J. Entomol. Res. Soc., 27(1): 151-162, 2025 *Research Article*
Doi: 10.51963/jers.v27i1.2776 *Online ISSN:2651-3579*

Efficacy of Different Entomopathogenic Fungal Isolates against the Pine Processionary Moths *Thaumetopoea pityocampa* Denis & Schiffermüller and *Thaumetopoea wilkinsoni* Tams

Elif F. TOPKARA[1*] Seda BİRYOL[2] Oğuzhan YANAR[1] İsmail DEMİR[3]

[1]Department of Biology, Faculty of Science, Ondokuz Mayıs University, Samsun, TÜRKIYE

[2]Department of Medical Services and Techniques, Tonya Vocational School, Trabzon University, Trabzon, TÜRKIYE

[3]Department of Biology, Faculty of Science, Karadeniz Technical University, Trabzon, TÜRKIYE

e-mails: [1a]topkaraelif@hotmail.com, [2]sedabiryol@trabzon.edu.tr, [1b]oyanar46@gmail.com, [3]idemir@ktu.edu.tr

ORCID IDs: [1a]0000-0002-4743-2914, [2]0000-0003-0881-5004, [1b]0000-0003-3457-0768, [3]0000-0001-6227-0039

*Corresponding Author

ABSTRACT

Pine processionary moths *Thaumetopoea pityocampa* and *Thaumetopoea wilkinsoni* are among the most harmful insects that cause severe damage to pine forests in many countries, including Türkiye. The aim of this study was to evaluate the efficacy of entomopathogenic fungi (EPF) isolates of *Beauveria bassiana* (PA-4 and KTU-24), *Metarhizium anisopliae* (KTU-51), and *M. floviridae* (AS-2) against the 2nd instar larvae of *T. pityocampa* and *T. wilkinsoni* under laboratory conditions. *T. wilkinsoni* eggs were collected from pine trees at Ondokuz Mayıs University Kurupelit Campus in Samsun, Türkiye, in 2021, while *T. pityocampa* eggs were collected from Kahramanmaraş province, Türkiye, in 2021, and the 2nd instar larvae were used for the experiment. Four fungal isolates were sprayed on the larvae at 2 mL for each concentration (1×10^5 - 1×10^8 conidia mL^{-1}). At a concentration of 1×10^8 conidia mL^{-1}, mortality rates for *T. pityocampa* larvae were 55.6-100%, while mortality rates for *T. wilkinsoni* larvae were 56.8-100%. It was found that two isolates of *B. bassiana* were virulent for both *T. pityocampa* and *T. wilkinsoni*, and the KTU-24 isolate was the most virulent isolate, causing the lowest LC_{50} values (2.2×10^5 for *T. pityocampa* and 4.3×10^5 for *T. wilkinsoni*) and the shortest mean survival time for both larval species. It is suggested that the KTU-24 isolate can be used in the biological control of pine processionary moth species.

Keywords: *Beauveria bassiana,* biological control, Metarhizium, pine pest.

Topkara, E.F., Biryol, S., Yanar, O., & Demir, İ (2025). Efficacy of Different Entomopathogenic Fungal Isolates against the Pine Processionary Moths *Thaumetopoea pityocampa* Denis & Schiffermüller and *Thaumetopoea wilkinsoni* Tams. *Journal of the Entomological Research Society*, 27(1), 151-162.

Received: November 06, 2024 *Accepted: February 04, 2025*

INTRODUCTION

Forests are critical to the terrestrial ecosystem due to their rich biodiversity. They provide ecological services for the protection of human health and ecosystems (Gilhen-Baker, Roviello, Beresford-Kroeger, & Roviello, 2022) due to their features such as climate regulation, air filtering, soil and water protection, habitat provision, and wood supply (Uribe-Toril, Ruiz-Real, Haba-Osca, & Valenciano, 2019). *Pinus* species, which comprise a large part of the trees in the forest ecosystem, are economically and ecologically valuable. Products obtained from *Pinus* species are important in terms of both providing raw materials for the furniture industry and being used in traditional medicine. *Pinus* species, a valuable part of the ecosystem, are threatened by invaders. Chief among these enemies is the pine processionary moths (PPM) species, which are among the most harmful species that threaten pine trees.

Pine processionary moths (PPM) *Thaumetopoea pityocampa* Denis & Schiffermüller 1775 and *Thaumetopoea wilkinsoni* Tams 1926 (Lepidoptera: Notodontidae) are among the most important forest pests in the Mediterranean basin (Yüksel, İpekdal, & Toper Kaygın, 2019). These two species, which are closely related but genetically very differentiated (Yüksel et al., 2019), are also distributed in Türkiye. While *T. wilkinsoni* is mostly found in pine forests in the north, south, and west of Anatolia, *T. pityocampa* is generally found in Thrace and northwestern Anatolia (İpekdal, Burban, Kerdelhué, & Çağlar, 2015). PPM larvae live gregariously throughout the winter in conspicuous silk webs woven into the crowns of host plants. Larvae feed on the needles of pine trees and cause significant leaf loss in plants, weakening the pines and thus making the plants vulnerable to secondary pests (Erkaya, 2020). Both *T. pityocampa* and *T. wilkinsoni* larvae have setae that are highly allergenic to humans and animals (Bruchim, Ranen, Saragusty, & Aroch, 2005; Olivieri, Ludovico, & Battisti, 2023). Due to their setae containing thaumetopoein, an urticarial protein, individuals exposed to the larvae may experience symptoms such as urticaria and local swelling, conjunctivitis, erythema, cough, and shortness of breath (Moneo et al., 2015). PPM invasion in forests causes serious problems in terms of both the environment and human health and economy. Due to all these harmful effects of PPM larvae, which spread to upper latitudes and higher altitudes due to climate change (Roques, 2015), combating these pests is inevitable.

Mechanical (removal of larval nests), chemical, and biological control methods are used against PPM larvae. Although mechanical control is still a valuable practice in small areas such as parks and gardens (Bouzar Essaidi, Rahim, Meftahi, & Benfekih, 2023), this practice is inadequate in large forest ecosystems. Although the use of chemical insecticides seems to work at first glance in combating the species, considering the negative effects of chemical insecticides on both humans and other living creatures and the environment, scientists have turned to researching different methods of combating this harmful species. Biological control is one of the most promising alternatives for non-chemical pest management, especially in forest ecosystems where chemicals are often completely prohibited (Lagogiannis, Mantzoukas, Eliopoulos, &

Poulas, 2023). Various studies have been carried out within the scope of biological control using different bioagents against PPM larvae (Alberghini, Filippini, Shevelev, Squartini, & Battisti, 2006; Gözel & Gözel, 2019; Triggiani & Tarasco, 2002; Topkara, Yanar, Tuncer, Özdemir, & Yıldırım, 2022; Yanar, Topkara, & Doruk, 2022; Tarasco et al., 2023). Entomopathogenic fungi (EPF) are among the environmentally friendly species used against PPM larvae within the scope of biological control.

EPF has an advantage over entomopathogenic viruses and bacteria as it can infect its hosts not only through diet but also directly from spikes and insect cuticles (Şahin & Yanar, 2021). At the same time, EPF can specifically control host pests, pose no risk to mammals, do not cause insect resistance and environmental pollution, and provide long-term control of the host (Wan, 2003). Among the EPFs, *Beauveria bassiana* (Bals. -Criv.) Vuill (Hypocreales: Cordycipitaceae), *Metarhizium anisopliae* (Metch) Sorokin (Hypocreales: Clavicipitaceae), and *Metarhizium flavoviride* Gams & Roszypal (Deuteromycotina: Hyphomycetes) are among the most widely used mycoinsecticides (Rosell, Quero, Coll, & Guerrero, 2008). The effectiveness of EPF against PPM larvae has been recorded in various studies (Tarasco, Triggiani, Zamoum, & Oreste, 2016; Topkara et al., 2022; Lagogiannis et al., 2023; Yanar et al., 2023).

Since the effectiveness of different EPF isolates against the target host varies from one isolate or strain to another (El Husseini, 2019), selecting and testing different EPF isolates with different properties increases the chance of obtaining a successful biocontrol agent to control insect hosts. Both *T. pityocampa* and *T. wilkinsoni* are common in Türkiye, and these two species have also shown hybridization in Türkiye (İpekdal et al., 2015). Therefore, it is important to compare these two larval species for biocontrol studies. The aim of this study was to evaluate the efficacy of *B. bassiana* (PA-4 and KTU-24), *M. anisopliae* (KTU-51), and *M. floviridae* (AS-2) isolates against the 2nd instar larvae of *T. pityocampa* and *T. wilkinsoni* under laboratory conditions.

MATERIALS AND METHODS

Sampling

Thaumetopoea wilkinsoni eggs were collected from the needles of *Pinus sylvestris* species at Ondokuz Mayıs University Kurupelit Campus in Samsun, Türkiye (N 41°22´ E 36°13´), in 2021, while *T. pityocampa* eggs were collected from the needles of *P. brutia* at Kahramanmaraş, Türkiye (N 37°14´ E 36°46´), in 2021. The eggs were brought to the laboratory, and they were disinfected with 10% sodium hypochlorite for about 7 min, and then washed with distilled water for about 7 min and rinsed. The disinfected eggs were placed in an air-conditioning room at 20 °C, 70 ± 5% RH, and a 12:12 h (L:D) photoperiod.

Fungal cultures

KTU-51 (*M. anisopliae*), PA-4 (*B. bassiana*), KTU-24 (*B. bassiana*), and AS-2 (*M. flavoviride*) were obtained from the entomopathogenic culture collection of Karadeniz

Technical University, Faculty of Science, Department of Biology, Microbiology Laboratory. The isolates were diagnosed by sequence analysis and recorded in the GenBank database (Table 1). When the studies previously conducted using these fungi were examined, these isolates had an effect of over 80% on different pests (Sevim, Demir, & Demirbağ, 2010a; Sevim, Demir, Höfte, Humber, & Demirbağ, 2010b; Biryol, Efe, Eski, Demirbağ, & Demir, 2020; Biryol, Demirbağ, Erdoğan, & Demir, 2022).

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

Species / Isolate	Genbank accession numbers	Host	Locality	References
Metarhizium anisopliae / KTU-51	FJ177506	Soil	Gümüşhane, Türkiye	Sevim et al. (2010)
Beauveria bassiana / PA-4	MK544080	*Pristiphora abietina*	Artvin, Türkiye	Biryol et al. (2021)
Beauveria bassiana / KTU-24	FJ177449	*T. pityocampa*	Samsun, Türkiye	Sevim et al. (2010)
Metarhizium flavoviride / AS-2	KY348739	*Amphimallon solstitiale*	Trabzon, Türkiye	Biryol et al. (2020)

Preparation of conidial suspension

Fungi isolates revived from stock culture were first inoculated on a Potato dextrose agar (PDA; Merck Ltd., Darmstadt, Germany) medium for sporulation. The conidial suspensions of fungal isolates for bioassays were prepared by adding 10 mL of sterile 0.01% Tween 80 (AppliChem, Darmstadt, Germany) to 4-week-old cultures. The spore suspensions were filtered into sterile 50 mL plastic tubes (Falcon, Franklin Lakes, NJ, USA) using a sterile cotton gauze to remove fungal residue and were vortexed for 5 min to homogenize the preparations. To determine the viability of conidia, the suspensions were spread on PDA medium, and germination was assessed after 24 h of incubation at 25 °C in the dark. After 24 hours, the ratio of germinated spores on each plate was evaluated using a microscope, with positive germination defined as germ tube length that is at least half the spore length. Cultures with conidia viability above 95% were used for bioassay experiments.

Experimental setup

The newly hatched larvae of *T. pityocampa* and *T. wilkinsoni* were fed with *P. sylvestris* needles (disinfected with 50% ethanol and then washed with distilled water), and the 2nd instar larvae were placed in plastic containers. Sterilized *P. sylvestris* needles were given to the larvae in the control groups. For the infected groups, the larvae were contaminated with different concentrations (1×10^5, 1×10^6, 1×10^7, and 1×10^8 conidia mL^{-1}) of *M. anisopliae* (KTU-51), *B. bassiana* (PA-4, KTU-24), and *M. floviridae* (AS-2) isolates at 2 mL for each concentration with a hand sprayer, and the *P. sylvestris* needles were given to the 2nd instar larvae. Thirty larvae were placed in each group to determine the survival rates, and each experiment was repeated three times. A total of 3060 larvae, 90 in each group, were used for each concentration to be applied to the 2nd instar larvae of both *T. pityocampa* and *T. wilkinsoni*. In this experiment, the larvae were observed for 15 days. The dead larvae were placed in Petri dishes with soaked paper, spore formations were followed, and fungal infection was checked.

Statistical analysis

The efficacy of four different fungal isolates (KTU-51, PA-4, KTU-24, and AS-2) against the 2nd instar larvae of *T. pityocampa* and *T. wilkinsoni* was compared with the control group. Cox-Regression analysis was used to calculate the risks of death of *T. pityocampa* and *T. wilkinsoni* larvae exposed to different fungal concentrations. In addition, the lethal doses (LC_{50}) were determined by the Probit analysis. SPSS version 21.0 was used for these tests.

RESULTS

Mortality rates of *T. pityocampa* larvae are shown in Fig. 1. The mortality rate in the control group was found to be 7.8%. Since there was more than 5% mortality in the control group in the experiment conducted with larvae, correction was made with the Abbott formula (Abbott, 1925). According to increasing concentrations, the mortality rates for AS-2 infection were 8.4%, 18.5%, 34.6%, 55.6%; for KTU-51 infection, 14.8%, 25.9%, 56.8%, 72.8%; for PA-4 infection, 27.2%, 39.5%, 75.3%, 100%; and for KTU-24 infection, 41.9%, 53.1%, 82.7%, and 100%, respectively.

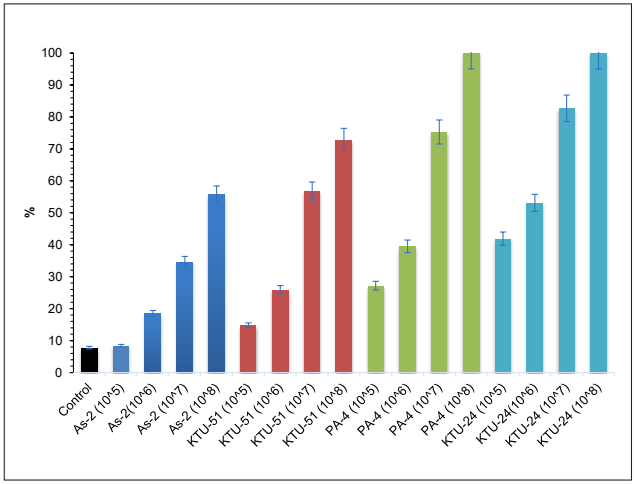

Figure 1. Mortality rates of *Thaumetopoea pityocampa* larvae.

Mortality rates of *T. wilkinsoni* larvae are shown in Fig. 2. Since there was more than 5% mortality in the control group, correction was made with the Abbott formula (Abbott, 1925). The mortality rate in the control group was 10%. According to increasing concentrations, the mortality rates for AS-2 infection were 6.1%, 17.2%, 38.2%, 56.8%; for KTU-51 infection, 16.1%, 27.1%, 56.8%, 71.6%; for PA-4 infection, 22.2%, 33.3%, 66.7%, 100%, and for KTU-24 infection, 35.8%, 43.2%, 75.3.7%, and 100%, respectively.

According to the Log-Rank test analysis results, it was determined that all groups of *T. pityocampa* and *T. wilkinsoni* larvae exposed to fungal infection were statistically different from the control. The infected groups were also found to be statistically different from each other (Table 2).

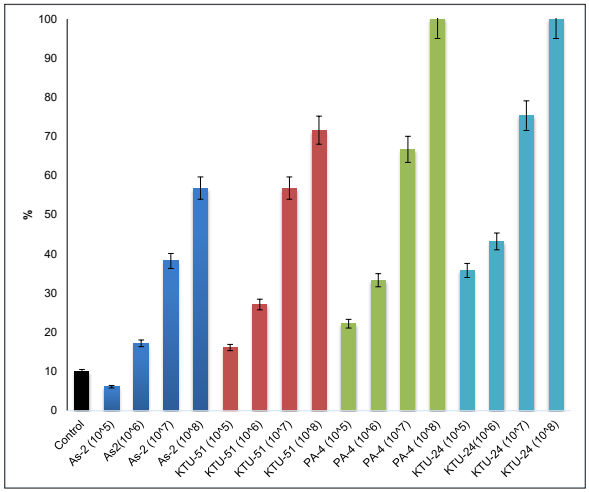

Figure 2. Mortality rates of *Thaumetopoea wilkinsoni* larvae.

Table 2. Log-Rank test results of *Thaumetopoea pityocampa* and *Thaumetopoea wilkinsoni* larvae infected with AS-2, KTU-51, PA-4, and KTU-24 isolates at 1 × 10^8 conidia mL^{-1} concentration.

Species	Groups	Control		AS-2		KTU-51		PA-4		KTU-24	
		X2	p	X2	p	X2	p	X2	p	X2	p
T. pityocampa	Control			25.58	.000	44.63	.000	79.43	.000	103.25	.000
	AS-2	25.58	.000			12.29	.000	71.41	.000	123.74	.000
	KTU-51	44.63	.000	12.29	.000			26.07	.000	62.73	.000
	PA-4	79.43	.000	71.41	.000	26.07	.000			8.66	.003
	KTU-24	103.25	.000	123.74	.000	62.73	.000	8.66	.003		
T. wilkinsoni	Control			25.58	.000	44.73	.000	78.02	.000	101.28	.000
	AS-2	25.58	.000			12.83	.000	64.03	.000	111.95	.000
	KTU-51	44.73	.000	12.83	.000			19.52	.000	50.18	.000
	PA-4	78.02	.000	64.03	.000	19.52	.000			7.95	.005
	KTU-24	101.28	.000	111.95	.000	50.18	.000	7.95	.005		

X2: Chi-square test, p: Significant

According to the results of Cox-Regression analysis, the AS-2 isolate increased the risk of death of *T. pityocampa* larvae by 5.2 times, the KTU-51 isolate by 7.6 times, the PA-4 isolate by 12.1 times, and the KTU-24 isolate by 15.5 times. It was determined that the AS-2 isolate increased the risk of death of *T. wilkinsoni* larvae by 4.2 times, the KTU-51 isolate by 6 times, the PA-4 isolate by 8.4 times, and the KTU-24 isolate by 10.5 times (Table 3).

The mean survival time for *T. pityocampa* larvae was 14 days in the control group. The mean survival time was 12 days for isolate AS-2, 11 days for the KTU-51 isolate, 9 days for the PA-4 isolate, and 8 days for the KTU-24 isolate. For *T. wilkinsoni* larvae, the mean survival time was recorded to be 14 days in the control group, 12 days for the AS-2 isolate, 11 days for the KTU-51 isolate, 10 days for the PA-4 isolate, and 9 days for the KTU-24 isolate (Table 4).

Table 3. Cox-regression analysis results of *Thaumetopoea pityocampa* and *Thaumetopoea wilkinsoni* larvae infected with AS-2, KTU-51, PA-4, and KTU-24 isolates at 1 × 10^8 conidia mL^{-1} concentration.

Species	Groups	B	E	Wald	df	P	Exp(B)	95.0% CI for Exp(B) Lower Upper
T. pityocampa	Control			160.36	4	.000		
	AS-2	1.662	.388	18.342	1	.000	5.271	2.463 11.278
	KTU-51	2.036	.386	27.887	1	.000	7.660	3.598 16.307
	PA-4	2.501	.384	42.466	1	.000	12.195	5.748 25.873
	KTU-24	2.744	.383	51.276	1	.000	15.554	7.339 32.965
T. wilkinsoni	Control			117.356	4	.000		
	AS-2	1.437	.345	17.397	1	.000	4.208	2.142 8.267
	KTU-51	1.806	.342	27.894	1	.000	6.083	3.113 11.888
	PA-4	2.132	.340	39.222	1	.000	8.428	4.325 16.422
	KTU-24	2.353	.340	47.982	1	.000	10.517	5.404 20.466

B: Coefficient of regression, SE: Standard error, Wald: Significance of the regression coefficients, df: Degree of freedom, p: Significant, Exp(B): Hazard proportion.

Table 4. Means for survival time of *Thaumetopoea pityocampa* and *Thaumetopoea wilkinsoni* larvae infected with AS-2, KTU-51, PA-4, and KTU-24 isolates.

Species	Groups	Estimate	Std. Error	Mean 95% Confidence Interval	
				Lower Bound	Upper Bound
T. pityocampa	Control	14.711	.138	14.440	14.982
	AS-2	12.50	.199	12.109	12.891
	KTU-51	11.494	.221	11.062	11.927
	PA-4	9.633	.236	9.170	10.097
	KTU-24	8.611	.236	8.149	9.074
T. wilkinsoni	Control	14.711	.136	14.445	14.977
	AS-2	12.522	.197	12.136	12.908
	KTU-51	11.444	.221	11.011	11.878
	PA-4	10.211	.235	9.751	10.671
	KTU-24	9.278	.237	8.813	9.742

According to the Probit analysis results, LC_{50} values for the fungal isolates applied to the 2nd instar larvae of *T. pityocampa* and *T. wilkinsoni* are shown in Table 5. The AS-2 isolate caused the highest LC_{50} value for both *T. pityocampa* (2.9 × 10^7 conidia mL^{-1}) and *T. wilkinsoni* (2.3 × 10^7 conidia mL^{-1}), while the KTU-24 isolate caused the lowest LC_{50} value for both *T. pityocampa* (2.2 × 10^5 conidia mL^{-1}) and *T. wilkinsoni* (4.3 × 10^5 conidia mL^{-1}).

Table 5. Probit regression estimates for the multiple-concentration bioassays performed with AS-2, KTU-51, PA-4, and KTU-24 isolates against larvae of *Thaumetopoea pityocampa* and *Thaumetopoea wilkinsoni*.

Species	Groups	LC50 (fungus/mL) (FL, 95%)	Slope±SE	Intercept	X2	df b
T. pityo-campa	AS-2	2.9×107	0.5 ± 0.1	-3.1 ± 0.1	0.1	2
	KTU-51	3.9×106	0.7 ± 0.1	-3.3 ± 0.7	1.5	2
	PA-4	6.5×105	0.7 ± 0.1	-4.5 ± 0.1	11.9	2
	KTU-24	2.2×105	0.7 ± 0.1	-3.7 ± 0.1	9.3	2
T. wilkinsoni	AS-2	2.3×107	0.5 ± 0.1	-3.3 ± 0.1	0.2	2
	KTU-51	3.8×106	0.5 ± 0.1	-3.1 ± 0.7	1.4	2
	PA-4	1.1×106	0.6 ± 0.1	-4.6 ± 0.1	15.8	2
	KTU-24	4.3×105	0.5 ± 0.1	-3.8 ± 0.1	14.2	2

X2: Chi-Square test, df: Degree of freedom.

DISCUSSION

EPF, targeting different insect species from various taxa, are considered environmentally friendly alternatives to chemical insecticides for pest control. EPF belonging to the *Beauveria* and *Metarhizium* genera are the most common environmentally friendly bioagents used for pest control. In this study, the efficacy of *B. bassiana* (PA-4 and KTU-24), *M. anisopliae* (KTU-51), and *M. floviridae* (AS-2) isolates against the 2nd instar larvae *T. pityocampa* and *T. wilkinsoni* was evaluated.

Mortality rates of both *T. pityocampa* and *T. wilkinsoni* larvae increased with increasing conidial concentrations of all four isolates. Lagogiannis et al. (2023) applied different EPF to *T. pityocampa* larvae; they noted that mortality rates increased with increasing EPF concentrations, and the mortality rates of larvae exposed to the highest dose 144 hours after application varied between 71-91%. In our study, AS-2 infection caused the lowest mortality in both *T. pityocampa* and *T. wilkinsoni*. In this case, it can be said that both larval species are resistant to *M. floviridae*. Furthermore, in larvae exposed to the highest concentration (1×10^8 conidia mL^{-1}) of the PA-4 and the KTU-24 isolates, the mortality rates were maximum (100%) for both species. Since these two isolates that caused maximum mortality in both larval species belong to the *B. bassiana* genus, it can be said that, in this case, the *B. bassiana* species is highly virulent for *T. pityocampa* and *T. wilkinsoni* larvae compared to the other two fungal species. This is consistent with Pour, Zibaee, Rostami, Hoda, & Shahriari (2021) who emphasized that *B. bassiana* is among the most virulent EPF against insects and recorded 100% larval mortality on day 8 in their study. At the same time, as a result of our study, it was determined that the AS-2 isolate had the least effect on the mortality risk of both *T. pityocampa* and *T. wilkinsoni* larvae, while the KTU-24 isolate had the maximum effect on the mortality risk of both larval species. This situation proves that among all the isolates, the KTU-24 isolate is the most lethal isolate for *T. pityocampa* and *T. wilkinsoni* larvae.

The effects of different EPF species on PPM larvae were investigated in various studies. Er, Tunaz, & Gökçe (2007) showed that *T. pityocampa* larvae infected with different EPF species were extremely sensitive to the applied fungi. Bonnet, Martin, Mazet, Correard, & Besse (2013) noted that *B. bassiana* was effective in the control of *T. pityocampa*, and larval mortality rates varied between 82-86%. Sönmez, Demir, Bull, Butt, & Demirbağ (2017) infected first to fourth instar *T. pityocampa* larvae with *M. brunneum* (V275, ARSEF 4556) and *B. bassiana* (KTU-24) isolates ranging from 1×10^5 to 1×10^8 conidia mL^{-1}, and they found that the strains caused 100% mortality for all instars at the highest dose. Akıncı et al. (2017) applied four EPF species, including *B. bassiana* against *T. pityocampa* larvae, and as a result of the study, they suggested that *B. bassiana* is one of the most lethal species and can be used to control the species. Saidi et al. (2023) found that the EPF they applied against *T. pityocampa* caused 92% mortality in larvae at the highest concentration 11 days after application. Compared to *T. pityocampa*, studies on *T. wilkinsoni* are more limited. Güven, Aydin, Karaca, & Butt (2021) determined that two *M. brunneum* isolates they applied to *T. wilkinsoni* caused 100% mortality in the laboratory. Topkara et al. (2022) investigated the effectiveness

of different *B. bassiana* isolates against *T. wilkinsoni* under laboratory conditions; they noted that all isolates caused a 100% mortality rate at the highest dose (1×10^8 conidia mL^{-1}). Yanar et al. (2023) applied *B. bassiana* (GOPT-331) and *M. brunneum* (ORP-13 and ORP-18) isolates against the 2nd instar larvae *T. wilkinsoni*, and they reported that the mortality rates varied between 91.1-100% for 1×10^8 conidia mL^{-1}. The results obtained from our study and the findings of the studies conducted with PPM proved that EPF leads to high mortality rates in *T. pityocampa* and *T. wilkinsoni*.

In the current study, it was determined that the mean survival time of larvae exposed to fungal infection was shorter than that of the control. It was determined that the shortest mean survival time for *T. pityocampa* larvae was in larvae infected with the KTU-24 isolate. A similar situation applies to *T. wilkinsoni* larvae. The mean survival time of *T. pityocampa* larvae is shorter than that of *T. wilkinsoni*, which we consider an important result. Considering that fungal isolates are negatively affected by ultraviolet light, especially in biological control studies with fungi, the shortened mean survival time is extremely important. Studies have documented that the effectiveness of EPF against *T. pityocampa* larvae is significantly affected by conditions such as larval instar, EPF isolate, applied doses, and the EPF application method (Sevim et al., 2010a; Sönmez et al., 2017), which coincides with the result of our study.

The AS-2 isolate caused the highest LC_{50} value for both *T. pityocampa* and *T. wilkinsoni*, while the KTU-24 isolate caused the lowest LC_{50} value for both larval species. Since the low LC_{50} value means that the applied fungal isolate is effective in low amounts, it can be said that the KTU-24 isolate is the most effective among all isolates. Yanar et al. (2023) applied different *B. bassiana* and *M. brunneum* isolates to *T. wilkinsoni* larvae. They noted that the LC_{50} values for the 2nd instar larvae were the highest for the *B. bassiana* isolate, while the values were the lowest for the *M. brunneum* isolates. This result is opposite to the result of our current study because even if the same species of EPF were used, different results were obtained because different isolates were applied. This result also supports the conclusion that different strains or isolates of the same fungal species may differ in the virulence of the fungi even if the same concentration is used (Fite, Tefera, Negeri, Damte, & Sori, 2020).

CONCLUSIONS

In conclusion, it is extremely essential to combat PPM larvae, which, in addition to being important forest pests, are highly allergenic to humans and animals. Our aim is to contribute to control of these extremely harmful insects by assessing the efficacy of several EPF isolates. In this study, the PA-4 and KTU-24 isolates of the *B. bassiana* genus were found to be virulent for both *T. pityocampa* and *T. wilkinsoni* larvae at the highest concentration (1×10^8 conidia mL^{-1}).

Entomopathogenic fungi are stated to be effective *in vitro* in this study and in the literature and various further studies can be carried out to increase their effectiveness as potential biological control agents. Field studies are critical to validating their effectiveness on target organisms in the natural environment. In addition, formulation studies that

will increase the resistance of these fungi to environmental conditions (temperature, humidity, UV light) and research on their interactions with microbial consortia constitute the steps of converting them into commercial products. At the genetic and molecular level, elucidation of pathogenicity mechanisms and development of host-specific strains can provide valuable information to optimize biological control effectiveness. Such multidisciplinary approaches will allow entomopathogenic fungi to be used more widely among sustainable and environmentally friendly methods of control in agriculture.

REFERENCES

Abbott, W.S. (1925). A method of computing the effectiveness of insecticides. *Journal of Economic Entomology*, 18, 265-267.

Akıncı, H.A., Ozman-Sullivan, S.K., Diler, H., Celik, N., Sullivan, G.T., & Karaca, G. (2017). Entomopathogenic fungi isolated from *Thaumetopoea pityocampa* and their efficacies against its larvae. *Fresenius Environmental Bulletin*, 26(8), 5251-5257.

Alberghini, S., Filippini, R., Shevelev, A.B., Squartini, A., & Battisti, A. (2006). Extended plant protection by an epiphytic *Pseudomonas* sp. derivative carrying the cry9Aa gene from *Bacillus thuringiensis galleriae* against the pine processionary moth *Thaumetopoea pityocampa*. *Biocontrol Science and Technology*, 16(7), 709-715.

Biryol, S., Demirbağ, Z., Erdoğan, P., & Demir, I. (2022). Development of *Beauveria bassiana* (Ascomycota: Hypocreales) as a mycoinsecticide to control green peach aphid, *Myzus persicae* (Homoptera: Aphididae) and investigation of its biocontrol potential. *Journal of Asia-Pacific Entomology*, 25(1), 101878.

Biryol, S., Efe, D., Eski, A., Demirbağ, Z., & Demir, I. (2020). Fungal pathogens of *Amphimallon solstitiale* Linnaeus, 1758 (Coleoptera: Scarabaeidae). *Turkish Journal of Entomology*, 44(3), 375-384.

Bonnet, C., Martin, J. C., Mazet, R., Correard, M., & Besse, S. (2013). *Beauveria bassiana* (Bals.-Criv) Vuillemin: un agent entomopathogène pour réduire l'extension de la processionnaire du pin par les plants transportés en conteneur. 3. AFPP. Conférence sur l'Entretien des Espaces Verts, Jardins, Gazons, Forêts, Zones Aquatiques et Autres Zones Non Agricoles, Toulouse, France. HAL Id: hal-02749656 https://hal.inrae.fr/hal-02749656.

Bouzar Essaidi, K., Rahim, N., Meftahi, B., & Benfekih, L. (2023). Ecological characteristics of pine processionary moth *Thaumetopoea pityocampa* (Lepidoptera: Notodontidae) larval nests in pine and cedar forests of Algeria. *Biodiversitas*, 24(4), 2107-2113.

Bruchim, Y., Ranen, E., Saragusty, J., & Aroch, I. (2005). Severe tongue necrosis associated with pine processionary moth (*Thaumetopoea wilkinsoni*) ingestion in three dogs. *Toxicon*, 45(4), 443-447.

El Husseini, M.M. (2019). Efficacy of the entomopathogenic fungus, *Metarhizium anisopliae* (Metsch.), against larvae of the cotton leaf worm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae), under laboratory conditions. *Egyptian Journal of Biological Pest Control*, 29, 50.

Er, M.K., Tunaz, H., & Gökçe, A. (2007). Pathogenicity of entomopathogenic fungi to *Thaumetopoea pityocampa* (Schiff.) (Lepidoptera: Thaumatopoeidae) larvae in laboratory conditions. *Journal of Pest Science*, 80, 235-239.

Erkaya, I. (2020). Predators and parasitoids of pine processionary moth (*Thaumetopoea wilkinsoni* Tams) in Western Mediterranean region in Turkey. *Bilge International Journal of Science and Technology Research*, 4(1), 7-13.

Fite, T., Tefera, T., Negeri, M., Damte, T., & Sori, W. (2020). Evaluation of *Beauveria bassiana*, *Metarhizium anisopliae*, and *Bacillus thuringiensis* for the management of *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae) under laboratory and field conditions. *Biocontrol Science and Technology*, 30(3), 278-295.

Gilhen-Baker, M., Roviello, V., Beresford-Kroeger, D., & Roviello, G.N. (2022). Old growth forests and large old trees as critical organisms connecting ecosystems and human health. A review. *Environmental Chemistry Letters*, 20, 1529-1538.

Gözel, Ç. & Gözel, U. (2019). The efficacy of native entomopathogenic nematodes against the pine processionary moth, *Thaumetopoea pityocampa* Den. & Schiff. (Lepidoptera: Thaumetopoeidae). *Turkish Journal of Biological Control*, 10(2), 118-126.

Güven, Ö., Aydin, T., Karaca, I., & Butt, T. (2021). Biopesticides offer an environmentally friendly solution for control of pine processionary moth (*Thaumetopoea wilkinsoni* Tams) larvae and pupae in urban areas. *Biocontrol Science and Technology*, 31(1), 35-52.

İpekdal, K., Burban, C., Kerdelhué, C., & Çağlar, S.S. (2015). Distribution of two pine processionary moth species in Turkey evidences a contact zone. *Turkish Journal of Zoology*, 39, 868-876.

Lagogiannis, I., Mantzoukas, S., Eliopoulos, P.A., & Poulas, K. (2023). First record of *Beauveria varroae, Cordyceps blackwelliae,* and *Purpureocillium lavendulum* from Greece and their pathogenicity against *Thaumetopoea pityocampa*. *Diversity*, 15, 312.

Moneo, I., Battisti, A., Dufour, B., Garcıa-Ortiz, J.C., Gonzalez-Munoz, M., Moutou, F., Paolucci, P., Petrucco-Toffolo, E., Rivière, J., Rodrıguez-Mahillo, A.-I., Roques, A., Roques, L., Vega, J.M., & Vega, J. (2015). Medical and veterinary impact of the urticating processionary larvae. In A, Roques (Ed.). *Processionary moths and climate change: an update* (pp. 359-410). *Springer-Quae*: Dordrecht, the Netherlands; Versailles, France.

Olivieri, M., Ludovico, E., & Battisti, A. (2023). Occupational exposure of forest workers to the urticating setae of the pine processionary moth *Thaumetopoea pityocampa*. *International Journal of Environmental Research and Public Health,* 20(6), 4735.

Pour, S.A., Zibaee, A., Rostami, M.G., Hoda, H., & Shahriari, M. (2021). Mortality and immune challenge of a native isolate of *Beauveria bassina* against the larvae of *Glyphodes pyloalis* Walker (Lepidoptera: Pyralidae). *Egyptian Journal of Biological Pest Control*, 31, 37.

Roques, A. (2015). *Processionary moths and climate change: an update*. Springer-Quae, Dordrecht-Versailles.

Rosell, G., Quero, C., Coll, J., & Guerrero, A. (2008). Biorational insecticides in pest management. *Journal of Pesticide Science*, 33(2), 103-121.

Saidi, A., Mebdoua, S., Mecelem, D., Al-Hoshani, N., Sadrati, N., Boufahja, F., & Bendif, H. (2023). Dual biocontrol potential of the entomopathogenic fungus *Akanthomyces muscarius* against *Thaumetopoea pityocampa* and plant pathogenic fungi. *Saudi Journal of Biological Sciences*, 30(8), 103719.

Sevim, A., Demir, I., & Demirbağ, Z. (2010a). Molecular characterization and virulence of *Beauveria* spp. from the pine processionary moth, *Thaumetopoea pityocampa* (Lepidoptera: Thaumetopoeidae). *Mycopathologia*, 170(4), 269-277.

Sevim, A., Demir, I., Höfte, M., Humber, R.A., & Demirbağ, Z. (2010b). Isolation and characterization of entomopathogenic fungi from hazelnut-growing region of Turkey. *Biocontrol*, 5, 279-297.

Sönmez, E., Demir, I., Bull, J.C., Butt, T.M., & Demirbağ, Z. (2017). Pine processionary moth (*Thaumetopoea pityocampa*, Lepidoptera: Thaumetopoeidae) larvae are highly susceptible to the entomopathogenic fungi *Metarhizium brunneum* and *Beauveria bassiana*. *Biocontrol Science and Technology*, 27, 1168-1179.

Şahin, F. & Yanar, Y. (2021). Pathogenicity of some local entomopathogenic fungus isolates on the cotton leafworm larvae, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae). *Egyptian Journal of Biological Pest Control*, 31, 150.

Tarasco, E., Fanelli, E., Salvemini, C., El-Khoury, Y., Troccoli, A., Vovlas, A., & De Luca, F. (2023). Entomopathogenic nematodes and their symbiotic bacteria: from genes to field uses. *Frontiers in Insect Science*, *3*, 1195254.

Tarasco, E., Triggiani, O., Zamoum, M., & Oreste, M. (2016). Natural enemies emerged from *Thaumetopoea pityocampa* (Denis & Sciffermüller) (Lepidoptera Notodontidae) pupae in Southern Italy. *Redia-Giornale Di Zoologia, 98*, 103-108.

Topkara, E.F., Yanar, O., Tuncer, C., Özdemir, I.O., & Yıldırım, E. (2022). Efficacy of *Beauveria bassiana* and *Beauveria pseudobassiana* isolates against the pine processionary moth, *Thaumetopoea wilkinsoni* Tams, 1926 (Lepidoptera: Notodontidae). *Egyptian Journal of Biological Pest Control*, 32, 3.

Triggiani, O. & Tarasco, E. (2002). Efficacy and persistence of entomopathogenic nematodes in controlling larval populations of *Thaumetopoea pityocampa* (Lepidoptera: Thaumetopoeidae). *Biocontrol Science and Technology*, 12(6), 747-752.

Uribe-Toril, J., Ruiz-Real, J.L., Haba-Osca, J., & Valenciano, J.D.P. (2019). Forests' first decade: a bibliometric analysis overview. *Forests*, 10, 72.

Wan, H. (2003). Molecular biology of the entomopathogenic fungus *Beauveria bassiana*: insect-cuticle degrading enzymes and development of a new selection marker for fungal transformation. Ph. D Thesis, Combined Faculties for the Natural Sciences and for Mathematics of the Ruperto-Carola University of Heidelberg, Germany.

Yanar, O., Topkara, E.F., & Doruk, T. (2022). Efficacy of Diptera-targeted *Bacillus thuringiensis* var. *israelensis* against the pine processionary moth, *Thaumetopoea wilkinsoni* (Lepidoptera: Notodontidae). *Archives of Phytopathology* and Plant Protection, 55(13), 1530-1541.

Yanar, O., Topkara, E.F., Şahin, F., Yanar, Y., Yanar, D., & Terzi, Y. (2023). Efficacy of *Beauveria bassiana* and *Metarhizium brunneum* isolates against the pine processionary moth, *Thaumetopoea wilkinsoni* Tams, 1926 (Lepidoptera: Notodontidae). *Egyptian Journal of Biological Pest Control*, 33, 32.

Yüksel, H., İpekdal, K., & Toper Kaygın, A. (2019). Comparison between egg batch and egg characteristics of the two pine processionary moth species, *Thaumetopoea wilkinsoni* and *T. pityocampa* in Turkey. *Journal of Bartin Faculty of Forestry*, 21(2), 534-542.