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Lethal effect of native *Metarhizium rileyi* (Farlow) Samson isolate to invasive fall armyworm, *Spodoptera frugiperda* (J.E. Smith), infesting corn in the Philippines

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Abstract Detection of fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), in the Philippines in 2019 prompted research on pest management solutions aside from insecticide application. This paper presents the bioefficacy of an entomopathogenic fungus in controlling *S. frugiperda*. A native *Metarhizium rileyi* Farlow (Samson) was successfully isolated from naturally infected *S. frugiperda* larvae collected in a corn field in Lucena, Quezon province in the Philippines. Laboratory bioassays were conducted to elucidate the virulence of *M. rileyi* to different life stages of *S. frugiperda*. Based on *t*-test, *M. rileyi* had no ovicidal activity. The larval instars of *S. frugiperda* were susceptible to *M. rileyi* with higher mortalities in early instars. Mycosed larvae were covered with white fungal growth and light olive green conidia. The mean time to larval death ranged from 5.10 to 8.67 days depending on conidial concentration and larval instar while lethal concentration (LC 50) was computed from 7.30×10^5 to 3.81×10^{13} conidia/ml. Based on *t*-test, reduced pupation and adult emergence were observed in fungal treated prepupa. However, there was no effect on the adult emergence of fungal treated pupa. Virulence of *M. rileyi* to *S. frugiperda* implies its potential use and integration in the Integrated Pest Management for *S. frugiperda* in the Philippines.

Keywords: Biocontrol, Pest management, Fall armyworm

Introduction

Biological control with the use of entomopathogens is a promising strategy against insect pests. Entomopathogenic fungi are very useful in pest management since indigenous isolates occur in the agricultural fields all over the world. These beneficial fungi cause infection to their host insect by initiating conidial attachment and penetration to the insect cuticle resulting to insect death. They subsist as saprophytes in organic matter and may also dwell as endophytes of several plants (Vega, 2008) making them valuable in pest

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management. Ramos *et al*. (2020) studied the endophytic establishment of *Beauveria bassiana* in different parts of the corn plant such as roots, stems, and leaves while *Metarhizium anisopliae* only colonized the roots of corn plant. Ahmad *et al*. (2020) noted the benefits of the systemic colonization of *M. robertsii* in host plants such as significant increase in height and above-ground biomass of Austrian winter pea and cereal rye.

With the detection of fall armyworm in the Philippines in 2019, crop protection solutions must be identified to mitigate this insect pest. Fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), is a biosecurity threat due to its polyphagous nature infesting more than 350 plant species (Rwomushana, 2019). This lepidopteran pest breached the biosecurity and archipelagic nature of the Philippines with reported infestation in corn (Navasero *et al*., 2019), sugarcane (Ocampo, 2020), and rice (Valdez *et al*., 2021). It can cause extensive defoliation and corn ear damages that may potentially contribute to yield loss. Hence, crop protection solutions beside insecticide application are warranted to lower the potential damage of this insect pest.

In the Philippines, laboratory bioassays have elucidated the virulence of several entomopathogenic fungi against *Spodoptera* species. A local isolate of *M. rileyi* isolate from *S. exigua* caused significant mortalities to 3rd larval instar of *S. exigua* (Montecalvo and Navasero, 2020) and cross infected to *S. frugiperda* larvae (Montecalvo and Navasero, 2021a). *M. anisopliae* and *B. bassiana* also exhibited varying degree of pathogenicity against different life stages of *S. frugiperda* (Montecalvo and Navasero, 2021b). Several research also reported the bioefficacy of entomopathogenic fungi against various insect pests, such as *M. rileyi* against *S. frugiperda* in Colombia (Bosa *et al*., 2004), *M. rileyi* isolates against *S. litura* (Rajan and Muthukrishnan, 2009), and *M. anisopliae* against *S. exigua* (Han *et al*., 2014). Induced mortalities due to entomopathogenic fungi can be attributed to the mechanical damage, depletion of nutrient resources and toxicosis, and production of toxin in the insect body (Sandhu *et al*., 2012).

Field survey and collection of mummified *S. frugiperda* were conducted with the hypothesis that a native isolate of entomopathogenic fungus occurred in the Philippines with a considerable virulence against this lepidopteran pest. This research work aimed to isolate a native entomopathogenic fungus and determine its bioefficacy against *S. frugiperda*. Susceptibility of eggs, larval instars, prepupa, and pupa were determined through mortality assays under laboratory condition.

Materials and methods

This research was conducted from November 2020 to February 2021 at the Mycology and Biological Control Laboratories at the National Crop Protection Center under the College of Agriculture and Food Science, University of the Philippines Los Baños, College, Laguna, Philippines.

Isolation from mummified S. frugiperda larvae

S. frugiperda larvae were collected in a corn field in Lucena, Quezon province, Philippines. The mummified larvae were subjected to fungal isolation. The larvae were surface sterilized in 1% (v/v) sodium hypochlorite for 1min and washed twice in sterile distilled water. Afterwhich, the larvae were blot dried in sterile tissue and placed in Petri plates with potato dextrose agar (PDA). The fungus was directly isolated from the sporulating larvae and transferred to PDA slants. Cultural and morphological characterizations were done to identify the fungus. Molecular identification of the isolate was also done to support the morphological and cultural characteristics. The DNA sequence of the fungal isolate was compared to known sequences by BLASTn program.

Mass rearing of S. frugiperda

The insect pest was collected in Barangay Patel, Gonzaga, Cagayan province, Philippines. It was reared in the laboratory and fed with fresh corn leaves. Several adults of *S. frugiperda* were paired to mass rear the insect pest. Neonates were harvested and fed with fresh corn leaves daily. Bioassays were conducted when the desired stage and number of individuals for testing were achieved.

Preparation of conidial suspension of M. rileyi

M. rileyi was cultured in PDA with 1% yeast extract (PDAY). Conidia were harvested by scraping the fungal growth from the culture and submerging to 0.1% Tween 80 solution. Conidial concentration was counted under compound microscope using Neubauer hemocytometer. The concentration of the conidial suspension was adjusted using 0.1% Tween 80 solution.

Bioassay with S. frugiperda eggs

Freshly laid egg masses of *S. frugiperda* were gathered from the insect mass rearing. Each egg mass approximately had 91-122 eggs. Egg masses were

treated with 1 x 10^9 conidia/ml of *M. rileyi* while the egg masses in control were treated with 0.1% Tween 80 solution. After treatment, the egg masses were incubated inside the Petri plate with moistened cotton. The Petri plates were sealed with parafilm and incubated in ambient condition. Neonates emerging from the egg masses were counted daily. Hatchability of eggs was also recorded daily. Percent egg hatchability was computed using the following formula: (hatched eggs in egg masses / total number of eggs in egg masses) x 100%. Each treatment was replicated four times.

Dose-mortality assays in S. frugiperda larvae

Larval instars of *S. frugiperda* were also exposed to *M. rileyi* using different conidial concentrations (1 x 10^5 to 1 x 10^9 conidia/ml). Corn leaves were surface sterilized and inoculated with conidial concentrations. After airdrying, these leaves were fed to larval instars. The larvae in control were fed with corn leaves sprayed with 0.1% Tween 80 solution. First and second larval instars were cultured at 10 individuals per Petri plate with moistened cotton. They were cultured singly as they molted to 3^{rd} larval instar. The other larval instars (3^{rd} to 6^{th}) were cultured individually in each Petri plate after treatment. Fresh corn leaves were fed to test larvae daily. The Petri plates were sealed with parafilm. The set-up was incubated in ambient condition. For each larval instar, each conidial concentration was replicated thrice with 10 larvae per replicate.

Mortality of the larvae were recorded daily. Percentage mortality was corrected using the equation: $M\ (\%) = [(t - c) / (100 - c)] \times 100$, where: M = corrected mortality; c = percentage mortality in control; t = percentage mortality in treatments (Abbott, 1925). Mean time to larval death was calculated using the formula of El-Hawary and Abd El-Salam (2009) wherein mean time to death (d) = $[(x_1y_1)+(x_2y_2)+(x_ny_n)]$ / total mortality, where: x = number of larvae that died on a given day; y = number of days of which the observation was made considering the time when the trial was initiated.

Effect of M. rileyi on adult emergence of S. frugiperda pupa and prepupa

Prepupa and 2-day old pupa of *S. frugiperda* were surface sterilized in 0.5% (v/v) sodium hypochlorite and two washes of sterile distilled water (Asi *et al*., 2013). After airdrying, the prepupa and pupa were dipped in *M. rileyi* suspension at 1 x 10^9 conidia/ml for 2 min with gentle shaking. In the control set-up, 0.1% Tween 80 solution was applied. The prepupa and pupa were kept inside a Petri plate with moistened cotton. The Petri plates were sealed with parafilm and incubated in ambient condition. Each treatment was replicated

three times with ten samples in each replicate. Pupation and adult emergence of prepupa were recorded daily. Adult emergence was noted daily for treated pupa.

Statistical design and analysis

The experimental set-up was arranged in CRD. The effect of *M. rileyi* to the different life stages of *S. frugiperda* was analyzed by performing One-way ANOVA and statistical analysis. Its effect on hatchability of eggs, pupation and adult emergence of prepupa, and adult emergence of pupa was compared using *t*-test ($P<0.05$). The virulence of *M. rileyi* to larval instars was analyzed using Tukey's Honestly Significant Difference test. Lethal concentration (LC) values were calculated using PriProbit ver. 1.63.

Results

Isolation of entomopathogenic fungus

An entomopathogenic fungus was successfully isolated from *S. frugiperda* larvae. Based on cultural and morphological characteristics, the fungus was identified as *M. rileyi* (Figure 1). The fungus has white to olive green colonies in PDAY. Its conidia were hyaline and ellipsoidal to cylindrical. Molecular characterization further confirmed the identity of the isolate.

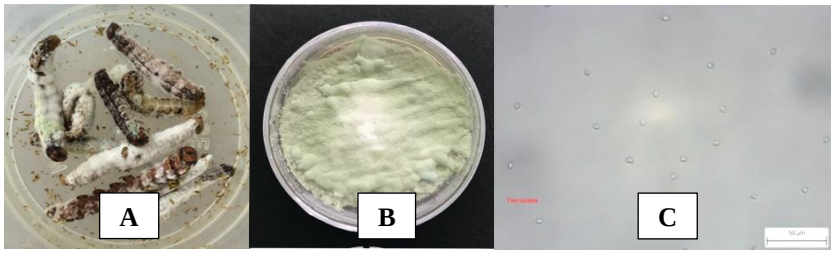

Figure 1. Native isolate of *Metarhizium rileyi* recovered from *Spodoptera frugiperda* larvae: mummified larvae collected in the corn field (a); fungal growth of *M. rileyi* in PDAY plate (b); and conidia of *M. rileyi* at 400x magnification (c)

Bioassay with S. frugiperda eggs

Based on *t*-test, *M. rileyi* had no ovicidal activity (Figure 2). *M. rileyi* did not affect hatchability of *S. frugiperda* eggs.

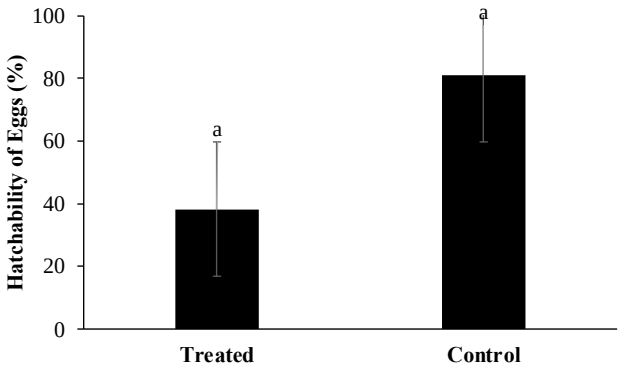

Figure 2. Effect of the native isolate of *Metarhizium rileyi* to hatchability of *Spodoptera frugiperda* eggs. Columns with the same letters are not significantly different by *t*-test ($P<0.05$). Bars represent standard error of the mean

Dose-mortality assays against S. frugiperda larvae

Cadavers of *S. frugiperda* larvae were initially stiff and subsequently covered with fungal growth (Figure 3). Mycosis started as white fungal growth covering the insect body and later with olive green sporulation. Partial and complete mummification of the larval body except the head and anal segments were observed.

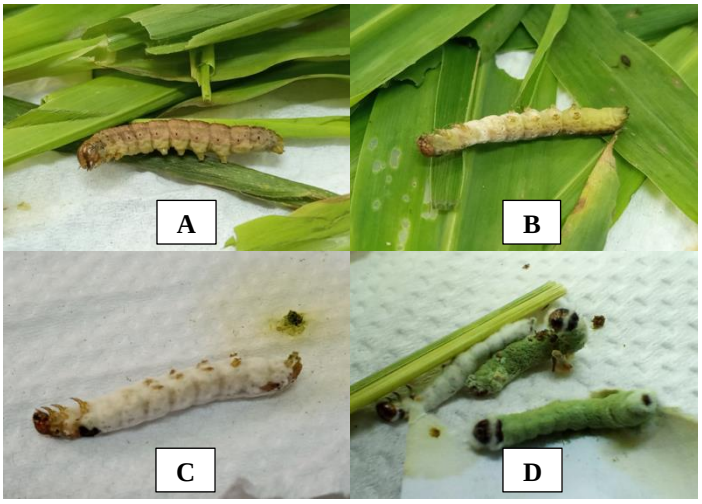

Figure 3. Progression of mycosis in *Spodoptera frugiperda* larvae due to infection by the native isolate of *Metarhizium rileyi*: stiff insect body (a), light fungal growth (b), white fungal growth covering the entire insect body (c), and light olive green conidial growth (d)

Larval instars of *S. frugiperda* had different sensitivity to *M. rileyi* (Figure 4a). Infection was evident at 1-2 days after treatment (DAT) with considerable increase in mortality starting at 6 DAT. *M. rileyi* induced faster mortality in early larval instars. Figure 4b shows the effect of using varying conidial concentrations. A general trend in disease progression was observed such that increasing conidial concentrations caused subsequent rise in larval mortalities.

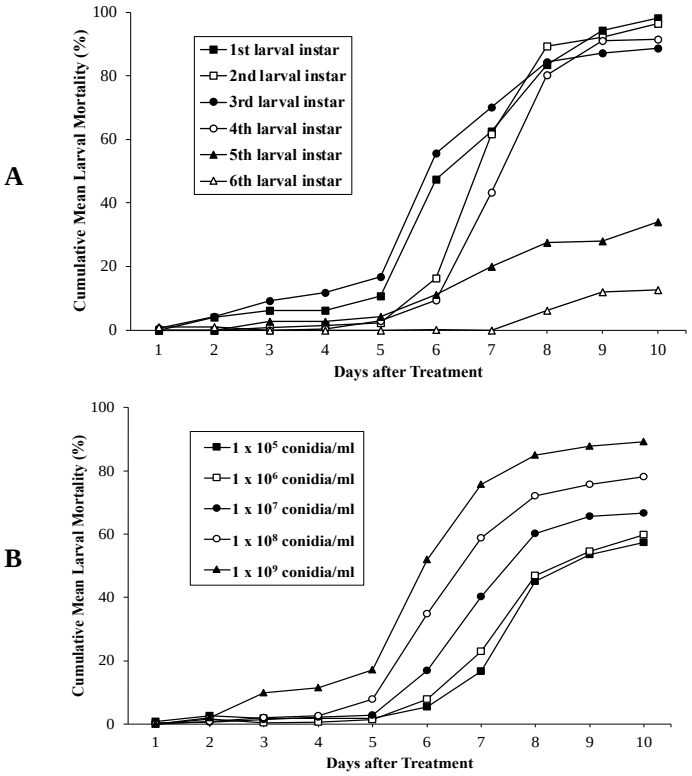

Figure 4. Cumulative mortality of larval instars of *Spodoptera frugiperda* that succumbed to the native isolate of *Metarhizium rileyi*: disease progression per larval instar (a) and effect of conidial concentrations on disease progression (b)

Based on Figure 5a, the larval instars had almost similar susceptibility to *M. rileyi* at 5 DAT with less than 20% mortality, however, after further incubation, the graph shows that early instars were more susceptible to fungal infection. At 7 DAT, mortalities in early instars reached up to 70% while 4th and 5th larval instars had 20-43% mortality and no mortality in 6th larval instar. At 10 DAT, 1st-4th larval instars had similar degree of susceptibility to *M. rileyi* with mortalities ranging from 89-98% while less than 40% mortality was recorded in 5th and 6th larval

instars. The conidial concentrations of *M. rileyi* also affected the larval mortality (Figure 5b). At 5 DAT, 1 x 10^8 and 1 x 10^9 conidia/ml caused considerable lethal infection. Interestingly, the conidial concentrations induced larval mortalities at the same rate at 7 and 10 DAT.

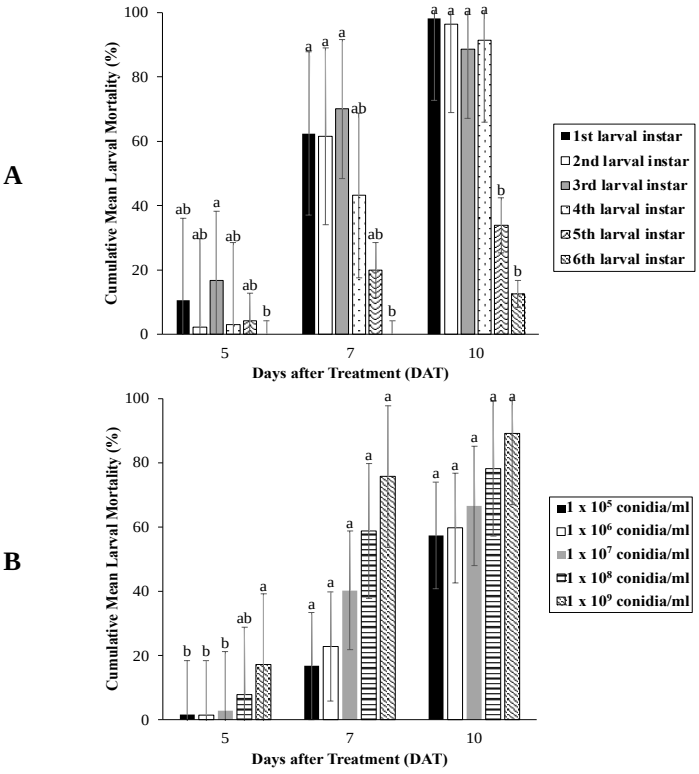

Figure 5. Cumulative mortality of larval instars of *Spodoptera frugiperda* that succumbed to the native isolate of *Metarhizium rileyi* at 5, 7, and 10 days after treatment: mortality per larval instar (a) and mortality as affected by conidial concentrations (b). Means with the same letter are not significantly different at Tukey's HSD (P<0.05). Bars represent standard error of the mean.

Mean time to larval death per instar ranged from 6.44 to 7.81 days (Figure 6). The highest conidial concentration (1 x 10^9 conidia/ml) in 1st larval instar resulted in faster mean time to larval death of 5.10 days while lowest conidial concentration (1 x 10^5 conidia/ml) resulted in 8.67 days in late larval instar (data not shown). Calculated lethal concentration 50 (LC50) at 7 DAT was also increasing from early to late larval instars ranging from 7.30 x 10^5 to 3.81 x 10^{13} conidia/ml, which can be correlated to the decreasing trend in mortality as the larva becomes older.

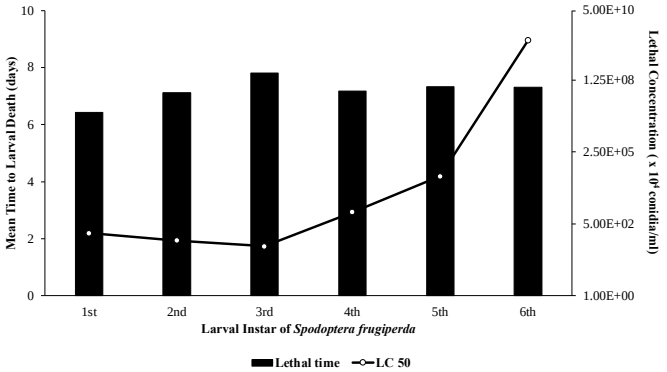

Figure 6. Mean time to larval death (days) and lethal concentration 50 (conidia/ml) estimates of the native isolate of *Metarhizium rileyi* infection to *Spodoptera frugiperda* larvae

Effect of M. rileyi on adult emergence of S. frugiperda pupa and prepupa

Lethal infection to prepupa and pupa was also assessed through laboratory bioassays. Based on *t*-test, *M. rileyi* significantly lowered the pupation and adult emergence of prepupa (Figure 7). Fungal infection resulted in 50% and 68% reduction in pupation and adult emergence, respectively. However, the fungus did not influence the adult emergence of treated pupa (Figure 8).

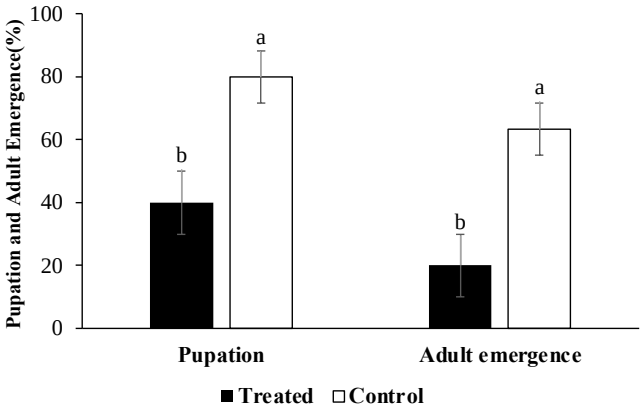

Figure 7. Pupation and adult emergence of *Spodoptera frugiperda* prepupa treated with the native isolate of *Metarhizium rileyi*. Pairs of columns with the same letters are not significantly different by *t*-test (P<0.05). Bars represent standard error of the mean

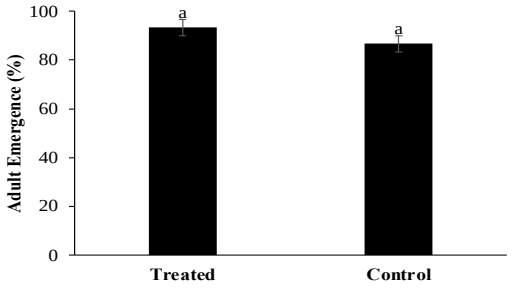

Figure 8. Adult emergence of *Spodoptera frugiperda* pupa treated with the native isolate of *Metarhizium rileyi*. Columns with the same letters are not significantly different by *t*-test ($P<0.05$). Bars represent standard error of the mean

Discussion

The present study highlighted the occurrence of a native isolate of *M. rileyi* against the invasive *S. frugiperda* in the Philippines. This entomopathogenic fungus was successfully isolated from the mycosed *S. frugiperda* larvae collected in a corn field in Quezon province, Philippines. A local isolate of *M. rileyi* infecting *S. exigua* was previously reported in Nueva Ecija province, Philippines (Montecalvo and Navasero, 2020). This entomopathogenic fungus is also indigenous in Colombia (Bosa *et al*., 2004), Cuba (Alvarez *et al*., 2018), and Mexico (Lezama-Gutierrez *et al*., 2001). Among the native isolates of *M. rileyi*, the isolate Nm-07 caused 100% mortality in second instar and lowest lethal times (Bosa *et al*., 2004). *Metarhizium* spp. were also recovered from soil in South Sumatra, Indonesia and found pathogenic to 3rd larval instar of *S. frugiperda* (Herlinda *et al*., 2020). These findings suggest that entomopathogenic fungi are naturally occurring, hence, they are considered valuable biological control agents against insect pests. *M. rileyi* infects 60 lepidopteran species that attack cotton, sunflower, corn, and soybean (Fronza *et al*., 2017). The production of enzymes, secondary metabolites, and large amount of extracellular polysaccharides contribute to its virulence and mycosis particularly during fungal adhesion and growth in insect cuticle.

In this research, laboratory bioassays were conducted to determine the bioefficacy of this native isolate of *M. rileyi* against the different biological stages of *S. frugiperda*. Results suggest that *M. rileyi* had no ovicidal activity. This finding conforms with the observation of Ramanujam *et al*. (2020) wherein *M. anisopliae* ICAR-NBAIR Ma-35 and *B. bassiana* ICAR-NBAIR Bb-45 did not show ovicidal activity. However, this observation contradicts our earlier result on the efficacy of *M. anisopliae* and *B. bassiana* in lowering the hatchability of

S. frugiperda eggs (Montecalvo and Navasero, 2021b) and to the findings of Akutse *et al*. (2019). This finding suggests that the fungus may not kill the eggs due to short period for fungal infection.

The symptomatology of fungal infection in larvae observed in this study was similar to the observations in *M. rileyi* infection in *S. exigua* (Montecalvo and Navasero, 2020) and its cross infection to *S. frugiperda* (Montecalvo and Navasero, 2021a). The research has also shown that larval instars of *S. frugiperda* had different sensitivity to native *M. rileyi* isolate with higher infection in early larval instars. Our results clearly showed the susceptibility of early larval instars to fungal infection caused by *M. rileyi* as supported by prior researches (Montecalvo and Navasero, 2021a and 2021b; Asi *et al*., 2013). Higher mortalities in early larval instars may be attributed to a more susceptible cuticular structure of the larvae. Fungal emergence occurs in less sclerotic regions of integument including intersegmental membranes and depends on the host insect and developmental stage during fungal infection (Mora *et al*., 2017).

This study also noted that increasing conidial concentrations caused subsequent rise in larval mortalities. In addition, it was apparent that high conidial concentrations resulted in faster mortality. Our results conform with the findings of Han *et al*. (2014) that larval mortalities in *S. exigua* increased with high conidial concentrations of *M. anisopliae* FT83 and *P. fumosoroseus* FG340 and median lethal times decreased with increase in conidial concentration. Similarly, Montecalvo and Navasero (2021a) reported significant lethal infection in higher conidial concentrations during cross infection of local *M. rileyi* from *S. exigua* to *S. frugiperda* larvae. Aside from causing larval mortality, *Metarhizium* spp. infection affected larval feeding, resulting in reduction in larval weight in *S. frugiperda* (Herlinda *et al*., 2020). They noted that the area of leaves eaten by treated *S. frugiperda* larvae significantly decreased, particularly at 5 DAT coinciding with the infection process of the fungus.

Calculated LC50 was also increasing from early to late larval instars, which can be correlated to the decreasing trend in mortality as the larva matures. The calculated LC50 (7.30×10^5 to 3.81×10^{13} conidia/ml) and median lethal time (5.10 to 8.67 days) in this study vary with the results in the previous research with LC50 of 1.44×10^5 to 9.36×10^8 conidia/ml and median lethal time of 4.51 to 8.89 days during the cross infection of *M. rileyi* from *S. exigua* to *S. frugiperda* (Montecalvo and Navasero, 2021a). It should be noted that different conidial concentrations were previously assayed such that conidial concentrations of 1×10^3 to 1×10^8 conidia/ml were used against 1st to 3rd larval instars while higher conidial concentrations for 4th to 6th larval instars (1×10^5 to 1×10^{10} conidia/ml). Difference in the results of the bioefficacy tests of *M.*

rileyi from *S. exigua* and native isolate of *M. rileyi* from *S. frugiperda* can also be due to the varying time when the experiments were conducted. These experiments were done in different months and different years, which may have variances in ambient temperature and relative humidity. Han *et al*. (2014) stressed that differences in bioefficacy of the entomopathogenic fungi can be attributed to the conidial concentration, temperature, and relative humidity. Likewise, same population but advanced generation of *S. frugiperda* was used in the bioassays, which may have differences in susceptibility to *M. rileyi* isolates. Likewise, genetic diversity and characteristic of the insect cuticle contribute to the varying effect of *M. rileyi* (Fronza *et al*., 2017).

This research also presented the significant effect of the native isolate of *M. rileyi* on *S. frugiperda* prepupa in terms of pupation and adult emergence but no effect on adult emergence of treated pupa. Montecalvo and Navasero (2021b) discovered that *M. anisopliae* and *B. bassiana* also caused slight effect on mortality and adult emergence of treated *S. frugiperda* prepupa. They also noted that these fungal isolates did not inhibit adult emergence of treated pupa but caused deformities in adults. Similarly, Asi *et al*. (2013) noted that *S. litura* pupae were less susceptible to entomopathogenic fungi, however, these biological control agents delayed the adult emergence. Herlinda *et al*. (2020) also observed unhealthy pupae and adults of *S. frugiperda* caused by *Metarhizium* spp. Schneider *et al*. (2013) discovered the morphological alterations in the pupae of *Diatraea saccharalis* during *M. anisopliae* infection through stereomicroscopic analysis. The interior of the prepupae after 72 h was empty with only cuticle and trachea suggesting that this integument is not totally chitinized making them vulnerable to fungal penetration through instar changes. They further suggested that the mechanical pressure and enzymatic degradation cause abnormalities in the integument of *D. saccharalis* pupae during *M. anisopliae* infection. These findings revealed that infection caused by entomopathogenic fungi does not affect the pupa but may affect the resulting adults that may have implication on the flight, mating, and oviposition behavior of the succeeding generation.

In summary, this research discovered a native *M. rileyi* isolate that has varying effect on the biological stages of *S. frugiperda*. Larval instars and prepupa were the susceptible stages for fungal infection. Prior studies have noted the bioefficacy of *M. rileyi* and other entomopathogenic fungi against several insect pests. Further research should be undertaken to investigate the persistence and survival of *M. rileyi* in the agroecosystem. A comparative study on the genetic difference and bioefficacy of native isolates of *M. rileyi* from *S. exigua* and *S. frugiperda* can also be undertaken. Diversity studies on *M. rileyi* are necessary to better understand the distribution and genetic variation in the Philippines. Likewise,

studies on mass production and formulation are needed so that this entomopathogenic fungus can be utilized in the agricultural field.

Acknowledgements

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References

Abbott, W. S. (1925). A method of computing the effectiveness of an insecticide. Journal of Economic Entomology, 18:265-267.

Ahmad, I., Jimenez-Gasco, Md. M., Luthe, D. S. and Barbercheck, M. E. (2020). Systemic colonization by *Metarhizium robertsii* enhances cover crop growth. Journal of Fungi, 6:64. http://dx.doi.org/10.3390/jof6020064.

Akutse, K. S., Kimemia, J. W., Ekesi, S., Khamis, F. M., Ombura, O. L. and Subramanian, S. (2019). Ovicidal effects of entomopathogenic fungal isolates on the invasive fall armyworm *Spodoptera frugiperda* (Lepidoptera: Noctuidae). Journal of Applied Entomology, 143:626-634.

Alvarez, S. P., Guerrero, A. M., Duarte, B. N. D., Tapia, M. A. M., Medina, J. A. C. and Rodriguez, Y. D. (2018). First report of a new isolate of *Metarhizium rileyi* from maize fields of Quivican, Cuba. Indian Journal of Microbiology, 58:222-226.

Asi, M. R., Bashir, M. H., Afzal, M., Zia, K. and Akram, M. (2013). Potential of entomopathogenic fungi for biocontrol of *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae). Journal of Animal and Plant Sciences, 23:913-918.

Bosa, C. F., Chavez, D., Torres, L., Paris, A., Villamizar, L. and Cotes A. M. (2004). Evaluation of native isolates of *Nomuraea rileyi* for the control of *Spodoptera frugiperda* (Lepidoptera: Noctuidae). Revisita Colombiana de Entomologia, 30:93-97.

El-hawary, F. M. and Abd El-salam, A. M. E. (2009). Laboratory bioassay of some entomopathogenic fungi on *Spodoptera littoralis* (Boisd.) and *Agrotis ipsilon* (Hufn.) larvae (Lepidoptera: Noctuidae). Egyptian Academic Journal of Biological Sciences, 2:1-4.

Fronza, E., Specht, A., Heinzen, H. and De Barros, N. M. (2017). *Metarhizium* (*Nomuraea*) *rileyi* as biological control agent. Biocontrol Science and Technology, 27:1243-1264.

Han, J. H., Jin, B. R., Kim, J. J. and Lee, S. Y. (2014). Virulence of entomopathogenic *Metarhizium anisopliae* and *Paecilomyces fumosoroseus* for the microbial control of *Spodoptera exigua*. Mycobiology, 42:385-390.

Herlinda, S., Octariati, N., Suwandi, S. and Hasbi, H. (2020). Exploring entomopathogenic fungi from South Sumatra (Indonesia) soil and their pathogenicity against a new invasive maize pest, *Spodoptera frugiperda*. Bioversitas Journal of Biological Diversity, 21:2955-2965.

Lezama-Gutierrez, R., Hamm, J. J., Molina-Ochoa, J., Lopez-Edwards, M., Pescador-Rubio, A., Gonzalez-Ramirez, M. and Styer, E. L. (2001). Occurrence of entomopathogens of

Spodoptera frugiperda (Lepidoptera: Noctuidae) in the Mexican States of Michoacan, Colima, Jalisco and Tamaulipas. Florida Entomologist, 84:23-30.

Montecalvo, M. P. and Navasero, M. M. (2021a). *Metarhizium* (=*Nomuraea*) *rileyi* (Farlow) Samson from *Spodoptera exigua* (Hübner) cross infects fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) larvae. Philippine Journal of Science, 150:193-199.

Montecalvo, M. P. and Navasero, M. M. (2021b). Comparative virulence of *Beauveria bassiana* (Bals.) Vuill. and *Metarhizium anisopliae* (Metchnikoff) Sorokin to *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) in the laboratory. Journal of International Society for Southeast Asian Agricultural Sciences, 27:15-26.

Montecalvo, M. P. and Navasero, M. M. (2020). Effect of entomopathogenic fungus *Metarhizium* (*Nomuraea*) *rileyi* (Farl.) Samson on the third instar larvae of the onion armyworm, *Spodoptera exigua* Hübner (Lepidoptera: Noctuidae), under laboratory conditions. Philippine Agricultural Scientist, 103:140-145.

Mora, M. A. E., Castilho, A. M. C. and Fraga, M. E. (2017). Classification and infection mechanism of entomopathogenic fungi. Arquivos do Instituto Biologico, 84.

Navasero, M. V., Navasero, M. M., Burgonio, G. A. S., Ardez, K. P., Ebuenga, M. D., Beltran, M. J. B., Bato, M. B., Gonzales, P. G., Magsino, G. L., Caoili, B. L., Barrion-Dupo, A. L. A. and Aquino, M. F. G. M. (2019). Detection of the fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) using larval morphological characters, and observations on its current local distribution in the Philippines. Philippine Entomologist, 33:171-184.

Ocampo, K. R. (2020). Fall armyworm pest threatens PH corn production. Retrieved from https://business.inquirer.net/

Rajan, T. S. and Muthukrishnan, N. (2009). Pathogenicity of *Nomuraea rileyi* (Farlow) Samson isolates against *Spodoptera litura* (Fabricius). Journal of Biological Control, 23:17-20.

Ramanujam, B., Poornesha, B. and Shylesha, A. N. (2020). Effect of entomopathogenic fungi against invasive pest *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) in maize. Egyptian Journal of Biological Pest Control, 30:1-5.

Ramos, Y., Taibo, A. D., Jimenez, J. A. and Portal, O. (2020). Endophytic establishment of *Beauveria bassiana* and *Metarhizium anisopliae* in maize plants and its effect against *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) larvae. Egyptian Journal of Biological Pest Control, 30:1-6. https://doi.org/10.1186/s41938-020-00223-2

Rwomushana, I. (2019). *Spodoptera frugiperda* (fall armyworm). Invasive species compendium. Wallingford, UK: CABI: DOI:10.1079/ISC.29810.20203373913.

Sandhu, S. S., Sharma, A. K., Beniwal, V., Goel, G., Batra, P., Kumar, A., Jaglan, S., Sharma, A. K. and Malhotra S. 2012. Myco-biocontrol of insect pests: factors involved, mechanism, and regulation. Journal of Pathogens, 2012:1-10.

Schneider, L. C. L., Silva, C. V., Pamphile, J. A. and Conte, H. (2013). Infection, colonization and extrusion of *Metarhizium anisopliae* (Metsch) Sorokin (Deuteromycotina: Hyphomycetes) in pupae of *Diatraea saccharalis* F. (Lepidoptera: Crambidae). Journal of Entomology and Nematology, 5:1-9.

Valdez, E. M., Rillon, G. S., Donayre, D. K., M., Martin, E. C., dela Cruz, K. B., Sandoval, F. R., Joshi, R. C., Quilang, E. J. P., Faheem, M. and Annamalai, S. (2021). Fall armyworm (*Spodoptera frugiperda*) (J.E. Smith): A first record of damage on rice in the Philippines. Poster paper presented during the Online Conference "Developing smallholder-oriented IPM strategies for fall armyworm (*Spodoptera frugiperda* Smith) management" on 24-26 August 2021.

Vega, F. (2008). Insect pathology and fungal endophytes. Journal of Invertebrate Pathology, 98: 277-279.

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