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Pathogenicity of some local entomopathogenic fungus isolates on the cotton leafworm larvae, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae)

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Abstract

Background: Pathogenicity of the entomopathogenic fungi (EPF), isolated from soil samples collected from Ordu Province, Turkey, was evaluated on the second-instar larvae of the cotton leaf worm *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae) under laboratory conditions.

Results: Firstly, single-concentration response tests were conducted in order to determine the efficacy of the 64 isolates on *S. littoralis* larvae at the concentration of 1×10^8 conidia/ml. The five isolates displaying the highest mortality rates in single-concentration response tests, ORU-50, ORM-40, ORP-13, ORP-27 and ORM-48 (which included *Beauveria bassiana, Metarhizium brunneum and Clonostachys rogersoniana*), were subjected to concentration–response tests at the concentrations of 1×10^5–1×10^9 conidia/ml. The lowest LC_{50} and LC_{90} values were recorded at ORP-27 with 1.68×10^7 and 4.60×10^8 conidia/ml, respectively, followed by ORP-13 and ORM-40.

Conclusions: Accordingly, it was found that *M. brunneum* isolates were more effective than *B. bassiana* and *C. rogersoniana* against *S. littoralis* larvae. ORP-27, ORP-13 and ORM-40 of *M. brunneum* isolates can be a potential biological control agent used against *S. littoralis* larvae.

Keywords: Entomopathogenic fungi, *Spodoptera littoralis*, *Metarhizium brunneum*, *Beauveria bassiana*, *Clonostachys rogersoniana*, Pathogenicity

Background

The cotton leaf worm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae), is a polyphagous pest that damages several crop plants and spreads in the Mediterranean, Aegean, and Southeastern Anatolia regions of Turkey, and many countries of the temperate zone (CABI 2021). Numerous studies showed that there have been many reports of effective parasitoids, predators, and pathogens in controlling this pest.

Entomopathogenic fungi (EPF) have an important place among pathogens, playing a role in controlling many pest groups naturally. Their infection process begins with the penetration stage following contact of fungal propagules to the host cuticle. After this stage, the fungus attacks the host through vegetative growth, using their enzymes or toxins, and causes damage or death of their host (Ortiz-Urquiza and Keyhani 2013).

EPF have an advantage over other insect pathogens like entomopathogenic bacteria and viruses as they can infect their host not only through diet, but also directly from the spiracles and insect cuticle. EPF can control their host pests specifically without harming biocontrol agents, do not pose a risk on mammals, do not cause pollution of

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the environment as encountered with pesticide applications, do not cause insect resistance, and provide long-term struggle with the host (Wan 2003). They can lead to death directly or weaken vital activities of their host by means of consuming the nutrient content, causing mechanical damage to host tissues and producing some toxic metabolites (Kulkarni 2015). Some abiotic factors such as temperature and humidity play an important role in the germination and development of fungi and may limit the pathogenicity of even a strong pathogen under insufficient conditions (Mishra et al. 2015). Efficacy of different EPF isolates varies from isolates/strain to another (even though in the same species) against the target host because their biological activities such as virulence, germination rate, mycelial growth, spores production and enzyme activity are different from each other (El Husseini 2019). Therefore, selecting and testing different EPF isolates with different characteristics increases the chance of getting a successful biocontrol agent for controlling host insects.

Various studies have reported that some EPF isolates including *Beauveria bassiana*, *B. brongniartii, Metarhizium anisopliae*, *M. flavoviride*, *Paecilomyces fumosoroseus* and *Lecanicillium lecanii* showed different activities at varying rates against *S. littoralis* larvae (Kılıç et al. 2019). The aim of the present study was to evaluate pathogenicity of the EPF isolates against *S. littoralis* larvae under laboratory conditions.

Methods

Rearing of *Spodoptera littoralis* larvae

Spodoptera littoralis larvae were reared on artificial diet under the laboratory conditions of 25 ± 1 °C and 65 ± 5% (Güney et al. 2019). Emerged adults were fed on honey solution in impregnated cotton in plastic boxes (36 × 23 cm). Egg masses were collected on paper band regularly and transferred into new storage boxes including artificial diet for maintaining the colony and for experimental uses. The second-instar larvae (L2) were used for testing the pathogenicity of the fungal isolates in the experiments.

Fungal isolates

In the experiment, 64 EPF isolates were isolated from soil samples taken from Ordu Province in Turkey in 2019–20 using the *Galleria* bait method (Zimmermann 1986). DNA extraction of isolated fungi was performed for the identification of the isolates. Then, the polymerase chain reaction (PCR) was carried out to genomic DNA amplify, using ITS4/ITS5 primers. Finally, the obtained PCR products were subjected to sequence analysis. As a result of the sequence analysis, it was determined that the isolates were 23 *Beauveria bassiana*, 11 *Metarhizium brunneum*, 8 M. *anisopliae*, 6 M. *robertsii*, 4 *Purpureocillium lilacinum*, 4 *Clonostachys rogersoniana*, 3 *Fusarium solani*, 1 *Clonostachys rossmaniae*, 1 *Aspergillus flavus*, 1 *Cordyceps cicadae*, 1 *C. fumosorosea* and 1 *F. oxysporum* isolates. All isolates were grown on potato dextrose agar (PDA) medium in incubator at 25 ± 1 °C for 15–30 days.

Preparation of fungal inoculum

To produce inoculum, the fungi were subcultured to PDA plates by conidial transfer. Fungal spores were harvested by scraping using scalpel to falcon tubes after getting sporulation by adding 10 ml of 0.02% Tween 80 solution. The conidial suspension was mixed for 1–2 min and filtered through four layers of cheesecloths to eliminate hyphal fragments. The suspension was diluted to a concentration of 1×10^8 conidia/ml, using a hemocytometer. The isolates displaying the highest mortality rates as a result of single-concentration response tests were adjusted to 1×10^5, 1×10^6 1×10^7, 1×10^8 and 1×10^9 conidia/ml by dilution with the same technique. All suspensions were stored at + 4 °C to be used within 3 days.

Single-concentration response tests

Second-instar larvae of *S. littoralis* were placed in petri dishes containing artificial diet and sprayed with 1×10^8 conidia/ml concentrations of all isolates. The larvae in control treatment were sprayed by 0.02% Tween 80 solution. Each treatment had a batch of 10 larvae and replicated six times. Mortality rates of the larvae were recorded daily from the 3rd day up to the 13th day of incubation.

Concentration–response tests

Second-instar larvae of *S. littoralis* were placed in petri dishes as noted above and sprayed with different spore concentrations (1×10^5, 1×10^6 1×10^7, 1×10^8 and 1×10^9 conidia/ml). Mortality rates of the larvae were recorded daily from the 3rd day up to the 13th day of incubation.

Statistical analysis

Average values of larvae mortality data were subjected to probit analysis for calculating LC_{50} and LC_{90}. Data were processed by one-way analysis of variance (ANOVA), followed by Tukey's post hoc test for comparison of means using the SPSS (Statistical Package of Social Sciences) software version 22.

Results

Single-concentration response tests

The results in Table 1 show the mortality percentages of *S. littoralis* larvae treated with 1×10^8 conidia/ml concentrations of each isolate.

Table 1 Mortality % of *Spodoptera littoralis* larvae treated with 1×10^8 conidia/ml concentrations of the EPF isolates

Species	Isolates	Mortality % in days post-treatment				Species	Isolates	Mortality % in days post-treatment			
		3	5	9	13			3	5	9	13
Control	0	0	0	0	0	*B. bassiana*	ORP-15	0	0	0	56.67
Beauveria bassiana	ORU-11	1.67	23.33	43.33	46.67	*M. robertsii*	ORP-16	5	8.33	10	58.33
B. bassiana	ORU-21	1.67	15.3	53.48	64.55	*Metarhizium brunneum*	ORP-17	0	0	0	68.33
B. bassiana	ORU-23	3.33	46.67	60	60	*M. brunneum*	ORP-18	0	6.67	75	75
Metarhizium robertsii	ORU-25	16.67	60	60	60	*M. brunneum*	ORP-22	0	21.67	90	90
M. robertsii	ORU-40	18.33	60	65	65	*B. bassiana*	ORP-24	0	0	0	48.33
B. bassiana	ORU-50	49.09	98.33	100	100	*Paecilomyces lilacinum*	ORP-26	0	0	1.67	83.33
B. bassiana	ORF-3	10	20	31.67	43.33	*M. brunneum*	ORP-27	0	1.67	100	100
B. bassiana	ORF-8	4.85	21.52	78.79	78.79	*Clonostachys rogersoniana*	ORP-29	4.17	4.17	3.52	10
B. bassiana	ORF-9	5	23.33	73.33	73.33	*M. anisopliae*	ORP-30	5	45	71.67	71.67
B. bassiana	ORF-11	10	10	43.64	56.97	*Cordyceps fumosorosea*	ORP-34-a	1.82	5.64	32	32
B. bassiana	ORF-17	0	60	66.67	66.67	*M. brunneum*	ORP-34-b	1.67	3.33	30	55
B. bassiana	ORF-22-a	16.67	29.7	53.94	67.27	*B. bassiana*	ORP-35	5	5	45	58.33
B. bassiana	ORF-23	1.67	6.67	65	65	*P. lilacinum*	ORP-36	3.33	6.67	10	23.33
B. bassiana	ORF-25	5	6.67	46.67	50	*M. brunneum*	ORP-37	8.33	8.33	31.67	36.67
M. brunneum	ORF-30	1.67	13.33	38.33	38.33	*M. anisopliae*	ORP-39	0	13.33	86.67	86.67
B. bassiana	ORF-42	1.67	35.76	68.79	68.79	*M. anisopliae*	ORP-40	13.33	16.67	33.33	35
B. bassiana	ORF-43	0	3.33	64.39	67.73	*B. bassiana*	ORP-46	0	3.33	45.91	50.91
M. anisopliae	ORM-8	14.85	39.85	70.45	72.12	*M. robertsii*	ORP-48	0	6.67	68.33	73.33
Aspergillus flavus	ORM-14	3.33	5	60	61.67	*B. bassiana*	ORG-1	0	0	30	30
P. lilacinum	ORM-21	9.39	19.39	58.94	58.94	*B. bassiana*	ORG-2	1.67	1.67	33.33	33.33
B. bassiana	ORM-39	3.33	3.33	6.67	20	*C. rogersoniana*	ORG-5	10	15	35	35
M. brunneum	ORM-40	50	98.33	98.33	98.33	*M. anisopliae*	ORG-6	23.33	33.33	56.67	56.67
B. bassiana	ORM-45	54	66	76	76	*M. anisopliae*	ORG-21	15	23.33	56.67	56.67
M. anisopliae	ORM-47	1.67	1.67	46.82	55.15	*C. rossmaniae*	ORG-24	26.67	28.33	58.33	58.33
C. rogersoniana	ORM-48	66.67	85	91.67	91.67	*C. rogersoniana*	ORG-35	23.33	28.33	63.33	63.33
B. bassiana	ORM-50	26.67	28.33	63.33	63.33	*M. robertsii*	ORG-42	35	38.33	61.67	61.67
M. brunneum	ORP-1	18.33	28.33	33.33	43.33	*P. lilacinum*	ORG-48	1.67	3.33	31.67	36.67
M. anisopliae	ORP-2	3.33	50	85	85	*Fusarium solani*	ORM-7	5	11.67	30	30
M. robertsii	ORP-4	1.67	60	90	90	*F. solani*	ORU-10	8.03	19.7	57.12	65.45
Cordyceps cicadae	ORP-9	5	5	43.33	65	*F. solani*	ORU-39	0	1.67	46.67	58.33
M. brunneum	ORP-13	43.33	71.67	85	93.33	*Fusarium oxysporum*	ORF-22-b	3.33	10	43.33	46.67
M. brunneum	ORP-14	50	65	88.33	88.33						

According to the findings of the single-concentration response test, it was generally reported that all the tested isolates differed in their virulence to the larvae. Most of the isolates were not different from the control group for 3 days after treatment. However, ORP-13, ORP-14, ORM-48, ORU-50, ORM-40 and ORM-45 isolates belonging to *Beauveria*, *Metarhizium* and *Clonostachys* genera showed a mortality rate of around 50%. Even though the mortality rates showed an increase for 5 days after treatment compared to 3 days, the most significant rise in mortality rates occurred on the 9th day of treatment; the mortality rate of over 50% was recorded in half of the isolates. Except for ORP-13, all the isolates had the highest mortality rates on the 13th day. Therefore, the isolates displaying the highest mortality rates ORU-50 (*B. bassiana*) (100%), ORM-40 (*M. brunneum*) (98.33%), ORM-48 (*C. rogersoniana*) (91.67%), ORP-13 (*M. brunneum*) (93.33%) and ORP-27 (*M. brunneum*) (100%) were subjected to concentration–response tests (1×10^5–1×10^9 conidia/ml).

Concentration–response tests

The results in Table 2 show the mortality rates of *S. littoralis* larvae treated with different concentrations of the

Table 2 Mortality % of *Spodoptera littoralis* larvae treated with different concentrations of the five isolates displaying the highest mortality rates in single-concentration response tests

Isolates	Mortality % in days post-treatment						
	Concentrations (conidia/ml)	3	5	7	9	11	13
Control	–	0a*	0a	0a	0a	0a	0a
ORU-50 *Beauveria bassiana*	1×10^5	0a	0a	0a	0a	0a	0a
	1×10^6	0a	0a	0a	0a	0a	0a
	1×10^7	0a	1.51a	1.51a	3.03a	7.57ab	7.57a
	1×10^8	0a	0a	1.51a	33.18a	43.18abc	43.18ab
	1×10^9	0a	0a	15.00a	36.66a	50.00bc	53.33b
ORM-40 *Metarhizium brunneum*	1×10^5	0a	0a	3.33a	3.33a	3.33a	6.66a
	1×10^6	1.66a	1.66a	1.66a	1.66a	16.06a	24.24ab
	1×10^7	2.00a	2.00a	2.00a	2.00a	30.00a	46.00bc
	1×10^8	2.00a	2.00a	2.00a	18.00ab	62.00b	64.00cd
	1×10^9	0a	0a	3.33a	50.30b	75.15b	80.15d
ORP-13 *M.brunneum*	1×10^5	0a	0a	0a	0a	0a	0a
	1×10^6	1.66a	1.66a	1.66a	1.66ab	10.00a	10.00a
	1×10^7	0a	0a	1.66a	32.27bc	57.12b	58.78b
	1×10^8	3.33a	5.00a	20.00a	61.66cd	70.00b	70.00b
	1×10^9	4.00a	4.00a	60.00b	84.00d	84.00b	84.00b
ORP-27 *M.brunneum*	1×10^5	2.00a	2.00a	2.00a	2.00a	3.81a	3.81a
	1×10^6	1.81a	1.81a	3.81a	3.81a	3.81a	3.81a
	1×10^7	5.00a	5.00a	12.22a	43.28b	44.79b	49.79b
	1×10^8	0a	0a	14.54a	76.91c	84.72c	84.72c
	1×10^9	0a	0a	16.00a	78.00c	88.00c	88.00c
ORM-48 *Clonostachys rogersoniana*	1×10^5	0a	0a	0a	0a	0a	0a
	1×10^6	1.66a	1.66a	10.00a	10.00a	10.00a	10.00a
	1×10^7	1.66a	1.66a	28.33a	33.33ab	48.33b	48.33b
	1×10^8	1.66a	3.33a	43.33a	61.66bc	63.33b	63.33b
	1×10^9	0a	7.50a	32.95a	75.45c	75.45b	75.45b

*Means in a column followed by the same letter for each isolate group are not statistically significant different ($P < 0.05$)

five isolates displaying the highest mortality rates in single-concentration response tests.

According to the findings of the concentration–response test, it was indicated that mortality rates on larvae were not different than the control group on the 3–5 days post-inoculation. On the 7th day of treatment, the highest concentration-dependent mortality was observed in ORP-13, followed by ORM-48, even though the increase in mortality rate was dependent. The highest mortality rates were obtained at 1×10^9 conidia/ml concentrations on the 9th day post-treatment for ORP-13 and ORM-48, on the 11th day post-treatment for ORP-27. On the 13th day post-inoculation, the mortality rates caused by *M. brunneum* isolates were around 80–88% and ORM-48 was also close to this percentage with a 75% mortality rate. On the other hand, *B. bassiana* isolate (ORU-50) caused the lowest mortality rate in concentration–response test (53%). The mortality rate of the isolates generally increased with concentration increase although the isolates were ineffective on larvae at 1×10^5 and 1×10^6 conidia/ml concentrations.

Data obtained from the dose–response tests were subjected to probit analysis. The concentrations which killed 50% (LC_{50}) and 90% (LC_{90}) of the population on the 13th day of treatment and the analysis parameters are given in Table 3. The lowest LC_{50} and LC_{90} values were recorded in ORP-27 with 1.68×10^7 and 4.60×10^8 conidia/ml, respectively. Accordingly, the most virulent isolate was determined as ORP-27, followed by ORP-13.

Discussion

Amer et al. (2008) reported higher mortality rates with the treatment of 1×10^5 and 1×10^6 conidia/ml concentrations and lower mortality rates with the treatment of 1×10^7, 1×10^8 and 1×10^9 conidia/ml concentrations of *B. bassiana* isolates on *S. littoralis* larvae compared to the

Table 3 LC_{50} and LC_{90} values of the isolates against second-instar larvae of *Spodoptera littoralis* at 13 days after treatment

Isolates	Slope ± SE	X^2	LC_{50} (conidia/ml) (95% Confidence limits lower–upper)	LC_{90} (conidia/ml) (95% Confidence limits lower–upper)
ORU-50	0.8333 ± 0.05	17.595	7.65×10^7 (1.09×10^7–2.36×10^9)	7.38×10^9 (5.28×10^8–7.08×10^{14})
ORM-40	0.75 ± 0.04	1.945	2.25×10^7 (1.35×10^7–3.83×10^7)	4.36×10^9 (1.67×10^9–1.59×10^{10})
ORM-48	0.8333 ± 0.05	18.708	4.07×10^7 (5.39×10^6–6.25×10^8)	2.96×10^9 (2.8×10^8–2.85×10^{13})
ORP-13	0.8333 ± 0.05	25.070	2.10×10^7 (1.74×10^6–3.04×10^8)	9.29×10^8 (1.03×10^8–1.13×10^{13})
ORP-27	1 ± 0.06	23.755	1.68×10^7 (1.97×10^6–1.47×10^8)	4.60×10^8 (6.92×10^7–2.78×10^{11})

present study. Contrary to this, Asi et al. (2013) recorded higher efficiency than these studies against *S. litura* larvae on the same dosages. In other study, a strain of *B. bassiana* showed high mortality rates with the treatment of 1×10^6, 1×10^7 and 1×10^8 conidia/ml concentrations, whereas another strain had not caused mortality on *S. littoralis* larvae (El-Katatny 2010). Similarly, some researchers determined that *M. anisopliae* showed around 88–90% mortality rate at 2×10^6 and 1×10^7 conidia/ml concentrations on *S. littoralis* larvae; nevertheless, the mortality rates of *M. anisopliae* on *S. litura* larvae were found below 5% even in the highest concentration (El Husseini 2019). In another study by Fite et al. (2020), *M. anisopliae* isolates showed a wide range of mortality rates between 20 and 70% on *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae) larvae. Several studies tested the efficacy of *Clonostachys* spp. against various target pests, yet no studies found on *S. littoralis* (Kim et al. 2020). These studies showed that *Clonostachys* spp. were not common in insects but may be possible in pathogens. Consequently, many studies on different target pests proved that different strains or isolates of the same fungal species even though using the same concentration may differ in virulence of the fungi (Fite et al. 2020).

Dhanapal et al. (2020) calculated the LC_{50} values of eight different *M. anisopliae* isolates on *S. litura* between 1.24×10^5 and 3.45×10^6 conidia/ml and concluded that the virulence of the fungus varied according to the strain. Asi et al. (2013) determined the LC_{50} value of a *B. bassiana* isolate on *S. litura* larvae as 1.11×10^7 conidia/ml at 10th days. El Husseini (2019) calculated the LC_{50} and LC_{90} values of a *M. anisopliae* isolate as 6.2×10^4 and 1.4×10^7 conidia/ml, respectively, on *S. littoralis* larvae at 7th days. The lethal concentration values in this study were lower than other previous studies on *Spodoptera* species and the present study; therefore, the death effects of this isolate were high. In contrast, El-Hawary and Abd El-Salam (2009) determined the LC_{50} and LC_{90} values of a commercial *B. bassiana* isolate on *S. littoralis* as 0.2×10^9 and 1.5×10^9 conidia/ml, respectively. The LC_{50} values were higher than other studies. These results can be interpreted that those local isolates may be more efficient on *Spodoptera* species than commercial isolates.

The mortality rates occurred at 1×10^8 conidia/ml concentration in the concentration–response tests of the five isolates which were lower than in the single-concentration tests. Similarly, Çerçi (2010) also stated that the findings of the same concentration obtained from the single-concentration and concentration–response tests differ. These variations may be due to the difference in application date or the continuous passing of fungus cultures to purify and reproduce them. Butt and Goettel (2000) argued that when an isolate is to be used in biological activity tests, its virulence should be increased by passing it through an insect host before culturing on media.

Generally, it was found that M*etarhizium* spp. (especially *M. brunneum*) showed a high mortality rate on *S. littoralis* larvae, whereas *B. bassiana* isolates showed lower mortality rate than *Metarhizium* spp. Besides that, *Cordyceps* spp. and *P. lilacinum* isolates showed a weak activity on the larvae. *C. rogersoniana* species generally showed a low mortality rate, but one isolate of this species managed to control *S. littoralis* larvae. Similarly, Amer et al. (2008) reported that *M. anisopliae* and *M. flavoviride* were highly effective on *S. littoralis*, whereas *B. bassiana* and *B. brongniartii* showed the lowest rate. El-Katatny (2010) revealed that one strain of *B. bassiana* isolates caused a high mortality rate on *S. littoralis* larvae, while the others did not show virulence. Similarly, El-Hawary and Abd El-Salam (2009) argued that *B. bassiana* was more effective than *P. fumosoroseus* against *S. littoralis.* Contrary to these, Cırbın (2017) inferred that *B. bassiana* isolates were more effective on *S. littoralis*

larvae than *M. anisopliae* ones. These findings confirm that different results can be obtained using different isolates of the same species as observed in the present study.

Conclusions

In conclusion, *M. brunneum* isolates (ORP-27, ORP-13 and ORM-40) had good potential as possible biocontrol agents against *S. littoralis* larvae. Additional studies to investigate the biological activity of the EPF isolates against *S. littoralis* and its natural enemies using various formulation techniques under field conditions are still needed.

Abbreviations

S. littoralis: *Spodoptera littoralis*; *S. litura*: *Spodoptera litura*; *B. bassiana*: *Beauveria bassiana*; *B. brongniartii*: *Beauveria brongniartii*; *M. anisopliae*: *Metarhizium anisopliae*; *M. brunneum*: *Metarhizium brunneum*; *M. flavoviride*: *Metarhizium flavoviride*; *C. rogersoniana*: *Clonostachys rogersoniana*; *P. fumosoroseus*: *Paecilomyces fumosoroseus*; *P. lilacinum*: *Purpureocillium lilacinum*; PDA: Potato dextrose agar; PCR: Polymerase chain reaction; ITS: Internal transcribed spacer.

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Authors' contributions

FS performed the experiment, analysis of data and manuscript writing; YY contributed to technical assistance, analysis of data, supervision and manuscript writing. Both authors read and approved the final manuscript.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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