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Conidial mass production of entomopathogenic fungi and tolerance of their mass-produced conidia to UV-B radiation and heat

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ABSTRACT

We investigated conidial mass production of eight isolates of six entomopathogenic fungi (EPF), *Aphanocladium album* (ARSEF 1329), *Beauveria bassiana* (ARSEF 252 and 3462), *Lecanicillium aphanocladii* (ARSEF 6433), *Metarhizium anisopliae* sensu lato (ARSEF 2341), *Metarhizium pingshaense* (ARSEF 1545), and *Simplicillium lanosoniveum* (ARSEF 6430 and 6651) on white or brown rice at four moisture conditions (75–100%). The tolerance of mass-produced conidia of the eight fungal isolates to UV-B radiation and heat (45 °C) were also evaluated. For each moisture content compared, a 20-g sample of rice in a polypropylene bag was inoculated with each fungal isolate in three replicates and incubated at 28 ± 1 °C for 14 days. Conidia were then harvested by washing the substrate, and conidial concentrations determined by haemocytometer counts. Conidial suspensions were inoculated on PDAY with 0.002% benomyl in Petri plates and exposed to 978 mW m^{-2} of Quaite-weighted UV-B for 2 h. Additionally, conidial suspensions were exposed to 45 °C for 3 h, and aliquots inoculated on PDAY with benomyl. The plates were incubated at 28 ± 1 °C, and germination was assessed at 400 × magnification after 48 h. Conidial production was generally higher on white rice than on brown rice for all fungal species, except for *L. aphanocladii* ARSEF 6433, regardless of moisture combinations. The 100% moisture condition provided higher conidial production for *B. bassiana* (ARSEF 252 and ARSEF 3462) and *M. anisopliae* (ARSEF 2341) isolates, while the addition of 10% peanut oil enhanced conidial yield for *S. lanosoniveum* isolate ARSEF 6430. *B. bassiana* ARSEF 3462 on white rice with 100% water yielded the highest conidial production (approximately 1.3 × 10^{10} conidia g^{-1} of substrate). Conidia produced on white rice with the different moisture conditions did not differ in tolerance to UV-B radiation or heat. However, high tolerance to UV-B radiation and heat was observed for *B. bassiana*, *M. anisopliae,* and *A. album* isolates. Heat-treated conidia of *S. lanosoniveum* and *L. aphanocladii* did not germinate.

1. Introduction

Concerns about the negative effects of synthetic insecticides on human health and the environment have encouraged use of alternative strategies for pest control, including developing entomopathogenic fungi (EPF) as biological control agents (BCA) (Acheampong et al., 2020a; Faria and Wraight, 2007; Feng et al., 1994; Hatting et al., 2019; Lacey et al., 2015; Li et al., 2010; Rangel et al., 2022; van Lenteren et al., 2018). Roberts (1973) mentioned several potential benefits of EPF for regulation of insect populations. Moreover, fungi are unique among insect pathogens, in that they infect through the insect cuticle and do not need to be ingested; therefore, they are the only microorganism group that can infect sucking insects such as aphids and leafhoppers (Roberts and Hajek, 1992; Sayed et al., 2019).

The genera *Metarhizium* and *Beauveria* are well-known EPF because of their wide geographical distribution and host ranges (Hall and Papierok, 1982; Roberts and St. Leger, 2004). They have been intensively studied to develop commercial mycopesticides

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(Faria and Wraight, 2007; Feng et al., 1994; Hatting et al., 2019; Kassa et al., 2004; van Lenteren et al., 2018). The fungal species *Metarhizium anisopliae*, *Beauveria bassiana*, *Verticillium lecanii,* and *Aphanocladium album* are BCA used in Integrated Pest Management (IPM) in the Philippines to control mango-crop pests. The first three species have been produced and commercialized worldwide for arthropod pest control (Butt et al., 2001; Faria and Wraight, 2007; Hatting et al., 2019; van Lenteren et al., 2018). *Lecanicillium aphanocladii* (formerly *A. album*) and *Simplicillium lanosoniveum* (formerly *V. lecanii*) has also been produced in Brazil to control the rubber tree pest, *Leptopharsa heveae* (Hemiptera: Tingidae) (Rangel and Correia, 2003).

Diverse techniques for mass production of fungal mycelium or conidia have been reported, including *in vivo*, submerged and surface culture (Feng et al., 1994; Mascarin and Jaronski, 2016). The method used depends on the growth requirements of the fungus and the desired end product (Goettel and Roberts, 1992; Lomer et al., 2001). The most common systems worldwide for conidial production utilize sterile rice grains as the growth substrate (Cebrail and Mehmet, 2021; Jaronski, 2014; Jenkins et al., 1998; Kruger et al., 2014; Loera-Corral et al., 2016; Mathulwe et al., 2022; Muñiz-Paredes et al., 2017; Pham et al., 2010; Roswanjaya et al., 2022; Taylor et al., 2013). In Brazil, commercial production of *M. anisopliae* to control spittle bugs on sugarcane and *B. bassiana* to control grasshoppers utilizes rice as the substrate because it is simple, nutritive, and provides a large surface area for aeration and conidia production, resulting in high yield (Alves and Pereira, 1989; Aquino et al., 1975; Mascarin et al., 2010; Mendonça, 1992). Although promising rice byproducts, such as husk (Mishra et al., 2016; Sala et al., 2020) and bran or bran−husk combinations, which are cheaper substrates than rice grains, have resulted in greater yield than the grains (Dorta et al., 1990), white or brown rice grains are usually favored as substrates due to their higher nutrient contents (Bich et al., 2018).

Besides the type of substrate used, the moisture content and C/N ratio of substrates are reported to influence mass conidial production of EPF (Aregger, 1992; Camara et al., 2022; Jenkins et al., 1998; Muñiz-Paredes et al., 2017; Sala et al., 2020; Teja and Rahman, 2017). High moisture (>90%) enhances *B. bassiana* and *M. anisopliae* conidial production on rice substrates (Aregger, 1992, 1992a; Dorta et al., 1990; Taylor et al., 2013). However, optimal conidial production has also been obtained for some EPF (including *B. bassiana* and *M. anisopliae*) cultivated on rice grains or husk with 40−70% moisture (Camara et al., 2022; Pham et al., 2010; Sala et al., 2020). Furthermore, the addition of oils and supplementary carbon sources (such as glucose, yeast extract, and coconut water) to substrates improves conidial production of EPF (Aregger, 1992; Camara et al., 2022; Kim et al., 2019; Safavi et al., 2007; Shah et al., 2005; Teja and Rahman, 2017).

Ultimately, the choice of an entomopathogenic fungal isolate for IPM should rely on (1) higher virulence; (2) greater efficiency in mass production; and (3) performance under challenging environmental conditions (heat, UV radiation, dry conditions, etc.) (Acheampong et al., 2020a; Acheampong et al., 2020b; Dias et al., 2018; Lacey et al., 2001; Licona-Juárez et al., 2023; Rangel et al., 2005, 2015). Therefore, the current study investigated conidial mass production of eight EPF isolates, *A. album* (ARSEF 1329), *B. bassiana* (ARSEF 252 and 3462), *L. aphanocladii* (ARSEF 6433), *M. anisopliae* (ARSEF 2341), *Metarhizium pingshaense* (ARSEF 1545), and *S. lanosoniveum* (ARSEF 6430 and 6651) on white or brown rice as substrate at four different moisture conditions. In addition, this study evaluated the tolerance of mass-produced conidia of the eight fungal isolates to UV-B radiation or heat (45 °C).

2. Materials and methods

2.1. Fungal isolates

Eight fungal isolates were obtained from the USDA-ARS Collection for Entomopathogenic Fungal Cultures (ARSEF), US Plant, Soil, and Nutrition Laboratory, Ithaca, New York, USA. The geographic origin and the insect host from which they were isolated are listed in Table 1.

2.2. Mass production of conidia

2.2.1. Conidial production and inoculum preparation

The fungal isolates were cultured on 23 mL potato dextrose agar (PDA, Difco Laboratories, Detroit, MI, USA) supplemented with 1% yeast extract (1 g L^{-1}) (Technical, Difco) (PDAY) in Petri dishes (polystyrene, 95 × 15 mm, Fisherbrand® Pittsburg, PA, USA). The isolates were incubated in the dark at 28 ± 1 °C for 14 days. Conidia were harvested from the medium surface and suspended in 0.1% Tween 80 solution. The suspension was filtered through four layers of sterile cheesecloth and immediately used for substrate inoculation.

2.2.2. Substrate for conidial mass production

White Basmati rice (Shangri-la Health Foods, Logan, UT, USA) or premium short grain brown rice (Premium short grain, Shangri-la Health Foods) was used as a substrate. Four moisture conditions were also compared using 20-g samples of rice in three replicates: 1) 100% distilled water (20 mL); 2) 100% distilled water, plus 5% pure peanut oil (Planters®, Nabisco, East Hanover, NJ, USA); 3) 100% distilled water plus 10% peanut oil; and 4) 75% distilled water and 25% coconut milk (Coco Premium Coconut Milk, Shangri-la Health Foods). The peanut oil was used because we hypothesized that the oil would prevent clumping of the rice. In addition, Aregger (1992) studied the growth and sporulation in 25 different combinations of water and oil, and found for *Beauveria brongniartii* that when oil was added, the conidial production was generally higher, probably due to a better granular structure of the medium. Furthermore, growth of *Isaria fumosorosea* on ground corn mixed with corn oil as a substrate produced conidia more tolerant to heat (Kim et al., 2010). Agricultural byproducts including coconut water has also been used in EPF mass production as nutritive additive and to increase moisture content (Sahayaraj and Namasivayam, 2008); therefore, in this study, we supplemented the rice grains with coconut milk.

This method was adapted from Daoust and Roberts (1983a; 1983b). Each mixture was autoclaved in a polypropylene bag (20 × 30 cm, Fisherbrand®), and the top of the bag was closed with a cotton plug held in place with a tie wire, and autoclaved at 121 °C for 20 (white rice) or 25 (brown rice) min. The brown rice cooked completely only after 25 min in the autoclave. Once cooled, the grains were squeezed to reduce clumps. Each bag was inoculated with 4 mL of the conidial suspension of each isolate (*ca.* 10^7 conidia mL^{-1}) using a sterile syringe and needle, and the point of inoculation was sealed with tape. The inoculated rice bags were incubated at 28 ± 1 °C for 14 days an optimum temperature for several insect-pathogenic fungal species (Dimbi et al., 2004; Fargues et al., 1997; Rangel, 2000; Rangel et al., 2010; Roberts and Campbell, 1977; Yeo et al., 2003).

2.2.3. Conidial yield and viability

Conidia were harvested after 14 days by washing the substrate in 100 mL Tween 80 (0.1%). Two washings were done with 50 mL of Tween 80 (0.1%). The rice grains were squeezed in the plastic bag to dislodge conidia from the substrate. The suspension was passed through two layers of sterile cheesecloth to separate mycelia and

Table 1
List of Isolates, their hosts, and geographic origin.

Species/Isolates	Host/Substrate	Geographic origin
Beauveria bassiana (Balsamo-Crivelli) Vuillemin		
ARSEF 252	*Leptinotarsa decemlineata*[Coleoptera: Chrysomilidae]	Orono, Maine, USA
ARSEF 3462	Soil	Canada
Metarhizium anisopliae sensu lato (Metschniko) Sorokin		
ARSEF 2341	*Scotinophara coarctata* [Hemiptera: Pentatomidae]	Philippines
Metarhizium pingshaense QT Chen & HL Guo		
ARSEF 1545	*Scotinophara coarctata* [Hemiptera: Pentatomidae]	Philippines
Simplicillium lanosoniveum (F. H. Beyma) Zare & W. Gams		
ARSEF 6430	*Leptoharsa hevea* [Hemiptera: Tingidae]	French Guiana
ARSEF 6651	*Leptoharsa hevea* [Hemiptera: Tingidae]	Brazil
Lecanicillium aphanocladii Zare & W Gams		
ARSEF 6433	*Leptoharsa hevea* [Hemiptera: Tingidae]	Brazil
Aphanocladium album (Preuss) Gams		
ARSEF 1329	egg of *Hypera postica* [Coleoptera: Curculionidae]	France

aggregates of the substrate. To determine conidial production, an aliquot of 10 μL was suspended in 990 μL of 0.1% Tween 80, and the number of conidia was determined by hemacytometer counts. The viability was assessed by inoculating 20 μL conidial suspension on 10 mL PDAY supplemented with 0.003% gentamycin (150 mg L^{-1}) (Sigma Chemical Company, Irvine, UK) in three replicate Petri dishes (Polystyrene, 60 × 15 mm), and incubation at 28 ± 1 °C for 12 h. Conidia were stained with a drop of methyl blue solution [13 g l^{-1} in a 85% (w/w) lactic acid solution]. Germination was assessed at 400 × magnification; conidia were considered germinated when the germ tube was longer than the diameter of the conidium (Rangel et al., 2005). A total of 300 conidia per plate were evaluated and viability was calculated. The conidial viability of some isolates grown on white rice with 100% moisture and stored for 2 years at −20 °C were also determined as above.

2.3. Effect of UV-B and heat on conidial germination

The effects of UV-B and heat on conidial germination were evaluated for all eight isolates of the EPF produced on white rice with the four moisture combinations previously mentioned. The inoculum was prepared by suspending conidia (*ca.* 1 × 10^5 conidia mL^{-1}) in 10 mL 0.1% Tween 80. The suspensions were vigorously shaken, and 2 mL was filtered through a polycarbonate membrane (25 mm diam., 8 μm pore size, Whatman® Nucleopore®, Clifton, NJ, USA). A drop of 20 μL conidial suspension was inoculated on 4 mL PDAY + 0.002% Benomyl [25% active ingredient (Hi-Yield Chemical Company, Bonham, TX, USA)] in three replicate Petri plates (polystyrene, 35 × 10 mm), and immediately exposed to UV-B irradiation for 2 h. PDAY plates with conidia were exposed to 978 mW m^{-2} of Quaite-weighted UV-B radiance (Quaite et al., 1992a;b) produced by two TL 20 W/12 RS fluorescent lamps (Philips, Eindhoven, Holland) [with primarily UV-B (peak at 313 nm) with minimal UV-A radiation output], providing a total dose of 7.04 kJ m^{-2}, in a Percival growth chamber (Boone, IA, USA) at 28 ± 1 °C. Plates were covered with cellulose diacetate filters (JCS Industries, Le Miranda, CA, USA) to exclude UV-C and short wavelength UV-B radiation provided by two TL 20 W/12 RS fluorescent lamps as described by Rangel et al. (2004). Control plates were covered with aluminum foil to block all UV radiation. Spectral irradiance was measured as done in Rangel et al. (2004). The DNA-damage (cyclobutane pyrimidine dimer formation) action spectrum developed by Quaite (Quaite et al., 1992a;b) and normalized to unity at 300 nm was used to calculate the weighted UV irradiances in mW m^{-2}. The reasons for using this action spectrum and selecting this biological spectral weighting function (BSWF) are discussed in Rangel et al. (2006b).

For heat treatment, the conidial suspension (2 mL) in 20 mL test tubes (Pirex®, NY, USA) was exposed in a water bath at 45 ± 1 °C for 3 h, an established heat-stress condition according to (Rangel et al., 2005; Souza et al., 2014). Then, 20 μL of the conidial suspension was inoculated on 4 mL PDAY + Benomyl (0.002%) in three replicate Petri plates (polystyrene, 35 × 10 mm). Immediately after treatments, the plates were inoculated with a drop of 20 μL and incubated at 28 ± 1 °C. The conidial germination in the plates exposed to UV-B or heat was assessed after 24 h of incubation for control (non-exposed plates) and 48 h of incubation for the treatments. Relative germination was calculated according to Rangel et al. (2005). The experiments were repeated three times.

2.4. Conidial survival after two years under freezing temperatures

Mass-produced conidia (on white rice with 100% moisture) of the isolates ARSEF 1545 (*M. pingshaense*), 2341 (*M. anisopliae*), 6651 (*S. lanosoniveum*), 6430 (*S. lanosoniveum*), and 6651 (*L. aphanocladii*) were stored at −20 °C. Then two years after production, the conidial viability was evaluated following the method above on PDAY medium. The conidial germination was counted after 16 h at 28 °C. Neither of the two *B. bassiana* isolates (ARSEF 252 and ARSEF 3462) nor the *A. album* isolate (ARSEF 1329) germinated following the 2-y storage at −20 °C.

2.5. Statistical analyses

Differences among isolates in conidial production and germination on white and brown rice under different moisture content, as well as the viability of two-year-old stored conidia among isolates, and conidial tolerances to heat and UV were assessed using an analysis of variance of a one-way factorial in a randomized block design in which experimental trials defined blocks. Assessment of the effects of substrate and moisture combination on conidial viability for each isolate were assessed using an analysis of variance of a two-way factorial in a randomized block design in which experimental trials defined blocks.

Pairwise comparisons of isolate means were calculated using Tukey–Kramer adjustment to control experiment–wise Type I error at the 0.10 level. Data were square root transformed prior to analysis to better meet assumptions of normality and homogeneity of variance. Calculations were done using Proc MIXED in the SAS System for Windows Version 9.0.

3. Results and discussion

Conidia produced by the eight fungal isolates were generally higher on white rice than on brown rice substrate, regardless of the moisture levels, except for *L. aphanocladii* ARSEF 6433, which had similar production on both substrates (Fig. 1). Even though this

result was unexpected, as brown rice (with its nutritious bran component) (Dorta et al., 1990) is more nutritious than white rice, a similar finding was reported by Pham et al. (2010). Similar results were observed with *M. anisopliae*, in which white rice was more productive than brown rice (Daoust and Roberts, 1983a). However, Sujatha et al. (2016) reported higher conidial production for an isolate of *L. lecanii* on brown rice compared to white rice. However, white rice is relatively cheaper than brown rice, hence more economical for use as a substrate.

Conidial production on white rice with 100% water varied among isolates ($F_{7,22} = 43.28$; $P < 0.001$). This moisture condition provided higher conidial production for *B. bassiana* (ARSEF 252 and ARSEF 3462) and *M. anisopliae* (ARSEF 2341) isolates. In contrast, the addition of 10% peanut oil enhanced conidial yield for one isolate of *S. lanosoniveum* (ARSEF 6430) (Fig. 1). This result was not entirely surprising given that previous mass production studies for several EPF species (including *B. bassiana* and *M. anisopliae*) using rice and other cereals as substrates reported higher conidial production at higher moisture conditions (substrate to water ratio generally $\geq$ 1:1) (Aregger, 1992; Damir, 2006, 2006a; Daoust and Roberts, 1983b; Dorta et al., 1990; Magalhães and Frazão, 1996). Conidial production has also been optimized for several EPF under similar mass production systems, at lower moisture level of substrates (40–70%) than the present study (Camara et al., 2022; Pham et al., 2010; Sala et al., 2020). Similarly, the insignificant effect of oil on conidial production, with the exception of *S. lanosoniveum* ARSEF 6430, contradicts findings from previous mass production systems, where the addition of oil, even at concentrations lower than those used in this study, enhanced conidial production (Camara et al., 2022; Dorta et al., 1990). These results, although with few isolates, support the isolate-dependent moisture and nutrient

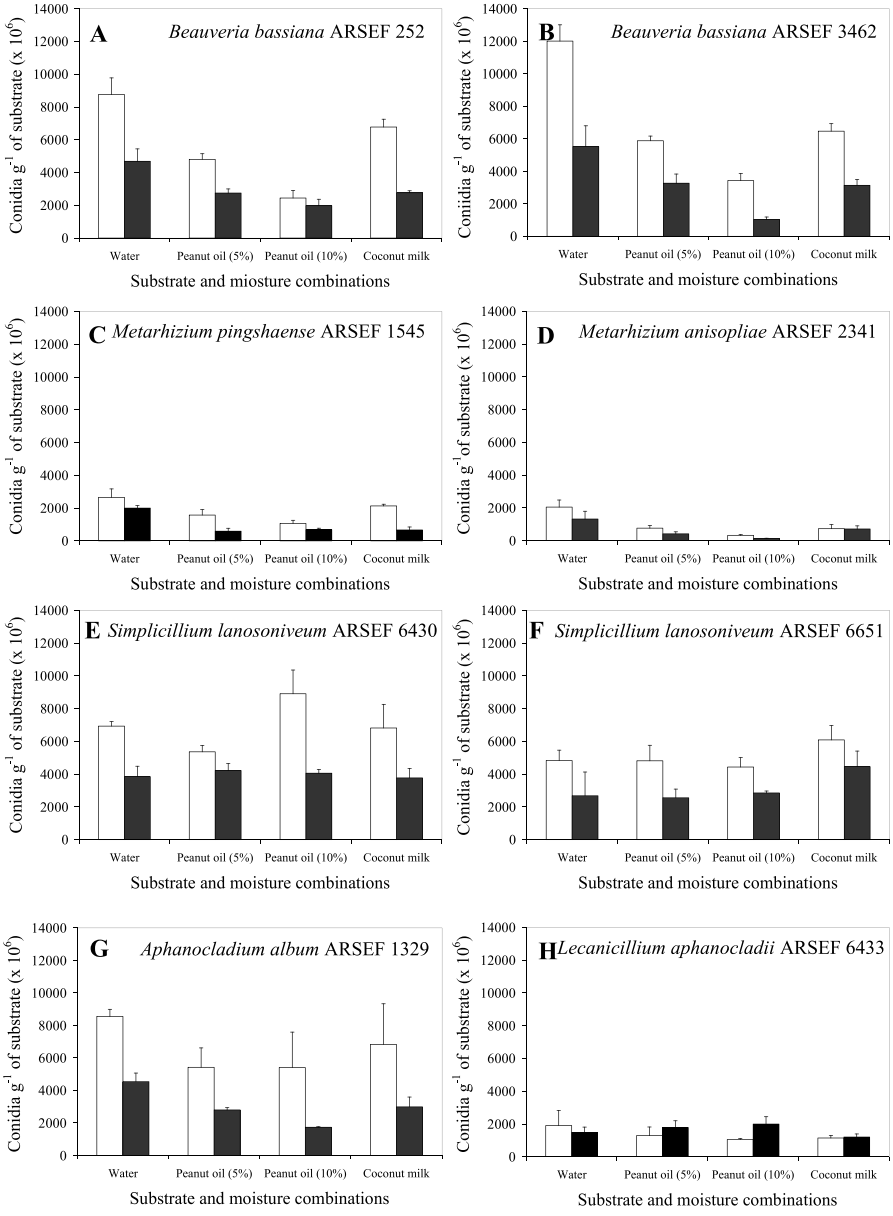

Fig. 1. Production of entomopathogenic fungi grown on white rice (open bars) and brown rice (closed bars) with different moisture combinations at 28 ± 1 °C for 14 days. Conidial production on white rice and 100% water varied among isolates ($F_{7,22} = 43.28$; $P < 0.001$).

requirements of EPF (Muñiz-Paredes et al., 2017; Shah et al., 2005; Taylor et al., 2013; Teja and Rahman, 2017).

Among the isolates, conidia produced per gram of rice substrate were higher for *B. bassiana* (ARSEF 252 and ARSEF 3462), *S. lanosoniveum* (ARSEF 6430 and ARSEF 6651), and *A. album* (ARSEF 1329) but lower for *M. anisopliae* (ARSEF 2341), *M. pingshaense* (ARSEF 1545), and *L. aphanocladii* (ARSEF 6433). The isolate with the highest conidial production was *B. bassiana* ARSEF 3462 on white rice with 100% moisture condition, obtaining approximately 1.3×10^{10} conidia g^{-1} of the substrate. This result concurs with the generally higher conidial production of *Beauveria* than *Metarhizium* species, under the same growing conditions (Liu et al., 2003; Petlamul and Prasertsan, 2012). Conversely, higher conidial production has been noted for *M. anisopliae* compared to those of *B. bassiana* using the same substrates and water volumes (Damir, 2006) and on cadavers of insect hosts (Marques et al., 2000). Nonetheless, few isolates were used in this study; thus, the observed differences in conidial yield between isolates may be ascribed to the inherent characteristics of each isolate.

For *B. bassiana* ARSEF 252, conidial production was affected by the interaction of substrate and moisture combination ($F_{3,19} = 3.17$; $P = 0.048$) (Fig. 1A). Production was higher on white rice than brown for water and coconut milk. White rice was not shown to be either better or worse than brown for peanut oil 10% or peanut oil 5%, although the white rice mean was greater than the brown rice mean. On brown rice, 100% water was significantly higher than peanut oil 10%; neither peanut oil 5% nor coconut milk could be distinguished from any other moisture combination. On white rice, water, coconut milk, and peanut oil 5% all are significantly higher than peanut oil 10%; water is significantly higher than peanut oil 5%; and coconut milk cannot be distinguished from either water or peanut oil 5%.

For *B. bassiana* ARSEF 3462, the conidial production was greater on white rice ($F_{1,19} = 73.74$; $P < 0.001$) (Fig. 1B). Production varied

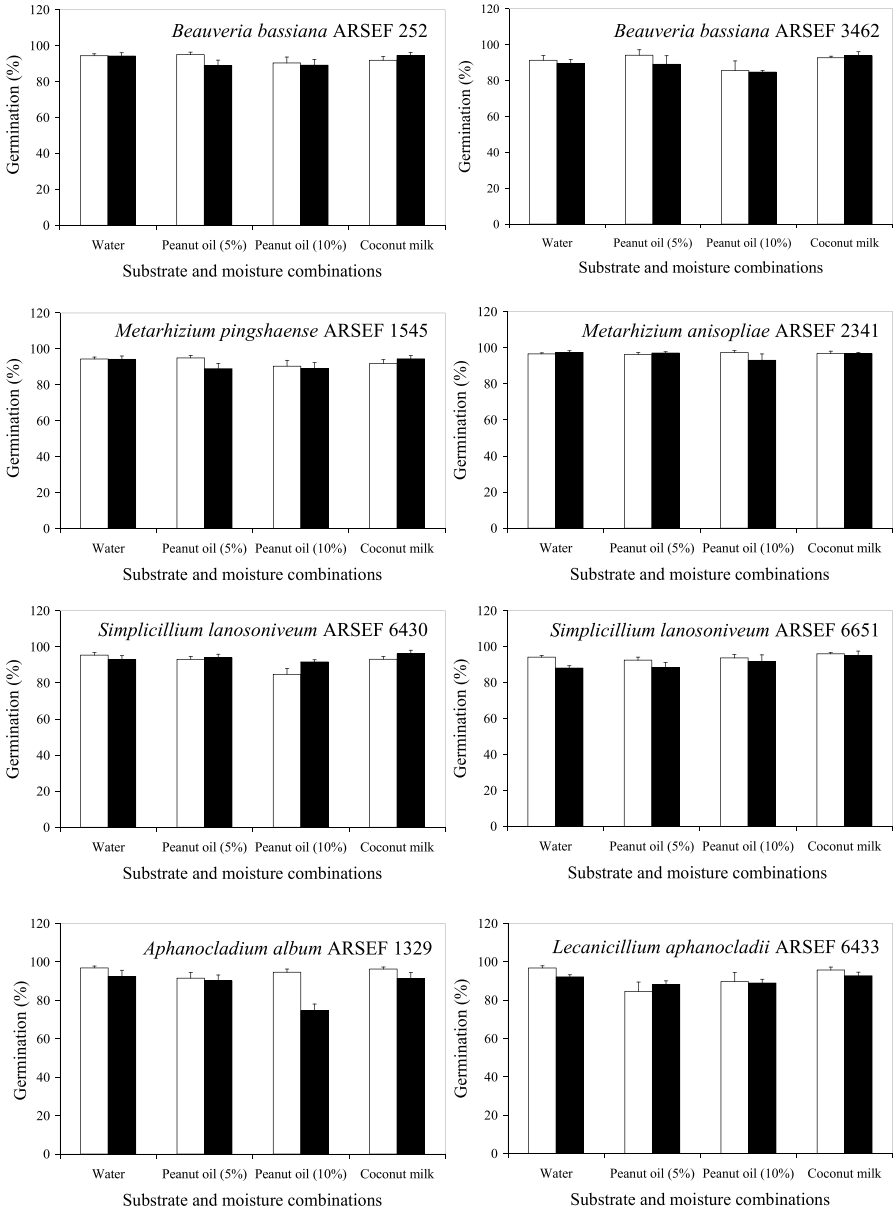

Fig. 2. Conidial viability of entomopathogenic fungi grown on white rice (open bars) and brown rice (closed bars) with different moisture combinations at 28 ± 1 °C for 14 days.

due to moisture combination and the production was greatest on water ($F_{3,19} = 35.53$; $P < 0.001$). There was no evidence of interaction between substrate and moisture combination effects (interaction; $F_{3,19} = 1.17$; $P = 0.346$).

For *M. pingshaense* ARSEF 1545, evidence of an interaction between substrate and moisture combination effects on production was found ($F_{3,14} = 3.10$; $P = 0.061$) (Fig. 1C). Conidial production was higher on white rice than brown for peanut oil 5% and coconut milk. White rice was not shown to be either better or worse than brown for water or peanut oil 10%; however, the white rice mean was greater than the brown rice mean. The figure also illustrates little difference in production among coconut milk, peanut oil 5%, and peanut oil 10% on brown rice, while production with water was significantly higher (based on pairwise mean comparisons). Production on white rice, water, and coconut milk was significantly higher than peanut oil 10%; peanut oil 5% cannot be distinguish from any other moisture combination. Main effects of both substrate and moisture combination were significant ($P < 0.001$ for both). Production was greater on white rice. Production was also greater for water compared to the other three moisture combinations (based on pairwise mean comparisons); differences among peanut oil 5%, peanut oil 10%, and coconut milk could not be distinguished.

For *M. anisopliae* ARSEF 2341, the conidial production varied due to moisture combination ($F_{3,7.08} = 16.95$; $P = 0.001$) (Fig. 1D). There was weak evidence that production was greater on white rice than on brown ($F_{1,11.1} = 4.31$; $P = 0.062$). No evidence was found of an interaction between substrate and moisture combination effects ($F_{3,7.08} = 0.42$; $P = 0.746$).

For *S. lanosoniveum* ARSEF 6430, the conidial production was greater on white rice with peanut oil 10% ($F_{1,16} = 25.61$; $P < 0.001$) (Fig. 1E). There was no evidence of production differences among the studied moisture combinations ($F_{3,16.1} = 0.42$; $P = 0.742$) or of interaction between substrate and moisture combination effects ($F3,16.1 = 0.85$; $P = 0.489$).

For *S. lanosoniveum* ARSEF 6651, the conidial production was greater on white rice ($F_{1,16} = 11.54$; $P = 0.004$) (Fig. 1F). The statistical evidence of differences in production among moisture combinations are weak. Coconut milk was greater than either peanut oil 5% or water; no other distinctions among moisture combination means were apparent ($F_{3,15.1} = 3.02$; $P = 0.063$). There was no evidence of interaction between substrate and moisture combination effects ($F_{3,15.1} = 0.71$; $P = 0.561$).

For *A. album* isolate ARSEF 1329, the production was greater on white rice ($F_{1,11.8} = 18.97$; $P = 0.001$) (Fig. 1G). Production varied due to moisture combination ($F_{3,11.8} = 3.39$; $P = 0.055$). There was no evidence of interaction between substrate and moisture combination effects ($F_{3,11.8} = 0.12$; $P = 0.946$).

For *S. lanosoniveum* ARSEF 6433, the conidial production was greater on white rice ($F_{1,21} = 8.87$; $P = 0.007$) (Fig. 1H). There was no evidence of differences in production among moisture combinations (main effect of moisture combination); ($F_{3,21} = 0.97$; $P = 0.426$) or of interaction between substrate and moisture combination effects ($F_{3,21} = 0.91$; $P = 0.452$).

The conidial germination after 12 h of incubation did not vary greatly between isolates grown on either type of rice or moisture combination, with relative germination exceeding 80%. The exception was *A. album* ARSEF 1329, whose germination on brown rice and 10% peanut oil was lower than conidia produced on white rice (Fig. 2). Conidial germination was tested to determine the culture conditions affected viability. We demonstrated in our previous studies that conidial viability can change when conidia are produced on certain culture medium or physical conditions (Oliveira et al., 2018; Oliveira and Rangel, 2018; Rangel et al., 2004), for example, conidia of *Metarhizium robertsii* produced on cadavers of *Zophobas morio* (Coleoptrea: Tenebrionidae) germinated less than conidia produced on PDAY medium. In addition, conidia of *M. robertsii* (ARSEF 23 and ARSEF 2575) germinated faster when produced on Emerson or Czapek media (Rangel et al., 2004). ARSEF 2575 also germinated faster on minimal medium (Czapek medium without sucrose) supplemented with lactose (Oliveira et al., 2018) or when conidia were produced on PDA medium under the white or blue light (Oliveira et al., 2018).

The viability of conidia stored at −20 °C for 2 years decreased significantly for *M. pingshaense* and *M. anisopliae* (ARSEF 1545 and ARSEF 2341) but not *S. lanosoniveum* (ARSEF 6651 and ARSEF 6430) and *L. alphanocladii* (ARSEF 6433) (Fig. 3). The result of the 2-y conidial viability test broadly matches those of other authors: Conidial viability of EPF species are lost over time depending on storage temperature, but effects are slower for some isolates (Roswanjaya et al., 2022; Sy et al., 2016; Taylor et al., 2013). The conidial viability of EPF was better maintained at lower temperatures (typically -20–4 °C), if long-term storage (≥1 y) is required (Daoust and Roberts, 1983b; Kim et al., 2019; Marques et al., 2000; Oliveira et al., 2011; Sy et al., 2016). Although the *B. bassiana* isolates in the present were not viable after the 2-y storage, the viability of millet and rice-mycotized grains of an isolate of this species exceeded 85%, when stored for 2 years at 4 °C (Kim et al., 2019). Similarly, pure conidia of *B. bassiana* isolates remained viable (100%) for 24 and 80 months when glycerol-frozen at −20 °C and stored at −7 °C, respectively (Marques et al., 2000; Oliveira et al., 2011).

Mycelial growth on different culture media (Rangel et al., 2006a; Rangel et al., 2004, 2008, 2012, 2015) or mycelial growth exposed to certain biotic or abiotic stress conditions (Dias et al., 2020, 2021, 2022; Medina et al., 2020) can greatly influence the stress tolerance of the produced conidia. However, all isolates exhibited no evident differences between the moisture combinations studied with white rice for conidial relative germination when exposed to heat ($F_{4,8} = 1.48$; $P = 0.295$) (Fig. 4) or UV-B radiation (Fig. 5). The exception was *A. album* (ARSEF 1329) whose conidia were more tolerant to UV-B radiation when white rice was moistened with water or peanut oil 0.5% than conidia produced on white rice moistened with peanut oil 0.5% or coconut milk (Fig. 5) ($F3,6 = 14.17$; $P = 0.004$).

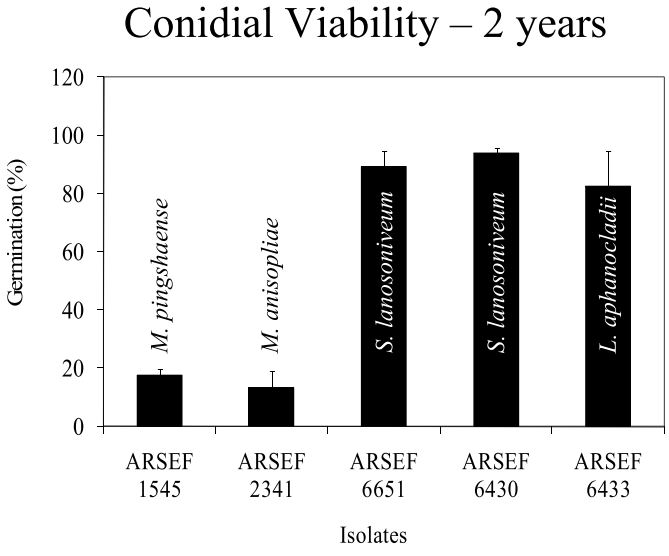

Fig. 3. Conidial viability of entomopathogenic fungi grown on white rice with 100% moisture at 28 ± 1 °C and stored for 2 years at −20 °C.

High tolerance to heat was observed for the *B. bassiana* (ARSEF 252 and 3462), *M. anisopliae* (ARSEF 2341), *M. pingshaense* (ARSEF 1545), and *A. album* (ARSEF 1329) (Fig. 4), with no differences in relative germination ($F_{4,8}$ = 1.48; P = 0.295). However, *S. lanosoniveum* (6430 and 6651) and *L. aphanocladii* (6433) were very vulnerable to heat at 45 °C and did not germinate (Fig. 4).

Conidial UV tolerance varied among isolates ($F_{7,14}$ = 11.22; $P < 0.001$) (Fig. 5). Accordingly, the isolates ARSEF 1329 (*A. album*), ARSEF 3462, ARSEF 252 (*B. bassiana*), ARSEF 2341 (*M. anisopliae*), and ARSEF 1545 (*M. pingshaense*) are considerably more UV tolerant than ARSEF 6651 and 6430 (*S. lanosoniveum*), and ARSEF 6433 (*L. aphanocladii*), and within each of these two groups, differences among moisture conditions could not be distinguished. Dias et al. (2018) reported that ARSEF 252 and ARSEF 6651 exhibited similar UV tolerance when conidia are produced on PDA, and exposed to simulated solar radiation at a Quaite-weighted irradiance of 1335 mW m^{-2} for 2 h.

In summary, conidial productions were generally higher on white rice than on brown rice for all fungal species, except for *L. aphanocladii* (ARSEF 6433), regardless of moisture combinations. The 100% moisture condition provided higher conidial production for *B. bassiana* (ARSEF 252 and ARSEF 3462) and *M. anisopliae* (ARSEF 2341) isolates, while the addition of 10% peanut oil enhanced conidial yield for *S. lanosoniveum* ARSEF 6430. *B. bassiana* ARSEF 3462 showed greater promise for future studies, as this isolate yielded the highest conidial production (approximately 1.3×10^{10} conidia g^{-1} of substrate) on white rice with 100% water and exhibited greater tolerance to UV radiation and heat. *S. lanosoniveum* isolates ARSEF 6430 and 6651 also yielded high quantity of conidia, remained viable for 2 years, but were extremely sensitive to UV radiation and heat. The virulence of these isolates

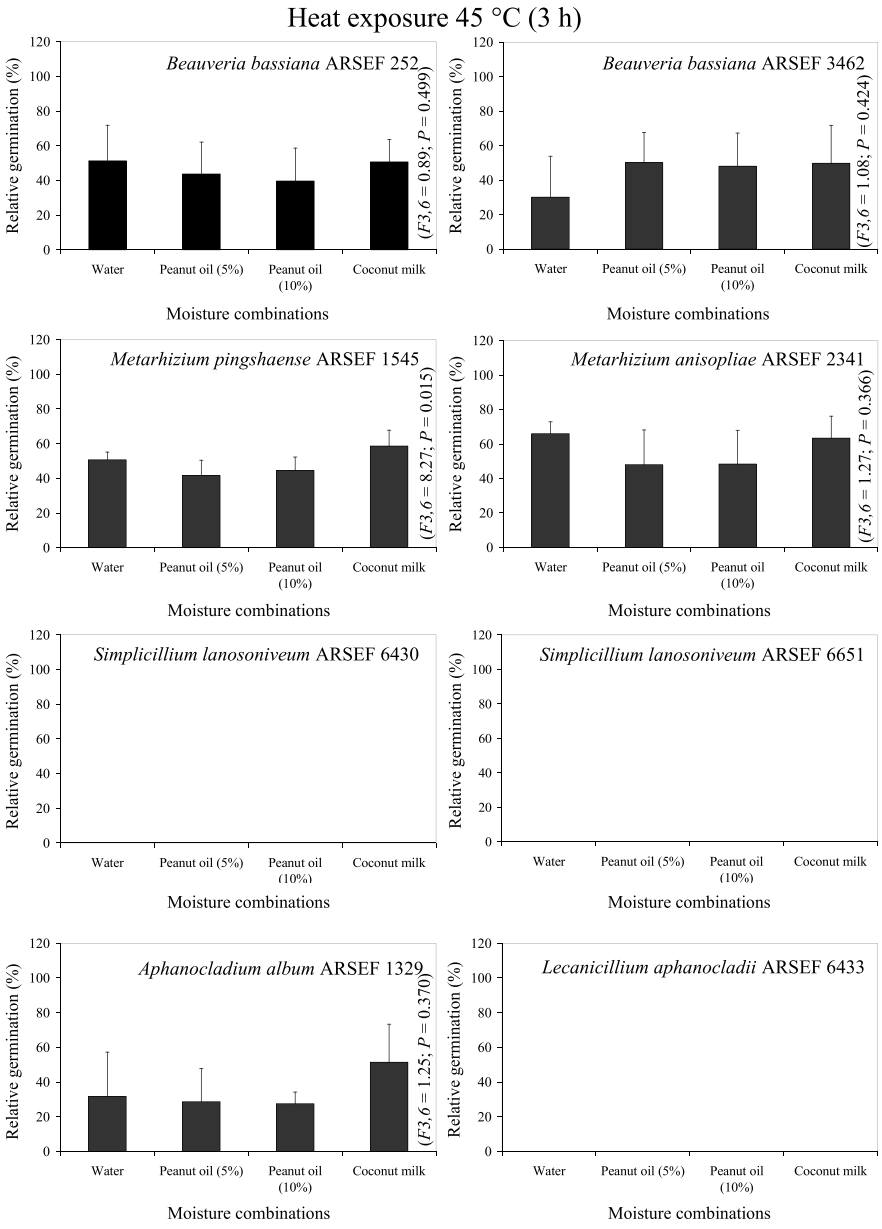

Fig. 4. Relative germination of conidia produced on white rice with different moisture combinations at 28 ± 1 °C exposed to wet heat (45 °C) for 3 h. Tolerance to heat among treatments within each isolate did not differ statistically ($F_{4,8}$ = 1.48; P = 0.295).

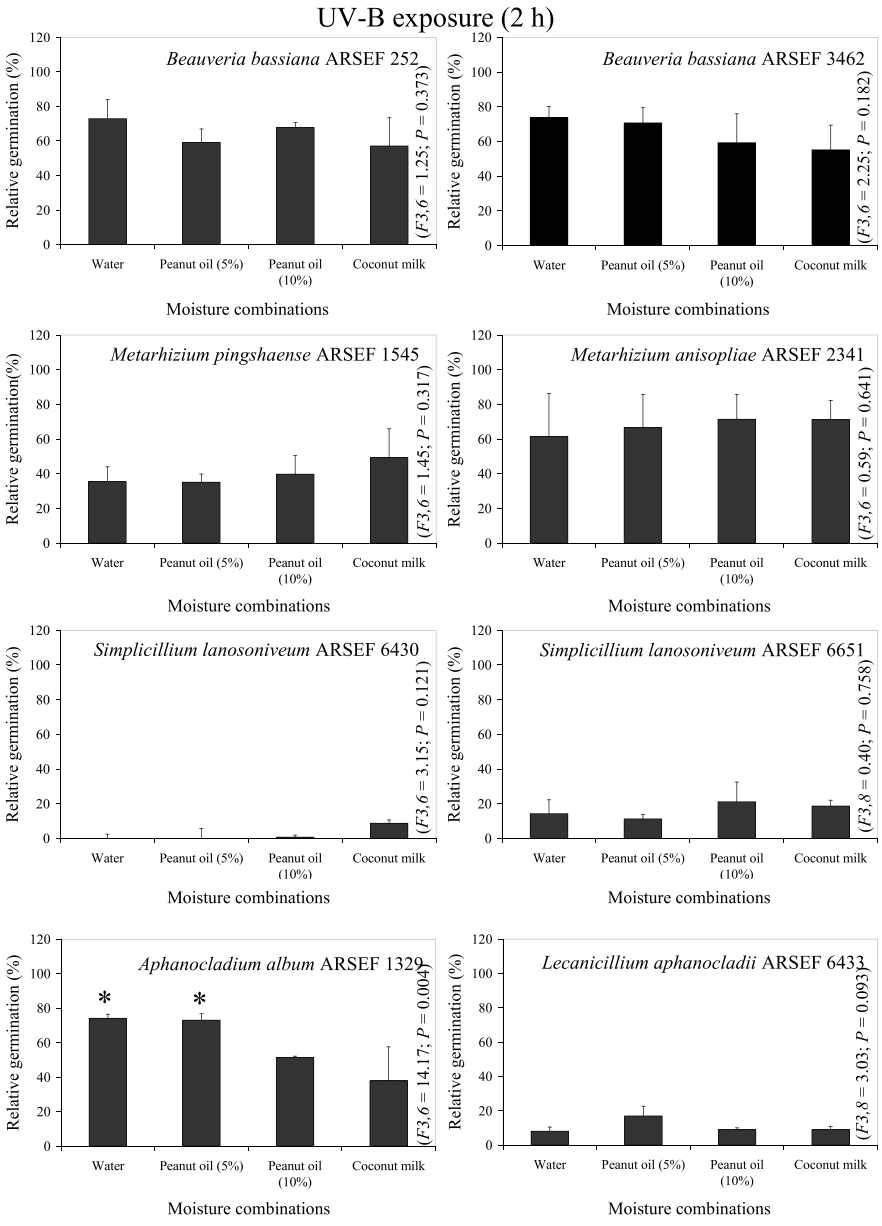

Fig. 5. Relative germination of conidia produced on white rice with different moisture combinations at 28 ± 1 °C were exposed for 2 h to 978 mW m^{-2} of Quaite-weighted UV-B radiance [with primarily UV-B (peak at 313 nm) and minimal UV-A radiation output] providing total dose of 7.04 kJ m^{-2}. Variation in UV-B tolerance was observed among isolates ($F_{7,14} = 11.22$; $P < 0.001$). The isolates ARSEF 1329, ARSEF 3462, ARSEF 252, and ARSEF 2341 were more UV-B tolerant than the others.

toward targeted insect pests ought to be prioritized in future development of these isolates into mycopesticides, followed by the biological traits obtained in this study.

Declaration of competing interest

The authors declare that they have no conflict of interest.

Author statement

Relevant CRediT roles: Conceptualization: DWR, HGG, and DENR; Data curation: DENR; Formal analysis; DENR, DWR, HGG, HGB, and MAA; Funding acquisition: DWR; Investigation: HGG, HGB, MAA, and DENR; Methodology: HGG, HGB, MAA, and DENR; Project administration: DWR; Resources: DWR; Supervision: DWR; Validation: DENR; Roles/Writing - original draft: DENR, MAA, and HGB; Writing - review & editing: DENR, MAA, and HGB. The authors HGB, MAA, and DENR read and approved the manuscript, the authors DWR and HGG are deceased.

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