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

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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 LC_{50} 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

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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 Kızılırmak 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. Dürdane 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. For

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}) was 100%. While the mortality rate in larvae exposed to the lowest concentration (1×10^6 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 of ORP-13 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 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).

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 ınterval	
			Lower bound	Upper bound
Control	14.867	.092	14.687	15.046
ORP-13 (1×10^6)	11.267	.433	10.417	12.116
ORP-13 (1×10^7)	9.200	.412	8.392	10.008
ORP-13 (1×10^8)	7.022	.186	6.658	7.386
GOPT-301-2 (1×10^6)	12.889	.334	12.234	13.544
GOPT-301-2 (1×10^7)	9.756	.367	9.036	10.475
GOPT-301-2 (1×10^8)	8.244	.267	7.720	8.768

[a] Estimation is limited to the largest survival time if it is censored

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		Abbott formula %
			N	Survival (%)	Mortality (%)
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 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)	
	X^2	*p*	X^2	*p*	X^2	*p*	X^2	*p*	X^2	*p*	X^2	*p*	X^2	*p*
Log-Rank (Mantel–Cox)														
Control			52.4	.000	91.8	.000	187	.000	37.3	.000	123.3	.000	196.3	.000
ORP-13 (1×10^6)	52.4	.000			9.2	.002	62.6	.000	3.5	.006	13.5	.000	46.9	.000
ORP-13 (1×10^7)	91.8	.000	9.2	.002			18.4	.000	26.7	.000	.000	1.0	8.2	.004
ORP-13 (1×10^8)	187	.000	62.6	.000	18.4	.000			129.3	.000	42.4	.000	16.2	.000
GOPT-301-2 (1×10^6)	37.3	.000	3.5	.06	26.7	.000	129.3	.000			38.9	.000	102.4	.000
GOPT-301-2 (1×10^7)	123.3	.000	13.5	.000	.000	1.0	42.4	.000	38.9	.000			15.7	.000
GOPT-301-2 (1×10^8)	196.3	.000	46.9	.000	8.2	.004	16.2	.000	102.4	.000	15.7	.000		

X^2 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			
ORP-13 (1×10^6)	2.620	.470	31.066	1	.000	13.741	5.468	34.530
ORP-13 (1×10^7)	3.239	.465	48.534	1	.000	25.498	10.252	63.418
ORP-13 (1×10^8)	4.019	.464	75.165	1	.000	55.651	22.432	138.060
GOPT-301-2 (1×10^6)	2.236	.474	22.212	1	.000	9.356	3.692	23.711
GOPT-301-2 (1×10^7)	3.240	.463	49.028	1	.000	25.540	10.311	63.258
GOPT-301-2 (1×10^8)	3.676	.462	63.381	1	.000	39.482	15.973	97.591

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

The LC_{50} values for both fungal isolates of *M. neustria* larvae are shown in Table 6. The LC_{50} 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

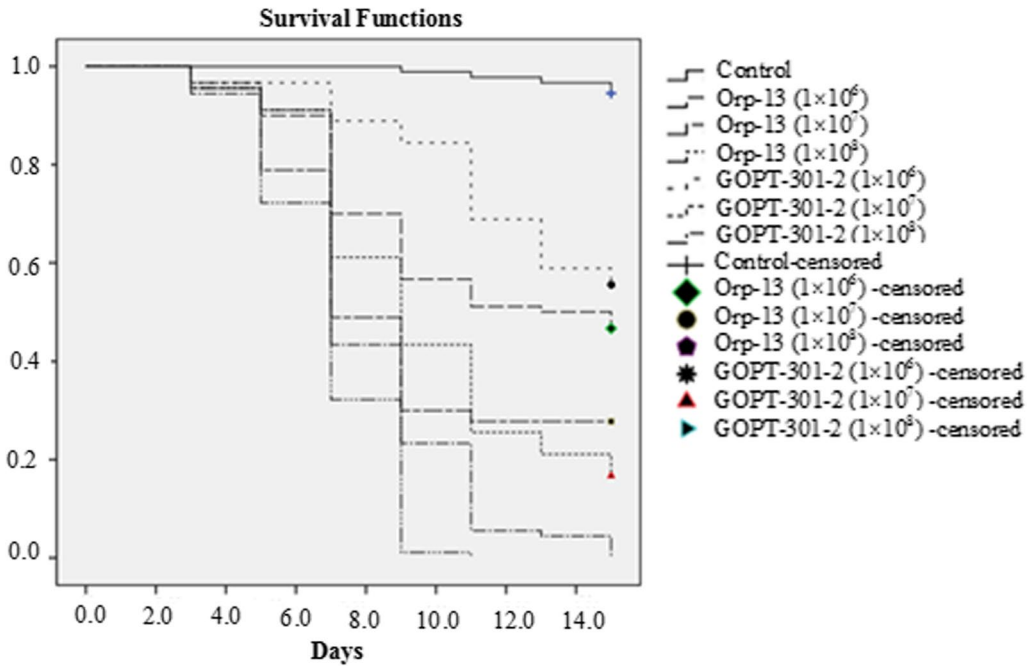

Fig. 1 Survival curve according to Kaplan–Meier analysis of *Malacosoma neustria* larvae exposed to different concentrations of ORP-13 and GOPT-301-2 isolates

Table 6 Median lethal concentration (LC_{50}) of *Malacosoma neustria* larvae exposed to different concentrations of ORP-13 ve GOPT-301-2 isolates according to the Probit analyses

Isolate	LC_{50} (conidia ml^{-1})	Intercept	Slope ± SE	*df*	X^2	95% CI	
						Lower bound	Upper bound
ORP-13	8.2×10^5	− 4.3 ± 0.5	0.6 ± 0.08	3	9.2	0.65	0.97
GOPT-301-2	9.0×10^5	− 5.8 ± 0.6	1.0 ± 0.1	3	5.5	0.8	1.2

X^2 Chi-Square test, *df* Degree of freedom, *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 LC_{50} 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 LC_{50} 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 LC_{50} 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.

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

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

Declarations

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Not applicable.

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Not applicable.

Competing interests
The authors declare no conflict interests.

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