JSAT
Journal of Science and Agricultural Technology
Research Article
https://doi.org/10.14456/jsat.2020.10 e-ISSN 2730-1532, ISSN 2730-1524

Compatibility of entomopathogenic fungi with Derris extracts

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Received: October 23, 2020. Revised: November 16, 2020. Accepted: November 19, 2020.

ABSTRACT

Integrated pest management (IPM) strategies have been suggested for the control of various insect pests. Entomopathogenic fungi and botanical insecticides such as derris extract have considerable potential to become significant components in sustainable IPM. However, the compatibility of entomopathogenic fungi with derris extracts is still unknown. Therefore, the present study was evaluated the compatibility of *Beauveria* sp., *Metarhizium* spp., and *Paecilomyces* spp. with derris extracts under *in vitro* conditions. The derris extract was used at 1% and 0.1% incorporated to a culture medium (malt extract peptone agar) and distributed into petri dishes for their effects on vegetative growth and conidia viability. The vegetative growth of all fungi tested was inhibited by 1% and 0.1% of derris extracts, but they did not affect the viability of conidia.

Keywords: entomopathogenic fungi, biological control, Derris extracts, IPM

INTRODUCTION

Integrate Pest Management (IPM) to be the basis for pest control. There is no single criterion that guarantees the successful uptake of biological control agents, and the difficulties that need to be overcome include scientific, economic, social, and political aspects. However, entomopathogenic fungi have considerable potential to become major components in sustainable IPM. Entomopathogenic fungi are important as natural control agents of many insect pests (St Leger and Wang, 2009). Previous research reported that many isolates of entomopathogenic fungi have effectively controlled various insect pests and were relatively safe on non-target beneficial insects (Malee and Sirinun, 2002, Sengonca et al., 2006, Thungrabeab et al., 2006, Thungrabeab, 2007). Integrated pest management with entomopathogens should be considered as an important reduction factor in insect pest population density. Therefore, we must understand the compatibility of entomopathogenic fungi with other strategies for sustainable pest control, such as botanical insecticides, which may inhibit to a smaller or larger extent the development and reproduction of entomopathogenic fungi.

Derris extract has insecticide activity against a wide variety of insect pests (Aroon et al. 1997, 2001, 2006). *Derris malaccensis* (Benth.) Prain is a climbing leguminous plant found in the southeastern parts of Asia. The root of *D. malaccensi*, which contains rotenone used as a source of natural insecticide. It can be lethal to insects and even fish. Rotenone affects not only the stomach of an insect that may digest some of it but also can transmit poison on contact. Derris has been used for years against fleas and lice, as well as it is also effective against aphids, beetles, caterpillars, maggots, bagworms, cabbage worms, thrips, leafhoppers, Japanese beetles, vegetable weevils, codling moths, sawflies, and slug sawflies (Rey, 1991). Additionally, it is also safe to use on grain, fruits, and vegetables. Rotenone may take a few days to terminate insects, but the insects will die, and feeding on the plants will end. Rotenone is biodegradable, so with the presence of the sun and heat, the chemicals will break down in about a week, as well as, it also rapidly breaks down in soil and water. Rotenone, although widely used, happens to be a very safe natural insecticide. The only warning that is more of a safety principle is not to enter a treated area for about 12 hours after using rotenone. Compatibility of entomopathogenic fungi with derris extract was never observed. The compatibility data are essential before such associations are employed in the field. Therefore, the objective of this study was to evaluate the compatibility between entomopathogenic fungi with derris extract *in vitro*, using concentrations that showed potential for control insect pests under field conditions.

https://doi.org/10.14456/jsat.2020.10

MATERIALS AND METHODS

Fungi

The following fungi isolates were used; *Beauveria bassiana* 5 isolates (Bb. 2637, Bb. 4591, Bb. 5335, Bb. 5736, Bb. 6241), *Metarhizium anisopliae* 5 isolates (Ma. 6071, Ma. 6079, Ma. 6171, Ma. 7965, Ma. 6491), Metarhizium flavoviride isolate Mfl. 1164, as well as *Paecilomyces fumosoroseus* isolate Pfu. 2507 and *Paecilomyces tenuipes* 3 isolates (Pt.7968, Pt. 7996, Pt. 8003).

Preparation of derris extracts and incorporation to the culture medium

To prepare derris extract, the roots of *Derris malaccensis* were harvested from 2 years of age. A hundred grams of fresh root were crushed and was then added to 1,000 ml of ethanol 95%. The extract remained at rest for approximately 24 h at room temperature, and it was then filtered through filter paper No.1 to separate the solid components. The concentration of rotenone obtained from the derris extracts was analyzed by HPLC (High-Performance Liquid Chromatography) method according to Aroon, 2008

After that, a concentration of derris extract recommended for control insect in the field, including 1 ml of derris extract was added to 100 ml of malt extract peptone agar (MEA, 3% malt extract; 0.5% Soybean peptone; 1.5% Agar), for the concentration to 1%. To obtain the 0.1 % concentration, 100 µl of derris extract was added to 100 ml MEA. The culture media treated after homogenization was dispensed in 90 mm diameter petri dish. There were five plates per treatment; each plate served as a replication. There were two controls, the first was untreated MEA, and a second was added 1% and 0.1% ethanol in MEA (diluted from ethanol 95%). The experiment consisted of 75 treatments (15 isolates × 2 concentrations of derris extract × 2 concentrations of ethanol 95% × control).

Vegetative fungal growth

For recording vegetative fungal growth, the mycelial mats were obtained by using water agar (WA, 15 g agar in 1000 ml H_2O). WA was dispensed in 90 mm diameter petri dish; after solidification, 100 µl of conidial suspension containing 1×10^6 conidia/ml of each isolate was spread on water agar. Plates were then incubated at 25±2°C, 12 h photophase for 3 days. Mycelial mats were cut from culture plates into round agar plugs using a 7 mm. diameter cork borer. Each agar plug was then transferred singly onto the center of media Petri dishes, prepared as the procedure described in capital 2.2. Plates were sealed with Parafilm membrane and incubated at 25±1°C under continuous light conditions. Colony growth was recorded daily for 14 days using two cardinal diameters through two orthogonal axes previously drawn on the bottom of each petri dish to serve as a reference. The diameter of the colonies was estimated by calculating the mean of two perpendicular measurements.

Conidia viability assessments

In order to determine conidia viability, conidial suspension (100 µl of 1×10^6 conidia/ml) was spread on each media plate, prepared as the procedure described in capital 2.2. Plates were then incubated individually at 25±1°C, 12 h photophase. After 24 h inoculation, germination was halted by transferring 1 ml of 0.5% formaldehyde onto each plate. A drop of lactophenol cotton blue stain was dropped into the plate. Germination and non-germination conidia in three separated (1×1 cm^2) squares of agar (100 conidia per square of agar) cut from WA were counted under the microscope by using microscope slides through moving the field of view of the microscope in order to cover a large area of agar square. Three counts were made for each replication for every treatment. Each plate was served as a replication; six replications were used for each treatment.

Statistical analysis

The experimental design for all trials was completely randomized. The data were submitted to ANOVA, and the mean values were compared by using Duncan's multiple range test at a probability level of < 0.05. Statistical analyses were performed using the Statistical Analysis System software (SAS).

RESULTS

Productive performance

Percent of rotenone content derived from the root extracts of *Derris malaccensis* was 7.41%, which analyzed by Aroon (2008)

Vegetative fungal growth

Derris extracts affected the colony growth of all *B. bassiana* isolates tested. Significant inhibition was observed in colony growth as compared with control. No significant difference was observed between the concentration of derris extract at 1% and 0.1%. No significant inhibition of colony growth with derris extract was observed between the third and the seventh days of inoculation (Table 1).

https://doi.org/10.14456/jsat.2020.10

Table 1. Colony growth (diameter± SE) of *Beauveria bassiana* cultured on MEA medium amended with different concentration of derris extract, at 25 ± 2 °C and 12 h photophase

Isolates	Concentration	Colony growth			
		3 days (cm ± SE)	Inhibition (%)	7 days (cm ± SE)	Inhibition (%)
Bb. 2637	Control	2.782 ± 0.086 a	00.00 A	5.026 ± 0.120 a	00.00 A
	0.1%	1.844 ± 0.026 b	33.81 A	3.392 ± 0.143 b	32.95 A
	1%	1.706 ± 0.065 b	38.66 A	2.702 ± 0.187 b	46.60 A
Bb. 4591	Control	2.610 ± 0.087 a	00.00 A	4.472 ± 0.235 a	00.00 A
	0.1%	1.962 ± 0.037 b	24.90 A	3.418 ± 0.099 b	23.77 A
	1%	1.762 ± 0.031 b	32.57 A	2.832 ± 0.152 b	36.83 A
Bb. 5335	Control	2.738 ± 0.067 a	00.00 A	4.912 ± 0.447 a	00.00 A
	0.1%	1.652 ± 0.055 b	39.67 A	2.848 ± 0.118 b	39.71 A
	1%	1.536 ± 0.044 b	43.87 A	2.582 ± 0.078 b	47.45 A
Bb. 5736	Control	2.522 ± 0.098 a	00.00 A	4.186 ± 0.183 a	00.00 A
	0.1%	1.966 ± 0.056 b	19.84 A	3.402 ± 0.173 a	18.89 A
	1%	1.978 ± 0.022 b	21.62 A	3.372 ± 0.032 a	25.93 A
Bb. 6241	Control	2.520 ± 0.034 a	00.00 A	4.062 ± 0.033 a	00.00 A
	0.1%	1.656 ± 0.073 b	34.32 A	2.980 ± 0.118 b	26.60 A
	1%	1.466 ± 0.036 b	41.86 A	2.698 ± 0.193 b	33.57 A

Means in a column with different small letters indicated significant differences among different concentrations of derris extract within the same isolate (one way ANOVA, P ≤ 0.05; Duncan's multiple range test). Means in the same row followed by the same capital letters indicate no significant difference between two days after inoculation within the same isolate at P ≤ 0.05 (T-test)

Effect of derris extract on colony growth of *Metarhizium* spp. showed the same tendency as *Beauveria bassiana*, derris extract inhibited the colony growth of all fungi test, but percentage inhibition was isolates – dependent. (Table 2)

Table 2. Colony growth (diameter ± SE) of *Metarhizium* spp. cultured on MEA medium amended with different concentration of derris extracts, at 25 ± 2 °C and 12 h photophase

Isolates	Concentration	Colony growth			
		3 days (cm ± SE)	Inhibition (%)	7 days (cm ± SE)	Inhibition (%)
Ma. 6071	Control	2.386 ± 0.063 a	00.00 A	4.758 ± 0.100 a	00.00 A
	0.1%	1.382 ± 0.033 b	42.13 A	2.728 ± 0.043 b	42.69 A
	1%	1.112 ± 0.026 b	53.45 A	2.112 ± 0.064 b	55.62 A
Ma. 6079	Control	1.766 ± 0.041 a	00.00 A	3.002 ± 0.079 a	00.00 A
	0.1%	1.606 ± 0.034 ab	9.06 B	2.270 ± 0.046 ab	21.66 A
	1%	1.386 ± 0.024 b	21.82 A	2.214 ± 0.032 b	26.33 A
Ma. 6171	Control	2.616 ± 0.042 a	00.00 A	4.836 ± 0.062 a	00.00 A
	0.1%	1.792 ± 0.054 b	31.55 A	3.488 ± 0.025 b	27.92 A
	1%	1.506 ±0.006 b	42.44 A	2.588 ± 0.078 b	46.54 A
Ma. 6491	Control	3.022 ± 0.107 a	00.00 A	5.580 ± 0.137 a	00.00 A
	0.1%	2.236 ± 0.043 b	25.99 A	3.908 ± 0.085 b	30.02 A
	1%	1.956 ± 0.028 b	35.26 A	3.478 ± 0.029 b	37.72 A
Ma. 7965	Control	2.618 ± 0.045 a	00.00 A	4.728 ± 0.060 a	00.00 A
	0.1%	1.762 ± 0.025 b	32.67 A	3.152 ± 0.086 b	33.33 A
	1%	1.416 ±0.032 b	45.89 A	2.422 ± 0.033 b	48.78 A
Mfl. 1164	Control	1.512 ± 0.032 a	00.00 A	2.762 ± 0.080 a	00.00 A
	0.1%	1.360 ± 0.046 a	9.93 A	2.512 ± 0.060 a	9.05 A
	1%	1.202 ± 0.022 a	20.52 A	2.268 ± 0.052 a	17.93 A

Means in a column with different small letters indicated significant differences among different concentrations of derris extract within the same isolate (one way ANOVA, P ≤ 0.05; Duncan's multiple range test). Means in the same row followed by the same capital letters indicate no significant difference between two days after inoculation within the same isolate at P ≤ 0.05 (T-test)

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The effect of derris extract on colony growth of *Paecilomyces* spp is illustrated in table 3. The colony in culture media containing derris extract had their vegetative growth reduced compared to control, even though the difference was not always significant.

Table 3. Colony growth (diameter ± SE) of *Paecilomyces* spp. cultured on MEA medium amended with different concentration of derris extracts, at 25 ± 2 °C and 12 h photophase

Isolates	Concentration	Colony growth			
		3 days (cm ± SE)	Inhibition (%)	7 days (cm ± SE)	Inhibition (%)
Pfu. 2507	Control	1.436 ± 0.021 a	00.00 A	3.142 ± 0.069 a	00.00 A
	0.1%	1.224 ± 0.034 a	14.98 A	2.866 ± 0.037 a	8.75 B
	1%	1.178 ± 0.026 a	18.11 A	2.620 ± 0.040 a	16.71 A
Pt. 7968	Control	1.9080 ± .037 a	00.00 A	3.642 ± 0.121 a	00.00 A
	0.1%	1.302 ± 0.041 a	32.41 A	2.930 ± 0.025 a	19.29 B
	1%	1.082 ± 0.021 a	43.30 A	2.236 ± 0.066 a	38.59 A
Pt. 7996	Control	2.498 ± 0.024 a	00.00 A	4.742 ± 0.040 a	00.00 A
	0.1%	1.808 ± 0.035 ab	27.65 A	3.614 ± 0.147 ab	21.20 A
	1%	1.566 ± 0.025 b	37.27 A	3.444 ± 0.324 a	34.28 A
Pt. 8003	Control	2.378 ± 0.070 a	00.00 A	4.546 ± 0.187 a	00.00 A
	0.1%	1.906 ± 0.089 b	19.79 B	3.068 ± 0.097 b	32.56 A
	1%	1.730 ± 0.035 b	27.37 A	3.058 ± 0.102 b	32.78 A

Means in a column with different small letters indicated significant differences among different concentrations of derris extract within the same isolate (one way ANOVA, $P \leq 0.05$; Duncan's multiple range test). Means in the same row followed by the same capital letters indicate no significant difference between two days after inoculation within the same isolate at $P \leq 0.05$ (T-test)

Conidia viability

The results showed that the conidia germination was isolates–dependent (Table 4). The germination varied among fungi species and extract concentrations. Mostly, no significant differences were observed between control and treatment.

Table 4. Percentage germination of *Beauveria bassiana* conidia at 24 h on MEA medium amended with different concentration of derris extracts, at 25 ± 2 °C and 12:12 h (L:D) photoperiod

Isolate	Percentage germination (% ± SE)		
	control	0.1% Derris extract	1% Derris extract
Beauveria bassiana			
Bb. 5335	100 ± 0.00 a	100 ± 0.00 a	100 ± 0.00 a
Bb. 4591	100 ± 0.00 a	100 ± 0.00 a	87.38 ± 1.35b
Bb. 6241	100 ± 0.00 a	100 ± 0.00 a	100 ± 0.00 a
Bb. 2637	100 ± 0.00 a	100 ± 0.00 a	100 ± 0.00 a
Bb. 5736	100 ± 0.00 a	100 ± 0.00 a	74.09 ± 1.67b
Metarhizium anisopliae			
Ma. 6071	100 ± 0.00 a	94.7 ± 5.30 a	55.08± 0.00b
Ma.6079	100 ± 0.00 a	100 ± 0.00 a	100 ± 0.00 a
Ma.6171	100 ± 0.00 a	100 ± 0.00 a	96.78 ± 3.22a
Ma.6491	100 ± 0.00 a	80.53 ± 4.91b	67.84 ± 5.65c
Ma.7965	100 ± 0.00 a	100 ± 0.00 a	100 ± 0.00 a
Metarhizium flavoviride			
Mfl.1164	100 ± 0.00 a	100 ± 0.00 a	100 ± 0.00 a
Paecilomyces fumosoroseus			
Pfu. 2507	100 ± 0.00 a	100 ± 0.00 a	100 ± 0.00 a
***Paecilomyces tenuipes*:**			
Pt.7968	100 ± 0.00 a	100 ± 0.00 a	100 ± 0.00 a
Pt. 7996	100 ± 0.00 a	100 ± 0.00 a	100 ± 0.00 a
Pt. 8003	100 ± 0.00 a	100 ± 0.00 a	100 ± 0.00 a

Means in a column with different small letters indicated significant differences among different concentrations of derris extracts within the same isolate (one way ANOVA, $P \leq 0.05$; Duncan's multiple range test).

https://doi.org/10.14456/jsat.2020.10

DISCUSSION

The roots of derris contain rotenone, in which rotenone is a botanical pesticide. It has pesticide activity against a wide variety of insects and arachnids encountered in both domestic and commercial horticulture and field crop. It has been shown a smaller negative effect on mycelial growth of plant disease fungi (Sookchaoy, 2007). However, the mechanism of action of derris on vegetative growth and conidia viability of entomopathogenic fungi is still unknown. The results of these experiments demonstrated that the derris extract did not affect the germination of conidia of entomopathogenic fungi but inhibited the colony growth of all fungi test. In fact, spores of the entomopathogenic fungi have been used successfully to control insects. High toxicity *in vitro* did not always mean that the same will occur in the field, but it shows a possibility for this to occur. These corroborate with Depieri et al. (2005), who stated that extracts had high toxicity *in vitro* does not mean that the extracts will always be toxic for the fungi in the field. This might be explained by the fact that fungi vegetative growth will occur or will be inhibited only inside the host. Concentration inside the insect is probably smaller than that used in in vitro tests. Thus, in vitro results represent what happens with the vegetative growth of fungi inside the insect body in a more drastic way. Moreover, under field conditions, environmental factors decrease the impact of toxic components on the fungus. Furthermore, Wood et al. (2005) reported that rotenone rapidly decomposes upon exposure to light and air. Its persistence is therefore limited to 2-3 days. In this situation, inhibition of vegetative growth might be a less representative indication of fungitoxicity than the viability of conidia or the effect of germination; due to under field conditions, compatibility between derris extracts and germination was necessary because insects become infected by means of conidia germination, by contact. The compatibility in the field should consider the effect of the extract on conidia germination as one of the most critical factors. This was due to the fact that fungi infect insects through the conidium germination by contact. In addition, the inoculum survival of the entomopathogenic fungus in the field was made by conidia. At the beginning of an epizootic, the conidia were responsible for the occurrence of the first disease focuses. Thus, if germination inhibition occurs, the pathogen control efficiency will be committed if the fungi have been applied in inundative form, together or separately with derris extracts, or the fungi was natural present in the insects and contacts the derris extracts.

CONCLUSIONS

This study had identified that derris extract had no negative effect on germination of promising entomopathogenic fungi; *Beauveria* sp., *Metarhizium* spp., and *Paecilomyces* spp. as biological control agents, even though effects on vegetative growth. Therefore, in the IPM program, they can be recommended for pest control where these entomopathogenic fungi are important natural enemies, and derris extracts are important botanical insecticides.

ACKNOWLEDGMENTS

The authors thank Asst. Prof. Aroon Sottikul for providing derris extract used in this study.

REFERENCES

Aroon, S. 2001. Efficiency of some local plant extractions to control flea beetles (*Phyllotreta* spp.) In Proceeding 5th National *conference on plant Protection* 21-23 November 2001 Kanchanaburi, 2001. p. 47-54.

Aroon, S. 2008. Product development from derris roots to control cabbage aphid (*Lipaphis erysimi* (Kaltenbach) Ph.D. Dissertation. Chiang Mai University, Chiang Mai, Thailand.

Aroon, S., Thungrabeab, M., Aemprapa, S., and Suteekan S. 1997. Study on using botanical insecticide to control mango leafhopper. In *Proceeding 3th National conference on plant Protection* 18-20 November 1997, Bangkok, p. 326-329.

Aroon, S., Pitaya, S., and Prachaval S. 2006. Formula development of derris root extract to controlled cabbage aphids In *Proceeding 6th National Horticulture Congress* 7-10 November 2006 Chiangmai, p. 130.

Depieri, R.A., Martinez, S., and Menezes, A.O. 2005. Compatibility of fungus *Beauveria bassiana* (Bals.) Vuill. (Deuteromycetes with extracts of neem seeds and leaves and the emulsible oil, Neotropical Entomology 34(4): 601-606.

Malee, T., and Sirinun A. 2001. Efficacy of *Beauveria* spp. to control aphids, In *Proceeding 5th National conference on plant Protection* 21-23 November 2001 Kanchanaburi, p. 63-70.

Malee, T., and Sirinun, A. 2002. Screening of entomopathogenic fungi *Beauveria* spp. against the aphid, *Myzus persicae* and *Macrosiphum euphorbiae*. In *Proceeding 3rd International conference on Biopesticides* 22-26 April 2002 Kuala Lumper, Malaysia, p. 157-160.

Rey, D.E. 1991. Pesticide derived from plants and other organisms. In Hayes, W.J.. and Laws, E.R. (eds.) Hand book of pesticide Toxicilogy, Academic Press, New York, 2-3 p.

Sengonca, C., Thungrabeab, M., and Blaeser, P. 2006. Potential of the different isolates of entomopathogenic fungi from Thailand as biological control agents against western flower thrips, *Frankliniella occidentalis* (Pergande) (Thys., Thripidae). *Journal of Plant Disease and Protection*, *113(2)*, 74-80.

Sookchaoy, K. 2007. Efficacy of plant extracts to control some important plant disease under laboratory and greenhouse conditions. Research Report, Lampang Agricultural Research and Training Centre Rajamangala University of Technology Lanna, 25p.

https://doi.org/10.14456/jsat.2020.10

St Leger, R.J., and Wang, C. 2009. Entomopathogenic fungi and the genomic era. In: Stock, S.P., Vandenberg, J., Glazer, I., and Boemare, N. (eds.), Insect Pathogens: Molecular Approaches and Techniques. CABI, Wallingford, UK, p.366-400 http://dx.doi.org/10.1079/9781845934781.0365

Thungrabeab, M., Blaeser, P., and Sengonca, C. 2006. Possibilities for biocontrol of the onion thrips *Thrips tabaci* Lindeman (Thys., Thripidae) using different entomopathogenic fungi from Thailand. *Mitt. Dtsch. Ges. allg. angew. Entomol. Mitt. Dtsch. Ges. allg. angew. Entomol 15*, 299-304.

Thungrabeab, M. 2007 Comparison of the efficacy of entomopathogenic fungi on insect pests and non-target insects. *In Proceeding 5th International Symposium on Biocontrol and Biotechnology* 1-3 November Nong Khai.

Wood, D.M., Alsahaf, H., Streete, P., Dargan, P.I., and Jones, A.S. 2005. Fatality after deliberate ingestion of pesticide rotenone: a case report. Licenses BioMed Central Ltd., p. 280-284.