OCCURRENCE AND CHARACTERIZATION OF *Metarhizium rileyi* (FARL.) KEPLER, S.A. REHNER AND HUMBER FROM INVASIVE ARMYWORM SPECIES IN THE PHILIPPINES WITH POTENTIAL AS A BIOPESTICIDE

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ABSTRACT

The entomopathogenic fungus, *Metarhizium rileyi* (Farl.) Kepler, S.A. Rehner and Humber, is an environmentally friendly organism with a global distribution that is beneficial in controlling transboundary pests. This research documented the presence of *M. rileyi* in selected onion and corn farms located in the Philippines. This paper also assessed mass production protocols to produce a significant number of infective propagules. Insect collections of invasive armyworm species infesting onion and corn were conducted in several production areas in the Philippines. Based on polyphasic identification, 11 isolates of *M. rileyi* were isolated from armyworm species. The fungus was successfully recovered from armyworm or *Spodoptera exigua* (Hubner), infesting onion fields in Nueva Ecija. Isolates of this fungus were also recovered from *S. frugiperda* (J.E. Smith) attacking corn plants in the provinces of Isabela, Laguna, and Quezon. Further characterization of the *M. rileyi* isolate from *S. exigua* was conducted including identification of suitable and cost-effective artificial media. The artificial media with potato, maltose, and yeast extract were identified as possible alternatives to commonly used artificial media for the cultivation of this entomopathogenic fungus. Solid substrate fermentation indicated that rice supported profuse growth and sporulation of *M. rileyi*. The result of this research provided baseline information on the mass production of *M. rileyi* that is essential in successfully utilizing this beneficial fungus, particularly in product development and further bioefficacy evaluation.

Key words: biological control, mummification, invasive insect pest, microbial control, *Spodoptera exigua, S. frugiperda*

INTRODUCTION

The armyworm species are invasive insect pests that cause severe damage to crops leading to economic crop loss. Two (2) of the recent insect pest outbreaks in the Philippines were caused by armyworm species. The onion armyworm or *Spodoptera exigua* (Hubner, Lepidoptera: Noctuidae) has been reported in the Philippines, however, the pest had an outbreak in onion fields in three (3) provinces including Nueva Ecija, Tarlac, and Pangasinan resulting in massive losses in 2016 (Navasero et al. 2017). In Nueva Ecija alone, an estimated 5,000 ha of onion fields was damaged by this insect pest translating to PhP 1.6 billion production loss. Similarly, the fall armyworm, *S. frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), damaged corn plants and promptly spread to multiple corn-producing areas in the Philippines posing a significant threat to crops in the region in 2019 (Navasero et al. 2019).

Research on pest distribution and climatic suitability models shows that the environmental conditions needed for the pest to establish are present in the invaded countries in Africa and Asia. To mitigate these insect pests, farmers rely on fast-acting and available products such as synthetic insecticides. However, excessive use of these agrichemicals poses serious concerns including insecticide resistance, deleterious effects to natural enemies, and environmental pollution.

Entomopathogens and macrobials are effective and eco-friendly strategies for sustainable pest control, reducing the need for chemical treatments. Viruses, bacteria, nematodes, and fungi are among the effective entomopathogens against economically important insect pests of crops. Entomopathogenic fungi (EPF) are promising biological control agents against insect pests. These biological control agents cause infection and kill insect pests and arthropods making them essential agents in managing pest populations (Mantzoukas et al. 2022). Among these EPF, *Beauveria bassiana* (Bals.-Criv.) Vuill. is one of the most studied species and used for biological control of insect pests. On the other hand, *Metarhizium* species have a wide range of hosts with *M. anisopliae* (Metschn.) Sorokin as an infective generalist fungus while *M. rileyi* (Farl.) Kepler, S.A. Rehner and Humber attacks about 60 arthropod species (Fronza et al. 2017). In the Philippines, native isolates of this EPF have been tested against *S. frugiperda* (Montecalvo and Navasero 2021b). *Metarhizium rileyi* was also virulent to *Mythimna separata* (Walker)(Lepidoptera: Noctuidae) or paddy armyworm (Montecalvo and Navasero 2023). Indigenous isolates of *M. rileyi* were also infective to onion armyworm (Montecalvo et al. 2022a) and fall armyworm (Montecalvo and Navasero 2021a; Montecalvo et al. 2022b; Montecalvo et al. 2023).

An effective biological control agent should be efficiently multiplied leading to its eventual utilization in the farmers' field. For instance, the subsequent subculture of *M. rileyi* could reduce its virulence (Mohammadbeigi 2013), hence, the passage of EPF through a suitable host could improve its virulence but is a lengthy and laborious process. A more practical means to maintain its virulence is essential to its mass production process and storage to ensure consistent efficacy. Producing fungal inoculum at a competitive cost is an essential step to make any microbial agent popular and adaptable (Ingle 2014). The goal of mass production is to develop products that have a long shelf life, are comparatively inexpensive, and are consistently successful in the field (Mantzoukas et al. 2022). A crucial stage in the mass production process is determining the optimal technique and appropriate artificial media to minimize the attenuation of fungal pathogenicity (Mohammadbeigi 2013). Broth media can be mixed in carriers for formulation (Ingle 2014).

This paper reports the occurrence of indigenous isolates of the beneficial fungus *M. rileyi* infecting invasive armyworm species including *S. exigua* and *S. frugiperda*. This study also investigated the multiplication of *M. rileyi* on various semisynthetic media. Lastly, it identified possible grain substrates to mass produce a significant number of inocula for formulation and field sprays.

MATERIALS AND METHODS

Studies were done at the Mycology Laboratory of National Crop Protection Center – College of Agriculture and Food Science, University of the Philippines Los Baños, Laguna, Philippines.

Collection, isolation, and identification of entomopathogenic fungus. Insect samples of *S. exigua* and *S. frugiperda* were collected from selected onion and corn farms in the Philippines, respectively, and identified through their morphological and molecular characteristics. Onion armyworm larvae were identified with their characteristic body color, and pale to dark stripe. On the other hand, fall armyworm larvae were recognized with an inverted Y-mark on the head and four dark spots arranged in a square on top of the eighth abdominal segment. Mummified *S. exigua* larvae were sourced from infested onion fields in Nueva Ecija in 2016. On the other hand, infected larvae of *S. frugiperda* were gathered from various corn farms in Isabela, Laguna, and Quezon provinces since 2019. Fungal infection in the larval cadavers was characterized by fungal growth in the larval body.

Surface sterilization of the mycosed larvae was done by dipping in 1% sodium hypochlorite and rinsed two times in sterile distilled water (1 min). The larvae were blot-dried, sectioned, and inoculated in plated potato dextrose agar (PDA) or potato maltose agar + yeast extract (PMAY). Fungal growth was observed and transferred to PDA or PMAY slants. Apart from this method, conidia from the larval cadavers were observed in Zeiss Stemi 305 Stereo microscope and aseptically transferred in PMAY slants. Polyphasic identification was done by determining the cultural, morphological, and molecular characteristics. The shape and size of the fungus were examined using a Zeiss Primostar microscope. Sequencing was done by Macrogen, Inc. in Seoul, South Korea. BLASTn program was used to compare the DNA sequences of the fungal isolates with those in NCBI database. The internal transcribed spacer (ITS) gene sequences were aligned using MEGA X with phylogenetics constructed through maximum parsimony with 1000 bootstrap replications. The evolutionary history of the isolates was done using the Tamura-Nei model and maximum likelihood method.

Mass production of *M. rileyi*

Artificial solid media assay. Different artificial media were assessed to determine specific components that may support the cultural growth of the fungus. The artificial solid media were prepared based on the available literature, except for potato maltose agar (PMA) and PMAY. The following media were used: malt extract agar (MEA), MEA + yeast extract (MEAY), oatmeal agar (OA), OA + yeast extract (OAY), PDA, PDA + yeast extract (PDAY), PMA, PMAY, Sabouraud dextrose agar (SDA), SDA + yeast extract (SDAY), Sabouraud maltose agar (SMA), and SMA + yeast extract (SMAY). *Metarhizium rileyi* was inoculated per Petri plate with 0.1 ml of conidial concentration of 1 x 10^6 conidia/ml through spread plating. The experiment was incubated at room temperature. Data including sporulation time, biomass amount, and conidial concentration were recorded. The biomass was determined by weighing the fungal growth scraped aseptically in the culture plates. The biomass was then submerged in standard surfactant (0.1% Tween 80 solution). After filtering, the concentration of the conidia was determined using Neubauer improved hemocytometer.

Liquid media fermentation. Artificial media (100 ml) including MEY, OMY, PDY, PMY, and SMY were prepared in 250ml Erlenmeyer flasks. An aliquot (1 ml) of 1 x 10^8 conidia/ml of *M. rileyi* suspension was inoculated in each flask. The inoculated flasks were vigorously shaken once daily for the first five days to dislodge the fungal growth that may have attached to the sides of the flasks. Sporulation and weight of biomass were recorded.

Solid substrate fermentation. Several grains and by-products were assessed as possible substrates for mass production. Based on the published literature, the mass production method and the promising substrates in the multiplication of *M. rileyi* were considered in this study including crushed sorghum soaked in 1% yeast extract (Ingle 2014), crushed sorghum soaked in 0.5% yeast extract (Vimala Devi et al. 2000), crushed sorghum soaked in 1% yeast extract (Vimala Devi 1994), rice with predetermined water (Loureiro et al. 2019), precooked rice (Faria et al. 2022), and crushed rice soaked in 1% yeast extract (Thakre et al. 2011). Other substrates were also tested such as precooked ground mungbean, 1:1 w/w of rice hull and rice bran, and 1:1 w/w rice hull and palay.

The solid fermentation was carried out in polypropylene bags containing 30g each of the substrates. Transparent polypropylene bags (15 x 35 cm) with 0.2 µm filter were sterilized by autoclaving at 121°C (15 psi) for 15 min. All substrate bags were inoculated with *M. rileyi* at 1 x 10^6 conidia/ml. Inoculated substrate bags were stored at room temperature for 20 days. The total yield produced in the substrate bags was determined by collecting the dry conidia. The grains were transferred in trays lined with sterilized paper and dried in chamber at 12±4°C and 22±6% relative humidity. A random sample was taken to assess moisture content at 24 h interval until the moisture content was stable. The percent moisture was calculated using the formula: ((initial weight – oven dry weight) / oven dry weight) x

100%. After which, the substrates with 20% moisture content were sieved using 1 mm mesh to separate the dry conidia. To determine the conidial concentration, the dry conidia were suspended in 0.1% Tween 80 solution and counted using Neubauer improved hemocytometer. In the wet method, conidial suspension was made by suspending the substrates in 0.1% Tween 80 solution and filtering it through double-layer cheesecloth. Conidial concentration was also counted using hemocytometer.

Experimental design and analysis. The research experiments were arranged following Completely Randomized Design. Analysis of variance was done and treatment means were compared following Tukey's HSD test at $P<0.05$.

RESULTS AND DISCUSSION

Polyphasic identification of *M. rileyi* isolates. A total of 11 isolates of *M. rileyi* were recovered from larval cadavers of armyworms (Table 1). These insect cadavers were stiff, hard, mummified, and covered with white mycelial growth and light olive-green sporulation. One (1) *M. rileyi* isolate was retrieved from onion armyworm, *S. exigua*, larvae during the outbreak of this pest in onion fields in Nueva Ecija in 2016. Similarly, 10 isolates of *M. rileyi* were obtained from *S. frugiperda* larvae infesting corn fields in Laguna, Isabela, and Quezon provinces in the Philippines.

Table 1. Isolates of entomopathogenic fungi, host, and ITS gene sequences used in this study.

Species	Isolate	Host	Locality	GenBank Accession Number
Metarhizium rileyi	Metarhizium_rileyi_FAW_ Laguna1	Fall armyworm, *Spodoptera frugiperda*	Los Baños, Laguna	OR826612
Metarhizium rileyi	Metarhizium_rileyi_FAW_ Isabela1	Fall armyworm, *S. frugiperda*	Ilagan, Isabela	OR826613
Metarhizium rileyi	Metarhizium_rileyi_FAW_ Isabela2	Fall armyworm, *S. frugiperda*	Ilagan, Isabela	OR826614
Metarhizium rileyi	Metarhizium_rileyi_FAW_ Isabela3	Fall armyworm, *S. frugiperda*	Ilagan, Isabela	OR826615
Metarhizium rileyi	Metarhizium_rileyi_FAW_ Isabela4	Fall armyworm, *S. frugiperda*	Ilagan, Isabela	OR826616
Metarhizium rileyi	Metarhizium_rileyi_FAW_ Isabela5	Fall armyworm, *S. frugiperda*	Ilagan, Isabela	OR826617
Metarhizium rileyi	Metarhizium_rileyi_FAW_ Quezon1	Fall armyworm, *S. frugiperda*	Lucena, Quezon	OR826618
Metarhizium rileyi	Metarhizium_rileyi_FAW_ Isabela6	Fall armyworm, *S. frugiperda*	Ilagan, Isabela	OR826619
Metarhizium rileyi	Metarhizium_rileyi_FAW_ Laguna2	Fall armyworm, *S. frugiperda*	Los Baños, Laguna	OR826620
Metarhizium rileyi	Metarhizium_rileyi_FAW_ Quezon2	Fall armyworm, *S. frugiperda*	Lucena, Quezon	OR826621

Species	Isolate	Host	Locality	GenBank Accession Number
Metarhizium rileyi	Metarhizium_rileyi_OAW_Nueva_Ecija	Onion armyworm, *Spodoptera exigua*	San Jose City, Nueva Ecija	OR826622
Metarhizium rileyi	*Metarhizium rileyi*	-	Tamil Nadu, India	KY436756.1
Nomuraea rileyi	*Nomuraea rileyi isolate Korea*	-	Andong, Korea	FJ824809.1
Metarhizium anisopliae	*Metarhizium anisopliae*	*Scotinophora lurida* B.	Ho Chi Minh, Vietnam	EU530680.1
Beauveria bassiana	*Beauveria bassiana* isolate 5A	-	Tamil Nadu, India	KX255641.1

Fungal colonies had white mycelial growth with powdery light olive-green sporulation (Fig. 1). In reverse, the fungal culture was white. Its hyphae were hyaline and septated, measuring about 2-4 μm in width. Conidia were hyaline, smooth-walled, globular to ellipsoidal, measuring 2-3 μm in width and 5-6 μm in length, typical to *M. rileyi*.

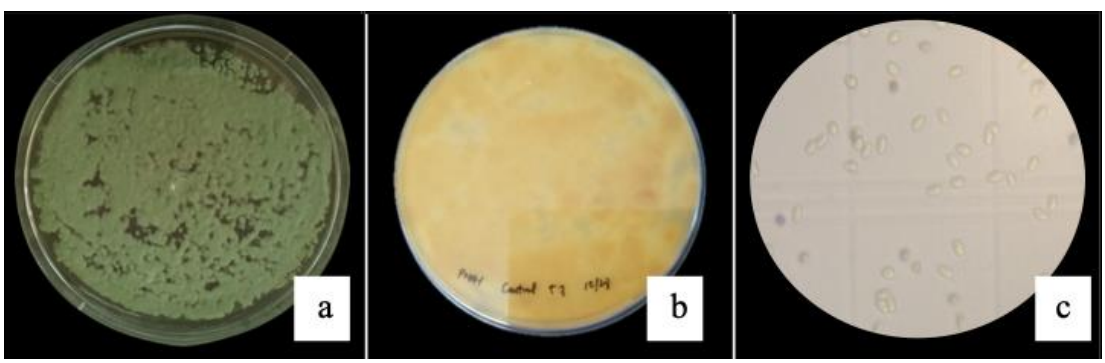

Figure 1. *Metarhizium rileyi* grown in potato maltose agar with yeast extract a) obverse, b) reverse, and c) its conidia under 400x magnification.

The DNA sequences of these *M. rileyi* isolates based on the ITS region were compared to known sequences of *M. rileyi*, *Nomuraea rileyi*, *M. anisopliae*, and *B. bassiana*. Using MEGA X, the phylogenetic tree showed 100% phylogenetic divergence of native *M. rileyi* isolates forming one clade with *M. rileyi* from Tamil Nadu, India; and *N. rileyi* isolate Korea from Andong, Korea with other sequences in the NCBI (Fig. 2). The *M. rileyi* isolates formed an outgroup with *M. anisopliae* from Ho Chi Minh, Vietnam; and *B. bassiana* isolate 5A from Tamil Nadu, India. These findings suggest that natural epizootics of *M. rileyi* occurred in onion and corn fields in the Philippines.

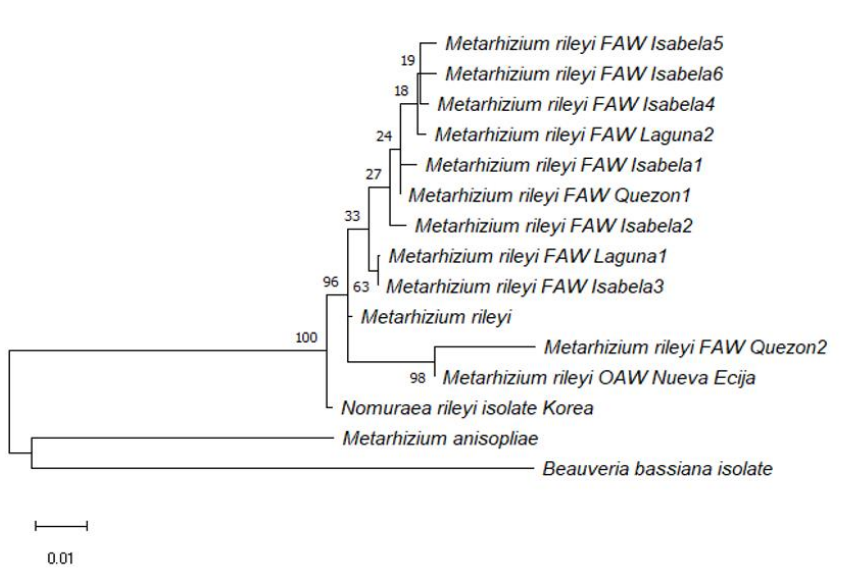

Figure 2. Phylogenetic tree based on ITS region of *Metarhizium rileyi*.

Similarly, it conforms to reports that *M. rileyi* is a cosmopolitan entomopathogenic fungus that infects lepidoptera and noctuids that damage crops (Fronza et al. 2017). More than 60 host species which are mainly lepidopterans particularly in the superfamily Noctuoidea, few beetles, hemipterans, and other insect orders are susceptible to this fungus (Fronza et al. 2017). It is useful in microbial control because, despite its limited host range, it has been found to effectively kill significant agricultural pests, indicating its potential for enhancing biological control in agricultural fields. This fungus was formerly known as *N. rileyi* (Farl.) Samson by Kepler and his co-workers (2014). *Nomuraea rileyi* was also among the entomopathogenic fungi recovered from soil and fungal-infected *S. frugiperda* larvae in Guanajuato, Mexico, and was discovered virulent against this insect pest (Cruz-Avalos et al. 2019). In *S. litura*, its infection starts upon contact of fungal inoculum with the insect host then conidia germinate and fungi grow further leading to insect death (Srisukchayakul et al. 2005).

In the Philippines, the beneficial fungus *M. rileyi* isolated from *S. exigua* was virulent to its original host (Montecalvo et al. 2022a), fall armyworm, *S. frugiperda* (Montecalvo and Navasero 2021a; Montecalvo et al. 2023), and paddy armyworm, *M. separata* (Montecalvo and Navasero 2023). Native isolate of *M. rileyi* from *S. frugiperda* was also pathogenic to fall armyworm (Montecalvo et al. 2022b). These records suggest that *M. rileyi* is a potential addition to the usual fungal species such as *B. bassiana* and *M. anisopliae* for polyphagous and migratory pests in the Philippines. These fungi were also virulent against fall armyworm under laboratory conditions (Montecalvo and Navasero 2021b). Effective multiplication is necessary for the successful application of this promising biopesticide in the field.

Mass Production of *M. rileyi*

Fungal growth in artificial media. The *M. rileyi* from *S. exigua* was utilized in the cultural characterization and mass production experiments. Growth and sporulation of *M. rileyi* were assessed in 12 artificial media with varying nutritional components. In general, fungal growth was initially white then sporulation occurred thereafter depending on the artificial media. Visible fungal growth was observed 3 to 5 days after incubation (DAI). Cottony growth was observed in PDAY, SMAY, OAY, and PMAY; while the most profuse mycelial growth was visible in SMA. On the other hand, *M. rileyi* had finer and lighter growth observed in other artificial media.

Among the artificial media, *M. rileyi* sporulated in PDAY, PMAY, SMAY, MEAY, and OAY at 8 to 10 DAI. Light olive-green conidia were visible in PMAY and SMAY at 8 DAI, followed by OAY (8.67 DAI), PDAY (9.67 DAI), and MEAY (10 DAI). PMAY, SMAY, and OAY were fully covered with dry conidia. The mass of mycelia and conidia and conidial yield of *M. rileyi* were determined in sporulated Petri plates (Fig. 3). No significant differences were observed in SMAY, MEAY, OAY, PDAY, and PMAY. However, it was evident that the biomass from PMAY had more conidia than SMAY and OAY which supported profuse mycelial growth. Conidial concentration ranged from 5.15 x 10^9 to 2.90 x 10^{10} conidia/ Petri plate.

The cost of these artificial media was also calculated. It was apparent that the most expensive among these five artificial media was SMAY amounting to 7.91$ as compared to OAY (4.48$), PDAY (5.19$), MEAY (5.58$), and PMAY (5.99$) per liter of artificial media.

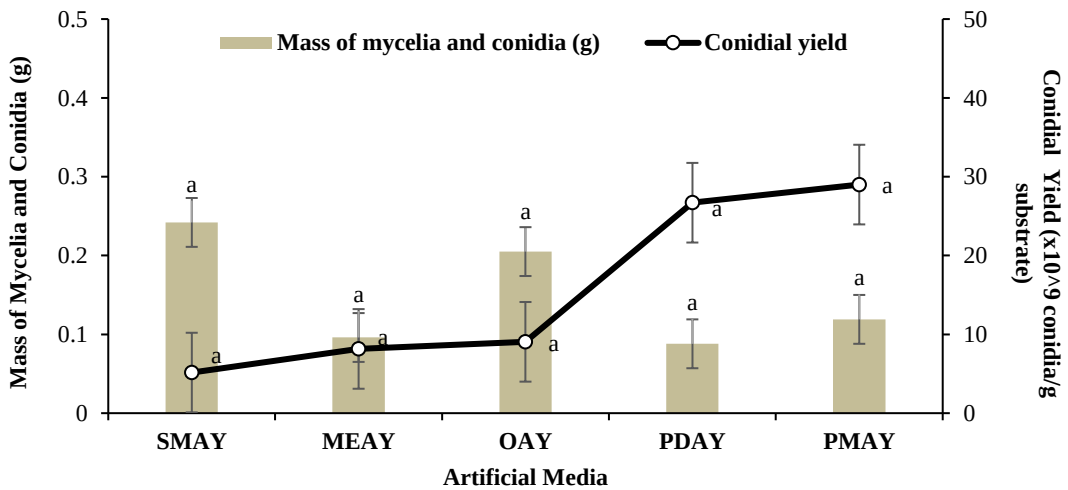

Figure 3. Growth and sporulation of *Metarhizium rileyi* in different solid media. SMAY= Sabouraud maltose agar + yeast extract, MEAY= maltose extract agar + yeast extract, OAY= oatmeal agar + yeast extract, PDAY= potato dextrose agar + yeast extract, and PMAY= potato maltose agar + yeast extract. Means that have a common letter are considered statistically the same at Tukey's HSD ($P<0.05$).

This study determined which artificial media allowed profuse growth and sporulation of *M. rileyi*. The artificial media provide ideal nutrients for fungal growth (Mohammadbeigi 2013). The most commonly used media is SMAY, which is made by amending the SMA medium with 1% and 2% yeast extract (Fronza et al. 2017). SMAY medium was found to support the highest fungal growth and abundant sporulation which initiated earlier than other artificial media followed by SDA and barley carrot yeast extract (BCY) (Ingle 2014). *Nomuraea rileyi* preferred SMAY medium than other artificial substrate (Vimala Devi et al. 2000). However, due to its high cost, mass multiplication media must be optimized to reduce the cost of production. Findings from this research indicate that aside from commonly used media (SMAY), this fungus can also be grown in MEAY, OAY, PDAY, and PMAY. Sporulation was also abundant in MEAY, OAY, PDAY, and PMAY which were relatively cost-effective artificial media. The results are consistent with earlier studies which identified PMAY and SMAY, respectively, as the best media to grow *M. rileyi* (Edelstein et al. 2004; Ingle 2014).

Fungal growth in the tested artificial media initiated at 3 to 5 DAI confirming earlier observations that mycelial growth and sporulation of *N. rileyi* started at 4 and 7 DAI in semisynthetic medium (Vimala Devi et al. 2000). Sporulation in this study occurred at 8 to 10 DAI wherein light

olive-green conidia were visible in PMAY and SMAY at 8 DAI, which was earlier than the sporulation of *N. rileyi* in SMAY at 9.43 days (Ingle 2014). Late sporulation was observed in other media such as SDA, BCY, and YPSS within 13.00 to 14.00 days. In contrast, Edelstein et al. (2004) noted that the lowest growth rates were recorded in SMAY for the tested *N. rileyi* isolated from larvae of *Anticarsia gemmatalis* Hübner and another Plusiinae larvae. The highest growth rate of the isolates was achieved with a medium containing potato and yeast extract. Similarly, this study showed that *M. rileyi* grew best in artificial media with maltose, potato, and yeast extract that favored fungal growth and sporulation. Edelstein et al. (2004) further noted that a cost-effective medium for mass production of *N. rileyi* can be created using potato extract or enriched slices. Maltose and sodium nitrate have been identified as the most proficient sources of carbon and nitrogen, respectively, for spore germination (Ingle 2014). These results are significant in the development of effective nutrient-rich media for spore germination. In addition, maltose is needed for sporulation (Vimala Devi et al. 2000). Mycelial growth of *N. rileyi* is also supported in media with peptone and yeast extract as nitrogen sources.

Liquid media fermentation. Those artificial media that induced sporulation were further tested in a liquid fermentation assay to determine fungal biomass in liquid media. Sporulation was evident from 7.60 to 9.80 DAI. No sporulation was observed in MEY. Based on biomass, PDY, PMY, and SMY were the promising media to produce a significant weight of mycelia and conidia (Fig. 4). The conidial yield did not vary significantly among the liquid media, which ranged from 5.02 x 10^{10} to 9.57 x 10^{10} in 100 ml liquid media.

These findings are consistent with previous research that SMY is one of the media that best supports the sporulation of *M. rileyi*. This study also presented that media with potato extract can be an alternative to SMY which has a relatively high cost of media components. Growth and sporulation of *M. rileyi* were almost similar in solid and liquid media which was in contrast with the findings of Ingle (2014) that *N. rileyi* grew slowly in liquid than in solid media. Culturing in broth composed of Sabouraud maltose with yeast extract (SMYB) resulted in a higher dry mycelial weight (0.680g) of *N. rileyi* isolates (Ingle 2014). This study recorded a biomass (0.741g) close to the findings of Ingle (2014). In contrast, another study observed that glucose and sucrose in liquid media increased conidiation on grains than maltose-rich liquid media (Faria et al. 2022).

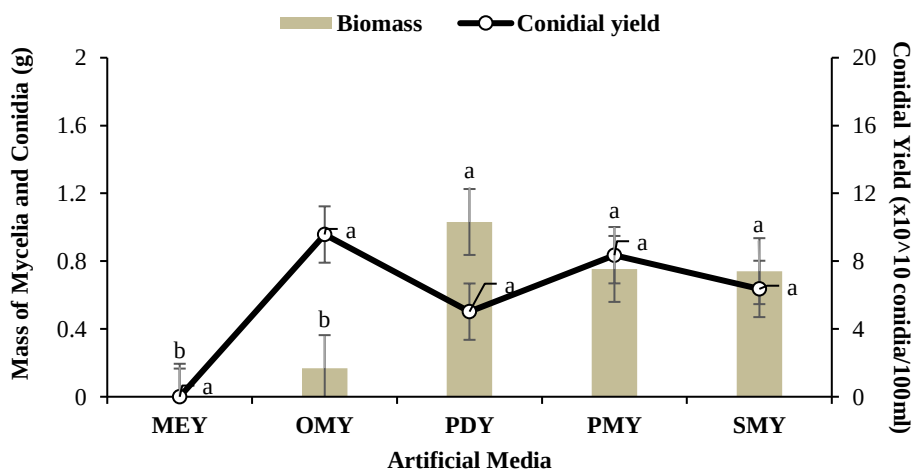

Figure 4. Sporulation of *Metarhizium rileyi* in various liquid media. MEY= maltose extract + yeast extract, OMY= oatmeal + yeast extract, PDY= potato dextrose + yeast extract, PMY= potato maltose + yeast extract, and SMY= Sabouraud maltose + yeast extract. Means that have a common letter are considered statistically the same at Tukey's HSD ($P< 0.05$).

Solid substrate fermentation. Various substrates were assessed in the mass production of *M. rileyi.* Visible growth initiated at 4.00 to 7.00 DAI (Table 2). *M. rileyi* had varying degrees of sporulation in various grain substrates. The methodology of Faria et al. (2022) allowed the earliest sporulation at 6 DAI in precooked rice, which also provided the highest conidial yield of 4.36 x 10^8 conidia/g substrate.

Rice substrate with 1% yeast extract (Thakre et al. 2011) yielded 8.72 x 10^7 conidia/g substrate. In other substrates, fungal mycelial growth was thin or thick with sparse sporulation such as in sorghum. Other substrates such as crushed sorghum with yeast extract, ground mungbean, and rice hull with rice bran or palay had statistically similar conidial yields ranging from 3.30 x 10^6 to 1.40 x 10^7 conidia/g substrate. The lowest sporulation (3.30 x 10^6 conidia/g) was recorded in crushed sorghum + 1% yeast extract following the method of Vimala Devi (1994). The use of by-products such as rice hull and rice bran (1.40 x 10^7 conidia/g) and rice hull and palay (1.14 x 10^7 conidia/g) also supported sporulation of *M. rileyi* but were significantly lower than the conidial yield produced in rice substrate.

Table 2. Sporulation of *Metarhizium rileyi* in solid substrate fermentation.

Substrate	Reference	First growth (DAI)	Initiation of Sporulation (DAI)	Conidial yield (conidia/g substrate)
Precooked rice	Faria et al. 2022	4.00	6.00	4.36 x 10^8a
Crushed rice + 1% Yeast extract	Thakre et al. 2011	6.63	8.67	8.72 x 10^7b
Crushed sorghum + 0.5% Yeast extract	Vimala Devi et al. 2000	4.90	10.58	5.60 x 10^6c
Crushed sorghum + 1% Yeast extract	Ingle 2014	5.20	9.08	4.57 x 10^6c
Precooked ground mungbean		4.00	15.75	5.65 x 10^6c
Rice	Loureiro et al. 2019	4.00	13.14	3.48 x 10^6c
Crushed sorghum + 1% Yeast extract	Vimala Devi 1994	7.00	8.00	3.30 x 10^6c
Rice hull + rice bran		4.00	15.10	1.40 x 10^7c
Rice hull + palay		4.00	8.20	1.14 x 10^7c

* DAI= days after incubation. Means that have a common letter are considered statistically the same at Tukey's HSD ($P< 0.05$).

Solid fermentation experiment suggests that rice supported the highest sporulation of *M. rileyi*. This grain substrate is commonly used in mass-producing commercial products of entomopathogenic fungi; however, their size and bulkiness are concerns in handling, transportation, and preparation (Fronza et al. 2017). *Metarhizium rileyi* grown in carbon-rich broth with nitrogen source and produced in precooked rice is used against *S. frugiperda* in greenhouse and field (Faria et al. 2022). Crushed sorghum + 0.5% yeast extract (Vimala Devi et al. 2000) had slightly similar visible growth, however, sporulation was delayed in this study (10.58 DAI) as compared with previous observation at 6 or 7 DAI. It has also been observed that mycelial growth was visible at 4 DAI while sporulation initiated at 6 or 7 DAI (Vimala Devi et al. 2000).

The conidial yield of *M. rileyi* in this study deviated from the previous findings which may be influenced by several factors such as the *M. rileyi* isolate, inoculum concentration and volume, and incubation time that were standardized in this study. Previously, 4.73 x 10^9 spore/ml of *N. rileyi* were produced using crushed sorghum + 1% yeast extract by inoculating a 6mm circular agar disc and stored

in BOD chamber at 25°C for 25 days (Ingle 2014). On the other hand, following the same protocol, this study only recorded a conidial yield of 4.57 x 10^6 conidia/g substrate when 1 ml of 1 x 10^6 conidia/ml was used to inoculate crushed sorghum + 1% yeast extract and stored at room temperature for 20 days. A previous study demonstrated a greater number of conidial yield produced ($1.34 \pm 0.23 \times 10^9$ and $1.23 \pm 0.08 \times 10^9$ conidia/g) inoculated with liquid media containing glucose (GY) and sucrose (MY), which promoted homogenous and fast conidiation (Faria et al. 2022). In contrast, this study only obtained 4.36 x 10^8 conidia/g following the same protocol but modifying the inoculum using a crude fungal suspension.

In addition, the conidial production of *M. anisopliae* is related to the amount of moisture, pH, and yeast extract concentrations in solid-state fermentation (Bhanu Prakash et al. 2008). Mycelial growth is favored with the addition of yeast extract (Vimala Devi et al. 2000) with profuse vegetative growth in the bags and increased sporulation (Bhanu Prakash et al. 2008). However, those substrates without yeast extract also had profuse fungal growth and higher sporulation including precooking rice and ground mungbean. Similarly, differences in conidial yield may also be due to the material used for incubating the substrate. In this study, grain substrates were stored in polypropylene bags (15 x 35cm) with 0.2 µm filter which would allow passive exchange of sterile air in bags facilitating sporulation of the fungus in solid substrates (Vimala Devi et al. 2000).

In terms of carbon source, these grain substrates had almost similar sugar components. Sorghum contains glucose, fructose, sucrose, raffinose, and stachyose (Nordin 1959). Rice also has sucrose as the major non-reducing sugar in the mature grain (Singh and Juliano 1977). Mungbean contains sucrose, fructose, and glucose (Wang et al. 2022). These carbon sources provide nutrition for the sporulation of *M. rileyi*.

Results also suggest that precooking may have contributed to promoting conidiation wherein conidial yield of precooked rice was considerably highest (Faria et al. 2022) as compared with those grains soaked in water or yeast extract before sterilization (Ingle 2014; Thakre et al. 2011; Vimala Devi et al. 2000; Vimala Devi 1994). Cooking rice releases starch such as amylose and amylopectin making the nutrients available for fungal growth. Aside from the substrates used in this study, other promising substrates tested in *M. rileyi* mass production were crushed maize with 1% yeast extract (Ingle 2014) and refuse raw bananas (Thakre et al. 2011).

CONCLUSIONS AND RECOMMENDATIONS

The paper reports for the first time the morphological, molecular, and cultural characteristics of indigenous isolates of *M. rileyi* isolated from armyworm species such as *S. exigua* and *S. frugiperda* larvae infesting onion and corn crops in the Philippines, respectively. Investigations were also conducted to identify this beneficial fungus through the polyphasic approach and determine the relatedness of the fungal isolates. The identification of cost-effective artificial media was studied as a necessary step in the mass production of this beneficial fungus. Liquid and solid substrate fermentations were also assessed to produce infective inoculum. With the high virulence of *M. rileyi*, further studies should optimize the cost-effective mass production techniques for the successful utilization of this fungus.

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