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by Susilawati Susilawati

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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

Siti Herlinda[1,3], Lilian Rizkie[2], Suwandi[1,3], Susilawati[3,4], Benyamin Lakitan[3,4], Marieska Verawaty[5], Hasbi[3,6]

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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).

[1]Department of Plant Pests and Diseases, College of Agriculture, Universitas Sriwijaya, Indralaya 30662, Indonesia.
[2]Department of Plant Pests and Diseases, College of Agriculture, Universitas Padjadjaran, Jatinangor, Indonesia 453633.
[3]Research Center for Sub-Optimal Lands (PUR-PLSO), Universitas Sriwijaya, Palembang 30139, Indonesia.
[4]Agronomy Program, College of Agriculture, Universitas Sriwijaya, Indralaya 30662, Indonesia.
[5]Department of Biology, College of Mathematic and Natural Sciences, Universitas Sriwijaya, Indralaya 30662, Indonesia.
[6]Department of Agricultural Technology, College of Agriculture, Universitas Sriwijaya, Indralaya 30662, Indonesia.

Unfortunately, the use of entomopathogenic fungi has some drawbacks, especially when they are applied in agroecosystems, due to their intolerance of high-energy light rays such as ultraviolet (UV). UV rays are known to affect the growth of entomopathogenic fungal conidia (Rahmatzadeh&Khara 2007). The effects of UV rays on entomopathogenic fungi have been discussed in detail by Rodrigues et al. (2016). UV-induced DNA damage leading to fungal inactivation is generally measured by conidial germination (Chelico et al. 2005). Studies of the negative effects of UV rays on these fungi have shown that UV-B radiation with an irradiance of 6153.3 mW/m2 has a damaging effect after only 5 minutes; conidial germination decreased from 96% to 54% for M. anisopliae and from 94% to 52% for B. bassiana (Rodrigues et al. 2016). Tolerance tests of B. bassiana isolates under UV-B irradiance at 978 mW m−2 were conducted and reported by Fernandes et al. (2007), and their experiment showed that 80% of the isolates tested were tolerant of UV-B.

In addition to UV irradiance, temperature also affects entomopathogenic fungal growth. The optimal temperature for the radial growth of fungal mycelium was 25–27°C. At 30°C, growth began to decline sharply (Pham et al. 2009). Lohse et al. (2014) reported that the ideal temperature for B. bassiana growth was approximately 25°C. A related study indicated that the number of B. bassiana and M. anisopliae colonies decreased significantly as incubation temperature increased from 30 to 35°C (Ottati-de-lima et al. 2014).

Research on UV-C- and high-temperature-resistant entomopathogenic fungi is crucial for selecting and collecting isolates resistant to high temperature and UV-C irradiance. These isolates carry a high potential as active ingredients for bio-insecticide products. Therefore, the aim of this research was to evaluate the conidial viability and density of B. bassiana and M. anisopliae isolates exposed to high temperatures and UV-C irradiance.

MATERIALS AND METHODS

Preparation of Entomopathogenic Fungi

This experiment was performed at the Laboratory of Entomology, Jurusan Hama dan PenyakitTumbuhan, FakultasPertanian, UniversitasSriwijayaIndralaya, from January to August 2016. B. bassiana and M. anisopliae samples were collected from the soil of freshwater swamps in Sumatera Selatan, Indonesia. The B. bassiana and M. anisopliae collection was performed using the insect bait method of Anwar et al. (2015). Both species of fungi were isolated and identified in the laboratory. The total number of fungal isolates used in this study was 28, consisting of 20 isolates of B. bassiana and eight isolates of M. anisopliae (Table 1).

Effect of Temperature on Conidial Viability and Density

All isolates of B. bassiana and M. anisopliae were cultured on Glucose Yeast Agar (GYA) medium using the method described in Herlinda (2010) with some minor modifications. GYA media was composed of 2% agar-agar, 0.6% sucrose, 0.52% flour of the third nymph of Gryllus sp. baked at 100°C for 12 h, and 0.4% yeast at pH 5.5. The B. bassiana and M. anisopliae cultures were incubated in plant growth cabinets (LEEC pL3) at 27, 30, 33, and 36°C for 7 d. After incubation, each isolate was subjected to the following treatment: 1 ml of each isolate was collected and transferred into a tube filled with 9 ml of sterile distilled water. This dilution was repeated three times for each of the isolate solutions.

Conidial density was observed based on the method of Gabarty et al. (2014), using a wooden drill with a 1-cm diameter to remove a section of fungal media. The fungal samples were diluted to make a suspension with a spore concentration of approximately 1/103 prior to viewing under a light microscope (Olympus BX51). The total numbers of conidia in these fungal cultures were counted using a haemocytometer (Gabarty et al. 2014).

Conidial viability was observed by diluting the fungal culture three times. The conidial dilution was transferred onto a glass slide (10 µL droplets); a cover glass was put on top of the specimen, and conidia were visualized under the light microscope with 400x magnification. The slide was protected against drying by applying nail polish around the cover glass.

The conidial germination of each isolate was calculated based on direct counting of viable and non-viable conidia after 24 and 48 h (Guilherme et al. 2015). Viability of conidia was determined based on the method of Xu et al. (2001); germination was observed for 100 conidia from each inoculum droplet, and the procedure was repeated three times. Signs of conidial viability were determined according to Guilherme et al. (2015). The signs of viable conidia included broken conidial walls, germ tube formation, elongation of the germ tube (beyond the normal conidium diameter), and clear enlargement of respective conidium size.

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

adaptable to extremely low soil pH conditions usually produced high chitinase and had the ability to activate this chitinase (Suryadi et al. 2013).

It is interesting to note that the high-temperature-tolerant BPcMs isolate was isolated from an insect host (Pseudoplusiachalcites) at Muarasiban village in the Pagaralam highland, while the other two high-temperature-tolerant isolates (BTmTs and BtmGa), as mentioned earlier, were collected from soils in lowland ecosystems in South Sumatra. The finding of the high-temperature-tolerant BPcMs isolate has opened a new horizon of knowledge by demonstrating that high-temperature-tolerant isolates can be found in tropical highlands. Prior to this finding, entomologists focused on lowland ecosystems in searching for high-temperature-tolerant entomopathogenic fungi. Tolerance of high temperatures is known to be associated with interspecific variation within fungal species rather than being due to other factors (Santoro et al. 2015).

Table 1: Beauveria bassianaand Metarhiziumanisopliaeisolates from South Sumatera, Indonesia

Species of fungi	Source host insects	Origin (village or city)	Isolate codes
B. bassiana	*Hypothenemushampei*	Jember **	BBY
B. bassiana	*Lipaphiserysimi*	Pagardin	BLePd
B. bassiana	*Pseudoplusiachalcites*	Muarasiban	BPcMs
B. bassiana	*Pseudoplusiachalcites*	Pagardin	BPcPd
B. bassiana	*Plutellaxylostella*	Soak	BPluS
B. bassiana	*Tenebrio molitor* *	Gandus	BTmGa
B. bassiana	*Tenebrio molitor*	Maryana	BTmMa
B. bassiana	*Tenebrio molitor*	Makarti Jaya	BTmMj
B. bassiana	*Tenebrio molitor*	Indralaya	BTmPc
B. bassiana	*Tenebrio molitor*	Pagardin	BTmPd
B. bassiana	*Tenebrio molitor*	Pemulutan	BTmPe
B. bassiana	*Tenebrio molitor*	Rambutan	BTmRa
B. bassiana	*Tenebrio molitor*	Saleh Mulya	BTmSm
B. bassiana	*Tenebrio molitor*	Soak	BTmSo
B. bassiana	*Tenebrio molitor*	Srikaton	BTmSr
B. bassiana	*Tenebrio molitor*	Indralaya	BTmTf
B. bassiana	*Tenebrio molitor*	TalangKelapa	BTmTk
B. bassiana	*Tenebrio molitor*	TelangRejo	BTmTr
B. bassiana	*Tenebrio molitor*	Mulya Sari	BTmTs
B. bassiana	*Leptocorisaacuta*	Pantura	BwsPantura
M. anisopliae	*Tenebrio molitor*	Indralaya	Ma
M. anisopliae	*Aphis gossypii*	Indralaya	MagIn
M. anisopliae	*Aphis gossypii*	Pagardin	MAgPd
M. anisopliae	*Tenebrio molitor*	Indralaya	MaMg
M. anisopliae	*Tenebrio molitor*	Jarai	MTmJr
M. anisopliae	*Tenebrio molitor*	Kenten	MTmKt
M. anisopliae	*Tenebrio molitor*	Muarasiban	MTmMs
M. anisopliae	*Tenebrio molitor*	Tanjung Raja	MTmTr

*) Tenebrio molitor was used for baiting the fungi from the soil,

**) used as reference isolate.

The ability of BPcMsto produce more conidia at high temperatures may be associated with its host insect species. Scully and Bidochka (2005) stated that selection pressures on host insects and fungal adaptability were highly related to the intraspecific strain characteristics of entomopathogenic fungi.

In this study, all isolates (100%) incubated at 36°C in vitro died (Table 2). Generally, a decline in conidial number was detected with increasing incubation temperatures from 27 to 33°C. Correspondingly, Luz and Fargues (1998) stated that conidium production by B. bassiana increased as temperature increased from 15 to 25°C but then declined at 28–30°C. Moreover, Ottati-de-lima et al. (2014) also reported that the number of B. bassiana and M. anisopliae colonies declined significantly at 30 to 35°C. Other studies have also reported similar phenomena; Pham et al. (2009) showed that conidium production decreased at temperatures of 27–33°C and at 36°C all fungal isolates died.

Conidial viability was calculated based on the percentage of germinated conidia, which was determined by conidial size changes. There was a clear difference between germinated and ungerminated

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.

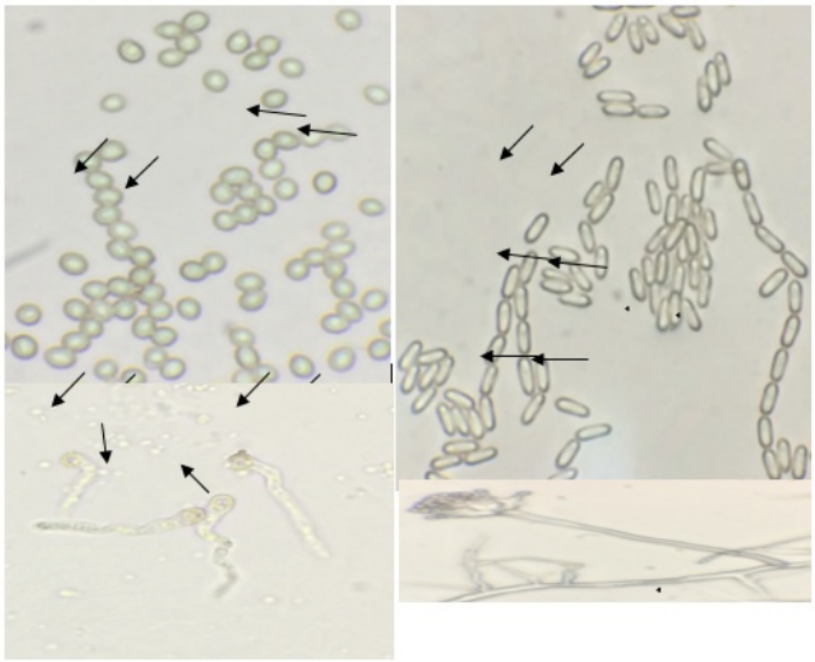

Fig. 1: Conidia of Beauveria bassiana (a) and Metarhiziumanisopliae (b); viable conidia after 48 h in culture medium, with germ tube elongations (arrows) (c); conidia after 72 h in culture medium, with an extending conidiophore tip (arrow) (d)

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/m² for 2 h exhibited variable results. The *B. bassiana* isolate BPcMs produced the highest conidial density (1.612

x 10^9 conidia/ml). The density of BPcMs was comparable to those of BTmTs (1.559 x 10^9 conidia/ml), BTmSr (1.410 x 10^9 conidia/ml), BPluS (1.315 x 10^9 conidia/ml), and BTmPd (1.293 x 10^9 conidia/ml) (Table 5), but BPcMs was significantly different from the other isolates. In general, conidial density declined as the isolates were exposed to higher irradiance intensities up to 20000 mW/m^2. Furthermore, at an UV-C irradiance of 30000 mW/m^2, none of the fungal isolates survived.

The viability of conidial isolates after 5000 mW/m^2 UV-C irradiance did not show a significant effect when examined at 24 h; however, after the suspensions had been incubated for 48 h, viability started to increase. The highest viability was found in the *B. bassiana* isolate BTmSr (14.64%). Other isolates of *B. bassiana* showing high viability comparable to BTmSr were BTmTs, BPcMs, BTmSr, BTmSm, BTmPd, BTmMj, BtmGa, and BPluS (Table 7). The conidial viability of isolates exposed to an UV-C irradiance of 5000 mW/m^2 was higher than those of isolates exposed to UV-C irradiances of 15000 and 20000 mW/m^2 after both 24 and 48 h (Tables 6 and 7).

At an UV-C irradiance of 20000 mW/m^2, three isolates (BTmTk, BWS Pantura of *B. bassiana*,and MaMg of *M. anisopliae*) produced conidia that did not germinate after 48 h of incubation (Table 7). In short, some fungi exposed to an UV-C irradiance of 20000 mW/m^2 produced conidia, but the conidia produced were not always able to germinate, depending on the isolate. Furthermore, exposure to an UV-C irradiance of 30000 mW/m^2 killed all of the fungal isolates.

A previous study showed that UV-C irradiance with 254-nm light for 7 h did not impair the germination of mycorrhizal fungal spores (Rahmatzadeh&Khara 2007). Similarly, UV-B irradiance at 978 mW/m^2 did not interfere with the viability of *B. bassiana*isolates (Fernandes *et al.* 2007). However, UV-B irradiance at 6153.3 mW/m^2 caused decreased conidial viability in *B. bassiana*and *M. anisopliae* (Rodrigues *et al.* 2016). The loss of viability was due to DNA damage in the fungi, which stopped them from actively growing (Chelico*et al.* 2005).

CONCLUSION

This study provides new information about variability in conidial viability and density among isolates of M. anisopliae and B. bassiana exposed to high temperatures up to 33°C and UV-C irradiances up to 20000 mW/m2, conditions which are commonly experienced in tropical lowland areas. These climatic conditions represent a challenge to the success of bio-insecticides that use entomopathogenic fungal conidia as the source of their active compounds. The isolates found in this study that are adaptable to high temperature and UV-C radiation will provide a potential source of active compounds and materials for bio-insecticide production to control insect pests in tropical lowland areas.

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