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RESEARCH **Open Access**

Efficacy of *Beauveria bassiana* and *Beauveria pseudobassiana* isolates against the pine processionary moth, *Thaumetopoea wilkinsoni* Tams, 1926 (Lepidoptera/Notodontidae)

Elif Fatma Topkara[1*], Oğuzhan Yanar[1], Celal Tuncer[2], Ismail Oguz Ozdemir[3] and Elif Yildirim[2]

Abstract

Background: The pine processionary moth, *Thaumetopoea wilkinsoni* Tams, 1926 (Lepidoptera/Notodontidae) is one of the most harmful insects that destroys pine ecosystems by feeding on pine leaves at its larval stage. Because of its urticating setae, the insect also causes severe skin reactions to animals and humans. Instead of chemical control, eco-friendly biological control methods are preferred to combat this species.

Results: The purpose of this study was to evaluate the efficacy of five different *Beauveria bassiana* Vuill, 1912 (Hypocreales/Cordycipitaceae) isolates (TR-SM-10, TR-SM-11, TR-SM-2, TR-SK-1 and TR-D-1) and one *B. pseudobassiana* Rehner & Humber (Hypocreales/Cordycipitaceae) isolate (TR-SM-1) against the fourth instar larvae of *T. wilkinsoni* under laboratory conditions. *T. wilkinsoni* larvae were collected from the Ondokuz Mayıs University Kurupelit Campus in Samsun, Turkey, in 2021, and the fourth instar larvae were used in the experiment. Two ml of spray of the six fungal isolates were applied to every ten larvae at each concentration (1×10^7 and 1×10^8 conidia ml^{-1}). The experiment was carried out in five replicates per group, and the larvae were observed for 10 days. As a result, all isolates of *B. bassiana* caused 100% mortality at 1×10^8 conidia ml^{-1} concentration. *B. pseudobassiana* isolate also caused 100% mortality at both concentrations. At 1×10^7 conidia ml^{-1} concentration, the larvae treated with the *B. pseudobassiana* isolate (TR-SM-1) had the lowest LT_{50} (2.89 days) and LT_{90} values (4.79 days), while the larvae treated with TR-SM-10 isolate had the highest LT_{50} (5.65 days) and LT_{90} values (9.39 days). At 1×10^8 conidia ml^{-1} concentration, the larvae treated with TR-SK-1 isolate had the lowest LT_{50} (2.89 days) and LT_{90} values (4.79 days), while those treated with TR-SM-10 isolate had the highest LT_{50} (3.95 days) and LT_{90} values (8.15 days).

Conclusion: It has been recommended that the five different isolates of *B. bassiana* and *B. pseudobassiana* isolates were virulent to *T. wilkinsoni* larvae and can be used for biological control of *T. wilkinsoni*.

Keywords: *Beauveria bassiana*, *Beauveria pseudobassiana*, Entomopathogenic fungi, Microbial control, *Thaumetopoea wilkinsoni*

Background

The Pinaceae family has many genera, one of the most important genera is *Pinus*. The needles of the *Pinus* genus contain essential oils. Because of their various pharmacological properties, such as anti-ageing and anti-inflammatory effects, the needles are widely used in folk medicine and as food additives.

*Correspondence: topkaraelif@hotmail.com
[1] Department of Biology, Science and Art Faculty, Ondokuz Mayıs University, 55139 Samsun, Turkey
Full list of author information is available at the end of the article

The *Pinus* species are distributed almost all over the world, including Turkey. Insect pests lead to the biological factors that threaten the continuity of pine forests in Turkey. The pine processionary moth, *Thaumetopoea wilkinsoni* Tams, 1926 (Lepidoptera/Notodontidae) is one of the most harmful needle-eating insects in pine ecosystems (Altunışık and Avcı 2016). The pest causes deformities in seedlings and stunting (Onaran and Katı 2010) and even tree deaths in young stands (Keleş et al. 2018). In addition, the larvae of the insect have urticating setae that cause skin reactions in both animals and humans (Rodriguez-Mahillo et al. 2012).

Both mechanical and chemical control methods are used to combat the pest in pine forests in Turkey. Given the adverse effects of chemical control on the ecosystem, biological control methods are preferred to combat the species. The use of entomopathogenic microorganisms as a biological control agent is critical in overcoming harmful species in terms of both targeting only a specific pest and being environmentally friendly. Entomopathogenic fungi (EPF) are one of the most effective biological control agent in pest control. *B. bassiana* Vuill, 1912 (Hypocreales/Cordycipitaceae), is the most common one (Zibaee et al. 2013). Studies on *B. bassiana* (Rehman et al. 2020) have revealed that the fungi are effective against a variety of insects. Furthermore, the effectiveness of *B. pseudobassiana* Rehner & Humber (Hypocreales/Cordycipitaceae), which is a cryptic species morphologically similar to *B. bassiana* but phylogenetically distant from *B. bassiana* (Rehner et al. 2011), has also been investigated against various insects (Bedini et al. 2018). There are various studies investigating the use of different *B.* strains to develop safe biological control strategies in the control of the pine processionary moth (Gök et al. 2018).

Combating with *T. wilkinsoni* is critical for protecting pine forests, which are important for both ecological and economic reasons, as well as reducing the threat to human and animal health. Therefore, in the present study, the efficacy of five different *B. bassiana* isolates was evaluated against the fourth instar larvae of *T. wilkinsoni* under laboratory conditions.

Methods

Sampling

The fourth instar larvae of *T. wilkinsoni* were collected from a pine forest at Ondokuz Mayıs University Kurupelit Campus in Samsun, Turkey (N 41° 22′ 26.5116″ E 36° 13′ 17.6340″), in 2021.

Preparation of entomopathogenic fungi

A total of six native EPF isolates (TR-SM-10, TR-SM-11, TR-SM-2, TR-SK-1, TR-D-1, and TR-SM-1) molecularly identified and isolated from infected *Palomena prasina* Linnaeus (Hemiptera/Pentatomidae) in hazelnuts orchards, Black Sea region of Turkey in 2018–2019, were used in this study (Table 1) (Ozdemir et al. 2021). All isolates were obtained from the same host and the same location.

The six native EPF isolates belong to *B. bassiana* (TR-SM-10, TR-SM-11, TR-SM-2, TR-SK-1, and TR-D-1) and *B. pseudobassiana* (TR-SM-1). These isolates were inoculated into potato dextrose agar (PDA; Merck Ltd., Darmstadt, Germany) and incubated in complete darkness at 25 ± 1 °C for one week (Incubator; Binder KBWF 240; Germany). At the end of the growth period, each Petri dish was filled with sterile distilled water containing 0.02% Tween 20 and scraped with a glass bag to pass the conidia into the water. Following that, the conidia suspensions were filtered through three layers of sterile cheesecloth to remove mycelium and homogenized for 3 min by vortexing. The resulting conidia suspensions were adjusted to concentrations of the 1×10^7 and 1×10^8 conidia ml^{-1} using a Neubauer hemocytometer under Olympus CX31 light microscope (Olympus America Inc., Lake Success, NY) (Tuncer et al. 2019).

Application of entomopathogenic fungi on *T. wilkinsoni*

In the experiment, two layers of sterile filter paper were lightly moistened and placed in 1-L plastic cups. Two ml of the 1×10^7 and 1×10^8 conidia ml^{-1} were sprayed on the fourth instar larvae placed in plastic cups (ten larvae per dish) using a Potter spraying tower (Burkard, Rickmansworth, Hertz UK), and the appropriate amount of sterilized pine leaves were placed in the dishes to feed the larvae. After each application of EPF suspension, the spray tower was cleaned by 70% ethanol and sterile distilled water. Subsequently, the fourth instar larvae of the insect were released in plastic cups. Only sterile-distilled water containing 0.02% Tween 20 was sprayed to the control group. For 10 days, all plastic cups were incubated at 25 ± 1 °C, 70 ± 5% RH, and a 16:8 h light/dark period. The mortality rates were monitored for

Table 1 Species of the entomopathogenic fungi isolates used in the study

Species/isolate denomination	Genbank accession numbers
Beauveria bassiana/TR-SM-10	MT102327
Beauveria bassiana/TR-SM-11	MT102328
Beauveria bassiana/TR-SM-2	MT102329
Beauveria bassiana/TR-SK-1	MT102330
Beauveria bassiana/TR-D-1	MT102331
Beauveria pseudobassiana/TR-SM-1	MT102333

ten consecutive days. The dead larvae in each cup were counted 10 days after the application, and the mortality rates were determined. The same procedure was repeated every day for the control groups (Ozdemir et al. 2019).

Statistical analyses

Daily mortality rates were corrected using the Abbott formula when the mortality rate in the control group exceeded 5% (Abbott 1925). The LT_{50} and LT_{90} values were determined by Probit analysis using the log-Probit method (POLO-PLUS ver.2.0). The slopes of the regression lines were compared to each. The LT_{50} and LT_{90} values of the isolates were compared using confidence intervals (95%).

Results

Before bioassays, the conidial viability of *B. bassiana* (5) and *B. pseudobassiana* (1) isolates was determined, as well as the germination rate (100%). *Beauveria* isolates were applied to the fourth instar larvae of *T. wilkinsoni* at different concentrations, and the LT_{50} and LT_{90} values for each isolates differed (Tables 1 and 2). The LT_{50} and LT_{90} values for TR-SM-1 isolate against *T. wilkinsoni* were 2.89 and 4.79 days, respectively, at 1×10^7 conidia ml^{-1} concentration, whereas they were 3.01 and 7.47 days at 1×10^8 conidia ml^{-1}. The LT_{50} and LT_{90} values for TR-D-1 isolate were 4.42 and 7.46 days at 1×10^7 conidia ml^{-1} while 3.27 and 6.39 days at 1×10^8 conidia ml^{-1}, respectively. While the LT_{50} and LT_{90} values of TR-SK-1 isolate were 4.23 and 8.68 days at 1×10^7 conidia ml^{-1} concentration, the LT_{50} and LT_{90} values were 2.89 and 4.79 days at 1×10^8 conidia ml^{-1} concentration, respectively. The LT_{50} values of the 1×10^7 and 1×10^8 conidia ml^{-1} concentration of TR-SM-2 isolate were 4.22 and 3.64 days, whereas the LT_{90} values of the same concentrations of the same isolate were 7.46 and 6.99 days, respectively. Similarly, the LT_{50} values of the 1×10^7 and 1×10^8 conidia ml^{-1} concentrations of TR-SM-10 isolate were 5.65 and 3.95 days, respectively. The LT_{90} value of the 1×10^7 conidia ml^{-1} concentration of the same isolate was 9.39 days, while the LT_{90} value for the 1×10^8 conidia ml^{-1} of the same isolate was 8.15 days. The LT_{50} and LT_{90} values for TR-SM-11 isolate at the 1×10^7 conidia ml^{-1} concentration were 4.01 and 8.57 days, whereas at 1×10^8 conidia ml^{-1} for the same isolate, they were 3.75 and 7.66 days, respectively (Tables 2 and 3).

It was determined that the two tested concentrations of nearly all isolates began to cause mortality 2 days after the application, and the mortality increased with time (Figs. 1 and 2). Except for TR-SM-10, TR-SM-11 and TR-SK-1 isolates, the 1×10^7 conidia ml^{-1} concentration of the isolates caused 100% mortality 10 days after the application, while the 1×10^8 conidia ml^{-1} concentration of all isolates caused 100% mortality. Although TR-D-1 and TR-SM-10 isolates caused 100 and 93.3% mortality

Table 2 Probit analysis data on the mortality time (days) of *Thaumetopoea wilkinsoni* after the applications of the 1×10^7 conidia ml^{-1} concentration of *Beauveria bassiana* and *B. pseudobassiana* isolates

Isolates	LT_{50} (95% CI)	LT_{90} (95% CI)	Slope ± SE	Regression	X^2	*Df*	Heterogeneity
TR-SM-1	2.89 (2.52–3.23)a*	4.79 (4.22–5.69)a*	5.84 ± 0.64	$y = -2.69 + 5.84x$	36.60	28	1.30
TR-D-1	4.42 (3.90–4.89)b	7.46 (6.60–8.93)b	5.63 ± 0.59	$y = -3.63 + 5.63x$	41.86	28	1.16
TR-SK-1	4.23 (3.71–4.73)b	8.68 (7.51–10.69)b	4.10 ± 0.43	$y = -2.57 + 4.10x$	32.60	28	1.09
TR-SM-2	4.22 (3.54–4.87)b	7.46 (6.26–10.79)b	8.40 ± 0.91	$y = -5.47 + 8.40x$	42.42	28	1.51
TR-SM-10	5.65 (5.10–6.21)c	9.39 (8.27–11.40)b	3.83 ± 0.39	$y = -2.78 + 3.83x$	362.63	28	12.95
TR-SM-11	4.01 (3.25–4.77)b	8.57 (6.91–12.30)b	3.88 ± 0.39	$y = -2.34 + 3.88x$	71.39	28	2.54

*Within columns, means followed by the same lower-case letters do not differ significantly at $p \leq 0.05$

Table 3 Probit analysis data on the mortality time (days) of *Thaumetopoea wilkinsoni* after the applications of the 1×10^8 conidia ml^{-1} concentration of *Beauveria bassiana* and *B. pseudobassiana* isolates

Isolates	LT_{50} (95% CI)	LT_{90} (95% CI)	Slope ± SE	Regression	X^2	*Df*	Heterogeneity
TR-SM-1	3.01 (2.20–3.78)ab*	7.47 (5.76–11.74)b*	3.25 ± 0.33	$y = -1.56 + 3.25x$	84.51	28	3.01
TR-D-1	3.27 (2.31–4.09)ab	6.39 (5.05–9.77)ab	4.42 ± 0.45	$y = -2.27 + 4.42x$	122.85	28	4.38
TR-SK-1	2.89 (2.52–3.23)a	4.79 (4.22–5.69)a	5.84 ± 0.64	$y = -2.69 + 5.84x$	36.60	28	1.30
TR-SM-2	3.64 (3.09–4.16)ab	6.99 (5.98–8.82)b	4.52 ± 0.45	$y = -2.54 + 4.52x$	49.09	28	1.75
TR-SM-10	3.95 (3.34–4.53)b	8.15 (6.86–10.57)b	4.07 ± 0.41	$y = -2.43 + 4.07x$	48.23	28	1.72
TR-SM-11	3.75 (2.70–4.81)ab	7.66 (5.82–13.35)b	4.14 ± 0.39	$y = -2.38 + 4.14x$	148.59	28	5.30

*Within columns, means followed by the same lower-case letters do not differ significantly at $p \leq 0.05$

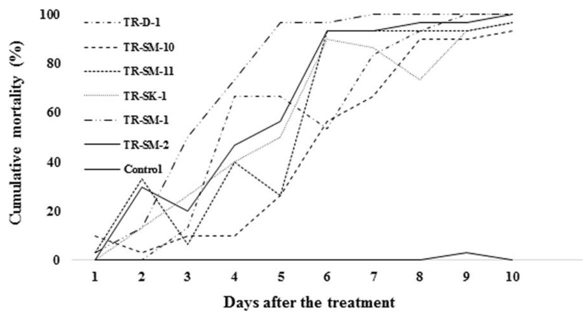

Fig. 1 Mortality rates of *Thaumetopoea wilkinsoni* treated with *Beauveria bassiana* and *Beauveria pseudobassiana* isolates at 1×10^7 conidia ml^{-1} concentration

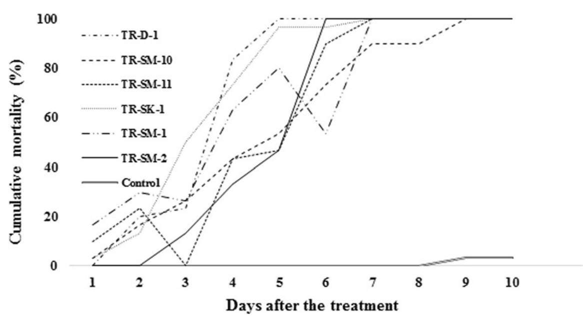

Fig. 2 Mortality rates of *Thaumetopoea wilkinsoni* treated with *Beauveria bassiana* and *Beauveria pseudobassiana* isolates at 1×10^8 conidia ml^{-1} concentration

(at 1×10^7 conidia ml^{-1} concentration), respectively, at 1×10^8 conidia ml^{-1} concentration, the two isolates caused 100% mortality 5 and 9 days after the treatment. Similarly, while the mortality rates by the two isolates, TR-SM-11 and TR-SK-1 at 1×10^7 conidia ml^{-1} concentration, were 96% within 10 days, these rates for the two isolates at 1×10^8 conidia ml^{-1} concentration were 100% 7 days after the treatment. The mortality rates in both concentrations of TR-SM-1 were recorded as 100% 10 days after the treatment. Similarly, the 1×10^7 and 1×10^8 conidia ml^{-1} concentrations of TR-SM-1 isolate caused 100% mortality within 10 and 6 days, respectively (Figs. 1 and 2).

Discussion

B. bassiana, as one of the EPFs, is one of the most preferred species in terms of its effectiveness in pest control. The efficacy of five different *B. bassiana* and one *B. pseudobassiana* isolates against the fourth instar larvae of *T. wilkinsoni* was investigated. Since the pine processionary moth often overwinters as the fourth instar larvae (Ozdemir et al. 2019), the fourth instar larvae were chosen in this study.

It is critical to determine the lethal times (LT_{50} and LT_{90} values) to reduce the overall cost of pest control while maintaining a high level of control. While the most effective isolate was TR-SM-1 at 1×10^7 conidia ml^{-1} concentration, TR-SK-1 was the most effective isolate at 1×10^8 conidia ml^{-1} concentration. Both the LT_{50} and LT_{90} values decreased with the increasing conidial concentrations in all isolates (except TR-SM-1). This situation has indicated that the highest conidial concentrations of *B. bassiana* isolates were more virulent for *T. wilkinsoni* larvae. The results of the previous studies showed that they were consistent with the findings obtained from this study (Tuncer et al. 2019). Ozdemir et al. (2019) determined that the *B. bassiana* strain applied to *T. pityocampa* Denis & Schiffermüller, 1775 (Lepidoptera/Notodontidae), larvae caused a decrease in LT_{50} values with increasing conidial concentration. Similar results were obtained in studies where *B. bassiana* was treated with insect species from other orders other than Lepidoptera.

Ten days after the application, it was determined that *B. bassiana* isolates, except TR-SM-10, TR-SM-11, and TR-SK-1, caused 100% mortality at 1×10^7 conidia ml^{-1} concentration. All three isolates led to high mortality rates of 93.3, 96, and 96%, respectively. At the end of the experiment, it was determined that all isolates caused 100% mortality at 1×10^8 conidia ml^{-1} concentration. This situation proved that the concentration of the 1×10^8 conidia ml^{-1} for all isolates was highly virulent to *T. wilkinsoni* larvae. There are several studies showing the effectiveness of different *Beauveria* strains against the pine processionary moth. Draganova et al. (2013) determined that the pine processionary moth was affected by the *B. bassiana* strain. Sönmez et al. (2017) applied *B. bassiana* KTU-24 strain to the pine processionary moth, and noted that the strain caused more mortality with increasing concentration. Gök et al. (2018) showed that the *B. bassiana* LD2016 strain sprayed on the pine processionary moth caused 100% death on the fifth day, while the *B. bassiana* BMAUMM6-4 strain caused 100% death on the third day. In studies with different species, the efficacy of *B. bassiana* isolates was evaluated.

Studies on the effectiveness of *B. pseudobassiana* in insects of the order Lepidoptera are few. Of these, Schemmer et al. (2016) reported that six different *B. pseudobassiana* strains were applied to *Cameraria ohridella* Deschka & Dimić (Lepidoptera/Gracillariidae), and all strains caused 100% mortality on the tenth day. Gürlek et al. (2018) stated that *B. pseudobassiana* caused death in *Cydia pomonella* Linnaeus (Lepidoptera/Tortricidae). In addition, the effectiveness of *B. pseudobassiana* against insects in other orders was also discussed in the studies. *B. pseudobassiana* has been reported to be an entomopathogen for *Hypera postica* Gyllenhal (Coleoptera/Curculionidae) (Yücel et al. 2018). Álvarez-Baz et al. (2015) demonstrated that *B. pseudobassiana* was highly virulent against *Monochamus galloprovincialis* Olivier (Coleoptera/Cerambycidae), the vector of pine wilt disease. *B. pseudobassiana* isolates have been reported to be virulent against the Eastern pine weevil *Pissodes nemorensis* Germar (Coleoptera/Curculionidae) (Romon et al. 2017).

Conclusions

T. wilkinsoni is an important forest pest that causes significant allergic reactions in humans and animals. Biological control has an important potential in order to protect the ecosystem against *T. wilkinsoni*, which is common in forests. The pest is a candidate for the use of EPF with high humidity and low average temperatures. *Beauveria* isolates obtained from this region were applied against the pest and five different isolates of *B. bassiana* and *B. pseudobassiana* isolate were found to be virulent against *T. wilkinsoni* larvae in this study. Moreover, the study revealed that *B. pseudobassiana*, which is less studied compared to *B. bassiana*, is a promising entomopathogen and can be used to control *T. wilkinsoni* larvae. As a result, these fungal isolates can be applied in the field under controlled conditions.

Abbreviations
EPF: Entomopathogenic fungi; PDA: Potato dextrose agar.

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Authors' contributions
EFT, OY, CT, IOO, and EY conceived, designed, analyzed, wrote, corrected and approved the final draft. All authors read and approved the final manuscript.

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

Declarations

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Not applicable.

Consent for publication
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Competing interests
The authors declare no competing interests.

Author details
[1]Department of Biology, Science and Art Faculty, Ondokuz Mayıs University, 55139 Samsun, Turkey. [2]Department of Plant Protection, Faculty of Agriculture, Ondokuz Mayıs University, 55139 Samsun, Turkey. [3]Department of Plant Protection, Faculty of Agriculture, Sakarya University of Applied Science, Sakarya, Turkey.

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