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Biocontrol of the Pine processionary moth, *Thaumetopoea wilkinsoni* (Tams.) (Lepidoptera: Notodontidae) by forest-derived entomopathogenic fungi

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Abstract

The pine processionary moth (PPM), *Thaumetopoea wilkinsoni* Tams. (Lepidoptera: Notodontidae), is an important forest pest in the Mediterranean region. This study demonstrated for the first time the biocontrol potential of entomopathogenic fungi (EPF) isolated from pine forest soils on *T. wilkinsoni*. The identity of five isolates obtained by the *Tenebrio*-bait method was established by macroscopic and microscopic features and ITS-rDNA sequencing. Pathogenicity and virulence of novel indigenous isolates on fourth stage larvae of *T. wilkinsoni* were then determined. PPM1, PPM3 and PPM5 were identified as *Beauveria bassiana* Vuill. (Hypocreales: Cordycipitaceae), PPM2 and PPM4 were identified as *Fusarium oxysporum* Schlecht. (Hypocreales: Nectriaceae). All isolates were found to be pathogenic at a concentration of 10^7 conidia/ml, with virulence varying between 15 and 100%. The pathogenicity of *F. oxysporum* on *T. wilkinsoni* was demonstrated for the first time in this study and its virulence was determined to be 76%. In addition, the virulence of *B. bassiana* PPM1 isolate reached 100% at a concentration of 10^7 conidia/ml 14 days after treatment. Moreover, the median lethal concentration (LC_{50}) required to kill 50% of the larval population was estimated to be 5.7×10^3 conidia/ml. Our results are important to improve the application of new EPF species as biological control agents in pest management.

Keywords: Pine processionary moth, *Tenebrio*-bait method, *Beauveria*, *Fusarium*, Biocontrol,

Introduction

The pine processionary moths include at least two major phytophagous insect species that defoliate Pinus and Cedrus forests in southern Europe and the Mediterranean region (Basso et al., 2017). *Thaumetopoea pityocampa* Denis & Schiffermüller, 1775 occurs from North Africa to southern Europe, while *Thaumetopoea wilkinsoni* Tams, 1925 occurs from western Turkey to southern parts of Israel (Petrucco-Toffolo et al., 2018). In many earlier studies on *Thaumetopoea* sp. in Turkey, the population was referred as *T. pityocampa* (Avcı, 2000; Kanat & Alma, 2004; Ince et al., 2008; Sevim et al., 2010; Sönmez et al., 2017). However, recent research utilizing molecular techniques has revealed that the species distributed in western Turkey is actually *T. wilkinsoni* (İpekdal et al., 2015; İpekdal et al., 2020; Basso et al., 2023). In addition, the pest is currently extending its geographical distribution in response to climate warming (İpekdal et al. 2015). *T. wilkinsoni* cause in the yield of trees by eating the needles in various pine species (*Pinus brutia* Ten., *P. nigra* Arnold., *P. sylvestris* L., *P. pinea* L., *P. halepensis* Mill.) during the larval phase in fall and winter. The pest causes deformities in seedlings and stunting and even tree mortality in young stands. The larval stages cause pines to shed their leaves and allow the invasion of other opportunistic pine pests, resulting in reduced viability of pines and lower yield of edible pine nuts (İpekdal and Çağlar 2014). In addition to its severe impact on biodiversity and the environment, the pest also has a dangerous effect due to its urticating hairs produced from the third instar onwards, which cause strong allergic reactions on contact with humans and animals. The urticating hairs are produced in large quantities in special abdominal pockets of the larvae and contain a proteinaceous toxin, thaumetopoein, which causes allergic reactions to the skin, respiratory tract, mouth and eyes (Lamy et al. 1986).

Chemical insecticides, especially insect growth inhibitors such as diflubenzuron, dimilin, decis and deltamethrin, have been used mainly to control *T. wilkinsoni* (Semiz et al.

2006). However, these agents have potentially undesirable side effects, particularly on predators and parasites of the pest, humans, plants and other non-target animals. Microbial control with entomopathogenic fungi (EPF) is an excellent alternative as they are not harmful to the environment and, unlike chemical pesticides, leave no significant residues on plants. In addition, EPFs have considerable host specificity with mild effects on non-target organisms (Vestergaard et al. 2003; Islam et al. 2021; Vivekanandhan et al. 2023).

Entomopathogenic fungi such as *Beauveria* spp., *Lecanicillium* spp., *Metarhizium* spp., and *Isaria* spp. are used worldwide to control numerous agricultural and forestry insect pests (Biryol et al. 2020; Eski et al. 2022; Swathy et al. 2024). Many of these fungi have been tested against *T. wilkinsoni* and have been shown to be useful for biocontrol. EPFs to be used to control an insect can be isolated using insect bait method as well as from insects showing disease symptoms. Abou-Jawdah et al. (2008) isolated *B. bassiana* from a dead larva of the cedar web-spinning sawfly, *Cephalcia tannourinensis* Chevin, collected from a cedar forest, and it was very effective against the early larval stage of *T. wilkinsoni*. Similarly, Topkara et al. (2022) tested the efficacy of five different *B. bassiana* isolated from infected *Palomena prasina* in hazelnut orchards in the Turkish Black Sea region against the fourth instar larvae of *T. wilkinsoni*, and *B. bassiana* TR-SM-1 killed faster than others. In addition, Yanar et al. (2023) tested isolates of *B. bassiana* and *M. brunneum* isolated from soil samples using the *Galleria*-bait method and showed that *M. brunneum* is more effective than *B. bassiana* on fourth larval stage. Despite all these studies, there is no study on the isolation of entomopathogenic fungi from pine forests and their virulence on the pine forest pest *T. wilkinsoni.*

The habitat and geographical origin of a fungus are important factors for its environmental competence. Many studies have shown that local entomopathogenic fungi isolated from the pest or its habitat are more effective against the pest than exotic strains. The selection of environmentally competent EPF strains is therefore the best option to ensure the

desired level of control and thus promote user acceptance and willingness to gradually replace chemical insecticides with EPF as microbial control agents (Quesada-Moraga et al. 2024).

Therefore, the aim of the study was to (1) isolate the local entomopathogenic fungi from pine forests using the insect bait method, (2) characterize them on the basis of their morphological and molecular properties, and (3) determine their insecticidal potential against the larvae of *T. wilkinsoni*.

Materials and Methods

Soil sampling

The soil samples were taken in November 2022 in the province of Bilecik in Turkey (40.1587°N, 29.9758°E and 850 m above sea level). The surface of the soil was scraped and approximately 500 g of soil was collected from a depth of 25 cm. To prevent possible contamination between successive samples, the soil sampler was washed with 5% sodium hypochlorite. The samples were placed in sterile glass jars (500 ml) and stored at 20°C for subsequent isolation of entomopathogenic fungi (Mantzoukas et al. 2020).

Isolation of entomopathogenic fungi

Entomopathogenic fungi were isolated from soil samples using the insect bait method described by Zimmermann (1986) with minor modifications (Wu et al. 2022). Ten larvae of Tenebrio molitor (4th instar, ≈5–7 mm body length) were placed in the jars containing the 250 g soil samples and incubated at 25 ± 2 °C. Mortality was monitored daily and the dead larvae were transferred to the humidity chamber at 25 ± 2°C and 70% relative humidity after surface sterilization with 1% sodium hypochlorite solution. Conidia from cadavers showing external mycelial growth were transferred to Sabouraud dextrose agar (SDA) plates and incubated at 25 ± 2°C until fungal growth was observed. The isolates were then subcultured several times on SDA plates to ensure their purity (Noman et al. 2020), and the pure fungal spores were stored in a 50% glycerol solution at −80°C.

Morphological characterization

For the morphological identification of the fungal strains, the macroscopic characteristics of the colonies were first observed. For each colony, characteristics such as the color on the upper and lower surface of the plates, shape, texture and growth pattern on SDA were observed. The microscopic features of the fungal isolates were characterized using slide culture techniques (Bello et al. 2021). A block of 1 cm2 SDA was placed in a sterile Petri dish with a glass slide. A two-week-old fungal culture was inoculated onto the four sides of the agar block and placed on a cover slide. The culture was incubated at 28 °C. When the fungal structure was visible, the slide was removed with tweezers and placed on a drop of lactophenol cotton blue on another slide. The size and arrangement of hyphae, conidiophores and conidia were then observed under a light microscope (Gebremariam et al. 2021).

Molecular characterization and phylogenetic analysis

Genomic DNA (gDNA) was isolated from fresh mycelium of each fungus using the Quick-DNA Fungal/Bacterial MiniPrep Kit (Zymo Research, Irvine, CA, USA) according to the manufacturer's instructions. The internal transcribed spacer (ITS) regions of the DNA of the fungal isolates were amplified with ITS5 (5'- GGAAGTAAAAAGTCGTAACAAGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') primers (Alfiky et al. 2022). The PCR mixture contained 200 µM of each dNTP, 50 pmol of each primer, 2.5 units of *Taq* DNA polymerase, 10× reaction buffer, 50 ng of genomic DNA and the final volume of sterile distilled water to 50 µl. The amplification protocol consisted of an initial denaturation step of 5 min at 96°C, 35 cycles of denaturation (96°C for 30 s), annealing (56°C for 30 s) and extension (68°C for 30 s) and a final extension step at 68°C for 5 min. PCR products were electrophoresed on a 1% agarose gel and stained with ethidium bromide. Approximately 500 bp products were purified using a Zymoclean Gel DNA Recovery Kit (Zymo Research, Irvine, CA, USA).

The purified PCR products were sent to Ficus Biotechnology (Ankara, Turkey) for Sanger sequencing and the sequences obtained were analyzed using the NCBI Basic Local Alignment Search Tool. The sequence data were also deposited in the NCBI database. Phylogenetic and molecular evolutionary analyzes were performed using MEGA X software (Kumar et al. 2018). The phylogenetic tree was constructed using the neighbor-joining clustering method (Saitou and Nei 1987) and the maximum composite likelihood distance model (Kimura 1980). The reliability of the dendrogram was tested by a bootstrap analysis with 1000 replicates.

Preparation of spore suspension

The spore suspensions used for the bioassay were prepared from 2-week-old cultures. Fungal spores were harvested from the agar surface with sterile 0.1% Tween 80 and the spore suspension was then filtered through four layers of sterile muslin into a 50 ml sterile Falcon tube to remove mycelium and agar pieces. The concentration of the suspensions was determined using a Neubauer hemocytometer and adjusted to 1×10^7 conidia/ml. Before the bioassay experiment, conidial germination was tested on SDA agar medium (Camara et al. 2022).

Screening experiment

The nests of *T. wilkinsoni* were collected in the pine forests of the province of Bilecik. In addition, the pest was verified as *T. wilkinsoni* by Prof. İpekdal. The silk nest was carefully opened with scissors and 30 larvae (4th instar) were transferred to a disinfected box with pine needles. The fungal suspensions (10^7 conidia/ml) were then applied with a mini hand sprayer and the boxes were incubated at 25±2ºC, 70±5% relative humidity and a 16:8 h light:dark photoperiod. The control group was sprayed with a 0.1% Tween80 solution. Each treatment was replicated four times with 30 individuals per treatment, and the experiments were conducted twice. Mortality was recorded 14 days after the applications. The dead larvae were placed in a Petri dish with moist filter paper to stimulate fungal sporulation after surface

sterilization with 1% sodium hypochlorite solution, and the mycosis rate was determined (Biryol et al. 2021).

Concentration response experiment

PPM1 isolate, which showed the highest insecticidal activity in the screening experiments, was used in the concentration response experiments. Spore suspensions of the isolate were prepared at concentrations of 10^7, 10^6, 10^5, 10^4 and 10^3 conidia/ml and bioassays were performed as indicated in the screening experiment.

Horizontal transmission experiment

The horizontal transmission ability of the PPM1 isolate was evaluated using larvae treated with the fungus and control larvae (untreated) at four vector ratios of 0, 25, 50, 75 and 100%. Each treatment was replicated three times. Fungus-treated larvae were air-dried at room temperature after being immersed for 10 seconds in the conidial suspension prepared at a concentration of 10^7 conidia/ml (Balachander et al. 2013). Both treated and untreated larvae were placed in the rearing cage with pine needles. After 14 days, mortality and mycosis were assessed as described in the screening experiments.

Statistical analysis

Mortality and mycosis rates were adjusted using Abbott's formula (Abbott 1925) and data were analyzed using one way ANOVA, after being subjected to studentized residual analysis to confirm the assumption of normality with a Shapiro–Wilk test and for homogeneity of variances with a Bartlett test. Means were separated using Tukey's HSD (honestly significant difference) test at 0.05 probability. The mortality data from the concentration–response experiment was subjected to probit regression analysis to estimate the median lethal concentration (LC_{50}) of the PPM1 isolate. Analyses were conducted using the statistical package SPSS 25.0 (IBM, Armonk, New York, USA).

Results

Morphological characterization of entomopathogenic fungi

Five fungal isolates (PPM1-PPM5) were obtained from forty soil samples using the *T. molitor* bait method. PPM1, PPM 3, PPM 4 showed white to yellowish-white colonies with irregular margins and powdery appearance, typical macroscopic features of the genus *Beauveria.* Microscopic observation revealed that all three isolates had similar conidial morphology producing hyaline, smooth and spherical to broadly ellipsoidal conidia. The other isolates (PPM2 and PPM5) showed a white, cotton-wool surface and dark purple underside, typical macroscopic features of the genus *Fusarium*. In microscopic observations of the isolates, the spores were oval to ellipsoid/kidney-shaped, tapering oval and segmented into three cells, while the chlamydospores were formed in chains.

Molecular characterization of isolates

For further characterization of the isolates, ITS1-5.8S-ITS2 region was amplified and sequenced. The sequence data were deposited at the GenBank with accession numbers OR150442-OR150446. Comparison of NCBI database revealed that PPM1, PPM3 and PPM4 showed high sequence similarity with *B. bassiana* isolates. *Fusarium* isolates PPM2 and PPM5 showed high sequence similarity with *F. oxysporum* isolates. Phylogenetic analysis also revealed that PPM1, PPM3 and PPM4 clustered with *B. bassiana*, PPM2 and PPM5 clustered with *F. oxysporum* isolates. (Fig. 1).

Screening experiment

Pathogenicity and virulence of the fungal isolates were tested on *T. wilkinsoni* larvae under laboratory conditions and significantly higher larval mortality was observed compared to the control ($F=193.7$; $df=4$; $P<0.05$) (Fig. 2). Although *B. bassiana* (PPM1 and PPM 3) isolates achieved 100% mortality at a concentration of 10^7 conidia/ml 14 days after treatment, the other *B. bassiana* isolate PPM2 caused 76% mortality, as did the *F. oxysporum* PPM4 isolate. The lowest mortality (15%) was obtained with *F. oxysporum* PPM5 isolate. No mortality was

observed in control groups. Mycosis development on cadaver surface was over 95% for all treatments.

Concentration response experiment

The experiment was carried out with different concentrations of *B. bassiana* (PPM1) that showed the highest virulence in the screening experiment. The mortality rate increased in a concentration dependent manner (Fig. 3). PPM1 caused 40% mortality at the lowest concentration (10^3 conidia/ml) and 97% mortality at the highest concentration (10^7 conidia/ml). Furthermore, median lethal concentration of the PPM1 isolate was estimated to be 5.7×10^3 conidia/ml (Table 1).

Horizontal transmission experiment

Horizontal transmission of conidia from treated to untreated larvae was evaluated for 14 days and it was found that there was no conidial transmission between larvae. It was found that there was no significant difference between larvae in terms of horizontal transmission ($P>0.05$) (Table 2). The mortality values of larvae contaminated at 0% (control), 25%, 50%, 75% and 100% with 10^7 conidia/ml suspension of *B. bassiana* (PPM1) were determined to be 0.6, 21.6, 49, 73.3, 97.6% after 14 days under laboratory conditions.

Discussion

This is the first study to demonstrate the virulence of entomopathogenic fungi isolated from pine forest soils against *T. wilkinsoni*, an important defoliating pest of pine forests. The fungi used in this study were recovered from forest soil samples using the insect-bait method. This method, which recommends the use of *G. mellonella* larvae or other insects such as *T. molitor* for the isolation of entomopathogenic fungi, is more common and a more sensitive method than the traditional approach of using selective medium (Sharma et al. 2018). Therefore, the method has been widely used to isolate indigenous species from soil samples around the world. Perumal et al. (2023) isolated entomopathogenic fungus *M. majus* from forest sample soil using

Galleria-bait method in India. They also isolated *B. bassiana* from soil samples using *Tenebrio*-bait method (Vivekanandhan et al. 2024). In this study, we isolated five indigenous fungal strains using *Tenebrio*-bait method.

Of these fungi, three isolates belonged to *Beauveria* (PPM1, PPM3 and PPM4) and other isolates belonged to *Fusarium* (PPM2 and PPM5), based on macroscopic and microscopic features described by Humber et al. (2012). However, identification of entomopathogenic fungi at the species level based on morphological properties alone is very difficult, which can lead to misidentification. Therefore, sequencing of ITS regions of rDNA, about 600 bp, allowed identification of fungal isolates at species level. The ITS region is often used for molecular identification because it has hypervariable sequences that allow recognition at both the species level and lower levels (Schoch et al. 2012). Comparison of the ITS sequence of *Beauveria* and *Fusarium* isolates deposited in the NCBI database showed high sequence similarity with *B. bassiana* and *F. oxyporum,* respectively. Phylogenetic analysis also confirmed the taxonomic identification.

Some researchers have studied the pathogenicity of *M. brunneum, B. bassiana and B. pseudobassiana* against *T. wilkinsoni*. However, there is no study on the pathogenicity of *F. oxysporum*, a known plant pathogen, on *T. wilkinsoni*. Therefore, this is the first study on the pathogenicity of *F. oxysporum* on *T. wilkinsoni*. Of the isolates identified as *F. oxysporum*, PPM2 caused 76% mortality, while PPM5 caused only 15% mortality on fourth stage larvae of *T. wilkinsoni*. Although *F. oxysporum* is widely known as a plant pathogen, it is often associated with insects from the orders Coleoptera (Caesar et al. 2002) and Hemiptera (Anwar et al. 2017) and Lepidoptera (Mantzoukas et al. 2022). *F. oxysporum* was previously isolated from irrigated fields in Palestine by Ali-Shtayeh et al. (2003) using the insect bait method and was shown to cause 30% mortality on *G. mellonella* larvae six days after application. In agreement with our

results, Ameen et al. (2012) isolated *F. oxysporum* strain from cultivated soil in Iraq and observed a mortality of 76% on *G. mellonella*.

Several studies reported that entomopathogenic fungi provided optimistic results in the biocontrol of *T. wilkinsoni.* Topkara et al. (2022) tested five *B. bassiana* strains isolated from infected *Palomena prasina* in Samsun, Turkey, on *T. wilkinsoni* larvae distributed in the same region and reported that the isolates caused a mortality of more than 90% at a concentration of 10^7 conidia/ml. On the other hand, Yanar et al. (2023) reported that the isolate of *B. bassiana* GOPT-331 at a concentration of 10^7 conidia/ml caused a mortality of 57.7% against the 4th larval stage of the pest. In our study, isolates of *B. bassiana* PPM1 and PPM3 caused complete mortality at a concentration of 10^7 conidia/ml. Studies have shown that the differences in fungal virulence depend on the speed of germination and hyphal growth (Talaei-Hassanloui et al. 2007). In addition, it is known that the secondary metabolites of *B. bassiana* play an important role in infection. Perumal et al. (2024) showed that 9,10-octadecadienoic acid produced by *B. bassiana* facilitated infection by significantly decreasing the levels of the enzymes acetylcholinesterase, α-carboxylesterase and β-carboxylesterase, which play an important role in combating oxidative stress in *T. absoluta* larvae.

Studies on the concentration-response relationship of entomopathogenic fungi on *T. wilkinsoni* are limited. Yanar et al. (2023) applied an indigenous isolate of *B. bassiana* GOPT-331 (isolated from agricultural soils) at six different conidial concentrations to fourth stage larvae of *T. wilkinsoni* and observed remarkable insecticidal activity that increased with application concentration. The highest mortality (93%) was observed at a concentration of 10^8 conidia/ml. They also determined the LC_{50} value of *B. bassiana* GOPT-331 to be 3.1×10^6 conidia/ml. In our study, *B. bassiana* PPM1 isolated from pine forest soils, which are the habitat of the pest, caused 40% mortality at a concentration of 10^3 conidia/ml and 97% mortality at the highest concentration, and the LC_{50} value was estimated to be 5.4×10^3 conidia/ml for the fourth

larval stage. The difference in LC_{50} value could be due to the isolation habitat of the fungus. Quesada Moraga et al. (2024) pointed out that fungi adapt to their natural habitat through physiological and ecological evolution, so their use in climatic and environmental conditions to which they are not adapted may affect their environmental competence. Therefore, geographical origin and isolation habitat are key factors for the development of an effective environmentally competent EPF.

Horizontal transmission by direct contact between fungus infected and uninfected insects is a mechanism of natural spread of conidia that controls insect populations (Hajek et al. 2018). Fungal transmission can occur through mating or physical contact. This has already been demonstrated in insect species such as the German cockroach, *Blatella germanica* (Quesada-Moraga et al. 2004), the European spruce bark beetle, *Ips typographus* (Kreutz et al. 2004), the beet webworm, *Spoladea recurvalis* (Opisa et al. 2019), the emerald ash borer, *Agrilus planipennis* (Srei et al. 2020), the lesser spruce sawfly, *Pristiphora abietina* (Biryol et al. 2021). In the study by Sönmez et al. (2017), it was shown that *M. brunneum* V275 and *B. bassiana* KTU-24 were successfully transmitted horizontally between the larvae of another pine processionary moth, *T. pityocampa*. Unfortunately, our isolate *B. bassiana* PPM1 could not be transmitted horizontally to the population of *T. wilkinsoni* within a certain time. This is thought to be due to insufficient physical contact between the larvae. As some studies have indicated the importance of mating for horizontal transmission (Toledo et al. 2007, Janmaat et al. 2022).

Conclusions

In conclusion, the isolation and characterization of entomopathogenic fungi from pine forest soils was carried out using *Tenebrio*-bait method. Among the isolates, *B. bassiana* PPM1 was found to have high virulence on *T. wilkinsoni* and could be used for its biological control. Since abiotic factors such as temperature, UV radiation and humidity influence the fungal infection

process under field conditions, further studies under field conditions should be carried out to verify our results.

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Competing interests

The authors declare no conflict of interest.

Data availability

All relevant data is contained within the article.

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547 **Table 1.** Lethal concentrations (LC_{50} and LC_{95}) of *Beauveria bassiana* PPM1 isolate against
548 fourth stage larvae of *T. wilkinsoni*.

Isolate	LC_{50} (conidia/ml) *(CL*, %95)	Slope ± *SE*	LC_{95} (conidia/ml) (*CL*, %95)	df	X^2
PPM1	5.7×10^3 $(2.4\times10^3 – 1.1\times10^4)$	0.625± 0.049	2×10^7 $(6.1\times10^6 – 1.1\times10^8)$	3	4.7

549 *CL*: confidence limit, *SE*: standard error, *df*: degree of freedom, X^2: chi-square

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568 **Table 2**. Horizontal transmission of fungal conidia of *B. bassiana* PPM1 from treated fourth-
569 stage larvae of *T. wilkinsoni* to untreated larvae at five infection rates 4 days after treatment.

Vector ratio (%)	Mortality (%) ± SD
0	0.66 ±0.57
25	21.6 ±3.05
50	49.0 ±2.64
75	73.3 ±4.93
100	97.6 ±2.51

570 The total number of larvae was 60; 0% vector ratio comprised untreated control insects, 25%
571 vector ratio comprised 20 fungus-treated and 40 untreated larvae, 50% vector ratio comprised
572 30 fungus-treated and 30 untreated larvae, 75% vector ratio comprised 40 fungus-treated and
573 20 untreated larvae, and 100% vector ratio comprised 60 fungus-treated larvae. Mortality
574 indicates the mean of four replicates, SD indicates the standard deviation.
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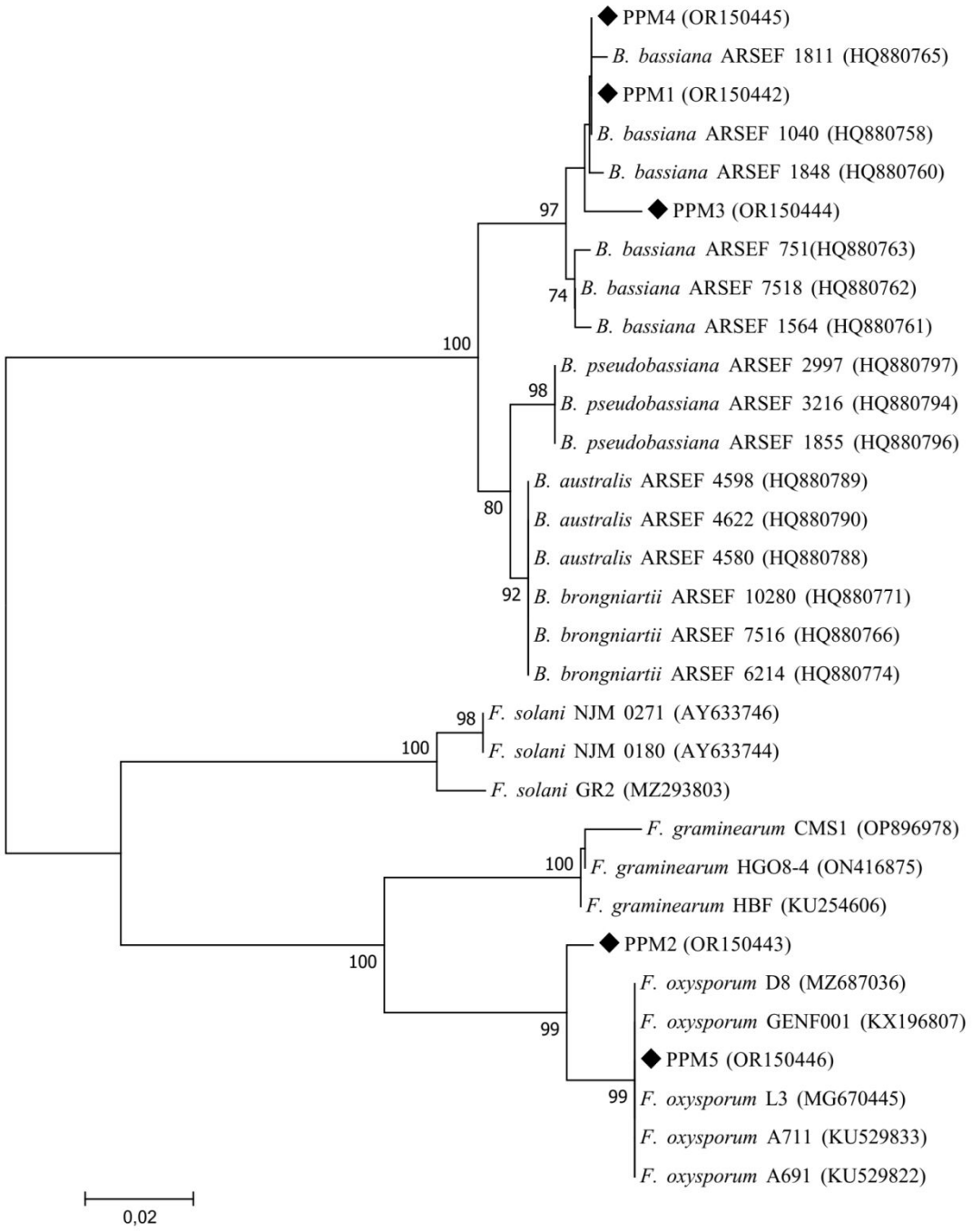

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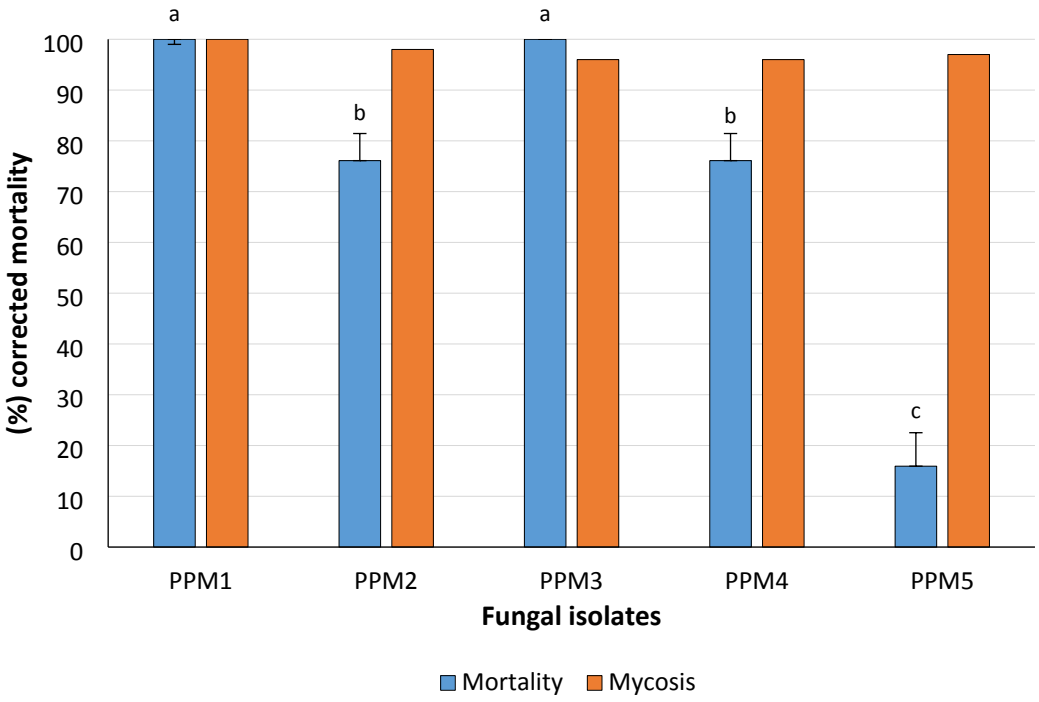
581 **Fig. 1.** Phylogenetic tree based on the sequence of the ITS1-5.8 S-ITS2 rDNA gene region of
582 *Beauveria* and *Fusarium* isolates. The tree was constructed using the neighbour-joining method
583 based on genetic distances calculated using the maximum composite likelihood method. The
584 reliability of the tree was assessed by bootstrap analysis with 1000 replicates. Only bootstrap
585 values above 70% are shown.



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587 **Fig. 2.** Screening experiment of local entomopathogenic isolates obtained from pine forest soils
588 at a concentration of 10^7 conidia/ml. Mortality represents the mean of mortality (%) obtained
589 from four dependent and two independent bioassays. The mortality data were corrected
590 according to the Abbott formula. Mean values labelled with different letters are significantly
591 different (Tukey's HSD, $p < 0.05$). The error bars show the standard deviation. Mycosis
592 indicates the percentage of mycosis in dead larvae.

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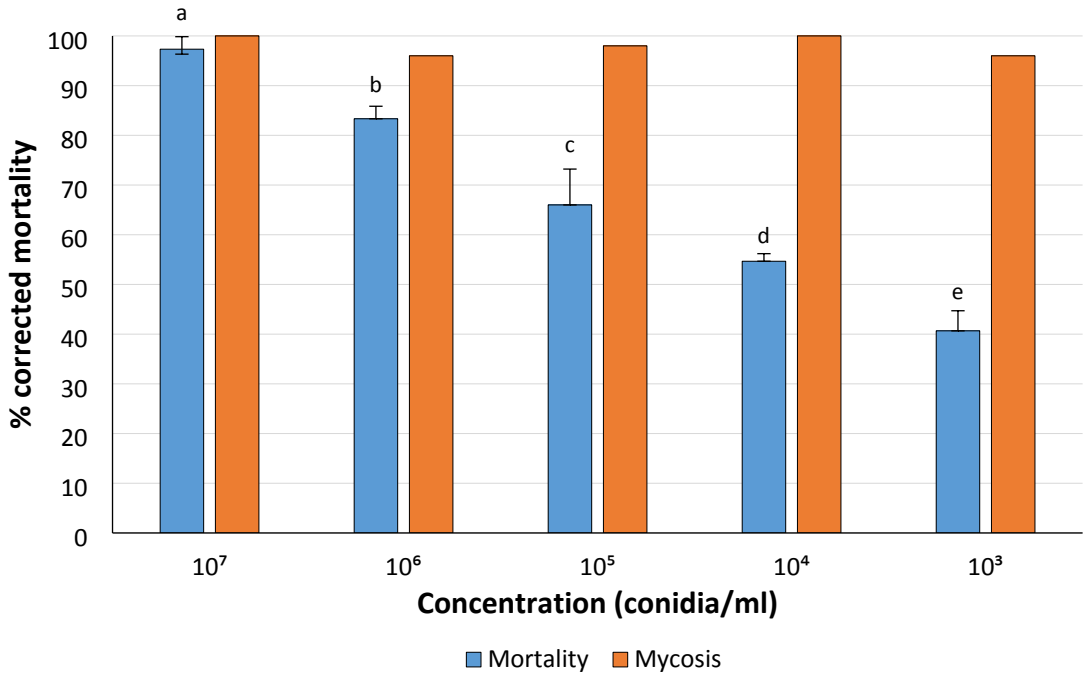

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601 **Fig. 3.** Concentration-response experiment of *B. bassiana* PPM1 isolate obtained from pine
602 forest soils. Mortality represents the mean of mortality (%) obtained from four dependent and
603 two independent bioassays. The mortality data were corrected according to the Abbott formula.
604 Mean values labelled with different letters are significantly different (Tukey's HSD, $p < 0.05$).
605 The error bars show the standard deviation. Mycosis indicates the percentage of mycosis in
606 dead larvae.

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