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EFFECT OF *Beauveria brongniartii* AND *B. bassiana* ON OIL PALM BAGWORM, *Pteroma pendula* (Joannis)

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ABSTRACT

Beauveria bassiana *and* B. brongniartii *strains 1, 2 and 3, were evaluated for their pathogenicity against the oil palm bagworm,* Pteroma pendula. *In a laboratory trial, the fungal isolates were found to cause mortality of up to 100% in the first and second larval instars of* P. pendula. *The lethal dose causing 80% mortality values (LD_{80}) for* B. bassiana *and* B. brongniartii *strains 1, and 2 was 1 x 10^7 spores ml^{-1}. The lethal time to 50% mortality value (LT_{50}) of all the isolates was seven days and the lethal time to 80% mortality value (LT_{80}) was 11 days. The results suggested that all four isolates were equally pathogenic to bagworm larvae at a high dose of spore concentrations.*

Keywords: entomopathogenic, efficacy, *Beauveria bassiana*, *Beauveria brongniartii*, *Pteroma pendula*.

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INTRODUCTION

Entomopathogenic fungi are found in the divisions Zygomycota, Ascomycota and Deuteromycota (Samsons *et al.*, 1988). Entomopathogenic fungi, in particular those belonging to the division Deuteromycota, including *Beauveria bassiana*, are potential candidates as microbial control agents because of their rapid rate of kill in laboratory assays, relative to other entomopathogens (Abdo *et al.*, 2008). According to Kessler *et al.* (2003), *B. brongniartii* (Saccardo) Petch is another entomopathogenic fungus which has been used for several years in Switzerland to control the European cockchafer larvae, *Melolontha melolontha* L.

B. brongniartii is thought to have a narrow host range (Traugott *et al.*, 2005), and it was mostly isolated from insects belonging to the Scarabaeidae family (Humber 1997). The use of *B. bassiana* together with *Metarhizium anisopliae* for controlling *Delia radicum* confirmed that insect pathogenic fungi are important in agricultural systems (Bruck *et al.*, 2005).

Bagworms (*Metisa plana*) have always been a major pest of the oil palm (*Elaeis guineensis* Jacquin) in Malaysia, and are becoming more serious in certain parts of Malaysia (Basri *et al.*, 1988). In recent studies, *B. bassiana* has been reported to infect this oil palm pest *Metisa plana* (Walker) (Ramle and Basri, 2004).

Previously, *B. bassiana* isolated from *M. plana* in Kapar, Selangor, was identified as one of the most important factors in controlling bagworm outbreaks (Ramlah Ali *et al.*, 1994). According to Ramlah Ali *et al.* (1993), *B. bassiana* is capable of infecting the second and fourth instars of *M. plana*.

This article reports on the effects of three *B. brongniartii* strains and of *B. bassiana* on the mortality of the oil palm bagworm, *Pteroma pendula.*

MATERIALS AND METHODS

Source of *Beauveria* spp.

Three strains, namely *B. brongniartii* strains 2 and 3 and *B. bassiana*, were isolated from dead larvae in an indoor insectory at the Malaysian Palm Oil Board, while *B. brongniartii* strain 1 was isolated from dead

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larvae collected at FELDA Besaut. *B. brongniartii* strains 1, 2 and 3 and *B. bassiana* were maintained in malt extract agar (MEA) at 28 ± 1ºC in the dark, and stocked in mineral oil.

Identification of *Beauveria* spp.

Microscopic identification. Morphological and microscopic observations on *Beauveria* spp. were made by light microscopy on the isolates cultured on MEA plates. The cellophane tape method was used for identification of the species (Forbes *et al.*, 2002).

RAPD-PCR. RAPD-PCR of total DNA using five different primers, D01 (CAGGCCCTTC), D02 (TGCCGAGCTG), D03 (AGTCAGCCAC), D04 (AATCGGGCTG) and D05 (AGGGGTCTTG) (1st Base Laboratories), were carried out for further confirmation of the species. The PCR reactions were performed in 25 μl reaction volumes containing 2 μl DNA, 2 μl primer, 0.4 μl taq polymerase, 0.5 μl dNTPs, 2.5 μl 10X complete PCR buffer and 17.6 μl distilled water. Amplifications were performed in a Eppendorf gradient PCR thermocycler at an initial 94ºC denaturation temperature for 2 min, followed by 35 cycles at 94ºC additional denaturation for 30 s per cycle, 60ºC annealing temperature for 30 s, 72ºC elongation for 30 s, and a final extension step at 72ºC for 2 min, and then held at 4ºC.

Bioassay of *Beauveria* spp. against *P. pendula*

The cultures were inoculated on malt extract (ME) solid medium, incubated for 10-15 days at 25ºC and 16L/8D photoperiod. Conidia which developed on the MEA were harvested directly from the fungal cultures by scraping the sporulating colonies and suspending them in 10 ml spore suspension solution containing 0.2% Tween 80 and 0.89% NaCl. The concentration of conidia was determined using an improved haemocytometer method with the aid of a light microscope. Three conidial suspensions, namely, 1 x 10^6, 1 x 10^7, 1 x 10^8 conidia ml^{-1}, were used in the bioassay. Both sides of the oil palm leaflets were sprayed to ensure that the conidia were evenly distributed. The control leaflets were sprayed with only the suspension solution without any conidia. Bioassay was conducted in four replicates with each replicate containing five larvae of *P. pendula* (20 larvae per concentration). These larval instars were obtained from MPOB Teluk Intan. Larval mortality was recorded daily over 13 days after treatment (DAT).

Statistical Analysis

Mortality data at 3, 7 and 11 DAT were analysed separately by the one-way analysis of variance (ANOVA) and by Fischer's least significant difference (LSD) test ($P<0.05$). All statistical analyses were carried out using the software SPSS version 11.5.

RESULTS AND DISCUSSION

Microscopic Identification of *Beauveria* spp.

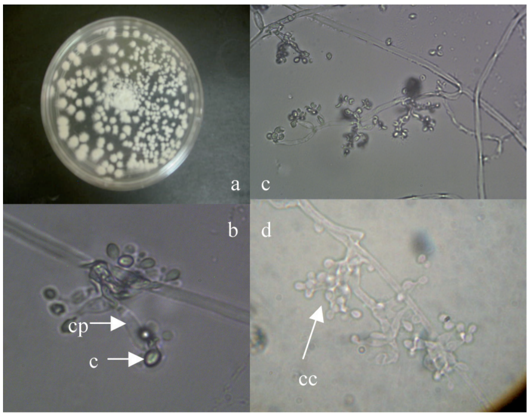
All isolates of the *Beauveria* spp. tested showed colonies that were powdery in texture on MEA. The colony surface was light yellowish to white, while the reverse was white or pale in colour. Although morphologically distinct as a genus, species identification in *Beauveria* is difficult because of its structural simplicity (Rehner and Buckley, 2005). *Beauveria* conidiophores consist of whorls and dense clusters of sympodial, short and globose or flask-shaped conidiogenous cells (Glare and Inwood, 1998). Microscopic observations showed the hyphae were hyaline, septate and narrow. The conidia were hyaline, one-celled and globose to ovoid in shape. The first, second and third isolates showing conidia which were more cylindrical in shape (*Figures 1, 2* and *3*) were identified as *B. brongniartii* and designated as strains 1, 2 and 3. The fourth isolate showed mainly spherical conidia, and was identified as *B. bassiana* (*Figure 4*).

Molecular Techniques

RAPD-PCR profiles of the three *B. brongniartii* strains and *B. bassiana* were conducted in order to confirm the species identification made by microscopy. From the following patterns generated by the RAPD primer profiles (*Figure 5*), B1 (*B. brongniartii* strain 1) and B2 (*B. brongniartii* strain 2) were quite similar because the band patterns produced were identical whereas for B3 (*B. brongniartii* strain 3), similar patterns as B1 and B2 were detected only by primers D01, D04 and D05. Band patterns of *B. bassiana* (B4) were quite different from the other three strains for all five primers. The results confirmed the identification made by microscopic observations.

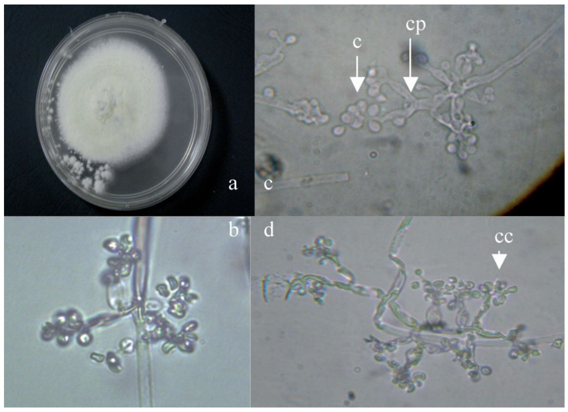
Bioassay

At the dose of 1 x 10^6 conidia ml^{-1}, *B. brongniartii* strain 1 reached LT_{70} at 11 DAT with a corrected mortality of 83.33% (*Figure 6a*). The corrected mortality induced by *B. brongniartii* strain 1 at seven DAT for all concentrations was significantly higher than the control ($P<0.05$) (*Figure 6a*). LT_{80} was achievable at 11 DAT for all concentrations, with the highest mortality of 100% at the dose of 1 x 10^8



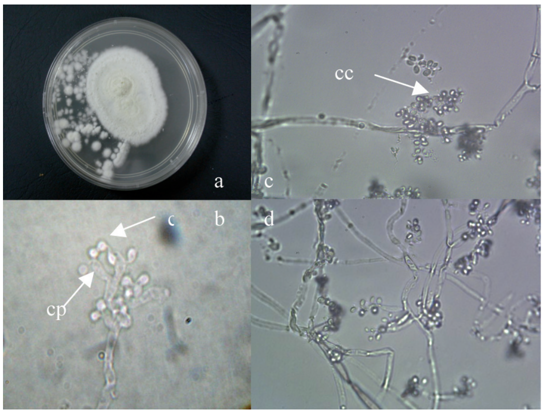
Note: c = conidium, cp = conidiophore, cc = conidiogenous cell.

Figure 1. Microscopic observations on B. brongniartii *strain 1 on malt extract agar (MEA). (a) Spores at 4000X magnification, (b), (c) and (d) conidia and conidiophores at 1000X magnification.*



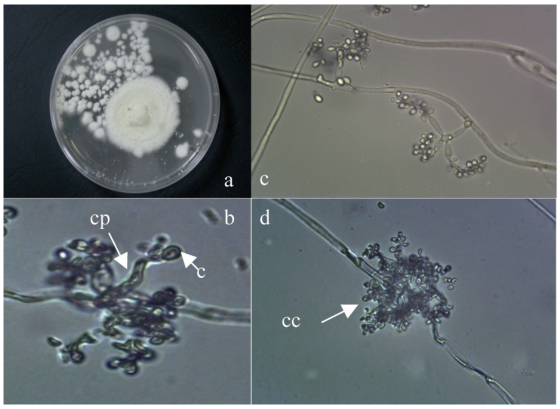
Note: c = conidium, cp = conidiophore, cc = conidiogenous cell.

Figure 2. Microscopic observations on B. brongniartii *strain 2 on malt extract agar (MEA). (a) Spores at 4000X magnification, (b), (c) and (d) conidia and conidiophores at 1000X magnification.*



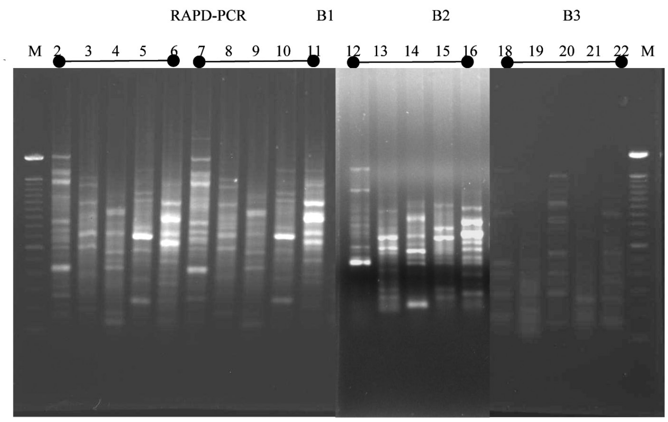
Note: c=conidium, cp=conidiophore, cc= conidiogenous cell.

Figure 3. Microscopic observations on B. brongniartii *strain 3 on malt extract agar (MEA). (a) Spores at 4000X magnification, (b), (c) and (d) conidia and conidiophores at 1000X magnification.*



Note: c=conidium, cp=conidiophore, cc= conidiogenous cell.

Figure 4. Microscopic observations on B. bassiana *on malt extract agar (MEA). (a) Spores at 4000X magnification, (b), (c) and (d) conidia and conidiophores at 1000X magnification.*



Note: Lanes 1 and 17 is ladder 100 bp (M), lanes 2 to 6 is *B. brongniartii* strain 1, lanes 7-11 is *B. brongniartii* strain 2, lanes 12 to 16 is *B. bassiana*, and lanes 18 to 22 is *B. brongniartii* strain 3. The five different primers were D01 (CAGGCCCTTC), D02 (TGCCGAGCTG), D03 (AGTCAGCCAC), D04 (AATCGGGCTG and D05 (AGGGGTCTTG).

Figure 5. RAPD profiles using five different primers of B. bassiana *and three strains of* B. brongniartii.

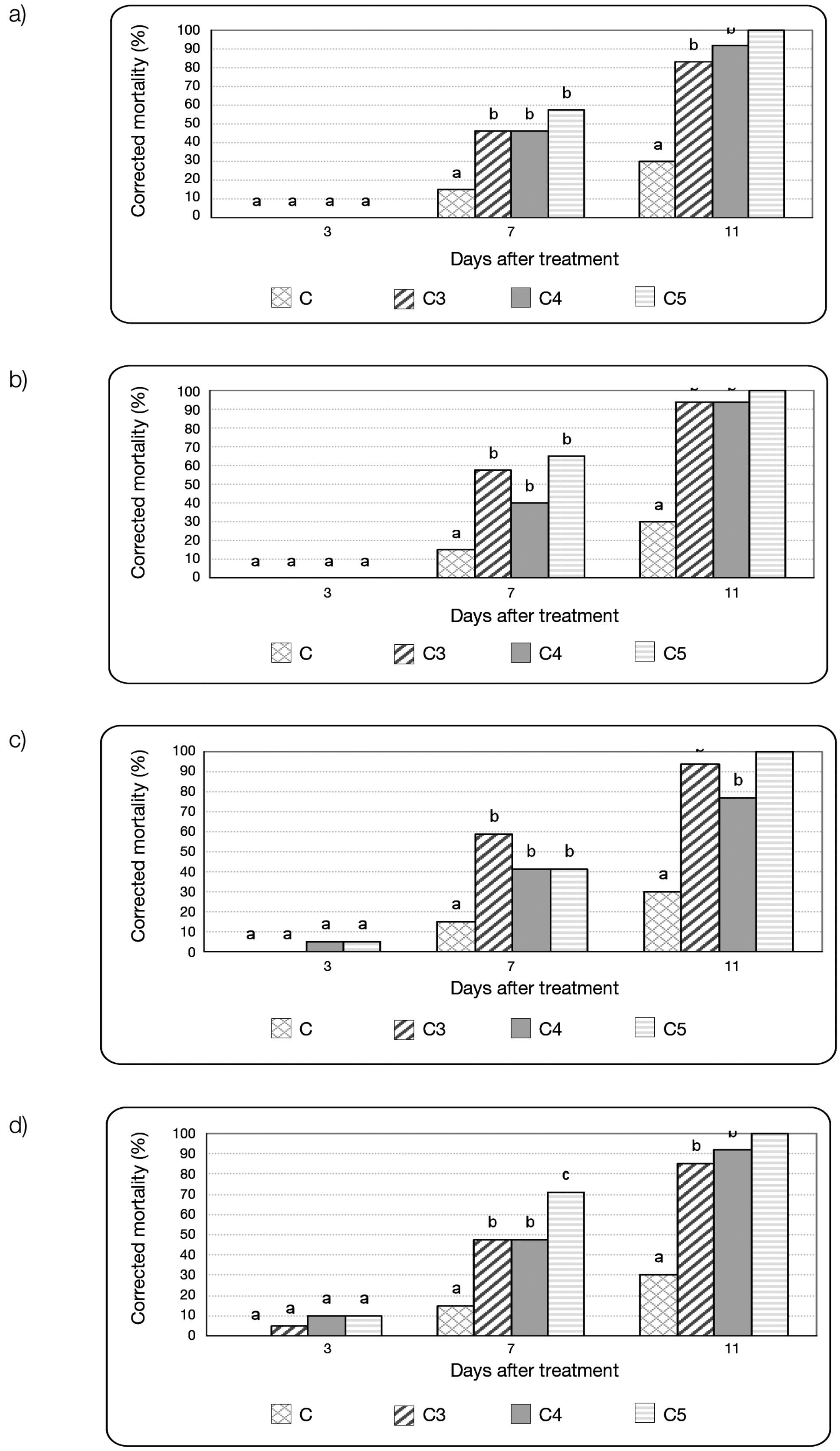
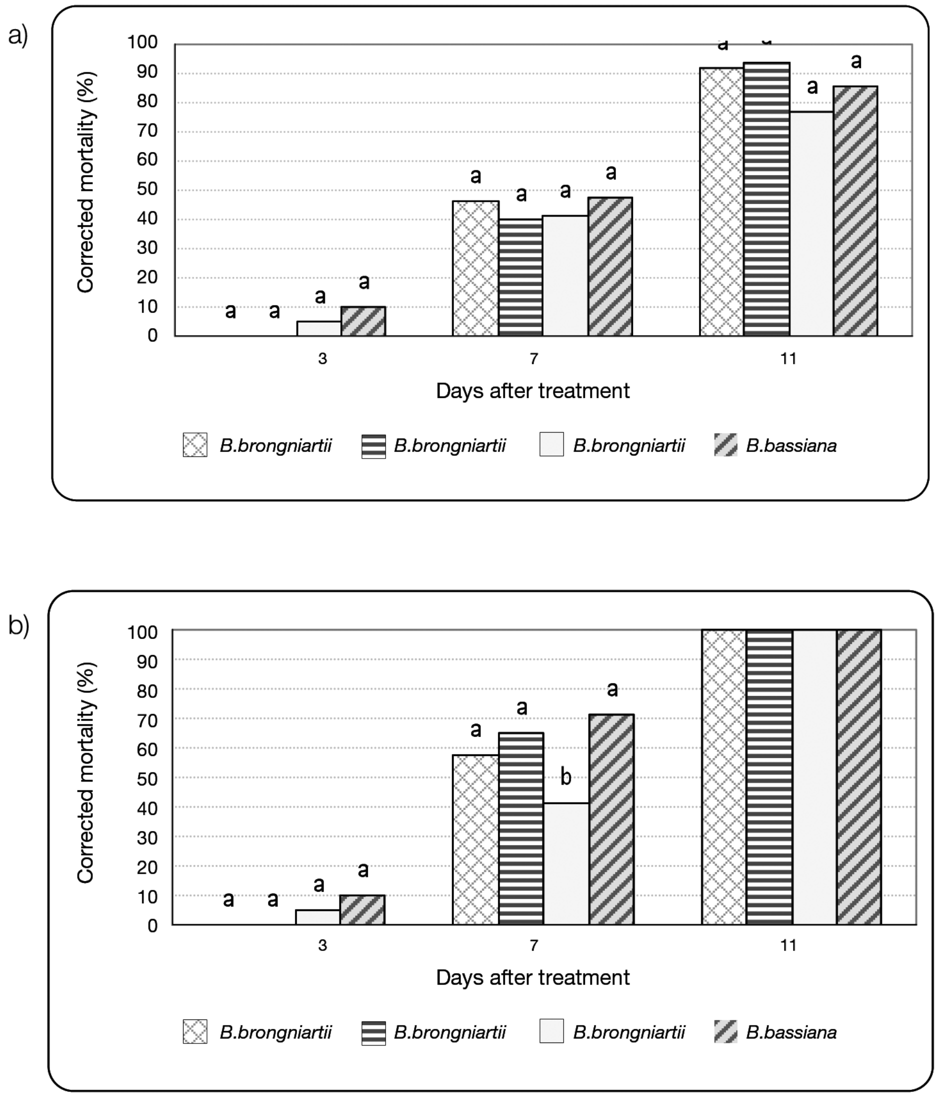


Figure 6. Corrected mortality of P. pendula *subjected to (a)* B. brongniartii *strain 1, (b)* B. brongniartii *strain 2, (c)* B. brongniartii *strain 3, and (d)* B. bassiana *propagated on malt extract agar (MEA). Concentrations used, c3= 1 x 10^6 spore ml^{-1}; c4= 1 x 10^7 spore ml^{-1}; c5= 1 x 10^8 spore ml^{-1}, and C= control without inoculum. Bars within a group with different letters are significantly different according to the LSD test ($P<0.05$).*



Note: Bars within a group with different letters are significantly different according to the LSD test (P<0.05).

Figure 7. Corrected mortality of P. pendula *subjected to* B. brongniartii *strains 1, 2, 3 and* B. bassiana *propagated on malt extract agar (MEA) using (a) 1 x 10^7 conidia ml^{-1} and (b) 1 x 10^8 conidia ml^{-1} concentrations.*

conidia ml^{-1}. Corrected mortality for the doses of 1 x 10^6 conidia ml^{-1} and 1 x 10^7 conidia ml^{-1} was 83.33% and 91.67%, respectively.

All the different concentrations of *B. brongniartii* strain 2 also caused significantly higher mortality than the control (P<0.05) (*Figure 6b*). At 11 DAT, using a dose of 1 x 10^8 conidia ml^{-1}, the mortality of *P. pendula* was 100%. The lower doses, 1 x 10^6 conidia ml^{-1} and 1 x 10^7 conidia ml^{-1}, resulted in 93.75% mortality.

At seven DAT with a dose of 1 x 10^6 conidia ml^{-1}, *B. brongniartii* strain 3 caused 58.75% mortality (*Figure 6c*). The corrected mortality induced by *B. brongniartii* strain 3 was significantly higher than the control (P<0.05). At 11 DAT, LT_{80} was achieved at doses of 1 x 10^8 conidia ml^{-1} and 1 x 10^6 spores ml^{-1} with 100% and 93.75% mortality, respectively. Mortality due to a concentration at 1 x 10^8 conidia ml^{-1} was significantly higher than those of the control and of the dose of 1 x 10^7 conidia ml^{-1}.

LT_{70} against *P. pendula* was achievable at 11 DAT for *B. bassiana* where the corrected mortality was 71.25% at a dose of 1 x 10^6 conidia ml^{-1} (*Figure 6d*). LT_{80} from solid fermentation was achievable for *B. bassiana* at 11 DAT with a corrected mortality of 91.67% for the dose of 1 x 10^7 conidia ml^{-1}. At 11 DAT, the highest dose, 1 x 10^8 conidia ml^{-1} of *B. bassiana*, led to 100% mortality which was significantly higher (P<0.05) than that of the control.

Unlike the *B. brongniartii* strains, a concentration of 1 x 10^7 conidia ml^{-1} for *B. bassiana* (*Figure 7a*) caused 10% mortality as early as three days after incubation. At 11 DAT, *B. brongniartii* strains 1 and 2 showed the highest mortality values of 91.67%

and 93.75%, respectively, while *B. bassiana* and *B. brongniartii* strain 3 showed 85.42% and 77.08% mortality, respectively. The results showed no significant difference in mortality between the two *Beauveria* species.

At the dose of 1 x 10^8 conidia ml^{-1} (*Figure 7b*), *B. bassiana* caused 71.25% mortality on day 7 compared to *B. brongniartii* strain 2 with 65% mortality, *B. brongniartii* strain 1 with 57.5% mortality and *B. brongniartii* strain 3 with 41.25% mortality. The corrected mortality values induced by *B. bassiana*, *B. brongniartii* strains 1 and 2 were significantly higher than that of *B. brongniartii* strain 3 ($P<0.05$). No significant difference was observed at 11 DAT among the treatments indicating that *B. bassiana* and the three strains of *B. brongniartii* were equally pathogenic and suitable for controlling the larvae of *P. pendula*.

All fungal isolates tested were able to infect the first and second larval instars of *P. pendula* in the laboratory, and were considered as pathogens of the bagworms. The highest mortality of 100% was obtained with the dose of 1 x 10^8 conidia ml^{-1} (*Figure 7b*). Abdo *et al.* (2008) reported that conidial concentrations from *B. bassiana* and *B. brongniartii* caused very high mortality rates of more than 90% after seven days when used against the third larval instar of *Cephalcia tannourinensis*, the cedar sawfly. High mortalities were also reported by Brockerhoff *et al.* (2002) who used *B. bassiana* to treat the spruce seed moth, *Cydia strobilella*, which resulted in 100% mortality under laboratory conditions.

CONCLUSION

The laboratory bioassay demonstrated that all four isolates of the two *Beauveria* spp. were pathogenic to *P. pendula* larvae. The results suggest that these four fungal isolates act as a larval pathogen by infecting the pest even when low doses of spores were applied. Thus, higher doses will cause higher rates of mortality and shorten the time to death. However, further studies need to be conducted to increase spore yield and to improve the efficacy of these entomopathogenic fungi.

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