Journal of Biological Control, 40(3): 315-329, 2026, DOI: 10.18311/jbc/2026/55710



Research Article

Field efficacy of entomopathogenic fungi against white grubs, *Anomala* spp. (Coleoptera: Scarabaeidae: Rutelinae: Anomalini) infesting peanut (*Arachis hypogaea* L.) (Fabaceae) in Surigao Del Sur, Philippines

Armando A. Ortiz[1], Maricel J. Didal[2], Abel F. Wagas[1], Orlando A. Calcetas[3]*, Joel L. Adorada[4], Michelle S. Guerrero[5], Rochelle A. Quiñones[5] and Lara Paul Ebal[6]

[1]*Department of Agriculture, Regional Field Office-XIII-Caraga, Agusan del Norte, Philippines*

[2]*Department of Agriculture, Regional Crop Protection Center-XIII, Agusan del Norte, Philippines*

[3]*Regional Crop Protection Centre, Regional Field Unit 4A–CALABARZON, Department of Agriculture, Marawoy, Lipa City, 4217 Batangas, Philippines*

[4]*Bureau of Plant Industry - Los Baños National Crop Research, Development and Production Support Centre, Timugan, Los Baños, Laguna, Philippines*

[5]*National Crop Protection Centre, College of Agriculture and Food Science, University of the Philippines Los Baños, College 4031 Laguna, Philippines*

[6]*Institute of Statistics (INSTAT) - University of the Philippines Los Baños, College, Los Baños, 4030 Laguna, Philippines*

**Corresponding author E-mail*: orly.calcetasgis@gmail.com

ABSTRACT: The potential of entomopathogenic fungi (EPF) for managing the different white grubs, *Anomala* spp. (Coleoptera: Scarabaeidae: Rutelinae: Anomalini) infesting peanut plantations in Surigao Del Sur was evaluated under field conditions. This investigation compared the field efficacy of *Beauveria bassiana* (Bals.-Criv.) Vuill. 1912, (Cordycipitaceae) and *Metarhizium aniso*pliae (*M. anisopliae*) (Metschn.) (Sorokin, 1883)) (Clavipitaceae) against white grubs, with an initial population of approximately six individuals per m^2 on peanut in the area. At 90 DAP, in trials evaluating EPF alone, *M. anisopliae* resulted in the highest mortality (29.43%), which was not significantly different from *B. bassiana* (27.63%). In combined trials involving biological control agents and insecticides, the highest white grub mortality at 90 DAP was recorded in plots treated with Carbofuran (Furadan 3G; 52.76%), which was not significantly different from Imidacloprid-treated plots (50.96%). The mortality in plots treated with *M. anisopliae* (47.16%) and *B. bassiana* (46.36%) was also high. All treatments were significantly different from the control plots, which exhibited zero mortality (0%). White grub mortality increased progressively from 35, 60, and 90 DAP across both biological and chemical treatments. The highest number of developed pods was recorded in Imidacloprid- treated plots (352.51 pods/m^2), followed by Furadan 3G (326.00 pods/m^2). Plots treated with *M. anisopliae* and *B. bassiana* produced 304.89 pods/m^2 and 281.44 pods/m^2, respectively. All treatments significantly outperformed the control plots, which produced only 173.56 pods/m^2. Peanut pod damage was significantly reduced in all treated plots. No significant differences were observed among *B. bassiana* (10.42%), *M. anisopliae* (11.05%), and Furadan 3G (9.36%), while Imidacloprid recorded the lowest pod damage (4.67%). All treatments differed significantly from the control plots at 31.92% damaged peanut pods. These results demonstrate that *B. bassiana* and *M. anisopliae* are effective and environmentally safer alternatives to chemical insecticides for managing scarab white grub pests population in peanuts. Molecular analyses were performed on white grub larvae. While species-level resolution was not achieved using the molecular markers, the analyses clearly discriminated two genetically distinct taxa, providing molecular evidence for the occurrence of different *Anomala* species infesting peanut fields in Surigao del Sur. However, seven species of *Anomala* were recorded in the Surigao provinces by Ohaus between 1923 and 1924, one of which is suspected to be a pest of peanuts in Surigao del Sur. Currently, no molecular marker data are available in the NCBI and BOLD databases for comparison.

KEYWORDS: Biological control, chafer, entomopathogens, insecticides, June beetle, scarabs

(Article chronicle: Received: 06-04-2026; Revised: 17-07-2026; Accepted: 18-09-2026)

INTRODUCTION

The different species of white grubs (Coleoptera: Scarabaeidae) are considered one of the major pest problems of peanut farmers and other important crops in Surigao del Sur in the vast island of Mindanao (Figure 1A). The white grub-infested municipalities reported in a survey are San Agustin, Lianga, Barobo, Tagbina, Hinatuan, and selected areas in Bislig City (Udarbe 2015 unpublished report) (Figure 1B). Other major crops infested with white grubs in the area are soybeans (*Glycine max* (L.) Merr.) (Fabaceae), corn (*Zea mays* L.) (Poaceae), rice (*Oryza sativa* L.) (Poaceae), coffee seedlings (*Coffea arabica* L.) (Rubiaceae), root crops, forage and other agricultural seedlings of fruit trees. Significant damage has also been observed in falcata tree (*Falcataria falcata* (L.) Greuter & R. Rankin) seedlings, indicating the broad host range and economic importance of white grub infestations if they remain unchecked (Udarbe 2015, unpublished report).

Peanut is one of the major crops in Surigao del Sur, especially in the municipality of Tagbina, where this leguminous crop is well adapted and widely cultivated (Figure 2A). The area has a warm, humid tropical rainforest climate with high annual precipitation and no pronounced dry season. Tagbina experiences relatively little seasonal variation in temperature, with mean temperatures generally around 25-27°C and daytime temperatures commonly reaching 28-32°C. Rainfall is distributed throughout the year at approximately 2,567 mm annually, and mean relative humidity is about 85%, although the amount varies considerably among months (PAGASA, 2026).

However, peanut yield was severely affected by white grub infestations, with reported yield losses of 40 to 80% (Udarbe & Jabao, 2017 unpublished report). White grub larvae actively feed on the root system, resulting in the death of the plant, thereby significantly reducing the crop yield. Gabriel (1997) reported three major root-feeding white grubs on peanut (under legumes), including *Anomala* spp., *Holotrichia* sp., and *Leucopholis* spp. White grubs are also recognised as troublesome pests in upland rice and rainfed ecosystems in Claveria, Misamis Oriental, and Northern Mindanao (Litsinger *et al.,* 2002). The rhizotrogine beetle species such as *Holotrichia mindanaoana* Brenske and *Amphitrichia (Holotrichia) flachi* Brenske have been recorded to cause infestations occurring every two years.

To combat the pest, insecticide application is usually the major strategy employed for managing white grub infestations (Veeresh, 1974; Raodeo *et al.,* 1976; Mane *et al.,* 2015). However, this reliance on chemical control has resulted in unintended problems, including the development



Figure 1. (A). Peanut pods infested with ruteline scarab white grubs, *Anomala* spp. **(B).** White grub larvae collected in the peanut plantation of Tagbina, Surigao Del Sur.

of insecticide resistance, accumulation of pesticide residues in crops, and destruction of the population of beneficial parasitoids and predators (Rao *et al.,* 2006). Thus, there is a need to utilise environmentally friendly and sustainable alternative strategies for the management of white grub pests.

Biological control plays a critical role in the management of white grub pests, which are widely infested under poor soil fertility and micronutrient-deficient conditions. In Surigao del Sur, no scientific studies have yet been done on the effects of *M. anisopliae* (=*M. anisopliae*) (Metschn.) (Sorokin, 1883) (Clavipitaceae) and *B. bassiana* (Bals.-Criv.) Vuill. 1912, (Cordycipitaceae) in managing the field population of white grubs on peanuts. The study is designed to describe the white grub populations; to test the efficacy of entomopathogens on the per cent infection in white grubs; to test the efficacy of entomopathogens and insecticides on the per cent mortality in white grubs; to determine if there are differences in peanut yield; and to test the per cent damage of entomopathogens and insecticides on peanut yield.

The use of microbial agents is a promising alternative to chemical control for managing white grub infestations. In natural ecosystems, microbial agents play an important role as mortality factors contributing to the regulation of pest populations (McCoy, 1990; Villacarlos *et al.,* 2004; Tabudlong & Estoy, 2015). Entomopathogenic fungi, particularly *M. aniso*pliae and *B. bassiana*, have been reported to exhibit potential for the control of white grub populations. Moreover, both entomopathogenic fungi have been evaluated against various scarab beetle species, with promising reports reported in earlier studies (Hurpin & Robert, 1972). These environmentally friendly fungi have a broad host range and have been widely used as microbial biocontrol agents on various types of pests, which include insects from different orders: Lepidoptera, Coleoptera, Hemiptera, Diptera, Orthoptera, Hymenoptera, as well as non-insect arthropods such as elm bark beetle, mosquito larvae, plant hoppers, coconut leaf beetle, rhinoceros beetle, onion thrips, storage pests, white grub and cattle tick (Shanmugam *et al.,* 2014; Chandel *et al.,* 2015). Several studies have shown the effectiveness of *M. anisopliae* in managing the white grub population of *Holotrichia serrata* F. in India on sugarcane fields (Srikanth *et al.,* 2011; Shanmugam *et al.,* 2014; Sahayaraj, 2008; Thamarai *et al.,* 2011; Pradnya *et al.,* 2014). The green muscardine fungus *M. anisopliae* was also used to manage *Oryctes rhinoceros* L. (Coleoptera: Scarabaeidae: Dynastinae) (Ramle *et al.,* 2009; Velavan *et al.,* 2017), a serious pest of oil palm in Malaysia (Bedford, 2014).

The study by Lacey *et al.* (1994) showed that 43% of the white grub larval population was effectively controlled by *Metarhizium* sp. Similarly, 79.8% and 71.6% of white grubs were infected by *B. bassiana and M. anisopliae*, respectively, under laboratory conditions (Fedai *et al.,* 2015). A laboratory bioassay on Holotrichia parallela Motschulsky demonstrated dose-dependent mortality with conidial densities of 5×10^6, 10×10^6, and 20×10^6 in soil, resulting in 22.4%, 41.1%, and 74.4% mortality, respectively (Liu *et al.*, 2011). An LD_{50} of 11.9 was recorded at the highest density tested. Furthermore, the field efficacy test of *M. anisopliae* strain was evaluated against Japanese beetle (*Popillia japonica*) (Coleoptera: Scarabaeidae) and annual bluegrass weevil (*Listronotus maculicollis*) (Coleoptera: Curculionidae) larvae and results showed that the mortality rate to varied concentrations ranged from 31-46% for *L. maculicollis* with the liquid formulation and 49% for *P. japonica* with the granular formulation on aerated turfgrass (Ramoutar *et al*., 2010).

Furthermore, according to Fernandez *et al.* (2010), the high infection was affected by the heat and cold temperatures of the surrounding environment. However, *M. anisopliae* detection was negatively associated with soil moisture, organic matter, zinc, sulfur, and copper concentrations in the soil (Jabbour, 2009), while low soil organic matter content is often associated with higher entomopathogenic fungal occurrence, creating favourable conditions for infection of insect pests (Oliveira, 2013).

MATERIALS AND METHODS

The efficacy trial was conducted at the Tagbina Research and Experiment Station (TRES), Bgy. Maglambing, Tagbina, Surigao del Sur, from May 2017 to August 2019. Although the experiment was established as a Randomised Complete Block Design (RCBD) to account for potential spatial gradients in soil fertility and moisture across the field, the study only focused on the effects of fertiliser on plants. Each block was divided into five experimental units (plots),

and the five treatments were randomly assigned to plots within each block. With the plot as the experimental unit, its size was 5m x 8m (40 m²), and it was then planted with two seeds of the NSIC Pn2 peanut variety per hill. Fertilisation was also applied based on a pre-trial soil analysis, with 14-14-14 complete fertiliser broadcast at a rate of one bag per application at 3 and 30 Days After Planting (DAP).

Pre-assessment of the White grub Population on peanut

Before the application of treatment to each experimental unit, three peanut plants were observed to assess the population of white grub larvae. The average population of white grub larvae was computed for three consecutive years (2017-2019).

Application of treatments on White grub population on peanut

The treatment levels in the study consist of entomopathogens or microorganisms, namely, (1) *M. anisopliae* and (2) *B. bassiana;* two chemical insecticides, namely, (3) Carbofuran (Furadan-3G) and (4) Neonicotinoids (Imidacloprid); and (5) the control or untreated level (Figures 2B and C). The microorganisms at 1 x 10^9 conidia mL^{-1} or 20 packs /ha, while Imidacloprid insecticides of 2 tbs/16 L or 10 tankload/ha were applied 1 week before planting, 14 days and 45 DAP by the spraying method. The maximum microbial dose (1x10^9 conidia mL^{-1}) was applied due to the high initial white grub population and favourable conditions for fungal infection. For the insecticide treatments, a total of



Figure 2. (A). Experimental field in Brgy. Maglambing, Tagbina Research and Experiment Station (TRES), Tagbina, Surigao del Sur; **(B).** *Metarhizium anisopliae* fungus used in the trial against scarab white grubs of peanut, **(C).** *Beauveria bassiana* fungus used in the trial against scarab white grubs of peanut.

3.5 kg/ha of Furadan 3G was applied 3X by the broadcasting method at the rate of 1.5 kg/ha one week before planting, 1 kg/ha 14 days after planting, and 1 kg/ha 45 days after planting. Per cent infection and mortality of white grub per m^2 were counted at 35, 60, and 90 DAP using destructive sampling.

From the five treatments mentioned, only the efficacy of entomopathogens and the control were compared to answer objective (1). Efficacy is based on mortality or infection rates over time (35, 60, and 90 DAP).

Number of peanut pods produced, developed and damaged by White grubs

During the crop maturity stage, the five treatment levels focused on objective (2): to determine the per cent damage by white grubs on peanuts and the per cent infection by entomopathogens. The per cent damage and per cent infection resulted from classifying the harvested peanut pods, which were placed inside the labelled nylon nets and which were sun-dried for two days. At the crop maturity stage, harvested peanuts were placed inside labelled nylon nets. Along with the per cent damage of white grubs on peanuts and the per cent infection of the entomopathogens, data on the number of pods per plant and yield per sample plot were collected to analyse objective (3) of the study, which is to determine the effects of white grubs on yield.

All data sets or responses, were statistically analysed using descriptive analysis that includes obtaining summary statistics and per cent distributions. Moreover, to compare the effects of the five treatments, an F-test using ANOVA technique for a Randomised Complete Block Design (RCBD) was used, and if there were significant differences among treatment means, pairwise comparison using Tukey's Test followed.

Molecular analysis of White grub larvae

White grub larvae infesting peanuts were collected in July 2024 from Maglambing, Tagbina, Surigao del Sur. Larval specimens were preserved and processed for molecular identification using mitochondrial Cytochrome *c* Oxidase Subunit I (COI) and nuclear 28S ribosomal RNA (28S rRNA) gene markers.

Genomic DNA was extracted from approximately three to four larval legs or, when necessary, a small portion of larval tissue, depending on specimen size and tissue availability, using the Promega Wizard® Genomic DNA Purification Kit (Promega Corporation, USA) following the manufacturer's protocol with minor modifications. The extracted DNA was rehydrated using the DNA Rehydration Solution provided with the kit and stored at −20°C until PCR amplification.

Molecular identification was performed using two genetic markers: the mitochondrial COI gene, commonly used for DNA barcoding of insects, and the nuclear 28S rRNA gene, which provided an additional marker for taxonomic identification. For amplification of the mitochondrial cytochrome c oxidase subunit I (COI) gene, the universal primer pair LCO1490 (GGTCAACAAATCATAAAGATATTGG) and HCO2198 (TAAACTTCAGGGTGACCAAAAAATCA) was used, targeting an approximately 650 bp fragment of the COI barcode region. PCR amplification consisted of an initial denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 94°C for 45 s, annealing at 52°C for 45 s, and extension at 72°C for 1 min, with a final extension at 72°C for 10 min. For amplification of the nuclear 28S ribosomal RNA (28S rRNA) gene, the universal insect primer pair, 28S3a (AGTACGTGAAACCGTTCAGG-3′) and 28Sb (TCGGAAGGAACCAGCTAC) was used to amplify was used to amplify an approximately 700 bp fragment of the 28S rRNA gene. PCR amplification consisted of an initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 45 sec, annealing at 52°C for 45 sec, and extension at 72°C for 1 min, followed by a final extension at 72°C for 10 min. Each PCR run included a no-template control to confirm the absence of potential reagent contamination.

PCR products were resolved by electrophoresis on a 1.5% agarose gel stained with GelRed® nucleic acid gel stain (Biotium, Inc., USA). Amplicons of the expected size were confirmed using a 100 bp Plus DNA Ladder (Vivantis Technologies Sdn. Bhd., Malaysia), visualised under UV illumination, and documented using an Enduro™ GDS Touch Gel Documentation System (Labnet International, Inc., USA). Successfully amplified PCR products were submitted to Macrogen Inc., Republic of Korea, for Sanger sequencing.

Raw sequence chromatograms were inspected for sequence quality, and low-quality regions and primer sequences were trimmed prior to analysis. The resulting sequences were compared with reference sequences deposited in the NCBI GenBank database using the Basic Local Alignment Search Tool (BLASTn) (Altschul *et al*., 1990) to identify closely matching nucleotide sequences.

Taxonomic assignments were evaluated based on percentage sequence identity, query coverage, E-value, sequence quality, and concordance between the COI and 28S rRNA results. Reference sequences showing the closest matches were retrieved for comparison, and the corresponding accession numbers were recorded. Molecular results were considered together with available morphological information to support the taxonomic identification of the white grub specimens.

RESULTS AND DISCUSSIONS

Occurrence and molecular characterisation of *Anomala* spp. associated with peanut

Field observations and collections in peanut-growing areas of Surigao del Sur revealed the occurrence of scarab beetles belonging to the genus *Anomala* (Coleoptera: Scarabaeidae: Rutelinae: Anomalini). Both adult beetles and soil-dwelling white grub larvae were encountered in association with peanut fields, suggesting that *Anomala* spp. constitute an important component of the scarab complex present in the study area.

Table 1. BLASTn results from the *COI* and *28S* nucleotide sequences of presumptive *Anomala* spp. (Coleoptera: Scarabaeidae: Rutelinae: Anomalini) specimens from Surigao Del Sur, Philippines, using the GenBank database from NCBI

Sample Code	Marker	Closest GenBank BLASTn Match	Identity (%)	Accession No.
AH3	COI	*Anomala dimidiata*	91.20%	KF939015.1
AH3	28S	*Anomala testaceipennis*	96.41%	KX278757
AH4	COI	*Anomala dimidiata*	91.20%	KF939015.1
AH4	28S	*Anomala testaceipennis*	96.41%	KX278757
AH8	COI	*Anomala orientalis*	88.95%	OQ551152
AH5	28S	*Anomala testaceipennis*	96.41%	KX278757

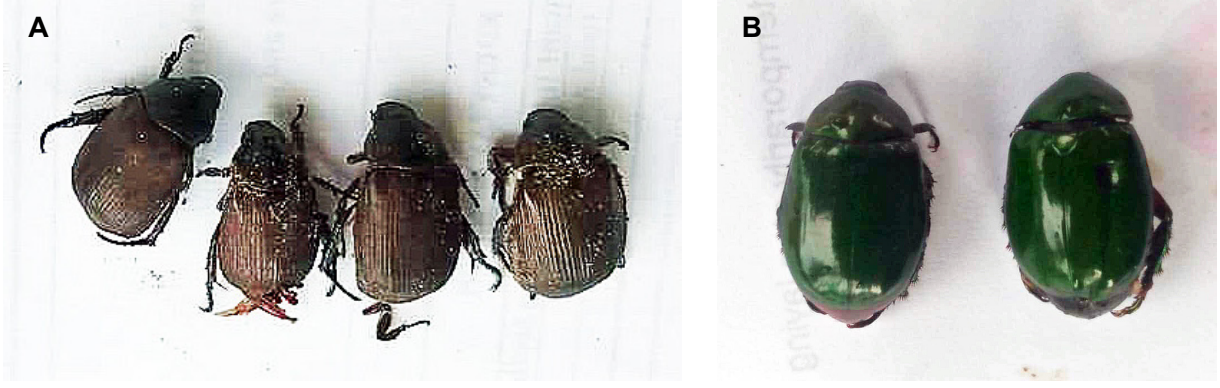


Figure 3. (A). Adults of small brown *Anomala* sp. (Coleoptera: Scarabaeidae: Rutelinae) collected in the peanut plantation in Surigao Del Sur, **(B).** Adults of green *Anomala* spp. collected in the peanut plantation in Surigao Del Sur.

Two visually distinct adult *Anomala* morphotypes were commonly collected from peanut fields (Figures 3A and B). The first morphotype was characterised by a predominantly dark brown to nearly black, relatively dull dorsal colouration, whereas the second exhibited a conspicuous shiny metallic-green colouration. These consistent differences in external appearance suggest the occurrence of more than one *Anomala* species in the study sites. However, because colouration alone is insufficient for reliable species-level identification in this taxonomically diverse group, the specimens are presently treated as *Anomala* spp. pending detailed morphological examination of diagnostic characters and molecular confirmation.

To complement the field observations, molecular characterisation was conducted on representative white grub larvae collected from peanut fields. Partial sequences of the mitochondrial *COI* gene and the nuclear 28S ribosomal RNA (28S rRNA) gene were generated and compared with available reference sequences in the NCBI GenBank database using BLASTn (Table 1).

BLASTn analysis showed high query coverage (99-100%) across all sequences. However, sequence identities with the closest available GenBank references were relatively low, ranging from 88.95% to 96.41%, and the closest taxonomic matches differed between the mitochondrial (COI) and nuclear (28S rRNA) markers. These results indicate that, despite extensive sequence alignment with available references, the current GenBank records provide insufficient resolution for confident species-level identification of the Philippine specimens.

For the *COI* marker, specimens AH3 and AH4 showed their closest BLASTn match to *Anomala dimidiata* (GenBank accession KF939015.1), both with 91.20% sequence identity, whereas specimen AH8 showed its closest match to *Anomala orientalis* (Waterhouse, 1875) (OQ551152), with only 88.95% identity. The occurrence of these two distinct *COI* sequence patterns is consistent with the field observation of two conspicuous adult *Anomala* morphotypes—a dark brown to nearly black morphotype and a metallic-green morphotype—suggesting that more than one *Anomala* taxon may be associated with peanut fields in the study area. It is therefore possible that the *COI* sequence represented by AH3 and AH4 and that represented by AH8 correspond to the two adult morphotypes. However, because the DNA sequences were generated from field-collected larvae rather than morphologically identifiable adults, the association of either *COI sequence* type with the brown or green adult morphotype cannot presently be established.

Analysis of the 28S rRNA marker produced a different pattern. Specimens AH3, AH4, and AH5 showed their closest BLASTn match to *Anomala testaceipennis* (accession KX278757), each with 96.41% sequence identity. Thus, AH3 and AH4 produced different closest species matches depending on the marker used: their *COI* sequences were closest to *A. dimidiata*, whereas their 28S sequences were closest to *A. testaceipennis*. Rather than indicating conflicting species identities, this pattern most likely reflects the limited representation of closely related Philippine *Anomala* species in public sequence databases and differences in the taxonomic resolution of the two markers.

The Philippines harbours a rich diversity of the genus *Anomala*, with approximately 76 species recorded nationwide, including 20 species known from Mindanao. Of these, seven species have been documented from the Surigao provinces: *Anomala apogonioides* Ohaus, 1924; *A. cassiana* Ohaus, 1923; *A. cucusa* Ohaus, 1923; *A. melitta* Ohaus, 1923; *A. samarensis* Ohaus, 1923; *A. surigana* Ohaus, 1923; and *A. volanda* Ohaus, 1923 (Magallanes *et al.,* 2025). These locally recorded taxa represent plausible candidates for the identity of the *Anomala* associated with peanut fields in Surigao del Sur. However, the absence or limited availability of corresponding *COI* and 28S reference sequences in GenBank and BOLD currently prevents direct molecular comparison with these Philippine species.

The molecular results should therefore be interpreted as supporting the identification of the collected white grubs as *Anomala* spp., rather than as definitive species-level assignments. The relatively low sequence identities and differences in the closest BLAST matches between *COI* and 28S suggest that the specimens may represent Philippine *Anomala* taxa that are poorly represented or entirely absent from existing molecular reference databases.

Field observations provide additional evidence of taxonomic diversity within the peanut-associated *Anomala* complex. Two readily distinguishable adult morphotypes were consistently observed: a dark brown to nearly black, relatively dull morphotype and a shiny metallic-green morphotype. The occurrence of these two morphotypes, together with the observed *COI* sequence differentiation among the larval specimens, suggests that at least two *Anomala* taxa may be associated with peanut fields in the study area. However, because the molecular analyses were conducted on field-collected larvae, a direct correspondence between the larval sequence types and the two adult morphotypes cannot yet be established.

Species-level resolution will therefore require an integrative taxonomic approach combining molecular data with adult morphology. Adult specimens representing both morphotypes should be examined using diagnostic morphological characters, particularly the male genitalia and other taxonomically informative structures, and reference *COI* and 28S sequences should be generated from morphologically identified adults for direct comparison with the larval sequences. Where feasible, field-collected larvae should also be reared until adult to establish a direct link between the damaging white grub stage and its corresponding adult morphotype.

The molecular results demonstrate the occurrence of *Anomala* white grubs associated with peanut production in Surigao del Sur and suggest that more than one *Anomala* taxon may be present in the study area. The inability of current database searches to provide confident species-level identification highlights a major gap in molecular reference data for Philippine Scarabaeidae. Establishing voucher-linked DNA barcode sequences for locally occurring *Anomala* species will be important for resolving the identity of peanut-associated white grubs and for improving the diagnosis, surveillance, and management of economically important scarab beetles in the Philippines.

Population prior to the application of treatments

The data on white grub population at pre-treatment application did not significantly differ among the three sample plots prior to the experiments conducted from 2017-2019. The average number of white grubs sampled was approximately 5 larvae/m^2. Active larvae were mostly found 10 to 20 cm below the soil surface in each experimental plot (Table 2).

Table 2. The population of white grub per m^2 before the application of the different treatments under field conditions in TRES, Tagbina, Surigao Del Sur

Treatments	Average population of white grub/m^2 before application			Mean
	2017	2018	2019	
Control	4.67 ± 0.58	6.33 ± 0.58	6.33 ± 0.58	5.78±0.96

Note: Means sharing a letter in the group label are not significantly different at the 5% level

Table 3. Per cent infection/m^2 of white grub at 35 days after planting applied with *B. bassiana* and *M. anisopliae* under field conditions in TRES, Tagbina, Surigao Del Sur

Treatments	Per cent infection/m^2 of white grub at 35 DAP			Mean
	2017	2018	2019	
Beauveria bassiana	20.63 ± 6.87	24.63 ± 5.87	22.63 ± 5.87	22.63±2.00
Metarhizium anisopliae	18.10 ± 3.29	20.10 ± 3.28	21.10 ± 3.28	19.77±1.53
Control	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00±0.00

Table 4. Percent infection/m^2 of white grub at 60 DAP applied with *B. bassiana* and *M. anisopliae* under field conditions in TRES, Tagbina, Surigao Del Sur

Treatments	Percent infection/m^2 of white grub at 60 DAP			Mean
	2017	2018	2019	
Beauveria bassiana	26.19 ± 8.58	29.19 ± 8.59	28.19 ± 7.59	27.86±1.53
Metarhizium anisopliae	28.89 ± 7.69	30.89 ± 6.69	28.89 ± 6.59	29.56±1.15
Control	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00±0.00

Table 5. Per cent infection/m^2 of white grub at 90 days after planting, applied with *B. bassiana* and *M. anisopliae* under field conditions in TRES, Tagbina, Surigao Del Sur

Treatments	Per cent infection/m^2 of white grub at 90 DAP			Mean
	2017	2018	2019	
Beauveria bassiana	20.63 ± 6.87 [a]	30.63 ± 5.87	31.63 ± 5.67	27.63±6.08
Metarhizium anisopliae	26.43 ± 7.43 [b]	30.43 ± 5.43	31.43 ± 5.33	29.43±2.65
Control	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00±0.00

Note: Means sharing a letter in the group label are not significantly different at the 5% level

Efficacy of entomopathogens on White grubs

Results showed that at 35 days after planting (DAP), the average per cent mortality and infection of white grubs were high in *B. bassiana-treated* plots at 22.63%, followed by *M. anisopliae* at 19.77% (Table 3). However, at 60 DAP, the highest infection was recorded in *M. anisopliae* treated plots at 29.56% infection, followed by *B. bassiana* at 27.86% mortality (Table 4). Similarly, at 90 DAP, *M. anisopliae*-infected larvae were high at 29.43%, while mortality due to *B. bassiana* infection was recorded at 27.63% (Table 5).

In testing for the efficacy of entomopathogens on white grubs, there are no significant differences in the results of the per cent infection on white grub larvae among the three treatments across 35, 60, and 90 DAP and years, except in 2017 at 60 DAP. However, these two entomopathogenic fungi are still virulent to infect the host larvae of the beetle and have the potential to control the white grub population in the field, where the average infection ranges from 18.01% to 31.43%. Similar results were obtained by Visalakshi *et al.* (2015) on *M. anisopliae* and *B. bassiana,* which decreased white grub damage in India.

Table 4 shows that the mean percentages of infection of white grubs differ between *B. bassiana* and *M. anisopliae*, with the latter having a higher percentage of infection on white grubs in 2017 at 90 DAP by around 6%.

Efficacy of entomopathogens and insecticides on White grubs population

To test the efficacy of entomopathogens and insecticides on the per cent mortality of white grubs, a separate trial of the different treatments was applied on white grubs, including *B. bassiana*, *M. anisopliae*, Imidacloprid, Furadan 3G, and control across 35, 60, and 90 DAP and years 2017, 2018 and 2019.

Results of three-year of consecutive trials showed that at 35 DAP observed that Furadan 3G showed the highest

per cent mortality rate (45.66%), followed by *Imidacloprid* (37.19%) (Table 6). However, *M. anisopliae* and *B. bassiana* had 9.77 % and 9.63%, respectively. After 60 DAP, it was observed that the percent mortality rate of white grub was increasing. The highest average of percent mortality was similarly noted in Furadan 3G at 45.77% followed by Imidacloprid at 40.63 %. These results on per cent mortality of the two insecticides are significantly different compared to the two entomopathogens, wherein *M. anisopliae* resulted in 27.89% while *B. bassiana* at 27.52% at 60 DAP (Table 7).

The highest percent mortality of white grub was noted in Furadan 3G at 52.76% (Table 8) but not significantly different from the results of Imidacloprid at 50.96% at 90 DAP. However, the results of the insecticide trial do not significantly differ from the results of the entomopathogens at 90 DAP. Results showed that the mortality recorded for both *entomopathogens* were nearly similar at 47.16% and 46.36%, respectively, and for insecticides at 50.96 % and 52.76%, respectively. It was observed that all four treatments except for the control plots showed no significant differences**.**

The above results concurred with Patel *et al.* (2010) and Mane *et al.* (2015) findings, wherein Neonicotinoids (Imidacloprid) and Carbofuran (Furadan 3G) are both effective in the management of white grubs. Similar results

Table 6. Per cent mortality/m^2 of white grub at 35 DAP as affected by application of different treatments under field conditions in TRES, Tagbina, Surigao Del Sur

Treatments	Percent mortality /m^2 of white grub at 35 DAP			Mean
	2017	2018	2019	
Beauveria bassiana	8.63 ± 5.57 ^{c}	10.63 ± 6.87 ^{c}	9.63 ± 6.57 ^{c}	9.63±1.00 ^{b}
Metarhizium anisopliae	9.10 ± 5.19 ^{c}	11.10 ± 3.29 ^{c}	9.10 ± 3.19 ^{c}	9.77±1.15 ^{b}
Imidacloprid	37.19 ± 4.58 ^{b}	36.19 ± 8.58 ^{b}	38.19 ± 6.58 ^{b}	37.19±1.00 ^{a}
Furadan 3G	40.19 ± 7.19 ^{a}	48.89 ± 7.69 ^{a}	47.89 ± 7.39 ^{a}	45.66±4.76 ^{a}
Control	0.00 ± 0.00 ^{d}	0.00 ± 0.00 ^{d}	0.00 ± 0.00 ^{d}	0.00±0.00 ^{c}

Note: This means sharing a letter in the group label is not significantly different at the 5% level.

Table 7. Per cent mortality/m^2 of white grub at 60 DAP as affected by application of different treatments under field conditions in TRES, Tagbina, Surigao Del Sur

Treatments	Percent mortality /m^2 of white grub at 60 DAP			Mean
	2017	2018	2019	
Beauveria bassiana	26.19 ± 3.58^{a}	27.19 ± 8.58^{a}	29.19 ± 3.58^{a}	27.52±1.53^{a}
Metarhizium anisopliae	26.89 ± 4.19^{a}	26.89 ± 7.69^{a}	29.89 ± 4.69^{a}	27.89±1.73^{a}
Imidacloprid	39.63 ± 3.87^{b}	40.63 ± 6.87^{b}	41.63 ± 5.87^{b}	40.63±1.00^{b}
Furadan 3G	42.10 ± 5.29^{a}	48.10 ± 3.29^{a}	47.10 ± 4.29^{a}	45.77±3.21^{b}
Control	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00±0.00^{c}

Note: This means sharing a letter in the group label is not significantly different at the 5% level.

Table 8. Per cent mortality/m^2 of white grub at 90 DAP as affected by application of different treatments under field conditions in TRES, Tagbina, Surigao Del Sur

Treatments	Percent mortality /m^2 of white grub at 90 DAP			Mean
	2017	2018	2019	
Beauveria bassiana	49.93 ± 5.86^b	41.63 ± 6.87^b	47.53 ± 6.86^b	46.36±4.27^a
Metarhizium anisopliae	49.63 ± 6.43^b	42.43 ± 7.43^b	49.43 ± 5.43^b	47.16±4.10^a
Imidacloprid	50.63 ± 4.87^a	50.63 ± 6.87^a	51.63 ± 5.87^a	50.96±0.58^a
Furadan 3G	53.43 ± 2.43^a	50.43 ± 7.43^a	54.43 ± 6.43^a	52.76±2.08^a
Control	0.00 ± 0.00^c	0.00 ± 0.00^c	0.00 ± 0.00^c	0.00±0.00^b

Note: This means sharing a letter in the group label is not significantly different at the 5% level.

in the studies of Fedai *et al.* (2015) and Visalakshi *et al.* (2015) showed that white grubs treated with *M. anisopliae and B. bassiana showed* 71.6% and 79.8% infection, respectively, under laboratory conditions.

The trial implies that chemical insecticides are fast and effective in managing the population of white grubs in the soil at 35 and 60 DAP. However, entomopathogenic fungi persist in the soil for a longer period at 90 DAP and are capable of infecting more white grubs similar to the effects of using insecticides on peanuts. The micro-organisms take time to multiply in the soil at the early stage of the crop but have a long-term efficacy against white grubs.

Peanut Yield

Peanut pods produced and developed

Results below (Table 9) show *B. bassiana*, *M. anisopliae*, Imidacloprid, Furadan 3G, and control across the years 2017, 2018, and 2019 as treatments in testing if at least one of the treatments has a different average yield in terms of the average number of peanut pods produced and developed.

Table 9 shows that there is at least one treatment that has a different mean number of pods among the rest of the treatments. The highest average number of pods produced was noted in a plot treated with Neonicotinoids

Table 9. Average number of pods per m^2 as affected by application of different treatments under field conditions in TRES, Tagbina, Surigao Del Sur

Treatments	Average number of pods (m^2)			Mean
	2017	2018	2019	
Beauveria bassiana	281.44 ± 7.13^{ab}	282.44 ± 7.12^{ab}	280.44 ± 7.13^{ab}	281.44±1.00^c
Metarhizium anisopliae	307.89 ± 42.32^a	304.89 ± 40.32^a	299.89 ± 20.32^a	304.22±4.40^b
Imidacloprid	352.28 ± 50.93^a	356.12 ± 40.93^a	349.12 ± 20.93^a	352.51±3.51^a
Furadan 3G	328.67 ± 45.84^a	329.67 ± 35.84^a	319.67 ± 25.84^a	326.00±5.51^b
Control	177.56 ± 56.39^b	174.56 ± 46.39^b	168.56 ± 36.39^b	173.56±4.58^d

Note: This means sharing a letter in the group label is not significantly different at the 5% level.

Table 10. Percent damage of peanuts as affected by application of different treatments under field conditions in TRES, Tagbina, Surigao Del Sur

Treatments	Per cent pest damage in pods (m^2)			Mean
	2017	2018	2019	
Beauveria bassiana	11.40 ± 2.00 ^{b}	10.40 ± 2.00 ^{b}	9.45 ± 1.00 ^{b}	10.42±0.98 ^{b}
Metarhizium anisopliae	13.42 ± 2.61 ^{b}	10.42 ± 2.51 ^{b}	9.32 ± 2.51 ^{b}	11.05±2.12 ^{b}
Imidacloprid	4.67 ± 0.51 ^{c}	4.16 ± 0.50 ^{c}	5.19 ± 0.50 ^{c}	4.67±0.52 ^{c}
Furadan 3G	10.39 ± 4.12 ^{b}	9.39 ± 4.22 ^{b}	8.29 ± 3.22 ^{b}	9.36±1.05 ^{bc}
Control	30.29 ± 5.05 ^{a}	35.29 ± 5.15 ^{a}	30.19 ± 6.15 ^{a}	31.92±2.92 ^{a}

Note: This means sharing a letter in the group label is not significantly different at the 5% level.

(Imidacloprid) insecticide at 352.51 pods/m^2. However, for Carbofuran (Furadan 3G), a value of 326.00 pods/m^2 was obtained but not significantly different from *M. anisopliae* at 304.89 pods/m^2. Much lower value was obtained on plots treated with *B. bassiana* at 281.44 pods/m^2. However, the lowest number of pods produced was obtained in the control plots with 173.56 pods/m^2 (Table 9).

Efficacy of entomopathogens and insecticides on peanut yield

To test efficacy of entomopathogens and insecticides on the per cent damage on peanuts yield, damaged and undamaged peanut pods were harvested at its maturity stage of 110 DAP., They were gathered, counted, and analysed. Results showed that significant differences in terms of the number of damaged pods were observed among treatments. The most serious damage was noted in the control plot at 31.92% pods/m^2. This indicates that higher damage is expected when no entomopathogens and insecticides are applied to peanut plants. Plots treated with Imidacloprid insecticide showed the lowest damage at 4.67% but were not significantly different from Furadan 3G at 9.36%. This indicates that insecticides can more effectively lessen the damage on peanuts. However, there is no significant difference in damage observed in plants treated with *B. bassiana* at 10.42%, *M. anisopliae* at 11.05%, and Furadan 3G at 9.36%, respectively (Table 10).

CONCLUSION

Based on the results of the study, the entomopathogens *B. bassiana* and *M. anisopliae* are both effective alternative Biocon agents for the management of several species of scarab white grubs on peanuts in Surigao Del Sur. This can be attributed to the high mortality rate of the white grub population on the entomopathogen-treated plots, which are not significantly different from insecticide-treated plots at 90 DAP. Also, the high number of developed and undamaged peanut pods produced is not significantly different on plots treated with Imidacloprid and Furadan insecticides. The white grub larvae mortality also increases as the number of entomopathogen applications increases (35, 60, and 90 DAP). *The entomopathogenic fungi M. anisopliae* and *B. bassiana* can be applied starting from the vegetative to flowering stages of the crop. The application of these Biocon agents can be done early in the morning or late in the afternoon at the rate of 20 packs/ha at 200g/ pack of each microorganism. It is also suggested that these biological control agents should be formulated into powder or pelletised form for ease of application at the farmer's field level. Furthermore, the application of these entomopathogenic fungi is highly recommended since they are a much safer and environmentally friendly alternative for the management of white grubs on peanuts. Furthermore, surveys and collection activities, coupled with morphological and molecular studies, are recommended

to determine which ruteline *Anomala* species are infesting peanut plantations in Surigao del Sur and some other areas in the Philippines.

ACKNOWLEDGEMENTS

The author gratefully acknowledges the Department of Agriculture, Research Division, Region-XIII- CARAGA for providing financial support to the project. Special thanks to Ms. Maricar A. Soberano of DA-RCPC-IVA CALABARZON for facilitating the connection between both DA offices and fostering this research collaboration.

REFERENCES

Altschul, S. F., Gish, W., Miller, W., Myers, E. W., and Lipman, D. J. 1990. Basic local alignment search tool. *J Mol Biol*, **215**: 403-410. https://doi.org/10.1016/S0022-2836(05)80360-2 PMid:2231712

Bedford, G. O. 2014. Biology and Management of Palm dynastid Beetles: Recent advances. *Annu Rev Entomol*, **58**: 353-372. https://doi.org/10.1146/annurev-ento-120710-100547 PMid:23317044

Chandel, R. S., Mandeep Pathania1, K. S., Verma, B. B., Sumit, V., and Kumar, V. 2015. The ecology and control of potato, whitegrubs of India. *Potato Res,* **58**: 147-164. https://doi.org/10.1007/s11540-015-9295-3

Fernandez, E. K. K., Keyser, C. A., Chong, J. P., Rangel, D. E. N., Miller, M. P., and Roberts, D. W. 2010. Characterization of *Metarhizium* species and varieties based on molecular analysis, heat tolerance, and cold activity. *J Appl Microbiol*, **108**(1): 115-118. https://doi.org/10.1111/j.1365-2672.2009.04422.x PMid:19664068

Fedai, E., and Osgur, A. 2015. The potential of two entomopathogenic fungi, *Beauveria bassiana* and *Metarhizium anisopliae* (Coleoptera: Scarabaeidae), as biological control agents against the June beetle. *J Insect Sci*, **15**(1): Article 44. https://doi.org/10.1093/jisesa/iev029 PMid:25881632 PMCid:PMC4535486

Gabriel, B. P. 1997. Insects and Mites Injurious to Philippine Crop Plants. National Crop Protection Center, University of the Philippines Los Baños, College of Agriculture, Laguna, p. 171.

Hurpin, B., and Robert, P. H. 1972. Comparison of the activity of certain pathogens of the cockchafer *Melolontha melolontha* in plots of natural meadowland. *J Invertebr Pathol*, **19**: 291-298. https://doi.org/10.1016/0022-2011(72)90225-X

Jabbour, R. 2009. Soil management effects on entomopathogenic fungi during the transition to organic agriculture in a feed grain rotation. *Biol Control,* **51**(3): 435-443. https://doi.org/10.1016/j.biocontrol.2009.08.004

Lacey, L. A., Martin, A., and Ribeiro, C. 1994. The Pathogenicity of *Metarhizium anisopliae* and *Beauveria bassiana* for Adults of Japanese beetle, *Papillia japonica* (Coleoptera: Scarabaeidae). *Eur J Entomol*, **91**: 313-319.

Litsinger, J. A., Libetario, E. M., Barrion, A. T. 2002. Population dynamics of white grubs in the upland rice and maize environment of Northern Mindanao, Philippines. *Int J Pest Manag,* **48**(3): 239-260. https://doi.org/10.1080/09670870210131861

Liu, X., Nong, X. Q., Liu, C. Q., Xi, G. C., Zhang, X., and Zhang, Z. H. **2011.** Biocontrol of Peanut White Grubs, *Holotrichia parallela,* using entomopathogenic fungus *Metarhizium anisopliae* at sowing period of peanut. Chinese Academy of Agricultural Sciences, 6p**.**

Magallanes, J. G., Nuneza, O. M., and Mondejar, E. P. 2025. First in 109 years: Catalogue of scarab beetles (Coleoptera: Scarabaeidae) of the Philippines. *ZooTaxa,* **5746**(1): 1-148. https://doi.org/10.11646/zootaxa.5746.1.1 PMid:42408322

Mane, B. P., and Mohite, B. P. 2015. Efficacy of newer molecules of insecticides whitegrub Leucopholis lepidophora infesting sugarcane in Western Maharashtra. *J Globebiosci***, 4:** 2431-2436.

McCoy, C. W. 1990. Entomogenous fungi as microbial pesticides. In: Baker, R.R., Dunn, P.E. (eds). New directions in biological control; Alternatives for suppressing agricultural pests and diseases. New York, Alan R. Liss, Inc., pp. 139-159.

Oliveira, I. 2013. Entomopathogenic fungi associated with *Prays oleae*: Isolation, characterization, and selection for biological control. [Doctoral dissertation, University of Minho, Portugal].

PAGASA. 2026. Philippine atmospheric, geophysical and astronomical services administration.

Patel, B. A., Patel, I. S., Pateland, P. S., and Patel, J. K. 2010. Efficacy of newer insecticidal formulations applied as soil drenching against white grub and termite in the groundnut. *Pestology,* **34**(7): 55-57.

Pradnya, B. M., and Pandurang, B. M. 2015. Efficacy of newer molecules of insecticides against white grub in sugarcane. *Asian J Biol Sci*, **9**: 173-177. https://doi.org/10.15740/HAS/AJBS/9.2/173-177

Ramle, M., Norman, K., and Mohd, B. W. 2009. Pathogenicity of granule formulations of *Metarhizium anisopliae* against the larvae of the oil palm rhinoceros beetle, *Oryctes rhinoceros* (L.). *J Oil Palm Res*, **2**: 602-612.

Ramoutar, D., Legrand, A. I., and Alm, S. R. 2010. Field performance of *Metarhizium anisopliae* against *Popillia japonica* (Coleoptera: Scarabaeidae) and *Listronotus* maculicollis (Coleoptera: Curculionidae) larvae in turfgrass. *J Entomol Sci*, **45**(1): 20-26. https://doi.org/10.18474/0749-8004-45.1.20

Rao, G. V., Ranga, N. T. L. G., Phan, L., and Nguyen, T. H. T. 2006. Occurrence of white grubs in groundnut crop in uplands of South Vietnam: A new report. *Insecta Mundi*, **26**: Article 31.

Raodeo, A. K., Deshapande, S. V., Puri, S. N., and Bilapate. 1976. A large-scale campaign for the control of whitegrub *Holotrichia serrata* F. in Maharashtra state. *Pestic Abstr News Summ*, **4**: 223-228. https://doi.org/10.1080/09670877609412368

Regniere, J., Rabb, R. L., and Stinner, R. E. 1979. *Popillia japonica* (Coleoptera: Scarabaeidae); A mathematical model of oviposition in heterogeneous agroecosystems. *Can Entomol*, **111**: 1271-1280. https://doi.org/10.4039/Ent1111271-11

Roger, J. D., Ward, A. L., and Wightman, J. A. 2000. Damage potential of two scarab species of groundnut. *Int J Pest Manag*, **51**(4): 305-312. https://doi.org/10.1080/09670870500399866

Rombach, M. C., and Roberts, D. W. 1994. Pathogens of rice insects. In: Biology and management of rice insects. Heinrichs, E. A. (ed.). Wiley Easter Limited. pp. 611-655.

Sahayaraj, K., and Karthick, S. 2008. Mass production of entomopathogenic fungi using agricultural products and by-products. *Afr J Biotechnol*, **7**(12): 1907-1910. https://doi.org/10.5897/AJB07.778

Shanmugam, P. S., Saravanan, N. A., and Tamilselvan, N. 2014. Assessment of bio fungicides, *Beauveria brongniartii* and *Metarhizium anisopliae* against sugarcane white grub, *Holotrichia serrata*. *Indian J Plant Prot*, **42**(4): 467-469.

Srikanth, J., Santhalakshmi, G., and Nirmala, R. 2011. An improved bioassay method for entomopathogenic fungi of sugarcane pests and its evaluation in studies of virulence in subcultures. *Sugar Tech,* **13**(2): 156-165. https://doi.org/10.1007/s12355-011-0080-6

Tabudlong, B. M., and Estoy, G. F. Jr. 2015. Field evaluation of entomopathogenic fungus, *Beauveria bassiana* (Balsamo) Vuillimin for the control of rice bug, *Leptocorisa oratorius* Fabricius. *Philipp Entomol*, **29**(2): 227.

Thamarai, C. C., Thilagaraj, R. W., and Nalini, R. 2011. Field efficacy of formulations of microbial insecticide *Metarhizium anisopliae* (Hypocreales: Clavicipitaceae) for the control of sugarcane white grub *Holotrichia serrata* F. (Coleoptera: Scarabaeidae). *J Biopestic*, **4**(2): 186-189. https://doi.org/10.57182/jbiopestic.4.2.186-189

Udarbe, R. D. 2015. Unpublished report. Department of Agriculture, Regional Field Office-XIII-Caraga, Agusan del Norte, Philippines. p. 2.

Udarbe, R. D., and Jabao, M. F. 2017. Unpublished report. Department of Agriculture, Regional Field Office-XIII-Caraga, Agusan del Norte, Philippines. p. 2.

Veeresh, G. K. 1974. Root grub control, a campaign in Kartanaka. *Whitegrub Newsl,* **1**: 17-18

Velavan, V., Sivakumar, G., Rangeshwaran, R., Sundararaj, R., and Sashidaran, T. O. 2017. *Metarhizium majus* and *Metarhizium robertsii* show enhanced activity against the coleopteran pests *Holotricha serrata* and *Oryctes rhinoceros*. *J Biol Control*, **31**(3): 135-145. https://doi.org/10.18311/jbc/2017/18078

Villacarlos, L. T., Mejia, B. S., and Patindol, R. A. 2004. Philippine entomopathogenic fungi II. Pathogenicity of common species to selected insect pests. *Philipp Agric Sci,* **87**(3): 266-275.

Visalakshi, M., Bhavani, B., and Rhao, S. G. 2015. Field Evaluation of entomopathogenic fungi against whitegrub *Holotrichia consanguinea* Blanch. in sugarcane. *J Biol Control*, **29**(2): 103-106. https://doi.org/10.18641/jbc/29/2/79824

AUTHOR CONTRIBUTION

Armando A. Ortiz – Field experiment, data gathering, and drafting of the manuscript. Maricel J. Didal – Field experiment data gathering, and drafting of the manuscript. Abel F. Wagas – Drafting of the manuscript. Orlando A. Calcetas – Morphological diagnosis, drafting, and revision of the manuscript. Joel L. Adorada – Morphological diagnosis and revision of the manuscript. Michelle S. Guerrero – Molecular diagnosis, drafting, and revision of the manuscript. Rochelle A. Quiñones – Molecular diagnosis and revision of the manuscript. Lara Paul Ebal – Statistical analysis and revision of the manuscript. All authors have read and agreed to the published version of the manuscript.