Fundamental and Applied Agriculture
Vol. 4(4), pp. 1032–1037: 2019
doi: 10.5455/faa.70566

Plant Protection
ORIGINAL ARTICLE

Compatibility of ten isolates of *Beauveria bassiana* (Balsamo) Vuillemin with four commonly used fungicides in Thailand

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ARTICLE INFORMATION

Article History
Submitted: 22 Oct 2019
Revised: 06 Nov 2019
Accepted: 18 Nov 2019
First online: 29 Nov 2019

Academic Editor
Md Harun Or Rashid
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ABSTRACT

The use of *Beauveria bassiana* (Balsamo) Vuillemin (Phylum: Ascomycota: and Order: Hypocreales) has greatly increased due to its broad spectrum of pathogenic activity. This fungi has been studied intensively for the purpose of developing mycoinsecticides that can be integrated with other agrochemicals in the management of insect pests in rice fields. Additionally, its use in crops other than rice is being studied. This study investigated the effect of four fungicides *viz.* mancozeb, propineb, propiconazole, and pifenoconazole, on the growth of 10 isolates of *B. bassiana* under laboratory conditions. Malt extract agar (MEA) amended with the fungicides at two rates (recommended and half-rate) where recommended rates were as follows: mancozeb (15 g 20 L^{-1} of water), propineb (55 g 20 L^{-1} of water), propiconazole (25 mL 20 L^{-1} of water) and difenoconazole (40 m 20 L^{-1} of water) were used to culture the isolates of the fungus. It was observed that propiconazole and difenoconazole consistently exerted a strong inhibitory effect on the mycelial growth of the *B. bassiana* isolates. On the other hand, the isolates of *B. bassiana* tested exhibited low to moderate sensitivity to mancozeb and propineb. *B. bassiana* isolate BCMU 2 displayed low to moderate sensitivity to mancozeb, propineb, and difenoconazole. This study suggests that mancozeb and propineb may have the potential for use in combination with *B. bassiana* in integrated pest management programs for simultaneous control of insect pests and plant diseases.

Keywords: *Beauveria bassiana*, entomopathogenic fungus, fungicides, difenoconazole, mancozeb, propiconazole, propineb

Cite this article: Krutmuang P, Pittarate S, Rahman A, Rajula J, Thungrabeab M. 2019. Compatibility of ten isolates of *Beauveria bassiana* (Balsamo) Vuillemin with four commonly used fungicides in Thailand. Fundamental and Applied Agriculture 4(4): 1032–1037. doi: 10.5455/faa.70566

1 Introduction

Beauveria bassiana (Balsamo) Vuillemin (Phylum: Ascomycota: Order: Hypocreales) was described 170 years ago for the first time and since then, it has been utilized the most in controlling insect pests. Muller-Kogler in 1965 reported that almost 28 species of insect pests of a variety of habitats namely; greenhouse, forestry, agriculture, orchards, and the tropics are susceptible to this pathogen (Zimmermann, 2007). This makes *B. bassiana* a very unique and valuable fungus in the EPFs family. In addition, *B. bassiana* has been shown to be a potential substitute or integrate for chemical insecticides. The use of this *B. bassiana* has greatly increased due to its broad spectrum of pathogenic activity against a variety of insects pests (Barnett and Hunter, 1988; Valero-Jiménez et al., 2016). This fungi has been studied intensively to develop mycoinsecticides for integration into management systems for insect pests in rice fields (Kaur et al., 2011; **?**; Reddy et al., 2013; Shakir et al., 2015) and they should be integrated with other agrochemical measures for additional crops (Tkaczuk et al., 2015). However, there is a likelihood of conidial survival being affected by the interaction of *B. bassiana* with agrochemicals in combination with environmental factors (Haseeb, 2009). In addition, the field efficiency of this fungi is influenced by compatibility with other chemical protection strategies employed. An evaluation of the effects of chemical pesticides on *B. bassiana* was undertaken to identify tolerant isolates (Feng and Pu, 2005). Evaluation of the effects of several fungicides on different parameters like mycelial growth of commercially available entomopathogenic fungi has also been carried out (Jaros-Su et al., 1999; Devi et al., 2005; Khan et al., 2012). It has also been established that *B. bassiana* has a great bioefficacy in terms of insect mortality along with mycelial growth and sporulation. Its compatibility with artificial insecticides causes amplified stress to insects, compromised immunity, and possible physiological and behavioral alteration (Haseeb, 2009). The objective of this study was to determine the effect of four fungicides (*viz.* mancozeb, propineb, propiconazole, and difenoconazole) commonly used in rice production on the mycelial growth of *B. bassiana* isolated locally in northern Thailand from infected field insects.

2 Materials and Methods

2.1 Fungal isolates collection and culture

Ten *B. bassiana* isolates collected from farmer and highland fields which are located in the same climatic region (tropical) of five provinces (Chiang Mai, Tak, Pitsanulok, Lampang and Nakhon Ratchasima) in Northern Thailand, and from insect cadaver hosts of different taxonomic groups were selected for screening. *B. bassiana* were identified from Rajamangala University of Technology Lanna, Lampang and Chiang Mai University, Chiang Mai as shown in Table 1. These isolates for biocontrol agents were cultured on 15 mL potato dextrose agar (PDA) in 9 cm Petri dishes and incubated at 25 ± 1 °C for 21 d with light 12:12 (dark:light). In order to confirm that the virulence of the isolates was not attenuated, original host insects from which the isolates were obtained were inoculated with these isolates (Thungrabeab and Krutmuang, 2017; Pittarate et al., 2017) and the fungi re-isolated on malt extract agar (3% malt extract and 0.5% peptone with 1.5% agar: MEA) medium and maintained at 25 ± 1 °C.

2.2 Mycelial growth bioassay

Compatibility testing was done on malt extract agar (MEA) using the poison food technique which was used by Khan et al. (2012). Ten mL of the autoclaved MEA was amended with the recommended rates of the fungicides as follows: mancozeb (15 g 20 L^{-1} of water), propineb (55 g 20 L^{-1} of water), propiconazole (25 mL 20 L^{-1} of water) and difenoconazole (40 mL 20 L^{-1} of water) while the half-rate used were as follows: mancozeb (7.5 g 20 L^{-1} of water), propineb (27.5 g 20 L^{-1} of water), propiconazole (12.5 mL 20 L^{-1} of water) and difenoconazole (20 mL 20 L^{-1} of water) in 90 mm culture plates (Table 2). After solidification, the agar medium in the culture plates were seeded with the *B. bassiana* isolates (5 mm culture disks from 7-day-old cultures) in the center of Petriplates; and three replications were maintained for each treatment. The plates without any amendment served as controls. The plates were incubated in the laboratory at an average ambient temperature of 28 °C. After 21 d of incubation, the colony diameter of *B. bassiana* was measured and the average colony diameter growth was determined. The data from the replicated plates were averaged and the result was expressed as percent inhibition of mycelial growth over the control.

The percentage growth inhibition of *B. bassiana* was obtained by using the formula (Khan et al., 2012):

$$I = \frac{(A - B)}{A} \times 100 \tag{1}$$

I = % inhibition, A = area covered by test organism in control (mm), and B = area covered by test organism in different treatments (mm).

2.3 Statistical analysis

Assays were set up in a completely randomized design with three replicates. The experiments were repeated three times. Percentage mycelial growth for each isolate was converted to percentages relative to the growth rate of the control (in medium with no fungicides). Standard procedures were followed to record the data. The data collected were analyzed statistically using Statistix 10 by Analytical Software, USA. The percentages of inhibition were analyzed based on the ANOVA. The treatment means were compared by the Least Significant Difference test (LSD) for their significance at the 5% probability level.

Table 1. Host insect and geographic origin of the isolates of *Beauveria bassiana*

Isolate†	Host insect	Taxonomic order	Geographic origin	Climatic zone
BCMU1	Unknown	–	Chiang Mai, Thailand	Tropical
BCMU2	Unknown	–	Chiang Mai, Thailand	Tropical
BCMU3	Lepidoptera -pupa	Lepidoptera	Tak	Tropical
BCMU4	Brown plant hopper	Hemiptera	Pitsanulok, Thailand	Tropical
BCMU5	Brown plant hopper	Hemiptera	Lampang	Tropical
BCMU6	Bug	Hemiptera	Lampang	Tropical
BCMU7	Aphid	Hemiptera	Lampang	Tropical
BCMU8	Unknown	Hemiptera	Nakhon Ratchasima	Tropical
BCMU9	Unknown	–	Lampang	Tropical
BCMU10	Unknown	–	Chiang Mai, Thailand	Tropical

† BCMU (3, 4, 5, 6, 8, 5, 7, 9) isolates were collected from Rajamangala University of Technology Lanna, Lampang; BCMU (1, 2,10) isolates were collected from Chiang Mai Province of Thailand.

Table 2. List and information of fungicides used in the study

Active ingred.	Brand name	Chemical group	Formulation	Recommended dose
Mancozeb	Dithane® M-45	Ethylenebis (dithiocarbamate)	WP 80%	15 g 20 L^{-1} water
Propineb	Antracol®	Propylenebis (dithiocarbamate)	WP 70%	55 g 20 L^{-1} water
Propiconazole	Saitill	Triazole	EC 25%	25 mL 20 L^{-1} water
Difenoconazole	Score 250 EC	Triazole	EC 25%	40 mL 20 L^{-1} water

WP = Wettable powder, EC = Emulsifiable concentrate

3 Results and Discussion

In the present study, four chemical fungicides were evaluated for their compatibility with 10 *B. bassiana* isolates by use of mycelial growth bioassays. Mycelial growth of the isolates was significantly inhibited to varying degrees by all the fungicides tested (Fig. 1 and Table 3). Propiconazole consistently exerted a strong inhibitory effect on the mycelial growth of the *B. bassiana*; all isolates except BCMU1, BCMU2, and BCMU7 were completely inhibited by the full rate of the fungicide. In addition, all isolates except BMCU 2 were totally inhibited by a half-rate of the fungicide. The full rate of difenoconazole completely inhibited the growth of BCMU1, BCMU8, and BCMU9; the half-rate generally caused about a 90% growth reduction. Full rates of mancozeb and propineb inhibited isolate growth by 28-61% and 33-59%, respectively. The mycelial growth in the control was full-plate (100%). This is in agreement with the study conducted by Pelizza et al. (2018) on the compatibility of chemical insecticides with *B. bassiana*, *M. anisopliae*, and *M. robertsii* for the control of soybean defoliating pest, *Rachiphusia nu*. Interestingly, they also observed reductions in conidial germination in all the fungal isolates that were tested *in vitro* for their compatibility with insecticides (Pelizza et al., 2018). Moreover, other researchers have reported similar fungicide inhibition of *B. bassiana*. Kouassi et al. (2003) and Challa and Sanivada (2014) reported that mancozeb inhibited the mycelial growth of all the isolates of *B. bassiana* tested in compatibility assays with insecticides and fungicides at recommended tank dosages. Similar results were also reported by Hsiao and Lin (1995) and Celar and Kos (2016). Pandey and Kanaujia (2009) indicated that propiconazole at eight concentrations strongly reduced the growth of the fungus. Khan et al. (2012) reported that nine fungicides *viz*. benomyl (0.1%), captan(0.2%), mancozeb (0.2%), tebuconazole (0.1%), pentachloronitrobenzene (0.1%), hexaconazole (0.2%), propiconazole (0.1%), difenoconazole (0.1%), and copper oxychloride (0.1%), were also not compatible with *B. bassiana* and caused complete or strong inhibition of mycelial growth similar to that seen with propiconazole and difenoconazole in the current research. On the flip side, the *in vivo* experiment carried out by Roberti et al. (2017) proved that the chemical-based commercial products for controlling pathogenic fungi were compatible with *B. bassiana* on zucchini plants infested with flies . Interestingly, *Beauveria bassiana* isolate BCMU2 displayed low to moderate sensitivity to mancozeb, propineb, and difenoconazole.

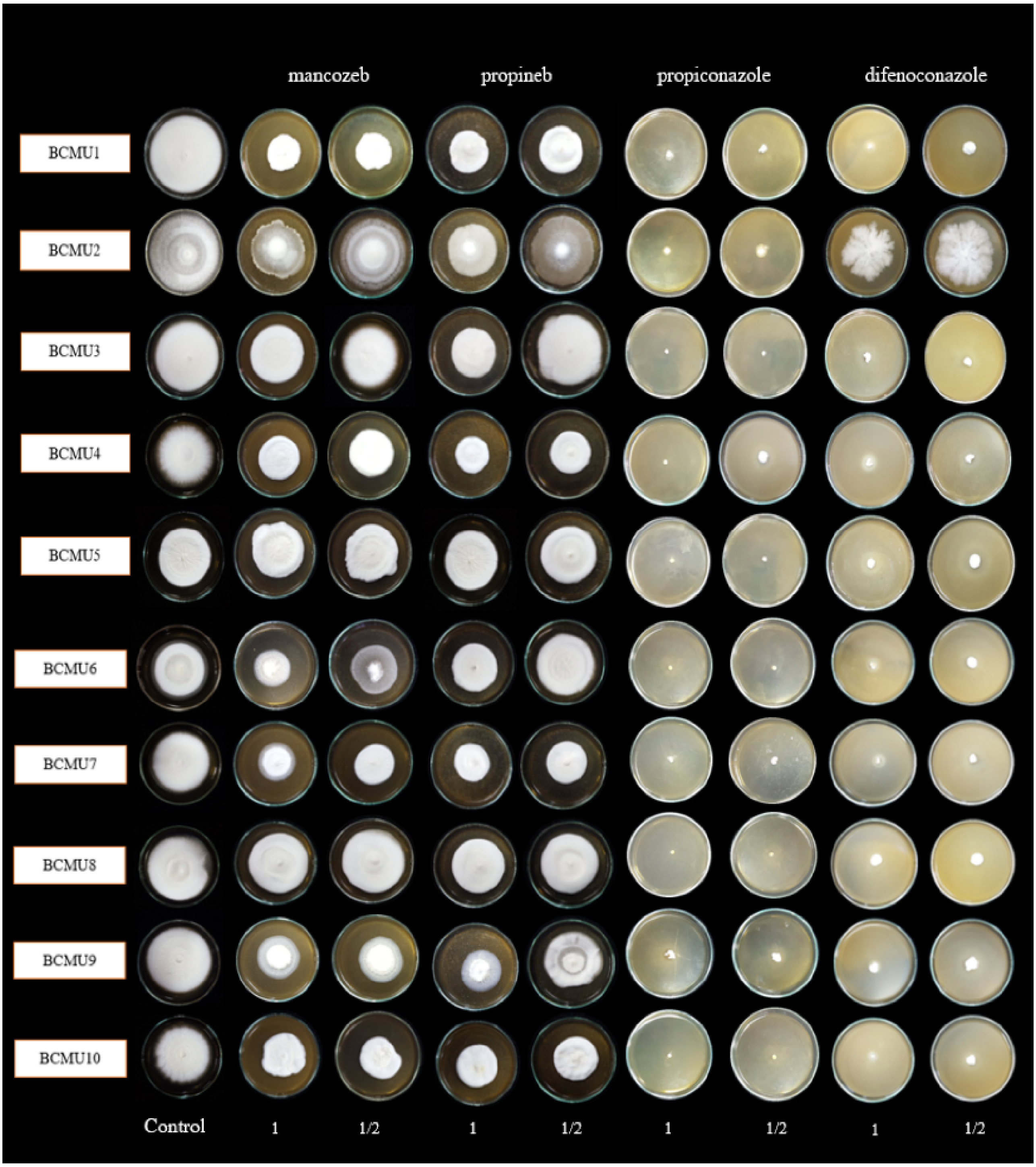

Figure 1. Colony pigmentations of *Beauveria bassiana* ten isolates after three weeks ofartificial media MEA) mixed with every four fungicides (mancozeb, propineb, propiconazole and difenoconazole) were used to culture fungus compare with control

Table 3. Mycelial growth bioassays of ten isolates of Beauveria bassiana in malt extract agar (MEA) amended with mancozeb, propineb, propiconazole, and difenoconazole after incubation for 21 d

	Mycelial growth inhibition (%) of *B. bassiana* isolates							
	Mancozeb		Propineb		Propiconazole		Difenoconazole	
Isolate	1	1/2	1	1/2	1	1/2	1	1/2
BCMU1	58.00 ab1,2	56 bc	52.67 b	44.67 cd	94.67 bc	93.33 c	100 a	90.67 efg
BCMU2	51.00 cd	11.00 i	35.33 efg	14.33 j	95.00 bc	90.67 d	52.33 h	32.33 i
BCMU3	34.00 f	17.00 h	44.67 cd	30.33 gh	100.00 a	100.00 a	96.67 ab	93.67 bcdef
BCMU4	46.00 e	45.00 e	59.00 a	45.67 cd	100.00 a	100.00 a	95.67 abc	95.00 bcde
BCMU5	34.00 f	27.00 g	37.00 ef	22.67 i	100.00 a	100.00 a	95.00 bcde	90.33 fg
BCMU6	47.00 de	48.00 de	45.00 cd	22.67 i	100.00 a	100.00 a	95.33 bcd	91.00 defg
BCMU7	61.00 a	54.00 bc	58.67 a	49.67 bc	97.00 b	96.33 b	97.00 ab	93.33 bcdef
BCMU8	28.00 g	23.00 g	33.67 fg	25.00 hi	100.00 a	100.00 a	100.00 a	87.33 g
BCMU9	47.00 de	46.00 e	45.00 cd	37.67 ef	100.00 a	100.00 a	100.00 a	91.33 cdefg
BCMU10	56.00 bc	55.00 bc	49.33 bc	41.00 de	100.00 a	95.00 bc	96.00 ab	87.67 g
Sig. level	***		***		***		***	
$LSD_{0.05}$	4.8		5.72		2.45		4.41	
CV(%)	6.85		8.47		1.52		3	

*** = significant at P < 0.001; 1 = Recommendation rate of fungicides and 1/2 = half-recommendation rate of fungicides; Means in the same column followed by the same letter are not significantly different by LSD (P < 0.05).

4 Conclusions

This study observed that fungicides used in the rice fields such as mancozeb and propineb have maximum percent mycelial growth inhibition at 61% and 59% respectively in all the isolates of *B. bassiana* except BCMU2. On the other hand, propiconazole and difenoconazole recommended rates completely inhibited mycelial growth by 100%. Importantly, it was found that *B. bassiana* isolate BCMU2 would be compatible with the fungicides that have mancozeb, propineb and difenoconazole as the active componets. Our findings are of great significance to guide and advice rice farmers to safely use these fungicides such as mancozeb and propineb in combination with *B. bassiana* for successful integrated pest management to control rice disease and insect pests in rice fields at the same time.

Acknowledgements

The authors are thankful to Dr. Robert J. McGovern for his comments on this manuscript. This study was supported by the Thailand Research Fund (TRF) and The Commission on Higher Education (CHE).

Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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The Official Journal of the
Farm to Fork Foundation
ISSN: 2518–2021 (print)
ISSN: 2415–4474 (electronic)
http://www.f2ffoundation.org/faa