RESEARCH PAPER

Innovating Liquid Beauveria Bassiana (LBb) and Testing Against Rice White Stem Borer (Scirpophaga Innotata Walker) Larvae

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ABSTRACT
Beauveria bassiana is a well-known entomopathogenic fungus that plays an important role in the biological control of many insect pests. As a microbial insecticide, it offers an environmentally safer alternative to chemical pesticides and helps minimize the accumulation of harmful residues in agricultural crops. Although this fungus is commonly produced using solid culture media, many farmers prefer liquid formulations because they are more convenient for field application. To address this objective, the research was conducted at Davao del Sur State College between January 2021 and May 2023 to examine the growth performance of B. bassiana grown in coco-water as a liquid medium under varying pasteurization periods. The study further evaluated whether liquidized B. bassiana (LBb) could effectively control the larvae of the rice white stem borer (Scirpophaga innotata Walker).
The findings showed that coco-water pasteurized for three to four hours created suitable conditions for the growth of B. bassiana within five days after inoculation. In contrast, no fungal growth was observed in unpasteurized coco-water. Full colonization of the medium was recorded in bottles pasteurized for three to four hours, while slower growth occurred in media pasteurized for shorter periods and in Potato Dextrose Agar. Furthermore, rice plants treated with LBb at rates of 100, 150, or 200 ml per liter of water applied weekly, biweekly, or monthly produced effects comparable to those obtained using granular insecticide applied monthly. These findings indicate that liquid formulations of B. bassiana using coco-water may provide a practical and environmentally friendly alternative for managing rice white stem borer infestation.
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INTRODUCTION

Rice farming plays an essential role in supporting agricultural livelihoods in the Philippines. and serves as one of the country's most important farming activities. Approximately one-third of the country's arable land, equivalent to about ten million hectares, is devoted to rice cultivation. Nearly 2.5 million farming households rely on rice production and related activities as their primary source of income. Filipinos also consume rice as a staple food, with an estimated annual consumption of around 114–120 kilograms per person, which is considerably higher than the global average of approximately 65 kilograms per capita.

Despite its importance, rice production is frequently affected by different insect pests that can significantly reduce crop yield. that can cause substantial yield losses. The rice white stem borer (*Scirpophaga innotata*) is widely known as a serious insect pest that causes major damage to rice crops. It is commonly found in several rice-producing regions, especially in Mindanao. During severe outbreaks, rice crops may suffer losses ranging from approximately 30% to almost 100%, highlighting the importance of implementing effective pest management measures to protect rice production.

Farmers often rely on synthetic insecticides to control stem borer infestations because these chemicals are readily available and provide rapid results. While insecticides can provide effective control, their excessive or improper use may lead to several adverse effects, such as environmental pollution, possible health hazards to humans, and the ability of insect pests to develop resistance over time.

Consequently, sustainable pest management practices are gaining greater attention.

Biological control methods, especially those involving microorganisms, are increasingly recognized as a sustainable option for reducing reliance on conventional chemical pesticides. Among the various microorganisms used in biological control, the entomopathogenic fungus *Beauveria bassiana* has received considerable attention due to its capacity to infect and suppress many insect pests. When the fungus infects an insect host, it produces several toxic compounds, including beauvericin, bassianolide, and oosporein, which ultimately cause the insect's death. Due

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to this capability, *B. bassiana* is widely recognized as an effective biological control agent for managing insect pests in rice cultivation.

Conventionally, mass cultivation of *B. bassiana* has been carried out on solid substrates, particularly corn grits. However, many users prefer formulations that are easy to prepare and apply, particularly liquid formulations. Developing a practical and cost-effective method for producing liquid *B. bassiana* could improve its adoption as a biological control agent in rice production systems. Therefore, this study explored the potential use of coco-water waste as a liquid medium for culturing *B. bassiana* and evaluated the effectiveness of the resulting formulation against larvae of the rice white stem borer.

METHODOLOGY

Activity 1: Production of Liquid *Beauveria bassiana* (LBb).

The laboratory portion of the research was conducted at the DSSC Laboratory of Davao del Sur State College, located in Matti, Digos City, Davao del Sur, between January and June 2021.

Collection of the Coco-water

Fresh coconut water was collected from a coconut grating shop located in the public market of Digos City. The coco-water was a 100% waste in the said store, only the grated coco meat was sold to costumers. The fresh coco-water was placed in clean plastic container and was brought to the DSSC Laboratory.

Dispensing of the Fresh Coco-water to Flat Bottles

Clean rhum flat bottles were used as the containers filled with 150 ml fresh coco-water and cotton plugged. The flat bottles were labelled based on the treatments and were arranged in the drum for pasteurization.

Pasteurization

Pasteurization was done using drum with water at the bottom levelled in its tray. Burning woods were done to bring and maintain the water boiling. One (1) hour from boiling, flat bottles labelled treatment 1 were taken from the drum, two (2) hours from boiling flat bottles labelled treatment 2 were taken from the drum, three (3) hours from boiling flat bottles labelled treatment 3, four (4) hours from boiling flat bottles labelled treatment 4.

Beauveria bassiana Inoculation/Planting

Seven (7) day old pure culture of *B. bassiana* grown in pasteurized corn grits was used. Two (2) corn grits were inoculated to pasteurized coco-water aseptically using sterilized transfer needle. Inoculated flat bottles were slanted to increase the surface for the *B. bassiana* to grow. Growing *B. bassiana* were incubated under room temperature.

Experimental Design

The study was conducted following a Completely Randomized Design (CRD) with five replications, and the experimental treatments were organized as follows:

T1 – pasteurization for **1 hour**
T2 – pasteurization for **2 hours**
T3 – pasteurization for **3 hours**
T4 – pasteurization for **4 hours**
T5 – **without pasteurization**

Treatment 6 – Potato Dextrose Agar (PDA)

Data Gathered

1. Number of flat bottles with pasteurized liquid medium (coco- water) had *B. bassiana* growth.
2. Percentage of the pasteurized liquid medium (coco-water) occupied by growth of *B. bassiana*.

Statistical Tool

The experimental data collected in this study were statistically evaluated through Analysis of Variance (ANOVA) under a Completely Randomized Design (CRD). When significant differences among treatments were detected, Tukey's HSD test was applied to compare the treatment means using the STAR statistical software.

Activity 2. Testing of LBb against larvae of white stem borer under pot experiments.

This experiment was implemented in Matti, Digos City, Davao del Sur over a five-month period spanning January to May 2023.

Experimental Layout

The experiment followed a split-plot arrangement under a Randomized Complete Block Design (RCBD) with four replications. Plastic basins were used as the experimental units, each containing four rice plants. The treatments included in the experiment were organized as follows:

Factor A – Application Rate

A1 – 100 ml mixed with 16 L of water
A2 – 150 ml mixed with 16 L of water
A3 – 200 ml combined with 16 L of water
A4 – RR granular insecticide formulation

Factor B – Frequency of Application

B1 – applied once per week

B2 – applied once every two weeks

B3 – applied once per month

Preparation of Basins and Soil Growing Medium

The experiment utilized a medium-sized plastic basin as the container. The soil medium was prepared by mixing garden soil and mud collected from a rice field in equal proportions (50:50). Both materials were thoroughly mixed to obtain a uniform medium before being placed in the

individual plastic basins. The basins were filled up to about three-fourths of their capacity to ensure adequate space for holding water.

Rice Seed Preparation and Planting Procedure
To initiate germination, rice seeds were immersed in clean water for about 12 hours and subsequently kept for incubation for an additional 24 hours. After sprouting, the seeds were sown in seedbeds. Twenty-five days after sowing, the young seedlings were transferred to the basins, Three seedlings were maintained in each hill after transplanting.

Fertilizer Application
A mixture of complete fertilizer (14-14-14) and urea (46-0-0) was applied seven days after sowing, using 10 g of each fertilizer per treatment. The same fertilizer combination and amount were reapplied 7 and 15 days after transplanting.

Preparation of LBb
Pure culture of *B. bassiana* used was from Regional Crop Protection Center 10. Mass propagation of *B. bassiana* was done using pasteurized coco-water in a flat bottle. After seven (7) days, the culture was blended to produce the LBb using portable blender. The LBb was stored in the chiller compartment of refrigerator.

Preparation of Stemborer Larvae and Infestation
Gravid white stem borers (WSBs) were collected from rice fields and kept overnight in a net cage containing rice plants. The egg masses deposited by the insects were then collected and placed in clean Erlenmeyer flasks lined with moistened cotton until they hatched. After hatching, ten newly emerged larvae were introduced to each plant at 50 days after transplanting. The infestation process was conducted in the late afternoon. For easier counting, the larvae were temporarily placed on cotton.

Procedure for Treatment Application
The application of liquid *B. bassiana* followed the assigned treatments and was carried out using a clean hand sprayer. The applications were performed in the late afternoon. Spray application began **15 days after transplanting** and continued throughout the entire duration of the study. The application frequency varied according to the treatment assigned in the experiment.

Statistical Analysis
The experimental data were subjected to statistical evaluation using Analysis of Variance (ANOVA) under a split-plot arrangement within a Randomized Complete Block Design (RCBD) to determine differences among treatments. Whenever significant effects were observed, Tukey's test was used to compare the treatment means.

Procedure for Data Collection
Percent White Head - This parameter was determined by recording the number of white head symptoms observed in each plant and then calculating the percentage using the formula presented below:

$$\text{Percent Infection} = \frac{\text{Number of tillers showing white head} \times 100}{\text{Total number of tillers}}$$

RESULTS AND DISCUSSION

Activity 1. Producing Liquid *Beauveria bassiana* (LBb)
Table 1 shows the number of flat bottles pasteurized liquid medium (coco-water) had *B. bassiana* growth five (5) days after planting.

With growth; only 2 out of 5 flat bottles with pasteurize coco-water as culture medium have been observed in T1 (1hr pasteurization) and 4 flat bottles in T2 (2 hrs pasteurization). All the 5 bottles with pasteurize coco-water as culture medium have been observed in T3 (3 hrs pasteurization), T4 (4 hrs pasteurization) and T6 (PDA medium). However, no growth had been observed in T5 (0 hr pasteurization).

REPLICATION

Table 1. Number of flat bottles with pasteurized liquid medium (coco-water) had *B. bassiana* growth five (5) days after incubation.

Treatment	Replication 1	Replication 2	Replication 3	Replication 4	Replication 5	Total	Mean**
T1 – 1-hour pasteurization	0	0	1	0	1	2	0.40^{bc}
T2 – 2-hour pasteurization	1	1	1	1	0	4	0.80^{ab}
T3 – 3-hour pasteurization	1	1	1	1	1	4	1.00^{a}
T4 – 4-hour pasteurization	1	1	1	1	1	4	1.00^{a}
T5 – no pasteurization	0	0	0	0	0	0	0.00^{c}
T6 – PDA medium	1	1	1	1	1	5	1.00^{a}

CV= 41.24%

** = *highly significant*

Treatment means sharing the same superscript letter indicate that there is no significant difference at the 5% level according to the HSD test.

Table 2 presents the percentage of the liquid medium (coco-water) colonized by *B. bassiana* five (5) days after incubation.

In T3 (3 hrs pasteurization) and T4 (4 hrs pasteurization), the surface of pasturized coco-water had fully colonized (100%) by *B. bassiana*. Only 38%, 8% and 5% in T2 (2 hrs pasteurization), T1 (1 hr pasteurization) and T6 (PDA medium), respectively.

No growth had been observed in T5 (0 hr pasteurization).

REPLICATION

Table 2. Percent of liquid medium (coco- water) occupied by growth of *B. bassiana* five (5) days after incubation.

Total	Treatment	Replication 1	Replication 2	Replication 3	Replication 4	Replication 5	Mean**
25	T1 – 1-hour pasteurization	0	0	10	0	15	5%[c]
190	T2 – 2-hour pasteurization	65	40	35	50	0	38%[b]
500	T3 – 3-hour pasteurization	100	100	100	100	100	100%[a]
500	T4 – 4-hour pasteurization	100	100	100	100	100	100%[a]
0	T5 – no pasteurization treatment	0	0	0	0	0	0%[c]
40	T6 – PDA culture medium	5	10	10	5	10	8%[c]

CV= 24.69%

** = *highly significant*

Means followed by the same superscript letter denote the absence of a significant difference at the 5% level based on the HSD test.

Activity 2. Testing of LBb against larvae of white stemborer under pot experiments.

Table 2 illustrates the mean percentage of white head symptoms observed in the rice plants. The results of the Analysis of Variance (ANOVA) revealed that the treatments differed significantly from one another.

Treatment A4B1 recorded no incidence of white head infection (0.00%), and its result was comparable with treatment A4B2 (0.12%). In contrast, the remaining treatments (A1B1, A1B2, A1B3, A2B1, A2B2, A2B3, A3B1, A3B2, A3B3, and A4B3) produced relatively similar outcomes, with mean infection percentages varying from 0.15% to 0.65%. These results show that white head symptoms were observed in treatments A1B1 through A3B3 as well as A4B3. In contrast, the weekly application of granular insecticide (A4B1) effectively prevented infestation by the white stem borer.

Table 2. Average percentage of white head symptoms observed in rice plants at the DSSC experimental field, Matti, Digos City, Davao del Sur, Philippines, during the period January–May 2023.

Treatment Code	Replication I	Replication II	Replication III	Replication IV	Total Value	Mean
A1B1	0.18	0.59	0.08	0.05	0.90	0.23[a]
A1B2	0.11	0.12	0.19	0.06	0.48	0.15[a]
A1B3	0.20	0.16	0.54	0.37	1.27	0.32[a]
A2B1	0.85	0.38	0.24	0.35	1.82	0.46[a]
A2B2	0.38	0.15	0.20	0.31	1.04	0.26[a]
A2B3	0.17	0.29	0.34	0.20	1.00	0.25[a]
A3B1	0.20	0.23	0.22	0.25	0.90	0.23[a]
A3B2	0.20	0.39	0.16	0.05	0.80	0.20[a]
A3B3	0.24	0.94	0.45	0.35	1.98	0.49[a]
A4B1	0.00	0.00	0.00	0.00	0.00	0.00[b]
A4B2	0.12	0.04	0.09	0.23	0.48	0.12[b]
A4B3	0.60	0.55	0.34	1.13	2.61	0.65[a]

CV (A) = 76.30%; CV (B) = 61.47%.

Means that share identical superscript letters are regarded as statistically similar at the 5% probability level based on Tukey's HSD test.

P-value (A) = 0.6910

P-value (B) = 0.0019

CONCLUSIONS

1. The findings revealed that the growth of B. bassiana in coco-water differed significantly according to the length of the pasteurization period.
2. There were no significant differences on average percent white heads occurrence regardless of the dosage and frequency of LBb application against granular insecticide applied monthly.

RECOMMENDATIONS

Considering the outcomes of this research, the following recommendations are proposed:

1. Collection of fresh coco water and pasteurization must be done on the same day for three (3) hours.
2. To evaluate the dosage and frequency of applications of LBb applied under field condition is also recommended.

REFERENCES

Balleras, G. D., Doverte, M. S., & Endonela, L. E. (2016). Alarmin g presence of three rice stem borer species in irrigated lowlan d rice agroecosystem in Midsayap, North Cotabato, Philippines. J. Biodivers. Environ. Sci, 9, 105-109.311966145_Alarming_presence_of_three_rice_stem_borer_ spe

cies_in_irrigated_lowland_rice_agroecosystem_in_Midsayap_ North_ Cotabato_Philippines

Bancole, W., Laing, M. D., Yobo, K. S., & Togola, A. (2020). Establishment of (*Beauveria bassiana*) isolates as endophytes in rice cultivars and their biocontrol efficacy against rice stem borer, Sesamia calamistis. *South African Journal of Science*, 116(11-12), 1-9.

http://dx.doi.org/10.17159/sajs.2020/7914doi: 10.1016/j.cropro.2020.105513

Cabasan, M.T.N. Tabora, J.A.G. Cabatac, N. Jumao-as, C.M. Soberano,

J.O. Turba, J.V. Dagamac, N.H.A. Barlaan, E. (2019). Economic and ecological perspectives of farmers on rice insect pest management. *Global Journal of Environmental Science and Management*. https://doi.org/10.22034/gjesm.2019.01.03

Chaudhary, K. K., (2019). Mass production of entomopathogens *Beauveria bassiana* and *Metarhizium anisopliae* using rice as a substrate by diphasic liquid-solid fermentation technique. Jayoti Vidyapeeth Women's University.

http://www.scienceandnature.org/IJABR/IJABR_Vol3(3)2013/IJABR_V3(3)1R.pdf

EL-HEFNY, A. S. (2016). Economic threshold and economic injury levels for rice stem borer, using simulated white heads in rice. *Egyptian Journal of Agricultural*

Research, 94(2), 353-364. DOI: 10.21608/EJAR.2016.152463

Fahad, S., Saud, S., Akhter, A., Bajwa, A. A., Hassan, S., Battaglia, M., & Irshad, I. (2021). Bio-based integrated pest management in rice: An agro-ecosystems friendly approach for agricultural sustainability. *Journal of the Saudi Society of Agricultural Sciences*, 20(2), 94-102. https://doi.org/10.1016/j.jssas.2020.12.004

Horgan F. G., Romena, A. M., Bernal, C. C., Almazan, M. L. P. & Ramal,

A. (2021) Stem borers revisited: Host resistance, tolerance, and vulnerability determine levels of field damage from a complex of Asian rice stemborers. Crop Prot.

Horgan, F. G., Romena, A. M., Bernal, C. C., Almazan, M. L. P., & Ramal, A.

F. (2021). Stemborers revisited: Host resistance, tolerance, and vulnerability determine levels of field damage from a complex of Asian rice stemborers. Crop Protection, 142, 105513./ https://doi.org/10.1016/j.cropro.2020.105513

January, B., Rwegasira, G. M., & Tefera, T. (2020). Rice stem borer species in Tanzania: a review. *The Journal of Basic and Applied Zoology*, 81(1), 36. https://link.springer.com/article/10.1186/s41936-020-

00172-0

Mascarin, G. M. & Jaronski, S.T. (2016). The production and uses of Beauveria bassiana as a microbial insecticide. *World J Microbial Biotechnology*. DOI: 10.1007/s11274-016-2131

Ndakidemi, B., Mtei, K., & Ndakidemi, P. A. (2016). Impacts of synthetic and botanical pesticides on beneficial insects. *Agricultural Sciences*, 7 (06), 364. DOI:10.4236/as.2016.76038

Ooi, P. A.C. (2015). Common insect pests of rice and their natural biological control an illustrated guide to the insect pests that feed on rice plants and the organisms that feed on and control those pests. *Agriculture Science Journal*. http://eprints.utar.edu

Rubia, E.G., Sanchez, D., Sigit, W., Nurhasyim, D., Heong, K.L., Zalucki, M.P., & Norton, G. A .(2015).Some factors affecting white stem *borer Scirpophaga innotata* (Walker) (Lepidoptera: Pyralidae) injury to rice. Crop Protection. https://doi.org/10.1016/S0261- 2194(98)00054-4.

Samanta, S., Mritunjoy, B., Nihal, R., & Samanta, A. (2020). Bio-efficacy trials of carbofuran 3% CG against insect pests of Rice. *Journal of Entomology and Zoology Studies*; 8(1): 1529-153. Retrieved from https://www.researchgate.net/publication/340548942_Bioefficacy_t
rials_of_carbofuran_3_CG_against_insect_pests_of_Rice

Shahriari, M., Zibaee, A., Khodaparast, S. A., & Fazeli-Dinan, M. (2021). Screening and Virulence of the Entomopathogenic Fungi Associated with *Chilo suppressalis* Walker. *Journal of Fungi*; 7(1): 34. Retrieved from
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7828016/Tacio, H. D. (2021). RICE: The staple food of Filipinos THE STAPLE FOOD OF FILIPINOS. https: //edgedavao.net/ agri-trends /2021/09/22/ rice-the staple -food-of-filipinos-the-staple- food-of-filipinos.

Allegrucci, N., Velazquez, M., Russo, M., Vianna, M., Abarca, C., & Scorsetti, A. (2020). Establishment of the entomopathogenic fungus *Beauveria bassiana* as an endophyte in Capsicum annuum and its effects on the aphid pest Myzus persicae (Homoptera: Aphididae). *Rev. biol. trop* vol.68 n.4. Retrieved from http://dx.doi.org/10.15517/rbt.v68i4.41218

Indriyanti, D., Alfien, M., Bintari, S., Setiati, N., Sumantri, G., & Prarastyani H. (2022). *Beauveria bassiana* Growth and Development in Various Liquid Media. *Journal of Biology & Biology Education*. Vol. 14 No.3. DOI: https://doi.org/10.15294/biosaintifika.v14i3.39705

Jaronski, S, T. & M.A. Jackson. (2012). Mass production of entomopathogenic Hypocreales. Manual of Techniques in Invertebrate Pathology (Second Edition)

Johnson, D., White, R., Pereira, R., & Geden, C. (2020). *Beauveria bassiana* Culturing and Harvesting for Bioassays With House Flies. *Journal of Insect Science*, Volume 20, Issue 6, 14, https://doi.org/10.1093/jisesa/ieaa072

Kovač , M., Lacković, N., & Pernek, M. (2020) . Effect of *Beauveria bassiana* Fungal Infection on Survival and Feeding Behavior of Pine-Tree Lappet Moth (Dendrolimus pini L.). *Forests*, 11(9), 974; Retrieved from https://doi.org/10.3390/f11090974

Norjmaa, U., Nasandulam, D., Enkhjargal, B., & Banzragch, D. (2019). Morphological and molecular identification of *Beauveria bassiana* from agricultural soils. *Mong. J. Agric. Sci.* Vol.27 (02), DOI: https://doi.org/10.5564/mjas.v27i02.1280

Pedrini, N. (2022). The Entomopathogenic Fungus *Beauveria bassiana* Shows Its Toxic Side within Insects: Expression of Genes Encoding Secondary Metabolites during Pathogenesis. *J. Fungi* , 8(5), 488; https://doi.org/10.3390/jof8050488

Savariya, K. (2022). Determination of an Effective Dose of *Beauveria bassiana* (Balsamo) Vuillemin against Garlic *Thrips tabaci* Lindeman. Fungal Genomics & Biology.Volume 12, Issue 1. Retrieved from https://www.longdom.org/open-access/determination-of-an-effective-dose-of-embeauveria-bassianaem-balsamo-vuillemin-against-garlic-emthrips-tabaciem-lindeman-89952.html

Wang, H., Peng, H., Li, W., Cheng, P. & Gong, M. (2021). The Toxins of *Beauveria bassiana* and the Strategies to Improve Their Virulence to Insects. Front Microbiol., 12: 705343. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8430825/

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