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Biocontrol Potential of Indigenous *Beauveria bassiana* Strain Against *Ips typographus* from Tara National Park Under Varying Temperature Regimes

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ABSTRACT

Entomopathogens are microbes that infect and cause pathogenesis in insects, often leading to the death of individual insects and, in some cases, the collapse of entire populations. More than 750 species of fungi have been documented as insect entomopathogens. Entomopathogenic fungi such as *Beauveria bassiana* play an important role in the biological control of insect populations. Environmental factors such as temperature and humidity significantly influence its growth and efficacy. This study focuses on *B. bassiana* isolated from *Ips typographus* populations in the Serbian Tara National Park. Laboratory experiments conducted at three constant temperatures (10°C, 20°C, and 30°C) demonstrated that fungal growth varied significantly across treatments, with the fastest growth recorded at 20°C. Furthermore, a mortality rate of 79.5% was observed when *B. bassiana* was applied to bark beetles, showing promising potential as a biological control agent under controlled conditions.

Keywords: biopesticides, climate, efficacy, entomopathogenic fungi, spruce bark beetle, *Picea abies*, pest management

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INTRODUCTION

Entomopathogenic fungi play a crucial role in regulating insect populations, sometimes leading to significant mortality and even the collapse of entire populations. More than 750 species of fungi have been identified as insect pathogens (Ramanujam et al. 2014) with prominent representatives from the divisions Ascomycota and Zygomycota, which are known to attack various insect groups (Hajek and Leger 1994, Landa 1994, Shah and Pell 2003, Zimmermann 2007, Pell et al. 2010, Hock 2012, Tabaković-Tošić et al. 2017, Kovač et al. 2021). Among these, the genera *Beauveria*, *Isaria* and *Metarhizium* are important biological control agents (Inglis et al. 2001).

The genus *Beauveria*, which belongs to the Ascomycota, the order Hypocreales and the family Cordycipitaceae, has undergone a comprehensive taxonomic revision thanks to advances in molecular genetics (Neelapu et al. 2009). The genus *Beauveria* currently comprises more than 80 described species according to the Index Fungorum database (Index Fungorum 2026), reflecting the discovery of numerous cryptic taxa in recent decades through advances in molecular phylogenetic analyses. The most well-studied and widely recognized species include *B. bassiana*, *B. amorpha*, *B. asiatica*, *B. australis*, *B. brongniartii*, *B. caledonica*, *B. kipukae*, *B. pseudobassiana*, *B. malawiensis*, *B. sungii*, *B. varroae*, and *B. vermiconia* (Rehner and Buckley 2005). In particular, *Beauveria bassiana* (Bals.-Criv.) Vuill. (1912) is one of the most commonly observed entomopathogens infecting a wide range of insect hosts, and its role in the natural regulation of pest populations is well documented (Wulf 1983, Uribe and Khachatourians 2004, Bugeme et al. 2008, Vega et al. 2008, Mishra et al. 2015, Matek and Pernek 2018, Dara et al. 2019, Sumikarsih et al. 2019, Zhang et al. 2020, Zemek et al. 2021). The sexual stage (teleomorph) *Cordyceps bassiana* Z.Z.Li, C.R.Li, B.Huang & M.Z.Fan 2001 was first described in China (Li et al. 2001).

This species occurs on all continents except Antarctica and plays an important role in regulating insect populations through epizootic outbreaks. It has been frequently reported from pests such as the spruce bark beetle *Ips typographus* (Linnaeus, 1758), although natural epizootics in bark beetle populations have not yet been clearly documented (Neuzilova 1956, Handel et al. 2003, Wegensteiner 2004, Takov et al. 2006, Mudrončeková et al. 2013, Wegensteiner et al. 2015). *Beauveria bassiana* is widely used for the biological control of economically important pests in forestry and agriculture (Shah and Pell 2003, Goble 2015). Successful infection of a host depends on the fungus overcoming the insect's immune defence. In insects, cellular immunity is mediated by hemocytes, among which granulocytes play a key role in defence mechanisms such as phagocytosis and encapsulation. During fungal infection, the number of granulocytes can be significantly reduced, with a marked decline observed three days after the infection (Hajek and St. Leger 1994). As early as 1935, *B. bassiana* was identified as a natural suppressor of the spruce bark beetle (*I. typographus*), which led to further research into its use and effects (Karpiński 1935, Siemaszko 1939, Neuzilova 1956, Kreutz et al. 2000, Landa et al. 2001, Takov et al. 2006, Takov et al. 2011, Draganova et al. 2017, Tabaković-Tošić and Milosavljević 2018).

Abiotic factors, such as temperature, humidity and sunlight, are crucial for the persistence and effectiveness of *B. bassiana* as an entomopathogen in the field (Bugeme et al. 2008, Sumikarsih et al. 2019). They influence the key stages of its life cycle, including spore germination, hyphal penetration and sporulation on the host (Mann and Davis 2020). Previous studies on the influence of temperature have shown that *B. bassiana* can grow at temperatures between 8°C and 37°C, with isolates from forest habitats thriving in cooler conditions, while isolates from agricultural habitats better tolerate higher temperatures (Bidochka 2002). Strains from different geographical regions have different temperature tolerances, with tropical strains tolerating higher temperatures better than those from cooler climates (Fargues and Luz 2000).

The first confirmed occurrence of *B. bassiana* in Serbian *I. typographus* populations was documented in the Tara National Park (Milosavljević et al. 2021). The isolates were identified as *B. bassiana sensu lato* and belong to the Bsh/Bsk group (regions with dry, arid and semi-arid climates), which occurs mainly in Italy and Spain (Milosavljević et al. 2021).

The objectives of this study were to evaluate the colony growth and development of this strain under different temperature regimes, and to evaluate the best growth temperature, as well as to test its biological effectiveness against *I. typhographus* under laboratory conditions.

MATERIALS AND METHODS

Study Area

A three-year study (from 2016 to 2018) on the presence of the entomopathogenic fungus in *I. typographus* populations at 20 selected sites on Mount Tara was carried out using the bait tree method. The sites were located in the eastern part of the Tara National Park (Figure 1), at an elevation of 953–1277 m above sea level, within the spruce forest belonging to the associations *Piceo-Fago-Abietetum* Čolić 1965. subass. *drymetosum* and *Piceo-Fago-Abietetum* Čolić 1965 subass. *typicum* (Gajić et al. 1992, Cvjetićanin and Novaković 2010, Knežević and Košanin 2010).

The climate in which the study was conducted (Mount Tara) can be described as continental with subalpine characteristics (Gajić 1989). It is characterized by fresh to cool summers and cold winters with little annual variation in air temperature. The average annual precipitation is 977.3 mm, with a maximum in July (104.0 mm) and a minimum in January (56.5 mm). The average annual air temperature in the wider Tara area is 7.9°C, with the lowest value in January (–3.5°C) and the highest in August (17.3°C). The amplitude of the annual temperature fluctuation is 20.8°C (Karaklić 2021). The climatic conditions vary in the different parts of the massif. The microclimatic conditions under the bark of the tree, where *I. typographus* develops, may be less suitable for the growth of the fungus. The temperature in the phloem can vary and even rise to 50°C.

Sampling and Identification

The study was conducted annually during the growing season (April–September) over a three-year period. Bait trees were established in two sessions each year: the first group in April and the second group in July. Sampling was carried out by debarking the trees at two time points: in July

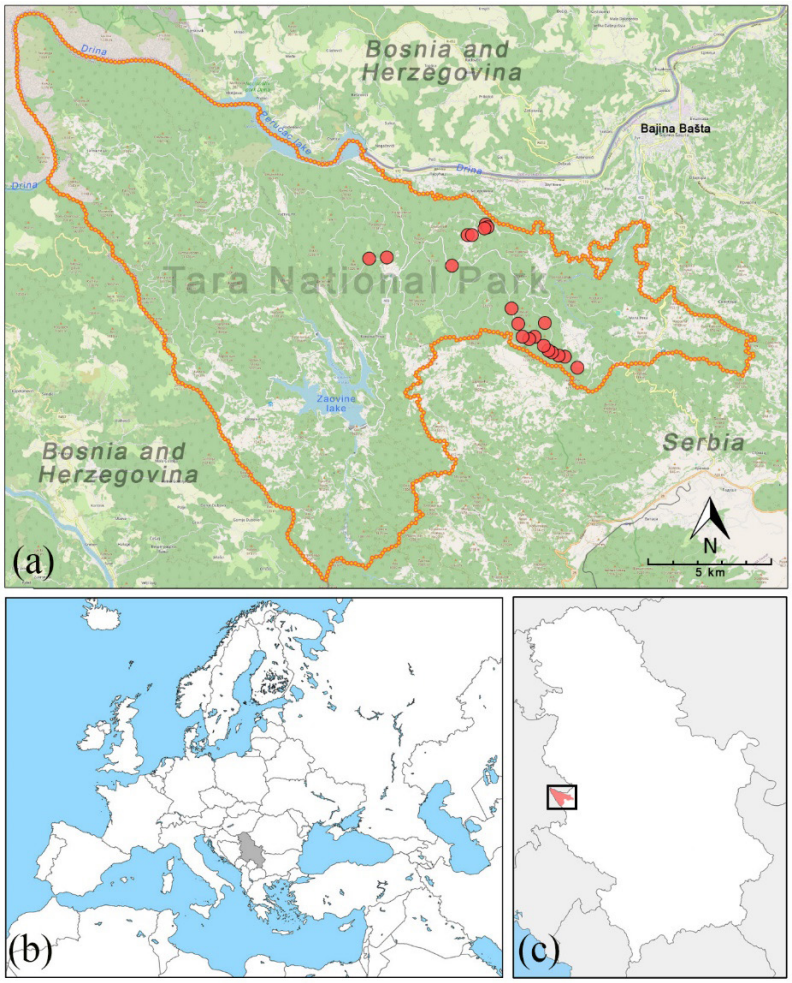

Figure 1 Study area and sampling locations in the Tara National Park, Serbia: v(a) map of the Tara National Park showing the park boundaries (orange line) and sampling sites (red dots), (b) location of the study area within Europe, and (c) location of the Tara National Park within Serbia.

(first group) and at the end of September (second group). Adults showing visible signs of fungal infection were collected from the debarked trees and transported to the laboratory for further analysis. Fungal isolation from field-collected *I. typographus* was performed following the protocol described in Milosavljević et al. (2021). The infected imagoes were incubated to promote fungal outgrowth, surface-sterilized in 70% ethanol, rinsed in sterile distilled water, and plated on PDA (Potato Dextrose Agar) medium. Emerging colonies were subsequently subcultured to obtain pure cultures. From 50 imagoes covered with fungal mycelium collected on Mount Tara, a total of 15 isolates were obtained, of which 10 were successfully purified into pure cultures. All isolates were identified as *B. bassiana* based on macro- and micromorphological characteristics (Koval 1974, Rehner et al. 2011, Beug et al. 2014). Colonies were snow-white, fluffy and powdery, completely covering the insect body. Conidiophores were straight, swollen at the base, with a characteristic zigzag (denticulate) rachis, while conidia formed round clusters measuring 2.1–3 × 1.8–2.2 µm. These procedures were conducted to confirm the presence of the entomopathogenic fungus in field samples. For further experiments (colony growth and insect mortality), a representative isolate from Mount Tara, previously DNA-confirmed based on ITS rDNA sequencing using primers ITS1 and ITS4 and deposited in GenBank under accession number MW774276 (Milosavljević et al. 2021), hereafter referred to as *Beauveria bassiana* isolate MW774276, was selected as a reference strain, while the remaining isolates were identified only morphologically.

The Impact of Temperature on Growth Rate

Physiological characterisation of the fungus was carried out by determining the effect of temperature on mycelial growth. Fungal isolate MW774276 was inoculated in the center of 90 mm diameter Petri dishes containing PDA medium by placing a drop of spore suspension (1×10^6 spores ml^{-1}) using a sterile micropipette. Conidia of the fungal isolate MW774276 were obtained from a developed colony by adding 5 ml of sterile 0.05% Tween® 80 solution and gently removing them with a sterile inoculation loop, followed by filtration through sterile gauze. A fungal suspension with a concentration of 1×10^6 spores ml^{-1} was prepared using a Neubauer chamber (Horňák 2004).

The inoculated plates were incubated at 10°C, 20°C, and 30°C, as described by Booth (1971), in separate thermostats set to the respective temperatures. The Petri dishes were wrapped in aluminum foil and maintained in darkness throughout the incubation period. For each temperature, ten replicates were prepared. Colony growth was measured after 7, 14, and 21 days along two perpendicular diameters, and the mean values were calculated.

Assessment of Biological Efficacy

The biological efficacy of *B. bassiana* isolate MW774276 was tested on adult individuals of *Ips typographus* collected in the Tara National Park area using pheromone traps (Milosavljević et al. 2025). The conidial suspension (1×10^6 spores ml^{-1}) was prepared as described in the previous subsection. The beetles were individually dipped into the fungal suspension for 20 seconds (dip test), while control individuals were dipped into a sterile 0.05% Tween® 80 solution.

After treatment, the beetles were placed in Petri dishes (90 mm diameter) lined with moist filter paper and maintained under controlled laboratory conditions (23 ± 1°C; 16L:8D photoperiod). The filter paper was moistened daily with distilled water to maintain high humidity. Insect mortality and mycosis development were recorded daily over a 15-day period. Only individuals with microscopically confirmed fungal sporulation were considered infected. The experiment was conducted with five replicates, each consisting of 10 insects.

The effectiveness of the treatment was evaluated using Abbott's formula (Abbott 1925). Since mortality in the control group exceeded 5%, the corrected formula was applied (WHO 2016).

$$\text{Corrected mortality} = ((\%\ \text{observed mortality} - \%\ \text{control mortality}))/((100 - \%\ \text{control mortality})) \times 100 \quad (1)$$

Statistical Analysis

Statistical analyses were performed in R (version 4.5.3) using the *nlme* and *emmeans* packages. The parameters of growth intensity of *B. bassiana* colonies depending on environmental temperature conditions were processed with the help of descriptive statistics and analysed using a Linear Mixed-Effects Model (LMM). LMM was used to account for the repeated measures design, with Temperature (10, 20, and 30°C) and Day (7, 14, and 21) treated as fixed factors, including their interaction term. To account for the non-independence of temporal measurements, Plate ID was included as a random effect. Initial diagnostic testing via Levene's test indicated a significant violation of the homogeneity of variance ($p < 0.001$). Consequently, the model was fitted using a variance identity structure (varIdent) to allow for heteroscedasticity across temperature treatments. Normality of residuals and random effects were confirmed through visual inspection of Q-Q plots and histograms. The significance of fixed effects was determined via Type III ANOVA, and where significant interactions were observed, post-hoc pairwise comparisons of means were conducted using the Tukey HSD adjustment. All results were considered significant at $\alpha = 0.05$.

Cumulative mortality data were analyzed using survival analysis centered on the Kaplan–Meier estimator, a non-parametric method used to calculate survival probabilities over time. We determined both the mean and median lethal time (LT_{50}), representing the interval required for 50% of the population to succumb, alongside 95% confidence intervals (CI). Differences in survival distributions between the *B. bassiana* treatment and the control were statistically evaluated using the log-rank (Mantel-Cox) test ($\alpha = 0.05$). Individuals that survived until the end of the 15-day observation period were treated as right-censored observations. Statistical analyses and high-resolution survival curves were performed in R using the *survival* and *survminer* packages.

RESULTS

Mycelial Growth and Thermal Preference

Over the course of three years of research, we collected 50 adult individuals of *I. typographus* that showed clear symptoms of infection with the entomopathogenic fungus *B. bassiana* (Figure 2). During the first inspection (July) in 2016, we discovered three infected beetles at two locations. The following year, in 2017, we found seven beetles at five sites

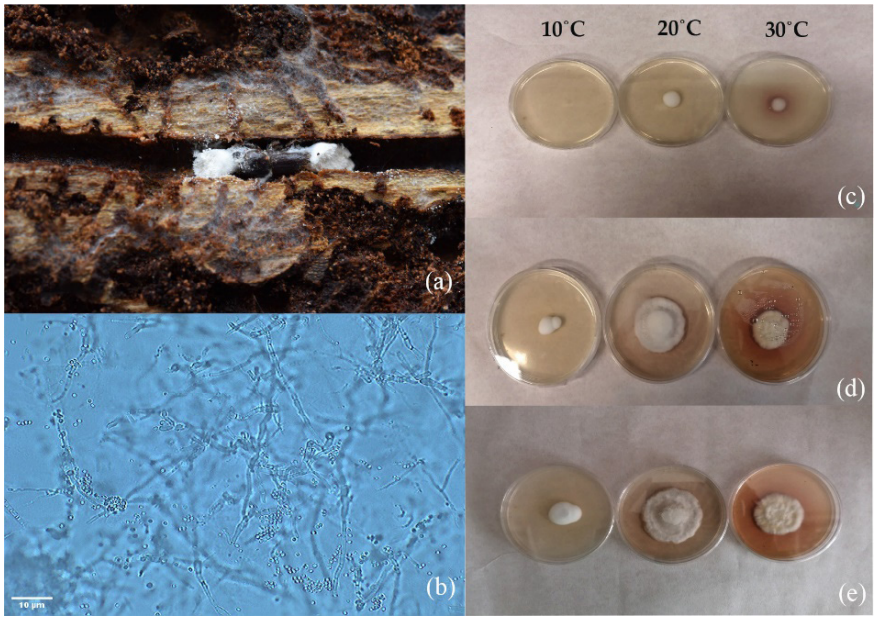

Figure 2 The process of fungal isolation: (a) infected adult with white mycelium, (b) identification under the microscope magnification 40X, and the effect of three constant temperature conditions (10, 20, and 30°C) on colony growth of Beauveria bassiana isolate MW774276 after (c) seven, (d) fourteen, and (e) twenty-one days.

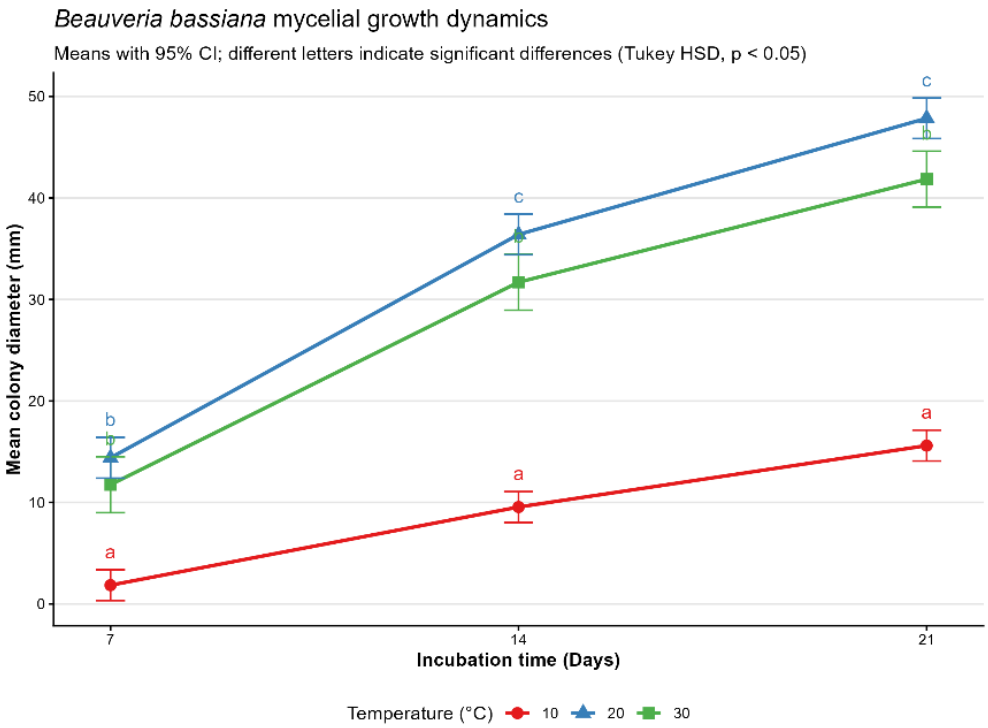

Figure 3 Thermal growth dynamics of *Beauveria bassiana*. Mean mycelial colony diameter (mm) of *B. bassiana* isolates cultured on PDA medium at three constant temperatures (10°C, 20°C, and 30°C) over a 21-day incubation period. Data points represent Estimated Marginal Means (EMMs) derived from a Linear Mixed-Effects Model (LMM) to account for repeated measures and heteroscedasticity. Error bars indicate the 95% confidence intervals (n = 10). Different lowercase letters at each time point denote significant differences between temperature treatments according to Tukey's HSD post-hoc test (α = 0.05).

during the first inspection (July) and 31 beetles at seven sites during the second inspection (September). In 2018, we found one beetle at one site during the first inspection and eight beetles at three sites during the second inspection.

Temperature is one of the key environmental factors influencing the vegetative growth of fungi. In our *in vitro* experiment, colony growth of *B. bassiana* varied significantly with temperature, with the highest growth observed at 20°C (Figure 3).

The Linear Mixed-Effects Model (LMM) confirmed that both temperature ($F_{2,27}$ = 598.98, p < 0.0001) and incubation time ($F_{2,54}$ = 917.77, p < 0.0001) had a highly significant effect on mycelial expansion. In addition, a significant interaction between temperature and incubation time was observed ($F_{4,54}$ = 91.39, p < 0.0001), indicating that the growth rate of *B. bassiana* varied depending on the thermal regime over time. The fixed effects of the LMM are summarised in Table 1.

Table 1 Results of the Type III Analysis of Variance (ANOVA) for the linear mixed-effects model evaluating the effects of temperature (10, 20, 30°C), incubation time (7, 14, 21 days), and their interaction on the mycelial growth of *Beauveria bassiana*.

Source of Variation	numDF	denDF	F-value	p-value
(Intercept)	1	54	4248.90	< 0.0001
Temperature	2	27	598.98	< 0.0001
Day	3	54	917.77	< 0.0001
Temperature × Day	4	54	91.39	< 0.0001

The isolate exhibited a clear thermal optimum at 20°C, reaching a maximum mean diameter of 47.85 mm by Day 21 (Figure 3). While growth at 20°C and 30°C was statistically comparable at the first measurement (Day 7; difference = 2.65 mm; p = 0.136), the treatments significantly diverged by Day 14 (p = 0.004) and Day 21 (p = 0.0003), where the 30°C group reached a lower final mean of 41.85 mm. The lowest growth was recorded at 10°C, which remained significantly lower than all other treatments at every time point (p < 0.0001), concluding with a mean diameter of 15.60 mm. Furthermore, higher thermal stress at 30°C was associated with increased biological variation; the within-group variance at 30°C was 2.24 times higher than that observed at 10°C, as specifically accounted for by the variance-weighted model structure.

A detailed summary of the estimated marginal means (EMMs), associated standard errors, and 95% confidence intervals for all treatment combinations is presented in Table 2. These values quantify the significant divergence in mycelial expansion observed between the three thermal regimes. Notably, the standard error increased progressively with temperature (SE_{10} = 0.60 vs. SE_{30} = 1.08), reflecting the higher phenotypic plasticity and physiological instability of the isolate when cultured at its upper thermal limit of 30°C. The statistical groupings within each time point further confirm the transition of 20°C from a shared growth rate with 30°C at Day 7 to a distinct, superior thermal optimum by the conclusion of the 21-day study.

Table 2 Estimated marginal means (EMM) of *Beauveria bassiana* colony diameter (mm) at different temperatures and incubation periods.

Day	Temperature (°C)	Mean Diameter (mm)	Standard Error (SE)
7	10	1.85	0.60
	20	14.40	0.79
	30	11.75	1.08
14	10	9.55	0.60
	20	36.40	0.79
	30	31.70	1.08
21	10	15.60	0.60
	20	47.85	0.79
	30	41.85	1.08

Biological Efficacy of *B. bassiana* Isolate from the Tara National Park

The biological effectiveness of a single *B. bassiana* isolate (MW774276) was tested on adult *Ips typographus* individuals collected from pheromone traps in the Tara National Park area. The Kaplan–Meier survival analysis was used to estimate the survival probability of the infected insects over time, as shown in Figure 4. The log-rank (Mantel-Cox) test indicated a highly significant difference in survival distributions between the fungal treatment and the control group ($\chi^2 = 63.1$, df = 1, $p < 0.0001$).

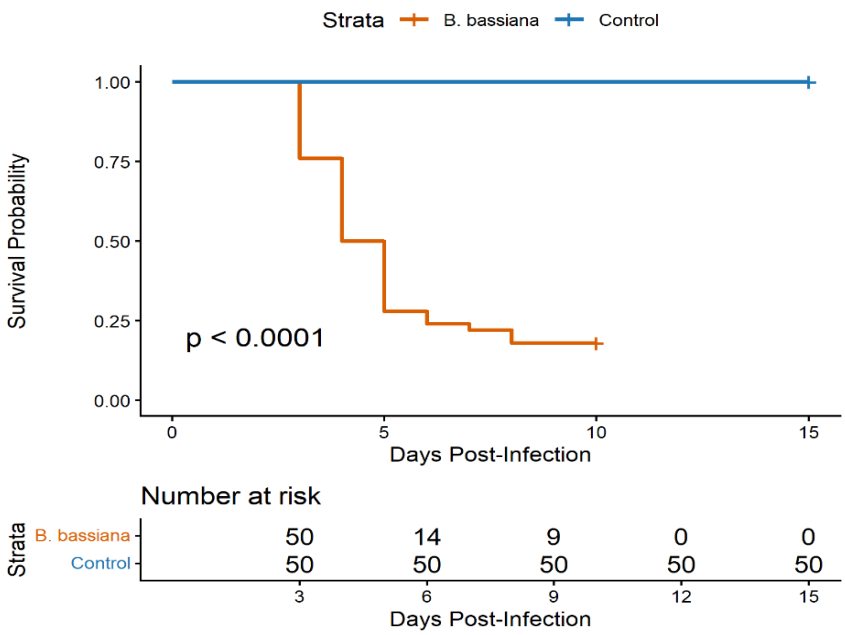

Figure 4 Kaplan–Meier survival curves of adult individuals of *Ips typhographus* in the control group and in the group infected with *Beauveria bassiana*. The red dashed lines represent 95% confidence intervals. The horizontal red line indicates a survival rate of 50%. Cross marks represent censored observations.

In the treatment group, pathogenicity was rapid, with the first mortality events recorded on day 3 post-inoculation. The median lethal time (LT_{50}) was reached at 4.0 days (95% CI: 4.0–5.0 days). The highest rate of attrition occurred between days 3 and 5, during which 72% of the treated population succumbed to mycosis. Following day 5, mortality slowed, with only an additional 10% of the population dying by the end of the experiment. By the conclusion of the bioassay, cumulative mortality in the *B. bassiana* group reached 82%, whereas the control group maintained 100% survival throughout the 15-day period (with all individuals right-censored at the end of the study). Based on Abbott's corrected formula, the overall biological efficacy of the isolate was estimated at 79.5%.

DISCUSSION

Temperature plays a decisive role in the growth and infection process of *B. bassiana*. It is typically a mesophilic fungus with a reported optimal temperature range between 20°C and 30°C (Fargues et al. 1997). Temperatures below this range significantly decelerate the development of mycosis, while temperatures above 30°C can inhibit mycelial growth, which typically ceases entirely above 37°C (Hywel-Jones and Gillespie 1990). However, microclimatic conditions within the host's habitat can be extreme; for instance, temperatures within the phloem of spruce trees have been recorded as high as 50°C during intense solar radiation (Annila 1969).

The thermal profile of the *B. bassiana* isolate reveals a distinct

preference for temperate conditions, with an optimal growth temperature of 20°C. The tested isolate MW774276 from Mount Tara shows comparable growth at 20°C to specific strains from France and Morocco also isolated from Curculionidae species (Fargues et al. 1997). While many entomopathogenic fungi exhibit a broad thermal breadth, the significant divergence in growth rates between 20°C and 30°C by the second week of incubation suggests that 30°C lies near the upper threshold of this isolate's metabolic efficiency. In this context, the increased variability observed at 30°C may indicate that this temperature approaches the upper limit of optimal growth for this isolate. However, further studies are needed to confirm whether this reflects physiological stress or reduced developmental stability.

At the point of origin in the Tara National Park, *Ips typographus* typically occupies sub-cortical environments that rarely sustain prolonged temperatures above 25°C. The observed growth pattern indicates a preference for moderate temperature conditions, with optimal growth at 20°C, while growth at lower temperatures (10°C) remained limited. Such adaptation makes this isolate a prime candidate for localized biocontrol programs targeting bark beetle outbreaks in high-value coniferous forests, where it may offer a competitive advantage over commercial biopesticide strains, which are often derived from isolates adapted to warmer, agricultural environments, particularly during the cooler periods of the beetle's active season.

The biological efficacy of the tested *B. bassiana* isolate MW774276 (79.5% corrected mortality) demonstrates its high virulence against adult *I. typographus*. This level of effectiveness aligns with the upper tier of results reported in the literature for entomopathogenic fungi targeting bark beetles. Previous studies investigating *B. bassiana* under various laboratory and semi-controlled conditions have yielded highly variable results, with efficacy rates ranging from negligible (18%) to total population suppression (100%) (Vaupel and Zimmermann 1996, Jakuš and Blaženec 2011, Grodzki and Kosibowicz 2015, Barta et al. 2020). Consistent levels of pathogenicity have been reported in recent laboratory studies on bark beetles, including work on *Ips duplicatus* Sahlberg (Coleoptera: Curculionidae, Scolytinae), where selected *B. bassiana* isolates caused high mortality, often exceeding 80–90%, depending on isolate virulence and experimental conditions (Vakula et al. 2025).

The rapid onset of mortality observed in our study, characterised by a median lethal time (LT_{50}) of 4.0 days, is particularly noteworthy. This swift pathogenic action is critical for effective bark beetle management, as it reduces the window of time available for the beetles to bore into the phloem and initiate brood production. Our findings are consistent with the laboratory dip-tests conducted by Draganova et al. (2017) and Mudrončeková et al. (2013), who also observed high susceptibility in *I. typographus* when exposed to concentrated conidial suspensions. Comparable LT_{50} values (3–5 days) have been reported in similar laboratory bioassays, indicating rapid host mortality following exposure to high conidial concentrations (Mudrončeková et al. 2013, Draganova et al. 2017).

The stark contrast between the high efficacy observed in laboratory bioassays and the lower performance (18–30%) often reported in field trials (e.g., Grodzki and Kosibowicz 2015) is likely due to environmental degradation of the conidia. In the forest environment, solar radiation and fluctuating humidity levels can significantly reduce spore viability. Consistent discrepancies between laboratory and field efficacy have been widely documented for *I. typographus*, where mortality rates are substantially reduced under natural conditions due to abiotic stress factors such as temperature variability and UV exposure (Jakuš and Blaženec 2011, Fora et al. 2022).

However, the high intrinsic virulence of the Tara National Park isolate MW774276, together with the evidence of horizontal transmission reported in previous studies (Kreutz et al. 2004), suggests that this isolate may represent a promising candidate for further investigation and development. Utilising an autochthonous isolate adapted to the temperate-cool microclimates of the Dinaric Alps may offer a competitive advantage over commercial products derived from agricultural strains adapted to warmer climatic conditions.

Our results indicate that *B. bassiana* is a promising agent against bark beetle pest species. A major advantage of this fungus is its efficacy as a biological insecticide (mycoinsecticide) which is environmentally friendly and has been approved for use in agriculture and forestry by the European Commission (EFSA 2020). This fungus is one of five biological pesticides that have been classified as safe for humans and the environment (Chiew et al. 2022). Further research will focus on the interaction between natural strains of *B. bassiana* and its bark beetle hosts along with outdoor experiments involving the strain from the Tara National Park and the *I. typographus* population.

CONCLUSIONS

Our research confirms the presence and activity of *Beauveria bassiana* as an entomopathogenic fungus in *Ips typographus* populations in the Tara National Park. Over the course of three years, 50 infected beetle individuals were identified, and the laboratory experiments showed that the fastest fungal growth occurred at 20°C compared to 10°C and 30°C. Although the selected strain of *B. bassiana* showed a biological efficiency of 79.5% under controlled laboratory conditions, the influence of environmental factors such as temperature and humidity on its pathogenicity remains to be investigated. While previous studies have reported variable efficacy in different geographical regions, our isolate showed strong potential as a biological control agent. However, natural epizootics caused by *B. bassiana* in bark beetle populations have not been documented, and further investigation into horizontal transmission of the fungus in natural populations is warranted.

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