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**Original article**

# Evaluation of *Beauveria bassiana* isolates from *Hypsipyla robusta* and *Zeuzera coffeae* for biological control of borer pests in *Chukrasia tabularis*

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**Abstract**

Indian mahogany (*Chukrasia tabularis*) is vulnerable to attack by insect borers. In Vietnam, serious damage is caused by the mahogany shoot borer (*Hypsipyla robusta*) and the coffee borer (*Zeuzera coffeae*). Biological control options are desirable for the future management of these pest species. This study aimed to evaluate the pathogenicity of seven wild indigenous *Beauveria bassiana* (Bals.-Criv.) Vuill, 1912 (Hypocreales: Cordycipitaceae) isolates obtained from mahogany and coffee borer larvae against the target pests. The fungi were identified by phylogenetic analysis of the Internal Transcribed Spacer, Large Subunit, and Translation Elongation Factor 1-alpha gene regions using sequence comparison. In laboratory tests, suspensions of *B. bassiana* conidia induced 71–89% mortality of *H. robusta* and *Z. coffeae* larvae after nine days. In nursery experiments, conidia were sprayed onto one-year-old *C. tabularis* seedlings infested with 1$^{st}$ instar larvae of *H. robusta* or *Z. coffeae* to confirm the pathogenicity of seven isolates. Isolates TP13, TP17, TP24, and TP46 reduced damage from *H. robusta* by 72–76% ($P < 0.001$); and TP14, TP19, and TP44 reduced damage from *Z. coffeae* by 74–77% ($P < 0.001$). In experiments in *C. tabularis* plantations, these *B. bassiana* isolates significantly ($P < 0.001$) reduced the incidence of borer damage from *H. robusta* and *Z. coffeae* by 71–73% and 72–75%, respectively. These results suggest that locally adapted isolates of *B. bassiana* can be developed as a biocontrol tool for future integrated pest management of *C. tabularis* borer pests. Further research is required to identify field protocols for commercial application.

**Keywords:** *Beauveria bassiana*, entomopathogenic fungi, insect pest, mahogany shoot borer, coffee borer

## Introduction

Indian mahogany *Chukrasia tabularis* A. Juss, 1830 (Sapindales: Meliaceae) is a tropical forest tree native to south, southeast, and east Asia (Ho and Noshiro 1995), and is cultivated in many countries such as Australia, China, Myanmar, South Africa, Sri Lanka and Vietnam for its high-quality wood (Pinyopusarerk and Kalinganire 2003). This tree species has high economic value, with sawn boards selling for approximately US$750–800/m$^3$ (Kien and Harwood 2017). Therefore, it is a priority species for reforestation in Vietnam (Chi 2022), where about 35,000 hectares have been established (Thu *et al.* 2021). However, the productivity of these plantations is threatened by the mahogany shoot borer *Hypsipyla robusta* Moore, 1866 (Lepidoptera: Pyralidae) with damage incidence of 25–50% (Chi *et al.* 2023; Chi 2024); and the coffee borer *Zeuzera coffeae* Nietner, 1861 (Lepidoptera: Cossidae) with damage incidence of 15–16% (Phuong *et al.* 2025). Larvae of *H. robusta* damage the shoots of 1 to 3 year-old trees (Do 2003) while *Z. coffeae* can damage stems of a wider range of tree ages (Phuong *et al.* 2025). The mahogany shoot borer has three life cycles/year, the coffee borer has five life cycles/year, and the period for control is from April to June (Do 2003; Phuong *et al.* 2025). Recently, dead larvae of these borer pests were observed covered in unidentified fungal mycelium in mahogany plantations (Chi 2024; Phuong *et al.* 2025).

Entomopathogenic fungi (EPFs) invade and kill susceptible insect hosts (Ma *et al.* 2024). The primary mode of infection is hyphal penetration of the cuticle (Mannino *et al.* 2019). EPFs are found mainly in the order Hypocreales, and less commonly in the Entomophthorales, Neozygitales and Onygenales (Boomsma *et al.* 2014). Many EPFs have been investigated and some isolates of *Beauveria* and *Metarhizium* have been promoted as commercial biological control agents (BCAs) (Toan *et al.* 2024; Hurley *et al.* 2025).

EPFs can be isolated from naturally infected pests (Abdo *et al.* 2008; Chen *et al.* 2013; Robène-Soustrade *et al.* 2015; Sutanto *et al.* 2022; Sutanto *et al.* 2023) or soil (Imoulan *et al.* 2016; Bustamante *et al.* 2019; Chen *et al.* 2019). Strains isolated from a particular pest may be more

effective in the control of that pest than EPFs isolated from other pest species (Abdo *et al.* 2008). Examples include *Isaria javanica* Samson & Hywel-jone, 2005 (Hypocreales: Cordycipitaceae) from the Asian citrus psyllid *Diaphorina citri* Kuwayama, 1908 (Hemiptera: Psyllidae) (Qasim *et al.* 2018), *B. bassiana* from the Asian hornet *Vespa velutina* Lepeletier, 1836 (Hymenoptera: Vespidae) (Poidatz *et al.* 2019), and *B. bassiana* from the ghost-moth *Endoclita salvazi* Tindale, 1958 (Lepidoptera: Hepialidae) (Chi and Anh 2025).

BCAs have been used for many decades in the management of forest pests (Goundar *et al.* 2025; Mahanta *et al.* 2025; Wang and Yang 2025). The adoption of biological control for pest management is necessary to meet the strict requirements of sustainable forestry and organic agricultural development (Zimmermann 2007; Mascarin and Jaronski 2016). Consequently, in Vietnam, the management of pests in forest plantations is starting to embrace integrated pest management using BCAs (Quang *et al.* 2021; Chi *et al.* 2023). The presence of wild EPFs infesting borers in *C. tabularis* plantations in Vietnam provides an opportunity to identify and select effective BCAs for these damaging pests. The aims of this study were to identify EPFs isolated from naturally infested *H. robusta* and *Z. coffeae* larvae, and to assess their pathogenicity against these pests in laboratory, nursery, and field experiments.

## Materials and Methods

### Sampling, isolation, and culture traits

Naturally infected larvae and pupae of *H. robusta* and *Z. coffeae* (Fig. 1) were collected from *C. tabularis* plantations in central Vietnam in August 2024. Twenty specimens of each pest were taken that were covered with white fungal mycelium or obviously discolored (brown to dark gray). Specimens were stored separately in 2 ml sterile plastic vials. In a laboratory in Hanoi, each specimen was scraped with a sterile knife, and conidia were transferred to potato dextrose agar (PDA) in 9 cm diameter Petri dishes (Beijing Labgic Technology Co., Ltd., China) and incubated at 25–26ºC in the

dark. After 5–6 days, when cultures had grown 0.8–1.0 cm in diameter, hyphal tips at the edge of colonies were sub-cultured and grown under the same conditions. Forty isolates with white fungal mycelium were obtained, one per specimen. The isolates are curated in the Forest Protection Research Centre of the Vietnamese Academy of Forest Sciences in Hanoi (21.072538, 105.776532). In a preliminary screening trial, all isolates were assessed for their entomopathogenicity in a laboratory experiment (data not presented), and the seven most pathogenic isolates (Table 1) were selected for molecular identification, and biocontrol potential of *H. robusta* and *Z. coffeae* in this study.

**Sequence and phylogenetic analysis**

The accurate classification of *Beauveria* species requires the integration of multiple genetic markers. The internal transcribed spacer (ITS) region facilitates preliminary species identification, whereas the translation elongation factor 1-alpha (EF1-α) and the large subunit nuclear ribosomal DNA (LSU) provide higher resolution for phylogenetic analyses. To identify fungal isolates, DNA was extracted and amplified using DreamTaq PCR Master Mix (2X) (Thermo Scientific, USA) as described previously (Chi and Anh 2025). Reactions were performed using a C1000 Touch™ thermal cycler (Bio-Rad, USA). PCR amplicons were purified using either a silica-based purification kit or AMPure beads according to the manufacturers' instructions. Amplicons were sequenced via Sanger sequencing by First BASE Laboratories Sdn Bhd (Selangor, Malaysia). Consensus sequences were generated from forward and reverse reads using BioEdit v7.1.9. Multiple sequence alignments were carried out using MAFFT v7, and a phylogenetic tree was produced in MEGA v7.0 (Kumar *et al.* 2016) using the Maximum Likelihood (ML) method with 1000 bootstrap replicates (Felsenstein 1985; Kumar *et al.* 2016).

**Inoculation of *Hypsipyla robusta* and *Zeuzera coffeae* with *Beauveria bassiana* in the laboratory**

*H. robusta* and *Z. coffeae* larvae have five and six instars, respectively. *H. robusta* was reared in a laboratory on artificial food developed by Chi *et al.* (2021). *Z. coffeae* was reared on artificial food developed by Thanh *et al.* (2024) and modified by the addition of 24% by weight of fresh plant powder from *C. tabularis* branches. Biological control is generally recommended when larvae are in their early growth stages; therefore, 2nd instar larvae for laboratory experiments were used.

Laboratory bioassays were set up to determine the efficacy of 7 *B. bassiana* isolates (Table 1) infecting the 2nd larval instars of *H. robusta* and *Z. coffeae* following Hussein *et al.* (2012) as modified by Chi and Anh (2025). The pests were single populations collected in Nghe An province. Conidia from 14-day-old fungal cultures were suspended in sterile distilled water + 1% Tween 80. The spore solutions were examined under a microscope using a hemocytometer and diluted to about $2 \times 10^6$ conidia/ml. A commercial *B. bassiana* product (Muskardin, CPC Co., Ltd., Vietnam, recommended dosage 3.75 g/l) was the positive control treatment. Sterile water + 1% Tween 80 was the negative control. Two separate experiments (one for each pest) were carried out, each comprising five blocks, nine treatments, and five replicates. There were seven larvae in each replicate, and a total of 315 larvae/pest/experiment. Larvae were dipped for 5 s in each treatment, then they were transferred to sterilized plastic containers (190 $cm^3$) containing 200 g of artificial diet [Chi *et al.* (2021) for *H. robusta*; Phuong *et al.* (2025) for *Z. coffeae*], and incubated at 25–26ºC. The number of infected larvae (discolored, engulfed with fungal mycelium) was recorded at 5, 7, and 9 days after inoculation.

**Inoculation of *Chukrasia tabularis* infested with *Hypsipyla robusta* and *Zeuzera coffeae* in the nursery**

The seven EPF isolates were assessed for their ability to reduce *H. robusta* and *Z. coffeae* damage to 1-year-old *C. tabularis* seedlings. There were two experiments, one for each pest. A randomized block design was used for each experiment, comprising five blocks, and nine treatments (7 EPFs, Muskardin and water, rates as in the laboratory experiment above, all with 1% Tween 80). There were five replicate plants/treatment, and a total of 315 plants/pest/experiment.

Open-pollinated seeds were collected from a single mother tree in a plantation in Nghe An province. The seeds were sown in containers (diameter 6 cm, height 12 cm) containing six parts ferrasol collected in Nghe An province and four parts composted elephant grass (*Pennisetum purpureum*). Plants were grown in netted nursery frames to exclude insect pests. They were watered daily, and inorganic complete fertilizer was applied quarterly. From one seedling batch, 630 uniform 1-year-old seedlings were selected for this study with a height of 90–95 cm, and a basal stem diameter of 1.2–1.3 cm.

Adult pests were produced in insect rearing cages using the artificial foods mentioned earlier. Eggs of *H. robusta* and *Z. coffeae* were taken within 48 hours of being laid. Three *H. robusta* eggs per seedling were placed near the terminal bud, and for *Z. coffeae*, the three eggs were placed on the stem 10–12 cm above the container surface. After 10 days for *H. robusta* or 20 days for *Z. coffeae*, plants with 1$^{st}$ instar larvae were selected for the experiments.

The treatments were applied in May 2025 with an electric sprayer (Mitsuyama TL-20SN, Binh Minh Co., Ltd., Vietnam) that delivered about 50 ml per plant. Treatments were reapplied after 7 days to ensure that all larvae were treated. The plants were checked for pest webs and external frass 15 days after the first treatments were applied. The stems were dissected to facilitate measurement of tunnel length and to count the number of infected larvae. In each experiment, damage was ranked into five categories: 0 = no tunnels; 1 = tunnel <5 cm; 2 = tunnels 5 to <10 cm; 3 = tunnels 10 to <15 cm; and 4 = tunnels >15 cm. As the tunnels lengthen, the foliage begins to senesce and wilt,

and severely damaged seedlings have broken stems.

**Inoculation of *Chukrasia tabularis* infested with *H. robusta* and *Z. coffeae* in the field**

To assess the biological control potential of the seven EPF isolates, two field experiments were established in 16-month-old *C. tabularis* plantations in Nghe An province. Trees were selected that were attacked only by *H. robusta* (P% = 22.1) or by *Z. coffeae* (P% = 19.7) using the following criteria. Trees damaged by *H. robusta* had active holes, tunnels, and frass on the terminal shoot and branches, and some wilted shoots were present. Trees damaged by *Z. coffeae* had active holes on the bark surface at a height of 15–25 cm above the ground with frass around the trunk base. The field sites for each pest species were about 3 km apart.

A randomized complete block design was used for each pest experiment, comprising five blocks, nine treatment plots, and five replicate trees. The 500 $m^2$ plots (each about 50 trees) were separated by 15–20 m buffers. The treatments were the same as applied in the nursery experiments; hence, the EPF treatments contained approximately $2 \times 10^6$ conidia/ml in sterile water + 1% Tween 80. Just prior to setting up the experiments, selected damaged trees were dissected to confirm the presence of larvae, most of which were in the range of the $2^{nd}$–$4^{th}$ instars. Treatments were applied in June 2025 with an electric sprayer that delivered about 200 ml/tree over the tree base, trunk, main branches, and foliage. The treatments were reapplied after 7 days to ensure that all larvae were treated.

The trees were assessed immediately before the treatments were applied and after 15 days. Damage was assessed in five categories: 0 = no active holes in shoots, branches or bole; 1 = one active borer hole in shoots, branches or bole; 2 = two active borer holes in shoots, branches or bole; 3 = three active borer holes in shoots, branches or bole, 4 = more than three active borer holes in shoots, branches or bole.

**Data analysis**

The infected rate (*D%*) was calculated using equation 1 (Chi *et al.* 2025):

$$D\% = (n/N) \times 100 \quad (1)$$

where: $n$ is the number of infected larvae; $N$ is the total number of larvae assessed.

Based on the results of damage classification, damage incidence was calculated using equation 2 (Phuong *et al.* 2025):

$$P\% = (n/N) \times 100 \quad (2)$$

where: $n$ is the number of trees attacked by the pests; $N$ is the total number of plants assessed.

The damage severity (*R%*) was calculated using equation 3 (Phuong *et al.* 2025):

$$R\% = ((\Sigma n_i \times v_i)/(N \times V)) \times 100 \quad (3)$$

where: $n_i$ is the number of trees infected at damage index $i$; $v_i$ is the damage index at level $i$; and $N$ is the total number of plants assessed; and $V$ is the maximum damage index used for classification.

The corrected efficacy ($E$) of each treatment in the nursery trials was calculated using equation 4 (Abbott 1925):

$$E = ((C_a - T_a)/C_a) \times 100 \quad (4)$$

where: $C_a$ is the damage severity of the control treatment at the end of the trial; and $T_a$ is the damage severity of the biocontrol agent at the end of the trial.

The corrected efficacy ($E$) of each treatment in the field trials was calculated with equation 5 (Henderson and Tilton 1955):

$$E = (1 - (C_b \times T_a)/(C_a \times T_b)) \times 100 \quad (5)$$

where: $C_b$ is the damage severity of the control treatment at the start of the trial; $T_b$ is the damage severity of the biocontrol agent at the start of the trial; $C_a$ is the damage severity of the control treatment at the end of the trial; and $T_a$ is the damage severity of the biocontrol agent at the end of the trial.

The correlation coefficient (r) of each treatment group in the trials was calculated from equation 6:

r = CORREL(array1, array2) (6)

where array1 and array2 are the data of each treatment in the trials, respectively.

The distribution of treatment, negative and positive control data of each pest in laboratory, nursery, and field experiments was assessed with the Kolmogorov-Smirnov Test, and the data for damage index and damage incidence were log-transformed before analysis (GenStat Release 12.1). Analysis of variance (ANOVA) was used to test for the significant effect of blocks (laboratory, nursery, and field experiments) and treatments, followed by Tukey's Multiple Range Test for comparisons of means. Correlation coefficients were calculated using Microsoft Excel.

## Results

### Phylogenetic analysis

The ITS, LSU, and TEF1-α gene sequences of the seven EPFs isolates closely matched sequences for eight taxa in GenBank (NCBI) using *Cordyceps militaris* as the outlier (Table 2). The consensus tree of the combined genes showed that all EPF isolates clustered with *B. bassiana* (Fig. 2) with >99.6% sequence similarities. The isolates presented here fell into two sub-clades: firstly, TP17 with *B. bassiana* ARSEF 2597 in the EPF collection of USDA-ARS (Valero-Jiménez *et al.* 2016) and *B. bassiana* B2 isolated from *Endoclita vietnamensis* larvae (Chi and Anh 2025); and secondly, TP13, TP14, TP19, TP24, TP44, and TP46 clustered with *B. bassiana* ATCC 74040 isolated from the commercial product naturalis® (Moro *et al.* 2025) and *B. bassiana* B3 isolated from *E. salvazi* larvae (Chi and Anh 2025).

**Laboratory experiments**

There were significant differences between the fungal treatments and the water control at 5, 7, and 9 days after inoculation. However, there were no block effects (P >0.05) in the experiments. The percentage of infected larvae in the seven EPF treatments and positive control (Muskardin) increased gradually from 5 to 9 days after treatments were applied. In addition, larvae in the water control had no symptoms of parasitism (Table 3).

For *H. robusta*, the percentage of infected larvae in the EPF treatments at 5, 7, and 9 days ranged from 40.0–54.3%, 51.4–68.6%, and 71.4–88.6%, respectively, while values for the positive control (Muskardin) were 37.1, 48.6, and 71.4%, respectively. In particular, isolates TP13, TP17, TP24, and TP46, that were originally isolated from *H. robusta*, gave the maximum infected larval rate of over 82% at 9 days after treatment (Table 3).

Turning to the *Z. coffeae* experiment, the percentage of infected larvae was quite similar to *H. robusta*, with ranges of 45.7–57.1%, 54.3–74.3%, and 71.4–84.7%, respectively, at 5, 7, and 9 days. The EPF isolates obtained from *Z. coffeae* (TP14, TP19, and TP44) were the most infectious (Table 3).

**Nursery trials**

There were significant differences (P <0.001) in infection rate and damage severity between the EPF treatments and the water control in the two nursery experiments at 15 days after spraying (Table 4). There were no significant block effects (P >0.05). The percentage of infected *H. robusta* larvae ranged from 68.6–82.9% in the EPF treatments, and it was 68.6% in the Muskardin treatment. Similar to the laboratory experiments, *B. bassiana* isolates from *H. robusta* were most effective with TP13 (Fig. 3a), TP17 (Fig. 3b), TP24, and TP46 giving infected larval rates ≥80%. The damage severity (R%) of the water control (Fig. 3d) was high (85.3%), and the R% of the seven EPF treatments and Muskardin were reduced to 20.2–31.8%. Furthermore, the damage

inhibition effect (E) of the seven EPF treatments and Muskardin (Fig. 3c) ranged from 62.7 to 76.3%. The TP13, TP17, TP24, and TP46 treatments had E values over 72% (Table 4).

With *Z. coffeae*, the percentage of infected larvae ranged from 65.7 to 80.0% in the EPF and Muskardin treatments (Fig. 4c). Isolates TP14 and TP44 showed the strongest pathogenicity in this trial, with 80% infected larvae. The R% of EPFs and Muskardin treatments ranged from 18.5 to 31.8%, which was much lower than the water control (R% = 81.3%, Fig. 4d). The E values of the fungal treatments ranged from 60.9 to 77.3%. The three *B. bassiana* strains isolated from *Z. coffeae* [TP14 (Fig. 4a), TP19 (Fig. 4b), and TP44] had control efficiencies greater than 73% (Table 4).

**Field bioassays**

There were no significant differences between blocks in the two field experiments (P = 0.285 and 0.452 for P% and R%, respectively, in the *H. robusta* trial; and P = 0.268 and 0.366 for P% and R%, respectively, in the *Z. coffeae* trial). In both experiments, damage incidence (P%) and damage severity (R%) did not differ between the plots (P = 0.998 and 1.000 for P% and R%, respectively, in the *H. robusta* trial and P = 0.796 and 0.962 for P% and R%, respectively, in the *Z. coffeae* trial) when assessed at the experimental setup, indicating homogeneity among plots. At 15 days after treatment application, there were significant differences (P <0.001) for P% and R% between the treatments in both field trials (Tables 5, 6).

In the *H. robusta* experiment, application of EPFs and Muskardin treatments resulted in P% of 19.2–20.5% and R% of 11.0–14.6% at 15 days; values for the water control (Fig. 3h) were 51.2 and 41.2%, respectively. The damage inhibition effect of the fungal treatments ranged from 64.1 to 72.7%. Notably, *B. bassiana* isolates from *H. robusta*, TP13, TP17, TP24 (Fig. 3e), and TP46 (Fig. 3f), had over 70% control efficiency, higher than Muskardin (Fig. 3g) with 64.1% (Table 5).

For *Z. coffeae*, P% and R% of the EPF isolates and Muskardin (Fig. 4g) were 16.5–18.1% and 10.0–12.6%, respectively, while in the water control (Fig. 4h) P% was 45.1% and R% was 36.4%.

The control efficacy (E) of *B. bassiana* isolates ranged from 69.2 to 74.7%, which was greater than 66.1% in the Muskardin treatment. In particular, *B. bassiana* isolates TP14, TP19 and TP44 (Fig. 4e) had high control efficiency, over 73% (Table 6).

## Discussion

This study showed that local Vietnamese isolates of *Beauveria bassiana* have potential for the biological control of two borer insect pests of *Chukrasia tabularis*. Seven isolates assessed in this study induced 65–74% mortality in *H. robusta* and *Z. coffeae* larvae. Moreover, *B. bassiana* isolates resulted in higher mortality when tested against the same insect species as the isolate source. Although *B. bassiana* has a broad host range, its pathogenicity on a particular target pest species is known to depend on the isolation source (Islam *et al.* 2023). Some isolates have been reported to have a narrow host range (Abdo *et al.* 2008; Imoulan *et al.* 2017; Rohrlich *et al.* 2018; Islam *et al.* 2021).

In the present study, the effectiveness of the EPF isolates was consistent across laboratory, nursery, and field experiments. Correlation coefficients between the indexes for infected rate, damage incidence, and damage severity were high in all *H. robusta* and *Z. coffeae* trials (Table 7). Reay *et al.* (2007) found that *B. bassiana* isolated from pinhole borers had the potential to control three pinhole borers, namely *Treptoplatypus caviceps* Broun, 1880 (Coleoptera: Curculionidae), *Platypus apicalis* White, 1846 (Coleoptera: Platypodidae), and *P. gracilis* Broun, 1893 (Coleoptera: Platypodidae) in laboratory log trials. However, they were ineffective in field trials in New Zealand. In a previous study in Vietnam, two *B. bassiana* strains isolated from ghost-moths *Endoclita salvazi* Tindale, 1958 (Lepidoptera: Hepialidae) and *E. vietnamensis* Buchsbaum & Grehan, 2022 (Lepidoptera: Hepialidae) caused high parasitism and lethality against the same pests in laboratory and field (in *Acacia mangium*) experiments with mortality rates of 80–81% and 75–77%, respectively (Chi and Anh 2025).

*Beauveria* species have been widely used for effective and environmentally friendly pest management (Zimmermann 2007; Mascarin and Jaronski 2016; Wang *et al.* 2021). In particular, *B. bassiana* has been deemed to be safe for biocontrol (Zimmermann 2007). The species has been extensively used as a commercial insecticide in agriculture (Hurley *et al.* 2025), including in Vietnam (Toan *et al.* 2024). In forestry, a few *B. bassiana* isolates have shown potential for biological control of borers and other pests, including the mountain pine beetle *Dendroctonus ponderosae* Hopkins, 1902 (Coleoptera: Curculionidae) (Li *et al.* 2023), ambrosia beetle *Platypus koryoensis* Murayama, 1893 (Coleoptera: Platypodidae) (Lee *et al.* 2025), and the agarwood defoliator moth *Heortia vitessoides* Moore, 1885 (Lepidoptera: Crambidae) (Rishi *et al.* 2016). So far in Vietnam, there has been limited application of *B. bassiana* in forest protection. Mostly locally-produced commercial *B. bassiana* products have been tested (Chi *et al.* 2021; Chi *et al.* 2022; Quang *et al.* 2022). Although commercial products isolated from field crop pests have been highly effective in managing pests in agricultural crops (Wang *et al.* 2021; Toan *et al.* 2024), they may be less effective in controlling forest pests.

Although this study showed that local *B. bassiana* isolates are pathogenic against *H. robusta* and *Z. coffeae*, further work is required before they can be used commercially. Areas that should be investigated include understanding the biology of local *B. bassiana* isolates, screening more effective isolates, developing methods for commercial inoculum production, defining protocols for field application, exploring inoculum persistence in plantations, quantifying impacts on non-target hosts, and establishing legal and ministerial requirements in Vietnam.

Regarding the biology of the species, it has recently been discovered that sexual reproduction is widespread in wild populations of *B. bassiana* in eastern China (Cai *et al.* 2022). This has not been explored for natural populations in Vietnam. Furthermore, it is not known whether the *B. bassiana* isolates tested in this study are effective pathogens against the complete life cycle of the lepidopteran borers of interest, and this should be appraised. For example, a commercial *B.*

*bassiana* strain was used to evaluate its ovicidal effect against the emerald ash borer (*Agrilus planipennis* Fairmaire, 1888 (Coleoptera: Buprestidae)) and found to have a high control efficacy against eggs and larvae (Simeto *et al.* 2024).

To date, the production of local *Beauveria* for commercial use has not been scaled up. However, methods of mass production of *B. bassiana* for agriculture have been perfected (Mascarin and Jaronski 2016; Seethapathy 2025), and these should prove useful for the production of target isolates for forestry operations in Vietnam. A wide range of substrates is available, from broken rice (Madhavi *et al.* 2020) to brewer's spent grain (Boggione *et al.* 2025), and recently, a canola oil-based *B. bassiana* product was tested for controlling forest pests (Biryol *et al.* 2025; Chi and Anh 2025; Niu *et al.* 2025).

In this study, $2 \times 10^6$ conidia/ml were applied but, it is not known if this is the optimal dosage. Previously, $5 \times 10^6$ conidia/ml of *B. bassiana* were used to control *Endoclita* larvae in planted forests and achieved 75–77% mortality (Chi and Anh 2025). Applications of *B. bassiana* at densities of $6.8 \times 10^5$ to $2.1 \times 10^6$ conidia/ml were most effective for controlling fruit flies (*Ceratitis rosa* Karsch, 1887 (Diptera: Tephritidae) and *C. capitata* Wiedemann, 1824 (Diptera: Tephritidae)) and the false codling moth (*Thaumatotibia leucotreta* Meyrick, 1913 (Lepidoptera: Tortricidae)) in citrus (Goble *et al.* 2011). Nevertheless, further research is required to establish the optimal spore density of each *B. bassiana* isolate for pest control. *B. bassiana* caused 100% larval mortality of the greenish silk-moth *Trilocha varians* Walker, 1855 (Lepidoptera: Bombycidae) at $2 \times 10^5$ conidia/ml (Ramzan *et al.* 2024). Similar efficacy was obtained with the pine processionary moth *Thaumetopoea wilkinsoni* Tams, 1926 (Lepidoptera: Notodontidae) using $1 \times 10^8$ conidia/ml (Topkara *et al.* 2022). As *H. robusta* and *Z. coffeae* were sometimes found damaging the same tree, it would be useful to explore the pathogenicity of mixtures of isolates from the two hosts. Resquín-Romero *et al.* (2016) showed that a combination of isolates of *B. bassiana* gave significantly higher larval control efficiency of the African cotton leafworm (*Spodoptera littoralis* Boisduval, 1833

(Lepidoptera: Noctuidae)) than when used separately.

To evaluate the *B. bassiana* isolates in the field, stands were selected where some trees had already been invaded by the borer larvae. *Hypsipyla robusta* has a life cycle of between 56 and 89 days and mainly causes serious damage in June (Do 2003). The life cycle of *Z. coffeae* is longer (about 120 days) and damage is prevalent in June and July (Phuong *et al.* 2025). To limit damage caused by these pests, trees should be treated well in advance of the peak damage periods. Therefore, field trials are necessary to establish the most effective months for treatment. For experimental purposes, manual spraying was used, but for large-scale deployment, alternative options such as the use of tractor-mounted rigs or drones need to be tested. Furthermore, since *B. bassiana* is known to persist as an endophyte in some plants (McKinnon *et al.* 2017), this raises the possibility of introducing the pathogen into *Chukrasia* prior to out-planting. Previously, bacterial endophytes were found in *C. tabularis* trees that could inhibit the growth and development of *H. robusta* (Tra *et al.* 2022). If this approach were to be successful, the mortality of non-target beneficial insects (Farrokhzadeh *et al.* 2025) might be reduced.

It has yet to be determined how long the test *B. bassiana* isolates can survive in *Chukrasia* plantations. Unsuitable temperatures and low moisture can reduce the survival of *B. bassiana* (Jaronski 2010). For example, Mann and Davis (2020) found that the pathogenicity of *B. bassiana* to bark beetles was reduced in the field and they attributed this to "low ambient temperatures and exposure to secondary metabolites". Hence, techniques to maintain the vitality of *B. bassiana*, such as using a polymeric matrix (Barta *et al.* 2018), are being explored. Since the *B. bassiana* isolates used in the present experiment were obtained locally, they should be well-adapted to the tropical environments where *Chukrasia* is being grown commercially.

The use of entomopathogenic bacteria, fungi, and viruses as biological control agents for pest management can reduce the risks of chemical pesticides causing environmental damage and entering the human food chain (Zimmermann 2007; Wang *et al.* 2021; Lopes *et al.* 2023; Hurley *et*

*al.* 2025). It is encouraging that the forestry sector is starting to replace traditional chemical pesticides with biological agents (Hurley *et al.* 2025). In Vietnam, this conversion trend has been prioritized for many years in the agricultural sector (Waterhouse 1998; Toan *et al.* 2024). However, the forestry sector in Vietnam is lagging behind and only recently have researchers begun to address the problem (Quang *et al.* 2021; Chi *et al.* 2023; Chi *et al.* 2025). The Vietnamese government is encouraging the development of commercial products from EPFs. However, all new EPFs need to be extensively tested and their biosafety assessed by independent assessment organizations managed by the Plant Protection Department of Vietnam. If they meet technical requirements and ensure biosafety, they can be licensed and become commercial products.

## Conclusions

This study found that four *B. bassiana* isolates obtained from *H. robusta* larvae and three from *Z. coffeae* larvae caused 71–87% mortality of larvae of the two pests in laboratory experiments. In nursery and field experiments, the seven isolates significantly reduced borer pests damage in *C. tabularis*. Although these findings are promising, further research and development are required before local *B. bassiana* strains can be made available for commercial production as biological control agents for borer control in *C. tabularis* plantations.

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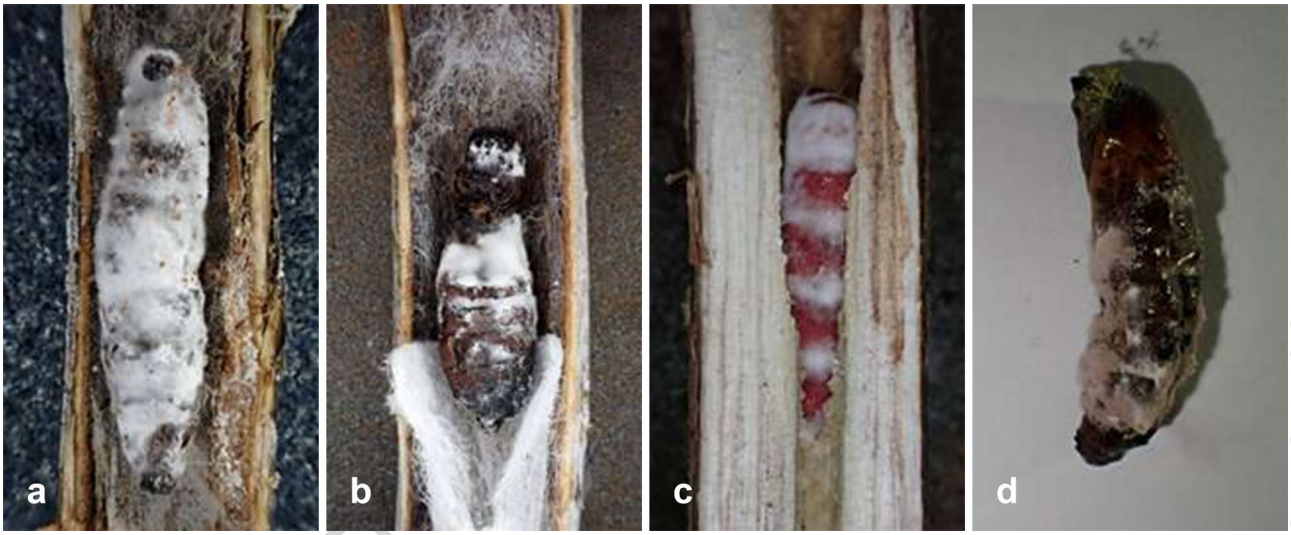


**Fig. 1.** Examples of naturally infected larvae and pupae from which *Beauveria* isolates were obtained: **a**, **b**. *Hypsipyla robusta*; **c**, **d**. *Zeuzera coffeae*; **a**, **c**. larvae; **b**, **d**. pupae

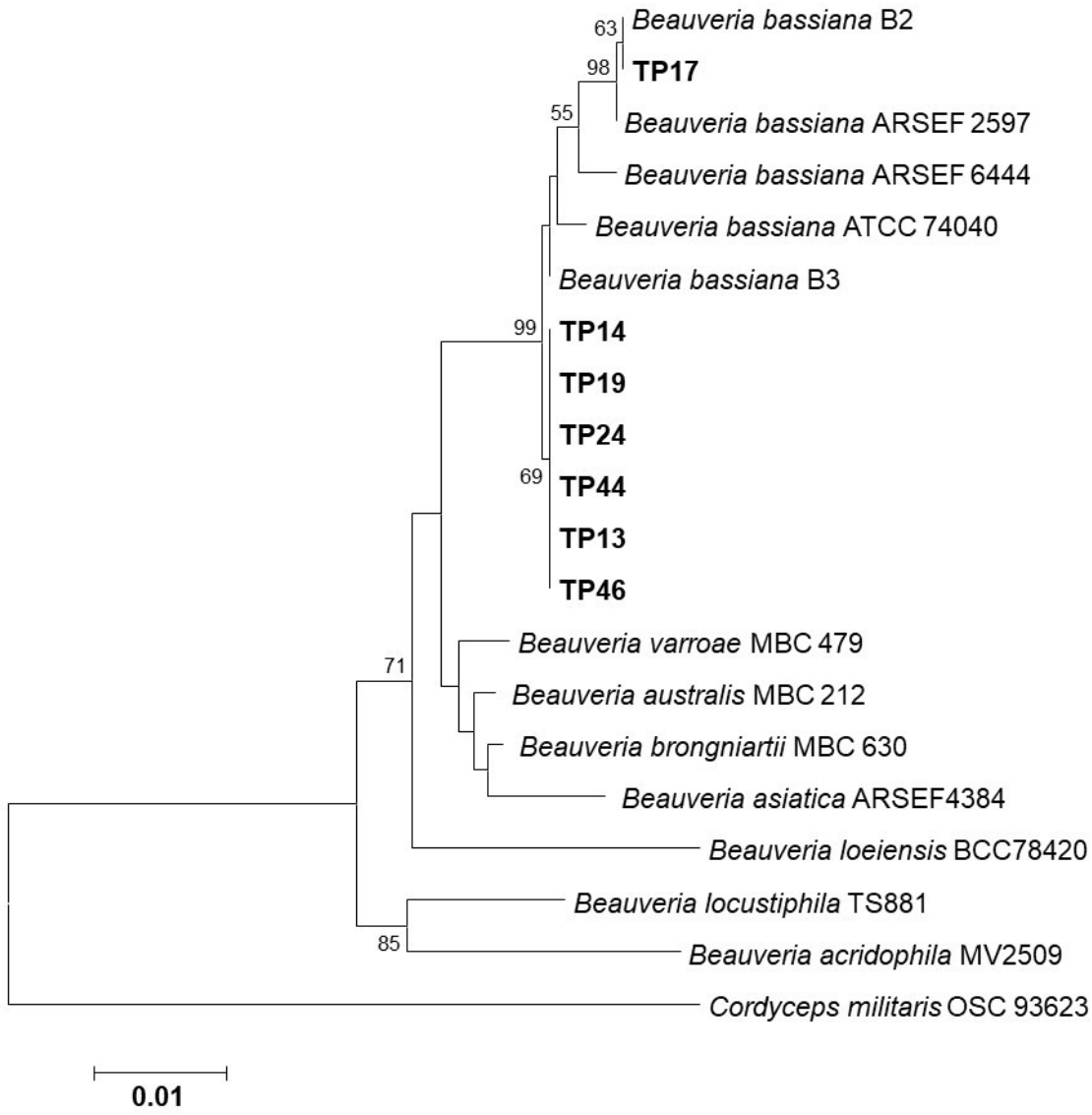


**Fig. 2.** Consensus tree of the concatenated sequences of ITS, LSU, and TEF1-α from species within the genus *Beauveria*. The Maximum Likelihood method was used to construct the tree. Numbers at branch points indicate bootstrap percentages from Maximum Likelihood analyses, and only values greater than 50% are shown. The bar represents an expected sequence variation of 1.0%. *Cordyceps militaris* was used as the outgroup taxon to root the tree


**Fig. 3.** Infected *Hypsipyla robusta* larvae 15 days after first spraying *Beauveria bassiana* conidia: **a**–**d**. larvae in the nursery trial; **e**–**h**. larvae in the field trial; **a**. isolate TP13; **b**. isolate TP17; **e**. isolate TP24; **f**. isolate TP46; **c**, **g**. Muskardin; **d**, **h**. water, note the healthy larvae. Infected larvae with discolored brown to dark gray bodies (a, b, c, e, f, g) and white fungal mycelium covering the head (a, b, c, e, f)

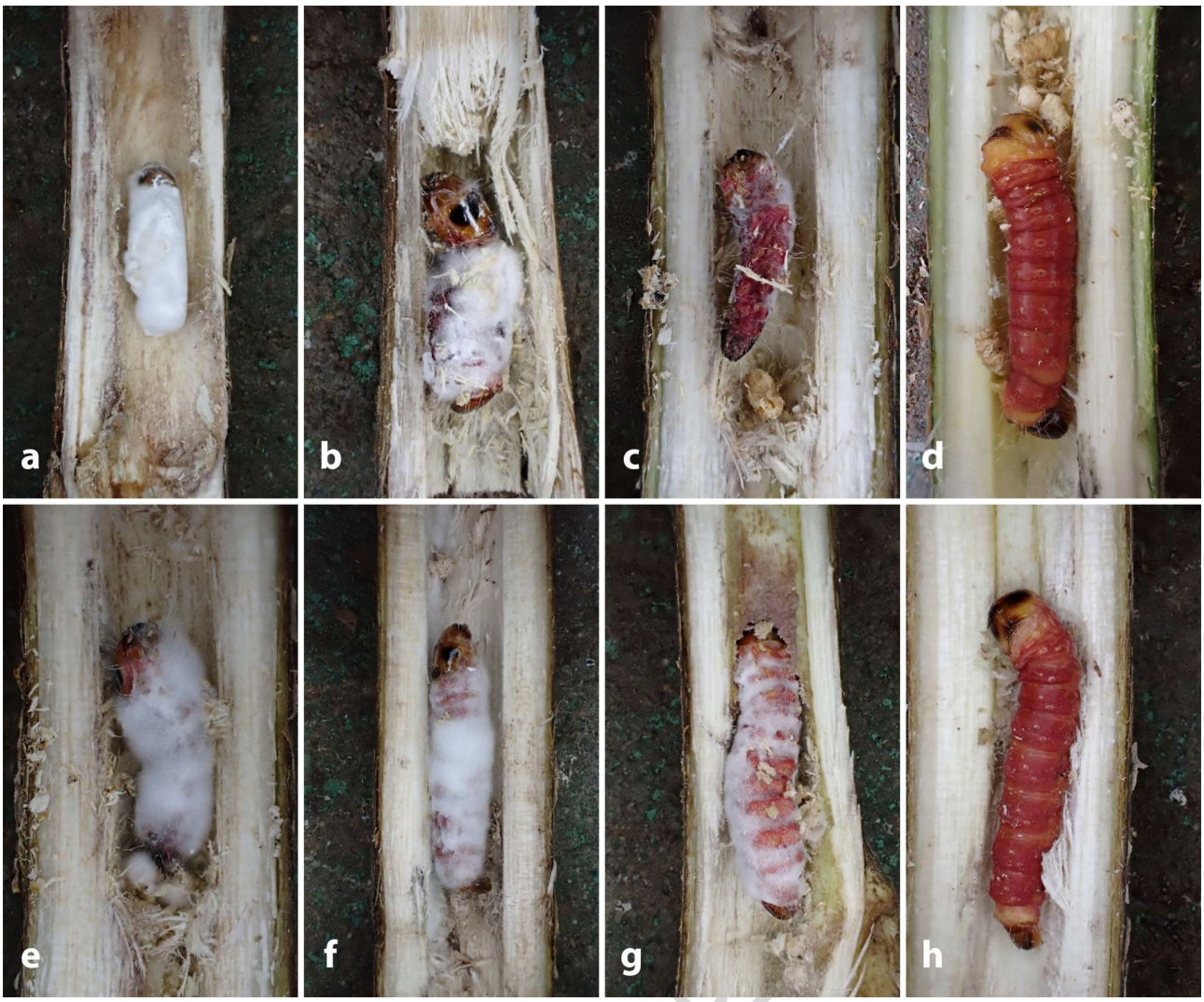


**Fig. 4.** Infected *Zeuzera coffeae* larvae 15 days after first spraying *Beauveria bassiana* conidia: **a**–**d**. larvae in the nursery trial; **e**–**h**. larvae in the field trial; **a**. isolate TP14; **b**. isolate TP19; **e**. isolate TP44; **f**. isolate TP46; **c**, **g**. Muskardin; **d**, **h**. water, note the healthy larvae. Infected larvae with white fungal mycelium covering the body (a, b, c, e, f, g)