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Susceptibility of Pre-adult Biological Stages of *Mythimna separata* (Walker) (Lepidoptera: Noctuidae) to Three Entomopathogenic Fungi (Hypocreales)

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Armyworms are polyphagous insect pests of agricultural crops. Their population is usually managed below threshold level using insecticides. However, a single control measure is insufficient to attain a sustainable pest management. Hence, this research was conducted to assess the three species of entomopathogenic fungi as potential biological control agents against *Mythimna separata* (Walker) or paddy armyworm. Dose mortality assays were conducted to determine the pathogenicity of *Beauveria bassiana* (Bals.) Vuill., *Metarhizium anisopliae* (Metschn.) Sorokin, and *Metarhizium rileyi* (Farlow) Kepler, S.A. Rehner and Humber against various biological stages of *M. separata* including egg, 1st to 6th larval instars, prepupa, and pupa. These entomopathogenic fungi did not have ovicidal activity but affected the survivorship of the resulting neonates. Exposure of eggs to *B. bassiana*, *M. anisopliae*, and *M. rileyi* infected 64, 60, and 70% of the resulting neonates, respectively. Adult emergence was also reduced in fungal-treated (29% in *B. bassiana*, 10% *in M. anisopliae*, and 10% in *M. rileyi*) as compared with 64% in control. At 7 d after treatment, *M. rileyi* applied at 1 x 10^5 to 1 x 10^9 conidia/mL was the most pathogenic causing a mean larval mortality of up to 70% as compared to *B. bassiana* and *M. anisopliae* with less than 10% mortality. Among the larval instars, the 1st, 2nd, and 3rd were the most susceptible to fungal infection. Conidial concentrations (1 x 10^8 and 1 x 10^9 conidia/ml) induced the highest larval mortalities as compared to the lower conidial concentrations (1 x 10^5 and 1 x 10^7 conidia/mL) of the entomopathogenic fungi. The prepupal and pupal stages were also slightly susceptible to the entomopathogenic fungi. These findings showed the pathogenic effect of the entomopathogenic fungi particularly *M. rileyi* to *M. separata*.

Keywords: biological control, mycobiocontrol, crop protection, paddy armyworm

INTRODUCTION

Agricultural pests affect productivity of crops. Armyworms are one of the devastating insect pests attacking diverse crops. Outbreaks of armyworms occur with heavy defoliation (University of Minnesota Extension 2022). The adults of armyworms also have a high migratory behavior which makes an agricultural field at high risk for larval feeding damage under favorable conditions. In corn, feeding of armyworms results in skeletonized leaves and damage in the tassels and ears (University of California Agriculture and Natural Resources 2019).

One of the important armyworm species is *Mythimna separata* (Walker) or paddy armyworm. *M. separata* occurs in Asia and Australian continents with confirmed outbreaks in India, China, Japan, Australia, New Zealand, Fiji, Bangladesh, and Thailand (CABI 2019; Sharma and Davies 1983). Uichangco recorded its first infestation in corn in the Philippines in 1959 (Cadapan and Sanchez 1972). This armyworm species infests about 100 plant species in the Philippines (Cadapan and Sanchez 1972; Catindig et al. 1994). The larvae damage the leaves and growing panicles of rice resulting in defoliation and panicle injury (Department of Agriculture Regional Field Unit IX 2012). Navasero et al. (2022) documented that the total development period from egg to adult is 28.95 ± 1.89 d and 28.43 ± 1.55 d for the male and female, respectively. A single generation of *M. separata* larvae causes injury to corn usually coinciding grain filling from January to March (Malik et al. 2013). It has been reported in several provinces in the Philippines, such as the infestation in several crops in Cebu (Letigio 2019) and rice in Nueva Ecija (Santiago et al. 1997). Biological control agents such

as *M. anisopliae, B. bassiana, Bacillus thuringiensis* Berliner, and nucleopolyhedrovirus (NPV) were recommended to manage this pest (Letigio 2019).

Lepidopteran species and other insect pests are primarily managed through insecticide application. However, the use of biological control agents offers a safer, sustainable, and more environment-friendly means to control insect pests. Natural enemies have also been documented such as parasites, predators, and pathogens against *M. separata* (Sharma and Davies 1983; CABI 2019). Various entomopathogenic fungi are pathogenic against armyworm species such as *Spodoptera exigua* (Hübner) and *S. frugiperda* (J.E. Smith) (Bosa et al. 2004; Montecalvo and Navasero 2020, 2021a, 2021b; Montecalvo et al. 2022a and 2022b) These beneficial fungi cause infection to their host insect by initiating conidial attachment and penetration to the insect cuticle resulting to insect death. They also subsist as saprophytes in organic matter and may also dwell as endophytes of several plants. Considering the potential of entomopathogenic fungi, this research work aimed to determine the entomopathogenic fungi bioefficacy against *M. separata*. In the Philippines, there are no published report on the efficacy of entomopathogenic fungi against *M. separata*.

MATERIALS AND METHODS

The study was conducted at the Mycology and Biological Control Laboratories of National Crop Protection Center (NCPC), College of Agriculture and Food Science, University of the Philippines Los Baños (UPLB), College, Laguna, Philippines.

Laboratory Rearing of *M. separata*

Larvae of *M. separata* were collected in the corn experimental field in NCPC. Male and female adults were paired and mated in mylar cages. Egg masses were harvested from the cages and placed in rearing pans with fresh corn leaves. Larvae were fed with fresh corn leaves daily. Bioassays were conducted when the desired stage and number of individuals for testing were attained.

Production of Entomopathogenic Fungi

The entomopathogenic fungi *B. bassiana, M. anisopliae*, and *M. rileyi* were revived and subcultured in PDA. *B. bassiana* was isolated from rice bug (*Leptocorisa oratoruis* (Fabricius), while *M. anisopliae* from rice black bug (*Scotinophara coarctata* (Fabricius), and *M. rileyi* from onion armyworm, *S. exigua*. Stock cultures of these fungal isolates are stored in the refrigerator and passed through insect (*M. separata*) to maintain virulence. These entomopathogenic fungi were reinfected to larvae and reisolated to ensure virulence of fungal cultures for the bioassays. Conidia were harvested from the fungal cultures by scraping the fungal growth and suspending in 0.1% Tween 80 solution. Conidial count was determined through microscopic observation using Neubauer improved hemacytometer. Several conidial concentrations were prepared for the bioassays by diluting the stock conidial suspension with 0.1% Tween 80 solution.

Laboratory Bioassays of Entomopathogenic Fungi Against Various Biological Stages of *M. separata*

Eggs, larvae, prepupae, and pupae of *M. separata* were subjected to dose-mortality assays. Egg masses that were freshly laid were counted under Zeiss stemi stereo microscope. Egg masses with approximately 60-80 eggs were used in the experiment. Egg masses were mist sprayed using hand-held sprayer with 1 x 10^9 conidia/ml of each entomopathogenic fungus while 0.1% Tween 80 solution was sprayed in control set-up. Each set-up was replicated with seven egg masses and incubated in a sterile Petri plate with moistened cotton at ambient condition. Hatchability of eggs was counted daily. Neonates hatched from these egg masses were transferred to a sterile Petri plate and fed with fresh corn leaves daily. Mummification of the cadavers infected with entomopathogenic fungi was also noted.

Larval instars (1^{st} to 6^{th}) were exposed to the entomopathogenic fungi using different conidial concentrations (1 x 10^5 to 1 x 10^9 conidia/ml) and 0.1% Tween 80 solution (control). These conidial concentrations were sprayed to surface sterilized corn leaves and fed to larval instars. Surface sterilization of corn leaves was done by washing in 0.05% sodium hypochlorite for 10 min followed by washing twice in sterile distilled water for 1 min each. The 1^{st} and 2^{nd} larval instars were cultured with 10 individuals and single cultured thereafter upon reaching the 3^{rd} larval instar to ensure proper growth and development. The 3^{rd} to 6^{th} larval instars were cultured singly in each Petri plate during the experiment. Those larvae which were fed with corn leaves treated with entomopathogenic fungi or 0.1% Tween 80 solution were placed in sterile Petri plates with moistened cotton. These larval instars were fed with fresh surface-sterilized corn leaves daily. Each treatment was replicated thrice with 10 individuals per replicate. Larval mortality was recorded daily and corrected using Abbott's formula (Abbott 1925).

Prepupa and pupa were also bioassayed following the methodology of Asi et al. (2013). These prepupa and pupa were surface-sterilized in 0.5% (v/v) sodium hypochlorite solution and in two washes of sterile distilled water. After airdrying, they were dipped in the fungal suspension at 1 x 10^9 conidia/ml of the entomopathogenic fungi or in 0.1%

Tween 80 solution (control). Treated individuals were placed in sterile cups with moistened cotton. Thirty (30) individuals were treated for each treatment. Adult emergence was recorded daily.

Lethal concentration values were calculated using PriProbit ver 1.63. Mean time to death was calculated using the formula: mean time to death (d) = $[(x_1y_1) + (x_2y_2) + (x_ny_n)]$/ total mortality, where: x = number of larvae died on a given day; y = number of days the observation was made considering the time when the trial was initiated (El-Hawary and Abd El-Salam 2009).

Statistical Design and Analysis

Laboratory bioassays were arranged in Completely Randomized Design. The virulence of the entomopathogenic fungi to various biological stages of *M. separata* were compared by analysis of variance using Tukey's honest significant difference (HSD) test.

RESULTS

Effect of Entomopathogenic Fungi on *M. separata* Eggs and Resulting Neonates

The three entomopathogenic fungi did not significantly affect the hatchability of *M. separata* eggs with 13 to 23% hatchability in fungal-treated egg masses in comparison with 24% hatchability in control (Fig. 1). Fungal growth was observed in unhatched egg masses treated with *B. bassiana* and *M. anisopliae*. The resulting neonates from the treated egg masses succumbed to fungal infection mostly at larval stage (65%) and very few successfully emerged into adults (16%) (Fig. 2). On the other hand, 64% of the resulting neonates from the control completed their life cycle and successfully emerged into adults. About 64% of those neonates from *B. bassiana* treated egg masses were infected at larval stage and only 29% emerged into adults. On the other hand, *M. anisopliae* and *M. rileyi* caused infection to 60 and 70% of the larvae, respectively. Only 10% of the resulting neonates emerged as adults in those treated with these *Metarhizium* spp. isolates. In contrast, 64% adult emergence was recorded in the resulting larvae in the control.

Effect of Entomopathogenic Fungi on Larval Instars of *M. separata*

The entomopathogenic fungi effectively caused infection against the larval instars of *M. separata*. The progression of fungal infection in 1st to 6th larval instars is shown in Fig. 3. Infection started at 2 d after treatment with varying trend among entomopathogenic fungi. *M. rileyi* was the most pathogenic causing significant mortalities to the larvae with increasing trend of disease progression up to 70% mortality at 7 d after treatment. Mortality in early larval instars (1st to 3rd) signficantly surged at 6 − 7 d after treatment. On the other hand, *B. bassiana* and *M. anisopliae* caused lower larval mortalities which were below 10%.

The effect of various conidial concentrations (1 x 10^5 to 1 x 10^9 conidia/ml) of entomopathogenic fungi on mean mortality of all larval instars was presented in Fig. 4. Conidial concentrations of *M. rileyi* consistently inflicted the highest mortality with a mean mortality of 13 to 47% from 1 x 10^5 to 1 x 10^9 conidia/ml, respectively. *B. bassiana* and *M. anisopliae* only caused 3 − 8% and 3 − 7% mortalities respectively, despite using high conidial concentrations. Among these conidial concentrations, 1 x 10^8 and 1 x 10^9 conidia/ml induced considerable larval mortalities as compared with lower conidial concentrations of the entomopathogenic fungi.

Various larval instars had varying degree of susceptibility to the entomopathogenic fungi (Fig. 5). Among the entomopathogenic fungi, *M. rileyi* was the most pathogenic causing the highest larval mortalities in 1st to 5th larval instars while *B. bassiana* caused significant mortalities in 6th larval instar although it was minimal

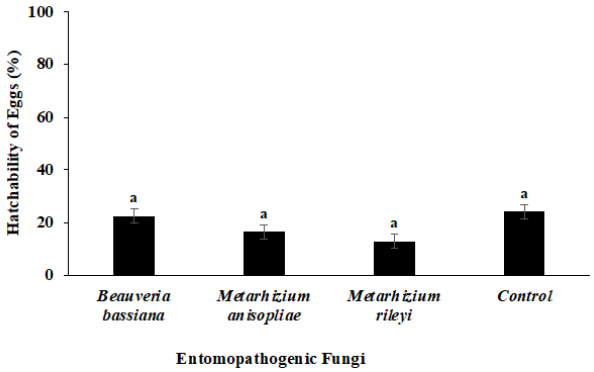

Fig. 1. Hatchability of *Mythimna separata* eggs as affected by entomopathogenic fungi. Bars represent the standard error of the means. Columns with the same letters are not significantly different in HSD ($P < 0.05$).

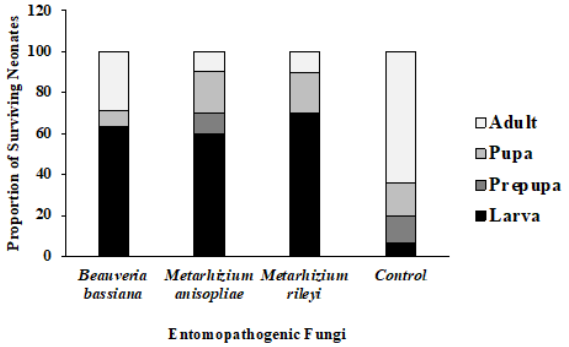

Fig. 2. Proportion of survivorship of larvae hatching from treated eggs.

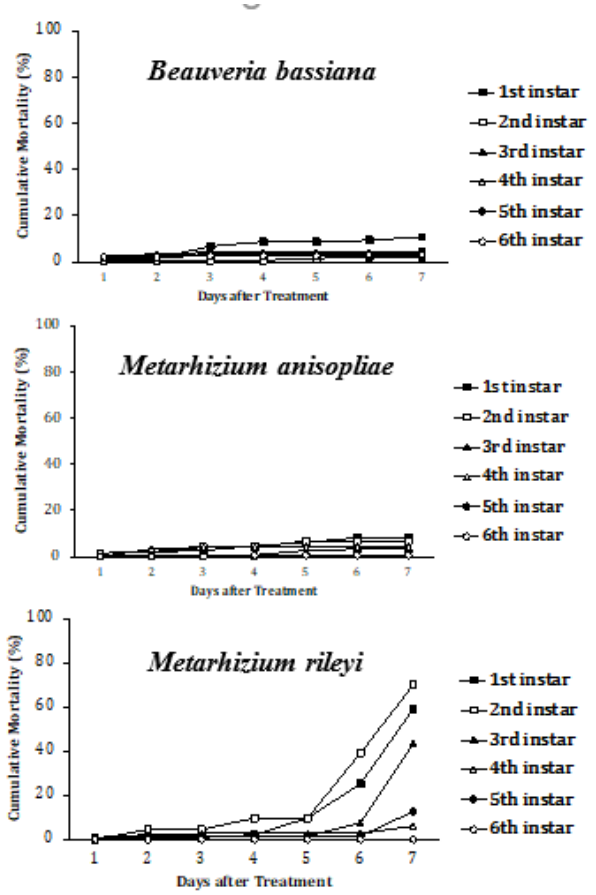

Fig. 3. Disease progression in larval instars of *Mythimna separata* as affected by three species of entomopathogenic fungi: *Beauveria bassiana*, *Metarhizium anisopliae*, and *Metarhizium rileyi* applied at 1 x 10^5 to 1 x 10^9 conidia/ml.

(3%). Among the larval instars, 1st, 2nd, and 3rd larval instars were the most susceptible to fungal infection.

Tables 1 and 2 summarize the calculated lethal concentration (LC_{50}) and mean lethal time due to exposure of *M. separata* larvae to entomopathogenic fungi. No trend was observed among the larval instars. LC_{50} values ranged from 2.07 x 10^{10} to 5.97 x 10^{141} in *B. bassiana*, 1.45 x 10^{11} to 9.82 x 10^{270} in *M. anisopliae*, and 2.09 x 10^5 to 7.16 x 10^{256} in *M. rileyi*. On the other hand, mean lethal time was calculated from 5.40 to 11.27 d in *B. bassiana*, 4.32 to 9.75 d in *M. anisopliae*, and 6.57 to 9.68 d in *M. rileyi*.

Effect of Entomopathogenic Fungi on Prepupa and Pupa of *M. separata*

The entomopathogenic fungi significantly reduced adult emergence of prepupa and pupa of *M. separata* (Fig. 6). *M. anisopliae* and *M. rileyi* applied at 1 x 10^9 conidia/ml lowered adult emergence in prepupa by 67 and 89%, respectively. Among these entomopathogenic fungi, only *M. anisopliae* significantly affected adult emergence in pupa with 88% reduction. Mummification of *M. separata* confirmed fungal infection (Fig. 7). Partial or complete mummification of larval cadavers were observed in larval instars infected with *M. rileyi* as compared with healthy larvae (Fig. 7a and b). These cadavers were covered with white fungal growth and light olive green sporulation. On the other hand, fungal infection in pupa due to *B. bassiana* and *M. anisopliae* exhibited white fluffy fungal growth (Fig. 7c) and green sporulation (Fig. 7d), respectively.

DISCUSSION

This paper presents the effect of entomopathogenic fungi to pre-adult biological stages of *M. separata*, which is an important pest of agricultural crops. The eggs of *M. separata* were not susceptible to the three entomopathogenic fungi tested (*B. bassiana*, *M. anisopliae*, and *M. rileyi*). This conforms to the previous findings that

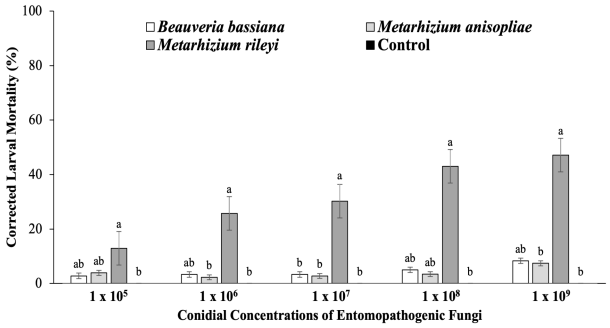

Fig. 4. Influence of various conidial concentrations of *Beauveria bassiana*, *Metarhizium anisopliae*, and *Metarhizium rileyi* on susceptibility of 1st to 6th larval instars of *Mythimna separata* in terms of mean larval mortality at 7 d after treatment. Bars represent the standard error of the means. Columns with the same letters are not significantly different in HSD (*P* < 0.05).

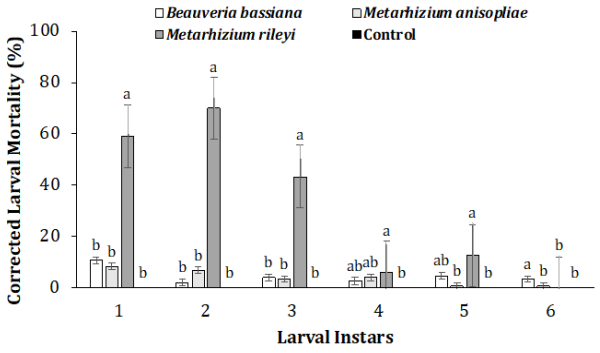

Fig. 5. Corrected larval mortality of *Mythimna separata* that succumbed to infection due to entomopathogenic fungi: *Beauveria bassiana*, *Metarhizium anisopliae*, and *Metarhizium rileyi* at 7 d after treatment. Bars represent the standard error of the means. Columns with the same letters are not significantly different in HSD (*P* < 0.05).

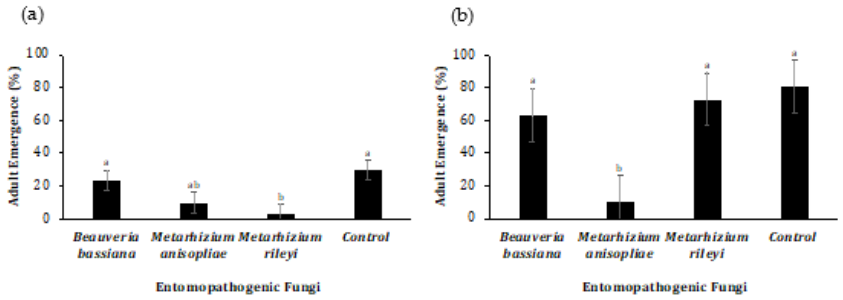

Fig. 6. Adult emergence of treated (a) prepupa and (b) pupa of *Mythimna separata* that were treated with entomopathogenic fungi: *Beauveria bassiana*, *Metarhizium anisopliae*, and *Metarhizium rileyi*. Bars represent the standard error of the means. Columns with the same letters are not significantly different in HSD ($P < 0.05$).

M. rileyi isolate did not have ovicidal activity on *S. exigua* (Montecalvo et al. 2022a), however, this contradicts the results that *B. bassiana* and *M. anisopliae* isolates slightly reduced hatchability of *S. frugiperda* eggs (Montecalvo and Navasero 2021a). Likewise, *M. anisopliae* L6, *Isaria fumosorosea* 32, and *B. bassiana* infected the freshly laid eggs of *S. litura* (Asi et al. 2013). Differences in the results suggest that egg mortalities depend on the conidial concentration and morphology of the eggs, which are covered with hydrophobic scales (Navon and Ascher 2000) that repel moisture needed for conidial germination. Higher mortality was observed in unscaled than scaled eggs of *S. litura* treated with entomopathogenic fungi (Anand and Tiwary 2009).

Even though the three entomopathogenic fungi tested against *M. separata* did not have ovicidal activity, a significant number of the resulting neonates from the fungal-treated egg masses succumbed to fungal infection at larval stage. This conforms with the findings on *S. exigua* eggs exposed to *M. rileyi* (Montecalvo et al. 2022a) and *S. frugiperda* exposed to *M. rileyi* (Montecalvo et al. 2022b), wherein larval instars hatched from the treated egg masses had fungal infection. Similarly, larval instars of *S. littoralis* emerging from treated egg mass succumbed to *M. rileyi* infection suggesting contamination of 1st larval instar with the fungus in the treated egg mass upon feeding in the chorions or maybe infected by the germinating fungus in the egg integument (Rodriguez-Rueda and Fargues 1980).

Table 1. Calculated lethal concentration (LC_{50}) values based on 95% fiducial limit of the laboratory bioassays of entomopathogenic fungi against larval instars of *Mythimna separata*.

Larval Instar	Calculated LC_{50} values (conidia/ml)		
	Beauveria bassiana	*Metarhizium anisopliae*	*Metarhizium rileyi*
1	2.07×10^{10}	4.59×10^{20}	3.23×10^{6}
2	4.29×10^{14}	1.45×10^{11}	2.09×10^{5}
3	4.66×10^{15}	1.92×10^{70}	2.04×10^{12}
4	1.01×10^{26}	2.72×10^{17}	5.43×10^{21}
5	5.97×10^{141}	4.73×10^{18}	3.38×10^{9}
6	2.05×10^{25}	9.82×10^{270}	7.16×10^{256}

Table 2. Calculated mean lethal time values of the laboratory bioassays of entomopathogenic fungi against larval instars of *Mythimna separata*.

Larval Instar	Calculated Mean Lethal Time (days ± SE)		
	Beauveria bassiana	*Metarhizium anisopliae*	*Metarhizium rileyi*
1	6.27 ± 1.68	5.65 ± 1.14	7.29 ± 0.57
2	8.26 ± 0.26	4.32 ± 0.96	6.57 ± 0.52
3	11.27 ± 1.09	6.46 ± 1.19	8.14 ± 0.24
4	9.72 ± 0.76	9.75 ± 1.35	9.68 ± 0.73
5	7.51 ± 1.21	8.68 ± 1.49	7.02 ± 1.48
6	5.40 ± 0.99	7.33 ± 1.14	8.16 ± 0.26

This paper also showed the susceptibility of larval instars of *M. separata* to the entomopathogenic fungi particularly to *M. rileyi*. It was also evident that early larval instars were more susceptible to fungal infection while higher conidial concentrations caused more severe infection. The results clearly showed the susceptibility of *M. separata* larval instars to fungal infection as supported by prior research (Montecalvo and Navasero 2020, 2021a, 2021b; Montecalvo et al. 2022a, 2022b). However, low mortalities were recorded in this bioassay in comparison with the previous bioassays using the same isolates against other lepidopteran species. In this study, infection of *B. bassiana* and *M. anisopliae* resulted only to below 10% mortality unlike in the previous experiment wherein 24 − 97% and 23 − 61% mortalities were recorded in the 1st to 6th larval instars of *S. frugiperda*, respectively (Montecalvo and Navasero 2021a). Likewise, even though *M. rileyi* was the most pathogenic among the three isolates, it only induced up to 70% mortality, which is quite low compared to the previous results. *M. rileyi* applied at 1×10^{7} and 1×10^{8} conidia/ml caused 100% mortality to 3rd larval instar of *S. exigua* (Montecalvo and Navasero 2020). This isolate applied at 1×10^{7} and 1×10^{8} conidia/ml also cross-infected to *S. frugiperda* larvae with up to 77 to 100% mortality in the 1st to 3rd larval instars and significant mortalities in late larval instars (Montecalvo and Navasero 2021b).

Lower mortalities in *M. separata* than in *S. exigua* (Montecalvo and Navasero 2020; Montecalvo et al. 2022a) and *S. frugiperda* (Montecalvo and Navasero 2021a, 2021b;

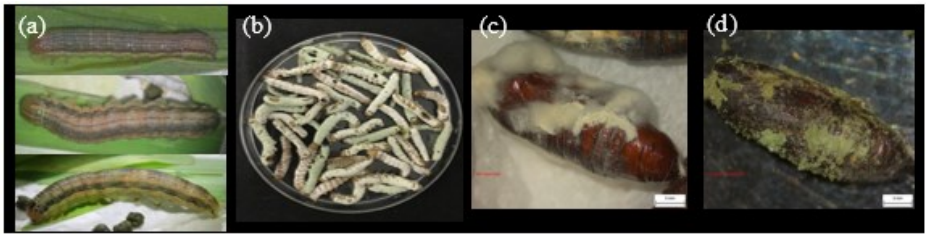

Fig. 7. Healthy (a) and mummified *Mythimna separata* (b) larvae due to *Metarhizium rileyi* and pupae mycosed with (c) *Beauveria bassiana* and (d) *Metarhizium anisopliae*.

Montecalvo et al. 2022b) may also be due to the larger size of *M. separata* larvae as compared with the two *Spodoptera* species. Larval size may have affected the susceptibility of *M. separata* to fungal infection since its larvae are larger compared with *S. exigua* and *S. frugiperda*, suggesting higher conidial dose maybe needed for successful fungal infection. The 5th and 6th larval instars of *M. separata* measure 23.96 and 41.40mm, respectively (Navasero et al. 2022) suggesting that the conidial concentrations tested used in this study maybe insufficient to induce mortality to these large larvae. However, the application of entomopathogenic fungi in the field should target the eggs and early instars to prevent severe crop damage. Targeting the large larval instars will be too late considering that the gross damage is already massive and the lethal effect of entomopathogenic fungi is not immediate. Previous research noted that early nymphal stages of cotton aphids evaded fungal infection due to low number of conidia attached to their cuticle, low levels of conidial germination, and rapid ecdysis which allow removal of conidia before the germ tube penetrated the insect hemolymph (Kim and Roberts 2012).

Other research documented the virulence of entomopathogenic fungi against other lepidopteran pests. *B. bassiana* and *I. fumosorosea* effectively induced 82.5 − 92.5% and 80 − 87.5% mortality to 1st to 5th larval instars of *M. separata*, respectively, using 1 x 10^8 to 6 x 10^8 conidia/ml under laboratory conditions (Malik et al. 2013). *S. litura* larvae were also vulnerable to *B. bassiana* and *M. anisopliae* at 1 x 10^7 conidia/ml with mortalities 27 − 59% and 14 − 41%, respectively (Asi et al. 2013). *Nomuraea rileyi* was also found effective against *S. frugiperda* (Bosa et al. 2004).

Larval mortality was observed to be dose dependent, wherein increasing fungal infection and reduced lethal time were recorded with higher conidial concentration (Malik at al. 2013; Montecalvo and Navasero 2020; Montecalvo and Navasero 2021a, 2021b; Montecalvo et al. 2022a, 2022b). However, in this study, no trend was observed in LC_{50} values and interestingly erratic values were calculated as depicted in very high concentrations. This observation can be attributed to the very low larval mortalities recorded particularly in late larval instars. This could also be associated with the larger size of the larval instars requiring high amount of inoculum for disease infection to occur.

Susceptibility of early larval instars of *S. frugiperda* has been observed in the previous research (Montecalvo and Navasero 2021a, 2021b; Montecalvo et al. 2022b). Higher mortalities in early instars may be attributed to a more susceptible cuticular structure of the larvae. Maturing larvae become more resistant to fungal infection due to the composition of the larval integument (Bosa et al. 2013). 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 research also presented data confirming the minimal susceptibility of prepupa and pupa to entomopathogenic fungi. The observed slight reduction in adult emergence conformed to the previous observation that *B. bassiana* and *M. anisopliae* also caused little effect on adult emergence of prepupa and abnormal adults from treated pupa of *S. frugiperda* (Montecalvo and Navasero 2021a). This abnormality may potentially affect the mobility and reproduction of the insect pest. In addition, prepupa molts to pupa in 1 − 2 d making them less susceptible to fungal infection. Pupa has thick and sclerotized cuticle which serve as barrier to fungal infection (Hajek and St. Leger 1994).

Varying virulence of the strains of entomopathogenic fungi may be attributed to the origin of strain. The *M. rileyi* isolate used in this study was isolated from *S. exigua* which is also a lepidopteran species, hence, may depict higher virulence to other lepidopteran species like *S. frugiperda* (Montecalvo and Navasero 2021b) and *M. separata*. On the other hand, *M. anisopliae* was isolated from rice black bug while *B. bassiana* from rice bug. The genetic diversity and insect cuticle characteristics also influence the pathogenicity of *M. rileyi* (Fronza et al. 2017). *M. rileyi* produces enzymes, secondary metabolites, and large amounts of extracellular polysaccharide during its growth that may contribute in the adhesion to host cuticle and mummification process. Other researches observed that *M. rileyi* isolates are more

virulent to the host species from which they were isolated (Fronza et al. 2017).

In summary, *M. separata* has varying susceptibility to entomopathogenic fungi depending on the biological stage, conidial dose, and fungal species. Field efficacy of these entomopathogenic fungi against *M. separata* should be established to further support its integration in pest management programs.

CONCLUSION

Dose-mortality assays elucidated the bioefficacy of entomopathogenic fungi namely *B. bassiana*, *M. anisopliae,* and *M. rileyi* against the different biological stages of *M. separata* such as eggs, larval instars, prepupa, and pupa. The pre-adult life stages of this insect pest had varying susceptibility to entomopathogenic fungi. Findings suggest that eggs were not susceptible to fungal infection, but the resulting larvae were severely infected with the fungi. Among the entomopathogenic fungi tested, *M. rileyi* caused the highest lethal infection to 1st to 5th larval instars while *B. bassiana* inflicted significant mortality in 6th larval instar. *M. anisopliae* and *M. rileyi* significantly reduced adult emergence in prepupa. *M. anisopliae* caused significant reduction in adult emergence in treated pupa. These findings confirmed the varying virulence of the entomopathogenic fungi against the different biological stages of *M. separata*.

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