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Potential of entomopathogenic fungi for controlling rice leafhoppers and lepidopterous larvae in northern Thailand

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Abstract The most virulent strain of *Beauveria bassiana* and of *Metarhizium anisopliae* was selected from each of ten indigenous strains, which had been collected from paddy fields. The collected strains were preliminarily screened in the laboratory and were then tested in greenhouse experiments for their pathogenicity against two species of rice pests: rice leafhopper (*Nephotettix virescens*) and rice leafroller (*Cnaphalocrocis medinalis*). The highest pathogenicity to *N. virescens* and *C. medinalis* at 7 days after inoculation was 78.9 ± 10.7% and 51.6 ± 12.0%, respectively, for *B. bassiana* isolate Bb06 was 86.3 ± 14.2% and 76.8 ± 7. 3% for *M. anisopliae* isolate Ma01, respectively. Dose-response studies revealed LC_{90} values ranging from 1.3×10^9 to 1.3×10^{11} and 1.25×10^7 to $1.5 \times 1\ 0^9$ spore mL^{-1} for Bb06 and Ma01, respectively. These two entomopathogens were subjected to another pathogenicity test in order to select the most suitable mycoinsecticide and media formulations. Spraying a 1:1 (v/v) mixture of the Bb06 and Ma01 spore suspensions at their highest LC_{90} value (24.8×10^{11} and 24.8×10^9 spore mL^{-1}, respectively) in water provided the highest mortality to *N. virescens* and *C. medinalis* at 81.2 ± 6.4 and 100.0%, respectively. Therefore, the combination of the Bb06 and Ma01 isolates was ultimately applied to rice plants at a field plot level. A direct effect of these two entomopathogenic fungi on rice pest insects, in terms of the corrected percent cumulative mortality and approximate population level, was considered as the effectiveness parameters. The mortality (%) in the treated and control plots was 18.9 ± 23.5 and 4.1 ± 10.7%, respectively, while the population density was 0.3 ± 0.3 and 0.4 ± 0.3 insects per evaluation time.

Keywords: *Beauveria bassiana*, Biological control, *Metarhizium anisopliae*, Pathogenicity test, Rice pest insects

Introduction

Entomopathogenic fungi are useful for the biological control of rice pests in paddy fields, especially in South East Asia, due to the suitable climate and geological landscape. The northern part of Thailand encompasses a major flood plain that has been used to grow rice for a

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thousand years. The rice pest insects and the native entomopathogenic fungi have co-adapted for their survival (Babendreier *et al*., 2020).

Evaluation of the pathogenicity of native fungi in this area is very important for screening and selection of highly pathogenic fungal strains (Rajula *et al*, 2021). *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metchnikoff) Sorokin (Hypocreales: Clavicipitaceae) are highly infective to several species of sucking pests and can cause more than 70% mortality (Feng *et al*., 2004). Several studies have reported on the use of these two entomopathogens to control rice leafhoppers (Bugti *et al*., 2018) and lepidopterous larvae under both laboratory and field conditions (Fite *et al*., 2019). Huynh *et al*. (1999) found that both *B. bassiana* and *M. anisopliae* isolated from the brown plant hopper [BPH; *Nilaparvata lugens* (Stål)] in Vietnam induced 60.3–70.7% host mortality at 7 days after treatment with fungal doses ranging from 6×10^{12} to 10×10^{12} spores mL^{-1}. The insects in the family Crambidae of the order Lepidoptera, such as the rice stem borer and rice leafroller (*Cnaphalocrocis medinalis*) are considered to be the important rice pests in Thailand (Wongsiri, 1991).

Beauveria bassiana has been used to control the European corn borer, *Ostrinia nubilalis* (Feng *et al*., 1994), as well as 1^{st} – 3^{rd} instars of the sugarcane stalkborer, *Eoreuma (Acigona) loftini* and sugarcane borer, *Diatraea saccharalis* (Lepidoptera: Pyralidae), which are the important pests that caused heavy damage in Texas, USA; where at a dose of 5×10^{13} spores mL^{-1} they caused a marked decrease in the population level of these insects in the field (Legaspi *et al*., 2000). In addition, *M. anisopliae* has also been evaluated for its potential to control rice or sugarcane pyralid larvae (Legaspi *et al*., 2000).

The objectives of this study were to screen for pathogenic strains of *B. bassiana* and *M. anisopliae* against two important rice pest insects: the rice leafhopper (*Nephotettix virescens*) and *C. medinalis* in the laboratory and then to further investigate efficacy of the most virulent strain of *B. bassiana* and *M. anisopliae* against important rice pest insects at the laboratory and field plot levels.

Materials and methods

Laboratory screening for the most virulent fungal isolates

The pathogenicity tests were conducted with ten *B. bassiana* isolates (Bb01 – Bb10) and ten *M. anisopliae* isolates (Ma01–Ma10). The chosen locations were in the three Districts of Wat Bot, Mueang Phitsanulok, and Wang Thong (16.60–17.50 ºN and 100.30–100.60 ºE). Each study site was divided into 10 subplots of 20 x 20 m. One each subplot was sampled monthly at 50 samples. All of fungal obtained from the Biological Control laboratory, Suandusit University, Thailand against *N. virescens* and *C. medinalis*. The tests were initially screened using a single fungal concentration

(10^7 spores mL^{-1}) as previously reported (Lacey, 1997) and the previously reported inoculation method (Goettel and Inglis, 1997). For each isolate, the conidia were harvested from 14-d-old *B. bassiana* and 10-d-old *M. anisopliae* that were cultured on potatoe-dextrose-agar medium, and then quantified and adjusted to 10^7 conidia mL^{-1} as previously reported (Lacey, 1997; Goettel and Inglis, 1997).

All insect species were obtained from the Maejo University Biological Control Laboratory (MJU – BCL), Department of Plant Protection, Faculty of Agricultural Production, Maejo University, Chiang Mai, Thailand.

The experimental design utilized a completely randomized design (CRD) with five replicates per isolate and 10 or 20 insects per experimental unit (depending upon the insect species). For the rice leafhopper, 20 adults per replication, collected from paddy fields, were released into a 10 cm × 20 cm (D × H) transparent plastic container with rice seedlings and covered with the respective cover with a 1 cm × 1 cm hold attached with a screen on the top (Figure 1), while first to second instars of 10 rice leafroller larvae were maintained on a rice leaf moistened by wet tissue paper in a Petri dish. All treatments were sprayed using a hand atomizer that could deliver an even volume diameter droplet spray of 75 μm before covering their respective caps with a 1 cm diameter hold attached with muslin cloth on the top (for the clear plastic container). Control insects were sprayed with 0.02% (v/v) Tween 80 in sterile distilled water instead of the conidia suspension. The boxes or petri dishes were placed on a plywood shelf (random position selection) and maintained at 25–28 ℃ and 40–60% relative humidity (RH). Mortality was observed at 24-h intervals for up to 2 weeks.

The criterion for evaluating mortality was to consider as dead those adults that presented neither movement nor the capacity to react. After being defined as dead, the dead insects were incubated in individual humid chambers in a Petri dish at 23–25 ℃ and 90 ± 1% RH to observe mycelia growth, while conidia formation was confirmed after further incubation of the cadavers. The evaluation of the insect mortality percentage was performed when the first treatment reached 100% mortality, and was corrected as suggested by Abbott (1925) and shown in Eq. (1):

$$\text{Corrected}\,(\%) = 1 - \frac{\text{n in T after treatment}}{\text{n in Co after treatment}} \times 100 \qquad (1),$$

where: n is the insect population size, T is the treated sample, and Co is the control sample.

The obtained results were compared as a 7-d percent cumulative mortality (PCM) of each strain using variance analysis and separation of means through the Duncan Multiple Range Test (DMRT) accepting significance at the $p \leq 0.05$ level (LeClerg *et al*., 1966).

The isolates of both *B. bassiana* and *M. anisopliae* that presented the highest mortality were selected for evaluation of their lethal concentrations at 90% (LC_{90}). The isolates were multiplied using solid substrates as

previously described (Lacey, 1997; Goettel and Inglis, 1997) and then conidia suspensions were prepared at 0, 10^2, 10^4, 10^6, 10^8, and 10^{10} spores mL^{-1} as previously reported (Goettel and Inglis, 1997). The tests were performed using 10 rice leafroller larvae or 20 rice leafhoppers of the same developmental stage per replication for five replications each. The insects were placed in containers at the previously described condition (above) used to screen for the most virulent isolate. The insect mortality was evaluated daily after incubation in individual humid chambers at 23–25 ℃ to determine the moment of development of the mycelium and conidia (Poinar and Thomas, 1984).

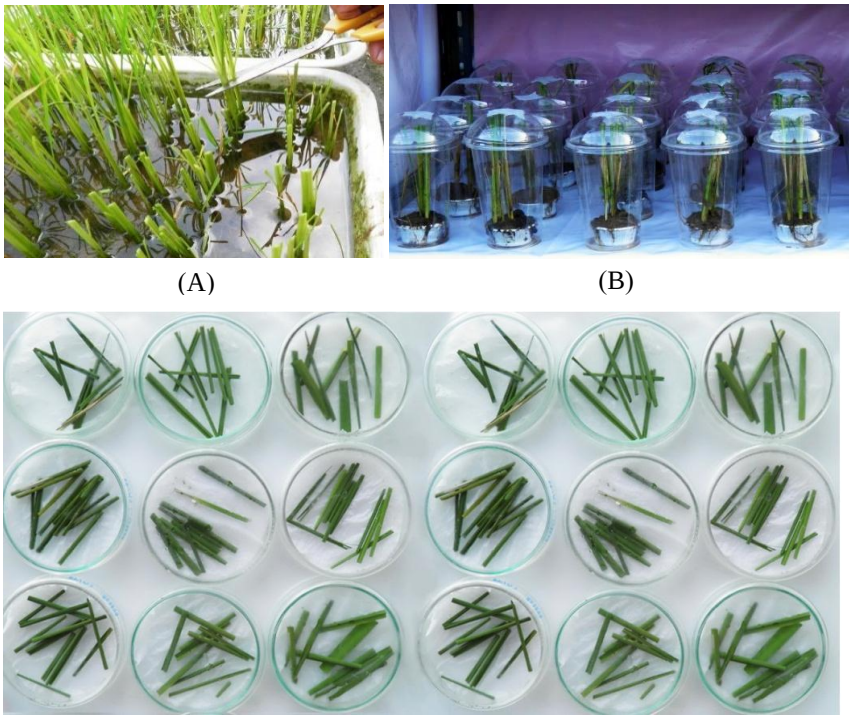
The insect mortality level (%) for different inoculum concentrations was evaluated and subsequently corrected as above (Abbott, 1925). The data were determined for probits by looking up the corresponding percentages in Finney's table (Finney, 1952), taking the log of the concentrations, plotting the probits versus the log of the concentrations, and then fitting a line of regression to compare the data with the relationship of the response variable or dependent variable (Y) to the independent variable (X). Next, the inverse of the log was taken to obtain the LC_{90} value, as previously described by Finney (1952).

Selection of the mycoinsecticide formulation

The most virulent isolates of both *B. bassiana* and *M. anisopliae*, selected as previously described, were evaluated for their most suitable mycoinsecticide formulation at the selected dose based on their LC_{90} value (Butt and Goettel, 2000; Poinar and Thomas, 1984). The pathogenicity tests with *B. bassiana* and *M. anisopliae* against the rice stem borer and *N. virescens* were conducted in the same manner as specified above. To evaluate the most suitable formulation of the entomopathogenic fungi, eight treatments were investigated as detailed: (1) control water (w), sprayed with only distilled water; (2) control vegetable oil (vo), sprayed with only vegetable oil; (3) *Beauveria* + w, sprayed with only *B. bassiana* suspended in distilled water at its LC_{90}; (4) Metarhizium + w, sprayed with only *M. anisopliae* suspended in distilled water at its LC_{90}; (5) *Beauveria* and *Metarhizium* + w, sprayed with both *B. bassiana* and *M. anisopliae* suspended in distilled water at their respective LC_{90} values; (6) *Beauveria* + vo, sprayed with only *B. bassiana* suspended in vegetable oil at its LC_{90}; (7) *Metarhizium* + vo, sprayed with only *M. anisopliae* suspended in vegetable oil at its LC_{90}; and (8) *Beauveria* and *Metarhizium* + vo, sprayed with both *M. anisopliae* and *B. bassiana* suspended in vegetable oil at their respective with LC_{90} values.

The experiment was set up as a CRD with a factorial arrangement (4 × 2). The treatments were the four different inoculations [control, *B. bassiana* only, *M. anisopliae* only, and *B. bassiana* mixed 1:1 (v/v) with *M. anisopliae*] and two formulations (water and oil). Each treatment combination was replicated

five times and each replicate consisted of one container with 10 or 20 insects for each experimental unit. The results were compared through the corrected mortality (Abbott, 1925) by the variance analysis and separation of means through the DMRT (LeClerg *et al*., 1966).



(A) (B)

(C)

Figure 1. Experimental units prepared for the laboratory pathogenicity test: cut seedlings (A), arrangement of experimental unit (B), and lepidopterous larvae (C)

Pathogenicity test in outdoor field pots

Rice seeds of Khao Hom Mali 105 variety were prepared for germination by soaking in water for 24 h. The soaked seeds were then wrapped in a moistened cloth for 2–3 d until germinated. The germinated seeds were grown on black clay loam soil in a 30 cm × 30 cm black plastic bag (five germinated seeds per bag, 430 bags) with the water level maintained to at least 10–20 cm depth above the soil surface. At the seedling stage (day 10), 16:16:8 NPK fertilizer was added at 10 g m^{-2}. At day 25, the seedlings were removed from the plot, the leaf was cut off at about 20 cm from the shoot and then the seedlings were transplanted to the same bag at 2–3 plants per bag. The bags were then placed in a warm and sunny outdoor condition and watered 3–4 times a day. At 15 days after transplanting, 16:16:8 NPK fertilizer was added at 25 kg

per rai. Post tillering (at about 30 days, before the milky stage), 40:0:0 fertilizer (urea) was added at 20 kg per rai. Thereafter the plants were watered twice a day.

Results

Screening of the fungal virulence at a laboratory level

The corrected PCM (cPCM) over 7 days for the field-collected rice leafhopper complex exposed to 10 different isolates of *B. bassiana* at doses of 1 $\times 10^7$ conidia mL^{-1} is shown in Figure 2A. The mortality of almost all *B. bassiana* treated insects was significantly higher than in the control. On the other hand, there was a variation in the effectiveness against rice leafhoppers among the different *B. bassiana* treated groups, ranging from 7.8 ± 3.0 to 78.9 ± 10.7% at 7 days after treatment. In this study, Bb06 was ultimately selected as the most virulent *B. bassiana* isolate for further study.

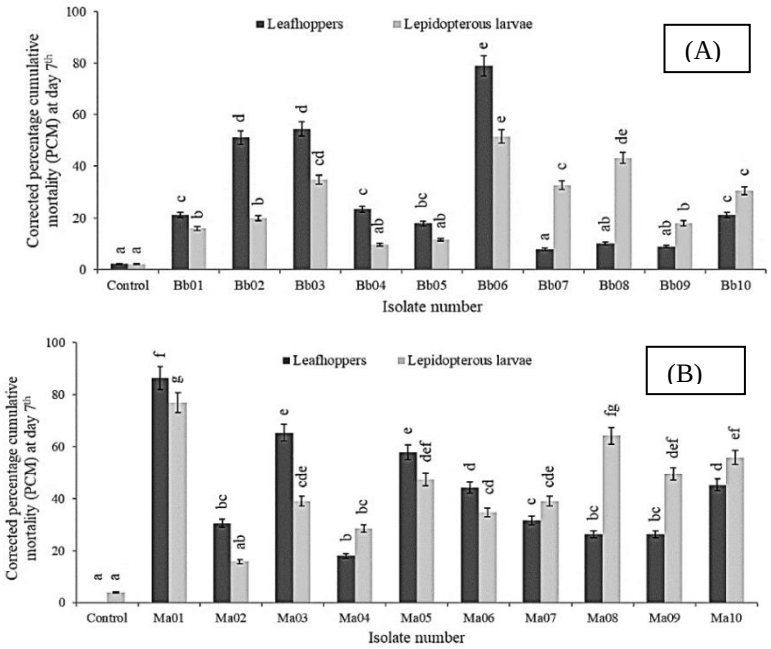


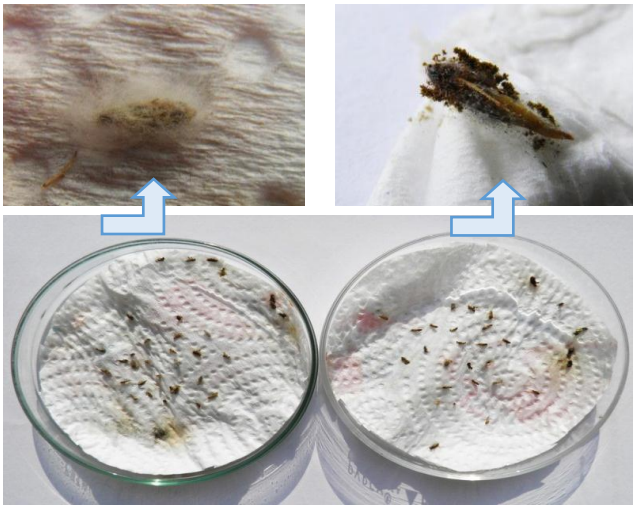
Figure 2. The cPCM of rice leafhopper (*N. virescens*) and rice leafroller (*C. medinalis*) larvae at 7 d after exposure to (A) *B. bassiana* or (B) *M. anisopliae* at 1.5 $\times 10^7$ spores mL^{-1} under laboratory condition. Data are shown as the mean ± 1 SD, derived from five replications. Means with a different letter are significantly different within each insect grouping ($p < 0.05$; DMRT)

All insects treated with *M. anisopliae* isolates at 1.5 $\times 10^7$ showed a significantly higher cPCM than the control, with isolate Ma01 showing the highest potential against rice leafhoppers (mortality of 86.3 ± 14.7) and it was

significantly higher than the other nine isolates (mortality ranging from 17.9 ± 2.9 to 65.3 ±10.9%) as shown in Figure 2B.

Representative images of *N. virescens* and *C. medinalis* infected with *B. bassiana* and *M. anisopliae* are shown in Figures 3 and 4, respectively.

The ten *B. bassiana* and ten *M. anisopliae* isolates were also screened for pathogenicity against the *C. medinalis*. When applied at 1.5 $\times 10^7$ conidia, the 7 days cPCM was significantly higher than in the untreated control (Figure 2). The mortality of the insects treated with *B. bassiana* or *M. anisopliae* varied between isolates, ranging from 9.5 ±5.8 to 51.6 ±12.0% and from 15.8 ±7.4 to 76.9 ± 17.3% respectively. Thus, the *B. bassiana* and *M. anisopliae* isolates with the highest potential against *C. medinalis* in this study were Bb06 and Ma01.



(A) (B)

Figure 3. Representative images of rice leafhoppers (*N. virescens*) infested with *B. bassiana* (A) and *M. anisopliae* (B) in laboratory pathogenicity tests

(A) (B)

Figure 4. Representative images of rice leafroller (*C. medinalis*) larvae infested with *B. bassiana* (A) and *M. anisopliae* (B) in laboratory pathogenicity tests

To be effective microbial insecticides for the control of rice insect pests in the field, the most effective formulation dose of Bb06 and Ma01 was evaluated in terms of their LC_{90} values against *N. virescens* and *C. medinalis* at formulation doses of 0, 10^2, 10^4, 10^6, 10^8, and 10^{10} spores mL^{-1}. The derived probit-log dosage regression coefficients and LC_{90} values are shown in Table 1. Generally, the fungi exhibited an insecticidal activity against the target insects at all tested doses compared to the control. The accumulated mortality over 7 and 10 days induced by isolates Bb06 and Ma01, respectively, against *N. virescens* and *C. medinalis* both dispalyed a dose-response relationship, with the susceptibility of *N. virescens* and *C. medinalis* to Bb06 and Ma01 increasing with increasing applied doses. The derived LC_{90} values ranged from 1.3×10^9 to 1.3×10^{11} for Bb06 and from 1.3×10^7 to 1.5×10^9 for Ma01. Thus, the *B. bassiana* Bb06 and *M. anisopliae* Ma01 formula doses that caused the optimal LC_{90} (1.3×10^{11} and 1.5×10^9 spore mL^{-1}, respectively) were ultimately selected for further studies as potential microbial control agents for some insect pests of rice.

Table 1. Determination of the LC_{90} value of *B. bassiana* isolate Bb06 and *M. anisopliae* isolate Ma01 to *N. virescens* and to *C. medinalis* larvae

Entomopathogenic fungi/insect	Regression analysis of probit mortality for toxicity bioassay		LC_{90}
	Regression Coef.	Chi Square (df)	
B. bassiana/ leafhoppers	0.28	43.14 (26)	1.3×10^9
B. bassiana/ leafroller larvae	0.46	9.97 (17)	1.3×10^{11}
M. anisopliae/ leafhoppers	0.23	86.83 (22)	1.3×10^7
M. anisopliae/ leafroller larvae	0.46	17.76 (21)	1.5×10^9

Note: 95% Confidence Limits

Selection of the mycopesticide formulations

The *B. bassiana* isolate Bb06 and *M. anisopliae* isolate Ma01 spores suspended separately or combined in either water or oil with 0.02% (v/v) Tween 80 were exposed directly to the test insects in the laboratory at their respective LC_{90} values (1.3×10^9 and 1.5×10^{11} spores mL^{-1} for Bb06 and Ma01, respectively). When applied individually or combined together, Ma01 and Bb06 induced a significantly increased insect mortality compared to other isolation numbers (Figure 2). The two different mycoinsecticide formulations were significantly differred in their efficacy at inducing mortality in both *N. virescens* and *C. medinalis*, while the two carriers displayed no significant effect on the mortality ($p = 95\%$). No interaction occured between applicator and carrier factors in the experiment (data not shown). The mortality of the treated *N. virescens* and *C. medinalis* ranged from 83.4 ± 8.5 to 100% and from 48.0 ± 8.4 to 88.0 ± 8.4%, respectively, which were significantly higher than in the control. Among the treated groups (Figure 5), *N. virescens* treated with Ma01 and Bb06 suspended in distilled water

showed the highest mortality (100 %). However, it was not significantly different from that for Ma01 and Bb06 suspended in vegetable oil (97.9 ± 4.6 %).

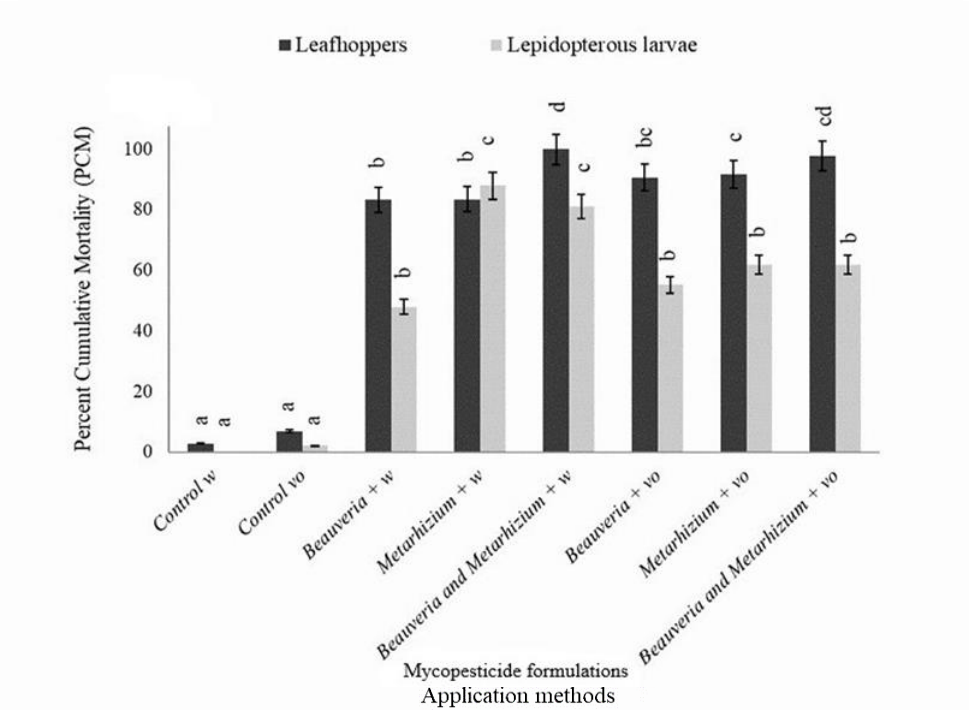


Figure 5. The cPCM of *N. virescens* and *C. medinalis* exposed to isolates Bb06 and Ma01 at their LC_{90} by different mycoinsecticide formulation. 1) control water (w), 2) control vegetative oil (vo), 3) *Beauveria* + w, 4) *Metarhizium* + w, 5) *Beauveria* and *Metarhizium* + w, 6) Beauveria + vo, 7) *Metarhizium* + vo, and 8) *Beauveria* and *Metarhizium* + vo

Pathogenicity test at the field pot level

Based on the laboratory studies, a 1:1 (v/v) mixture of Bb06 and Ma01 was consequently selected for evaluation in a field pot study with the spores suspended in water with 0.02% (v/v) Tween 80 at their highest LC_{90} value (1.25×10^{11} and 1.5×10^{9} spores mL^{-1} for Bb06 and Ma01, respectively). The mycoinsecticide medium was performed at 3-d intervals and compared with the control [water + 0.02% (v/v) Tween 80]. Direct effects of the entomopathogenic fungi on rice pests were evaluated using two indicators. Firstly, the cPCM of the rice pests in the treated and control pots were recorded daily until day 60 of the experiment. Rice pests found infected with entomopathogens included mainly the rice leaf hopper, rice planthopper, other hemipterans, and the Asian rice gall midge (*Orseolia oryzae*) (Figure 6). The cPCM was significantly (t = 5.31) higher in the treated pots (18.9 ± 23.5%) than in the untreated ones (4.1 ± 10.7%). Secondly, the approximate population size

of rice pests in both pots was evaluated. The population level in the treated pots (6.3 ± 0.3 insects per evaluation time) was significantly (t = 5.28) higher than in the control (0.4 ± 0.3 insects per evaluation time). Although the cPCM recorded in the treated pots was higher than that of the control (18.9 ± 23.6% vs. 4.1 ± 10.7%), it was not as high as that observed in the laboratory.

Data were pooled and a weekly cPCM dynamic graph of the insect pests found in the treated and control field pots was constructed. The mortality in the treated pots were higher than in the control pots at almost all experimental periods except for in week 5, where the percent mortality of the treatment was not significantly different from the control (Figure 7).

In addition to the direct effect on the rice pest mortality and population, this study also revealed that the rice plants in the field pots were infested with virus-induced diseases.

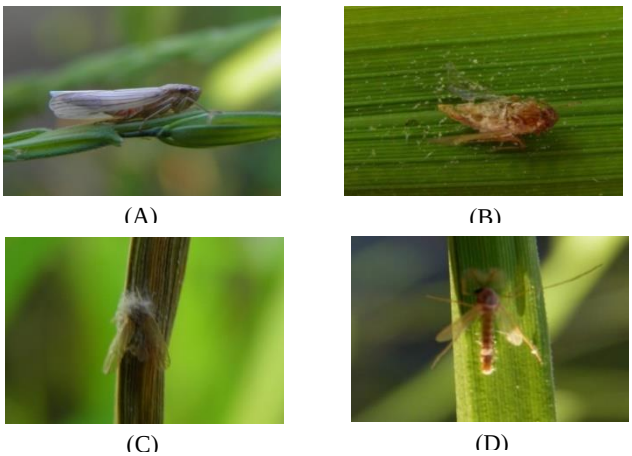


Figure 6. Examples of infected insects detected in entomopathogenic fungi treated pots, including the white leafhopper, *Cofana spectra* (A), planthopper (Hemiptera: Delphacidae) (B), unknown hemipteran (C), and Asian rice gall midge, *Orseolia oryzae* (D)

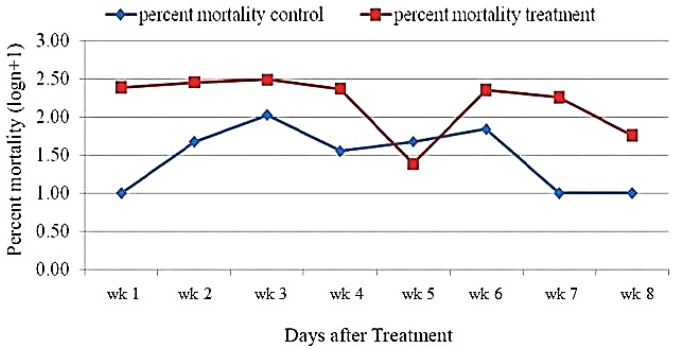


Figure 7. Weekly dynamics of the cPCM of rice pests in the untreated (control) and entomopathogenic fungi treated field pots

Discussion

Although the cPCM of the rice pests in the treated field pots was significantly higher than in the control pots (t = 5.31) the mortality in the treated field pots was nevertheless somewhat low (only 18.86 ± 23.53) compared to that observed in the laboratory study (81–100%). Several studies have reported that the efficacy of most entomopathogenic fungi increased with higher applied spore doses (Feng *et al*., 2004; Legaspi *et al*., 2000; Sharififard *et al*., 2011). This effectiveness result was similar to the report of *B. bassiana* application by spraying on green leaf hopper (Abdullah *et al*., 2020). Interestingly, there were five pots (2.5% of all pots) in the treated rice compared to 80% (160 pots) in untreated control that showed severe symptoms of viral disease, in terms of chlorosis of the plant, severe stunting, and profuse tillering. The chlorotic zone turning whitish-brown symptoms appeared initially on newly emerging leaves and spread thereafter to succeeding leaves. This symptom was found both in the tillers and the main stem. The leaf tips lost turgidity and became frilly acquiring a dirty brown colour. Panicles showed retarded emergence and the grain was often spotted and defective. Although the detection and identification of the virus is not within the scope of this study, the tentative identification based on the data available at www.doa.go.th and the symptoms was that of the yellow rice dwarf virus.

According to Murigu *et al.* (2016) who evaluated the efficacy of entomopathogenic fungi between laboratory and field conditions. The potential causes of this are discussed as follows. Several factors are known to influence the infection potential of entomopathogenic fungi, of which those that may have reduced the efficacy of these fungi in the field compared to the laboratory include the unfavourable high level (intensity) of sunlight, relative humidity, and temperature conditions in the outdoor field. Thus, the laboratory-based evaluation and prediction of the efficacy of these entomopathogenic fungi may not be realistically applicable to agro-ecosystems (Murigu *et al.*, 2016). This is in agreement with a previous study that reported the efficacy of entomopathogenic fungi towards various pests in the field depends on many factors, often related to the behaviour of the insect host in its natural habitat (Gindin *et al.*, 2006). In addition, Dorschner *et al*. (1991) claimed that the mortality in laboratory bioassays was much higher than in the control, yet these fungal microbial insecticides commonly failed to prevent epizootic outbreaks of the same pests in the field. This failure in the outdoor aerosol spray of entomopathogenic fungi could be due to the innate susceptibility of the target pest, degree of exposure to the pesticide, prevailing environmental conditions, and physiological interactions between the host and pathogen (Tanada and Kaya, 1993).

The medium type for entomopathogenic fungi is one of the most impartant factor to increase the infectivity of fungus. Normally the plant oil

is commonly to used due to its efficacy is higher than water. Ganga Visalakshy *et al.* (2006) reported the different enhancing results from seven plant oils viz; coconut, groundnut, gingili, sunflower, neem, pongamia and castor oil on the mycelial growth of *Paecilomyces farinosus*. In this study we chosen to use Thai commercial cooking oil (palm oil) due to its convenient and low price for the farmer. However, palm oil media showed not significantly higher efficacy than water especially for the Lepidopterous larvae.

Several hemipterous rice pests, such as the rice leafhoppers and rice planthoppers, are known to vector plant viruses. Hibino (1989) reported that several rice pathogenic viruses, such as rice grassy stunt virus and rice ragged stunt virus, are transmitted by the BPH (*N. Lugens*) and two other *Nilaparvata* spp.: *N. bakeri* and *N. muiri*. He also claimed that increased levels of BPH occasionally accompanied substantial losses of rice crops from virus infections. For example, more than 485,000 ha of rice production area in 2005–2006 in southern Vietnam was severely affected by viral diseases spread by BPH and resulted in the loss of 828,000 tons of rice valued at US$ 120 million.

Nephotettix virescens is reported to be a rice tungro virus (RTV) vector and the incidence of RTV-infected rice plants consistently increased with increases in the vector population (Rao and Hasanuddin, 1991). *Nephotettix cincticeps, N. apicalis, N.nigropictus, N.virescens,* and *Recilia dorsalis* (Hemiptera: Cicadellidae) have been confirmed as vectors of various pathogenic rice viruses. Among these vectors, *N. cincticeps* was considered as the principal natural vector transmitting the virus in a persistent manner (Ling, 1972). Moreover, *Nephotettix* spp. were also confirmed to be the most important vectors of yellow rice dwarf virus in a molecular analysis (Wei *et al*., 2007). Consequently, it could be implied that the aerosol spray of entomopathogenic fungi may play their role indirectly in reducing the incidence of pathogenic rice virus infections by causing the death of the insect vectors.

The success in the aerosol spray of entomopathogenic fungi as microbial insecticides for a sustainable agricultural practice involves several basic steps, including isolation from the environment or diseased insects; strain selection based on several selection criteria, such as growth potentials, inoculation productivity, and pathogenicity to targeted insects; dose or spore concentration; formulation; media solvent; and pathogen longevity and storage properties or shelf life. Although the application of entomopathogenic fungi in the field condition has been shown a lower efficacy than lab condition. It is still important to promote and develop this technique to farmer. It provides low cost, smallholder friendly and keep the genetic co-evolution between entomopathogenic fungi and host pest in term of agro-ecology under the natural condition.

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