**DOI:** https://doi.org/10.47773/shj.1998.041.6
SCIENCE AND HUMANITIES JOURNAL
Volume 4, 2004, pp. 63-80

# BIOLOGICAL CONTROL OF RICE BLACK BUG, *SCOTINOPHARA COARCTATA* (FAB.), IN LEYTE[1/]

**Mylene Milan Po**
Leyte State University
Visca, Baybay, Leyte

*and*

**Bimbo T. Mandras**
Department of Pest Management
Leyte State University, Visca, Baybay, Leyte

## ABSTRACT

*Metarhizium anisopliae (Metch.) Sorokin was applied in three different methods: spray, through irrigation and broadcast. Spraying was the most effective method of applying M. anisopliae to reduce rice black bug population as evidenced by the significantly higher percentage of infected rice black bugs collected from plots sprayed with spore suspension of M. anisopliae than in plots applied with same fungus through immersion of the medium and broadcasting them regardless of the time of sampling. The effectiveness of spraying over the other two methods was the efficient deposition of spores on the plant surface or on the cuticle of the rice black bug themselves during application allowing greater chances of pick up by the insect which resulted in higher percentage (90%) infection of rice black bug on the sprayed plot. M. anisopliae had no effect on fecundity and hatchability of the eggs of rice black bug suggesting that the fungus was not transovarially transmitted. Percentage nymphal infection by M. anisopliae was very low. The results of the study also indicate that application of M. anisopliae in the field did not affect the egg parasitoid.*

**KEY WORDS:** Rice black bug. Biological control. *Metarhizium anisopliae.* Application methods. *Scotinophara coarctata.*

1/ *Part of the M.S. thesis of the senior author*

## INTRODUCTION

Damage by pests has often been cited as one of the reasons for crop losses both in the field and in storage. In the Philippines, 10 adults per hill can cause losses of up to 35% in susceptible varieties (IRRI, 2001). Heinrichs et al. (1985) also reported that economic injury is estimated at 3 bugs per hill. More severe damage was observed during dry season cropping than wet season rice cropping. Moreover, synchronous double cropping of irrigated rice with high levels of nitrogen seems to favor black bug outbreaks.

The use of chemicals is one of the methods recommended in controlling rice black bugs. This practice, however is not popular to farmers who cannot afford to buy expensive pesticides. In addition, constant application of pesticides also leads to the development of resistant pests (Perez et al., 1989). In view of these drawbacks, other control measures, which are safe and effective, were looked into. Arida and Shepard (1985) pointed out that the use of natural enemies has some advantages over chemical control. It causes less disturbance to the environment, more economical and self-perpetuating, thus, providing a continuous control of the pests in the field.

This study was conducted to determine the percentage parasitism of rice black bug eggs in the field, and to determine the most appropriate method of application of *Metarhizium anisopliae* (Metchnikoff) Sorokin against rice black bug in the field.

## METHODOLOGY

### Mass Production of *Metarhizium anisopliae*

Infected black bugs were collected from the field and were brought to the laboratory. They were placed in a petri plate lined with moist tissue paper and were incubated at room temperature to allow massive growth of the pathogen. Mycelial growth at the side of the infected insect was picked up using a fine dissecting needle and was plated on PDA and incubated for 1-2 weeks. A pure culture of the pathogen was prepared by streaking a small portion of the colony on the agar slants in the test tubes (Figure 1). *M. anisopliae* was mass produced using corn as substrate with the fungal suspension (Figure 2).

## Evaluation of Different Methods of Application of *M. anisopliae*

### Field Experiment

The farmer's field, measuring approximately 900 $m^2$ located at Brgy. Guadalupe, Baybay, Leyte and planted with PSB Rc18 rice variety, was used in this experiment. The field was laid out in RCBD with six replications. The experimental field was divided into six plots with each plot serving as a replication. The plots were further divided into four sub-plots measuring 5 x 5 $m^2$ where the treatments were allocated. The three methods of fungal application such as broadcasting the culture medium with the fungus in the field, immersing the medium in the irrigation canals to allow distribution of the spores in the field and spraying the spore suspension were tested. Untreated control plots were provided for comparison. Mass cultured *M. anisopliae* was applied in the field twice following the methods mentioned above.

Twenty rice black bugs were collected at 4, 7, and 14 days after *M. anisopliae* application from each treated and untreated plots. These were applied to all methods of fungal applications used. The number of infected black bugs in each sampling period was counted and percentage infection was computed using the formula used by Estoy et al. (2000):

$$\text{Percentage Infection (\%)} = \frac{\text{Number of infected rice black bugs}}{\text{Total number of rice black bugs collected}} \times 100$$

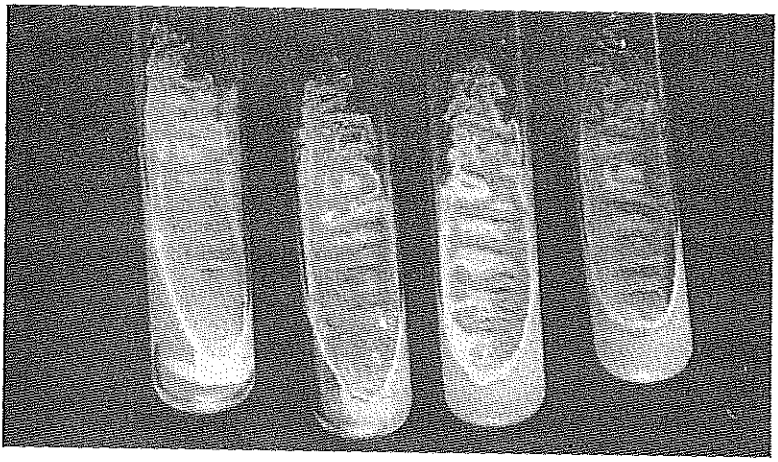

Figure 1. Pure culture of *Metarhizium anisopliae* grown on potato dextrose agar slants (1.2x).

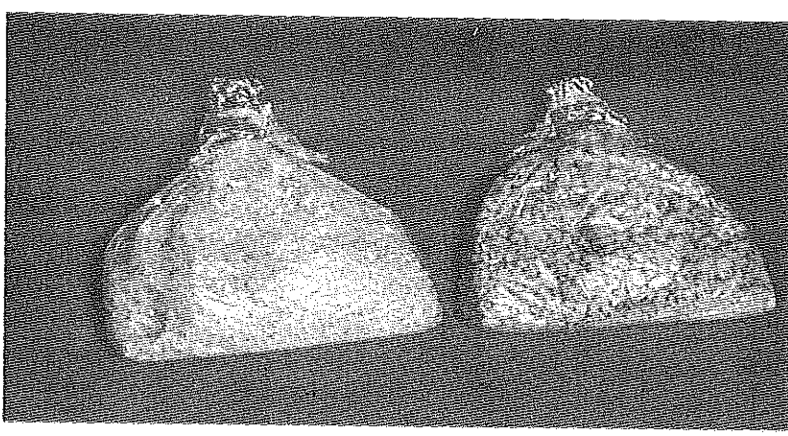

Figure 2. Cracked corn used in mass production of *Metarhizium anisopliae* (0.43x).

**Laboratory Experiment**

From the treated plots, five mating pairs of rice black bugs were collected approximately 8 hours after application to determine the mortality of the insect and the effect of the fungal pathogen on the fecundity, egg hatchability and nymphal infection. Assessment of rice black bug mortality was done by counting infected dead insects daily for 7 consecutive days. Percent mortality was computed using the formula:

$$\% \text{ Mortality} = \frac{\text{Number of infected dead insects}}{\text{Total number of insects}} \times 100$$

Female rice black bugs were allowed to lay eggs and newly laid eggs were placed in a petri plate. Number of eggs laid per female per day was recorded. Number of eggs hatched was counted and percent hatchability was computed following the formula:

$$\% \text{ Hatchability} = \frac{\text{Number of eggs hatched}}{\text{Total number of eggs laid}} \times 100$$

Infected rice black bug and nymphs that hatched from eggs laid were maintained in the same rearing container. In order to determine the development of infection in nymphs, daily mortality due to *M. ànisopliae* infection was recorded for 7 days and percent nymphal infection was computed using the formula:

$$\% \text{ Nymphal infection} = \frac{\text{Number of nymphs infected}}{\text{Total number of nymphs}} \times 100$$

## Egg Parasitism of Rice Black Bugs

Ten to twenty egg masses of rice black bug were collected from treated and untreated plots with *M. anisopliae* and were brought to the laboratory. The samples were observed daily until emergence of the parasitoid. Upon emergence the number of parasitized egg masses and parasitized eggs per egg mass were counted. The percentage parasitism of the egg masses and percentage parasitism of eggs per egg mass were computed using the formula:

$$\%\ \text{Parasitism of egg masses} = \frac{\text{Number of parasitized egg masses}}{\text{Total number of egg masses collected}} \times 100$$

$$\%\ \text{Parasitism of eggs/egg mass} = \frac{\text{Average number of parasitized eggs/egg mass}}{\text{Average number of eggs/egg mass}} \times 100$$

## Analysis of Data

Data gathered were tabulated and analyzed statistically using frequency, percentages, means and analysis of variance whenever applicable.

# RESULTS AND DISCUSSION

## Efficacy of *Metarhizium anisopliae* Against Rice Black Bugs as Influenced by Methods of Application

Field Experiment

Figure 3 presents the effects of *M. anisopliae* application on the infection of rice black bug. It could be noted that the percent infection of *M. anisopliae* was consistently higher in the treated than in the untreated plots. Of the three methods of application, spraying method was most effective in reducing the rice black bug population compared with

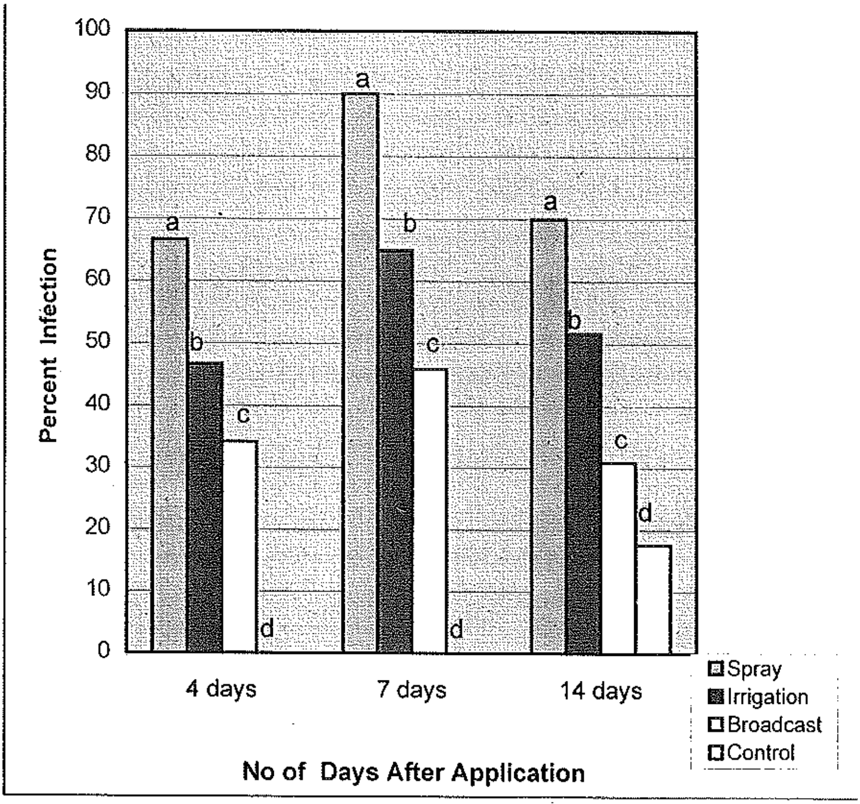


Figure 3.Percent infection of rice black bug at 4, 7 and 14 days after *Metarhizium anisopliae* application as influenced by method of application.

------------------------------

*Bars with a common letter are not significantly different using protected LSD at 5% level of significance* immersion of the medium with *M. anisopliae* in irrigation canal or broadcasting them on the rice black bug infected field. This supposition is evidenced by the significantly higher percentage of infected rice black bug collected from plots sprayed with spore suspension of *M. anisopliae* than in plots applied with the same fungus through immersion of the medium and broadcasting them regardless of the time of sampling. The effectiveness of spraying over the other two methods could be explained by the efficient deposition of the spores on the plant surface or on the cuticle of the rice black bug themselves during application. It is highly probable that spores of *M. anisopliae* were properly dispersed during spraying allowing greater chances of pick up by the insect. This in turn resulted in higher percentage (90%) infection of rice black bug on the sprayed pot.

Immersion of the medium in irrigation canal ranked next to spraying in reducing rice black bug population and the least effective was the broadcast application. The lower effect of these methods over the spraying method could be attributed to the inefficient dispersal of the fungus during application. Although the spores were spread as in the case of immersion of the medium in irrigation canal, distribution of the spores was limited only at the water level so that only those insects that may have concentrated at the basal portion of the rice plants had the chance of picking up the fungus. The ineffectiveness of the broadcast application compared with spraying and immersion methods in infecting rice black bug in the field was also attributed to the inefficient dispersal of the spores after application, similar to that of immersion of the medium in irrigation canal. However, unlike in the immersion method, where there was continuous flow of water in the inlet which allowed spreading of the fungal spores faster, in the broadcast method, dispersal of the fungus from the medium was slow. This was because movement of water within the paddy was nill, thus, limiting also the spread of the spores.

The percentage of infected rice black bug in plots applied with *M. anisopliae* increased during the first 7 days after application but declined

14 days thereafter. The earlier observation corroborates with the findings of Estoy et al. (1999). These results seemed to suggest that the virulence of *M. anisopliae* may decline with time. This attribute is of significant importance in biological control program since over production and accumulation of this entomopathogen in the field which later on might affect non-target organisms would not occur. The presence of infected rice black bug in the untreated plot 14 days after application might be attributed to spore dispersal or movement of the spore-contaminated rice black bugs. It must be noted that the experiment was conducted in an open field allowing the insects to move from plot to plot.

## Laboratory Observations

*Description of field collected rice black bug infected with M. anisopliae*

Based on infected rice black bugs collected from the field, the most frequent sites of infection were on the membrane between the head capsule and the thorax and also in between segments of the appendages (Figure 4 B and C). The mycelia of *M. anisopliae* appeared white during the early saphrophytic stage (Figure 4 B) and turned to green with age (Figure 4 C). On the nymphs (Figure 5), white mycelia were also observed during the early saphrophytic stage.

*Effect of M. anisopliae on the survival of rice black bug adults.* Table 1 shows the percent mortality and lethal time of rice black bugs collected from the field 8 hours after *M. anisopliae* application. Although differences in percent mortality and lethal time in days were observed in the laboratory set-up, test of means revealed no significant differences among treatments including the control. The results indicate that once the spores of *M. anisopliae* landed and infected the black bug, the insect was expected to die within 12.38 -31.56 days whether the fungus was applied as spray, immersion in the irrigation canal, or by broadcast. The effect of *M. anisopliae* against rice black bug took time. Burdeos and Gabriel (1995) explained that such differences in pathogenecity were indicative of

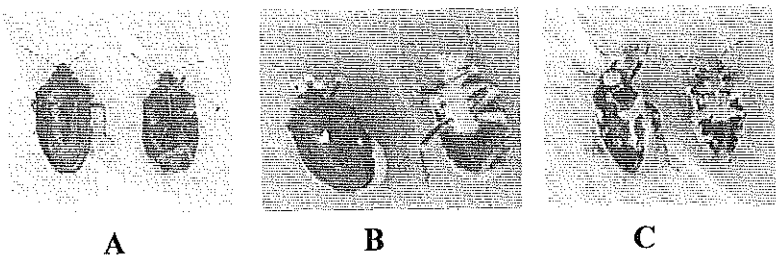

Figure 4. Rice black bug infected with *Metarhizium anisopliae* A) Control (8x), B) Early saphrophytic stage (8x), C) Late saphrophytic stage (8x ).

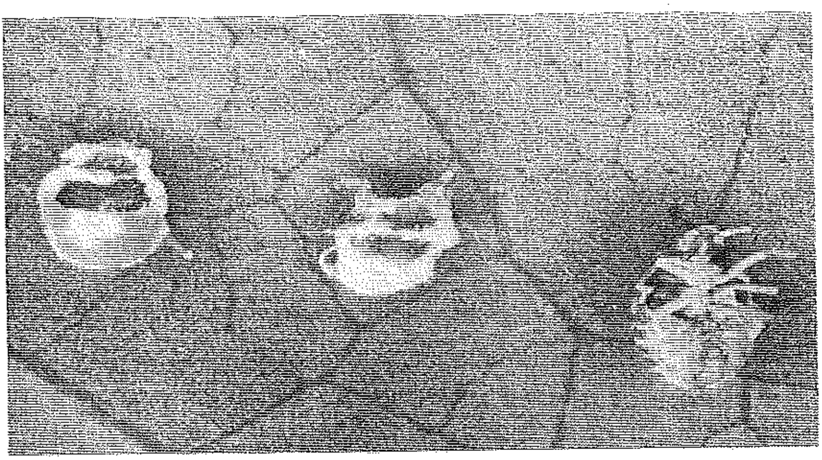

Figure 5. Rice black bug nymphs infected with *M. anisopliae (8x).*

Table 1. Percent mortality and lethal time of rice black bugs (RBB) collected from the field 8 hours after *Metarhizium anisopliae* application [1/].

| Method of Application | Trial 1 | | Trial 2 | |
|---|---|---|---|---|
| | % Mortality of RBB | Lethal Time (Days) | % Mortality of RBB | Lethal Time (Days) |
| Spray | 73.33[a] | 28.76[a] | 50.00[a] | 16.78[a] |
| Irrigation | 76.67[a] | 26.74[a] | 50.00[a] | 13.05[a] |
| Broadcast | 73.33[a] | 18.85[a] | 46.67[a] | 12.38[a] |
| Control | 60.00[a] | 31.56[a] | 30.00[a] | 21.50[a] |
| C.V. (%) | 48.91 | 52.24 | 79.25 | 62.68 |

[1/] *All means with in the same column followed by a common letter are not significantly different using protected LSD at 5 % level of significance*

naturally occurring genetic variation. This variation is also affected by other characters which included dimensions of conidia, growth rates and enzyme activities. Variation in pathogenecity could also be a function of the pathogens' ability to penetrate the host cuticle.

*Effect of M. anisopliae on the fecundity and egg hatchability.* Table 2 presents the average number of eggs laid, eggs hatched and percent hatchability of rice black bug eggs; test of means revealed no significant difference in all the treatments. This result indicates that *M. anisopliae* does not affect the fecundity and hatchability of the eggs of rice black bug, suggesting further that this fungus is not transovarially transmitted.

The percent hatchability in the 1st laboratory set-up was much lower than that of the 2nd laboratory set-up. This was because the female rice black bug was separated from their eggs immediately after laying. It

Table 2. Average number of eggs laid, eggs hatched and percent hatchability of rice black bug as influenced by method of application of *M. anisopliae* [1/].

| Method of Application | Trial 1 | | | Trial 2 | | |
|---|---|---|---|---|---|---|
| | Eggs Laid | Eggs Hatched | % Hatchability | Eggs Laid | Eggs Hatched | % Hatchability |
| Spray | 38.46ab | 7.46a | 14.17a | 33.90a | 26.96a | 80.91a |
| Irrigation | 63.03a | 14.63a | 25.93a | 29.66a | 22.93a | 77.88a |
| Broadcast | 34.10b | 8.56a | 21.78a | 31.63a | 24.66a | 77.32a |
| Control | 38.80ab | 10.40a | 26.23a | 34.70a | 30.70a | 88.44a |
| C.V. (%) | 46.45 | 80.89 | 71.04 | 21.14 | 22.78 | 9.73 |

[1/] *All means within the same column followed by a common letter are not significantly different using protected LSD at 5 % level of significance*

was observed that lesser number of eggs hatched when the female rice black bug was not guarding its eggs.

*Effect of M. anisopliae on nymphal infection. Table 3 presents* the percent infection of rice black bug nymphs that emerged from the laboratory laid eggs. The results show no significant difference on nymphal infection between treatments regardless of the set-up. It could also be noted that the nymphs produced by the insects in the laboratory had very much lower percentage of infection. This seemed to confirm the earlier statement that this fungus is not transmitted transovarially. It is also possible that the lower number of infected nymphs could be due to the shedding off of the cuticle during molting of the nymphs which Vey and Farques (1977) believed as an effective defense mechanism of rice black bug against infection by *M. anisopliae*.

Table 3. Percent infection of rice black bug nymphs that emerged from the laboratory laid eggs as influenced by method of application of *Metarhizium anisopliae* 1/.

| Method of Application | % Nymphal Infection | |
|---|---|---|
| | Trial 1 | Trial 2 |
| Spray | 5.56a | 1.66a |
| Irrigation | 0.78a | 0.00a |
| Broadcast | 1.31a | 0.79a |
| Control | 0.00a | 0.00a |
| C.V. (%) | 309.88 | 342.47 |

*1/ All means within the same column followed by a common letter are not significantly different using protected LSD at 5% level of significance*

*Effect of M. anisopliae on egg parasitization.* Table 4 shows the percent parasitism of egg masses while Table 5 presents percent parasitism of rice black bug eggs per egg mass at 4, 7 and 14 days after *M. anisopliae* application during the 1st and 2nd laboratory trials. As noted, no significant differences on the percent parasitism of the egg masses and percent parasitism of eggs were recorded regardless of the set-up. These results indicate that application of *M. anisopliae* did not affect the activity of the egg parasitoids.

Corbett and Yusope (1924) and Perez et al. (1989) mentioned that percent parasitism of rice black bug eggs by the parasitoid was affected by the "egg guarding" behavior of the female bug. Perez et al. (1989) also observed that *T. triptus* hardly parasitized all the eggs because female rice black bug used her legs and antennae to prevent parasitization.

Although it is likely that *T. triptus* found rice black bug egg masses more easily due to the olfactory cue emitted by the female rice black bug (IRRI, 1987), the egg guarding behavior was the reason for inadequate biological control of rice black bug by *T. triptus* under field condition.

## CONCLUSION AND RECOMMENDATION

### Conclusion

- Spraying of *M. anisopliae* for the control of rice black bug is the most effective method based on its higher percentage infection of rice black bugs.
- The fungus, *M. anisopliae* has no effect on the fecundity, hatchability and the egg parasitoid of rice black bug.

### Recommendation

Residual effect of *M. anisopliae* application in the field should be studied to determine the effect of the entomopathogen on the crop and to natural enemies present in the field.

## LITERATURE CITED

ARIDA, C. S. and B. M. SHEPARD. 1985. Biological control of insect pests of rice. IRRI Saturday Seminar. August 17, 1985. 26 pp.

BURDEOS, A. T. and B. P. Gabriel. 1995. Virulence of different *Metarhizium anisopliae* (Metsch.) Sorokin isolates against the rice bug, *Leptocorisa oratorius* Fabr. (Hemiptera: Alydidae). Philipp. Ent. 9 (5) 467-478.

CORBETT, G. H. and M. YUSOPE. 1924. *Scotinophara coarctata* (F.) The black bug of Padi. Malayan Agric. J. 12(4):91-106.

ESTOY, A. B., E. H. BATAY-AN, P. A. SORIA and G. B. AMAR. 1999. *Metarhizium anisopliae*: Microbial control agent against rice black bug, *Scotinophara coarctata* Fab. PhilRice Tech. Bull. 4:14-17.

HEINRICHS, E. A., I. T. DOMINGO, and E. H. CASTILLO. 1985. Levels of resistance and grain yield responses of rice cultivars when exposed to the black bugs, *Scotinophara coarctata* (F.) (Hemiptera:Pentatomidae). J. Plt. Prot. 4 (1): 55-64.

INTERNATIONAL RICE RESEARCH INSTITUTE (IRRI). 2001. Rice IPM. The University of Queensland. An Interactive Information and Identification System of IPM of Rice.

PEREZ, V., B. M. SHEPARD, and G. S. ARIDA. 1989. Indigenous natural enemies of the Malayan black bug, *Scotinophara coarctata* (Fab.) in Palawan. Phil. Ent. 7 (5): 485-490.

REISSIG, W. H., E. A. HEINRICHS, J. A. LITSINGER, K. MOODY, L. FIEDLER, T. W. MEW and A. T. BARRION. 1986. Illustrated Guide to Integrated Pest Management in Rice in Tropical Asia. IRRI. Los Baños, Philippines. 411 pp.

VEY, A. and J. FARQUES. 1977. Histological and ultrastructural studies of *Beuaveria bassiana* infections in *Leptenotarsa decemlineata* larvae during ecdysis. J. Inv. Path. 30: 207-215.

## ABOUT THE AUTHOR

The author obtained her Bachelor of Science in Agriculture major in Plant Protection (Entomology) from the then Visayas State College of Agriculture (ViSCA), now the Leyte State University (LSU), Cum Laude in 1999. After graduation, she pursued her graduate studies at LSU leading to the degree of Master of Science major in Entomology as Graduate Teaching Assistant (GRA) teaching General Biology laboratory classes until she was hired as Part-time instructor at the Department of Pest Management (DPM) teaching Plant Protection 21 laboratory classes.

The co-author is a professor at the Department of Pest Management and is the major adviser of the senior author.