Can Tho University Journal of Science
website: sj.ctu.edu.vn

DOI: 10.22144/ctu.jen.2018.032

Effectiveness of green muscardine fungus *Metarhizium anisopliae* and some insecticides on lesser coconut weevil *Diocalandra frumenti* Fabricius (Coleoptera: Curculionidae)

Nguyen Hong Ung[1*], Duong Kieu Hanh[2], Trinh Thi Xuan[2], Le Van Vang[2] and Tran Van Hai[2]

[1]*College of Agriculture and Aquaculture, Tra Vinh University, Vietnam*

[2]*College of Agriculture, Can Tho University, Vietnam*

[*]*Correspondence: Nguyen Hong Ung (email: hongungtv@gmail.com)*

Article info.

Received 31 May 2018
Revised 03 Aug 2018
Accepted 30 Nov 2018

Keywords

Coconut weevil, Diocalandra frumenti, insectisides, in the field, laboratory, Metarhizium anisopliae

ABSTRACT

This report was aimed to evaluate the effectiveness of treatments for lesser coconut weevil (Diocalandra frumenti) with green muscardine fungus (Metarhizium anisopliae) and some insecticides at the laboratory and farmer's coconut orchards. Results showed that the isolates of M. anisopliae at the concentration of 10^8 spores.mL^{-1} killed D. frumenti adults under laboratory condition (T = 28-31^{0}C; RH = 60-70%). In the laboratory, the corrected effectiveness of Ma5, Ma2 and Ma1 were 100%, 95.0% and 95.8% at day 15 after treating. Both Emamectin benzoate and Fipronil gave effective control of the beetles at 100% after 3 days of treating. Among four tested concentrations, the 10^9spores.mL^{-1}gave higher effectiveness up to 96.7% after 15 days of treating. In the farmer's coconut orchard, the results also showed coconut fruits damaged by the weevil clearly reduced from 35 days after treating. At 65th day, all of treatments applied with M. anisopliae gave effective control just at 4.00% and 5.60% (ratios of infected fruits). Meanwhile, insecticide applied with treatments had a higher ratio of coconut fruits infected by this weevil (14.7% and 11.4%).

Cited as: Ung, N.H., Hanh, D.K., Xuan, T.T., Vang, L.V. and Hai, T.V., 2018. Effectiveness of green muscardine fungus *Metarhizium anisopliae* and some insecticides on lesser coconut weevil *Diocalandra frumenti* Fabricius (Coleoptera: Curculionidae). Can Tho University Journal of Science. 54(8): 1-7.

1 INTRODUCTION

Lesser coconut weevil, *Diocalandra frumenti* Fabricius (Coleoptera: Curculionidae), which can cause death of the whole trees (Lever, 1979; Liao and Chen, 1997, Giblin-Davis, 2001), is one of the main pests on coconut (*Cocos nucifera*) (Hill, 1983). Damages caused by *D. frumenti* were firstly recognized in Kien Giang province, Vietnam, of which infected trees got their big fruits deformed, reduced in sizes while the small ones fell off their trees. Until 2015, its presence and damages were identified in many provinces and cities of the country (Nguyen Thi Thu Cuc, 2015). In addition, *D. frumenti* was recorded to cause damages on areca, nipa palm, and other plants belonging to the Palmae family at many places in the world (CABI, 2009; EPPO, 2012). The pest control and prevention by common chemicals have been very difficult since they cause environmental pollution and affect cultivators' health. In this paper, results of applying

entomopathogenic fungus *M. anisopliae* on the coconut weevil *D. frumenti* were reported.

2 MATERIALS AND METHODS

2.1 Time and locations

The experiments were carried out from April to October, 2015. The corrected effectiveness of *M. anisopilae* on *D. frumenti* were conducted in laboratory of Department of Plant Protection, College of Agriculture and Applied Biology, Can Tho University while the field experiments were done in coconut orchard of Ben Tre province.

2.2 Materials and tools

Equipment, chemicals and tools included culture cabinet, microscope, steam sterilization autoclave, electronic scale, and other lab equipment such as distilled water, alcohol 70%, petri dishes, culture rods, hemicytometer, trianglular flasks, 500 mL heat-resistant glass bottles, 1000-mL frozen test tubes, eppendorf tubes, stainless steel clips etc. *M. anisopliae* parasitizing on *D. frumenti* was collected in Soc Trang and Ben Tre (Figure 1).

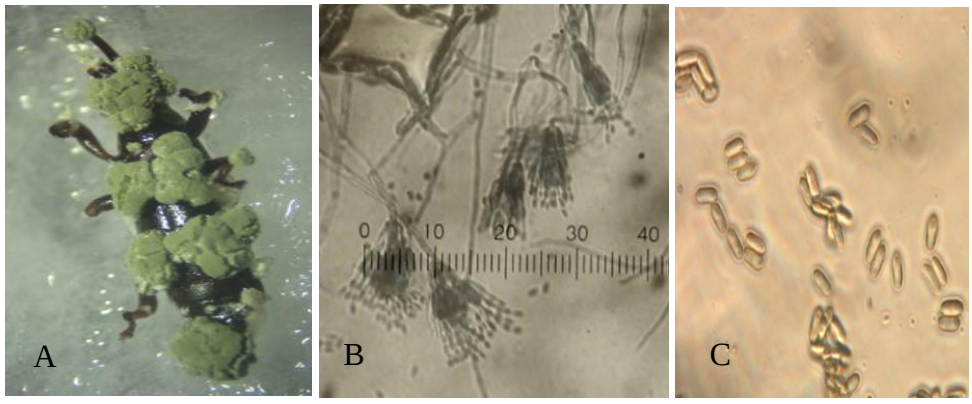

Fig. 1: *M. anisopliae* parasitizing *D. frumenti* (A), morphological structure of conidiophore branching (B) and spores after isolated (C)

2.3 Methodologies

2.3.1 Evaluating the efficacy of M. anisopliae on D. frumenti in laboratory condition

The experiment was conducted with 6 treatments, including 5 green muscardine fungus samples collected from local areas and one control with water. The treatments were specifically mentioned in Table 1.

Table 1: Treatments to evaluate the efficacy of the green muscardine fungus collected on *D. frumenti* adults in the laboratory condition at Can Tho University, 2015

No	Samples symbolized by	Samples collected in	Concentrations
1	*Ma1*	Giong Trom district, Ben Tre province	10^8 spores/mL
2	*Ma2*	Binh Dai district, Ben Tre province	10^8 spores/mL
3	*Ma3*	Binh Dai district, Ben Tre province	10^8 spores/mL
4	*Ma4*	Mo Cay Nam district, Ben Tre province	10^8 spores/mL
5	*Ma5*	Long Phu district, Soc Trang province	10^8 spores/mL
6	Distilled water	-	-

Ma: Metarhizium anisopliae

The utilized concentration of the *M. anisopliae* treatments was 10^8 spores.mL^{-1}; all treatments were added with siloxane alkoxylate as an adhesive substance. The experiment was arranged in a randomized complete block design, 4 replications with 30 *D. frumenti* adults for each treatment. The experiment was carried out by dipping the adults into the fungal spore solution for 30 seconds. Then the *D. frumenti* were transferred to petri dishes treated with alcohol 70% and contained small cuts of coconut petioles as their feed.

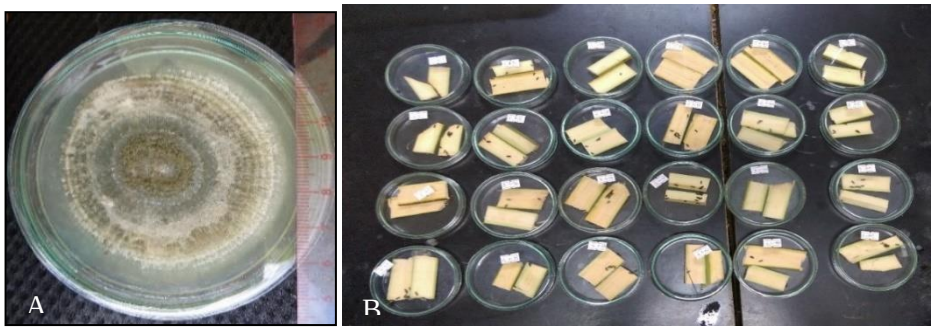

Fig. 2: Experiment to evaluate efficacy of *M. anisopliae* on *D. frumenti* adults in laboratory: *M. anisopliae* on petri dish (A) and experimental arrangements with coconut petiole as food supply (B)

Number of alive adults was recorded at the time of 1, 3, 5, 7, 9, 11, 13 and 15 days after treating.

2.3.2 Evaluating the efficacy of green muscardine fungus M. anisopliae and some insecticides on lesser coconut weevil adults in the laboratory condition

This experiment was conducted with seven treatments and four replications arranged in randomized complete block design. Each replication of one treatment was a petri dish small pieces of coconut ocrea and 30 adults. The treatments were treated by spraying solution over the experimented treatments with dosages and concentrations mentioned in Table 2.

Table 2: Treatments to evaluate efficacy of green muscardine fungus *M. anisopliae* and some insecticides on lesser coconut weevil *D. frumenti* adults in the laboratory condition at Can Tho University, 2015

Treatments	Concentrations
Ma1	10^8 spores/mL
Ma2	10^8 spores/mL
Ma5	10^8 spores/mL
Abamectin	1.25 gr/16L
Emamectin benzoate	1.25 gr/16L
Fipronil	1.5 gr/16L
Distilled water	-

Number of alive adults was recorded at 1, 3, 5, 7, 9, 11, 13 and 15 days after treating.

2.3.3 Evaluating the efficacy of green muscardine fungus at different concentrations on D. frumenti adults in the laboratory condition

The experiment was conducted with 5 treatments and 4 replications arranged in randomized complete design. Each treatment had a petri dish with **coconut petiole** and 30 *D. frumenti* adults with the specific concentrations as mentioned in Table 3.

Table 3: Treatments to evaluate efficacy of green muscardine fungus at different concentration on adults in the laboratory condition, Can Tho University, 2015

Treatments	Concentrations
Ma1	10^6 spores/mL
Ma1	10^7 spores/mL
Ma1	10^8 spores/mL
Ma1	10^9 spores/mL
Distilled water	-

Ma: Metarhizium anisopliae

Number of alive adults was recorded at 1, 3, 5, 7, 9, 11, 13 and 15 days after treating.

2.3.4 Evaluation of preventive efficacy of green muscardine fungus strains and some insecticides on D. frumenti in the field condition

Two isolates of muscardine fungi were *Ma1* and *Ma5* and two insecticides Emamectin benzoate and Fipronil were used. The controlled treatments were treated with distilled water and adhesive substances. The experienment was conducted at coconut orchards in Ben Tre province with the areas of 3,000 m^2. They were in the stage of blooming and bearing young fruits which were damaged by *D .frumenti*. The experiment was arranged in randomized complete block design with 5 treatments and 4 replications. Each treatment was 6 coconut trees with 3 times of application during an interval of 2 weeks per each time (Figure 3).

Fig. 3: Products of green muscardine fungus *M. anisopliae* (A) and spraying fungus on coconut trees (B)

The record of new infected coconut fruits was conducted at day 1 before being treated and 7, 21, 35, 50 and 65 days after treating.

2.4 Data analysis method

The corrected effectiveness in the laboratory was calculated using the Abbott's formula (1925):

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

Where: n = Insect population, T = treated, Co = control

Percentage (%) of infected coconut fruits at days after treating in the coconut orchard was calculated by formula:

$$\text{Infected Ratio (\%)} = \frac{\text{Number of infected fruits}}{\text{Total number of fruits}}) * 100$$

Data were collected, calculated on Excel program and analyzed via MSTATC and Duncan test.

3 RESULTS AND DISCUSSIONS

3.1 In the laboratory condition

3.1.1 Efficacy of green muscardine fungus collected on D. frumenti

Results recorded in Table 4 indicated that after 3 days of treatment, the efficacy of *M. anisopliae* on *D. frumenti* adults is not good enough, only 14.2% for *Ma3* and 6.70% for *Ma4* as highest.

At 5th day after treatment, the efficacy of all treatments increased and was statistically different compared to the control. From 13 to 15 days after treating, the treatments had high effective ratios (over 60%). Three treatments gave high controlling efficacy on *D. frumenti* adults were *Ma5* (100% at 13 days), *Ma1* (95.8% at 13 and 15 days) and *Ma2* (95.0% at 15th day after treatment), but not statistically different. Evidently, green fungus (*Ma)* was recorded to be able to kill many insects belonging to Coleoptera order (Zelazny, 1989; Nussenbaum and Lecuona, 2012), and *M. anisopliae* was also identified with abilities to parasitize on insects such as Isoptera, Orthoptera, Coleoptera, Hemiptera etc. (Pham Thi Thuy, 2004).

Table 4: Efficacy of green muscardine fungus at the concentration of 10^8 spores.mL^{-1} on *D. frumenti* adults in the laboratory condition, Can Tho University, 2015

Treatments	Corrected effectiveness (%) at the days after treated						
	3	5	7	9	11	13	15
Ma1	12.5	71. 7^{ab}	80.8^{ab}	85.0^{ab}	89.2^{ab}	95.8^{ab}	95.8^{ab}
Ma2	8.3	69.2^{b}	83.3^{ab}	89.2^{ab}	91.7^{ab}	94.2^{ab}	95.0^{ab}
Ma3	14.2	35.8^{c}	44.2^{c}	49.2^{c}	57.5^{c}	60.8^{c}	64.2^{c}
Ma4	6.7	50.0^{bc}	67,3^{b}	71.7^{bc}	75.8^{b}	85.0 ^{b}	89.2^{b}
Ma5	9.2	92.5^{a}	94.2^{a}	96.7^{a}	98.3^{a}	100^{a}	100^{a}
Control	0.0	0.0^{d}	0.0^{d}	0.0^{d}	0.0^{d}	0.0^{d}	0.0^{d}
CV (%)	14.5	19.3	17.8	16.2	13.1	9.9	8.9
Significance level	ns	**	**	**	**	**	**

*Means within the same column followed by the same letters are not significantly different according to Duncan's multiple range test; ns: not significant; **: significant at 1%; Ma: Metarhizium anisopliae*

3.1.2 Efficacy of green muscardine fungus and some insecticides on D. frumenti adults in the laboratory condition

At day 1 after being treated, the treatments with *Ma1*, *Ma2* and *Ma5* all did not indicate any efficacy while those with Emamectin benzoate and Fipronil got high ratios and were significantly different in comparison with the remaining treatments, with 62.5% and 63.3%, respectively (Table 5).

Table 5: Corrected effectiveness of green muscardine fungus and some insecticides on *D. frumenti* adults at the time of treatment in the laboratory condition, Can Tho University, 2015

Treatments	Concentrations	Corrected effectiveness (%) at days after being treated					
		1	3	5	7	11	15
Ma1	10^8 spores/mL	0.0^{c}	2.5^{c}	36.8^{c}	44.6^{bc}	55.1^{bc}	66.3^{b}
Ma2	10^8 spores/mL	0.8^{c}	7.5^{c}	11.9^{d}	22.8^{c}	39.8^{c}	60.7^{b}
Ma5	10^8 spores/mL	0.0^{c}	10.0^{c}	30.8^{cd}	53.4^{bc}	58.7^{bc}	63.3^{b}
Abamectin	1.25 gr/16L	18.3^{b}	52.5^{b}	66.6^{b}	67.1^{b}	74.5^{b}	77.7^{b}
Emamectin benzoate	1.25 gr/16L	62.5^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}
Fipronil	1.5 gr/16L	63.3^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}
Control		0.0^{c}	0.0^{d}	0.0^{e}	0.0^{d}	0.0^{d}	0.0^{c}
CV (%)		20.8	26.1	20.3	22.1	18.5	24.8
Significance level		**	**	*	**	**	*

*Means within the same column followed by the same letters are not significantly different according to Duncan's multiple range test; *: significant at 5%; **: significant at 1%; Ma: Metarhizium anisopliae*

From 3 days after being treated, Emamectin benzoateandFipronil gave highest effective ratio on *D. frumenti* adults at 100%, while that of Abamectinonly reached 52.5%. All treatments of muscardine fungus concentrations gave slow efficacy and reached highest at 15th day after treatment with 60.7 to 66.3% without significant difference between 3 isolates. Similarly, Tran Van Hai *et al.* (2009a) also showed that efficacy of *M. anisopliae* on *Lepidiota cochinchinae* causing damages on peanut and corn roots was of 70.8 – 79.2% and lasted until 28 days.

3.1.3 Influence of different concentrations of muscardine fungus on D. frumenti adults

Results in Table 6 showed 4 concentrations of the investigated green muscardine fugus with controlling efficacy on *D. frumenti* adults. Starting from 5 to 15 days after being treated, the effective ratios of all concentrations increased.

Table 6: Effective ratios of green muscardine fungus at different concentrations on *D. frumenti* adults at days after being treated in the laboratory condition, Can Tho University, 2015

Treatments	Concentrations	Corrected effectiveness (%) at days after being treated						
		3	5	7	9	11	13	15
Ma1	10^6 spores/mL	3.3^{b}	34.2^{b}	60.0^{b}	70.0^{b}	75.0^{b}	79.2^{a}	80.8^{a}
Ma1	10^7 spores/mL	3.3^{b}	44.7^{b}	64.8^{b}	72.0^{b}	76.7^{b}	79.2^{a}	83.2^{a}
Ma1	10^8 spores/mL	5.8^{b}	46.2^{b}	68.6^{b}	75.5^{b}	77.8^{b}	79.2^{a}	84.3^{a}
Ma1	10^9 spores/mL	10.0^{a}	80.0^{a}	90.0^{a}	93.3^{a}	95.0^{a}	95.0^{a}	96.7^{a}
Control		0.0^{c}	0.0^{c}	0.0^{c}	0.0^{c}	0.0^{c}	0.0^{b}	0.0^{b}
CV (%)		15.2	18.1	16.3	15.8	18.8	16.3	15.9
Significance level		**	**	**	**	**	**	**

*Means within the same column followed by the same letters are not significantly different according to Duncan's multiple range test; **: significant at 1%; Ma: Metarhizium anisopliae*

In the investigated treatments, green muscardine fungal treatment with the concentration of 10^9 spores.mL^{-1} gave the highest efficacy started from 5 days until 15 days after being treated (96.7%). Three treatments with the concentrations of 10^6, 10^7 and 10^8 spores.mL^{-1} had effective ratios at 80.8%, 83.2% and 84.3%, respectively at the time of 5 days after being treated. However, the ratios were lower compared to those of 10^9 spores.mL^{-1} but had no significant difference since 13 to 15 days after being treated. Another study (Tran Van Hai *et al.*, 2009b) on efficacy of green muscardine fungal concentrations on peanut leaf roller *Archips micacerana* Walker showed the highest effective ratio from 92.8 – 94.7 % which could last until 17 days after being treated (in the laboratory condition). The efficacy accordingly tended to increase strongly at 7th day after spraying and reached at 80% after 12 days. In addition, Pham Kim Son *et al.* (2016) also reported their results on efficacy of

M. anisopliae on sweet potato weevil *Cylas formicarius* at the concentrations from 10^6 to 10^9 spores.mL^{-1} with the effective ratios from 88.2% to 100%. This demonstrates that muscardine fungus have potentials to reduce damages caused by pests.

3.2 In field condition

The results indicated that at 7th day after being treated, damage percentage (%) of coconut fruits among treatments was similar, with no statistically significant differences. Until 21 days after being treated, such percentage increased significantly, especially at controlled treatments, percentage of infected fruits reached 17.4%. Those used Fipronil revealed remarkably high efficacy and total difference from the remaning treatments (the lowest percentage of infected fruits was at 2.3%) (Table 7).

Table 7: Preventive efficacy of green muscardine fungus and some insecticides on *D. frumenti* adults at days after being treated in the coconut orchards in Giong Trom district, Ben Tre province

Treatments	Concentrations	Infected ratio (%) of coconut fruits at days after being treated				
		7	21	35	50	65
Ma1	10^8 spores/mL	1.20	4.20^{bc}	5.95^{b}	7.30^{b}	4.00^{b}
Ma5	10^8 spores/mL	1.80	6.90^{b}	6.80^{b}	9.30^{ab}	5.60^{b}
Emamectin benzoate	1.25 gr/16L	1.10	9.10^{b}	7.50^{b}	16.5^{a}	14.7^{a}
Fipronil	1.5 gr/16L	1.20	2.30^{c}	5.40^{b}	8.5^{b}	11.4^{a}
Control	Water	0.00	17.4^{a}	14.6^{a}	15.8^{a}	11.2^{a}
CV (%)		24.9	23.4	16.4	37.7	34.6
Significance level		ns	*	**	*	**

*Means within the same column followed by the same letters are not significantly different according to Duncan's multiple range test; ns: not significant; *: significant at 5%; **: significant at 1%; Ma: Metarhizium anisopliae*

At 35th day after being treated, the percentage of infected coconut fruits at treatments applying with *Ma1*, *Ma5*, Emamectin benzoate and Fipronil all reduced and differentiated from the control ones. At 50th day after being treated, the percentage of fruits damaged by *D. frumenti* using *Ma1* was the lowest (7.3%) and differentiated from the controlled ones. Percentages of damaged fruits at two treatments using *Ma1* and *Ma5* were at 4.00% and 5.60% respectively while those at treatments using insecticides with Emamectin benzoate and Fipronil got back to increase. Therefore, the results showed that both insecticides and green muscardine fungus could reduce the percentage of infected by *D. frumenti* on fruits in laboratory and field conditions.

4 CONCLUSION AND RECOMMENDATIONS

All green muscardine fungal treatments gave effectiveness on *D. frumenti* adults from 5 days after being treated, and the effectiveness increased in parallel with the implementation time. Among which, 3 treatments giving the high efficacy from 5 days until 15 days after being treated were *Ma5*, *Ma2* and *Ma1* reaching 100, 95.0 and 95.8%, respectively. Both Emamectin benzoate and Fipronil gave higher effects comparing to fungal treatments.

Both Emamectin benzoate and Fipronil all had higher preventive effects than those of the experimented fungus with the effectiveness at 100%. Fungal treatments had effective ratios ranged from 63.3% to 66.3%.

Fungal treatments with the concentration of 10^9 spores.mL^{-1} gave highest efficacy from 5 until 15 days after being treated with the highest effective ratio (96.7%), compared to 80.8%, 83.2% and 84.3% of 10^6, $10^7$and 10^8spores.mL^{-1}, respectively at 15th day after treatment.

Under the field condition, percentages of infected fruits in treatments using green muscardine fungus and insecticides all decreased at 35th day after being treated. Especially, until 65 days after treament, all treatments of green muscardine fungus still remained lowest in percentages of infected fruits (4.00% to 5.60%) while those of insecticides started to increase (11.4 to 14.7%).

REFERENCES

CABI, 2009. Diocalandra frumenti (Fabricius). Distribution Maps of Plant Pests no. 249 1st revision. CABI. Wallingford. UK.

EPPO, 2012. EPPO Technical Document No. 1061. EPPO Study on the Risk of Imports of Plants for Planting. EPPO. Paris, 39 pages.

Giblin-Davis, R. M., 2001. Borers of palms. In: Howard, F.W., Moore, D. (Eds). Insects on palms. CABI Publishing. Wallingford. GB, pp. 267-304.

Hill, D. S., 1983. Agricultural insect pests of the tropics and their control. 2nd Edition. Cambridge University Press. Cambridge. UK, 704 pages.

Lever, R. J. A. W., 1979. Pests of the Coconut Palm. FAO Agric. Studies No 77. FAO Rome. 190 pages

Liao, C. T. and Chen, C. C., 1997. Primary study the insect pests, hosts and ecology of weevil attacking ornamental palm seedlings. Bulletin of Taichung District Agriculture Improvement Station. 57: 43-48.

Nguyen Thi Thu Cuc, 2015. Insects, spiders damage fruits in Vietnam and natural enemies. Can Tho University Publishing House. Can Tho, 623 pages (in Vietnamese).

Nussenbaum, A. L., and Lecuona, R. E., 2012. Selection of Beauveria bassiana sensu lato and Metarhizium anisopliae sensu lato isolates as microbial control agents against the boll weevil (Anthonomus grandis) in Argentina. Journal of Invertebrate Pathology. 110 (1): 1-7.

Pham Kim Son, Le Van Vang and Tran Van Hai, 2016. Disease causing abilities of parasitic fungus on sweet potato weevil Cylas formicarius Fabr. (Coleoptera: Curculionidae). Can Tho University Journal of Science. Part B: Agriculture, Fishery and Biotechnology. 44: 31-37 (in Vietnamese).

Pham Thi Thuy, 2004. Biotechnology in plant protection. Hanoi National University Publishing House. Ha Noi, 357 pages (in Vietnamese).

Tran Van Hai, Pham Kim Son and Trinh Thi Xuan, 2009a. Investigating bio-characteristics of Lepidiota cochinchinae Brenske causing damages on peanut and corn roots and efficacy of some Metarhizium anisopliae Sorokins, Beauveria bassiana Vuillemins on this pest. Can Tho University Journal of Science. 11: 63-70 (in Vietnamese).

Tran Van Hai, Trinh Thi Xuan and Pham Kim Son, 2009b. Investigating bio-characteristics of peanut leaf rollers Archips micacerana Walker and efficacy of Metarhizium anisopliae Sorokins on this pest in Tra Vinh province. Can Tho University Journal of Science. 11: 54-62 (in Vietnamese).

Zelazny, B., 1989. Biological control of Oryctes rhinoceros with Metarhizium anisopliae. CRI, 7 pages