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Nematicidal effect of *Beauveria* species and the mycotoxin beauvericin against pinewood nematode *Bursaphelenchus xylophilus*

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Introduction and main objective: *Bursaphelenchus xylophilus*, commonly known as pine wood nematode (PWN), is considered one of the greatest threats to European and Asian pines. Regarding its management, most efforts have been directed toward control measures for the major vector (*Monochamus* spp.) and screening for genetic resistance in its hosts. However, an integrated pest management strategy which also implements pinewood nematode control is currently lacking. The aim of this study was to evaluate the nematicidal effect of two *Beauveria* species, a genus well known for its entomopathogenic activity.

Summary methodology: For this purpose, *in vitro* antagonism tests of fungi (*Beauveria bassiana* and *B. pseudobassiana*) and the mycotoxin beauvericin ($C_{45}H_{57}N_3O_9$) on *B. xylophilus* populations were conducted. Finally, the production of beauvericin in *B. bassiana* and *B. pseudobassiana* strains was quantified by high-performance liquid chromatography - mass spectrometry (HPLC-MS).

Results and discussion: Both the *B. bassiana* and *B. pseudobassiana* fungal species and the mycotoxin beauvericin showed a clear nematicidal effect on *B. xylophilus* populations, substantially reducing their survival rate and even attaining 100% mortality in one case. HPLC-MS analysis confirmed and quantified the production of beauvericin by *B. bassiana* and demonstrated for the first-time beauvericin production in *B. pseudobassiana*.

Final conclusion: These findings highlight the potential of *Beauveria* species and the mycotoxin beauvericin to be implemented in an integrated pest management strategy to control both nematode and vector.

KEYWORDS

pine wilt disease, integrated management, biological control, nematophagous fungi, fungal toxins

1. Introduction

Bursaphelenchus xylophilus (Nematoda: Aphelenchoididae) poses a serious threat, endangering coniferous forests worldwide. This pathogen, commonly known as pine wood nematode (PWN) and belonging to the group of plant-parasitic nematodes (PPNs), causes decay of afflicted trees, leading to the so-called pine wilt disease (PWD) and eventually, tree mortality. Although PWN is thought to originate in North America, PWD was firstly detected in the early twentieth century in Japan and quickly spread to other Asian countries (Kim et al., 2020). In the late 1990s it was introduced into Europe via Portugal (Mota et al., 1999) where it has subsequently caused significant forest damage. PWD is now found in more than 30% of the Portuguese forest area (De la Fuente et al., 2018) and in several Spanish regions bordering Portugal: Extremadura, Galicia and Castilla y León (Zamora et al., 2015). Although the pathogen currently appears fairly contained, it is listed as a quarantine pest in Europe (List A2) (EPPO, 2016) due to its high pathogenicity and dispersive capacity. Moreover, the impact is predicted to worsen under future climate change scenarios (Hirata et al., 2017; De la Fuente et al., 2018).

The spread of PWN within Europe is linked to insect vectors belonging to genus *Monochamus*, and more specifically *M. galloprovincialis* (Coleoptera: Cerambycidae). The pathogen is transmitted via these beetles from dead or dying trees to healthy ones through feeding (Mamiya and Enda, 1972) or oviposition (Edwards and Linit, 1992). Once the nematodes have entered the tree, they lodge in the resiniferous canals and feed on epithelial and parenchymal cells, triggering a host response which leads to disruption of water transport and rapid death by cavitation (Fukuda and Suzuki, 1988; Hara et al., 2006). Thus far, disease control mitigation techniques have been mainly focused on the search for genetic resistance in the host organisms and controlling disease spread by reducing populations of the insect vector. The first reported program for breeding genetic resistance to PWD started in western Japan in 1978, on Japanese black and red pine (*Pinus thunbergii* and *P. densiflora*, respectively) and obtained high post-inoculation survival rates in both (Fujimoto and Ohba, 1981; Toda and Kurinobu, 2002). Since then, many resistance programs have been operational, such as on *P. massoniana* (Liu et al., 2017; Zhu et al., 2021), *P. pinaster* (Gaspar et al., 2017; Carrasquinho et al., 2018) and *P. radiata* (Zas et al., 2015; Menéndez-Gutiérrez et al., 2018, 2021) in China, Portugal and Spain, respectively.

In terms of insect vector control, several strategies have been developed to reduce *M. galloprovincialis* populations such as a trapping method combining pheromone and kairomones (Álvarez-Baz et al., 2016; Galloprotect 2D®), the use of entomopathogenic fungi (Naves et al., 2008; Álvarez-Baz et al., 2015; Petersen-Silva et al., 2015) and the development of an auto-infection device based on the combined use of the attractant and the entomopathogenic fungi (Sacristán-Velasco et al., 2018). To date, only the use of traps with attractants has been implemented more widely, but successful field trials have been performed combining this system with the auto-infection device and powdered formulations of an entomopathogenic fungus (Martín-García, Unpublished data).

Despite progress in other areas, an effective integrated pest management technique for this disease will require nematode control. Progress toward achieving this goal has been made with the effect of fungi and bacteria on different PPNs being studied (Mankau, 1980; Askary, 2015; Abd-Elgawad and Askary, 2018; Migunova and Sasanelli, 2021). For PWN specifically, the most studied antagonistic fungal species is *Esteya vermicola*, which was first found infecting PWN in Taiwan in 1999 (Liou et al., 1999) and reported as a natural enemy of the nematode. Subsequent laboratory and field tests have corroborated this efficacy as a biological agent against the pathogen (Kubátová et al., 2000; Wang et al., 2008, 2009, 2018; Lin et al., 2013; Pires et al., 2022). Other fungal genera such as *Verticillium* spp. or *Trichoderma* spp. have also exhibited nematicidal effect on PWN populations (Maehara and Futai, 2000). More recently, some fungal species within the genera *Leptographium* and *Graphilbum* have also been shown to have a nemastatic effect on this pathogen (Vicente et al., 2022). However, little attention has been paid to potential nematicidal effects of the genus *Beauveria* spp. on PWN, despite significant mortality effect on other nematodes species being thoroughly documented (Youssef et al., 2020; Ye et al., 2021; Karabörklü et al., 2022) and its entomopathogenic effect on *M. galloprovincialis* is well known (Naves et al., 2008; Álvarez-Baz et al., 2015; Petersen-Silva et al., 2015). Maehara et al. (2007) proved the reduction of PWN transmission from vectors due to *Beauveria bassiana* was possible; however, this potential control method has not been examined in depth.

Most fungi's nematicidal properties appear at least in part due to excreting secondary metabolites (mycotoxins) (Anke and Sterner, 1997, 2002; Li et al., 2007; Anke, 2011; Baazeem et al., 2021; Seong et al., 2021). However, some fungi (e.g., *E. vermicola*) appear to have alternate control mechanisms against the nematode, such as the emission of attractant volatiles similar to the host pine and subsequently the production of specific types of spores (lunate conidia) to trap the attracted nematodes (Kubátová et al., 2000; Lin et al., 2013). It is likely that the antagonistic effect of these fungi on the pathogen is the result of the combination of these two modes of action: nematode-trapping capacity and production of mycotoxins.

The nematicidal mycotoxin mechanism is probably the prime mode of action for the *Beauveria* genus and the most studied mycotoxin from this genus is beauvericin (BEA). Chemically, BEA is defined as a cyclic hexadepsipeptide consisting of alternating D-α-hydroxy-isovaleryl-(2-hydroxy-3-methylbutanoic acid) and amino acid units (Hamill et al., 1969; **Figure 1**) and is considered an emerging mycotoxin since it is still neither routinely determined, nor legislatively regulated (Jestoi, 2008; Vaclavikova et al., 2013; EFSA, 2014).

The compound is not only produced by *Beauveria* species such as *B. bassiana* (Hamill et al., 1969), but also by others, for example *Fusarium* spp (Gupta et al., 1991; Logrieco et al., 2002; Moretti et al., 2002, 2008). In comparison to *B. bassiana*, *B. pseudobassiana* has to date few studies identifying and assessing the toxins produced by this species. Berestetskiy et al. (2018) proved that *B. pseudobassiana* strain BCu22 produces different metabolites depending on the culture medium and demonstrated its insecticidal effect on grain aphid larvae. A subsequent study confirmed the existence of a gene cluster coding for BEA in the strain RGM 2184 of *B. pseudobassiana* (Altimira et al., 2022). However, none of them were able to corroborate the actual production of BEA by this fungal species. The toxicity of BEA was first tested on the crustacean *Artemia salina* (Hamill et al., 1969) but soon found to have insecticidal (Grove and Pople, 1980; Gupta et al., 1991; Ganassi et al., 2002; Fornelli et al., 2004; Leland et al., 2005) and nematicidal effects, tested first on *Meloidogyne incognita*

(Mayer, 1995) and later on *Caenorhabditis elegans* and PWN (Shimada et al., 2010). Additionally, according to the Technical Commission on Contaminants in the Food Chain, acute exposure to BEA does not indicate concern for human health (EFSA, 2014). This fact, together with the nematicidal activity, makes BEA one of the most promising mycotoxins that could be implemented in integrated pest management of PWN.

This evidence suggests that *Beauveria* species and the mycotoxin BEA could be a potential control option against not only the PWN insect vector (*M. galloprovincialis*), but also against the nematode itself. The goals of the work presented here were firstly to demonstrate the nematicidal effect of different strains of *B. bassiana* and *B. pseudobassiana* on PWN populations, secondly to prove whether BEA has nematicidal effect on PWN and lastly to test whether not only *B. bassiana* is able to produce BEA, but also potentially *B. pseudobassiana*.

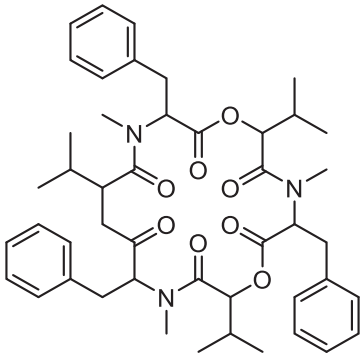

FIGURE 1
Chemical structure of BEA. Diagram created using the software ChemDraw®.

2. Materials and methods

2.1. Fungal and nematode strains for the *in vitro* assays

Two different strains of *B. bassiana* (EABps 11/01-Mg* and 95b) and one of *B. pseudobassiana* (MG-BU-17-001) were used for the assays.

*Note: EF1α and ITS classification of several strains of *Beauveria* species (*B. bassiana* and *B. pseudobassiana*) were modified in GenBank a few years ago (see accession numbers AY531938.1 and AY531931). So, the isolate EABps 11/01-Mg was wrongly identified by Álvarez-Baz et al. (2015) as *B. pseudobassiana* based on the homology of the isolates AY531938.1 and AY531931 (previously identified as *B. pseudobassiana* and currently *B. bassiana*). However, we have again analyzed the isolate EABps 11/01-Mg (EF1α and ITS) and checked in GenBank and it should be classified as *B. bassiana*.

Esteya vermicola CBS 115803 (supplied by the Czech Collection of Microorganisms), which has already demonstrated nematicidal effect on PWN (Wang et al., 2016; Pires et al., 2022) was used as negative-growth control. A non-sporulating *Botrytis cinerea* isolate, which is used to grow the PWN colonies for fostering its reproduction, was used as positive-growth control. All the isolates were cultivated using potato dextrose agar medium (PDA Scharlau, Spain).

The nematode strain was *B. xylophilus* CSF-N-1 and was grown using glass vials [28 mm (Ø), 43 mm (h), 25 mL (V)] with medium composed of hulled barley and non-sporulating *B. cinerea* (**Supplementary Figure 1A**). The vials were kept at room temperature with the cover ajar, always with oxygen flow inward, and under continuous darkness.

2.2. *In vitro* antagonism tests of *Beauveria* spp. on *B. xylophilus* populations

Thirty glass vials with 3 g hulled barley and 3 mL distilled water inside were autoclave sterilized and separated into 6 treatments (*B. bassiana* 95b, *B. bassiana* EABps 11/01-Mg, *B. pseudobassiana* MG-BU-17-001, *B. cinerea*, *E. vermicola* CCM 8247 and mock inoculated). A mycelium plug of each fungus was introduced into each vial (except for mock inoculated treatment, in which a plug of PDA was added) and left to grow for 13 days. After this growth period, a 100 μL aliquot with 270 nematodes (*B. xylophilus* CSF-N-1) was added to each vial and they were kept for a further 13 days at 25°C in continuous darkness.

The extraction of the nematodes from the vials was conducted according to a slightly modified version of the Baermann (1917) funnel technique. A tea filter (M-size, Finum®) was placed with the content of each vial on an autoclaved beaker (100 mL). The content was covered with autoclaved distilled water, so that the nematodes pass through the filter and remain in a clean aqueous suspension. After 24 h, they were washed with distilled water using a nylon sieve of 20 μm mesh size (NY-0073-Labopolis, Spain). The nematodes were retained by the sieve, washed with a washing bottle and finally resuspended into 55 mm (Ø) petri dishes. Three aliquots (100 μL) of each replicate were counted using a counting grid and binocular loupe to determine nematode concentration (**Supplementary Figures 1B, C, D**).

2.3. Effect of commercial BEA on *B. xylophilus* populations

Considering the nematicidal effect of the metabolite beauvericin naturally extracted from strains of *Fusarium bulbicola* on PWN is already published (Shimada et al., 2010), we decided to test the effect of BEA commercial (Merck Life Science S.L.U, USA) on populations of *B. xylophilus*. Solutions of 0.1 mM, 1 mM and 2 mM of BEA with 5% dimethyl sulfoxide (DMSO) were prepared with eight replicates for each concentration by dividing each stock solution into eight 1.5 mL Eppendorf tubes. For the negative controls, another eight replicates were prepared with 5% DMSO only. A 75 μL nematode suspension (400 nm/mL) was added to each tube and live nematodes were then counted. A small hole was made in the top of each tube (with a heated entomological pin) to ensure oxygen flow, and the rack was covered with aluminum foil for continuous dark conditions. The assay was incubated at 25°C for 48 h, each tube was vortex mixed once per day to resuspend the

nematodes. After 2 days, the contents of each tube were poured on a grid and the live nematodes counted.

2.4. Extraction and quantification of BEA produced by the strains of *Beauveria* spp.

Four replicates of each strain were grown on PDA plates and kept in an incubator at 28°C for 21 days. The PDA plates were subsequently cut into 2 cm cube pieces and all cubes from each PDA plate was placed in individual 150 mL conical flasks. MeOH (100 mL) was then added to each flask and the flasks sonicated for 15 min at 40°C. The organic extract was removed, and the solid media washed with MeOH (50 mL). The organic extracts were combined, filtered, and dried under vacuum. The subsequent oily extract was dissolved in 80% MeOH (7 mL), filtered again, and transferred to a sample vial. The organic solvent was removed under N_2 and the aqueous mixture was frozen at −75°C before being dried using a freeze drier. All extract samples were weighed and dissolved in 90% MeOH in a 20 mg/mL concentration.

2.4.1. HPLC protocol

Samples were analyzed using an Agilent 1260 series HPLC system with attached 1200 series diode array detector and MSD-XT single quad mass spectrometer with attached atmospheric pressure chemical ionisation (APCI) source. The LC-MS used a Phenomenex Kinetex, 2.6 μm, C18, 75 mm × 2.1 mm column with a three solvent system A: H_2O, B: acetonitrile and C: 2% formic acid in acetonitrile. The solvent method had a constant flow rate of 0.4 ml/min and started with 90/5/5 initial mix which increases to 45/50/5 over 1 min, then rises again to 5/90/5 over 7 min (8 min total) and held for a further 1 min. The system was allowed re-equilibrate to starting conditions before the next sample was run. BEA was identified in samples using retention time, UV spectra and mass spectrometry. BEA was quantified using UV absorbance (190–210 nm), with a calibration curve obtained using a beauvericin standard purchased from Cayman Chemical Company (concentrations 0.2, 0.1, 0.05 and 0.025 mg/mL).

2.5. Statistical analysis

One-way analysis of variance (ANOVA) and multiple comparison procedures were performed to test the effects of fungi and commercial BEA on nematode populations and to test the concentrations of BEA extracted from each *Beauveria* strain by HPLC. The ANOVA assumptions (normality and homogeneity of variances) were tested in each analysis by the Shapiro and Bartlett tests. When neither assumption was violated (concentrations of BEA extracted from each *Beauveria* strain), classical one-way ANOVA and Tukey's honestly significant difference (HSD) tests was applied. When at least one of these assumptions was violated (effects of fungi and commercial BEA on nematode populations) robust statistical methods were applied (García-Pérez, 2010). In particular, heteroscedastic one-way ANOVAs were performed using the generalized Welch procedure and a 0.1 trimmed mean transformation. These analyses were carried out using the Wilcox' robust statistics (WRS2) package implemented in the R software environment (R Core Team, 2021).

3. Results and discussion

3.1. *In vitro* antagonism tests of *Beauveria* spp. on *B. xylophilus* populations

Growth of *B. xylophilus* populations were strongly inhibited by the presence of *Beauveria* species ($F = 28.29$, $p < 0.001$). All three *Beauveria* strains showed a clear nematicidal effect on *B. xylophilus* populations, with no significant differences between the treatments (**Figure 2**). The nemastatic or nematicidal effect of *B. bassiana* has already demonstrated on other nematode species such as *M. incognita* (Kepenekci et al., 2017; Youssef et al., 2020; Karabörklü et al., 2022) and *C. elegans* (Ye et al., 2021). Thus, our results are congruent with previous evidence and confirm the potential biocontrol agent role of this fungus on *B. xylophilus*. To our knowledge, this is the first study that demonstrates the nematicidal activity of *B. pseudobassiana*. However, considering the phenotypic proximity of both *Beauveria* species (Rehner et al., 2011; Berestetskiy et al., 2018), this is not an unexpected result.

Growing methods for *B. xylophilus* are not standardized, but cultivation with *B. cinerea* (a non-sporulating isolate) grown on barley is the most widely used (Aikawa and Kikuchi, 2007; Espada et al., 2016; Pimentel et al., 2020) and the one recommended by EPPO (2013). Thus, as predicted, the nematode population in the presence of *B. cinerea* exponentially increased during the 13-day assay, obtaining a final population of approximately 165,000 individuals (**Figure 2**), corroborating its role as a positive-growth control. In contrast, the results obtained from the negative-growth control *E. vermicola* were unexpected. The final population (≈ 96,000 nematodes) from *E. vermicola* is much larger than obtained in the barley base-growth control (around 2,700 individuals) (**Figure 2**). The nematophagous effect of this fungus had been previously demonstrated, although this result could be due to the lack of lunate adhesive conidia detected in this study. Previous studies had linked the nematophagous effect to the production of this specific type of conidia (Liou et al., 1999; Kubátová et al., 2000; Wang et al., 2008, 2009, 2018; Lin et al., 2013; Pires et al., 2022).

3.2. Effect of commercial BEA on *B. xylophilus* populations

The results obtained show a clear nematicidal effect of beauvericin on populations of *B. xylophilus.* While the control treatment had a survival rate (after 48 h) of almost 60%, the BEA treatments survival rates were significantly lower ($F = 18.26$, $p < 0.001$). The 0.1 mM BEA treatment attained a survival rate of 35.1%, and 1 mM and 2 mM BEA treatments resulted in 23.5 and 20.8% survival rates, respectively, with no significant differences between them (**Figure 3**). Compared to control treatment (i.e., deducting the natural mortality), the mortality rates of each BEA treatment were 39.2, 59.2 and 63.9% (0.1, 1, and 2 mM, respectively), demonstrating the nematicidal activity of BEA.

The anthelmintic capacity of numerous compounds chemically close to BEA (cyclic-depsipeptides) have been quantified on many occasions (Scherkenbeck et al., 2002; Jeschke et al., 2005; Firakova et al., 2007; Prosperini et al., 2017). More specifically, the disruptive effect of BEA on the model nematode *C. elegans*

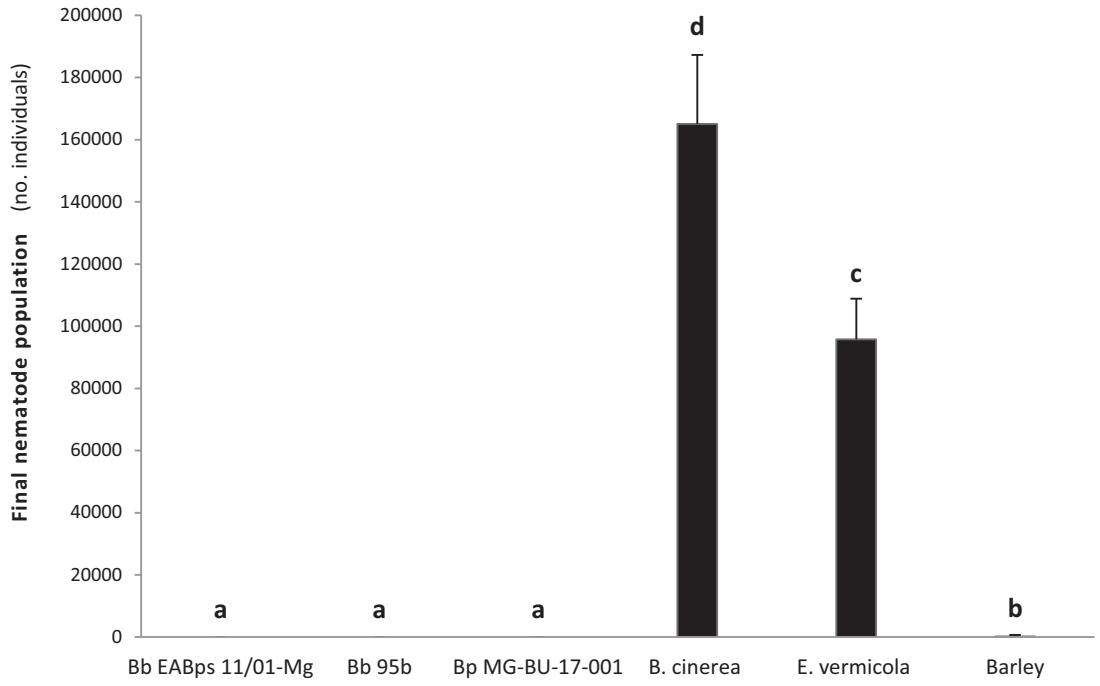

FIGURE 2
Effect on *B. xylophilus* populations from *B. bassiana* and *B. pseudobassiana* strains after 13 days. Bars with different letters indicate significantly different means (α = 0.05).

(Büchter et al., 2020) and on *B. xylophilus* (Shimada et al., 2010) has been confirmed. Comparing the results of beauvericin obtained from *F. bulbicola* (Shimada et al., 2010) with the commercial one used in this study, both exhibited similar linear trends: with higher concentrations of BEA resulting in higher mortality, although this effect seems to stabilize when the concentration approaches 2 mM. In the present study even the lowest BEA treatment (0.1 mM) showed a high nematode mortality (≈ 23% higher than mock treatment), however, Shimada et al. (2010) found only weak nematicidal activity using the same concentration. Of the concentrations of BEA tested, it appears the most suitable one for field application is 1 mM BEA, since it retained a similar nematicidal effect when compared with the 2 mM solution, but with lower product expenditure.

The mechanism of action of BEA on animal cells is still not completely understood, but seems linked to its ionophoric activity, which increases ion permeability in biological membranes and the consequent oxidative stress at molecular level (Mallebrera et al., 2018). In our trials, a brownish "encapsulation" was observed during the final visual assessment of BEA treatments individuals (**Figure 4**) which could be related to the combination of BEA molecules to the lipids of the nematode epicuticle.

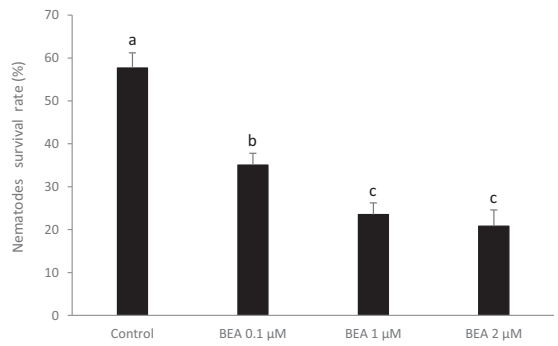

FIGURE 3
Survival rate of *B. xylophilus* in control (DMSO 5%) and treatments (BEA + DMSO 5%). Bars with different letters indicate significantly different means (α = 0.05).

3.3. Extraction, identification and quantification of BEA produced by our indigenous strains of *Beauveria* spp.

Beauvericin (BEA) was positively identified in all three strains using a mixture of UV, MS and HPLC column retention. Shown in **Figure 5**, is chromatograms of the BEA standard and *B. pseudobassiana* MG-BU-17-001, in both UV (200 nm) and single ion mode (SIM) MS (m/z = 784.3). BEA was assigned as a single peak [retention time (RT) = 6.85 min (UV) and 6.86 min (MS)] which was found in all experiments. This assignment was further confirmed by comparing the MS spectra (m/z = 100–1,050) of the BEA standard with all three strains showing we believe, for the first time the presence of BEA in *B. pseudobassiana* (**Figure 6**).

All three strains tested produced BEA, but significant differences were found between them (F = 10.06, p = 0.02). *B. bassiana* EABps 11/01-Mg exhibits the highest levels of BEA (151.4 µg/mL), followed by *B. bassiana* 95b (119.3 µg/mL) and lastly *B. pseudobassiana* MG-BU-17-001 (56.7 µg/mL) (**Figure 7**).

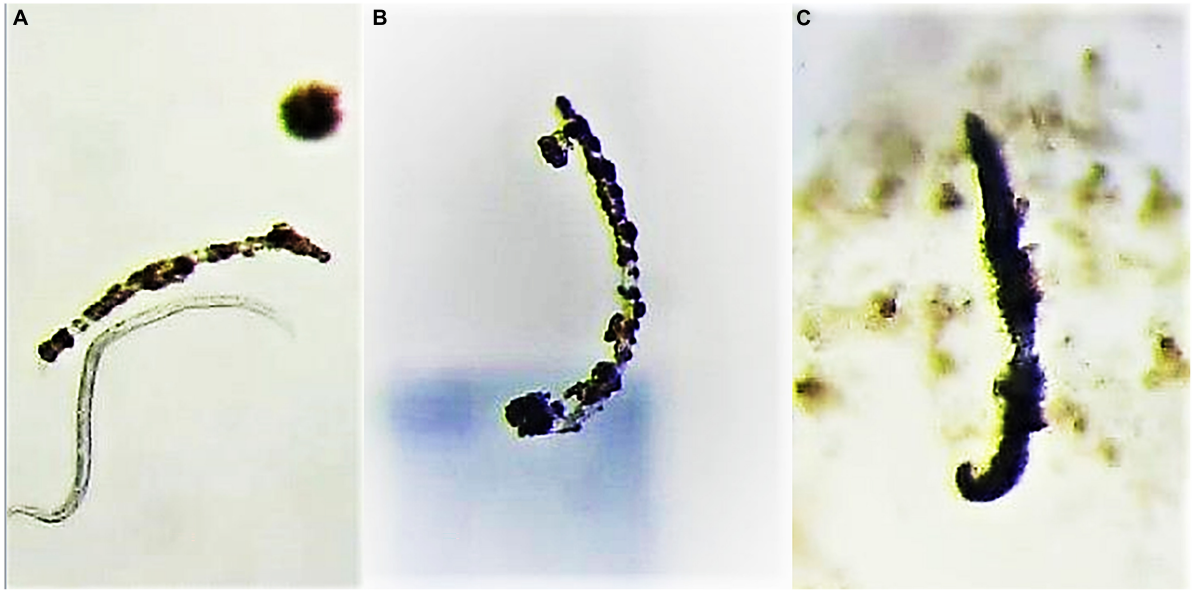

FIGURE 4
A nematode with a normal appearance together with a "encapsulated" one in 0.1 mM BEA **(A)** and other "encapsulated" nematodes found in 1 and 2 mM BEA treatments [**(B,C)**, respectively].

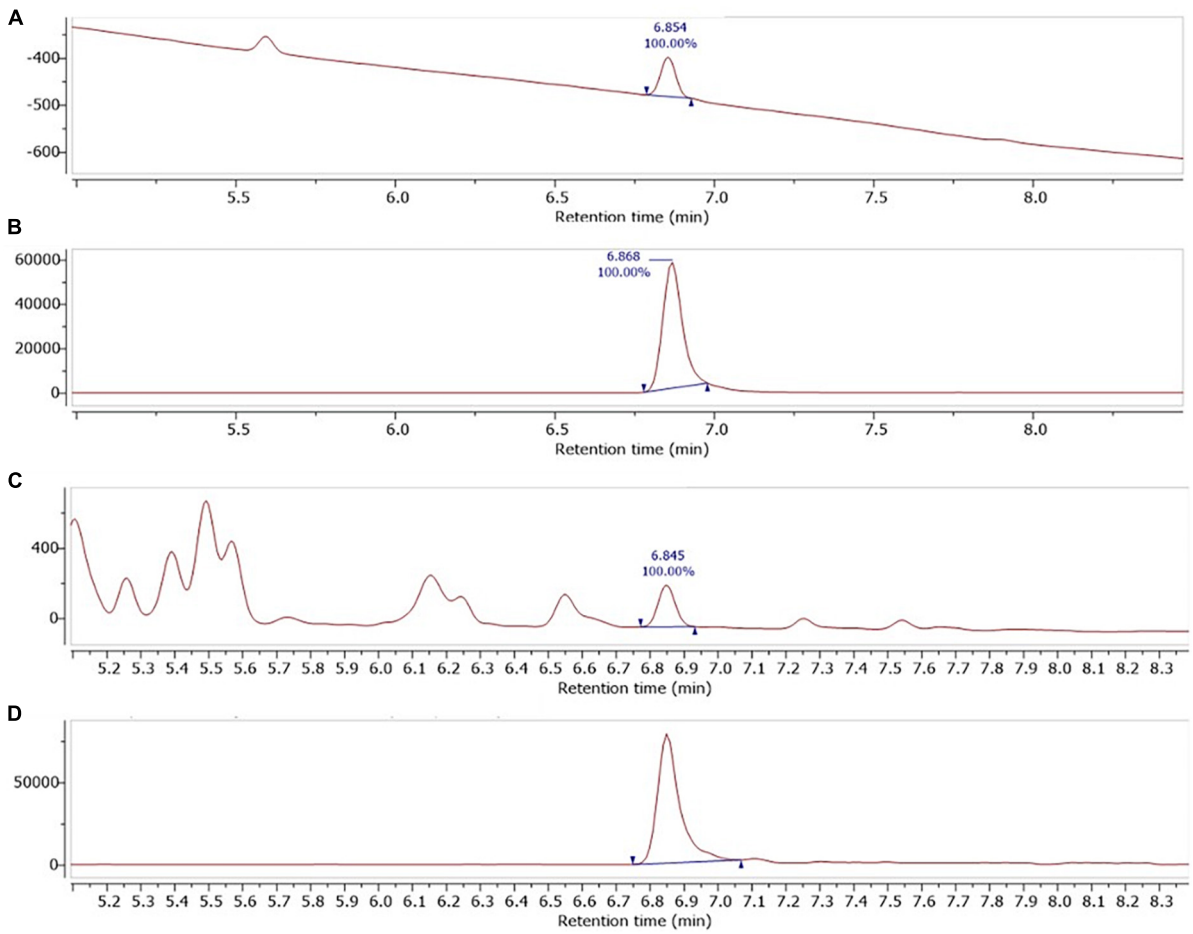

FIGURE 5
UV diode array detector (DAD) (200 ± 10 nm) and single ion mode (SIM) (784.3) atmospheric pressure chemical ionisation (APCI) chromatograms of, **(A,B)** BEA standard (10 μg/mL), and **(C,D)** *B. pseudobassiana* MG-BU-17-001.

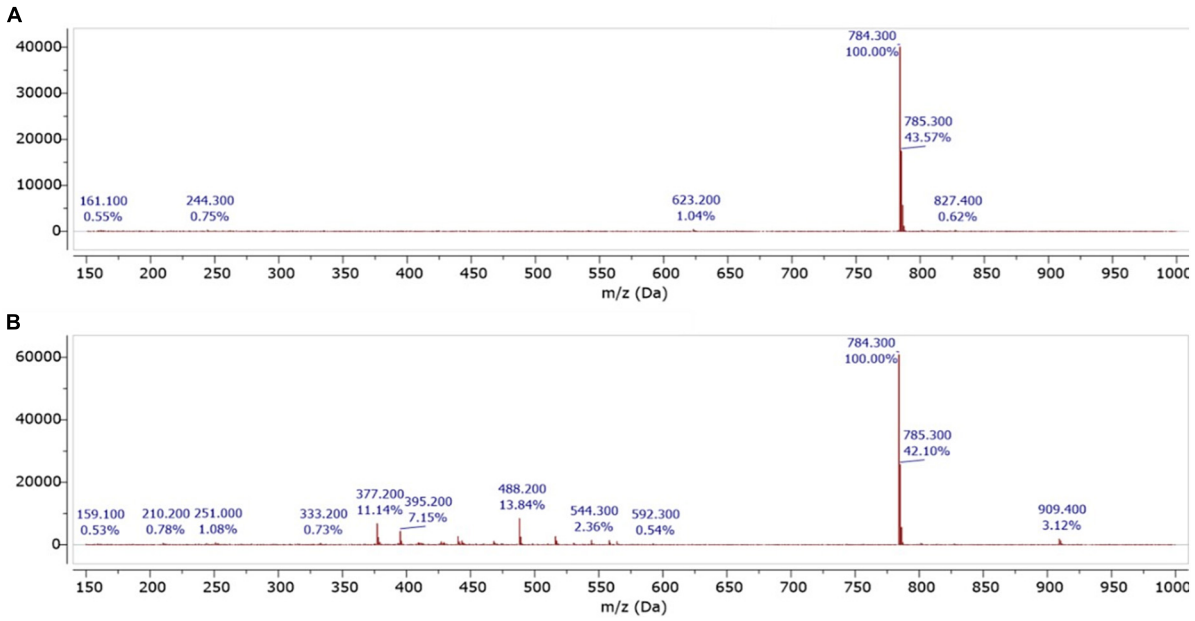

FIGURE 6
APCI MS spectra for **(A)** BEA standard (10 μg/mL), and **(B)** *B. pseudobassiana* "MG-BU-17-001."

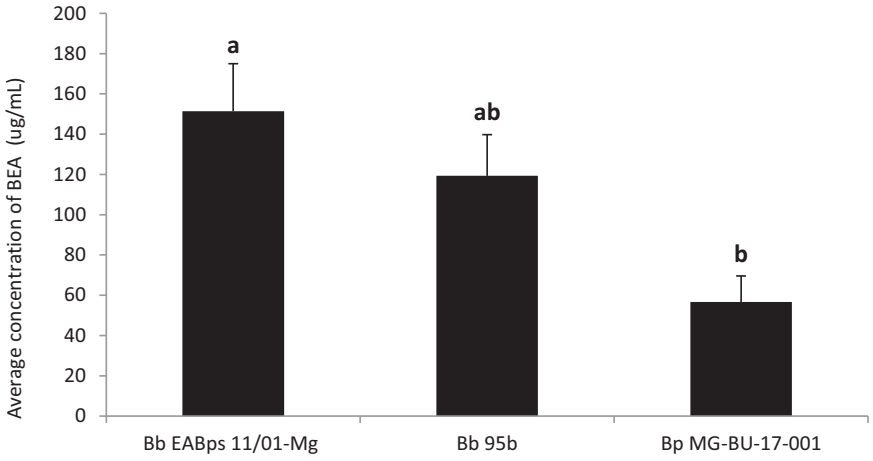

FIGURE 7
Average concentration of BEA (μg /mL) in extracts obtained from the three strains of *Beauveria* spp. Bars with different letters indicate significantly different means (α = 0.05).

While previous studies have confirmed the production of BEA by *B. bassiana* (Hamill et al., 1969; Valencia et al., 2011; Berestetskiy et al., 2018), this assay showed that the *B. bassiana* strains tested here produced a higher amount of beauvericin than other strains tested by Valencia et al. (2011), which should lead to stronger nematicidal and insecticidal effects. Moreover, to our knowledge, this is the first study that identified and quantified this mycotoxin in extracts of *B. pseudobassiana*. This finding opens the possibility of using both *Beauveria* species as potential antagonistic organisms against *B. xylophilus*. Further studies testing more strains of both species are needed to elucidate whether the differences found in this study are related to intraspecific or interspecific variability. Beyond the field of biopesticides, this outcome has parallel relevance for nutrition and human health, since BEA is classified as an emerging mycotoxin (Jestoi, 2008; Vaclavikova et al., 2013; EFSA, 2014) with high occurrence in food commodities (Al Khoury et al., 2021). It is therefore vital for food monitoring chains to have as much information as possible on all fungal species able to produce BEA.

4. Conclusion

Taking into account the high risk of spread of *B. xylophilus* and the serious threat that this species of nematode poses to European pines, the development of an effective tool for controlling the pine wilt disease is essential. Different antagonistic

fungi could exert biological control against the nematode or the beetle vector, although ideally a single fungus would be used to control both. This study found that *Beauveria* species, which had already demonstrated its entomopathogenic activity against *M. galloprovincialis* (Álvarez-Baz et al., 2015) also exhibited nematicidal activity against *B. xylophilus*. This finding paves the way for new research into the potential of these fungal strains to be tested in a self-infection device for PWN population control (Sacristán-Velasco et al., 2018) and their subsequent implementation in field conditions.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The manuscript presents research on animals that do not require ethical approval for their study.

Author contributions

JM-G, TS-G, and PZ conceived and designed the experiment. TS-G, PZ, MB, and SH performed the experiments. JM-G analyzed the data. TS-G and JM-G wrote the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The handling editor IM is currently organizing a research topic with the author JD.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/ffgc.2023.1229456/full#supplementary-material

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