Efficacy of *Metarhizium brunneum* and *Beauveria bassiana* isolates against the European tent caterpillar, *Malacosoma neustria* Linnaeus, 1758 (Lepidoptera: Lasiocampidae)

Elif Fatma Topkara1*, Oğuzhan Yanar1, Funda Sahin2, Yusuf Yanar2 and Dürdane Yanar2

Abstract

**Background:** The European tent caterpillar *Malacosoma neustria* Linnaeus, 1758 (Lepidoptera: Lasiocampidae), a worldwide pest, feeds on a wide variety of woody and shrub-like plants in its larval stage and causes extensive economic losses. In the fight against this species, environmentally friendly biological control methods should be preferred instead of chemical control. The aim of this study was to evaluate the efficacy of entomopathogenic fungi (EPF) *Metarhizium brunneum* (ORP-13) and *Beauveria bassiana* (GOPT-301-2) isolates against the fourth instar larvae of *M. neustria* under laboratory conditions.

**Results:** *M. neustria* eggs were collected from the Kızılırmak Delta of Samsun Province, Turkey, and the fourth instar larvae were used in the experiment. Larvae in the control group were fed on sterilized leaves of *Eleagnus rhamnoides*. Both fungal isolates were applied onto the larvae at 2 ml for each concentration (1 × 10^6, 1 × 10^7, and 1 × 10^8 conidia ml^-1). Ten larvae were placed in each group, and sterilized *E. rhamnoides* leaves were offered to the larvae. The study was carried out in 9 replicates for each group, and the larvae were observed for 14 days. As a result of the study, it was found that the survival rates of the larvae decreased as concentration increased. It was determined that both isolates caused 100% mortality at 1 × 10^8 conidia ml^-1 concentration. The lowest LC50 value was found in larvae exposed to the ORP-13 isolate.

**Conclusion:** It has been suggested that *M. brunneum* and *B. bassiana* isolates were virulent for *M. neustria* larvae and can be used for biological control of this species.

**Keywords:** Entomopathogenic fungi, *Beauveria bassiana*, *Metarhizium brunneum*, European tent caterpillar, Virulence

Background

The European tent caterpillar *Malacosoma neustria* Linnaeus, 1758 (Lepidoptera: Lasiocampidae) is one of the most important agricultural pests in Turkey. It causes various damages to many fruit and forest trees, particularly apple, pear, cherry, hazelnut, and oak (Kati et al. 2005). Although the outbreak periods are irregular, population explosion occurs at intervals of 3–7 years, and thus, many trees have been damaged (Özbek and Çoruh 2010). As a result of invasion, most host plants become completely leafless, so combating this species is inevitable. Chemical pesticides used to combat insect pests are also used to control this species (Kati et al. 2005). Given
the adverse effects of chemical pesticides in the ecosystem, biological control methods should be used to combat this species.

Compared to chemical pesticides, entomopathogenic microorganisms offer various advantages in biological control due to their fast generation times, safety, being environmentally friendly, and the ability to target a specific target organism. Entomopathogenic fungi (EPF); Beauveria bassiana Vuill and Metarhizium brunneum Petch are widely used biological control agents in pest control. EPF infects insects belonging to the orders Lepidoptera, Coleoptera, Hemiptera, Diptera, Orthoptera, and Hymenoptera have shown the effectiveness of these pathogenic fungi against various insects (Ozdemir et al. 2020).

In this study, the efficacy of EPF M. brunneum (ORP-13) and B. bassiana (GOPT-301-2) isolates against the fourth instar larvae of M. neustria was evaluated under laboratory conditions.

Methods

Sampling

Malacosoma neustria eggs were collected from E. rhamnoides in the Kizilirmak Delta of Samsun, Turkey, (N 41° 30’ E 36° 05’) and brought to the laboratory. The eggs were disinfected with 10% sodium hypochlorite for about 7 min and then washed and rinsed with distilled water for about 7 min. The disinfected eggs were taken to the air-conditioning room at 24 °C, 70±5% RH, at 16:8 h light/dark period. Hatching larvae were fed on E. rhamnoides leaves until the fourth instar. Each leaf sample was sterilized with 50% ethanol and then given to the larvae. Plant leaves used in feeding experiments were collected daily. The fourth instar larvae of M. neustria were used in this study.

Fungal cultures

The EPF Metarhizium brunneum (ORP-13) isolate isolated from soil samples at Ordu province was collected and identified by Prof. Dr. Yusuf YANAR and Prof. Dr. Durdane Yanar at Tokat Gaziosmanpasa University, Agricultural Faculty, Department of Plant Protection, Tokat/Turkey, and tested in the study. Beauveria bassiana (GOPT-301-2) isolate was isolated from Leptinotarsa decemlineata Say (Coleoptera: Chrysomelidae). For identification of the isolates, DNA extractions of fungi were performed. Genomic DNA amplification was carried out using ITS4/ITS5 primers. The isolates were diagnosed by sequence analysis and recorded in the GenBank database (Table 1). The isolates had already been tested for pathogenicity and were considered virulent. They were grown in an incubator at 24±2 °C in the dark on potato dextrose agar (PDA; Merck Ltd., Darmstadt, Germany) medium for 15–30 days.

Table 1 Species, hosts and locations of entomopathogenic fungi isolates used in the study

| Species/Isolate denomination | GenBank accession numbers | Host          | Locality |
|-----------------------------|---------------------------|---------------|----------|
| Metarhizium brunneum/ ORP-13| MW410195                  | Soil          | Ordu     |
| Beauveria bassiana/GOPT-301-2| MK411548                 | Leptinotarsa decemlineata | Tokat    |

Preparation of conidial suspensions

The fungi were subcultured by conidial transfer to PDA plates to produce inoculum for experiments. After getting sporulation, fungal conidia were harvested by scraping with a scalpel. The conidial suspension was prepared by adding 10 ml of sterile-distilled water containing 0.02% Tween 80. The conidial suspension was vortexed for 1–2 min and filtered through four layers of sterile cheesecloths to remove mycelial fragments. The resulting spore suspensions were adjusted to concentrations of 1 × 10^6 to 1 × 10^8 conidia ml⁻¹ using a hemocytometer. The viability of conidia was determined by applying 0.1 ml of the suspension to the PDA plates. A sterile microscope coverslip was placed on each plate and incubated at 27 °C. After 24 h, the percentage of germination was determined by counting 100 spores per dish.

Application of entomopathogenic fungi on M. neustria

Two layers of sterile filter paper were lightly moistened and placed in 1-L plastic cups for the experiment. 2 ml of 1 × 10^6, 1 × 10^7, and 1 × 10^8 conidia ml⁻¹ of both fungal isolates were sprayed on the fourth instar larvae placed in plastic cups (10 larvae per dish) using a Potter spraying tower (Burkard, Rickmansworth, Hertz UK), and the appropriate amount of sterilized E. rhamnoides leaves was placed in the cups to feed the larvae. After each application of EPF suspension, the spray tower was cleaned by 70% ethanol and sterile-distilled water. Only sterile-distilled water containing 0.02% Tween 20 was sprayed on the control group, and the larvae in the control group were fed on sterilized E. rhamnoides leaves. The study was carried out with nine repetitions for each group. A total of 630 larvae were used in the experiment. All plastic cups were incubated at 25 ± 1 °C, 70 ± 5% RH and a 16:8 h light/dark period for 14 days. The mortality rates were monitored for 14 consecutive days. The mycosed in each cup were counted 14 days after the application, and the mortality rates were calculated.

Samples were disinfected with 10% sodium hypochlorite for about 7 min and then washed and rinsed with distilled water for about 30 ± 2 °C in the dark on potato dextrose agar (PDA; Merck Ltd., Darmstadt, Germany) medium for 15–30 days.
the control groups, the same procedure was followed every day.

Statistical analyses
The daily mortality rates were corrected using the Abbott formula when the mortality rate in the control group exceeded 5% (Abbott, 1925). The Log-Rank test was used to compare the concentrations of the fungal isolates and the control group. The Cox regression analysis was used to compare the mortality risk of larvae exposed to two isolates. The survival curve was made according to the Kaplan–Meier analysis. The lethal concentration (LC_{50}) was calculated by Probit analysis. For these tests, SPSS version 21.0 was used.

Results
As a result of the experiment, it was determined that the mortality rate of *M. neustria* larvae in the control group was 5.6%. It was noted that the mortality rate in larvae exposed to the lowest concentration (1 × 10^6 conidia ml^{-1}) of ORP-13 isolate was 50.6%, while the mortality rate in larvae exposed to the highest concentration (1 × 10^8 conidia ml^{-1}) of GOPT-301-2 isolate was 41.2%, mortality rate in larvae exposed to the highest concentration (1 × 10^8 conidia ml^{-1}) was 100% (Table 2).

When the mean survival times were compared, it was found that the longest lifespan (14.8 days) was in the control larvae. Larvae exposed to the highest concentration (1 × 10^8 conidia ml^{-1}) of GOPT-301-2 isolate had the shortest lifespan (7 days). It was determined that the mean lifespan of the larvae treated with 1 × 10^8 conidia ml^{-1} concentration of the GOPT-301-2 isolate was 8.2 days (Table 3).

According to the Log-Rank test analysis results, *M. neustria* larvae infected with different concentrations of the ORP-13 and GOPT-301-2 fungal isolates were found to be statistically different from the control group. It was determined that there was no statistical difference between the 1 × 10^7 conidia ml^{-1} concentration of ORP-13 and GOPT-301-2 isolates, whereas the other groups were different from each other (Table 4).

The Cox regression analysis revealed that there was a statistical difference (p < 0.01) between the control group and each of the groups infected with different concentrations of the ORP-13 and GOPT-301-2 fungal isolates. The risk of death increased 55.6 times for larvae exposed to the highest concentration of ORP-13 isolate, while it increased 39.4 times for larvae exposed to the highest concentration of GOPT-301-2 isolate. The risk of death of *M. neustria* larvae increased with increasing conidial concentrations of both isolates (Table 5).

Figure 1 illustrates the survival curves of the control and the infected groups. The control group had the highest survival rate, while larvae exposed to the ORP-13 isolate at 1 × 10^8 conidia ml^{-1} concentration had the lowest mortality rate.

### Table 2 Survival rates of *Malacosoma neustria* larvae and mortality confirmed by Abbott formula

| Groups                | Total N | N of events | Censored N | Survival (%) | Abbott formula % |
|-----------------------|---------|-------------|------------|--------------|------------------|
| Control               | 90      | 5           | 85         | 94.4         | 5.6              |
| ORP-13 (1 × 10^6)     | 90      | 48          | 42         | 46.7         | 50.6             |
| ORP-13 (1 × 10^7)     | 90      | 65          | 25         | 27.8         | 70.6             |
| ORP-13 (1 × 10^8)     | 90      | 90          | 0          | 0.0          | 100              |
| GOPT-301-2 (1 × 10^6) | 90      | 40          | 50         | 55.6         | 41.2             |
| GOPT-301-2 (1 × 10^7) | 90      | 75          | 15         | 16.7         | 82.4             |
| GOPT-301-2 (1 × 10^8) | 90      | 90          | 0          | 0.0          | 100              |

### Table 3 Mean lifespan of *Malacosoma neustria* larvae exposed to different concentrations of ORP-13 and GOPT-301-2 isolates

| Groups                | Mean^a Estimate (days) | SE | 95% confidence interval |
|-----------------------|------------------------|----|-------------------------|
| Control               | 14.867                 | 0.92 | 14.687 - 15.046         |
| ORP-13 (1 × 10^6)     | 11.267                 | 0.433 | 10.417 - 12.116        |
| ORP-13 (1 × 10^7)     | 9.200                  | 0.412 | 8.392 - 10.008         |
| ORP-13 (1 × 10^8)     | 7.022                  | 0.186 | 6.658 - 7.386          |
| GOPT-301-2 (1 × 10^6) | 12.889                 | 0.334 | 12.234 - 13.544        |
| GOPT-301-2 (1 × 10^7) | 9.756                  | 0.367 | 9.036 - 10.475         |
| GOPT-301-2 (1 × 10^8) | 8.244                  | 0.267 | 7.720 - 8.768          |

^a Estimation is limited to the largest survival time if it is censored.
The LC50 values for both fungal isolates of *M. neustria* larvae are shown in Table 6. The LC50 value of the ORP-13 isolate was low compared to the GOPT-301-2 isolate.

### Discussion

Given the harmful effects of chemical insecticides on the environment, relatively safer and environmentally friendly pest control methods such as EPF, which is effective against a wide range of insect pest species, should be preferred. This laboratory study evaluated the pathogenicity of promising isolates of *B. bassiana* and *M. brunneum* against the insect pest *M. neustria*.

As a result of the treatment of *M. neustria* larvae with two different isolates, it was determined that the control larvae had the lowest mortality rate. Larval mortality rates increased as conidial concentrations of both isolates increased. The highest mortality rate was determined in larvae exposed to the highest concentration (1 × 10^8 conidia ml^-1) of both isolates, which was 100% in both. In the studies (Machowicz-Stefaniak 1979), it was determined that EPF significantly reduced moth populations from the Lasiocampidae family, and these results are consistent with the findings of our study.

Although *M. neustria* causes severe damage to many fruits and forest trees, studies on its control with EPF are limited. Studies have evaluated the efficacy of EPFs against *M. neustria*. In the studies, conducted by Draganova et al. (2013), it was announced that *M. neustria* was infected by the genus *Beauveria*. Machowicz-Stefaniak (1979) showed that *M. neustria* was infected by *B. bassiana* and *M. anisopliae*. Wang et al. (2014) stated that *B. bassiana* showed a high insecticidal activity against *M. neustria*. *B. bassiana* and *M. brunneum* isolates were found to have a high insecticidal activity against *M. neustria* in this study.

When the mean lifespan was evaluated, it was determined that the control larvae had the longest lifespan. Leathers and Gupta (1993) treated *M. americanum* Fabricius, 1793 (Lepidoptera: Lasiocampidae) with different isolates of *B. bassiana* and found that all of the treated larvae died at the end of the fourth day. In the present study, it was determined that increasing conidial

**Table 4** Log-Rank (Mantel–Cox) test results of *Malacosoma neustria* larvae exposed to different concentrations of ORP-13 and GOPT-301-2 isolates

| Groups          | Control | ORP-13 (1 × 10^6) | ORP-13 (1 × 10^7) | ORP-13 (1 × 10^8) | GOPT-301-2 (1 × 10^6) | GOPT-301-2 (1 × 10^7) | GOPT-301-2 (1 × 10^8) |
|-----------------|---------|-------------------|-------------------|-------------------|----------------------|----------------------|----------------------|
|                 | χ²      | p                 | χ²                | p                 | χ²                  | p                    | χ²                  | p                    |
| Control         | 52.4    | .000              | 91.8              | .000              | 187                 | .000                 | 37.3                | .000                 |
| ORP-13 (1 × 10^6) | 52.4    | .000              | 9.2               | .022              | 62.6                | .000                 | 3.5                 | .006                 |
| ORP-13 (1 × 10^7) | 91.8    | .000              | 9.2               | .002              | 18.4                | .000                 | 26.7                | .000                 |
| ORP-13 (1 × 10^8) | 187     | .000              | 18.4              | .000              | 129.3               | .000                 | 42.4                | .000                 |
| GOPT-301-2 (1 × 10^6) | 37.3    | .000              | 3.5               | .06               | 26.7                | .000                 | 129.3               | .000                 |
| GOPT-301-2 (1 × 10^7) | 123.3   | .000              | 13.5              | .000              | 1.0                 | .000                 | 42.4                | .000                 |
| GOPT-301-2 (1 × 10^8) | 196.3   | .000              | 46.9              | .000              | 8.2                 | .004                 | 16.2                | .000                 |

χ² Chi-square test, p Significant

**Table 5** Cox regression analysis results of *Malacosoma neustria* larvae exposed to different concentrations of ORP-13 and GOPT-301-2 isolates

| Groups          | B      | SE     | Wald   | df   | p       | Exp(B) | 95.0% CI for Exp(B) |
|-----------------|--------|--------|--------|------|---------|--------|---------------------|
|                 |        |        |        |      |         |        | Lower               |
|                 |        |        |        |      |         |        | Upper               |
| Control         | 163.616| 6      | .000   |      |         | 13.741 | 5.468               |
| ORP-13 (1 × 10^6) | 2.620  | .470   | 31.066 | 1    | .000    | 13.741 | 5.468               |
| ORP-13 (1 × 10^7) | 3.239  | .465   | 48.534 | 1    | .000    | 25.498 | 10.252              |
| ORP-13 (1 × 10^8) | 4.019  | .464   | 75.165 | 1    | .000    | 55.651 | 22.432              |
| GOPT-301-2 (1 × 10^6) | 2.236  | .474   | 22.212 | 1    | .000    | 9.356  | 3.692               |
| GOPT-301-2 (1 × 10^7) | 3.240  | .463   | 49.028 | 1    | .000    | 25.540 | 10.311              |
| GOPT-301-2 (1 × 10^8) | 3.676  | .462   | 63.381 | 1    | .000    | 39.482 | 15.973              |

B Coefficient of regression, SE Standard error, Wald Significance of the regression coefficients, df Degree of freedom, p Significant, Exp(B) Hazard proportion, CI Confidence interval
concentration reduced life expectancy. Larvae infected with the ORP-13 isolate were found to die in a shorter time, indicating that this isolate was more effective than the GOPT-301-2 isolate.

When LC50 values were compared, it was noted that larvae exposed to the GOPT-301-2 isolate had the highest value, while larvae exposed to the ORP-13 isolate had the lowest value. Therefore, the ORP-13 isolate was more effective than the GOPT-301-2 isolate, since a low LC50 value indicated that the applied fungal isolate was effective even in low amounts.

Larvae infected with the ORP-13 isolate had a shorter life expectancy, a high mortality risk, and a low LC50 value, indicating that the ORP-13 isolate was more effective than the GOPT-301-2 isolate. It has been also reported that M. neustria larvae infected with B. bassiana in the early stages have higher mortality rate than those in the late stages (Machowicz-Stfaniak 1979).

Although the fourth instar M. neustria larvae was used, both isolates were extremely virulent against M. neustria larvae, causing 100% mortality at the highest concentration (1 × 10^8 conidia ml^-1).

**Conclusions**

The two isolates (ORP-13 and GOPT-301-2) of B. bassiana and M. brunneum were found to be virulent against M. neustria larvae in this study, and these isolates could be used for biological control of this species. Understanding the insect-EPF related parameters is critical because it will provide us with preliminary evaluation results in field applications for the biological control of insect pests. These effects may differ in field applications. As a result, it is crucial to apply these fungal isolates in the field under controlled conditions.
Abbreviations
EPF: Entomopathogenic fungi; PDA: Potato dextrose agar.

Acknowledgements
Not applicable.

Author Contributions
EFT, OY, FS, YY, and DY conceived, designed, analyzed, wrote, corrected and approved the final draft. All authors have read and approved the manuscript.

Funding
Not applicable.

Availability of data and materials
The data generated and/or analyzed during the current study are available from the corresponding author.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no conflict interests.

Author details
1 Department of Biology, Science and Art Faculty, Ondokuz Mayis University, 55139 Samsun, Turkey. 2 Department of Plant Protection, Faculty of Agriculture, Tokat Gaziosmanpaşa University, 60250 Tokat, Turkey.

Received: 22 April 2022   Accepted: 23 July 2022

Published online: 29 July 2022

References
Abbott WS (1925) A method of computing the effectiveness of insecticides. J Econ Entomol 18:265–267. https://doi.org/10.1093/jee/18.2.265a
Draganova S, Takov D, Pilarska D, Doychev D, Mirchev P, Georgiev G (2013) Fungal pathogens on some Lepidopteran forest pests in Bulgaria. Acta Zool Bulg 65(2):179–186
Kati H, Sezen K, Belduz AO, Demirbag Z (2005) Characterization of a Bacillus thuringiensis subsp. kurstaki strain isolated from Malacosoma neustria L. (Lepidoptera: Lasiocampidae). Biol Sect Cell Mol Biol 60:301–305
Leathers TD, Gupta SC (1993) Susceptibility of the eastern tent caterpillar (Malacosoma americanum) to the entomogenous fungus Beauveria bassiana. J Invertebr Pathol 61(2):217–219
Machowicz-Stefaniak Z (1979) Objawy chorobowe występujące u gąsienic Malacosoma neustria L. (Lepidoptera) zakażonych przez niektóre grzyby [Disease symptoms in the caterpillars of Malacosoma neustria L. (Lepidoptera) infected by some fungi]. Acta Mycol 15(1):145–149 (in Polish)
Özbek H, Çoruh S (2010) Egg parasitoids of Malacosoma neustria (Linnaeus, 1758) (Lepidoptera: Lasiocampidae) in Erzurum province of Turkey. Turk Entomol Derg 34(4):551–560
Ozdemir IO, Tuncer C, Erper I, Kushiye R (2020) Efficacy of the entomopathogenic fungi; Beauveria bassiana and Metarhizium anisopliae against the cowpea weevil, Callosobruchus maculatus F. (Coleoptera: Chrysomelidae: Bruchinae). Egypt J Biol Pest Control 30:24. https://doi.org/10.1186/s41938-020-00219-y
Wang Z, Sun L, Zhang J, Cao C (2014) Preparation and insecticidal efficacy of wettable powder formulations of Bacillus thuringiensis and Beauveria bassiana. J Beijing For Univ 36(3):34–41. https://doi.org/10.13332/j.cnki.jbfu.201403.005

Publisher's Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.