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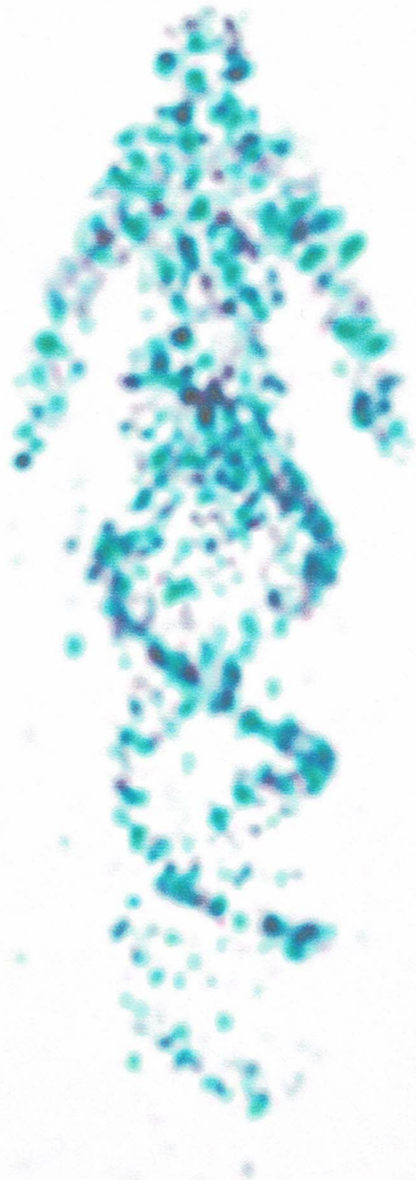

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Effects of High Temperature and Ultraviolet-C Irradiance on Conidial Viability and Density of Beauveria Bassiana and Metarhizium Anisopliae Isolated from Soils of Lowland Ecosystems in Indonesia

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Effects of High Temperature and Ultraviolet-C Irradiance on Conidial Viability and Density of Beauveria Bassiana and Metarhizium Anisopliae Isolated from Soils of Lowland Ecosystems in Indonesia

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Abstract: *Beauveria bassiana and Metarhiziumanisopliae are the most common entomopathogenic fungi used as biocontrol agents for controlling insect pests. Entomopathogenic fungi have some drawbacks in the field due to their intolerance of high temperatures and ultraviolet-C (UV-C) irradiance. The objective of this research was to evaluate the conidial viability and density of B. bassiana and M. anisopliae isolates when exposed to high temperature and UV-C irradiance. The first experiment, isolates were incubated for 7 d at temperatures of 27, 30, 33, and 36°C. The second one, four intensity levels of UV-C irradiance tested were 5000, 15000, 20000, and 30000 mW/m2. Both B. bassiana and M. anisopliae isolates displayed high conidial viability and density at temperatures of 27, 30, and 33°C, but at 36°C, all isolates died. All isolates tolerated UV-C irradiances of 5000 to 20000 mW/m2, but three of the 18 B. bassiana isolates (16.67%) died at 20000 mW/m2. Three isolates of B. bassiana produced conidia at a UV-C irradiance of 20000 mW/m2, and viable conidia were found after 48 h of incubation. All isolates died after exposure to a UV-C irradiance of 30000 mW/m2. In conclusion, both M. anisopliae and B. bassiana showed high conidial viability and density at temperatures up to 33°C and were tolerant of UV-C irradiance up to 20000 mW/m2.*

Keywords: Bio-Insecticides, Entomopathogenic Fungi, Suboptimal Lands.

INTRODUCTION

Bio-insecticides containing entomopathogenic fungi have become a primary option for controlling insect pests, because bio-insecticides are effective and do not induce resistance in the pests (Salim et al. 2015). Beauveria bassiana (Bals.) Vuill. and Metarhiziumanisopliae (Metch.) Sor. are the most common entomopathogenic fungi used as biocontrol agents (Sevİm et al. 2012). Their ability to control various insect pests, such as Aphis gossypii (Herlinda et al. 2008; Herlinda 2010; Herlinda et al. 2010), Plutellaxylostella (Loc & Chi 2007; Godonou et al. 2009), Nilaparvatalugens (Lee et al. 2015; Chinniah et al. 2016), Leptocorisaacuta (Singh et al. 2015), and Scirpophagaincertulas (Thalib et al. 2013; Chatterjee & Mondal 2014) has been proven and described. Rates of insect death caused by B. bassiana in laboratories can reach 98% (Herlinda et al. 2010), and those caused by M. anisopliae can reach 84% (Rodrigues et al. 2016). Fungi can also be combined with the non-repellent chemical termiticide imidacloprid (Wright & Lax 2013).

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Effect of Ultraviolet-C (UV-C) Irradiance on Conidial Density and Viability

The fungal isolates used in this study were cultured using the method of Wongjiratthiti and Yottakot (2017) in Glucose Yeast Broth (GYB) on a rotary shaker (120 rpm) at 29°C for 7 d. After 7 d of growth in GYB medium, the fungal culture was diluted to prepare a fungal suspension with a concentration of 103 conidia/ml. The diluted suspension (100 µL) was transferred to GYA agar and then spreaded before incubation according to the following experimental treatments. For the UV resistance experiment, four different UV-C irradiance dosages were applied to the collected isolates: (1) 5000 mW/m2 (providing a total dose of 18 kJ/m2); (2) 15000 mW/m2 (providing a total dose of 54 kJ/m2); (3) 20000 mW/m2 (providing a total dose of 72 kJ/m2); and (4) 30000 mW/m2 (providing a total dose of 108 kJ/m2). The distance between the media exposed and the UV-C radiation source was 25 cm, and the exposure duration was 2 h.

The procedures used for the conidial viability and density measurements after UV exposure were similar to those used in the high-temperature experiment.

Statistical Analysis

The differences in conidial viability and density among isolates were analysed based on a completely randomized design, and then Least Significant Difference (LSD) analyses were used to compare the means of all possible pairs of isolates at the 5% significance level using SAS/STAT 6.12 software (Microsoft Inc.).

RESULTS AND DISCUSSION

Conidial Viability and Density of Entomopathogenic Fungi Exposed to High Temperatures

The results showed that the only isolates that could survive on media exposed to a temperature of 27°C were B. bassiana. The isolate of B. bassiana that exhibited the highest conidial density was BTmTs, at 7.072 x 109 conidia/ml. However, it was not significantly different from the conidial densities of the BPcMs (6.955 x 109 conidia/ml), BPluS (6.595 x 109 conidia/ml), and BtmGa (6.701 x 109 conidia/ml) isolates (Table 2).

The entomopathogenic fungus with the highest number of conidia after exposure to a temperature of 30°C was also the BTmTs isolate (6.669 x 109 conidia/ml). A similar phenomenon occurred at 33°C, with the BTmTs isolate still showing the highest conidial density (6.001 x 109 conidia/ml) among all treated isolates. This experiment indicated that the BTmTs isolate of B. bassiana was consistently well adapted to higher temperatures of up to 33°C. In addition, two other promising isolates were BPcMs and BtmGa; the conidial density of these two isolates was not significantly lower than that of BTmTs.

Many factors affected the ability of entomopathogenic fungi to produce conidia, such as isolate origin (Constanski et al. 2015), in vitro culture medium (Indrayani& Prabowo 2010), and incubation temperature (Constanski et al. 2011). In this study, three B. bassiana isolates (BTmTs, BtmGa, and BPcMs) produced more conidia under stress conditions (high temperature and UV-C irradiance). This finding was significant because high temperature frequently occurs in tropical regions, especially in rice fields and other ephemeral agroecosystems. Therefore, the discovery of high-temperature-resistant entomopathogenic fungal isolates will allow their use as microbial agents for controlling insect pests, because these isolates can grow and produce spores inside host insects in high-temperature ecosystems.

The origin of isolates is often a defining factor in producing conidia or spores during growth in vitro, especially when the temperature and in vitro media match the collection conditions. In this study, however, the similarly performing B. bassiana isolates BTmTs, BtmGa, and BPcMs were collected from different ecosystems (Table 1). The BTmTs isolate was collected from the tidal swamp of Mulya Sari village, Sumatera Selatan, Indonesia, while BtmGa was collected from a freshwater swamp near the village of Gandus, South Sumatra. Both locations have acidic soil (pH < 6). Isolates with the ability to produce high conidial density that originate from acidic soils are considerably important for bio-insecticide production, especially in Indonesia. Agricultural lands in Indonesia mostly have acidic soils, whereas the most suitable pH for B. bassiana spores to germinate has been reported to be between pH 6 and pH 8 (Karthikeyan et al. 2008), although entomopathogenic fungal spores can survive in the pH range of 4-7 (Constanski et al. 2011) and even as high as pH 8 (Fan et al. 2011).

The discovery of acid-tolerant isolates that are able to produce high spore densities in acidic soils creates an opportunity for using these isolates for biological control of insect pests in acidic wetland ecosystems. Imanudin and Armanto (2012) reported that soils at depths up to 10 cm in the lowlands and tidal swamps of South Sumatera are very acidic, with pH values less than 4.04. Entomopathogenic fungi entomopathogenic fungal conidia. Before germination, B. bassiana conidia were single spherical cells that appeared on sterigmata and were hyaline in colour, whereas M. anisopliae conidia had a distinctive rod shape. When the fungal conidia began to germinate, which was observed at 24 hours or at 48 hours of culture time in this experiment, germ tube elongation greater than the conidial diameter became visible, and then, after 72 hours, a new conidium on the extending tip of a conidiophore appeared (Figure 1).

The conidial viability of entomopathogenic fungi decreased with increasing temperatures up to 33°C during the incubation period. Conidial viability was different among the isolates (Table 3 and 4) after 24 and 48 h in suspension culture. After 24 h in suspension culture, the highest conidial viability was observed at 27°C. In contrast, at 36°C, all isolates were dead. Among the isolates studied, the isolate with the highest viability at 27°C was the BTmTs isolate (32.31%). However, it was not significantly different from some of the other isolates of B. bassiana, including BTmSr, BTmSm, BtmGa, BPluS, and BPcMs (Table 3). After 48 h at 27°C, this similar group of isolates exhibited higher viability than the rest of the isolates evaluated.

Constanski et al. (2011) showed that the conidia of B. bassiana could grow well at 32°C, but no isolates could grow and develop at temperatures of 35 or 40°C. Factors that affect the viability of entomopathogenic fungi include temperature (Constanski et al. 2011), humidity (Luz &Fargues 1999), pH (Indarmawan et al. 2016), and light intensity (Ottati-de-lima et al. 2014; Rodrigues et al. 2016). The ideal temperature for the growth of B. bassiana was 25-27°C (Pham et al. 2009), and that of M. anisopliae was 28°C (Alves et al. 1984).

There is one important results of this study related to insect pest control in high-temperature agroecosystems, namely, success in identifying several isolates of B. bassiana and M. anisopliae that can produce considerably high conidial density and viability at temperatures up to 33°C (Salim et al. 2015). This finding can be further explored to identify active chemical compounds and for producing bio-insecticides to control insect pests in high-temperature (up to 33°C) regions such as the tropical lowlands of Indonesia.

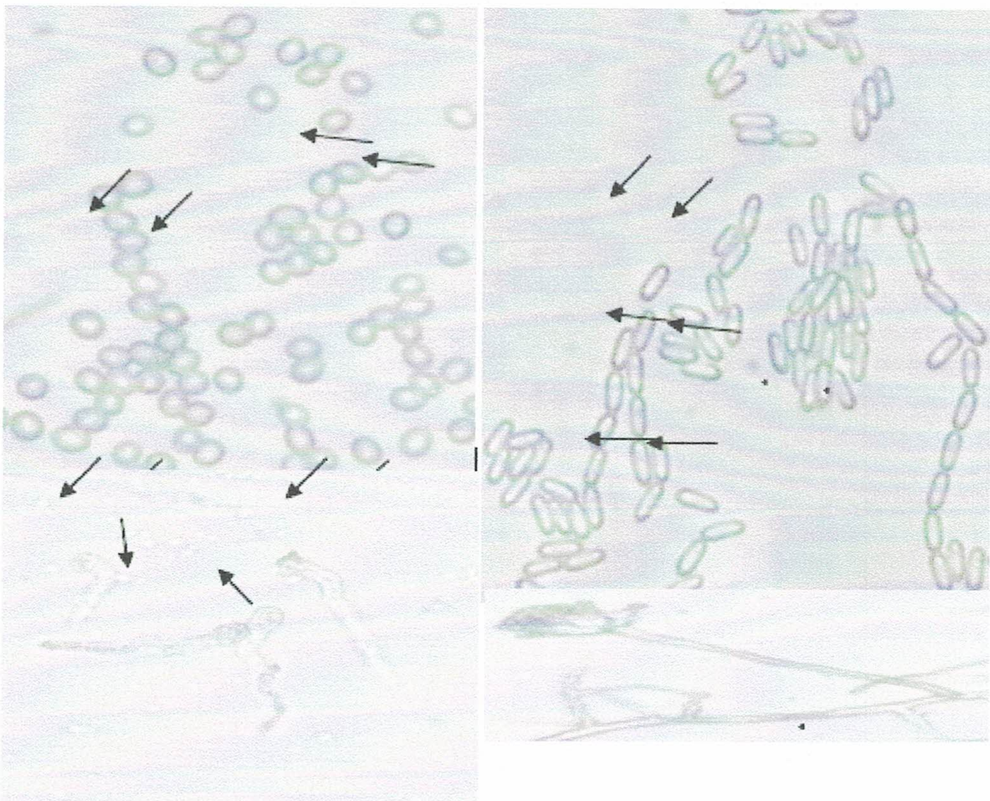

Fig. 1: Conidia of Beauveria bassiana (a) and Metarhiziumanisopliae (b); viable conidia after 48 h in culture medium, with germ tube elongations (arrows) (c); conidia after 72 h in culture medium, with an extending conidiophore tip (arrow) (d)

Conidial Viability and Density of Entomopathogenic Fungi Exposed to UV-C Radiation

This study showed that isolates grown *in vitro* and exposed to an UV-C irradiance of 5000 mW/m^2 for 2 h exhibited variable results. The *B. bassiana* isolate BPcMs produced the highest conidial density (1.612

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