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EFFICACY OF SOME NEW ISOLATES OF *Metarhizium anisopliae* and *Beauveria bassiana* AGAINST RICE EARHEAD BUG, *Leptocorisa acuta.*

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ABSTRACT

Rice earhead bug, Leptocorisa acuta Thunberg (Hemiptera: Alydidae) is found in almost of countries where rice crop is grown. It, however, becomes more prevalent in rainfed wetland or upland rice. In Cuu Long Delta of Vietnam, rice earhead bug (REB) is one of the major pests of rice crop which leading to considerable field losses. Experiments were conducted in the greenhouse and field to evaluate the efficacy of some new isolates of Metarhizium anisopliae and Beauveria bassiana against REB, L. acuta. The results in greenhouse showed that all of 12 selected isolates of M. anisopliae and B. bassiana which have been isolated from naturally infected insects during 2003-2005 were found to be pathogenic to the tested REB. The mortality percentage of L. acuta caused by B. bassiana and M. anisopliae isolates ranged from 57.5 to 77.7%, and from 74.7 to 87% at 10 DAT, respectively. Among 12 new selected isolates of M.a and B.b have been tested, M.a (OM$_3$-BD), M.a (HG$_3$-B) and M.a (HG$_5$-BD) exhibited higher pathogenicity to REB as compared to the rest. In field experiments, all of 12 selected isolates of M. anisopliae and B. bassiana were found to be effective for controlling REB, the efficacy could be seen from 7 DAT and reached to its highest peak at 14 DAT. The field mortality of L. acuta caused by B. bassiana and by M. anisopliae isolates ranged from 45.3 to 74.9%, and from 63.6 to 86.6% at 10 DAT, respectively. M. anisopliae showed better efficacy against REB as compared to B .bassiana. Among 12 new selected isolates of M.a and B.b have been tested, M.a (OM$_3$-BD), M.a (HG$_3$-B) and M.a (HG$_5$-BD) exhibited higher efficacy against the REB as compared to the rest.

Key words: *Beauveria bassiana*, entomopathogens, *Leptocorisa acuta*, *Metarhizium anisopliae*, pathogenicity,

INTRODUCTION

Rice earhead bug, *Leptocorisa acuta* Thunberg (Hemiptera: Alydidae) is found in almost of countries where rice crop is grown such as India, Bangladesh, Bhutan, Burma, Indonesia, Cambodia, Laos, Malaysia, Nepal, Pakistan, Philippines, Thailand, South of China, Japan, Korea and Vietnam. Rice earhead bug (REB) is more prevalent in rainfed wetland or upland rice. In Cuu Long Delta of Vietnam, rice earhead bug (REB) is one of the major pests of rice crop which leading to considerable field losses. Intensity and type of damage caused by REB depends on stage of rice crop, population density of the pest and ecological conditions. Both nymphs and adults are destructive to the crop, even though the damage by nymphs is more severe. Nymphs prefer grains at milky stage for feeding. They feed by the insertion of proboscis at points where glumes meet. During the process, the bug secretes a proteinaceous stylet sheath to form a feeding canal for its sucking mouthparts. Removal of stored assimilates from developing grain may result in either unfilled or partially filled grains with damage symptoms. Quality of grains is also reduced by the insect attack. Smell of the infected grains lowered market value. Damaged grains even after cooking retain the buggy smell (Dale 1994).

The major measure to control this insect pest depends upon application of chemical pesticides. However, insecticidal control has led to several problems in insect pest management like insecticide resistance in pests, pest resurgence, undesirable toxic effects to natural enemies of pests and toxic residues in crop plants and environmental pollution. Consequently, the search for new environmentally safe methods is being intensified. Among the various methods, biological control has shown considerable promise.

Microbial control aims at biological suppression of insect pests, by the use of entomopathogens like viruses, fungi, bacteria, protozoa and nematodes which usually posses the special features required for implementation of IPM system *viz*., host specificity, high virulence, safety to natural enemies of the target pest and ecologically non-disruptive.

We have studied to exploit the biocontrol potential of *Metarhizium anisopliae* and *Beauveria bassiana* against REB, the rerults indicated that *M.anisopliae*(OM_2-B) and *B. bassiana* (OM_1-R) were effective in controlling this insect pest (Loc *at al,* 2001). *M.a*(OM_2-B) which was isolated from naturally infected rice bug that collected from OMon exhibited the highest infectivity to this insect. We have used *M.a*(OM_2-B) isolate for producing the new bioinsecticide that got a Trade name as Ometar. Ometar is now using by many farmers as effective bioinsectide in controlling the rice insect pests and it is very effective against REB and brown planthopper (BPH).

However, the data of previous studies were limited to only one isolate of *M. anisopliae* and one isolate of *B. bassiana.* Since there is much scope for study of various isolates of *M. anisopliae* and *B. bassiana* in controlling the REB. Moreover, we have isolated and selected some new isolates of *M.a* and *B.b*. The present study has been taken up to evaluate the efficacy of some new isolates of *Metarhizium anisopliae* and *Beauveria bassiana* against rice bug, *L. acuta*.

MATERIALS AND METHODS

1- Materials

- Equipments for fungal study in laboratory; and necessary tools for different experiments in greenhouse and field were used.

- We selected new isolates of *M. anisopliae* and *B. bassiana,* then Ometar and Fastac were used as control chemical.

- Materials were used for multiplication of different isolates of the *M.a* and *B.b* fungi and production of bioinsecticide (Ometar) such as potato, dextrose, agar, rice bran, corn powder and rice husk...

2- Methods

a. Pathogenicity tests in the greenhouse

Pathogenicity tests were also done in the greenhouse of Insect Ecology and Biocontrol Department. The nymphs of REB were used for pathogenicity tests. The pure fungal culture of each fungal isolate was multiplied on potato-dextrose-agar (PDA) medium for 10 days. Conidia were harvested from the surface of the petri dishes by washing with sterile distilled water containing 0.02 percent Tween 80® surfatant. The conidial suspension was agitated in household mixer for 5 minutes and then filters through double-layered muslin cloth. Conidial concentration was 10^7 conidia ml^{-1} in the prepared suspension. This suspension was applied directly on the third and fourth instar nymphs of REB in a closed net cage by spraying with a sterilized atomiser at the rate of 6 ml per a flowering Jasmine potted rice plant. Control insects were sprayed with 0.02 percent Tween 80® solution (Loc 1995). Only 20 insects were retained on each rice plant and the net cage were closed after spraying. There were four replications. The mortality was recorded after 3, 5, 7 and 10 days and the per cent mortality of insect were corrected by a formula as suggested by Abbott (1925).

b. Field efficacy of *M. anisopliae*, *B. bassiana* against rice bug

To confirm the efficacy of some new isolates of *M. anisopliae* and *B. bassiana* against REB, some field experiments were conducted under experimental field condition of Cuu Long Delta Rice Research Institute (CLRRI) during 2003, 2004 Summer-Autumn and 2005 Winter-Spring seasons. The experiments were laid out in a randomized complete block design with three replications; the plot size was 40 m^2. The conidial concentration of different fungal isolates were standardized at 10^7conidia ml^{-1} with 0.02% cooking oil which was a surfatant. The conidial dose was used 6 x 10^{12} conidia ha^{-1} (Loc 1995). The fungal suspension was sprayed with a sprayer. The control plot was not sprayed. The concentration of chemical control was prepared according to the recommendation in the label of it. The count of live REB was taken 1 day before treatment and at 3, 7 and 14 days thereafter. Number of live insects in 5 points on 2 crisscross lines of plot were recorded. For each point, the number of live insects on 40 rice plants was counted. The average number of live insects per square meter was calculated (Plant Protection Department 2004). The field efficacy of different B.b and M.a isolates against REB were calculated by formula as suggested by Henderson – Tilton.

RESULTS AND DISCUSSIONS

1. Pathogenicity of certain *M. anisopliae* and *B. bassiana* isolates against REB

In laboratory, pathogenicity studies in table 1 indicated that all of the four different isolates of *M. anisopliae* and *B. bassiana* which selected in 2003 and bioinsecticide Ometar were found to be pathogenic to the REB. However, there was a variation in their infectivity against REB. The mortality (%) of REB ranged from 16.7 to 40% at 3 days after treatment (DAT) and all of them were significantly lower than the mortality of REB in the chemical control that was 81.7. At 5 DAT, the mortality of REB ranged from 34.3% to 78.8% and there were not significantly different between Ometar and chemical control treatments. The mortality of REB in four new isolate treatments ranged from 59.3 to 87.0 at 10 DAT and the mortality of REB of *M.a(*OM_3-BD) treatment was not significantly different as compared to that of chemical treatment. These results showed that *M.a (*OM_3-BD) isolate which was isolated from naturally infected coconut leaf beetle (CLB) exhibited very high infectivity which was not significantly different as compared to that of Ometar at 5 DAT, 7 DAT and 10 DAT. Among four new isolates of *M.a* and *B.b, M.a (*OM_3-BD) isolate was the best, next is *B.b*(ST_1-R) and then *M.a*(AG_3-R).

Table 1. Pathogenicity of different isolates of *M. anisopliae* and *B. bassiana* to REB nymph, *L.acuta (*CLRRI Greenhouse, *2003)*

No	Treatment	Concentration	Corrected motality (%)			
			Days after treatment			
			3	5	7	10
1	*B.b* (OM_5-R)	10^7conidia /ml	16.7 c	33.4 d	43.0 d	59.3 d
2	*B.b* (ST_1-R)	10^7conidia /ml	25.0 c	61.3 c	65.9 bc	77.7 bc
3	*M.a* (AG_3-R)	10^7conidia /ml	20.0 c	63.1 c	64.0 c	75.7 c
4	*M.a (*OM_3-BD)	10^7conidia /ml	28.3 bc	72.0 b	75.0 ab	87.0 ab
5	Ometar	10^7conidia /ml	40.0 b	78.8 ab	80.4 ab	90.9 a
6	FASTAC 5EC	0,1%	81.7 a	85.8 a	87.4 a	96.1 a
	CV (%)		20.0	6.1	11.8	6.4

Means followed by a common letter are not significantly different at the 5% level by DMRT

The results from table 2 showed that the chemical treatment could give a quick effect against REB, however, at 7 DAT and 10 DAT the mortality of REB in Ometar and chemical treatments were not significantly different. All of the four different new isolates of *M. anisopliae* and *B. bassiana* which selected in 2004 and the bioinsecticide Ometar were found to be pathogenic to the REB, but *M. anisopliae* was more pathogenicity to REB than *B. bassiana.* The mortality of REB in four new isolate treatments ranged from 65 to 81.5% at 10 DAT and the mortality of REB of *M.a* (HG_3-B) treatment was not significantly different as compared to that of chemical treatment. The mortality of REB in *M.a* (HG_3-B) treatment was not significantly different as compared to that in Ometar treatment at 5, 7 & 10 DAT. The results indicated that among four new isolates of *M.a* and *B.b* selected in

2004, *M.a* (HG_3-B) isolate which was isolated from naturally infected REB was the best isolate, next was *M.a* (OM_6-R) which was isolated from naturally infected BPH.

Ignoffo and Garcia (1985) reported that two cultures of the same insect species obtained from different sources also responded differently to the same fungal biotype. In nature, living organisms, particularly the microbes, undergo selection, recombination and mutation depending upon the ecological situations which ultimately influence their genetic make up. Sikura and Bevzenco (1972) found variations in toxin production in different strains of *B. bassiana* which could be correlated with their virulence. In the present investigation, the *M.a* (HG_3-B) isolate was most infective to REB which may be due to its' origin as this isolate was obtained from naturally infected REB .

Table 2. Pathogenicity of different isolates of *M. anisopliae* and *B. bassiana* to REB nymph, *L.acuta (*CLRRI Greenhouse, *2004)*

No	**Treatment**	**Concentration**	**Corrected motality (%)**			
			Days after treatment			
			3	5	7	10
1	*B.b* (OM_7-R)	10^7conidia /ml	21.7 d	31.5 d	46.5 cd	65.0 cd
2	*B.b* (HG_2-CL)	10^7conidia /ml	23.3 cd	24.5 d	42.8 d	57.5 d
3	*M.a* (OM_6-R)	10^7conidia /ml	26.7 cd	48.8 c	62.2 bc	75.3 bc
4	*M.a* (HG_3-B)	10^7conidia /ml	31.7 c	54.3 bc	71.3 b	81.5 ab
5	Ometar	10^7conidia /ml	43.3 b	63.0 b	78.7 ab	85.2 ab
6	FASTAC 5EC	0,1%	86.7 a	89.5 a	91.0 a	94.2 a
	CV (%)		14.0	12.7	15.6	11.7

Means followed by a common letter are not significantly different at the 5% level by DMRT

The results in Greenhouse of 2005 also indicated that all of the four selected isolates of *M. anisopliae* and *B. bassiana* and the bioinsecticide Ometar were found to be pathogenic to the REB, but *M. anisopliae* was more pathogenicity to REB than *B. bassiana.* The mortality of REB in four new isolate treatments ranged from 59 to 76.9% at 10 DAT. Among 4 new isolates of *M.a* and *B.b* have been tested, *M.a* (HG_5-BD) & *M.a* (OM_7-STO) exhibited higher pathogenicity to REB which was not significant different as compared to that of Ometar treatment (table 3)

Table 3. Pathogenicity of different isolates of *M. anisopliae* and *B. bassiana* to REB nymph, *L.acuta (*CLRRI Greenhouse, *2005)*

No	Treatment	Concentration	Corrected motality (%)			
			Days after treatment			
			3	5	7	10
1	*B.b* ($ĐT_{14}$-R)	10^7conidia /ml	21.7 cd	40.0 cd	47.9 d	59.0 c
2	*B.b* (OM_{10}-STO)	10^7conidia /ml	18.3 d	33.3 d	51.9 cd	60.7 c
3	*M.a* (HG_5-BD)	10^7conidia /ml	38.3 b	58.3 b	67.8 bc	78.5 b
4	*M.a* (OM_7-STO)	10^7conidia /ml	35.0 bc	51.7 bc	65.7 bc	76.9 b
5	Ometar	10^7conidia /ml	36.7 b	63.3 b	71.2 b	85.6 ab
6	FASTAC 5EC	0,1%	90.0 a	91.7 a	92.9 a	92.9 a
	CV (%)		18.7	13.3	13.8	9.7

Means followed by a common letter are not significantly different at the 5% level by DMRT

b. Field efficacy of certain *M. anisopliae*, *B. bassiana* isolates against rice bug

Based on successful control of REB in greenhouse, the field experiments were conducted in 2003 and 2004 Summer-Autumn, 2005 Winter-Spring to evaluate the efficacy of 12 new isolates of *M. anisopliae* and *B. bassiana* against the REB.

In 2003 Summer-Autumn (table 4), chemical treatment (Fastac) gave quick effective to REB as mortality of REB. In this treatment mortality offered 68,3 % at 3 DAT (significantly higher than the fungal treatments). However, the mortality of REB of chemical treatment was not significantly different as compared to that of Ometar, *M.a(*OM_3-BD), other fungal treatments at 7 DAT and 14 DAT. Moreover, at 14 DAT the mortalities of REB in Ometar and *M.a(*OM_3-BD) treatments were 86.6% and 89.4%, respectively. They were higher than that of chemical treatment (82.0%), even though significant differences in the mortalities of these three treatments were not observed. The mortality of REB in four new isolate treatments ranged from 53.4 to 86.6% at 14 DAT and the mortality of REB of *M.a (*OM_3-BD) treatment was not significantly different as compared to that of chemical treatment. The results also indicated that *M.a (*OM_3-BD) isolate exhibited very high field efficacy which was not significantly different as compared to that of Ometar at all the times of observation. Among 4 new isolates of *M.a* and *B.b, M.a (*OM_3-BD) isolate gave the best field efficacy in controlling REB.

Table 4: Field efficacy of different isolates of *M. anisopliae* and *B. bassiana* against REB, *L.acuta (*CLRRI, 2003 Summer-Autumn*)*

No	Treatment	Dose/ha	Corrected motality (%)		
			Days after treatment		
			3	7	14
1	*B.b* (OM_5-R)	6 x 10^{12}conidia	8.6 d	36.2 d	53.4 c
2	*B.b* (ST_1-R)	6 x 10^{12}conidia	24.4 bc	57.5 bc	74.9 b
3	*M.a* (AG_3-R)	6 x 10^{12}conidia	10.7 cd	52.8 cd	74.7 b
4	*M.a (*OM_3-BD)	6 x 10^{12}conidia	25.4 b	69.0 abc	86.6 a
5	Ometar	6 x 10^{12}conidia	36.5 b	74.8 ab	89.4 a
6	FASTAC 5EC	500ml	68.3 a	83.2 a	82.0 ab
	CV (%)		26.2	15.9	8.0

Means followed by a common letter are not significantly different at the 5% level by DMRT

The results of 2004 Summer-Autumn indicated that Fastac showed good initial control against the REB but subsequently declined from 7 DAT onwards. Contrarily, the efficacy of all the fungal treatments was not high at 3 DAT, but subsequently increased from 7 DAT onwards. The mortality of REB in four new isolate treatments ranged from 45.3 to 79.3% at 14 DAT and the mortality of REB of *M.a* (HG_3-B) treatment was not significantly different as compared to that of chemical treatment. The mortalities of REB in *M.a* (HG_3-B) treatment were 70.2% and 79.3% at 7 DAT and 14 DAT, respectively. They were not significantly different as compared to those of Ometar treatment. These results showed that among four new isolates of *M.a* and *B.b* which selected in 2004, *M.a* (HG_3-B) was the best one (table 5). The results of our previous study also indicated that *M.a (*OM_2-B) isolate which was one from naturally infected REB gave very high efficacy to control REB (Loc *et al*. 2001). We have used M.a *(*OM_2-B) isolate for producing Ometar bioinsecticide to control REB.

Table 5: Field efficacy of different isolates of *M. anisopliae* and *B. bassiana* against REB, *L.acuta (*CLRRI, 2004Summer-Autumn*)*

No	Treatment	Dose/ha	Corrected motality (%)		
			Days after treatment		
			3	7	14
1	*B.b* (OM_7-R)	6 x 10^{12}conidia	24.4 b	39.4 c	52.6 cd
2	*B.b* (HG_2-CL)	6 x 10^{12}conidia	29.2 b	42.6 c	45.3 d
3	*M.a* (OM_6-R)	6 x 10^{12}conidia	30.0 b	52.4 c	63.6 bc
4	*M.a* (HG_3-B)	6 x 10^{12}conidia	23.7 b	70.2 b	79.3 a
5	Ometar	6 x 10^{12}conidia	32.2 b	71.8 ab	82.0 a
6	FASTAC 5EC	500ml	87.7 a	86.7 a	74.7 ab
	CV (%)		23.8	14.8	11.9

Means followed by a common letter are not significantly different at the 5% level by DMRT

Table 6. Field efficacy of different isolates of *M. anisopliae* and *B. bassiana* against REB, *L.acuta (*CLRRI, 2005 Winter-Spring*)*

No	Treatment	Dose/ha	Corrected motality (%)		
			Days after treatment		
			3	7	14
1	*B.b* ($ĐT_{14}$-R)	6 x 10^{12}conidia	19.8 c	43.0 c	50.8 d
2	*B.b* (OM_{10}-STO)	6 x 10^{12}conidia	24.7 bc	51.1 bc	62.4 cd
3	*M.a* (HG_5-BD)	6 x 10^{12}conidia	32.7 b	58.3 b	76.1 bc
4	*M.a* (OM_7-STO)	6 x 10^{12}conidia	29.7 bc	54.8 bc	72.6 bc
5	Ometar	6 x 10^{12}conidia	34.3 b	73.1 ab	81.3 ab
6	FASTAC 5EC	500ml	91.1 a	93.1 a	89.6 a
	CV (%)		16.8	13.3	10.2

Means followed by a common letter are not significantly different at the 5% level by DMRT.

The field experiments during 2005 Winter-Spring indicated that all of 4 selected isolates of *M. anisopliae* and *B. bassiana* were found to be effective to control REB at 7 DAT onwards and the mortality of REB in these four new isolate treatments ranged from 50.8 to 72.6% at 14 DAT. However, the mortality caused by these isolates was lower than that caused by Ometar in almost the times of observation, even though significant differences in the mortalities of these five treatments were not observed in all times of observation. The results also showed that M. *anisopliae* was more effective in controlling of REB than *B. bassiana.* Among four new isolates have been tested, *M.a* (HG_5-BD) & *M.a* (OM_7-STO) exhibited better efficacy in controlling REB which is not significant different as compared to that of Ometar treatment (table 6)

The results of the field experiments were in accordance with those obtained in Greenhouse. The three new isolates of *M. anisopliae*: *M.a (*OM_3-BD), *M.a* (HG_3-B), and *M.a* (HG_5-BD) in 2003, 2004 and 2005, offered high efficacy to control REB at the dose of 6 x 10^{12}conidia /ha.

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Hiệu lực của một số isolates mới của nấm xanh, *Metarhizium anisopliae* và nấm trắng, *Beauveria bassiana* đối với bọ xít hôi hại lúa, *Leptocorisa acuta*.

Bọ xít hôi hại lúa, *Leptocorisa acuta* Thunberg (Hemiptera: Alydidae) xuất hiện ở hầu hết các nước có trồng lúa, nhưng mật số của loài sâu hại này tăng cao ở những vùng trồng lúa nước trời. Tại Đồng Bằng Sông Cửu Long, bọ xít hôi là một trong những côn trùng chính hại lúa dẫn tới sự thiệt hại đáng kể trên đồng ruộng. Mười hai isolates mới của 2 loài nấm xanh *Metarhizium anisopliae* và nấm trắng *Beauveria bassiana* được phân lập từ bọ xít hôi hại lúa và một số loài sâu hại khác ở một số địa phương đã được thử nghiệm trong nhà lưới và ngoài đồng để đánh giá hiệu lực của chúng đối với bọ xít hôi hại lúa. Kết quả trong nhà lưới cho thấy cả 12 isolates mới nói trên đều có khả năng gây bệnh đối với bọ xít hôi hại lúa. Tỷ lệ chết của bọ xít hôi do nấm trắng gây ra biến động từ 57.5 tới 77.7% và do nấm xanh gây ra biến động từ 74.7 tới 87% sau xử lý nấm 10 ngày. Trong số 12 isolates mới này thì *M.a* (OM_3-BD), *M.a* (HG_3-B) và *M.a* (HG_5-BD) tỏ ra có khả năng gây bệnh đối với bọ xít hôi cao hơn so với các isolates còn lại. Sử dụng các isolates này để trừ bọ xít hôi ngoài đồng ruộng thì hiệu lực của chúng thể hiện từ 7 ngày sau khi phun và tăng dần tới 14 ngày sau phun. Các isolates nấm xanh tỏ ra có hiệu lực trừ bọ xít hôi cao hơn so với các isolates nấm trắng. Trong số 12 isolates mới đã được khảo nghiệm và đánh giá thì *M.a* (OM_3-BD), *M.a* (HG_3-B) và *M.a* (HG_5-BD) tỏ ra có hiệu lực trừ bọ xít hôi cao hơn hẳn so với các chủng nấm còn lại.