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ENTOMOPATHOGENIC FUNGI SCREENING AGAINST FRUIT FLY IN THAILAND

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ABSTRACT

The screening of entomopathogenic fungi, 7 isolates of *Metarhizium* spp., 12 isolates of *Beauveria* spp., and 1 isolate of *Hirsutella citriformis* against fruit fly was performed in Lampang Agricultural Research and Training Centre, Lampang, Thailand. The efficacy test was done by spraying suspension of 10^8 spores per ml of fungus on *Bactrocera dorsalis* in completely randomized design experiments. The numbers of the dead fruit flies which were covered by fungus was recorded and percent mortality was calculated by Abbott's formula. The results showed that the percentage of fruit fly mortality was from 2 to 68%. *Beauveria* sp. isolate 6241 which killed 50% of fruit flies was selected and virulent level was checked by calculating the LC_{50} using completely randomized design experiments. The results showed that the numbers of the fruit flies killed by fungi were correlated with the spore concentration. Its LC_{50} was $7.36x10^7$spores/ml respectively.

KEYWORDS: friut fly, entompathogenic fungi, biological control

1. INTRODUCTION

Mango is an important fruit in Thailand due to high consumption of both fresh and processing products. Furthermore it can be exported in high value to Japan, Malaysia, Australia etc. In year 2005, Thailand exported 12,206 tons of mango, in value of 547.73 million bath [1]. Expert from C.P. company, Thailand [2] said that the first policy for exported fruits is to improve the fruit quality especially pesticide contamination.

In central and northern parts of Thailand, insecticide application for fruit fly control is very costly, and is not affordable to small-scale farmers. Furthermore unilateral use of insecticides could not effectively control fruit fly, hence, an integrated management system is necessary. Nowadays the controls used in the integrated management system are fruit bagging, protein bait spraying, methyl eugenol trap, parasitoid, predator and sterile male release which affect the fruit fly adults [3-5]. The alternative way by controlling the fruit fly at the late larva and pupa stages to reduce the fly population was considered. The possible way is the biological control using entomopathogenic fungi to apply for the fruit plantation. To get the effective entomopathogenic fungal strain, the screening of entomopathogenic fungi on the oriental fruit fly, *Beauveria dorsalis* Hendel, was investigated.

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2. MATERIALS AND METHODS

2.1 Insect

Fruit flies were collected from mango crops and other fruit trees in Lampang Agricultural Research and Training Centre, Lampang province, Thailand and has been maintained in raring cage, size 2x2x3 ft^3. The adult fruit flies were kept at room temperature and were supplied with sugar : yeast = (3 : 1). Fruit flies laid eggs in plastic bottles with inner nylon sheet soaked with guava juice. Larvae were rear in artificial food (Methyl-p-hydroxybenzoate 0.1%, sodium benzoate 0.1%, suger 12.0%, dried yeast 3.6%, wheat bran 26.0%, HCl (conc.) 0.2%, water 58.0%). Larvae (8 days after emerging) were used in the experiment.

2.2 Fungi

Seven isolates of *Metarhizium* sp. and 12 isolates of *Beauveria* spp. and 1 isolate of *Hirsutella citriformis* from the culture collection of the National Center for Genetic Engineering and Biotechnology, Thailand were used for screening. Malt peptone agar (MA) containing 3% malt extract and 0.5% peptone solidified with 1.5% agar was used for cultivation of entomopathogenic fungi. The fungi were kept at 4°C as stock culture. Conidia development on MA at 25°C from the stock culture were used for the bioassays.

2.3 Bioassay

For the first screening, the fungi were grown on MA plates and incubated at room temperature (25°C) for 7-10 days. Then spores were collected as spore suspension in sterile distill water with Tween 80 0.1% and determined the spore concentration by a Haemacytometer. The spore concentration of $1x10^8$ spores per ml was used in the bioassay on *Bactrocera dorsalis*. The sterile distill water with Tween 80 0.1% was used as control. The number of infected pupae was recorded everyday after spraying until 2 weeks was passed. The adults that emerged from pupae were checked for their death. For the second screening, the isolates which have % mortality higher than 50% in the first screening were selected for virulent test. The experimental design was Factorial in Completely Randomized Design (Factorial in CRD) with isolate as factor A and concentration levels, $1x10^3$, $1x10^5$, $1x10^7$, $1x10^9$, $1x10^{11}$ spores/ml, as factor B. The sterile distill water with Tween 80 0.1% was used as control. The data was recorded the same way as the first screening. Then calculated LC_{50} by MstatC program using probit analysis of Finney [6]. The virulence levels were performed by calculating the Log LC_{50} and then leveling by Duncan's multiple renge test (DMRT) and normal distribution.

3. RESULTS AND DISCUSSION

The result showed that all fungi had pathogenicity against *B. dorsalis* (Table 1) and can be classified into three groups according to mortality percentage (Table 2). Fungal isolates in group 1 were selected for their high pathogenicity. *Beauveria bassiana* isolate 6241 with 68 % pupa mortality was tested for virulence as second screening. The virulence level was classified by the LC_{50} of the fungi. Compared to the work of Konstantopoulou *et al.* [7] which showed that a strain of *Mucor hiemalis* isolated from *Sesamia nonagrioides* larvae was the most toxic resulting in 85.2% mortality to the olive fruit fly adults. *Beauveria brongniartii* and *B. bassiana* were the most pathogenic to *Ceratitis capitata* adults causing 97.4 and 85.6% mortality. In another work, the measurement of the pathogenicity of 10 autochthonous isolates of *Beauveria bassiana* and of five *Metarhizium anisopliae* toward the pupa and adult of Mediterranean fruit fly, *Ceratitis capitata*, were done by Quesada-Moraga *et al.* [8]. The result showed that the mortality rates ranging from 30 to 100% and average survival times (ASTs) from 6.5 to 8.6 days, when *C. capitata* pupae were

immersed in the conidial suspensions, only *B. bassiana* Bb-1333 and EABb 01/103-Su and *M. anisopliae* EAMa 01/58-Su isolates caused >50% mortality of pupae. The median lethal concentrations (LC_{50}) of the four most virulent isolates ranged from 4.9×10^5 to 2.0×10^6 cfu/ml with estimated time to kill 50% of the insects ranging from 4.6 to 5.3 days. In this experiment, the result showed the same way of the later experiment of Quesada-Moraga *et al*. [8] that only *B. bassiana* Bb6241 caused >50% mortality of the pupae (Figure 1). The adults that emerged from pupae in the pathogenicity test were not only laying eggs but also died.

Table 1 Mortality of fungi against oriental fruit fly

Isolate	% Mortality
control	0.00 E
H662.1	9.07 CDE
M.2787	5 CDE
M 3000	1.67 DE
M 3021	3.73 CDE
M 6071	6.67 CDE
M 6171	16.73 BCDE
M 7527	7.33 CDE
M 7965	6.67 CDE
B 6988	10.43 BCDE
Bb 2605	3.3 CDE
Bb 2637	7 CDE
Bb 3073	25.00 BC
Bb 4871	8.3 CDE
Bb 5335	19.67 BCDE
Bb 5736	22.3 BCD
Bb 6241	68.03 A
Bb 6942	8.73 CDE
Bb 6966	8.3 CDE
Bb 7757	30.3 B
Bb 7772	7.3 CDE

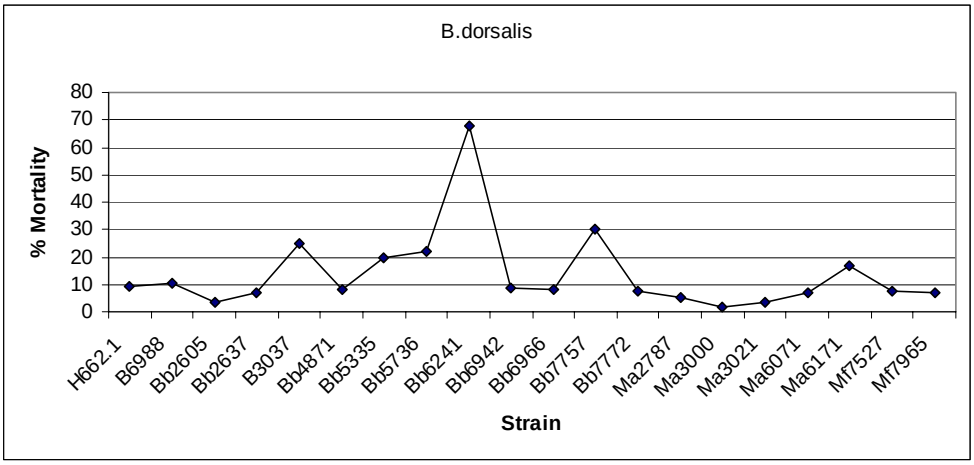

Figure 1 Mortality percentage of fungi against *B. dorsalis*

Table 2 Pathogenicity level of insect fungi on aphids

% Mortality	Strain
>50%	6241
49-10%	6171, 3073, 5335, 5736, 6988, 7757
<10%	H662.1, M.2787, 3000, 3021, 6071, 7527, 7965, 2605, 2637, 4871, 6942, 6966, 7772

The LC_{50} values of *Beauveria bassiana* strain Bb 6241 against *B. dorsalis* was $7.36x10^7$spores/ml. or Log LC_{50} was 7.86.

4. CONCLUSIONS

The origin of entomopathgenic fungi was from insects. There was no isolate from Dipteran insect origin. This suggested that fungi from any insect origin would have potential of high pathogenicity against the host insect and a wide variety of isolates should be examined for the screening of useful fungi. The potential to adopt entomopathogenic fungi for reducing the oriental fruit fly population was obvious. Among the screened fungi, the isolate 6241was the most suitable fungus because of high pathogenicity and also easy culturing and quick growing. This biological control agent can be a tool for integrated pest control, supporting organic farming for mango and other fruits crops in Thailand.

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