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EXPLOITATION OF *Beauveria bassiana* AND *Metarhizium anisopliae* AS POTENTIAL BIOCONTROL AGENTS IN INTEGRATED PEST MANAGEMENT (IPM) ON CITRUS

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ABSTRACT

Studies were conducted on white muscardine fungus, Beauveria bassiana and green muscardine fungus, Metarhizium anisopliae to exploit their potentiality for using in Integrated Pest Management (IPM) on citrus. The result of survey, collection, isolation and domestication determined sixteen isolates of B. bassiana and nineteen isolates of M. anisopliae in Can Tho and Vinh Long. Ten good isolates were selected, in which, two isolates of B. bassiana such as B.b (VL_3-RMCQ), B.b (CT_{11}-RMCQ) and two isolates of M. anisopliae such as M.a (VL_2-RMCQ), M.a (CT_{15}-RCC) had high effectiveness to black citrus aphid and citrus pyrilla in Greenhouse tests. Two bioinsecticides which produced from M.a (CT_{15}-RCC) isolate and B.b (VL_3-RMCQ) isolate had very high effectiveness to black citrus aphid and citrus pyrilla in Greenhouse tests as well as the field experiments. Laboratory assessment of safety of M.a(CT_{15}-RCC) and B.b(VL_3-RMCQ) bioinsecticides indicated that direct conidial application at a concentration 10^7 conidia/ml of M.a(CT_{15}-RCC) or B.b(VL_3-RMCQ) had no adverse effects on the predatory potential of Lynx spider, Oxyopes sp. Both these bioinsecticides got very high efficiency on management of black citrus aphid, citrus pyrilla and citrus leafminer when they were applied in the models of 50 ha in Can Tho city and Vinh Long province. The economic efficiency was compared between demonstration garden, which was applied with the bioinsecticide M.a (CT15-RCC) /B.b (VL3-RMCQ), and the control garden, which was done with chemical insecticides periodically. It was indicated that net profit from the demonstration garden was higher than that from the control garden, with VND 2,834,000 /ha for the model of four-year-old King orange in Tam Binh (Vinh Long province). It also gained VND 3,915,000 for the model of five to six-year-old orange in Phong Dien (Can Tho city), and VND 4,217,000 for the model of five to six - year - old Nam Roi pomelo in Binh Minh (Vinh Long province).

Key words: *Beauveria bassiana*, Black citrus aphid, Citrus pyrilla, Entomogenous fungi, insect pests, *Metarhizium anisopliae*

INTRODUCTION

Insecticides are the only tool in the pest management strategy that is reliable for emergency action when insects at the times of blooming. However, insecticidal control has led to several problems in insect management such as appearance of insecticide resistance pests, pest resurgence, undesirable toxic effects to natural enemies of target pests, disruption of the ecosystem, toxic residues in crop plants and environmental problems. Consequently, the research for new environmentally safe method is being intensified. Among the various methods in IPM program, biological control has shown considerable promise. Microbial control agents obviously should have characteristics that on one hand can be integrated in the IPM politics and support the role of natural enemies, but on the other hand provides farmer a tool to reduce the damage of major insect pests.

The two entomogenous fungi (*Metarhizium anisopliae* and *Beauveria bassiana)* have been

studied and applied to manage various insect pests on many crops around the world (Rombach *et al.* 1986a and b; Aguda *et al.* 1987, 1988; Ramamohan Rao 1989; Butt and Copping 2000; Goettel *et al*. 2000; Nugroho and Ibrahim 2007 and Yubak *et al*. 2008.

In Vietnam, *Metarhizium anisopliae* and *Beauveria bassiana* have been studied and applied in controlling of rice Brown planthoppers, rice bug, coconut beetle, grasshoppers, termite etc. (Chinh *et al*. 2001; Thuy e*t al.* 2001; Loc 1995, 1997a and b; Loc *et al.* 1999, 2001, 2002, 2004, 2005).

Citrus are main fruit in agriculture production in the Mekong Delta. The Citrus pyrilla (*Diaphorina citri)* is a most serious sucking insect pest of citrus-tree in the world as well as in Vietnam. It is a vector transferring *Liberobacter asiaticum* bacterium causes greening disease, the main damage of citrus-tree group. The Black citrus aphid (*Toxoptera sp.)* is also an important pest sucking the sap of the citrus young shoots and leading deformation of citrus shoot. Thereafter, leaves are twisted, stunted and no fully development is expected. Black citrus aphid is a vector to transfer the "Tristeza" disease of citrus. The farmers in Cuu Long delta who are cultivating citrus-tree group always spray insecticides every 15 days to control these two serious sucking insects.

The microclimate occurred in the citrus gardens would be quite suitable for the fungal disease development and moreover the cadaver of insect is followed by external sporulation which helps fungus to be spread in very high levels to kill insects. Unlike bacteria or virus, fungi penetrate actively and do not require ingestion to infect, thus, the fungi have a better prospect in the microbial control of sucking insect pests of citrus. Therefore, the project **"**Exploitation of *Beauveria bassiana* and *Metarhizium anisopliae* as potential biocontrol agents in integrated pest management (IPM) on citrus" was conducted. The specific objective of this study deals with isolating the *Beauveria bassiana (B.b)* and *Metarhizium anisopliae (M.a)* from naturally infected insects to select those having high insecticidal activity against target insects of citrus. The formulation will target two serious sucking insect pests of black citrus aphid, *Toxoptera citricidus* and citrus pyrilla, *Diaphorina citri* and eventually it will be incorporated into IPM program of citrus in the Mekong Delta of Vietnam.

MATERIALS AND METHODS:

1.1. Collection, isolation and purification the entomogenous fungi (B. bassiana and M. anisopliae):

The fungal infected insects, soil samples were collected from different locations of Can Tho and Vinh Long, particularly at some gardens where the farmers were less applying the pesticides regularly. The insects were found to be sticking to the leaves of citrus, and overgrown by chalky white or green mass of conidia. The cadavers were collected in sterile glass tube for isolating the causal organisms in the laboratory. The fungus culture was purified by single conidium culture on potato dextrose agar (PDA) medium and subsequently subculturing was done as Loc (1995).

1.2. Inoculation and identification:

The pure fungal culture was multiplied on PDA medium for 10 days. Conidial concentration was adjusted to 10^7 conidia ml^{-1} in suspension and was applied directly on the insects in plastic jars. Control insects were sprayed with 0.02 percent Tween 80® solution instead of conidia suspension. Thirty insects were retained on each citrus shoot in a plastic jar and covered with muslin cloth. There were 4 replications. The mortality was recorded after 5 days and the death insects were collected and incubated in BOD incubator for 3-4 days and examined under microscope (Rombach *et al*., 1986a)

The fungus was reisolated from infected insect and single conidium culture was purified and compared with original isolate. The single conidium culture was identified for confirmation of the pathogen (Loc, 1995).

1.3. The pathogenicity tests

The pathogenicity tests with different isolates of *B. bassiana* and *M. anisopliae* against nymphs of black citrus aphids and citrus pyrilla were done in the same way as specified above (Rombach *et al*.,

1986a). From the mortality recorded, we selected the best isolates of *B.b* and *M.a* which had high insecticidal activity against target insects for further study. Percent mortality of insect was corrected by a formula as suggested by Abbott (1925).

2.1. Mass production of selective B.b and M.a isolates to produce B.b and M.a wettable powder formulation.

In previous research, we have established best medium formulas for multiplying the *B.b* and *M.a,* Those were rice bran, corn powder and rice husk, and very cheap and available at everywhere in the Mekong delta. After inoculating, at about 2 week, we got the maximum number of fungus conidia and dried at 40 ^{0}C for 40 hours, and then ground to get B.b and M.a wettable powder formulation. The number of conidia per gram of product was determined with haemocytometer.

2.2. Pathogenicity tests of selected B.b/M.a isolates against the target insects in the Greenhouse

The pathogenicity tests of *B.b*/*M.a* bioinsecticide against nymphs of black citrus aphids and citrus pyrilla were done in the Greenhouse to determine the efficacy against target insects. Conidial concentration in the suspension is 10^7 conidia/ml and applied directly to the nymphs of Black citrus aphids/Citrus pyrilla in a closed net cage with spraying by a sterilized atomizer at 5 ml per a potted citrus small plant. Control insects were sprayed with 0.02 % Tween®80 solution. Thirty insects were retained on each potted citrus plant and the net cage was closed after spraying. There are 4 replications. The mortality was recorded after 3, 7 and 10 days and the percent mortality of insect were corrected by a formula as suggested by Abbott (1925).

2.3. Laboratory assessment of safety of B.b and M.a bioinsecticides on Lynx spider, Oxyopes sp.

Safety tests were conducted with *B.b*/*M.a* bioinsecticide by direct conidial application as suggested by Magalhaes *et al* (1988) at 10^8 conidia/ml to the Lynx spider, *Oxyopes sp* collected from the field and kept in plastic jar with citrus shoot. It is one of dominant natural enemies of the citrus insect pests. Each treatment and control was replicated four times with 5 spiders per each replication. Hundred fifty Black citrus aphid nymphs were released daily as food for spiders. The feeding potential was calculated on daily consumption rate of Black citrus aphid nymphs. The mortality (if any) of spider was also recorded.

2.4. Field efficacy of **new** *B.b/ M.a bioinsecticides against Black citrus aphids and Citrus pyrilla in Phong Dien and Binh Minh district.*

To confirm the activity of *B.b/M.a* formulations, field bioassays against Black citrus aphids and Citrus pyrilla on citrus gardens were conducted. The experiments were laid out in a randomized complete block design with three replications. The field efficacy of *B.b* and *M.a* products against these target insects will be calculated by formula as suggested by Henderson –Tilton (1955)

3.1. Conducting training courses for Plant Protection staffs, Agriculture Extension workers to organize formulation into IPM program

In province and district, advanced farmers were introduced with how to incorporate these *B.b* and *M.a* bioinsecticides in IPM program of citrus. All the techniques of Citrus IPM were taught in the training course; however, the technique how to use *B.b* and *M.a* formulation in management of citrus insect pests was most emphasized.

3.2. Conducting the on-farm demonstration models.

After performance of 2 training classes in 2 provinces, 12 advanced farmers from 6 villages of Phong Dien, Binh Minh and Tam Binh were selected for the incorporation of on-farm demonstration models in which new *M.a/B.b* formulation was applied to control citrus insect pests for the dissemination and extension of the new bioinsecticides.

The garden of farmer grouped into 2 plots for demonstration model and control. Each area was about 3,000m^2. In the control plot, the farmer employed the ordinary methods to control the insect pests. The observation was done regularly to record the fluctuation of number of each target insect species.

In the demonstration plot, the farmer used *B.b* and *M.a* bioinsecticides to manage black citrus aphids and citrus pyrilla, and fluctuation of number of each insect species was regularly recorded. The *M.a/B.b* formulations were sprayed when the population of black citrus aphids and citrus pyrilla reach to the economical threshold. Other cultivating techniques are followed the citrus IPM program.

+ The input and the yield of orange or pomelo in each plot should be also recorded for economic analysis.

4.1. Statistical analysis

The data obtained were statistically analyzed following the methods of Gomez and Gomez (1993). Percent mortality of insect (in the laboratory and green house experiments) was corrected by a formula as suggested by Abbott (1925). The field efficacy of *B.b /M.a* bioinsecticides against the target insects were calculated by formula as suggested by Henderson – Tilton (See above)

RESULTS AND DISCUSSIONS:

Studies were mainly conducted the white muscardine fungus, *Beauveria bassiana* (Bals.) Vuill and green muscardine fungus, *Metarhizium anisopliae* (Metchnikoff) Sorokin to exploit their potentiality in controlling black citrus aphid and citrus pyrilla in citrus. To achieve the objective, studies were carried out on collection, isolation, purification and selection of *B.b* and *M.a* isolates having high insecticidal activity against two serious sucking insect pests of citrus to develop bioinsecticides having high insecticidal activity for controlling them in IPM program of citrus. The results obtained are presented and discussed in the following paragraphs.

3.1. Collection, isolation, purification and selection of *B.b* and *M.a* isolates having high insecticidal activity against target insects of citrus.

The fungal infected insects, soil samples were collected from different locations of Can Tho and Vinh Long, particularly at some gardens where the farmers were less applying the pesticides regularly. The result of collection, isolation and domestication determined sixteen isolates of *B. bassiana* and nineteen isolates of *M. anisopliae* in Can Tho and Vinh Long.

The pathogenicity tests with these 35 isolates of *B. bassiana* and *M. anisopliae* against nymphs of Black citrus aphids and Citrus pyrilla have been done in the green house by the same way as specified above (Rombach *et al*., 1986), from these tests then ten good isolates have been selected for further study in greenhouse. From the results of this study, we have selected two isolates of *B. bassiana* such as *B.b* (VL_3-RMCQ), *B.b* (CT_{11}-RMCQ) and two isolates of *M. anisopliae* such as *M.a* (VL_2-RMCQ), *M.a* (CT_{15}-RCC) had high effectiveness to black citrus aphid and citrus pyrilla in greenhouse tests. These four fungal isolates were selected for further study.

3.2. Development of new *B.b* and *M.a* bioinsecticides having high insecticidal activity for controlling two serious sucking insect pests of citrus.

Four selected fungal isolates such as *B.b* (VL_3-RMCQ), *B.b* (CT_{11}-RMCQ), *M.a* (VL_2-RMCQ) and *M.a* (CT_{15}-RCC) have been used to produce four new bioinsecticides, conducted pathogenicity tests in the Greenhouse to evaluate their efficacy against the black citrus aphid and citrus pyrilla.

3.2.1. Pathogenicity tests of the new B.b/M.a bioinsecticide which produced from selected B.b/M.a isolates against the target insects in the greenhouse

The pathogenicity tests of the new *B.b*/*M.a* bioinsecticide which produced from selected *B.b/M.a* isolates against nymphs of black citrus aphids and citrus pyrilla have been done in the Greenhouse to determine whether these new *B.b*/*M.a* bioinsecticide having high insecticidal activity against target insects or not. The results in table 1 indicated that M.a bioinsecticide which produced from *M.a*(CT_{15}-RCC) isolated from fungal infected citrus pyrilla which collected in Can Tho had very high efficacy against nymphs of black citrus aphids, the efficacy of this *M.a*(CT_{15}-RCC) bioinsecticide reached 72.4 % at 7 days after treatment (DAT). At 10 DAT the efficacy of *M.a*(CT_{15}-RCC) bioinsecticide have reached 86.8 % and significantly higher as compared to the efficacy of three bioinsecticides which produced from three other fungal isolates and particularly significantly higher than efficacy of two bioinsectides Ometar and Biovip. Immediately

after was the bioinsecticide which produced from *M.a*(VL_2-RMCQ) isolated from fungal infected black citrus aphid which collected in Vinh Long province had quite high efficacy against black citrus aphids, efficacy of *M.a*(VL_2-RMCQ) bioinsecticide reached 57.4 % at 7 DAT and 72.2 % at 10 DAT. The efficacy of *B.b*(VL_3-RMCQ) bioinsecticide against black citrus aphid was higher as compared to the efficacy of *B.b*(CT_{11}-RMCQ) bioinsecticide. The efficacy of *B.b*(VL_3-RMCQ) bioinsecticide reached 62.8 % and was not significantly different as compared to the efficacy of *M.a*(CT_{15}-RCC) and *M.a*(VL_2-RMCQ) bioinsecticides at 7 DAT, at 10 DAT the efficacy of this bioinsecticide reached 70.3 % .

Table 1: Efficiency of new *B.b/M.a* bioinsecticides, which produced from fungal isolates, selected in Can Tho and Vinh Long to black citrus aphid *(Greenhouse of CLRRI, 2006)*

No	Treatment	Corrected mortality (%)		
		Days after treatment (DAT)		
		3 DAT	7 DAT	10 DAT
1	*B.b*(VL_3-RMCQ)	31.1 bc	62.8 abc	70.3 bc
2	*B.b*(CT_{11}-RMCQ)	22.7 c	49.6 c	66.4 c
3	*M.a*(VL_2-RMCQ)	27.7 c	57.4 abc	72.2 bc
4	*M.a*(CT_{15}-RCC)	42.0 a	72.4 a	86.8 a
5	Biovip	30.2 bc	54.1 bc	67.8 c
6	Ometar	39.4 ab	66.4 ab	75.2 b
	CV (%)	26.3	20.6	9.7

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

The efficacy of four new bioinsecticides which produced from 4 selected isolates from Can Tho and Vinh Long against citrus pyrilla in the Greenhouse was recorded in table 2. The bioinsecticide, which produced from *M.a* (CT_{15}-RCC) isolated from fungal infected citrus pyrilla in Can Tho, exhibited high efficacy against to citrus pyrilla. It reached 63.4 % and 78.6 % at 7 DAT and 10 DAT, respectively. The efficacy of *M.a* (CT_{15}-RCC) bioinsecticide was the highest as compared to other three new bioinsectides in the experiment at 7 DAT and 10 DAT. The efficacy of *B.b* bioinsecticide, which produced from *B.b*(VL_3-RMCQ) isolated from *Beauveria bassiana* infected black citrus aphid in Vinh Long, and the efficacy of *B.b*(VL_3-RMCQ) bioinsecticide against citrus pyrilla reached 55.8 % and 69.4 % at 7 DAT and 10 DAT, respectively. Especially, there were not significantly different between efficacy of *B.b*(VL_3-RMCQ) bioinsecticide, *M.a*(CT_{15}-RCC) bioinsecticide and Ometar against citrus pyrilla.

Table 2: Efficiency of 4 new fungal isolates selected in Can Tho and Vinh Long to citrus pyrilla *(CLRRI greenhouse, 2006)*

No	Treatment	Corrected mortality (%)		
		Days after treatment (DAT)		
		3 DAT	7 DAT	10 DAT
1	*B.b*(VL_3-RMCQ)	28.7 a	55.8 ab	69.4 ab
2	*B.b*(CT_{11}-RMCQ)	26.3 a	41.1 c	55.3 c
3	*M.a*(VL_2-RMCQ)	28.0 a	40.4 c	62.8 bc
4	*M.a*(CT_{15}-RCC)	32.2 a	63.4 a	78.6 a
5	Biovip	27.9 a	50.8 bc	66.5 bc
6	Ometar	33.1 a	57.7 ab	68.2 ab
	CV (%)	21.9	16.1	14.3

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

From the results of this experiment, we have selected a *Beauveria bassiana* isolate such as *B.b* (VL_3-RMCQ). It was isolated from fungal infected black citrus aphid in Vinh Long. Two *Metarhizium anisopliae* isolates such as *M.a* (CT_{15}-RCC) were isolated from *Metarhizium anisopliae* infected citrus pyrilla in Can Tho and *M.a* (VL_2-RMCQ) from *Metarhizium anisopliae* infected black citrus aphid in Vinh Long to produce the bioinsecticides for the next greenhouse experiments

Table 3 indicated that *M.a* bioinsecticide produced from M.a (CT_{15}-RCC) exhibited very high efficacy against black citrus aphid, reached 74.6 % at 7 DAT. At 10 DAT the efficacy of *M.a* (CT_{15}-RCC) bioinsecticide reached 84.8%. It was significantly higher than the efficacy of Ometar, Biovip and *M.a* (VL_2-RMCQ) bioinsecticide. The *B.b* bioinsecticide *B.b*(VL_3-RMCQ) also had quite high efficacy against black citrus aphid. It reached 63.8 % and 77.8 % at 7 DAT and 10 DAT, respectively. Especially, there were not significantly different between efficacy of *B.b* (VL_3-RMCQ) bioinsecticide and *M.a* (CT_{15}-RCC) to black citrus aphid at 7 DAT and 10 DAT.

Table 3: Efficiency of three new fungal isolates selected in Can Tho and Vinh Long to black citrus aphid *(CLRRI Greenhouse 2006)*

No	Treatment	Corrected mortality (%)		
		Days after treatment (DAT)		
		3 DAT	7 DAT	10 DAT
1	*B.b*(VL_3-RMCQ)	29.2 a	63.8 ab	77.8 ab
2	*M.a*(VL_2-RMCQ)	26.8 a	58.4 b	67.2 c
3	*M.a*(CT_{15}-RCC)	34.6 a	74.6 a	84.8 a
4	Biovip	28.4 a	55.1 b	66.8 c
5	Ometar	30.2 a	65.4 ab	73.2 bc
	CV (%)	10.6	12.4	9.6

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

Table 4 indicated that bioinsecticide *M.a* (CT_{15}-RCC) had very high efficacy against black citrus aphid, reached 68.4 % and 80.2 % at 7 DAT and 10 DAT, respectively. The efficacy of *M.a* (CT_{15}-RCC) was the highest as compared to the efficacy of others in this experiment. The bioinsecticide *B.b* (VL3-RMCQ) also had quite high efficacy against citrus pyrilla, reached 58.2 % and 72.4 % at 7 DAT and 10 DAT, respectively. There were not significantly different between efficacy of *M.a* (CT_{15}-RCC), Ometar and *B.b* (VL_3-RMCQ) against citrus pyrilla.

Table 4: Efficiency of three new fungal isolates selected in Can Tho and Vinh Long to citrus pyrilla *(*CLRRI greenhouse 2006)

No	Treatment	Corrected mortality (%)		
		Days after treatment (DAT)		
		3 DAT	7 DAT	10 DAT
1	*B.b*(VL_3-RMCQ)	29.2 a	58.2 ab	72.4 ab
3	*M.a*(VL_2-RMCQ)	27.4 a	42.8 c	60.2 b
4	*M.a*(CT_{15}-RCC)	34.6 a	68.4 a	80.2 a
5	Biovip	26.8 a	52.4 bc	65.4 b
6	Ometar	30.6 a	56.8 ab	69.2 ab
	CV (%)	13.2	14.4	12.8

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

3.2.2. Laboratory assessment of safety of B.b and M.a bioinsecticides on *Lynx* spider, *Oxyopes* sp.

It is essential that a candidate fungal pathogen/fungal bioinsecticide should not be inimical to the natural enemies of the target pests.

The present investigation showed that in all safety tests with the bioinsecticides, which produced from *M.a* (VL_2-RMCQ), *M.a* (CT_{15}-RCC) and *B.b* (VL_3-RMCQ), no mycosis was noticed on the Lynx spider, *Oxyopes sp.* Direct conidial application at a concentration 10^7 conidia/ml had no adverse effects on the predatory potential of Lynx spider, *Oxyopes sp.* (table 5).

Ramamohan Rao (1989) reported that conidial application of *M. anisopliae*, *M. flavoviride, B. bassiana* and *P. lilacinua* at a dose of 10^8 conidia/ml had no adverse affect on predatory cocinellids, spider, mirid bug and on parasitoids like *Trichogramma japonicum* Ashmead, *platygaster oryzae* Cameron and *Tetrastichus schoenobii* Ferriere.

Loc *et al* (2002) also reported that *M. anisopliae* and *B. bassiana* used at the dose of 6 x 10^{12} conidia/ha in the rice fields had no adverse effect on predatory wolf spider as *Lycosa peudoannulata*, *Araneus inustus*, *Tetragnatha maxillosa*; mirid bug, *Cyrtohinus lividipennis* and predatory bug, *Polytoxus fuscovittatus.*

Table 5: Effect of *B. bassiana* and *M. anisopliae* on Lynx Spider, *Oxyopes sp. (CLRRI greenhouse, 2006)*

No	Fungal isolate (concentration:10^7 conidia/ ml)	Number of black citrus aphid consumed			
		1DBT	3DAT	5DAT	7DAT
1	*B.b*(VL_3-RMCQ)	20.5 ± 1.9	21.0 ± 2.4	21.5 ± 1.7	19.5 ± 2.5
2	*M.a*(VL_2-RMCQ)	20.0 ± 1.6	19.3 ± 2.2	20.8 ± 1.0	21.0 ± 0.8
3	*M.a*(CT_{15}-RCC)	18.5 ± 0.6	19.5 ± 3.1	19.0 ± 2.2	19.8 ± 1.7
4	Biovip	19.3 ± 3.2	22.0 ± 2.4	22.0 ± 2.3	21.3 ± 1.9
5	Ometar	18.3 ± 1.7	20.5 ±2.1	21.8 ± 2.9	20.5 ± 1.0
6	Control	20.3 ± 2.6	21.3 ± 2.2	21.5 ± 1.7	22.0 ±1.2

DBT: days before treatment; DAT: days after treatment

From the results of greenhouse experiments, we decided to select *B.b*(VL_3-RMCQ) isolated from a *Beauveria bassiana* infected black citrus aphid in Vinh Long and *M.a* (CT_{15}-RCC) isolated from a *Metarhizium anisopliae* infected citrus pyrilla in Can Tho, to produce the bioinsecticides for further studies in the fields.

***3.2.3. Field efficacy of* new *B.b/ M.a bioinsecticides against Black citrus aphids and Citrus pyrilla* in Phong Dien and Binh Minh district.**

To confirm the activity of B.b/M.a bioinsecticides formulations, some field bioassays against Black citrus aphids and Citrus pyrilla on citrus gardens were conducted.

The results were recorded in table 6 indicated that M.a bioinsecticide, which produced from *M.a* (CT_{15}-RCC) isolated from *Metarhizium anisopliae* infected citrus pyrilla collected in the orange garden at Nhan Ai - Phong Dien - Can Tho had very high efficacy against black citrus aphid under field conditions. It reached 79.6 % at 7 DAT and significantly higher as compared to the efficacy by Ometar (65.7 %) and not significantly different to Bassa 50 EC insecticide (82.5 %) and DC Tron Plus 98,8 EC mineral oil (76.2 %). The efficacy of *B.b* (VL_3-RMCQ) isolated from *Beauveria bassiana* infected black citrus aphid collected in orange garden at Tam Binh - Vinh Long, reached 71.7 % and not significantly different to *M.a* (CT_{15}-RCC), Bassa 50 EC insecticide, DC Tron Plus 98,8 EC mineral oil. At 10 DAT and 14 DAT, *M.a* (CT_{15}-RCC) still showed the highest efficacy against black citrus aphid. It reached 85.7 % and 76.5 % at 10 DAT and 14 DAT, respectively. It was significantly higher than the efficacy of Bassa 50 EC and DC Tron Plus 98.8 EC mineral oil.

Table 6: Field efficiency of two new bioinsecticides produced from two selected fungal isolates to black citrus aphid *(orange garden at Nhon Ai - Phong Dien – Can Tho city, 2006)*

No	Treatment	Corrected mortality (%)			
		Days after treatment (DAT)			
		3 DAT	7 DAT	10 DAT	14 DAT
1	*B.b* (VL3-RMCQ)	43.7 c	71.7 ab	76.6 ab	65.8 ab
2	Biovip	34.0 d	53.2 c	58.3 c	56.2 b
3	*M.a* (CT15-RCC)	44.2 c	79.6 a	85.7 a	76.5 a
4	Ometar	32.4 d	65.7 b	74.5 ab	64.8 ab
5	Bassa 50 EC	74.5 a	82.5 a	70.5 b	40.5 c
6	DC Tron Plus 98,8 EC	60.6 b	76.2 a	66.1 bc	38.5 c
	CV (%)	10.7	11.0	11.3	14.4

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

The efficacy of four new bioinsecticides, which produced from 4 selected isolates from Can Tho and Vinh Long against citrus pyrilla in the field was recorded in table 7. They indicated *M.a* (CT_{15}-RCC) isolated from fungal infected citrus pyrilla colleted in the orange garden at Nhon Ai and Phong Dien - Can Tho had high efficacy against to citrus pyrilla in the field test. Efficacy of *M.a* (CT_{15}-RCC) against citrus pyrilla reached 69.7 % at 7 DAT and not significantly different to Bassa 50EC insecticide (71.1%) and DC Tron Plus 98.8 EC mineral oil (65.6 %). The B.b (VL_3-RMCQ) isolated from *Beauveria bassiana* infected black citrus aphid collected in orange garden at Tam Binh - Vinh Long, also had quite high efficacy against citrus pyrilla, reached 59.5 % and not significantly different to *M.a* (CT_{15}-RCC), Ometar, Bassa 50EC, DC Tron Plus 98.8 EC.

At 10 DAT and 14 DAT, *M.a* (CT15-RCC) bioinsecticide still showed the highest efficacy against citrus pyrilla, reached 86.4 % and 72.7 %, and significantly higher than Bassa 50EC, DC Tron Plus 98.8 EC and Biovip.

Table 7: Field efficiency of two new bioinsecticides products to Citrus pyrilla *(orange garden at Nhon Ai and Phong Dien – Can Tho city, 2006)*

No	Treatment	Corrected mortality (%)			
		Days after treatment (DAT)			
		3 DAT	7 DAT	10 DAT	14 DAT
1	*B.b* (VL_3-RMCQ)	24.6 bc	59.5 ab	72.5 ab	64.2 ab
2	Biovip	32.0 b	52.3 b	68.8 b	57.1 b
3	*M.a* (CT_{15}-RCC)	24.6 bc	69.7 a	86.4 a	72.7 a
4	Ometar	22.6 c	61.3 ab	74.5 ab	62.9 ab
5	Bassa 50 EC	60.1 a	71.1 a	63.1 b	47.5 bc
6	DC Tron Plus 98,8EC	28.6 bc	65.6 ab	62.8 b	40.5 c
	CV (%)	18.3	12.9	15	14.4

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

Similarly, we have conducted field experiments at My Thanh Trung - Tam Binh – Vinh Long province, 2006.

The *M.a* (CT_{15}-RCC) had very high efficacy against black citrus aphid under field experiments at My Thanh Trung - Tam Binh. It reached 72.4% at 7 DAT and and not significantly different to Bassa 50EC (83.7%) and DC Tron Plus 98.8 EC (80.3%). M.a (CT_{15}-RCC) bioinsecticide still showed the highest efficacy against black citrus aphid. It reached 84.2 % and 78.4 % at 10 DAT và

14 DAT, respectively. It was significantly higher than Bassa 50 EC and DC Tron Plus 98.8 EC. However, efficacy of M.a (CT_{15}-RCC) bioinsecticide against black citrus aphid was not significantly different as compared to Ometar bioinsecticide and B.b (VL_3-RMCQ) bioinsecticide at 10 DAT and 14 DAT.

The *M.a* (CT_{15}-RCC) had high efficacy against to citrus pyrilla under the field test at My Thanh Trung. Efficacy of *M.a* (CT_{15}-RCC) against citrus pyrilla reached 71.2 % at 7 DAT and not significantly different to Bassa 50 EC (75.4 %) and DC Tron Plus 98,8 EC (74.2 %). It was significantly higher than three remain other bioinsecticides as Ometar, Biovip and *B.b* (VL_3-RMCQ). At 10 DAT, *M.a* (CT_{15}-RCC) had the highest efficacy against citrus pyrilla. It reached 82.5 %, and was not significantly different to *B.b* (VL_3-RMCQ) bioinsecticide (76.9 %). It was significantly higher than four remained treatments. At 14 DAT, M.a (CT_{15}-RCC) still showed the highest efficacy against citrus pyrilla. It reached 71.8 %, and was not significantly different to *B.b* (VL_3-RMCQ), Ometar, but significantly higher than Bassa 50 EC insecticide, DC Tron Plus 98.8 EC and Biovip.

So *M.a* (CT_{15}-RCC) and *B.b* (VL_3-RMCQ) were considered to gain the high efficacy against two sucking insect pests, which have been tested. *M.a* (CT_{15}-RCC) isolate and *B.b*(VL_3-RMCQ) isolate have been selected for mass production. These two bioinsecticides will be recommended to use on-farm demonstration models in Can Tho Vinh Long.

3. Incorporating the new B.b and M.a bioinsecticides in IPM program on citrus production

Two training courses in two provinces were implemented. Then 12 advanced farmers from six villages of Phong Dien, Binh Minh and Tam Binh were selected to create twelve on-farm demonstration models to apply new *M.a/B.b* bioinsecticides in management of citrus insect pests.

The demonstration models conducted at Nhon Ai, Truong Long - Phong Dien (Can Tho); My Hoa, Dong Binh - Binh Minh and My Thanh Trung, Ngai Tu - Tam Binh (Vinh Long) exhibited very good results. They reduced pesticide cost, labor and brought higher profits for the farmers.

Table 8 indicated that the models conducted in four- year- old King orange at My Thanh Trung – Tam Binh – Vinh Long, exhibited the average pesticide cost reduced with VND 1,417,000 /ha (57.8 %) as compared to the control. The average fertilizer cost in demonstration reduced VND 498,000 /ha (11.5 %) as compared to the control. Similarly, the average labor cost reduced VND 355,000 /ha (4.8 %) as compared to the control. Therefore, total cost in the demonstration models reduced VND 2,270,000 /ha (15.9 %) as compared to the control. The production cost of King orange in the demonstration models reduced VND 124 /kg (16.5 %) as compared to the control. Average gross income of demonstration models was higher than control as VND 564,000 /ha (0.6 %). The average profit increased VND 2,834,000 /ha (3.8 %) as compared to the control.

Table 8: Comparison of economic efficiency when applied bioinsecticides (*M.a/B.b*) to control **(**King orange at My Thanh Trung - Tam Binh – Vinh Long, Jan to Dec, 2007)

Items	Average of models (1)	Average of Controls (2)	Difference	
			Amount 3 = (1)-(2)	Ratio (%)
Cost of pesticides (VND/ha)	1,035,000	2,452,000	- 1,417,000	-57.8
Cost of fertilizer (VND/ha)	3,825,000	4,323,000	- 498,000	-11.5
Cost of hired labor(VND/ha)	7,095,000	7,450,000	- 355,000	-4.8
Total costs (VND/ha)	**11,955,000**	**14,225,000**	**- 2,270,000**	**-15.9**
Pomelo yield (kg/ha)	19,035	18,915	120	0.6
Cost price (VND/kg)	628	752	- 124	-16.5
Selling price (VND/kg)	4,700	4,700	0	0
Gross income (VND/ha)	**89,464,000**	**88,900,000**	**564,000**	**0.6**
Profits (VND/ha)	**77,509,500**	**74,675,500**	**2,834,000**	**3.8**

Four- year- old King orange

Similarly, looking on-farm demonstration models conducted in six - year - old orange garden at Nhon Ai – Phong Dien – Can Tho, the average pesticide cost in demonstration models reduced VND 1,338,000 /ha (56.9 %) as compared to the control. Similarly, the average fertilizer cost in demonstration models reduced VND 902,000 /ha (10.3 %). The average labor cost reduced VND 730,000 /ha (9.6 %). Therefore, the total costs of the demonstration models reduced VND 2,970,000 /ha, (15.8 %) as compared to the control. Average orange yield of demonstration models was higher than the control as much as 120 kg/ha. There fore, average gross income of demonstration models was higher than the control as VND 945,000 /ha (1.4 %). The average profits in demonstration models increased VND 3,915,000 /ha (8.3 %) as compared to the control.

Table 9: Comparison of economic efficiency between demonstration model garden which applied bioinsecticides (*M.a/B.b*) and control garden (Nam Roi pomelo garden at My Hoa - Binh Minh – Vinh Long, Jan to Dec 2007)

Items	Average of models (1)	Average of controls (2)	Difference	
			Amount 3= (1)-(2)	Ratio (%)
Cost of pesticides (VND/ha)	1,205,000	2,312,000	- 1,107,000	- 47.9
Cost of fertilizer (VND/ha)	19,012,000	19,721,000	- 709,000	-3.6
Cost of hired labor (VND/ha)	5,668,000	6,273,000	- 605,000	- 9.6
Total costs (VND/ha)	**25,885,000**	**28,306,000**	**- 2,421,000**	**- 8.6**
Pomelo yield (kg/ha)	42,744	42,295	449	1.1
Cost price (VND/kg)	605	669	- 64	-9.6
Selling price (VND/kg)	4,000	4,000	0	0
Gross income (VND/ha)	**170,976,000**	**169,180,000**	**1,796,000**	**1.1**
Profits (VND/ha)	**145,091,000**	**140,874,000**	**4,217,000**	**3.0**

Six- year- old Nam Roi pomelo

Table 9 indicated that when on-farm demonstration models conducted on five to six - year-old Nam Roi pomelo at My Hoa - Binh Minh, the average pesticide cost reduced VND 1,107,000 /ha (47.9 %) as compared to the control. The average fertilizer cost in demonstration models reduced VND 709,000 /ha (3.6 %) as compared to the control. The average cost of hired labor in demonstration models reduced VND 605,000 /ha (9.6 %) as compared to the control.

Therefore, total costs of the demonstration models reduced VND 2,421,000 /ha (8.6 %) as compared to the control. Average pomelo yield of demonstration models was higher than control as much as 449 kg/ha. The average gross income of demonstration models was higher than control as VND 1,796,000 /ha (1.1 %). The average profits at demonstration models increased VND 4,217,000 /ha (3 %) as compared to the control.

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Khai thác tiềm năng phòng trừ sinh học của nấm trắng, *Beauveria bassiana* và nấm xanh, *Metarhizium anisopliae* trong việc quản lý dịch hại tổng hợp (IPM) trên cây có múi

Sự phát triển tính kháng thuốc của sâu hại cũng như ảnh hưởng có hại của thuốc hóa học lên sức khoẻ của con người, hệ sinh thái và môi trường đã tạo áp lực mạnh cho sự phát triển các loại thuốc trừ sâu sinh học để sử dụng trong phòng trừ tổng hợp côn trùng gây hại cây trồng. Đề tài này được thực hiện nhằm khai thác tiềm năng sinh học của nấm trắng, *Beauveria bassiana* và nấm xanh, *Metarhizium anisopliae* để sử dụng trong chương trình quản lý dịch hại tổng hợp (IPM) trên cây có múi tại ĐBSCL. Đề tài được tiến hành tại ba huyện: Phong Điền (TP Cần Thơ), Bình Minh và Tam Bình (tỉnh Vĩnh Long) từ tháng 01 năm 2006 đến tháng 12 năm 2007.

Kết quả đã điều tra thu thập, phân lập và tạo thuần được 16 dòng nấm trắng và 19 dòng nấm xanh từ Cần Thơ và Vĩnh Long. Tuyển chọn được 10 dòng nấm tốt, trong đó 2 dòng nấm trắng *B.b*(VL_3-RMCQ), *B.b*(CT_{11}-RMCQ) và 2 dòng nấm xanh *M.a*(VL_2-RMCQ), *M.a*(CT_{15}-RCC) có hiệu lực rất cao đối với rầy mềm và rầy chổng cánh hại cây có múi khi khảo nghiệm trong nhà lưới. Hai loại thuốc trừ sâu sinh học sản xuất từ dòng nấm xanh, *M.a*(CT_{15}-RCC) và dòng nấm nấm trắng, *B.b*(VL_3-RMCQ) có hiệu lực rất cao đối với rầy mềm, rầy chổng cánh hại cây có múi, khi đánh giá trong nhà lưới cũng như khảo nghiệm ngoài vườn. Kết quả thí nghiệm trong nhà lưới cho thấy: thuốc trừ sâu sinh học mới được sản xuất từ dòng nấm xanh, *M.a*(CT_{15}-RCC) và dòng nấm nấm trắng, *B.b*(VL_3-RMCQ) không gây ảnh hưởng tới khả năng ăn mồi của nhện linh miêu *Oxyopes*. Cả hai loại thuốc trừ sâu sinh học này đã đạt hiệu quả cao trong việc quản lý rầy chổng cánh, rầy mềm và sâu vẽ bùa hại cây có múi khi ứng dụng vào 50 ha mô hình ở Cần Thơ và Vĩnh Long. So sánh hiệu quả kinh tế giữa mô hình trình diễn (ứng dụng chế phẩm *M.a*(CT_{15}-RCC)/*B.b*(VL_3-RMCQ)) với vườn đối chứng của nông dân (sử dụng thuốc hóa học và phun định kỳ) tại các điểm thực hiện mô hình cho thấy lợi nhuận thu được từ các vườn mô hình cao hơn so với vườn đối chứng của nông dân là 2.834.000 đ/ha (mô hình trồng cam 4 tuổi tại Tam Bình – Vĩnh Long); 3.915.000 đ/ha (mô hình trồng cam mật 5-6 tuổi tại Phong Điền - Cần Thơ) và 4.217.000 đ/ha (mô hình trồng bưởi Năm roi 5-6 tuổi tại Bình Minh – Vĩnh Long). Hai chế phẩm vi nấm *M.a*(CT_{15}-RCC) và *B.b*(VL_3-RMCQ) có tiềm năng rất lớn trong quản lý dịch hại tổng hợp (IPM) trên cây có múi tại ĐBSCL.