EFFICACY OF ENTOMOPATHOGENIC FUNGI, *Paecilomyces* spp., IN CONTROLLING THE OIL PALM BAGWORM, *Pteroma pendula* (Joannis)

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ABSTRACT

Laboratory efficacy of the entomopathogenic fungi, Paecilomyces carneus *and* P. farinosus*, was assessed against the first larval instars of* Pteroma pendula*, using conidia generated on solid fermentation media. Three isolates of* P. carneus *and three isolates of* P. farinosus *were infective on the first larval instars of* P. pendula*. Most of the* Paecilomyces *spp. caused a significant increase in mortality between seven days after treatment (DAT) and 11 DAT. However, a dramatic increase in mortality was recorded with isolate PC2 of* P. carneus *whose effectiveness increased nearly three-fold over this period. The highest corrected mortality after 11 DAT varied from 75% for* P. farinosus *to 93.8% for* P. carneus*. Among the tested isolates,* P. carneus *caused the highest corrected mortality of 93.8% at 11 DAT, while a corrected mortality of only 75% was induced by* P. carneus *isolated from the soil. The most successful isolate was of* P. carneus *which controlled over 80% of the first larval instars of the* P. pendula *population at 11 DAT. This indicates that the efficacy of P. carneus is superior to that of* P. farinosus *against the first larval instars of* P. pendula*, and can be exploited as biological control agents of the oil palm pest bagworm.*

Keywords: entomopathogenic fungi, *Paecilomyces* spp., efficacy, bagworms, oil palm.

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INTRODUCTION

The continuous use of chemicals has affected beneficial insects such as the pollinators and natural enemies of pests, as well as the environment. Biological control comprises the more target-specific components of integrated pest management programmes under oil palm. Bagworms are common and serious pests of oil palm (*Elaeis guineensis* Jacq.) in Malaysia (Basri *et al*., 1988). The bagworms, *Pteroma pendula* (Joannis) and *Metisa plana* (Walker) (Lepidoptera Psychidae), are among the most important occasional pests in oil palm plantations in Malaysia since 1956 (Wood, 1968). During the period from 1981 to 1985, more than 10 000 ha of oil palm were seriously attacked by bagworms (Basri *et al.,* 1988). MPOB data in 2005 recorded that bagworms were a serious problem in the oil palm industry as 35 657 ha were attacked by these pests (Norman and Basri, 2007). Although bagworms are presently under control by *Bacillus thuringensis*, they still pose a major threat to the oil palm industry, capable of causing serious outbreaks in Malaysia. Apart from *B. thuringensis*, many other microbes can possibly be exploited as environmentally safe biocontrol agents.

Species of *Paecilomyces*, such as *P. fumosoroseus*, *P. carneus* and *P. farinosus,* are common entomogenous fungi, geographically widespread and can also be isolated from soil samples (Samson, 1974). *Paecilomyces* spp. infect more than 40 insect species (Smith, 1993), and produce blastospores in liquid media (Jackson *et al*., 1997; Vandenberg *et al*., 1998) and conidia on solid media (Fernando *et al*., 1999).

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P. fumosoroseus has been known to infect the whitefly, *Bemisia tabaci* (Smith, 1993; Wraight *et al*., 2000). Some strains of *P. lilacinus* frequently isolated from soil, particularly in samples originating from warmer regions, have been shown to be mycoparasitic, capable of penetrating the exoskeleton (cuticle) of the insect, and proceeding to grow until the insect dies (Gupta *et al*., 1993). These fungi are being developed for use against a range of agricultural pests, where biocontrol may offer an effective alternative for the control of insects which have increasingly become resistant to chemical pesticides.

In previous years, an entomopathogenic fungus, *P. farinosus*, has been reported to attack *Mahasena corbetti* in an insectary in MPOB, Bangi (Ramlah *et al*., 1994), suggesting that it could be an alternative biological agent against the bagworm. In recent studies, other entomopathogenic fungi attacking oil palm insect pests have also been reported, *e.g. Beauveria bassiana* against *Metisa plana* (*Walker*) (Ramle and Basri, 2004) and *Metarhizium anisopliae* against *Oryctes rhinoceros* (Ramle *et al*., 2006). This article aims to report the results of screening the efficacy of two species of the entomopathogenic *Paecilomyces* spp. as alternative biocontrol agents against bagworms.

MATERIALS AND METHODS

Origin of *Paecilomyces* spp.

Six species of *Paecilomyces* were isolated on malt extract media and identified from samples of soil collected from under oil palms and samples from cadavers of bagworm larvae. These isolates were maintained on malt extract agar (MEA) at 28°C in the dark and stocked in mineral oil. Two isolates each of *P. carneus* (PC1 and PC2) and of *P. farinosus* (PF1 and PF2) were from cadavers of bagworm larvae, whereas *P. carneus* isolate PC3 and *P. farinosus* isolate PF3 were from soil sampled under oil palms from MPOB, Keratong.

Morphological Identification of *Paecilomyces* spp.

Morphological and microscopic observations on the isolated fungal strains were conducted using the cellophane tape method (Forbes *et al*., 2002), and studied under a light microscope. The isolates were cultured on MEA plates and incubated at 25°C-30°C.

Identification of Strains of *Paecilomyces* spp. by Using RAPD-PCR

DNA of the *Paecilomyces* spp. were extracted using the boiling method (Millar *et al*., 2000). Cultures of the *Paecilomyces* spp. were scraped and the scrapings were placed in an eppendorf tube with 500 μl of double distilled water, and boiled for 20 min. After boilling, the cultures were put on ice for 5 min, and centrifuged at high speed for another 5 min. The supernatant was used in the PCR mixture as DNA template. The RAPD primers used were DO1 (5′-TGCCGAGCTG-3′), DO2 (5′-AGTCAGCCAC-3′), DO3 (5′AATCGGGCTG-3′) and DO4 (5′-AGGGGTCTTG-3′) (1^{st} Base Laboratories, Malaysia). The amplification mixture with the RAPD primers had a final volume of 25 μl, and contained 10 pmol of each primer, 100 m*M* dNTPs, 1X PCR buffer, 50 m*M* Mg_2Cl, 0.3% BSA and 2.5 units of Taq polymerase. The reaction began with an initial 94°C denaturation for 2 min, followed by 35 cycles at 94°C for 30 s, 60°C for 30 s, 72°C for 30 s, a final extension at 72°C for 2 min, and then held at 4°C. PCR products were run on 2% agarose gel at 80V for 1 hr.

Propagation of Entomopathogenic Fungi *Paecilomyces* spp.

Solid media were used to cultivate the *Paecilomyces* cultures. The cultures were inoculated on MEA solid agar, incubated for 10-15 days at 25°C and 16 L/8D photoperiod. The conidia which developed on the MEA agar plates were suspended in 10 ml of spore suspension solution containing 0.2% Tween 80 and 0.89% NaCl. The concentration of conidia from both media was determined by a hemocytometer and examined under a light microscope.

Bioassay of *Paecilomyces* spp.

Two conidial suspension concentrations, 1×10^7 and 1×10^8 cfu 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 0.2% Tween 80 solution only. A bioassay was conducted in four replicates, each with five larvae. The first larval instars of *P. pendula* were obtained from an oil palm plantation in Jeram, Kuala Selangor, Malaysia. Larval mortality was recorded every two days over 13 days after treatment (DAT). The bioassay on *P. pendula* was repeated with the same number of replicates.

Data Analysis

Corrected mortality was calculated as in the following formula:

$$\%\text{ corrected mortality} = \frac{\%T - \%C}{100\% - \%C} \times 100$$

where %T = percentage of dead test organisms.
%C = percentage of dead control organisms.

Corrected mortality data at 3 to 13 DAT were analysed separately with the LSD test and a one-way analysis of variance (ANOVA) (P= 0.05) by using the software SPSS 11.0 for Windows.

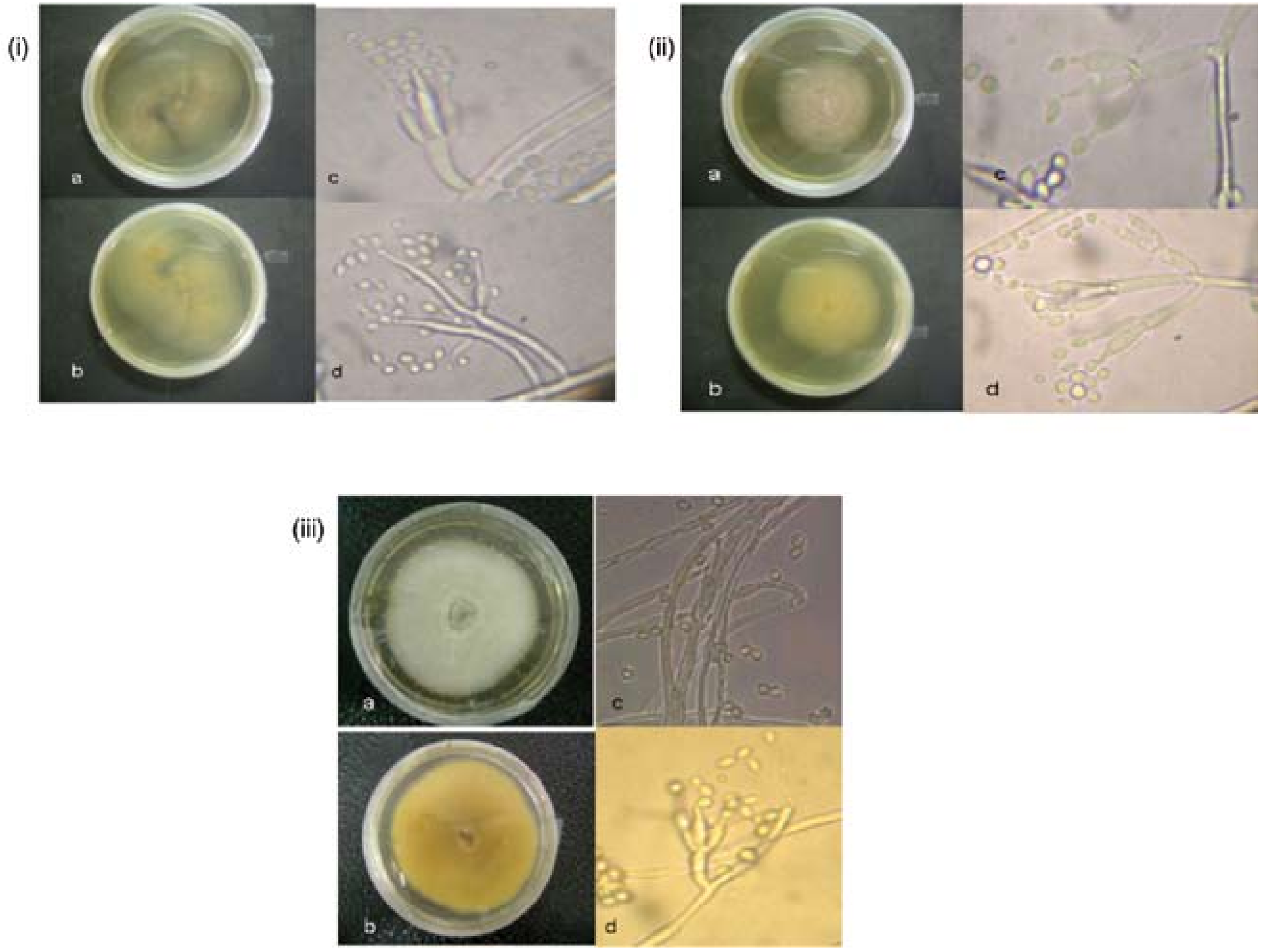
RESULTS AND DISCUSSION

Identification of *Paecilomyces* spp.

The first isolate of the *Paecilomyces* spp. showed cottony textured colonies on MEA. The colonies were white to dark grey on the surface while the bottom of the colonies was pink to brown. Microscopic observations showed that the mycelia were hyaline and septate. The conidiophores had phialides that were swollen at their bases and tapered towards their apices. The conidia were globose and measured from 2 to 3 μm at the highest magnification of 1000X. The isolate was identified as *P. carneus* [*Figure 1(i)*, *1(ii)* and *1(iii)*]. The second isolate showed colonies that were downy and cottony in texture on MEA. The colonies were white to yellow on the surface while the bottom was colourless to light yellow. The conidiophores had phialides that were swollen at their bases and tapered towards their apices. The ellipsoidal to fusiform conidia measured 2 to 3 μm and were produced on 110 to 300 μm-long conidiophores bearing several groups of phialides measuring 7 to 14 μm long. According to Samson (1974) and Domsch and Gams (1980), these morphological patterns are characteristic of *P. farinosus*, which is an ubiquitous entomopathogenic fungus [*Figure 2(i)*, *2(ii)* and *2(iii)*].

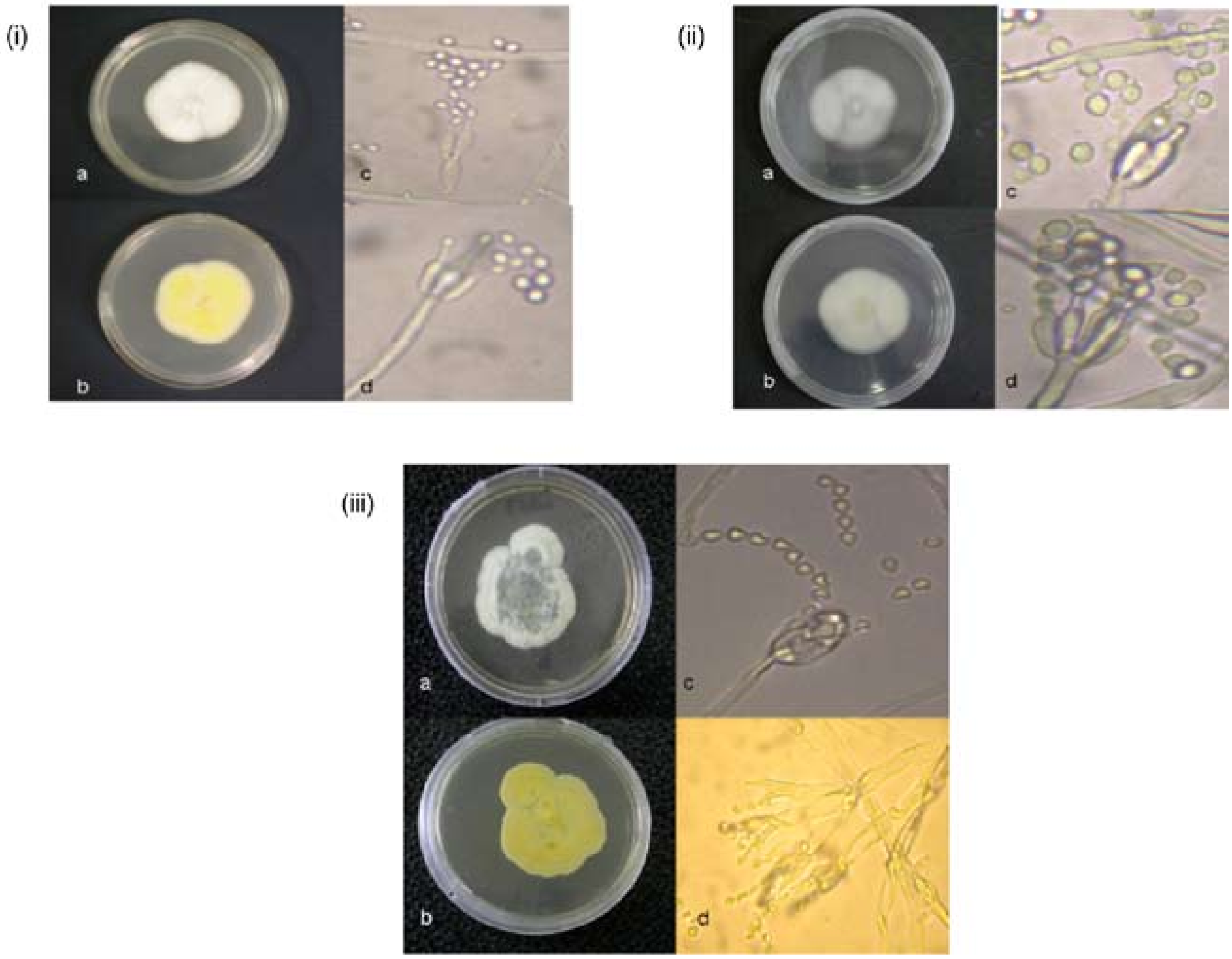
Identification of Strains of *Paecilomyces* spp. by Using RAPD-PCR

Amplification of the PCR products were obtained for all strains of *Paecilomyces* spp. using the four RAPD primers, namely, D01, D02, D03 and D04. Similar patterns were shown among the *Paecilomyces* spp. isolates except for isolate PF2, when using primers D01, D02 and D03. Primer D04 showed a similar pattern with isolates PF1, PF2 and PC1, but showed dissimilar band patterns with isolates PC3 and PF3 (*Figure 3*).



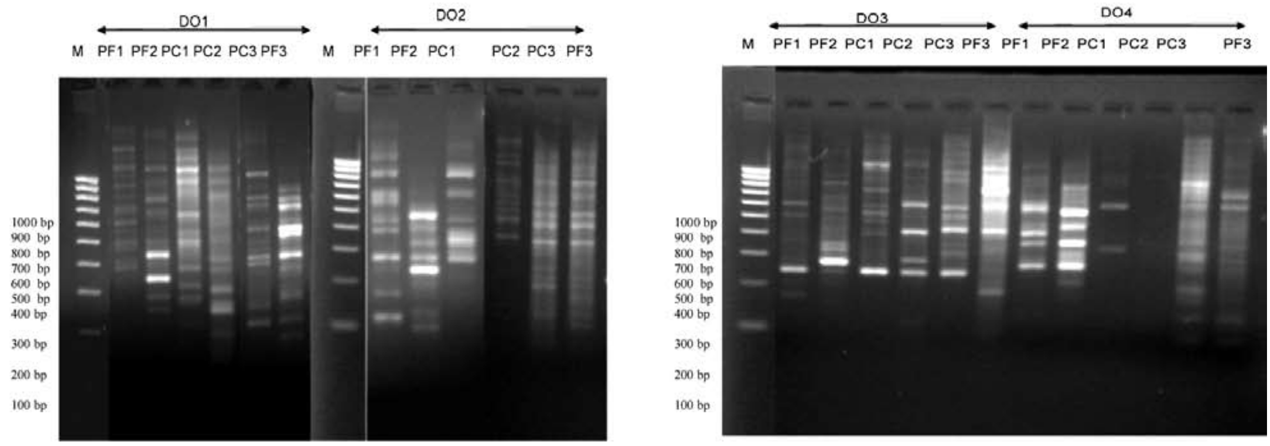
Note: *P. carneus isolates:* (i) PC1, (ii) PC2 and (iii) PC3.

Figure 1. (a) Paecilomyces carneus *on malt extract agar (MEA), (b) bottom of MEA was pink to brown, (c) conidia at 1000X magnification, and (d) conidiophore at 1000X magnification.*



Note: *P. farinosus isolates:* (i) PF1, (ii) PF2 and (iii) PF3.

Figure 2. (a) Paecilomyces farinosus *cultures on malt extract agar (MEA), (b) bottom of MEA was colourless to yellow, (c) conidia at 1000X magnification, and (d) conidiophore at 1000X magnification.*



Note: M: 100 bp DNA ladder; *P. farinosus* isolates PF1, PF2 and PF3; *P. carneus* isolates PC1, PC2 and PC3.

Figure 3. RAPD-PCR of Paecilomyces *spp. cultures (PF1-PF3) using primers: D01, D02, D03 and D04.*

Bioassay of *Paecilomyces* spp.

Three isolates of *P. carneus* (PC1, PC2 and PC3) and three of *P. farinosus* (PF1, PF2 and PF3) at different concentrations were tested against the first larval instars of *P. pendula*, using solid malt extract medium. Lethal time, $LT_{80,}$ for isolates PC1 and PC2 at 11 DAT was at the concentration of $1x10^7$ CFU ml^{-1} at a corrected mortality of 87.5%. For PF1, LT_{80} at 11 DAT was achieved at a higher concentration of $1x10^8$ CFU ml^{-1} at a corrected mortality of 81.3%.

The lowest lethal dose, LD_{80}, was for PF2 at 13 DAT, at the concentration of $1x10^6$ CFU ml^{-1} (*Figure 4*). The results for LT_{80} show that the isolates of *P. carneus* achieved higher LT_{80} compared to those of *P. farinosus*. The corrected mortality induced by *P. carneus* isolates PC1 and PC2 at the concentration of $1x10^8$ CFU ml^{-1} was significantly higher than that of *P. farinosus* at 11 DAT ($P < 0.05$). At 13 DAT, isolates PF1 to PC2 caused a mean mortality ranging from 80% to 100% except for isolate PC1 which did not reach the 80% corrected mortality (*Figure 4a*).

LC_{80} was highest for the isolates of *P. carneus*, PC1 and PC2, at the concentration of $1x10^7$ CFU ml^{-1} at 11 DAT with a mean mortality of 81.3% (*Figure 4a*). However, the soil isolate of *P. carneus*, PC3, did not reach the satisfactory value of corrected mortality for LC_{80} (*Figure 4a*).

Figure 4b shows that the *P. farinosus* isolate, PF1, reached LC_{80} after 11 DAT at a higher concentration of $1x10^8$ CFU ml^{-1}, with also a corrected mortality of 81.3%. For the soil isolate of *P. farinosus* PF3, LC_{80} was achievable at the concentration of $1x10^8$ CFU ml^{-1} when at 13 DAT the corrected mortality reached of 85.7%. The results for LC_{80} show that isolates from cadavers of bagworm larvae were more virulent than soil isolates.

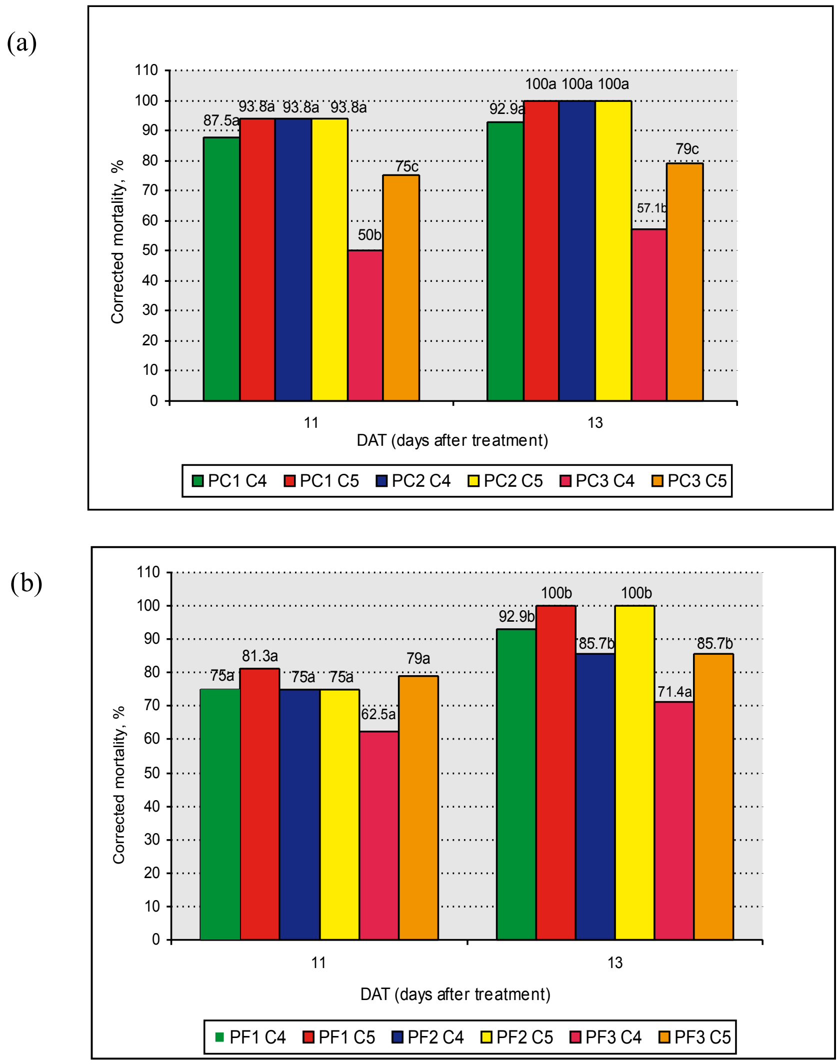


Figure 4. Corrected mortality of first larval instars of Pteroma pendula *subjected to isolates of (a)* Paecilomyces carneus *and (b)* Paecilomyces farinosus *propagated via solid state fermentation. [Values above bars within a group with the same letter are not significantly different according to the LSD test (P>0.05).* P. carneus *isolates: PC1, PC2, and PC3;* P. farinosus *isolates: PF1, PF2 and PF3. Each conidial suspension was prepared in two concentrations: C4 = $1x10^7$ cfu ml^{-1} and C5 = $1x10^8$ cfu ml^{-1}].*

In this study, most of the *Paecilomyces* isolates caused a significant increase in mortality between day 11 and day 13, but a more dramatic increase was recorded with isolates of *P. carneus* compared to isolates of *P. farinosus* (*Figures 4a* and *4b*). The least virulent isolate was from the soil, namely, isolate PC3. The highest corrected mortality by *Paecilomyces* isolates after 11 DAT varied from 75% for *P. farinosus* PF1 isolate to 93.8% for *P. carneus* PC1 isolate. Among the tested isolates, *P. carneus* PC1 and PC2 isolates (*Figure 4a*) caused the highest corrected mortality of 93.8% at 11 DAT, while only 75% corrected mortality was induced by *P. carneus* soil isolate PC3. The most successful isolates, PC1 and PC2, were ultimately able to kill over 80% of the first larval instars of the *P. pendu*la population at 11 DAT (*Figure 4*).

CONCLUSION

Most of the *Paecilomyces* spp. caused a significant increase in mortality between 7 and 13 DAT, but a dramatic increase was recorded especially with the *P. carneus* isolate PC2 whose effectiveness increased nearly three-fold over this period. The most successful isolates were of *P. carneus* which controlled over 80% of the first larval instars of the *P. pendula* population at 11 DAT. Among the test isolates, *P. carneus* isolate PC2 was the most successful, causing the highest corrected mortality of 93.8% at 11 DAT. This seems to indicate that the efficacy of *P. carneus* is superior to *P. farinosus* against the first larval instars of *P. pendula* and that this entomopathogenic fungus could be exploited as a biological control agent against the bagworm pest.

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