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On the efficiency of entomopathogenic nematodes (Rhabditida: Heterorhabditidae and Steinernematidae) on rust red flour beetle, *Tribolium castaneum* (Herbst.) (Coleoptera: Tenebrionidae)

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Abstract

Background: The rust red flour beetle, *Tribolium castaneum* (Herbst.) (Coleoptera: Tenebrionidae) is a serious pest of stored grains and grain products across the world. This beetle is hold a significant place in Turkey by causing damages on stored products. *T. castaneum* primarily attacks milled grain and its derivates. Entomopathogenic nematodes (EPNs) are regarded as extremely an important biological control agent. EPNs kills their hosts within 48 h by the bacteria they carry.

Results: Efficacies of 4 isolates of EPNs *Steinernema carpocapsae* (Tokat Bakisli 05), *S. feltiae* (Tokat-Emir), *Heterorhabditis bacteriophora* (TOK-20) and *H. bacteriophora* (11-KG) against *T. castaneum* was investigated under laboratory conditions. The experiments were carried out thrice with 10 replicates at 2 different temperatures (15 and 25 ºC). EPNs isolates were tested at 3 different concentrations (250, 500 and 1000 IJs/ml) with a pure water as control. The overall mortality caused by *H. bacteriophora* (Kg11) was significantly higher than the other EPN species. At 25 ºC, *H. bacteriophora* (Kg11) at the highest concentration (1000 IJs/ml) caused 87.6% mortality after 120 h., followed by *S. carpocapsae* and *S. feltiae* with 79.22 and 75.3% mortality rates, respectively. The mortality percentages exhibited by all nematodes' species at both temperatures were lowest at the concentration of (250 IJs/ml). At 15 °C, *H. bacteriophora* (Kg11) caused (55.2%) mortality rate at the highest concentration (1000 IJs / ml) after 120 h.

Conclusion: The study suggested that these nematodes were efficient and could be recommended to control *T. castaneum* in its biological control programs.

Keywords: Entomopathogenic nematodes, *Tribolium castaneum*, Efficiency, Biological control

Background

The red flour beetle, *Tribolium castaneum* (Herbst.) (Coleoptera: Tenebrionidae) is a cosmopolitan pest of stored products (Nenaah 2014), with a polyphagous feeding habit (Bachrouch et al. 2010), attacking widespread variety of stored products and their by-products causing a loss in both quantity and quality (Obeng-Ofori and Reichmuth 1999). Product attacked by *T. castaneum* usually contains carcasses, and exuviae. Both larvae and adults cause serious losses by feeding on product and processed foods. The damage of larvae are mostly confined to the germ of the grains in wheat (White and Lambkin 1988). Fumigation by chemical pesticides still the main method for control stored grain pests. These

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chemical pesticides have hazard effects on both the environment and the consumers, on the other hand caused insect resistance (Lu and Wu 2010), therefore, scientists work to use other methods to control store insects rather than chemical pesticides.

Entomopathogenic nematodes (EPNs) are an important biological control agent found in soil all over the world. Today, in many countries, EPNs are used in biological control programs of many economic insect pests. They are a group of soil dwelling nematodes that parasitize insects pests. Use of these nematodes is economical and eco-friendly, since they are harmless to non-target organisms, human health and the environment (Gulcu et al. 2017). These organisms infect insects in soil through natural openings or thin parts of the cuticle and release their symbiont bacteria in insect hemolymph. Death occurs within 16–24 h due to the bacteria reproducing in the insect's body (Kepenekci 2012).

This study aimed to evaluate the virulence of 4 EPNs' isolates against *T. castaneum* under laboratory conditions.

Methods

EPNs, *T. castaneum* adults, the great wax moth, *Galleria mellonella* L. cultures constituted the main materials of the study. Experiments carried out under laboratory conditions in the Nematology Laboratory of the Central Research Institute of Plant Protection (Ministry of Agriculture and Forestry of the Republic of Turkey) in 2020.

Nematode culture

Infective juveniles of *Steinernema carpocapsae* (Tokat, Bakışlı 05), *S. feltiae* (Tokat, Emir), *Heterorhabditis bacteriophora* (TOK-20), *H. bacteriophora* (11-KG) were obtained from Tokat Gaziosmanpaşa University. All nematode species were reared on *G. mellonella* mature larvae as described by Kaya and Stock (1997).

Rearing *Galleria mellonella* larvae

A special nutrient diet, containing 890 g of flour, 222 g of dry baker's yeast, 500 g of glycerin, 500 g of honey, 445 g of milk powder and 445 g Flour, bran, milk powder and yeast, mixed and then poured on a mixture of honey and glycerin, was prepared (Mohammed and Coppel, 1983). *Galleria mellonella* eggs were placed on a food medium in 1 lt glass jars and then placed in the incubator (16/8 h lighting set at 23–24 °C) for hatching and development of the larvae.

Rearing of entomopathogenic nematode species

Mature instar larvae of *G. mellonella* were used for rearing the EPNs. The larvae were placed on Whatman paper (White, 1927), soaked in sterile water, in small Petri dishes (diameter of 6 cm). The 2nd and 3rd stages of infective nematode were collected from the water by a dropper and applied on the *G. mellonella* larvae. Then, the lids of the Petri dishes were wrapped by a parafilm and placed in the incubator (20–23 °C). The Petri dishes were inspected every 10 days. The obtained juveniles were kept in a refrigerator (10 °C).

Rearing of *T. castaneum*

Tribolium population grown in the Stored Crop Pests Unit of the Entomology Department, Ankara Plant Protection Central Research Institute, Turkey was used in the study. A mixture of dry yeast and wheat flour (1: 3) was used in the cultivation of *Tribolium*. 250 g of the nutrient mixture, sterilized at −18 °C for 120 h, was placed in a 1 L glass jar with a perforated lid for ventilation. Glass jars were kept in climate chambers at 25 ± 1 °C and 60 ± 5% RH. 500–750 adults were placed in each jar for oviposition and after a week, adults were removed from by passing through a 30 mesh sieve. The screened adult individuals were placed to a new jar containing food, and the continuity of the cultures was ensured.

Laboratory experiments of EPNs

For each nematode species, the experiments were carried out in plastic Petri dishes (9 cm) under the same conditions 3 times on different dates with 10 replicates per repeat and at 2 different temperatures (25 and 15 °C) in a climate chamber. *T. castaneum* adults (10 individuals) were placed by the help of soft forceps into Petri dishes with 5 g sterilized wheat crumbs bedding. Then, the EPN isolates prepared, using distilled water at 250, 500 and 1000 IJs/ml, were applied directly into the Petri dish. After the application, the Petri dishes were covered by a parafilm. Only pure water was used in the control group. The vitality of the adult individuals in the Petri dishes was counted regularly at the end of 48, 72 and 120 h and the mortality % were calculated. Cadavers were taken place "White trap" and were examined under a stereomicroscope. After one week, EPNs juveniles were obtained from infected *T. castaneum* adults.

Statistical analysis

Duncan multiple comparison test was performed with SPSS statistics 17. Square root transformation was applied to non-normally distributed data, followed by ANOVA (Duncan test).

Results

Statistical evaluations of the activitiy of the EPNs (48, 72 and 120 h post-inoculation), all the 3 parameters (nematode species, levels and temperatures) and their interactions were significant. The of all EPNs species was

directly proportional to the mortality percentage of pest adults as presented in tables (1 and 2). The lowest mortality % was recorded at the lowest applied concentration (250 IJs/ml) and at 15 °C for the 4 EPNs species.

H. bacteriophora (Kg11) isolate was the most effective one at 15 °C causing (55.2%) mortality at the highest level (1000 IJs/ml) 120 h post-treatment, followed by *H. bacteriophora* (Tok 20) with (43.2%), *S. feltiae* with (34.7%), and *S. carpocapsae* with (33*%)*, respectively. No mortality occurred in the control treatments, pure water-only (Table 1).

At 25 °C, *H. bacteriophora* (Kg11) caused (87.6%) insect mortality at the highest concentration (1000 IJs/ml) after 120 h., followed by *S. carpocapsae* (79.22%), *S. feltiae* (75.3%) and *H. bacteriophora* (Tok 20) (Table 2).

Discussion

Although EPNs are most effective in the larval stage, they can be effective on all insect stages. They enter the host's hemocell through the host's natural openings such as the mouth, anus, stigmas, hair follicles, or through thin parts of the cuticle (only in Heterorhabdites) (Bedding and Molyneux 1982; Wang and Gaugler 1998). EPNs are in a symbiotic relationship with bacteria of the genus Xenorhabdus (in Steinernematids) and Photorhabdus (in Heterorhabdites). IJs entering

Table 1 Mortality (%) of *Tribolium castaneum* adult caused by EPN isolates at 48, 72 and 120 h (15 °C)

Concentrations	*Steinernema carpocapsae* (Tokat Bakışlı 05)	*S. feltiae* (Tokat Emir)	*Heterorhabditis bacteriophora* (Tok 20)	*H.bacteriophora* (Kg11)
48 h				
250	0.00c	1.50c	00.00c	0.00c
500	0.00c	4.50bc	1.00c	1.50c
1000	6.00b	4.50bc	30.00a	26.56a
72 h				
250	0.00f	1.50ef	0.00f	7.00ef
500	0.00f	5.50cde	1.00f	41.61b
1000	6.00cd	7.50c	38.33b	46.00b
120 h				
250	8.50e	6.50ef	0.00f	2.50ef
500	20.33 ± 2.82d	30.72c	2.00def	18.61d
1000	33.22c	34.67c	43.17a	55.17a

$F_{15.3}$ = 23, 48,104 and 71 for 48, 72, 120 h, respectively. $P < 0.01$

Table 2 Mortality (%) of *Tribolium castaneum* adult caused by EPN isolates at the end of 48, 72 and 120 h (25 °C)

Concentrations	*Steinernema carpocapsae* (Tokat Bakışlı 05)	*S. feltiae* (Tokat Emir)	*Heterorhabditis bacteriophora* (Tok 20)	*H.bacteriophora* (Kg11)
48 h				
250	14.6g	19.39fg	24.06f	21.06fg
500	17.00fg	39.83cd	31.56e	24.50f
1000	58.78b	38.67d	46.61c	66.33a
72 h				
250	27.17h	25.11h	28.94gh	36.11fg
500	44.50de	47.61cde	40.61ef	29.83gh
1000	65.17b	55.22c	52.06cd	81.56a
120 h				
250	34.28g	32.83g	40.61fg	39.44fg
500	52.11de	54.45d	47.11def	45.67ef
1000	79.22b	75.33b	65.33c	87.61a

$F_{15.3}$ = 67, 77 and 108 for 48, 72, 120 h, respectively. $P < 0.01$

the host, change their coats and deposit the symbiotic bacteria they carry into the host hemocell. Bacteria that reproduce by breaking down the insect tissue cause the host to die within 48 h (Constant and Boven 2000, Glazer and Lewis 2000).

Some studies have assessed the potentials of EPNs on *T. castaneum*. For instance, the study of Ramos-Rodr quez et al. (2007) investigated the efficacy of *Steinernema riobrave* against *T. castaneum.* In laboratory bioassays, *S. riobrave* reduced survival of *T. castaneum*, larvae, pupae and adults from 77.9 ± 3.2% in the controls to 27.4 ± 2.5% in treatments.

The study also stated that the larval stage were the most susceptible to EPNs and that temperature (25 and 30 °C) and RH (43, 57, 75 and 100%) had non-significant effects on the efficiency of *S. riobrave*. Erturk et al. (2013) evaluated the activities of Aydın isolates of *S. feltiae, S. carpocapsae* and *H. bacteriophora* against *T. castaneum* and *T. confusum* adults under laboratory conditions. The results showed non-significant difference between *S. feltiae* and *H. bacteriophora* applications, while *S. carpocapsae* was found to be the most effective isolate against *T. castaneum* (86.47% mortality) and *T. confusum* (85.35% mortality) adults at a concentration of (2000 IJs).

Javed et al. (2020) evaluated the efficacy of *Steinernema pakistanense* (LM-07), *S. bifurcatum* (LM-30), *S. affinae* (GB-14) and *S. cholashanense* (GB -22) against *T. confusum* and *Rhyzopertha dominica* adults at 3 different concentrations (50, 100 and 150 IJs / insect) and 3 different temperatures (20, 25 and 30 °C) under laboratory conditions. *S. pakistanense* at (150 IJs/insect) concentration caused (100%) death at 30 °C. Another study for the virulence of Pakistani isolates of *S. bifurcatum* and *S. affine* against *T. castaneum* and *Lasioderma serricorne* under laboratory conditions found that the insect larvae were more sensitive than adults to nematode infection, and that at the highest concentration (200 IJs/insect) used in the trial, *S. bifurcatum* caused (92%) *L. serricorne* and 93% *T. castaneum* larval mortality, while *S. affine* caused (90 and 95%) mortality in *L. serricorne* and *T. castaneum*, respectively (Khanum and Javed 2021).

Conclusions

All EPN species tested in this study were found to be effective against the *T. castaneum* adults at 25 °C. But *H. bacteriophora* (Kg11) was the most effective species against the flour beetle adults under controlled conditions. The isolates *H. bacteriophora* (Kg11), *S. carpocapsae* (Tokat Bakışlı 05) and *S. feltiae* (Tokat Emir) can be recommended to be used in biological control programs against *T. castaneum*.

Abbreviations

EPNs: *Entomopathogenic nematodes*; IJs: *Infective juveniles*; *T. castaneum*: Tribolium castaneum; *H. bacteriophora*: *Heterorhabditis bacteriophora*; *S. carpocapsae*: *Steinernema carpocapsae*; *S. fertilae*: *Steinernema feltiae*; *G. melonella*: *Galleria melonella*; Rust red flour beetle: *Tribolium castaneum*.

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Authors' contributions

F.D.E. She carried out all the stages of the study.

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Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

None.

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