile silver sand (particle size variable between 300 and 400 µm), 285 ± 28 g of sterilized or non-sterile clay-loam soil (33% sand, 41% silt, and 26% clay), and 210 ± 12 g of sterilized or non-sterile compost (50-300 mg/l N, 80-300 mg/l P2O5, 80-400 mg/l K2O, and salt, < 1.5 g/l). The described soil, sand, and compost were sterilized according to Kung et al. (13).

The soil in each experimental box was adjusted to 10% (w/w) water content by adding sterile distilled water. The water content of the sand in the boxes was checked with an electronic moister analyzer (Sartorius MA 40, Göttingen, Germany). After 1 h acclimatization, 10 *T. molitor* larvae were placed at the bottom of each experimental box. This was followed by the addition of 10 ml of nematode inoculum suspension containing approximately 1000 ± 45 infective juveniles per box. Control boxes without nematodes (10 ml of distilled water only) were included in the study to evaluate spontaneous mortality of the test insect (11). The boxes were incubated at 12, 18, and 24 °C. There were 10 replications per nematode species and temperature. To determine the effectiveness of the infective juveniles, dead *Tenebrio* larvae were assessed 3 days after inoculation with the entomopathogenic nematodes. For *H. bacteriophora*, color change of the infected larvae from yellow to red indicated nematode infection. Moreover, each dead larva was checked for luminescence using a luminometer (wavelength: 390-620 nm) (Lumat LB 9501-Berthold, Wildbad, Germany). For *S. feltiae*, nematode infection was assessed by dissecting the dead mealworms and screening for nematodes under the microscope at 120 × magnification.

**Statistical analysis**

All data were corrected for control mortality using Abbott’s formula (14). Corrected mortality data and interactions between factors were analyzed by ANOVA (breakdown one-way ANOVA) and followed by the least significant difference (LSD) test for post-hoc comparison of the mortality means. The minimal level of significance was *P* ≤ 0.05. The analyses were performed using the Statistica program (15).

**Results**

Effect of temperature on the mortality of *T. molitor*:

Temperature had a statistically significant effect on the mortality of *T. molitor* larvae caused by both nematode species (Figure 1). The differences in mortality rate caused by *S. feltiae* and *H. bacteriophora* at each temperature were significant (F = 130.04; df = 17, 162; *P* < 0.01 for *H. bacteriophora* and *F* = 15.14; df = 17, 162; *P* < 0.001 for *S. feltiae*). This difference was highest at 12 °C and lowest at 24 °C. At 12 °C, *S. feltiae* caused greater mortality (72.8 ± 4.0%) than *H. bacteriophora* (14.5 ± 3.0%). *S. feltiae* caused 90.3 ±
3.2% mortality at 18 °C, whereas *H. bacteriophora* caused 73 ± 3.8% mortality. At 24 °C, *H. bacteriophora* caused a higher mortality (86.5 ± 1.63%) than at 12 and 18 °C, but mortality at 24 °C was lower than that caused by *S. feltiae* (97.6 ± 1.5%; *F* = 71.85; df = 11, 108; *P* < 0.01).

**Results at 12 °C**

There was no significant difference in the mortality of *T. molitor* larvae caused by either nematode in sterile and non-sterile silver sand or in sterile and non-sterile compost. In clay-loam, the mortality rate was higher in sterile (21 ± 3.13%) than in non-sterile soil (11 ± 2.33%) conditions.

*Steinernema feltiae* caused the same mortality rate in sterile and non-sterile sand (72 ± 5.3%). Though mortality was 67 ± 4.5% in non-sterile clay-loam and 75 ± 3.4% in sterile clay-loam, this difference was not significant (*F* = 14.90; df = 11, 108; *P* < 0.0001) (Figure 1B). However, mortality in non-sterile compost (71 ± 2.1%) was significantly lower than in sterile compost (80 ± 2.0%). Among the soil types, the
mortality of the insect was the lowest in non-sterile clay-loam soil (67 ± 4.5%) and the highest (80 ± 2.0%) in sterile compost soil at 12 °C.

Results at 18 °C

Heterorhabditis bacteriophora exhibited no significant difference in mortality effectiveness in non-sterile and non-sterile sand conditions, and the same tendency was observed in non-sterile and sterile clay-loam; however, in sterile compost, the mortality of the insect was significantly higher (82 ± 2.9%) than in non-sterile compost (75 ± 3.4%).

Steinernema feltiae showed no significant difference in non-sterile and sterile sand, and non-sterile and sterile compost, but a significant difference was observed in non-sterile (80 ± 2.6%) and sterile (90 ± 2.3%) clay-loam (Figure 1A and B).

Results at 24 °C

The difference of mortality of T. molitor caused by H. bacteriophora in non-sterile (84 ± 2.6%) and in sterile sand (85 ± 2.2%) was not significant. Additionally, no significant difference was observed in the mortality of the insect larvae in non-sterile (86 ± 1.6%) and sterile compost (91 ± 2.3%). In contrast, in sterile clay-loam, mortality (90 ± 2.6%) was significantly higher than in non-sterile clay-loam (83 ± 2.6%). Among soil types, H. bacteriophora caused the lowest mortality rate in non-sterile clay-loam soil (83%) and the highest rate in sterile compost soil (91%) (Figure 1A).

The mortality caused by S. feltiae in non-sterile (95 ± 1.6%) and in sterile sand (97 ± 1.5%) was similar; however, in clay-loam soil, the mortality in non-sterile conditions (94 ± 2.2%) was significantly higher than in sterile (100%) conditions. In the composts, the mortality of T. molitor larvae was 100%, both in sterile and non-sterile conditions. There were no significant differences in the mortality rate observed in different soil types (F = 10.54; df = 11, 108; P < 0.0001) (Figure 1B).

Interactions among factors

In the present study, interactions are statistically summarized in the Table. The interaction among the factors indicated that each factor had a significant effect on the mortality of the insect larvae, but the combinations of different factors had no significant effect. The most effective factor was temperature, followed by nematode species.

Discussion

The present study demonstrated that temperature had a direct and strong effect on the efficiency of H. bacteriophora and S. feltiae to eliminate larvae of T. molitor. The effect of different soil types, on the other hand, was of minor significance. As the experimental temperatures increased from 12 to 24 °C, the mortality

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rate of *Tenebrio* larvae also increased. Entomopathogenic nematodes generally become sluggish at low temperatures (< 10-15 °C) and inactive at high temperatures (> 30-40 °C) (16). Temperature is one of the most important factors affecting the infectivity of entomopathogenic nematodes (11,17-19); however, the effect of temperature on nematode survival and infectivity varies with nematode species and strain (16,17,19,20). New species and isolates of entomopathogenic nematodes are recovered during soil surveys conducted throughout the world. These surveys usually demonstrate a prevalence of *S. feltiae* under temperate and cold climates and of *H. bacteriophora* in tropical and subtropical coastal regions (9,17,19,21,22).

The present results support these findings. As *S. feltiae* was more effective than *H. bacteriophora* at 12 °C the observed difference became smaller with an increase in temperature. The results also agree with Csontos (23), who found that temperatures < 10 °C depressed the movement of *H. bacteriophora* more than that of *S. glaseri* in sand, and Aydin and Susurluk (24) similarly reported that, when *S. feltiae* and *H. bacteriophora* were applied against *T. molitor* larvae in sand at 12, 18, and 25 °C, *S. feltiae* was more effective only at 12 °C, while at 18 and 25 °C the differences in effectiveness between the 2 nematode species were not significant.

Soil texture is another important factor determining the abundance and survival of nematodes. Sandy to sandy loam soils have the greatest abundance of nematodes, whereas clay soils have the lowest (13,25,26). In the present study, *H. bacteriophora* produced significantly higher mortality of *T. molitor* in sterile clay-loam than in the other 5 soil types at 12 °C. In sterile compost, at 18 and 24 °C, *H. bacteriophora* caused the highest insect mortality. For *S. feltiae*, the highest mortality was observed in sterile compost at all 3 temperatures. Compared with the other soil types, both *H. bacteriophora* and *S. feltiae* had the lowest mortality in non-sterile clay-loam, at all used temperatures. These results can be explained by the effect of soil texture. Clay content soils adversely affect mobility and parasitism of nematodes (13,27).

In sterile soils, the mortality rate of the insect was higher than in non-sterile soils, at all temperatures tested. In contrast, Buchhop (28) and Susurluk (29) reported no significant differences in the number of infective juveniles of *H. bacteriophora* recovered from sterile and non-sterile soils at 15 and 25 °C. These authors also concluded that when the number of infective juveniles of the nematodes recovered from soil stored at 15 and 25 °C is compared, it is always lower at the highest temperature. Ishibashi and Kondo (30,31) stated that entomopathogenic nematodes were affected by natural enemies; therefore, when they placed infective juveniles in sterilized and non-sterilized soils, the infective juveniles survived longer in sterile soils than in non-sterile soils. Kaya (16) explained that predation by invertebrate predators and parasitism by microorganisms can reduce the effectiveness of infective nematode juveniles. Bacterial symbionts also have natural enemies that can negatively affect nematodes.

Possible reasons for the observed effect of varying temperatures and soil types on the efficiency of the entomopathogenic nematodes against *Tenebrio* larvae are that the metabolism of the nematodes and their associated bacteria increase with temperature. As a result, not only does the nematodes’ penetration efficiency increase, but the growth and toxin production of symbiotic bacteria increase as well (32-34). Moreover, non-sterile soils may contain natural enemies of the nematodes (i.e. nematophagous fungi, protozoans, mites, and collembolans) (16,30,31). Finally, in non-sterile soils there might be competition between entomopathogenic nematodes and other pathogens, such as entomopathogenic fungus, protozoa, turbellerians, or other nematode species (7,16).

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