

## BIOCONTROL EFFICACY OF NATIVE *Metarhizium rileyi* TO VARIOUS LIFE STAGES OF *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae)

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### ABSTRACT

The efficacy of an entomopathogenic fungus (EPF) was investigated as a potential biological control agent against onion armyworm, *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae), which is an important lepidopterous insect pest infesting onion crop in the Philippines. The pathogenic effect of a native isolate of *Metarhizium rileyi* was assessed against the pre-imaginal stages of *S. exigua* such as eggs, various larval instars, prepupa, and pupa by exposure to conidial concentrations of *M. rileyi* in laboratory bioassays. The EPF showed no significant effect on the hatchability of *S. exigua* eggs, but later caused mortality to the neonates from hatched treated eggs. Various instars of *S. exigua* larvae were all susceptible to *M. rileyi* with infection usually initiated at 4 days after treatment (DAT) and peaked at 6 to 7 DAT. Higher conidial concentrations caused higher and faster larval mortality as compared to lower concentrations with mean time to mortality of 6.2 to 7.1 days. There was no trend observed on the lethal concentration (LC<sub>50</sub>) values that ranged from 5.59 x 10<sup>5</sup> to 5.95 x 10<sup>6</sup> conidia/ml relative to various instars. Adult emergence of prepupae and pupae was not significantly affected but abnormalities were observed in adults. Our findings revealed the infectivity of native *M. rileyi* against *S. exigua* and suggest its potential as biological control agent against this major economic insect pest of onion.

**Key words:** biological control, entomopathogenic fungus, bioassays, mummification, onion armyworm

### INTRODUCTION

*Spodoptera exigua* (Hübner), locally known in the Philippines as *harabas* or onion armyworm, is a prolific, destructive, and polyphagous insect pest. A female adult lays 829-1,675 eggs in its lifetime while the hatched neonates are known to be gregarious feeders (Navasero et al. 2019). *S. exigua* infests more than 90 plant species in Asia including ornamental crops, weeds, and agricultural crops (Fu et al. 2017). In the Philippines, an outbreak of *S. exigua* was first reported in 2016 affecting onion fields in the provinces of Tarlac, Nueva Ecija, and Pangasinan. In Nueva Ecija, the infestation in 5,000 ha of onion field resulted to an estimated crop loss of PhP 1.6 billion. Chemical pesticides were extensively used in attempting to salvage the fields (Navasero et al. 2017). Aside from causing harm to the environment and imposing health risks, reliance to and misuse of chemical pesticide lead to its inefficacy and may induce resistance to target pests. Several studies noted resistance development of *S. exigua* against various synthetic pesticides (Che et al. 2013). In addition, chemical pesticides commonly used against *S. exigua* were found to be toxic to its known predators and parasitoids (CABI 2019; de

Castro et al. 2018; Liu et al. 2016). Hence, a safer, sustainable, and more environment-friendly means to control this insect pest must be explored.

The use of entomopathogens such as virus and fungi are being explored to control this insect pest (Navasero et al. 2017). These microorganisms are safe to use as these are naturally found in the environment (Dara 2017). *S. exigua* multiple nucleopolyhedrovirus (SeMNPV) was found infective to five larval instars of *S. exigua*, inflicting high mortality and reduced growth and development of the insect pest (Montecalvo and Navasero 2019). Likewise, a previous research reported successful isolation of entomopathogenic fungus (EPF) *Metarhizium rileyi* from field collected *S. exigua* that significantly caused mortality and affected development of the 3<sup>rd</sup> larval instar of the insect pest (Montecalvo and Navasero 2020). In this research, this *M. rileyi* isolate was further studied against *S. exigua* considering its biocontrol potential and being a native isolate from this insect pest.

The EPFs are beneficial fungi that cause disease and mortality to its host insect characterized by stiffening of the insect body with subsequent growth of mycelia and production of conidia. The most studied EPFs are *Beauveria bassiana* and *M. anisopliae*, which are both generalists infecting approximately 700 and 300 arthropod species, respectively (Rohrlich et al. 2018; Sbaraini et al. 2016). *M. rileyi*, on the other hand, is known to infect only around 60 species of insects, which are mainly lepidopterous insects that belong to the Noctuidae family (Fronza et al. 2017). These EPFs pose less threat to the natural enemies in the field while maintaining their virulence to their host.

*M. rileyi* is a dimorphic hyphomycete that causes epizootic mortality to various lepidopterous pests (Sinha et al. 2016). This EPF infects its host by adhesion to the cuticle, tissue invasion, enzymatic activity, and toxicosis (Fronza et al. 2017; Mengzhao et al. 1992). Some lepidopterous pests infected by *M. rileyi* are *Helicoverpa armigera* (da Costa et al. 2015), *Anticarsia gemmatalis* and *Chrysodeixis includens* (Lopes et al. 2020), *S. litura* (Liu et al. 2019; Namasivayam and Bharani 2015), and *S. frugiperda* (Cruz-Avalos et al. 2019; Montecalvo and Navasero 2021b; Ramanujam et al. 2020).

Higher virulence of *M. rileyi* is expected against *S. exigua* than other EPFs such as *M. anisopliae* and *B. bassiana* since *M. rileyi* was isolated from *S. exigua*. Considering the virulence of *M. rileyi* to *S. exigua*, this study aimed to further assess the potential of this EPF as a biological control agent against *S. exigua*. Bioassays were conducted to elucidate the pathogenicity of this isolate to the different life stages of *S. exigua* particularly eggs, 1<sup>st</sup> to 5<sup>th</sup> larval instars, prepupa, and pupa. Likewise, its biocontrol efficacy was determined based on calculation of lethal concentration (LC) and time.

## MATERIALS AND METHODS

**Laboratory rearing.** *S. exigua* was obtained from the existing culture at the Biological Control Laboratory of the National Crop Protection Center, College of Agriculture and Food Science, University of the Philippines Los Baños, Laguna, Philippines. The parental stock was originally collected from an infested onion field in Nueva Ecija, Philippines. Adults were mated in Mylar cages and fertilized egg masses were harvested. Homogenous larvae were obtained from eggs hatched on the same day. Newly hatched larvae were fed daily with fresh castor oil leaves in plastic pans until appropriate larval instar was reached. Consequently, homogenous prepupae and pupae were obtained from larvae that reached prepupal stage and pupated on the same day, respectively.

**Preparation of conidial suspensions.** The native *M. rileyi* was originally isolated from a naturally infected larvae of *S. exigua* collected from an infested onion field in Nueva Ecija, Philippines (Montecalvo and Navasero 2020). The fungus was revived and subcultured in potato dextrose agar (PDA). This isolate was previously characterized based on cultural and morphological characteristics. Molecular identification through comparison of the DNA sequence of the fungal isolate to known sequences by BLASTn program confirmed the identity of the isolate.

Sporulated cultures of *M. rileyi* were used to reinfect larvae. *M. rileyi* was reisolated in PDA with yeast extract (PDAY) from mummified larvae to enhance virulence. Conidia were harvested from the cultures by scraping fungal growth and were suspended in 0.1% Tween 80 solution. Conidia in the suspension were quantified using a Neubauer improved hemocytometer (Blaubrand 717805, GMBH + CO KG, Germany). Conidial suspensions with various concentrations ( $1 \times 10^5$  to  $1 \times 10^9$  conidia/ml) were prepared by diluting the conidial stock suspension in 0.1% Tween 80 solution.

### **Bioassay of *M. rileyi* against various life stages of *S. exigua***

**Hatchability of eggs and mortality of hatched larvae.** Freshly laid egg masses of *S. exigua* were counted under a dissecting microscope. Egg masses with nearly similar counts were randomly distributed in UV-sterilized Petri plates with damp sterile cotton. Egg masses were mist-sprayed with  $1 \times 10^9$  conidia/ml of *M. rileyi*, while 0.1% Tween 80 solution was sprayed in the control set-up. Each treated and control set-up was replicated four times, with approximately 97 to 112 eggs per egg mass.

Fresh castor oil leaves were surface-sterilized by washing in 0.05% sodium hypochlorite for 10 min followed by washing twice in sterile distilled water for 1 min each. These castor oil leaves were placed inside the Petri plates, which were sealed using Parafilm to prevent the escape of neonates. Neonates and unhatched eggs were counted daily. Ten (10) newly hatched larvae per replicate were transferred in sterile Petri plates and were fed daily until 3<sup>rd</sup> larval instar was reached. Surviving 3<sup>rd</sup> instar larvae were single cultured by transferring and feeding one larva per Petri plate until the adult stage was reached. Mortality and mycosis were recorded daily. The experiment was done in four replicates.

**Dose-mortality of various larval instars.** Leaf discs of fresh castor oil leaves were prepared and surface-sterilized as previously described. Various suspensions of *M. rileyi* ( $1 \times 10^5$  to  $1 \times 10^9$  conidia/ml) were mist-sprayed on both surfaces of the leaves and were air dried. Tween 80 (0.1% v/v) solution was sprayed for control. Two inoculated leaves, one on top of the other, were placed in a sterile Petri plate containing a sterile damp cotton ball. For the 1<sup>st</sup> and 2<sup>nd</sup> larval instars, 10 larvae were placed in a Petri plate and single cultured upon reaching 3<sup>rd</sup> larval instar. The 3<sup>rd</sup>, 4<sup>th</sup>, and 5<sup>th</sup> larval instars were cultured singly in a Petri plate. Fresh surface-sterilized castor oil leaves were fed to the larvae daily. Each treatment was done in triplicates with 10 individuals per replicate. Mortality was noted daily and mycosis of the larvae was documented. The percentage of mortality was corrected (Abbott 1925) and mean time to mortality was calculated (El-Hawary and Abd El-Salam 2009).

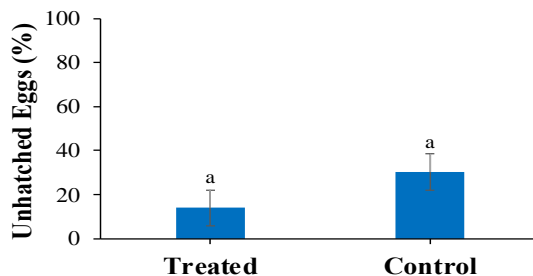
**Adult emergence of prepupa and pupa.** Prepupa and pupa were surface-sterilized by washing in 1% sodium hypochlorite for 1 min then washing twice in sterile distilled water for 30 sec then air dried. These prepupae and pupae were treated by submerging in  $1 \times 10^9$  conidia/ml of *M. rileyi* for 1 min. For the control, prepupae and pupae were submerged in 0.1% Tween 80 solution. After air-drying, treated prepupae and pupae were distributed singly to sterile specimen cups. Adult emergence was counted daily. The control and treated set-ups were replicated thrice with 10 prepupae or pupae per replicate.

**Statistical design and analysis.** All experiments were arranged in CRD. T-test was conducted to compare the results of fungal treated versus the control set-up in the bioassays of *S. exigua* eggs, prepupa, and pupa. Treatment means in bioassays conducted in larval instars were compared by analysis of variance using Tukey's honest significant difference test. Lethal concentration (LC) values were calculated using Probit software ver. 1.63.

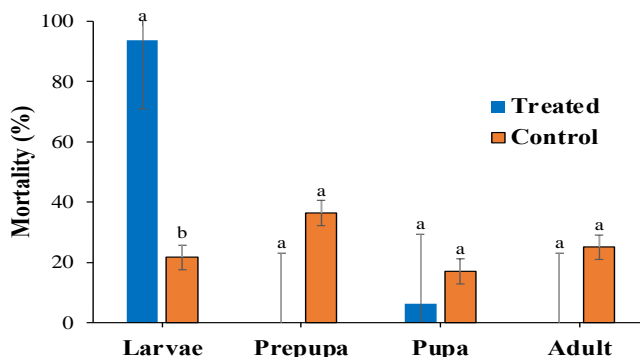
## **RESULTS AND DISCUSSION**

**Hatchability of eggs and mortality of neonates.** The native isolate of *M. rileyi* had no significant ovicidal effect on *S. exigua* (Fig. 1). Interestingly, 93.75% of the larvae that hatched from the treated egg masses succumbed to fungal infection, while the remaining 6.25% reached pupal stage, but no

longer emerged into adult (Fig. 2.). Some pupae were observed to exhibit incomplete pupation. On the contrary, *S. exigua* in the control set-up pupated successfully and emerged into adults.



**Fig. 1.** Unhatched eggs of *Spodoptera exigua* treated with *Metarhizium rileyi*. Bars represent the standard error of the means. Those with same letters are not significantly different by t-test ( $P < 0.05$ ).



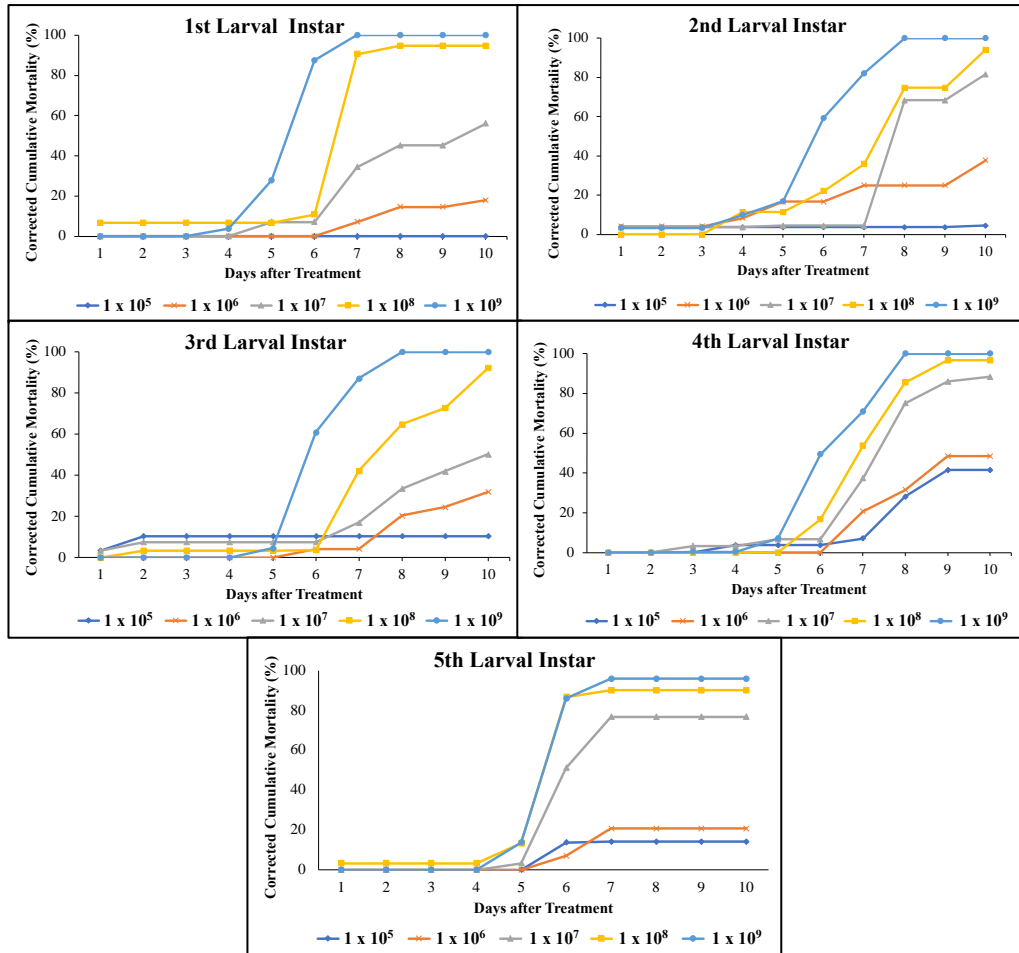
**Fig. 2.** Mortality in different life stages of *Spodoptera exigua* which hatched from egg masses treated with *Metarhizium rileyi*. Bars represent the standard error of the means. Pairs of bars with the same letters are not significantly different by t-test ( $P < 0.05$ ).

Our observations suggest that the ovicidal activity of *M. rileyi* was not apparent based on its effect on hatchability. This result agrees with previous studies wherein eggs of *Spodoptera* spp. were exposed to *M. rileyi* but did not show significant effect in terms of hatchability (Montecalvo et al. 2022). Both studies by Cruz-Avalos et al. (2019) and Ramanujam et al. (2020) reported that *M. rileyi* was one of the strains which did not infect the eggs of fall armyworm (*S. frugiperda*) after treatment with various strains of EPF. In contrast, *B. bassiana* and *M. anisopliae* reduced hatchability of *S. frugiperda* eggs (Montecalvo and Navasero 2021a). *M. anisopliae*, *Isaria fumosorosea*, and *B. bassiana* also infected freshly laid *S. litura* eggs (Asi et al. 2013).

Although the hatchability of eggs was not affected, this research also presented the lethal effect on the larvae hatched from the egg masses exposed to *M. rileyi*. These neonates may have acquired *M. rileyi* from the surface of the treated egg masses upon emergence, hence, these larvae succumbed to fungal infection. A similar study in *S. littoralis* showed that *M. rileyi* caused larval mortality after contamination of first instar larva from treated egg mass, which might be infected by the germinating fungus in the egg integument or the larva acquired the conidia from the egg cuticle which fed upon chorions (Rodriguez-Rueda and Fargues 1980).

**Dose-mortality of various larval instars.** Based on laboratory bioassays against various larval instars of *S. exigua*, the mortality across time points revealed that all larval instars of *S. exigua* were susceptible

to *M. rileyi* with infection initiating at 4 days after treatment (DAT) and significantly increased at 6 to 7 DAT (Fig. 3).



**Fig. 3.** Corrected cumulative mortality of larval instars of *Spodoptera exigua* exposed to conidial concentrations of *Metarhizium rileyi*:  $1 \times 10^5$  conidia/ml (A);  $1 \times 10^6$  conidia/ml (B);  $1 \times 10^7$  conidia/ml (C);  $1 \times 10^8$  conidia/ml (D); and  $1 \times 10^9$  conidia/ml (E).

Mycosis was confirmed in *S. exigua* cadavers having stiff bodies with white fungal growth and olive green sporulation (Fig. 4). This finding is consistent with the initial results where 3<sup>rd</sup> instar larvae of *S. exigua* were treated with various conidial concentrations ( $1 \times 10^4$  to  $1 \times 10^8$  conidia/ml) of the same *M. rileyi* isolate (Montecalvo and Navasero 2020).



**Fig. 4.** Larval cadaver of *Spodoptera exigua* mycosed by *Metarhizium rileyi*.

Increasing conidial concentration resulted in higher mortality that rose significantly days after exposure to *M. rileyi* (Fig. 5). Conidial concentrations of  $1 \times 10^8$  and  $1 \times 10^9$  conidia/ml induced the highest lethal infection up to 100% mortality in all larval instars of *S. exigua*, while lower conidial concentrations induced lower and slower mortalities at 10 DAT (up to 41.48% in  $1 \times 10^5$  conidia/ml; 45.19% in  $1 \times 10^6$  conidia/ml; and 88.33% in  $1 \times 10^7$  conidia/ml).

Increasing trend in fungal infection with increasing conidial concentrations was also observed in *S. exigua* treated with various conidial concentrations ( $1 \times 10^4$  to  $1 \times 10^9$  conidia/ml) of *M. anisopliae* and *Paecilomyces fumosoroseus* (Han et al. 2014) and 3<sup>rd</sup> instar larvae of *S. exigua* treated with various conidial concentrations ( $1 \times 10^4$  to  $1 \times 10^8$  conidia/ml) of *M. rileyi* (Lee et al. 2012). Likewise, significant number of early larval instars of *S. frugiperda* succumbed to a native isolate of *M. rileyi* (Montecalvo et al. 2022).

Larval mortalities are influenced by molting, pupation, and conidial concentration. Inoculum may be lost during molting (Meeke 2001). Kim and Roberts (2012) observed low fungal infection of early nymphal stages of cotton aphids due to low number of conidia attached to the insect cuticle, low levels of conidial germination, and rapid ecdysis that may result in removal of conidia before the germ tubes penetrated the host hemolymph.

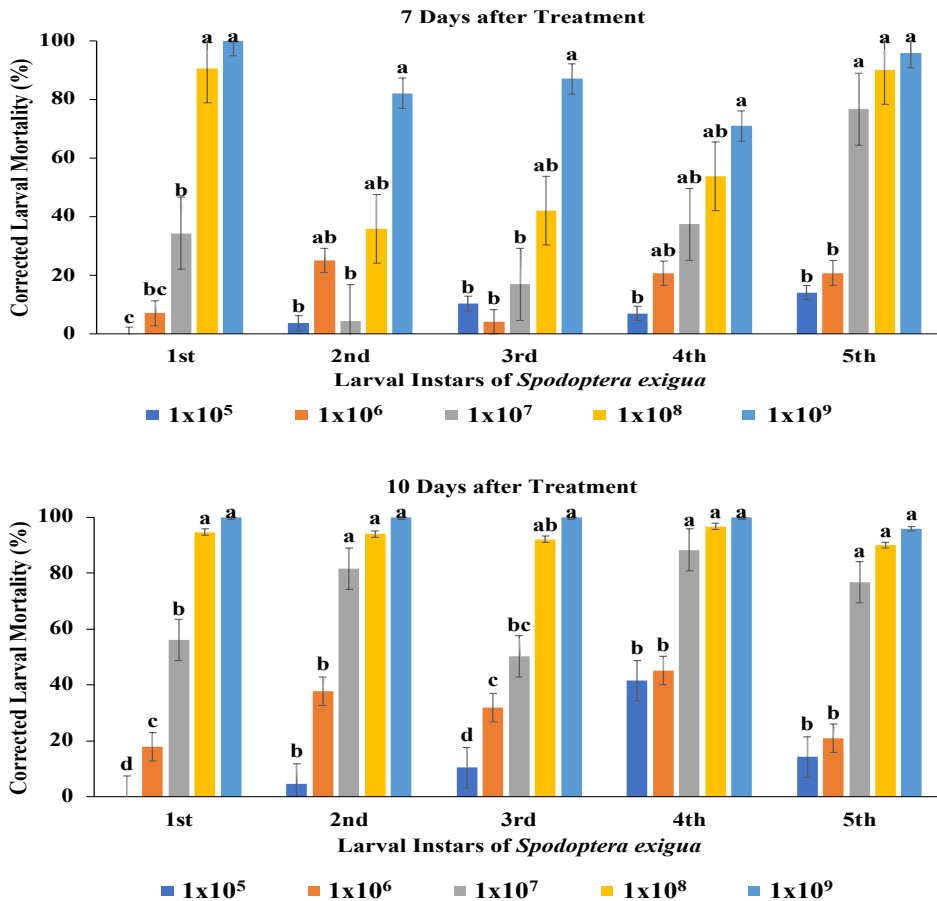


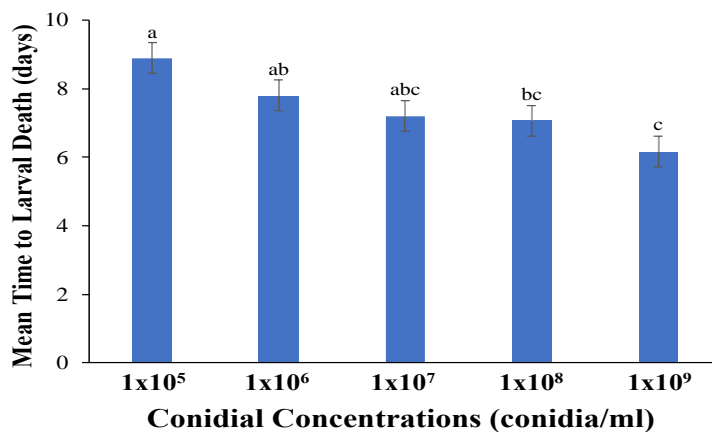
Fig. 5. Corrected cumulative mortality of larval instars of *Spodoptera exigua* exposed to conidial concentrations of *Metarhizium rileyi*. Bars represent the standard error of the means. Pairs of columns with the same letters are not significantly different in HSD ( $P < 0.05$ ).

This study also presented the calculated LC<sub>50</sub> of each larval instar using probit analysis at 95% fiducial limit (Table 1). The LC<sub>50</sub> values revealed that different conidial concentrations of *M. rileyi* were required for 50% of each larval instar to be infected with no distinct trend. Calculated LC<sub>50</sub> at 10 DAT ranged from 5.59 x 10<sup>5</sup> to 5.95 x 10<sup>6</sup> conidia/ml. This is contrary to the findings of Kaur et al. (2011) wherein LC<sub>50</sub> values increased with the larval stage when *S. litura* was treated with *B. bassiana*. Likewise, increasing LC<sub>50</sub> conidial concentrations of this *M. rileyi* isolate was observed when it cross-infected *S. frugiperda* with increasing LC<sub>50</sub> values ranging from 1.44 x 10<sup>5</sup> to 9.36 x 10<sup>8</sup> conidia/ml for young to old larval instars (Montecalvo and Navasero 2021b). Larval size may also have contributed to its susceptibility to EPF with larger larvae requiring more fungal inoculum.

**Table 1.** Lethal concentration of *Metarhizium rileyi* against *Spodoptera exigua* larvae at 95% fiducial limit.

| Larval instar   | LC <sub>50</sub><br>(conidia/ml) | 95% Fiducial Limit<br>(Lower-Upper)             |
|-----------------|----------------------------------|---|
| 1 <sup>st</sup> | 5.95 x 10 <sup>6</sup>           | 3.08 x 10 <sup>6</sup> - 1.09 x 10 <sup>7</sup> |
| 2 <sup>nd</sup> | 2.01 x 10 <sup>6</sup>           | 7.49 x 10 <sup>5</sup> - 4.02 x 10 <sup>6</sup> |
| 3 <sup>rd</sup> | 5.08 x 10 <sup>6</sup>           | 1.95 x 10 <sup>6</sup> - 1.07 x 10 <sup>7</sup> |
| 4 <sup>th</sup> | 5.59 x 10 <sup>5</sup>           | 1.63 x 10 <sup>5</sup> - 1.32 x 10 <sup>6</sup> |
| 5 <sup>th</sup> | 3.12 x 10 <sup>6</sup>           | 1.25 x 10 <sup>6</sup> - 6.71 x 10 <sup>6</sup> |

Mean time to larval mortality suggests that increasing conidial concentrations of *M. rileyi* resulted in earlier larval mortality (Fig. 6). The earliest larval mortality was observed with conidial concentrations of 1 x 10<sup>8</sup> conidia/ml (7.1 days) and 1 x 10<sup>9</sup> conidia/ml (6.2 days) which were significantly faster than lower conidial concentrations. Our findings conform with our earlier observations that increasing conidial concentrations of *M. rileyi* resulted in earlier larval mortality. There was no significant difference on the mean time to larval mortality among different larval instars (6.9 to 8.0 days). In a previous study, this isolate caused mean time to mortality of 4.5 to 8.9 days during cross infection to larval instars of *S. frugiperda* (Montecalvo and Navasero 2021b). Shorter lethal time due to higher conidial concentrations were observed in earlier studies (El-Hawary and Abd El-Salam 2009; Han et al. 2014).



**Fig. 6.** Mean time to larval mortality of *Spodoptera exigua* as affected by conidial concentrations of *Metarhizium rileyi*. Bars represent the standard error of the means. Bars with the same letters are not significantly different in HSD (P< 0.05).

**Adult emergence of prepupa and pupa.** Prepupa and pupa of *S. exigua* were not susceptible to *M. rileyi* (data not shown). *M. rileyi* had no effect on the emergence of adults from treated prepupae and pupae. This is different from the results obtained by Garrido-Jurado et al. (2020) where high mortalities were observed when *S. littoralis* prepupae and pupae were treated with various isolates of *B. bassiana* and *M. brunneum*. On the contrary, prepupa of *S. frugiperda* was affected by *M. rileyi* but pupa was not susceptible to fungal infection (Montecalvo et al. 2022). On the other hand, *B. bassiana* and *M. anisopliae* caused low mortalities in *S. frugiperda* prepupae but these EPF did not affect adult emergence of treated pupae (Montecalvo and Navasero 2021a). Nevertheless, the emergence of deformed adults such as reduced size of wings from treated prepupae and pupae in their study was also observed in this experiment implying the possible impact on the mating ability of the adults. Pupae may not be susceptible to fungal infection due to their thick and sclerotized cuticle that serves as barrier to fungal infection (Hajek and St. Leger 1994).

This paper presented the virulence of the *M. rileyi* isolate which also caused epizootics to several insect pests. This *M. rileyi* isolate cross infected two armyworm species including *S. frugiperda* (Montecalvo and Navasero 2021b) and true armyworm, *Mythimna separata* (Montecalvo et al. 2021). Likewise, a native isolate of *M. rileyi* naturally infected *S. frugiperda* collected in Quezon province in the Philippines (Montecalvo et al. 2022) suggesting that *M. rileyi* is a potent EPF against invasive insect pests such as *S. exigua* and *S. frugiperda*. The use of this EPF in the integrated pest management of *S. exigua* is promising since the fungus can establish endophytically in its host.

## CONCLUSION AND RECOMMENDATION

The native isolate *M. rileyi* caused lethal infection to its original insect host, *S. exigua*. Different life stages of *S. exigua* were exposed to local isolate of *M. rileyi*. This EPF did not affect hatchability of eggs, however, larvae hatched from the treated eggs succumbed to fungal infection. Larval instars succumbed to fungal infection, however, prepupa and pupa were not susceptible to *M. rileyi*. This EPF is a potent biocontrol agent against *S. exigua* larvae. Its biocontrol efficacy can be further assessed by conducting screenhouse and field trials implying the need to optimize mass production methods and to formulate this EPF.

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