



**Full Length Article**

# Field Evaluation of Metschnikoff (*Metarhizium anisopliae*) Sorokin in Controlling Cotton Jassid (*Amrasca biguttula biguttula*) in Aubergine (*Solanum aculeatissimum*)

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## ABSTRACT

Twelve entomopathogenic fungi were screened by dipping an aubergine leaf into each isolate suspension at the concentration of  $5 \times 10^6$  conidia mL<sup>-1</sup> and feeding to cotton jassid (*Amrasca biguttula biguttula* Ishida) in the laboratory. *Metarhizium anisopliae* CKM-048 was the most virulent strain with the percent mortality of  $73.33 \pm 10.00$ . The fungus was formulated into a wettable powder form at the concentration of  $1 \times 10^9$  conidia g<sup>-1</sup> and tested its efficacy in controlling cotton jassid in two aubergine plantations in central Thailand, which was compared with an insecticide, lambda-cyhalothrin 2.5% EC applied @ 31.25 g a.i.ha<sup>-1</sup>. After three consecutively sprayed treatments at seven day intervals, results from both locations were similar, i.e., *M. anisopliae* CKM-048 at the dosage of  $1.25 \times 10^{13}$  conidia ha<sup>-1</sup> showed good controlling efficacy. This was not significantly different from the chemical treatment but significantly different from the un-treated control.

**Key Words:** *Amrasca biguttula biguttula* Ishida; *Metarhizium anisopliae*; Screening; Aubergine; Management; lambda-cyhalothrin

## INTRODUCTION

Cotton leafhopper or cotton jassid (*Amrasca biguttula biguttula* Ishida), (Homoptera: Cicadellidae), has a broad host range including cotton, okra, brinjal, eggplant, jute and aubergine (Kittiboonya *et al.*, 2004). Both nymph and adult stages can destroy the plants by not only sucking into the leave tissues but also by transmitting different viruses, resulting in symptom and yield loss. The extent of jassid damage to number and weight of okra fruits could approach 54% (Rawat & Sahu, 1973). The insect pest management program still relies heavily on the chemical insecticides, which lead to a destabilization of ecosystem and enhanced resistance to insect pests (Kranthi *et al.*, 2001; Mohan & Gujar, 2003). An alternate to the conventional insecticide is the microbial control. Feng *et al.* (2004) developed an oil-based emulsifiable formulation of *Beauveria bassiana* conidia mixed with an insecticide, imidacloprid, at low rates for control of false-eye leafhopper *Empoasca vitis* on tea in southern China. Pu *et al.* (2005) tested the field efficacy of a *Beauveria bassiana*-based mycoinsecticide against the false-eye leafhopper *E. vitis* in the tea canopy in central China. Gandhi *et al.* (2006) revealed that neem oil could be used to decrease the population of leafhopper *A. biguttula biguttula* in okra. To the best of our knowledge, none of the

fungus has been tested against *A. biguttula biguttula*.

Aubergine (*Solanum aculeatissimum* Jacq.) is a native perennial plant distributed throughout Southeast Asian countries and also in South America as well. Similar to eggplant, its fruit has been utilized as food for centuries. Besides food, it is also classified as a type of medicinal plant, which contains some types of steroids i.e., saponins aculeatiside A and aculeatiside B (Ikenaga *et al.*, 1990).

In this study, we screened for the most virulent fungus from several local isolates against *A. biguttula biguttula* using aubergine leaves as a feeding source in the laboratory. The best strain was selected and formulated into a powder form and field experiments were compared with a recommended lambda-cyhalothrin 2.5% EC (Thai Department of Agriculture, 2002) in order to avoid over claim the fungal efficacy. Since there is an increasing demand of organic products annually when all chemical pesticides are prohibited for used in pest control. Therefore, microbial control agent should become ultimate effective choice.

## MATERIALS AND METHODS

### Laboratory Test

**Preparation of conidial suspension.** Sixty soil samples, 32

homopteran including leaf hopper, plant hopper and 24 hemipteran including stink bug, rice black bug and lygus bug cadavers were sampled from northern and central parts of Thailand. Twelve entomopathogenic fungi were isolated (Meikle *et al.*, 2005) and sent to Thailand Institute of Scientific and Technological Research (TISTR) for identification. *Beauveria bassiana* CKB-001, *B. bassiana* CKB-048, *B. bassiana* CKB-095, *Hirsutella citriformis* CKH-001, *Verticillium lecanii* CKV-053 [reclassified in part as *Lecanicillium muscarium* (Petch) Zare & Gams, 2001], *Metarhizium anisopliae* CKM-048, *M. anisopliae* CKM-051, *M. anisopliae* CKM-036, *M. anisopliae* CKM-007, *M. flavoviridae* CKM-083, *Paecilomyces lilacinus* CKP-012 and *P. fumosoroseus* CKPF-095 were identified.

All cultures were grown on potato dextrose agar (Difco, Becton-Dickson, Sparks, MD) at  $27 \pm 1^\circ\text{C}$  in the dark for 14 days. Conidial suspensions were made by lightly scraping the fungal culture surface with a sterile cell spreader into a 100 mL plastic container. The conidial clumps were suspended in distilled water with 0.01% Tween 80 (ICI Americas, Norwich, NY). The suspensions were vortexed for five minutes to dissociate conidial clumps and filtered through one layer of cheese cloth to remove remaining clumps and mycelial debris. The concentration of each suspension was diluted to  $5 \times 10^6$  conidia  $\text{mL}^{-1}$  determined by a Neubauer hemocytometer under a phase-contrast microscope. The suspensions were used within a 24 h period. The pure fungal culture of the most virulent strain was deposited at TISTR, Bangkok, Thailand.

**Collection and preparation of cotton jassid.** Cotton jassid (*A. biguttula biguttula*) nymphs and adults were collected from aubergine plantations in Ratchaburi and Kanchanaburi provinces, both are located in central Thailand.

**Screening for the most virulent fungus against cotton jassid.** Fourteen treatments of twelve entomopathogenic fungi with an untreated and water treated were performed with three replications. A fresh, clean aubergine leaf, approximately  $40 \text{ cm}^2$  in size, was dipped into the conidia suspension, resulting in a concentration of  $6.25 \times 10^5$  conidia per  $\text{cm}^2$  of leaf surface (Cuthbertson & Walters, 2002). The leaf was allowed to dry and placed into a plastic container ( $17.0 \times 25.0 \times 9.0 \text{ cm}$ ). Twenty cotton jassid nymphs were transferred into each container. Dead jassids were counted and removed everyday for seven days. The dead jassids from each fungal suspensions were confirmed by dipping the cadavers into a 10% sodium hypochlorite solution (Sigma-Aldrich, MO) for five minutes, allowed to dry and placed on Martin's streptomycin medium (Difco, Becton-Dickson, Sparks, MD) for any possible mycelial germination.

#### Fields Test

**Preparation of conidia into a wettable powder form.** After having screened for a fungus in controlling cotton jassid, a conidial suspension was subjected to freeze drying (Heto FD3, Denmark) using sterile attapulgite clay (AGSORB-325<sup>TM</sup> LVM-GA, OIL-DRI Corp., IL) as a filler

and 3% of Tween 80 as wetting agent. The final concentration was adjusted to  $1 \times 10^9$  conidia  $\text{g}^{-1}$  for ease of handling in field operations.

**Preparation for field experiments.** Two farmer's plantations in Kanchanaburi province were field tested. The experiments were planned as follows:

- i) Aubergine was planted in a subplot size of  $4 \times 7 \text{ m}$  with  $0.75 \times 1.00 \text{ m}$  intervals. Each subplot contained five rows with ten plants for each row with a distance between sub-plots of 1.5 m.
- ii) There were five treatments with four replications, using a backpack knapsack sprayer:
  1. The selected fungus at a dosage of  $1,000 \text{ g } 200 \text{ L}^{-1}$  water, which resulted in an approximately  $6.25 \times 10^{12}$  conidia  $\text{ha}^{-1}$ .
  2. The selected fungus at a dosage of  $1,500 \text{ g } 200 \text{ L}^{-1}$  water, which resulted in an approximately  $9.37 \times 10^{12}$  conidia  $\text{ha}^{-1}$ .
  3. The selected fungus at a dosage of  $2,000 \text{ g } 200 \text{ L}^{-1}$  water, which resulted in an approximately  $1.25 \times 10^{13}$  conidia  $\text{ha}^{-1}$ .
  4. Lambda-cyhalothrin (Karate<sup>TM</sup> 2.5% EC, Syngenta Corp., UK) at a dosage  $200 \text{ mL}$  water, which resulted in an approximately  $31.25 \text{ g a.i. ha}^{-1}$ .
  5. Control (untreated).
6. Before spraying, jassid nymphs were counted on the five top leaves on ten plants in each subplot. If the number of jassids exceeded two nymphs per leaf, which corresponded to the economic injury level for cotton jassid (Thai Department of Agriculture, 2002), then spraying was initiated and a count was done on third, fifth and seventh day. Application was performed consecutively for three times every seven days and the amount of water used was  $1,250 \text{ L h}^{-1}$  for each application.

**Statistical analysis.** If mortality of the control group was observed in the laboratory testing, the correction factor from fungal treatments was calculated using the formula of Abbott (1925). Data from each trial was analyzed using the analysis of variance (ANOVA) and means were calculated by Duncan's new multiple range test (DMRT) at  $p = 0.05$ . For field tests, the data was calculated using the analysis of variance if there was no significant difference before spray treatments on the number of jassids. If a significant difference was observed before spraying, then the data was analyzed by the analysis of covariance.

## RESULTS

**Laboratory tests.** *Metarhizium anisopliae* CKM-048 proved to be the most virulent fungus compared to the other eleven isolates. The percent mortality on the cotton jassid, *Amrasca biguttula biguttula*, was  $73.33 \pm 10.00$  and was significantly different from the other isolates. The percent mortality of the remaining strains was as follows: *Paecilomyces fumosoroseus* CKPF-095,  $43.33 \pm 5.77$ ; *M. anisopliae* CKM-051,  $36.67 \pm 5.77$ ; *M. anisopliae* CKM-

007,  $36.67 \pm 5.77$ ; *Beauveria bassiana* CKB-048,  $36.67 \pm 5.77$ ; *B. bassiana* CKB-001,  $26.67 \pm 5.77$ ; *M. anisopliae* CKM-036,  $26.67 \pm 5.77$ ; *M. flavoviridae* CKM-083,  $23.33 \pm 5.77$ ; *B. bassiana* CKB-095,  $23.33 \pm 5.77$ ; *Verticillium lecanii* CKV-053,  $16.67 \pm 5.77$ ; *Hirsutella citriformis* CKH-001,  $10.00 \pm 0.00$  and *P. lilacinus* CKP-012,  $10.00 \pm 0.00$  (Fig. 1 & 2). *M. anisopliae* CKM-048 was selected as a representative fungus to be tested in farmers' plantations in order to evaluate whether it could be used as a microbial insecticide. In addition, the lower concentration at  $1 \times 10^6$  conidia mL<sup>-1</sup> of all the fungi had been tested but the percent mortality of cotton jassids were too low to be used for further study. New mycelial growth that emerged after the cadavers were dipped into the sodium hypochlorite solution was confirmed that the fungus was relevant to the original isolate.

#### Field Test

**At Amphor Thamuang, Kanchanaburi province.** The population reduction of cotton jassid nymphs at Amphor Thamuang, Kanchanaburi Province is shown in Table I. Before the spray treatment, the average number of cotton jassid nymphs found from all treatments was 3.15 (range 2.95-3.30) nymphs per leaf and had non-significant difference among the treatments ( $p<0.05$ ). After the first spray, lambda-cyhalothrin showed the fastest and highest efficacies, followed by *M. anisopliae* CKM-048 at dosages of  $1.25 \times 10^{13}$ ,  $9.37 \times 10^{12}$ , and  $6.25 \times 10^{12}$  conidia ha<sup>-1</sup>, respectively. The average number of nymphs found at five and seven days after treatment follow the same trend as the three days results. This includes the control treatment as well. After the second spray, *M. anisopliae* CKM-048, at  $1.25 \times 10^{13}$  conidia ha<sup>-1</sup>, displayed an efficacy comparable to lambda-cyhalothrin, while the other two concentrations were inferior. The trend still remained for the third spray, but there was no statistical difference between treatments of *M. anisopliae* CKM-048 at  $1.25 \times 10^{13}$  conidia ha<sup>-1</sup> and lambda-cyhalothrin after seven days.

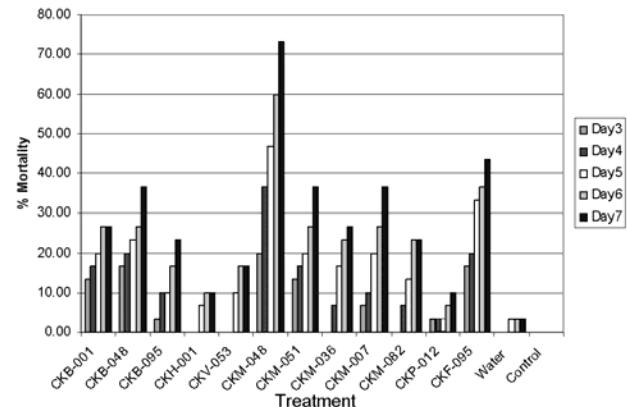
**At Amphor Thamaga, Kanchanaburi province.** Results of means and standard errors (in parenthesis) of cotton jassid nymphs from the second location are shown in Table II. Before treatment, the population densities of cotton jassid nymphs were almost similar to the first location. The average number of nymphs found from all treatments was 2.90 (range 2.56-3.18) nymphs per leaf. There was no significant difference among the treatments ( $p<0.05$ ). Similar results were obtained as shown for Amphor Thamuang, Kanchanaburi Province except the lowest fungal concentration displayed a slightly better efficacy. However, the fungal concentration at  $1.25 \times 10^{13}$  conidia ha<sup>-1</sup> is the minimum concentration required to control cotton jassid nymphs, which was as effective as lambda-cyhalothrin.

#### DISCUSSION

Monitoring the adult jassid populations in research fields with randomized spots is a questionable procedure.

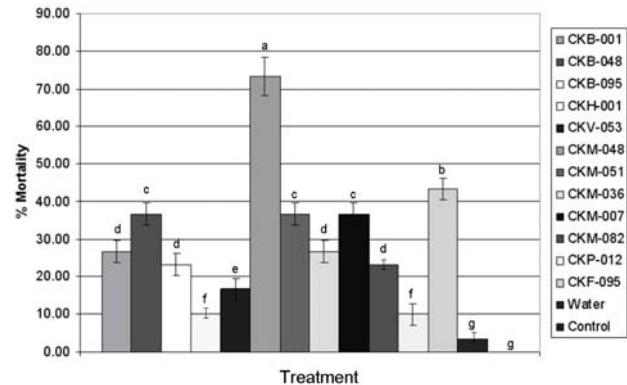
**Fig. 1. The average percent mortality of cotton jassids (*Amrasca biguttula biguttula* Ishida) treated with twelve entomopathogenic fungi, 3 - 7 days after treatment at  $27 \pm 1^\circ\text{C}$**

Remark: Control = untreated, Water = Water treated



**Fig. 2. The average percent mortality of cotton jassids (*Amrasca biguttula biguttula* Ishida) treated with twelve entomopathogenic fungi, 7 days after treatment at  $27 \pm 1^\circ\text{C}$ . Bars with different letters are significantly different ( $p=0.05$ , DMRT)**

Remark: Control = untreated, Water = Water treated



The adults fly freely from plot to plot and the extensive border areas associated with small-plot trials and areas neighboring the controls are especially vulnerable to population changes unrelated to the applied treatments. Other factors that influence the fungal activities are humidity and temperature. Landa *et al.* (1994) observed no growth of *P. fumosoroseus* on whitefly *Bemisia argentifolii* nymphs incubated at 80% relative humidity (RH). Their protocols involved removal of the fourth-instar nymphs from foliage and placing droplets of the conidial suspension on glass slides, where they were observed daily, but in our assays, nymphs were treated *in situ* on a living aubergine leaf. The results from these different assays suggest that the moisture required for fungal development was present in the boundary layers created by the leaf rather than the insect. On the other hand, the nymphs on the glass slides were unable to feed and almost certainly were not in a normal

**Table I.** The average number of cotton jassids (*A. biguttula biguttula*) and standard error (in parenthesis) on aubergine leaves before and after spray treatments at the first locations in Amphor Thamuang, Kanchanaburi province

Treatment	Dosage	Average number of cotton jassids (nymphs leaf <sup>-1</sup> ) <sup>1/</sup>								
		Before			After the first spray			After the second spray		
		Spray	3 D	5 D	7 D	3 D	5 D	7 D	3 D	5 D
<i>M. anisopliae</i>	$6.25 \times 10^{12}$	3.27 ns	2.75c	3.01c	3.33c	3.01b	3.20b	3.58c	3.09b	3.35c
CKM-048 WP	conidia ha <sup>-1</sup>	(0.27)	(0.24)	(0.42)	(0.31)	(0.40)	(0.10)	(0.44)	(0.35)	(0.26)
<i>M. anisopliae</i>	$9.37 \times 10^{12}$	3.30 ns	2.51bc	2.57bc	2.77b	2.59ab	2.71ab	2.85b	2.01a	2.30b
CKM-048 WP	conidia ha <sup>-1</sup>	(0.12)	(0.16)	(0.08)	(0.21)	(0.11)	(0.09)	(0.24)	(0.22)	(0.11)
<i>M. anisopliae</i>	$1.25 \times 10^{13}$	3.13 ns	1.97ab	2.18ab	1.92a	2.28a	2.37a	2.47ab	1.73a	1.89ab
CKM-048 WP	conidia ha <sup>-1</sup>	(0.33)	(0.18)	(0.16)	(0.08)	(0.12)	(0.04)	(0.13)	(0.14)	(0.17)
Lambda-cyhalothrin	31.25 g a.i ha <sup>-1</sup>	3.09 ns	1.54a	1.90a	1.69a	2.12a	2.10a	2.21a	1.46a	1.59a
(Karate 2.5% EC)		(0.26)	(0.20)	(0.28)	(0.10)	(0.07)	(0.22)	(0.08)	(0.10)	(0.15)
Control (untreated)	-	2.95 ns	3.40d	4.00d	4.08d	4.68c	4.63c	4.94d	4.18c	4.45d
		(0.48)	(0.36)	(0.67)	(1.21)	(1.36)	(0.98)	(0.12)	(1.17)	(0.96)
CV (%)		14.80	17.20	12.10	12.20	13.00	12.60	8.80	20.10	14.10
										13.00

1/means followed by the same alphabet in the same column showed no significant difference at 95% by DMRT

**Table II.** The average number of cotton jassids (*A. biguttula biguttula*) and standard error (in parenthesis) on aubergine leaves before and after sprays treatments at the second location in Amphor Thamaga, Kanchanaburi province

Treatment	Dosage	Average number of cotton jassids (nymphs leaf <sup>-1</sup> ) <sup>1/</sup>								
		Before			After the first spray			After the second spray		
		Spray	3 D	5 D	7 D	3 D	5 D	7 D	3 D	5 D
<i>M. anisopliae</i> CKM-	$6.25 \times 10^{12}$	3.18 ns	2.15bc	2.35b	2.54b	3.13c	3.31c	4.21c	3.59b	4.34d
048 WP	conidia ha <sup>-1</sup>	(0.11)	(0.12)	(0.31)	(0.06)	(0.26)	(0.30)	(0.80)	(0.31)	(0.27)
<i>M. anisopliae</i> CKM-	$9.37 \times 10^{12}$	2.90 ns	1.85b	1.66ab	2.28b	2.89bc	2.79b	3.05b	2.78b	3.63c
048 WP	conidia ha <sup>-1</sup>	(0.24)	(0.08)	(0.17)	(0.12)	(0.18)	(0.10)	(0.22)	(0.16)	(0.20)
<i>M. anisopliae</i> CKM-	$1.25 \times 10^{13}$	3.05 ns	1.48ab	1.42a	1.39a	2.45ab	2.34a	2.55ab	1.82a	1.85b
048 WP	conidia ha <sup>-1</sup>	(0.18)	(0.14)	(0.11)	(0.07)	(0.14)	(0.03)	(0.10)	(0.11)	(0.12)
Lambda-cyhalothrin	31.25 g a.i ha <sup>-1</sup>	2.82 ns	0.87a	1.26a	1.22a	2.05a	1.99a	2.01a	1.29a	1.60a
(Karate 2.5% EC)		(0.08)	(0.27)	(0.23)	(0.11)	(0.06)	(0.12)	(0.14)	(0.09)	(0.80)
Control (untreated)	-	2.56 ns	3.02c	3.40c	3.63c	4.80d	4.65d	4.83d	4.68b	5.05e
		(0.40)	(0.50)	(1.12)	(0.96)	(0.87)	(0.96)	(1.04)	(0.87)	(1.46)
CV (%)		14.10	31.40	22.40	17.60	12.80	8.40	11.10	27.70	17.10
										11.80

1/means followed by the same alphabet in the same column showed no significant difference at 95% by DMRT

state of hydration. Fargues *et al.* (1997) and Vidal *et al.* (1997) reported that most isolates of *B. bassiana* and *P. fumosoroseus* were inhibited at temperatures higher than 32–35°C. These temperature effects might also include *M. anisopliae* as well. However, the trials reported here were conducted during the months of August to January with the average temperature ranging between 26–36°C and the relative humidity of 70–80% in central Thailand. Temperature is therefore not considered to have been an important negative factor, especially since the leaf surface temperatures were lower than ambient (Willmer, 1986).

In field experiments, lambda-cyhalothrin 2.5% EC was employed, because there was no microbial control agent recommended for controlling cotton jassids for comparison. The spraying program was discontinued after three consecutive times in both locations to access whether the microbial control agent can be used as a plant protection tool for either organic farming or integrated pest management program. Even though the cotton jassids were high in number after each scouting time due to the rapid change of population dynamics of the insects (Mahmood *et*

*al.*, 2002), *M. anisopliae* CKM-048 sprayed at a dosage of  $1.25 \times 10^{13}$  conidia ha<sup>-1</sup> still gave a good control when compared with the chemical insecticide. In addition, less population of thrips and fruit borer were observed from the microbial sprayed treatments than the control, which *M. anisopliae* might also have some effect against those insects as well.

The application rates of fungal formulations for insect control are of primary interest for microbial control. A range of fungal rates from  $1 \times 10^{13}$  to  $1 \times 10^{14}$  conidia ha<sup>-1</sup> is usually considered in field trials for hyphomycetes-based mycoinsecticides (Bateman, 1997; Poprawski *et al.*, 1999; Wraight *et al.*, 2000; Vandenberg *et al.*, 2001; Malsam *et al.*, 2002). Our study of *M. anisopliae* CKM-048 at the rate of  $1.25 \times 10^{13}$  conidia ha<sup>-1</sup> has confirmed with these authors.

**Acknowledgements.** We would like to thank Ms. Larwan Chatanon, TISTR for her assistance in fungal identification.

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(Received 15 August 2007; Accepted 07 November 2007)