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BIOCONTROL POTENTIAL OF *Metarhizium anisopliae* AGAINST COCONUT BEETLE, *Brontispa longissima.*

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ABSTRACT

Studies were conducted on Metarhizium anisopliae to exploit their potential for controlling the coconut leaf beetle. All of three selected isolates of M.anisopliae which have been isolated and purified from naturally infected insects were found to be pathogenic to the tested coconut leaf beetle (CLB), Brontispa longissima. M.a (OM$_3$-BD) isolate, which was isolated from naturally infected CLB, exhibited the highest infectivity to both stages of the CLB. The larvae of CLB were more susceptible to M. anisopliae as compared to adults. Rotenone was very effective to CLB when used at 0.6% in the laboratory tests. In the field experiments, all of four selected isolates of M. anisopliae and one selected B. bassiana were found to be effective for controlling the CLB, the efficacy could be seen from seven DAT and it showed long persistence, even up to 21 DAT. The mortality caused by selected M. anisopliae isolates was significantly higher than that caused by B. bassiana isolate in almost the times of observation. Among four selected M. anisopliae isolates have been tested, M.a (OM$_3$-BD) and M.a (TG2-BD) exhibited higher efficacy against the CLB as compared to the rest. Rotenone with high concentration (0.8%) was very effective in controlling the CLB, however, at 14 DAT the efficacy of Rotenone and M.a (OM$_3$-BD) isolate were not significant difference.

Key words: biocontrol, *Brontispa longissima*, entomopathogens, *Metarhizium anisopliae*.

INTRODUCTION

Coconut leaf beetle (CLB) (*Brontispa longissima*, Coleoptera: Chrysomelidea), was first found to be a pest of coconut trees and ornament areca at Sadec town, Dong Thap province in April 1999. Since then it has spread widely and rapidly and caused severe damage in coconut plantations. In July 2000, CLB has been recorded to attack coconut trees in 18 provinces out of 21 Southern provinces (except three provinces as Lam Dong, Ninh Thuan and Binh Thuan). In August 2001, this insect has been recorded in all 21 southern provinces and 9 central provinces, so coconut has been attacked by coconut beetle. It spreads from Quang Nam to Ca Mau. Roughly, three million coconut trees have been seriously attacked. According to data by Plant Protection Department in June 2002, in all of provinces from Quang Nam to Ca Mau, CLB attacked 5,665,340 coconut trees and 12,857 ornamental trees, which belong to Palmae family.

Both larvae and adults of CLB impair young coconut leaves, epithelium of the leaves become brown, dry and die. Larvae eat stronger than adults. The younger the coconut trees were more heavily attacked. Dry season is more suitable for increasing population and damage by coconut beetle. Damages caused by CLB not only reduce coconut yields but also affect to its processing products such as coconut candy, oil, matting, etc…

Major measure to control this pest in Vietnam depends upon application of chemical pesticides actually. However, insecticidal control has led to several problems such as insecticide resistance by pests, pest resurgence, undesirable toxic effects to natural enemies, 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 is addressed as a considerable promise.

Microbial control aims at biological suppression of insect pests, by using entomopathogens like viruses, fungi, bacteria, protozoa and nematodes, which usually posses their 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 approaches.

More than 700 species of fungi, mostly Deuteromycetes and Entomophorales from about 90 genera are pathogenic to insects (Charnley 1989). The larvae, adults and pupae of CLB have been found to be infected by *Metarhizium anisopliae* and *Beauveria bassiana* in Taiwan, New Caledonia, American Samoa, Vanuatu. *Metarhizium anisopliae* were found in coconut fields in Japan. *M. anisopliaae* can kill about 65% of the fouth or fifth instar larvae and 27% of the adults (Chiu and Chien 1989). The *Brontispa* sp. can be controlled if we apply three times of *M. anisopliae*. It may be used in powder or suspension form (Liu *et al*. 1989, Liu 1994).

In Vietnam, some experiments on pathogenicity of *M. anisopliae* against CLB in the laboratory have been conducted. Some trials on the use of *M. anisopliae* fungus to control CLB in Ben Tre province were carried out. The results have shown that *M. anisopliae* was found to be pathogenic to tested CLB and *M. anisopliae* was effective to control CLB under field condition (Thuy *et al*. 2001). However, previously published data are limited due to mentioning only one given isolate of *M. anisopliae.* Since there is much scope for study of various *M. anisopliae* isolates to control CLB. The present study deals with exploit biocontrol potential of *Metarhizium anisopliae* against coconut leaf beetle, *Brontispa longissima.*

MATERIALS AND METHODS

1- Materials

Equipments for fungal study were used in CLRRI's laboratory with necessary tools for various experiments under laboratory, greenhouse and field conditions. Different isolates of *Metarhizium anisopliae* and *Beauveria bassiana* and botanical insecticide (Rotenone) were identified and used. Coconut leaf beetles and young coconut leaves were collected from many coconut gardens.

2- Methods

a. Collection, isolation and purification of the fungus from naturally infected CLB

The infected CLBs, which were found sticking to young coconut leaves, were overgrown in a green mass of conidia. The cadavers were collected in sterile glass tube to isolate causal organism. The fungus culture was purified by single conidium's culture on PDA subsequently subculturing.

b. The pathogenicity tests in the laboratory

The pathogenicity tests with different isolates of *M. anisopliae* against CLB larvae and adults were done in CLRRI's Insect Ecology and Biocontrol laboratory. Larvae and adults of CLB were used for pathogenicity tests. The conidial concentration of different *M. anisopliae* isolates were standardized at 1.5 x 10^7 conidia ml^{-1} with 0.02 percent Tween 80 (R) surfactant. Rotenone concentration was 0.6 per cent. Fifty CLB larvae or adults on a young coconut leaf were directly sprayed with the above fungal suspension or rotenone solution and transferred into glass jar. The jars were covered with muslin cloth for aeration. Control CLBs were sprayed with 0.02 percent of Tween 80 (R) surfactant. There were five replications. Mortality counts were taken at 3, 7 and 10 days after inoculating. Mortality percentage was corrected by Abbott protocol.

c. The pathogenicity tests in the green house:

The pathogenicity tests were also done under the green house condition. Larvae and adults of CLB were used for pathogenicity tests. The conidial concentration of the fungus and rotenone concentration were the same as used in laboratory pathogenicity tests. One hundred CLB larvae or adults on young coconut leaf were directly sprayed with the above fungal suspension or rotenone solution. Each young coconut leaf with the CLBs was fixed inside the jar including some water and transferred into green cage. Control CLBs were sprayed with 0.02 percent Tween 80 (R) surfactant. Data were collected from four replications. Mortality counts were taken at 3, 7, 10 and 14 days after inoculating. Mortality percentage was corrected by Abbott protocol.

d. Field efficacy of *M. anisopliae*, *B. bassiana* and rotenone against coconut leaf beetle

Field experiment was laid out in a randomized block design with 5 replications. Each replication included a four-year old coconut tree. The conidial concentration of different *M. anisopliae / B. bassiana* isolates were standardized at 1.5 x 10^7 conidia ml^{-1} with 0.1 percent U -Tron surfactant. Rotenone concentration was 0.8 per cent. The fungal suspension or rotenone solution was directly sprayed on the top of the coconut tree where the CLBs were damaging young leaves. Mortality counts were taken at 3, 7, 10 and 14 days after spraying. The efficacy of fungi / rotenone was calculated by Henderson Tilton's formula.

RESULTS AND DISCUSSIONS

1. Infectivity of certain *M. anisopliae* isolates against CLBs

In the laboratory pathogenicity studies, all of the available three selected different isolates of *M. anisopliae* were found to be pathogenic to the CLBs. However, variation in their infectivity against different stages of CLBs was recognized. Mortality percentage ranged from 38.3 to 42.5% at three days after treatment (DAT), 72.6 to 81.7 at seven DAT and 85.7 to 91.6 at ten DAT in CLB larvae (Table 1). Significant differences in mortality caused by different isolates were observed at seven DAT.

Mortality percentage ranged from 29.4 to 35.0 at three DAT, 73.2 to 78.6 at seven DAT and 84.6 to 87.9 at ten DAT in CLB adults (Table 2). The results from table 1 & 2 indicated that *M.a* (OM_3-BD) isolate, which was isolated from naturally infected CLB exhibited the highest infectivity to both stages of the CLB; the CLB larvae were more susceptible than CLB adults. Rotenone was very effective to CLB when used at 0.6% in the laboratory tests.

Table 1: Pathogenicity of three isolates of *M. anisopliae* and rotenone to CLB larvae, *Brontispa longissima (*CLRRI Laboratory, *2002)*

Treatment	Concentration	Corrected mortality (%) days after treatment		
		3	7	10
M.a(OM_1-R)	1.5 x 10^7 conidia ml^{-1}	38.3 b	72.6 c	85.7 b
M.a(OM_2-B)	1.5 x 10^7 conidia ml^{-1}	37.5 b	74.5 bc	88.5 b
M.a(OM_3-BD)	1.5 x 10^7 conidia ml^{-1}	42.5 b	81.7 b	91.6 ab
Rotenone	0.6 percent	92.5a	100 a	100.0 a
CV (%)		8.72	7.03	6.05

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

Table 2: Pathogenicity of three isolates of *M. anisopliae* and Rotenone to CLB adults, *Brontispa longissima (*CLRRI Laboratory, *2002)*

Treatment	Concentration	Corrected mortality (%) days after treatment		
		3	7	10
M.a(OM_1-R)	1.5 x 10^7 conidia l^{-1}	29.4 b	73.2 b	84.6 b
M.a(OM_2-B)	1.5 x 10^7 conidia ml^{-1}	25.0 b	72.8 b	85.4 b
M.a(OM_3-BD)	1.5 x 10^7 conidia ml^{-1}	35.0 b	78.6 b	87.9 b
Rotenone	0.6 percent	85.0 a	95.2 a	100.0 a
CV (%)		6.94	5.99	6.81

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

The results of the pathogenicity tests in Greenhouse were similar to those of the laboratory pathogenicity tests. The available three selected different isolates of *M. anisopliae* were found to be pathogenic to the CLBs. Mortality (%) ranged from 32.5 to 40.0 at three DAT, 67.5 to 72.5 at seven DAT, 74.2 to 81.9 at ten DAT, and 82.4 to 89.4 at 14 DAT in the CLBs (Table 3). The *M.a* (OM_3-BD) isolate which isolated from naturally infected CLB exhibited the highest infectivity to the CLBs. However, there was no significant difference in mortality caused by different isolates.

Table 3: Pathogenicity of three isolates of *M. anisopliae* and Rotenone to CLB, *Brontispa longissima (CLRRI Greenhouse, 2002)*

Treatment	Concentration	Corrected motality (%) Days after treatment			
		3	7	10	14
M.a(OM_1-R)	1.5 x 10^7 conidia ml^{-1}	34.7 b	70.2 b	76.8 b	82.4 b
M.a(OM_2-B)	1.5 x 10^7 conidia ml^{-1}	32.5 b	67.5 b	74.2 b	84.9 b
M.a(OM_3-BD)	1.5 x 10^7 conidia ml^{-1}	40.0 b	72.5 b	81.9 b	89.4 b
Rotenone	0.6 percent	82.5 a	90.0 a	94.7 a	100.0 a
CV (%)		7.48	8.21	9.09	7.45

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

Ignoffo and Garcia (1985) reported that two cultures of the same insect species obtained from different sources also responded differently to the same fungal isolate. In nature, living organisms, particularly microbes, undergo selection, recombination and mutation depending upon ecological situations, which ultimately influence their genetics make up. Sikura and Bevzenco (1972) found variations in toxin production in different strains of *B. bassiana,* which could be correlated to their virulence. In the present investigation, the *M.a* (OM_3-BD) isolate was the most infective to CLB, which may be due to its origin, for example the isolate obtained in Omon CLBs.

Based on successful control of CLB in laboratory and greenhouse, subsequently field experiments were conducted at different places to assess the efficacy of some *M. anisopliae* isolates and *B. bassiana* against CLBs. Results from Thoi Long, Omon, Can Tho indicated that all of the available three selected different isolates of *M. anisopliae* were found to be effective to control CLBs. The mortality (%) ranged from 62.4 to 80.7 at seven DAT, 70.6 to 83.1 at ten DAT and 75.2 to 86.4 at 14 DAT on CLB. Significant differences on mortality caused by different isolates were recorded through many observations. The *M.a* (OM_3-BD) isolate, which was identified from naturally infected CLBs exhibited the highest efficacy against CLBs. The efficacy of *M.a* (OM_3-BD) isolate was significantly higher than *M.a* (OM_1-R) and *M. a*(OM_2-B) (table 4).

Table 4: Field efficacy of three isolates of *M. anisopliae* against CLB**,** *Brontispa longissima (Thoi Long, Omon, Can Tho, 2003)*

Treatment	Concentration	Corrected mortality (%) days after treatment		
		7	10	14
M.a(OM_1-R)	1.5 x 10^7 conidia ml^{-1}	62.4 b	70.6 b	75.2 b
M.a(OM_2-B)	1.5 x 10^7 conidia ml^{-1}	69.0 b	72.6 b	77.0 b
M.a(OM_3-BD)	1.5 x 10^7 conidia ml^{-1}	80.7 a	83.1 a	86.4 a
CV (%)		14.32	12.39	13.80

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

Results of field experiments at Thoi Thanh, Omon, Can Tho also showed that all of the three selected different isolates of *M. anisopliae* were found to be effective to control CLBs. The mortality (%) ranged from 20.2 to 24.2 at three DAT, 63.2 to 68.3 at seven DAT, 65.3 to 74.6 at ten DAT and 71.6 to 79.1 at 14 DAT on CLBs. The *M.a* (OM_3-BD) also exhibited the highest efficacy against CLBs. However, significant differences in mortality caused by different isolates were recorded only at ten DAT. Rotenone with high concentration (0.8 %) exhibited its very effective effect to control CLBs. However, at 14 DAT the efficacy of rotenone and *M.a* (OM_3-BD) isolate were not significantly different (table 5).

Table 5: Field efficacy of three isolates of *M. anisopliae* and rotenone against CLB, *Brontispa longissima (Thoi Thanh, Omon, Can Tho, 2003)*

Treatment	Concentration	Corrected mortality (%) Days after treatment			
		3	7	10	14
M.a (OM_1-R)	1.5 x 10^7 conidia ml^{-1}	20.2 b	63.2 b	65.3 c	71.6 b
M.a (OM_2-B)	1.5 x 10^7 conidia ml^{-1}	23.0 b	65.2 b	69.5 bc	74.8 b
M.a(OM_3-BD)	1.5 x 10^7 conidia ml^{-1}	24.2 b	68.3 b	74.6 b	79.1 ab
Rotenone	0.8 percent	94.3 a	95.5 a	95.6 a	85.6 a
CV (%)		10.26	9.31	9.42	6.64

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

Recently, we have isolated a new *M. anisopliae* isolate from naturally infected CLBs at Trung An, My Tho, Tien Giang where we have conducted under field experiments to test the efficacy of three selected isolates of *M. anisopliae* and one selected isolate of *B. bassiana* against CLB. Results indicated that all of the selected different isolates of *M. anisopliae* and *B. bassiana* were found to be effective to control CLBs. However, mortality caused by *B. bassiana* isolate was significantly lower than those caused by three selected *M. anisopliae* isolates through almost observations (Table 6). Among three selected *M. anisopliae* isolates, *M.a* (TG2-BD) isolate exhibited the highest efficacy against the CLB. However, significant differences in mortality by the three *M. anisopliae* isolates, were recorded only at ten DAT. Particularly, no significant difference in mortality by *M.a* (OM_3-BD) and *M.a* (TG2-BD), which were isolated from naturally infected CLB, was recorded. The controlling effect of these two fungi prolonged up to 21 DAT. *M. anisopliae* offered higher efficacy i.e. 77.2% - 85.1% reduction of CLBs was recorded at 21 DAT (Table 6).

Table 6: Field efficacy of three isolates of *M. anisopliae* and *B. bassiana* against CLB, *Brontispa longissima (Trung An, My Tho, Tien Giang, 2004)*

Treatment	Concentration	Corrected mortality (%) Days after treatment			
		5	10	14	21
M.a(OM_1-R)	1.5 x 10^7 conidia ml^{-1}	45.1 a	65.3 b	78.5 ab	77.2 a
M.a(OM_3-BD)	1.5 x 10^7 conidia ml^{-1}	49.2 a	79.9 a	86.0 a	83.5 a
M.a(TG2-BD)	1.5 x 10^7 conidia ml^{-1}	51.2 a	83.2 a	89.4 a	85.1 a
B.b(OM_1-R)	1.5 x 10^7 conidia ml^{-1}	32.20 b	63.2 b	67.8 b	58.9 b
CV (%)		15.41	10.56	17.88	11.31

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

Waterhouse and Noris (1987) indicated that *Brontispa* sp. in young coconut trees could be controlled by using *Metarhizium anisopliae* in suspension form. This is a fungus, which can quickly distribute in the wet condition and kill more than a half of larvae and adults. After three applications of *M. anisopliae* var *anisopliae* formulated as homogenous biomass, in granules or in a conidial suspension, *B. longissima* could not be detected (Liu *et al.* 1996). Results of the present study are in accordance with the results by the above authors. This indicated M. *anisopliae* having a good biological potential to control CLBs. Its efficacy could be seen from seven DAT and it showed long persistence, even up to 21 DAT.

Figure 1: Coconut leaf beetles were infected by *Metarhizium anisopliae*

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SUMMARY IN VIETNAMESE

Tiềm năng của nấm xanh *Metarhizium anisopliae* trong phòng trừ sinh học bọ cánh cứng hại dừa *Brontispa longissima*

Bọ cánh cứng hại dừa *Brontispa longissima* được phát hiện đầu tiên gây hại trên cây dừa và cau cảnh tại thị xã Sa Đéc, tỉnh Đồng Tháp vào tháng 4 năm 1999 và mau chóng trở thành dịch hại nguy hiểm trên cây họ Palmae. Nấm xanh, *Metarhizium anisopliae* được phân lập từ bọ cánh cứng hại dừa và một và loài sâu hại khác nhiễm bệnh tự nhiên ở một số địa phương và đã được thử nghiệm về hiệu lực trừ bọ cánh cứng hại dừa trong các điều kiện khác nhau: phòng thí nghiệm, nhà lưới và ngòai đồng ruộng. Các thí nghiệm trong phòng và nhà lưới cho thấy 3 chủng nấm *M. anisopliae* đều có khả năng gây bệnh cho bọ cánh cứng. Chủng M.a (OM3-BD), phân lập từ bọ cánh cứng hại dừa nhiễm bệnh tự nhiên, nó có hiệu lực cao nhất trên ấu trùng và bọ dừa trưởng thành. Ấu trùng bọ cánh cứng hại dừa mẫn cảm với nấm xanh hơn so với con trưởng thành. Xử lý nấm xanh trừ bọ cánh cứng hại dừa ngoài đồng ruộng có hiệu lực cao từ ngày thứ 7 trở đi và kéo dài đến ngày 21 sau khi phun nấm. Nấm xanh có hiệu lực cao hơn so với nấm trắng *Beauveria bassiana* trong tất cả những lần quan sát. Ở điều kiện tự nhiên, 2 chủng nấm xanh M.a (OM3-BD) and M.a (TG2-BD), phân lập từ bọ cánh cứng hại dừa nhiễm bệnh tự nhiên có hiệu lực cao nhất trong số 4 chủng nấm xanh đã thử nghiệm. Xử lý rotenone ở nồng độ cao (0,8%) trừ bọ cánh cứng hại dừa rất hiệu quả, nhưng hiệu lực không khác biệt với nghiệm thức xử lý M.a (OM3-BD) ở giai đọan 14 ngày sau khi phun thuốc.