Tạp chí KHLN Số 2/2025: 115 - 122
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DOI: https://doi.org/10.70169/VJFS.1043
ISSN: 1859 - 0373 Đăng tải tại: https://vjfs.vafs.gov.vn

FIRST RECORD OF *Beauveria bassiana*, A POTENTIAL BIOCONTROL AGENT FOR MANAGEMENT OF STEM BORERS

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ABSTRACT

Acacia and Eucalyptus hybrids are widely planted in Vietnam for wood products and rural development, and the total area is now about 2.75 million hectares. As the plantation area expands, insect pests have increased and they are causing serious damage to the forest estate. However, some parasitic entomopathogens have been recorded on pest species and these may have potential for future biocontrol. Entomopathogenic fungi isolated from parasitized larvae of *Endoclita salvazi* (isolate B3) and *E. vietnamensis* (isolate B2) were identified and their pathogenicity evaluated in trials. Based on the ITS, LSU and TEF1 - α sequences, isolates B2 and B3 are *Beauveria bassiana*. These isolates had high pathogenicity, with the percentage of parasitized larvae being 74.5 - 76.8%. These findings provide further evidence for the effectiveness of *B. bassiana* isolates for the development of biopesticides.

Keywords: *Beauveria bassiana*, biocontrol, *Endoclita*, entomopathogenic fungi, insect pest, pesticide, plantation forestry, stem borer

GHI NHẬN NẤM KÝ SINH CÔN TRÙNG *Beauveria bassiana* VỚI TIỀM NĂNG PHÒNG TRỪ SÂU ĐỤC THÂN

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TÓM TẮT

Các loài keo và bạch đàn lai đang được trồng rộng rãi ở Việt Nam để cung cấp gỗ nguyên liệu và phát triển sinh kế nông thôn với tổng diện tích đạt khoảng 2,75 triệu ha. Diện tích rừng rất lớn và trồng tập trung đã tạo điều kiện thuận lợi cho các loài sâu hại gia tăng nhanh và gây thiệt hại nghiêm trọng cho các chủ rừng. Tuy nhiên, nấm ký sinh côn trùng đã được ghi nhận trên một số loài sâu hại. Nghiên cứu này nhằm kiểm tra khả năng ký sinh, gây bệnh và định danh các mẫu nấm ký sinh côn trùng đã được phân lập từ sâu non của hai loài sâu đục thân *Endoclita salvazi* (mẫu B3) và *E. vietnamensis* (mẫu B2). Dựa trên các trình tự ITS, LSU và TEF1 - α, hai mẫu nấm ký sinh côn trùng B2 và B3 đã được xác định là *Beauveria bassiana*. Các mẫu nấm này có khả năng ký sinh và gây bệnh cao với tỷ lệ sâu non bị ký sinh khoảng 74,5 - 76,8%. Những phát hiện này cung cấp thêm dữ liệu cho việc phát triển chế phẩm trừ sâu sinh học từ hai mẫu *B. bassiana* nêu trên.

Từ khóa: *Beauveria bassiana*, *Endoclita*, nấm ký sinh côn trùng, phòng trừ sinh học, sâu đục thân, sâu hại, rừng trồng, thuốc trừ sâu

I. INTRODUCTION

Acacia and Eucalyptus plantations in Southeast Asia provide wood products and support rural development over very large areas (Nambiar *et al.*, 2015). In Vietnam, development of *Acacia* and *Eucalyptus* plantations for solid-wood utilisation is steadily progressing. Total areas of *Acacia* and *Eucalyptus* plantations have reached about 2.35 and 0.4 million hectares, respectively (To *et al.*, 2021). As exotic tree species have been planted on a large scale, potential threats to *Acacia* and *Eucalyptus* plantations have been investigated and identified. Leaf diseases, pink disease and wilt disease have been widely reported (Old *et al.*, 2000; Chi *et al.*, 2022c; Thu *et al.*, 2023; Chi & Thu 2024), and several insect pests have been recorded causing serious damage to plantations, including ambrosia beetles, leaf feeders, longhorn beetles, and stem borers (Thu *et al.*, 2021; Buchsbaum *et al.*, 2022; Chi *et al.*, 2022b; Hung *et al.*, 2022; Thu *et al.*, 2023; Pham *et al.*, 2024).

Several unidentified entomopathogenic fungi were collected from naturally parasitized larvae of the stem borers *Endoclita salvazi* and *E. vietnamensis* (Lepidoptera: Hepialidae) in a previous study (Chi *et al.*, 2022a). This study has two aims, first to identify the entomopathogens using gene sequences, and second to assess their pathogenicity in laboratory and field trials. The results will be useful in the development of biological agents to control stem borer infestations in forest plantations.

II. MATERIALS AND METHODS

2.1. Sampling and culture traits

In 2021, two potential entopathogenic fungal samples were isolated from parasitized larvae of *Endoclita vietnamensis* in an *Acacia mangium* plantation (B2) and *E. salvazi* in a *Eucalyptus* hybrid plantation (B3) by Chi *et al.*, (2022a). The *A. mangium* plantation was located in Tan Lac district, Hoa Binh province and the *Eucalyptus* hybrid plantation was located in Yen Lap district, Phu Tho province (Buchsbaum *et al.*, 2022; Chi *et al.*, 2022a). The isolates were maintained in the laboratory of the Forest Protection Research Centre at $5^{o}C$ and dark condition, and they were freshly subcultured before using.

2.2. Molecular identification

The primers ITS1/ITS4 5.8SR/LR7 and EF983F/EF2212R were used to identify the entomopathogenic fungal isolates. Amplifications were carried out using the method of Hall (1999). The ITS, LSU and TEF1 - α sequences of isolates B2 and B3 were compared with the GenBank database via the nucleotide-nucleotide BLAST search interface located at the National Center for Biotechnology Information, Bethesda, USA. Relevant sequences were transferred and then processed using BioEdit software (Hall, 1999).

Phylogenetic analyses were perfomed using the ITS, LSU and TEF1 - α sequences. The evolutionary history was inferred by using the Maximum Likelihood method based on the Kimura 2 - parameter model (Kimura, 1980). Consensus trees with the highest log likelihoods (1,158.71) were created. Evolutionary analyses were conducted in MEGA7 (Kumar *et al.*, 2016). *Ophiocordyceps sinensis* was used as the outgroup taxon to root the tree.

The ITS, LSU, and TEF1 - α gene sequences of two isolates in this study were compared with reference sequences obtaned from the National Center for Biotechnology (NCBI) GenBank for *Beauveria bassiana*, *B. brongniartii* and *B. varroae* (Table 1). Bootstrap values were equal to or greater than 50% derived from 1,000 iterations.

Table 1. GenBank accession numbers of *Beauveria* isolates and reference sequences

Species	Isolate	ITS	LSU	TEF1 - α	Reference
Beauveria bassiana	B2	ON876733	ON876740	ON815106	(Chi *et al.*, 2022a)
Beauveria bassiana	B3	ON876734	ON876741	ON815107	(Chi *et al.*, 2022a)
Beauveria bassiana	ATCC 74040	JBKFFR010000383	JBKFFR010000383	JBKFFR010000200	(Moro *et al.*, 2025)
Beauveria bassiana	ARSEF 2597	JTCX01001201	JTCX01001201	KV580567	(Valero-Jiménez *et al.*, 2016)
Beauveria bassiana	ARSEF 6444	JASJOK010000012	JASJOK010000012	JASJOK010000011	NA
Beauveria varroae	MBC 479	JAPFTO010000041	JAPFTO010000041	JAPFTO010000360	NA
Beauveria varroae	MBC 525	JAPFUP010000009	JAPFUP010000009	JAPFUP010000150	NA
Beauveria brongniartii	MBC 630	JARJPP010000009	JARJPP010000009	JARJPP010000589	NA
Ophiocordyceps sinensis	EFCC 7287	JN049854	EF468827	EF468767	(Sung *et al.*, 2007)

Note. NA not available

2.3. Pathogenicity tests on *Galleria mellonella* larva

An initial bioassay was conducted to evaluate the efficacy of B2 and B3 against the 4th larval instar of *Galleria mellonella* (Lepidoptera: Pyralidae) using the method of Hussein *et al.*, (2012). We used *G. mellonella* in this first screening because we did not want to damage plantation trees to source larvae of *E. vietnamensis*. Conidia were harvested from 14 day old fungal cultures grown on PDA at 25°C by scraping the sporulating colonies and suspending this in 1% Tween 80 in distilled water. The spore solution was diluted at a density of about 5×10^6 CFU/ml. *Beauveria bassiana* (Muskardin, CPC, Can Tho, Vietnam, dosage of 3.75 g/l) was included as the positive control. Sterile water + 1% Tween 80 provided the negative control. Larvae were submersed for 5s in each treatment. Four separate experiments were carried out using fifteen larvae per replicate. After treatment, the larvae from each treatment were placed in sterilized plastic circular boxes (9 cm diameter, 3 cm depth, 15 larvae per box) containing 15 g of artificial diet and wet filter paper, and incubated at 25 - 26°C. The number of parasitized larvae (discolored brown to dark gray bodies or covered with fungal mycelium) was recorded three, six, and nine days following fungal inoculation.

2.4. Field trial on *Endoclita vietnamensis* larva

The two isolates were evaluated for bioassay test in an *A. mangium* plantation at 16 - month-old growth stage in Tan Lac district, Hoa Binh province that were damaged by *Endoclita vietnamensis* with damage incidence of 19 - 20%. Muskardin and clean water were used as positive and negative controls, respectively. All treatments included 1% Tween 80. Spore solutions of approximately 5×10^6 CFU/ml were added to larval tunnels of the pest insect (about 15 ml/tunnel) using an electric spray machine on August 10, 2024. Five damaged trees with one active tunnel per tree were used for each treatment, and the experimental design was completely randomized. Each of the four treatments had five larvae and there were 5 replicates of each treatment. Thus, 25 larvae were used for each treatment. The total number of trees used in the trial was 100 trees, with tree spacing of 3 m and the distance between treatments being 15 - 20 m. Fifteen days after fungal inoculation, the trees were cut down, opened with a knife and the number of the healthy and parasitized/dead *E. vietnamensis* larvae was recorded.

2.5. Data analysis

From the parasitized insect data in the laboratory and field trials, the parasitized rate (*P%*) was calculated using equation:

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

Where: n is the number of parasitized larvae; N is total number of larvae assessed.

Data were analysed using GenStat Release 12.1 software package. The data were transformed and tested for normality. Analysis of variance (ANOVA) was used to test significant effects of blocks and treatments, followed by Tukey's Multiple Range Test for comparisons of means of different treatments at probability level of 0.05.

III. RESULTS

3.1. Phylogenetic analysis

The phylogenetic analysis (Figure 1) revealed that isolates B2 and B3 grouped with *Beauveria bassiana* in two sub-clades: B2 aligning with *B. bassiana* ARSEF 2597 in the entomopathogenic fungi collection of USDA-ARS (Valero-Jiménez *et al.,* 2016) and B3 with *B. bassiana* ATCC 74040 isolated from the commercial product naturalis® (Moro *et al.,* 2025).

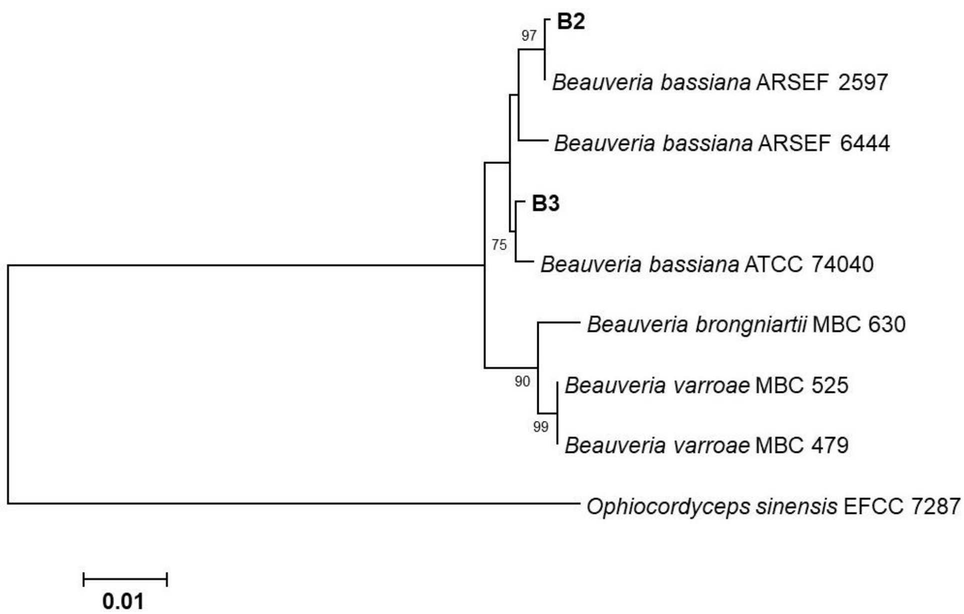

Figure 1. Phylogenetic tree based on mitochondrial cytochrome oxidase 1 gene region showing relationships between samples in this study (B2, B3) and other species within the GenBank database within the genus *Beauveria*. The phylogram was obtained using the Maximum Likelihood method. The bar represents an expected sequence variation of 1.0%

3.2. Pathogenicity on *Galleria mellonella* larvae

There were no significant differences between blocks (P>0.05) in the experiment (data not shown). The two entopathogenic fungal samples (*B. bassiana* B2 and B3) isolated from parasitized larvae of *Endoclita* spp. had strong pathogenicity on *G. mellonella* larvae (Table 2).

Table 2. Percentage of parasitized *Galleria mellonella* larvae in the inoculation experiment using *Beauveria* isolates (%)

Treatment	After three days	After six days	After nine days
B. bassiana B2	50.3^b	63.7^b	80.7^b
B. bassiana B3	48.7^b	63.3^b	80.3^b
Muskardin	15.3^a	56.7^b	75.7^b
Water	0.0^a	0.0^a	0.0^a
P	*<0.001*	*<0.001*	*<0.001*

Note. Means identified by the same letters in a column are not significantly different between treatments from Tukey's multiple range test (P = 0.05).

The percentage of larvae parasitized by entopathogenic fungal isolates after three, six and nine days were 48.7 - 50.3%, 63.3 - 63.7% and 80.3 - 80.7%, respectively. The corresponding percentages for the positive control using Muskadin was 15.3%, 56.7% and 75.7%, respectively. Meanwhile, there was no mortality in the negative control using water (Table 2).

3.3. Pathogenicity on *Endoclita vietnamensis* larvae

There were no significant differences between blocks (P>0.05) in the field experiment (data not shown). The *Beauveria* isolates were pathogenic on *Endoclita vietnamensis* larvae in the *Acacia mangium* plantation (Table 3).

Table 3. Percentage of parasitized *Endoclita* larvae 15 days after inoculation with *Beauveria* in an *Acacia mangium* plantation

Treatment	Sample size (n)	Percentage parasitized larvae (%)
B. bassiana B2	25	74.5^c
B. bassiana B3	25	76.8^c
Muskardin	25	48.3^b
Water	25	1.3^a
P		*<0.001*

Note. Means identified by the same letters in a column are not significantly different between treatments from Tukey's multiple range test (P = 0.05).

After 15 days, inoculation with *B. bassiana* B2 and B3 resulted in 74.5% and 76.8%, respectively, mortality of *Endoclita* larvae. There was less mortality (48.3%) with the commercial Muskadin. Almost all parasitized larvae were covered with fungal mycelia (Figure 2a, b).

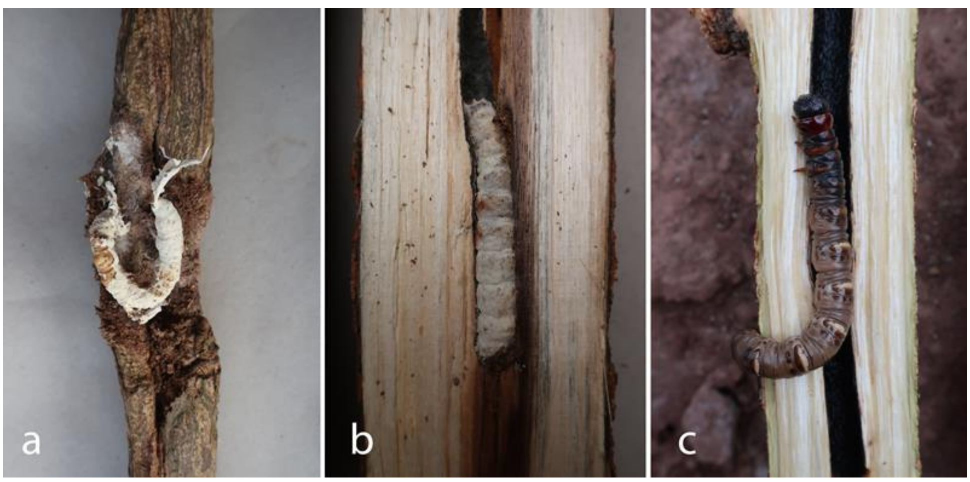

Figure 2. Parasitized *Endoclita* larvae 15 days after inoculation by *Beauveria*: **a**. isolate B3; **b**. positive control using Muskadin; **c**. negative control using water

IV. DISCUSSION

This is first report of *Beauveria bassiana* parasitisim of *Endoclita salvazi* (B3) and *E. vietnamensis* (B2) larvae. In the previous publication, these entomopathogenic fungi were suggested to be *Cordycep* spp. (Chi *et al.*, 2022a). In this study we have shown that the sequences of isolates B2 and B3 had 99.7 - 99.9% homology with published sequences for the isolates ARSEF 2597 (Valero-Jiménez *et al.*, 2016) and ATCC 74040 (Moro *et al.*, 2025) that were identified as *B. bassiana* (Moro *et al.*, 2025). *Beauveria bassiana* is extensively used as a commercial agricultural insecticide (Mascarin & Jaronski, 2016). It has been used for control of stem borer *Neurozerra conferta* (Chi *et al.*, 2022d), leaf feeders *Phalera grotei* and *Ericeia pertendens* (Thu *et al.*, 2023) in Vietnam. This fungal species was effective in the control of white grubs (*Brahmina coriacea*) with the mortality of larvae up to 80% (Chandel *et al.*, 2019), and *B. bassiana* strains 147 and 203 are widely used as a bio-insecticide in Europe (EFSA 2015, 2020).

Some *Beauveria* species used as biological agents in pest control have high efficiency (Zimmermann, 2007; EFSA, 2015; Mascarin & Jaronski, 2016). *Beauveria brongniartii* used for control of *Melolontha melolontha* larvae in Switzerland generated fungal infection rates of 59 - 86% (Enkerli *et al.*, 2004) while application to *Brahmina coriacea* resulted in larval mortality of 40 - 83% (Chandel *et al.*, 2019). The fungus could survive in soil after 16 months of application during control of *M. melolontha* (Kessler *et al.*, 2004). *Beauveria bassiana* have been shown to be a safe entomopathogenic fungus for biocontrol (Zimmermann, 2007). Some other species of the genus *Beauveria* have also been recommended for use as mycoinsecticides (Mascarin & Jaronski, 2016).

The use of biological agents in pest management in agricultural and forestry is being encouraged to limit the use of chemical pesticides (Zimmermann 2007; Mascarin & Jaronski, 2016). Pest control in planted forests in Vietnam is also being directed towards integrated pest management where the use of biological agents is emphasied (Quang *et al.*, 2021; Chi *et al.*, 2023). Experimental control of insect pests using biological agents in Vietnam has achieved promising results, such as the use of *Bacillus bombysepticus* to control *Ceracris kiangsu* and *Hypsipyla robusta* (Tra *et al.*, 2022; Chi *et al.*, 2025), and *Beauveria bassiana* and *Metarhizium anisopliae* for control of *Neurozerra conferta* (Chi *et al.*, 2022d). The results of our study showed that the two strains *Beauveria bassiana* B2 and B3 have potential for being developed into effective biocontrol agents of wood boring insects in forest planations of Vietnam. Further investigation is needed to determine the best method for delivering the fungus in the field and to identify a suitable carrier for the fungal spores. In addition, biosafety testing needs to be done before these entomopathogenic fungi can be used for biocontrol in forest plantations in Vietnam.

V. CONCLUSION

The entomopathogenic fungi isolated from parasitized larvae of *Endoclita salvazi* (B3) and *E. vietnamensis* (B2) are *Beauveria bassiana*. This is first report of *B. bassiana* parasitisim of *Endoclita* larvae in Vietnam. These isolates have potential for biocontrol of the pest species with the infestation of plantation *Endoclita* larvae being 74.5 - 76.8%.

Acknowledgments: *This work was supported by the Ministry of Agriculture and Environment. The authors would like to thank Dr. John Grehan and two reviewers for their revisions, suggestions and English language editing.*

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Ngày nhận bài: 10/03/2025

Ngày phản biện đánh giá và sửa chữa: 17/03/2025; 20/03/2025

Ngày duyệt đăng: 21/03/2025