

# Patogenicity of Entomopathogenic Fungi *Metharhizium rileyi* (Farlow) to *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae)

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**Abstract** – Isolates of the entomopathogenic fungus *Metharhizium rileyi* (Farlow) Samson of *Spodoptera litura* (Fabricius) origin were studied for efficacy against the host insects. Laboratory bioassays at a concentration of  $5,2 \times 10^8$  conidia ml<sup>-1</sup> indicated that *M. rileyi* isolates showed mortality (65.5–82.5% mortality in 9 days) in test larvae of *Spodoptera litura*. It can be inferred from this study that *M. rileyi* isolate has great potential for use in the control of *Spodoptera litura* at the recommended dose of  $5.2 \times 10^8$  causing the mortality around 60-80%. Factors such as virulence and spore viability must also be considered for commercial production of the biological product.

**Keywords** – Entomopathogenic Fungus, Biological Control, Sporulation, Virulence, Insecticides.

## I. INTRODUCTION

The tobacco caterpillar, *Spodoptera litura* (Fabricius), is one of the most destructive pests of various crops [1]. *Spodoptera litura* (F.) is an important insect pest of the South Asian region with a voracious leaf-feeding habit and a large host range of more than 112 plant species [2], [3]. It is responsible for indirect and direct damage to crops [4] and can cause considerable economic losses [5].

Among the insects, polyphagous pest *S. litura* (Lepidoptera: Noctuidae) is an important pest of groundnut besides affecting tobacco, cotton, pulses and several vegetables crops [6]. It was report that an infestation level of one larva per plant during the seedling or flowering stage resulted in 20% yield loss [4]. Severe outbreak of the pest results in 30-40% loss in pod formation [7]. *Spodoptera litura* infestations in Japan generally start at the summer. The pest is usually expose to insecticides from April to early November [8], after the hottest seasons, low temperatures difficult the development of insects affecting their circadian rhythm. The presence of this pest on different crops throughout the year has widely exposed it to insecticides and resulted in the rapid development of resistance to a range of these [9], causing major problems for farmers mainly related to integrated pest management programs.

Though many effective insecticide molecules are suggested to combat *Spodoptera* spp., they are not eco-friendly and add to the cost of cultivation especially in semi-arid tropics where farmers Grow groundnut as subsistence crop. In this regard, breeding for inbuilt resistance occupies importance and is an amenable approach [10].

One of the alternatives to this problem is the use of biological control to control these pest insects. Among the tools available in biological control programs is the use of entomopathogenic fungi. Among them, we can mention *Metarhizium anisopliae*, *Thichoderma harzianum* and *Beauveria bassiana*, which already have

commercial product registration in many countries. However, there are other fungi with the ability to control the insect population in crops. Many of these biological agents occur naturally in ecosystems, as is the case with the fungus *Metarhizium rileyi*. This fungus has a cosmopolitan occurrence infecting mainly of larvae of family Noctuidae, which attack crops; and it was reported to be pathogenic to several lepidopterous insect pests of economic importance of global [1].

*Metarhizium rileyi* (Farlow) Kepler (Hypocreales: Clavicipitaceae), previously known as *Nomuraea rileyi* [11], is a well-known entomopathogenic fungus (deuteromycete, Fungi Imperfecti belonging to the order Moniliales. Which can effectively control some of the pests in lepidopteran. Deuteromycete fungus (Fungi belonging to the order Moniliales is of cosmopolitan occurrence infecting mainly of the larvae of the family Noctuidae, which attack crops; and it was reported to be pathogenic to several lepidopterous insect pests of economic importance of global [1]. In Brazil, many research centers are aware of the potential use of the *Metarhizium rileyi* fungus in the natural control of soybean caterpillar (*Anticarsia gemmatalis*. Approximately 90% of *M. rileyi* hosts belong to the order Lepidoptera, but this entomopathogenic fungus can also occur in more than 30 species of insects of different orders like Coleoptera, and Orthoptera [12].

The entomopathogenic fungi *M. rileyi* is fastidious and has specific growth requirements in terms of temperature, humidity, good aeration for optimum sporulation. It is important to assess suitability and cost-effectiveness, and the virulence and viability of spores produced.

It is reportedly safe to human beings and other non-target organisms, thus being an excellent alternative from an environmental point of view, isolates of entomopathogenic fungi vary in their virulence to target insects. Parameters for assessing virulence of different fungal isolates include time of conidial germination, mortality of insects (efficacy in laboratory bioassays and other factors).

In this study, we exploited the isolate of collection available at the Laboratory of Biological Control - TUAT – Tokyo University of Agriculture and Technology, Tokyo - Japan. Laboratory bioassays with this isolate indicated efficacy against *S. litura*. We thus evaluating its biological potential to be used as a myco-insecticide on a commercial scale (Table 1).

Table 1. Some insect species of agricultural importance susceptible to *Metarhizium rileyi*.

Species	Host Plant	Occurrence	Country
<i>Alabama argilacea</i>	<i>Gossypium hirsutum</i>	Field	Brazil
<i>Agrotis ipsilon</i>	<i>Glycine max</i>	Nature	USA
<i>Anticarsia gemmatalis</i>	<i>Phaseolus vulgaris</i>	Nature	Brazil
	<i>Glycine max</i>		
<i>Cirphis latiuscula</i>	<i>Saccharum officinarum</i>	Nature	Brazil
<i>Diatrea saccharalis</i>	<i>Saccharum officinarum</i>	Nature	Brazil
<i>Helicoverpa zea</i>	<i>Zea mays</i>	Nature	USA/Brazil
	<i>Glycine max</i>		
	<i>Gossypium hirsutum</i>		
<i>Heliothis virescens</i>	<i>Glycine max</i>	Nature	USA

	<i>Gossypium hirsutum</i>		
<i>Plusia sp.</i>	<i>Glycine max</i>	Nature	Brazil
<i>Spodoptera frugiperda</i>	<i>Zea mays</i>	Laboratory	Brazil
<i>Trichoplusia ni</i>	<i>Gossypium hirsutum</i>	Nature	Brazil

Source: adapted Alves [12]; Ignoffo [13].

## II. MATERIAL AND METHODS

### A. Insects

*Spodoptera litura* were collected from crop fields in the Tokyo University of Agriculture and Technology Field Science Center, Tokyo, Japan and reared in laboratory under standard conditions. Larvae were maintained in plastic cages (30 x 22 x 6 cm) at  $25 \pm 1$  °C with a 16:8 (L:D) h photoperiod until pupation, and were reared on the artificial diet (Insecta: Nosan Corporation, Yokohama, Japan). Individual adults were transferred to paper bags (8 x 15 x 20 cm) with a 10% crude sugar solution for feeding following the model of Takatsuka et al. [14]. The reproduction of adults allowed using the 3<sup>rd</sup> and 5<sup>th</sup> instar larvae in this study.

### B. Sources of *M. rileyi* and Preparation of Spore Suspension

Pure cultures used of *M. rileyi* were obtained from collection available at the Laboratory of Biological Control – Tokyo University of Agriculture and Technology (TUAT), from different geographic locations of Japan. All isolates were maintained on SMAY medium [15]. [Peptone: 10g; Yeast Extract: 2g; Dextrose: 40g; Agar: 20g; Distilled water: 1000 mL (pH = 6)] and the spore suspension was prepared from 15-day old cultures of *M. rileyi*. The fungal surface was scraped using a sterile loop with 10 ml of sterile distilled water having 0.02% Tween 80 as a wetting agent [16]. The suspension was then filtered through sterile muslin cloth to eliminate the medium [17]. Spore concentration of the filtrate was determined using a Neubauer Hemocytometer. This served as the stock suspension. Different spore concentration was prepared by adding sterile 0.02% Tween 80 in distilled water. Spore suspension of *M. rileyi* at four different concentrations, conidia of *M. rileyi* were suspended in a 0.1% Tween-80 solution, counted with a hemocytometer, and adjusted to  $5 \times 10^8$ ,  $5 \times 10^7$ ,  $5 \times 10^6$ ,  $5 \times 10^5$  conidia/ml dilutions.

### C. Bioassays

Larvae that completely ingested the suspension were individually transferred into 30 ml plastic cups with a piece of fresh artificial diet (8 mm in diameter, 1 mm in thickness) and maintained at 25 °C under a 16L:8D photoperiod until death or pupation. The mortality of the larvae was checked daily. Experiments were replicated three times with 35 larvae for each dose.

### D. Lethal Effect of Conidia against Larvae

Conidia of *M. rileyi* were suspended in a 0.02% Tween-80 solution, counted with a hemocytometer, and adjusted to  $5 \times 10^8$ ,  $5 \times 10^7$ ,  $5 \times 10^6$ ,  $5 \times 10^5$  conidia/ml dilutions. Larvae of the 3<sup>rd</sup> instar were selected respectively. After immersion in spore suspensions for 10 seconds, their integuments dried on clean filter paper. The number of dead larvae and their death time were recorded every day. To make sure the death was induced by the infection of *M. rileyi*, only dead larvae exhibiting signs of *M. rileyi* mycosis were recorded. Assay was

repeated three times. Data were analyzed with SPSS 22.0 to calculate the LT<sub>50</sub> and LC<sub>50</sub> values.

#### E. Isolation from infected cadavers

Sabouraud's Maltose Agar medium supplemented with 2% Yeast extract (SMAY) medium was used to isolate the fungus. Conidia of *M. rileyi* formed on the cadavers were taken by a mycological loop and streaked on SMAY medium. After incubation at room temperature ( $25 \pm 1^\circ\text{C}$ ) for a week, the colonies obtained were subcultured on SMAY slants for preservation. The isolates were identified by microscopic observation of the conidia forming mycelia for conidiogenous structure and conidial morphology [18], [19], according Fig. 1.; and *M. rileyi* isolates were refrigerated at  $4^\circ\text{C}$ .

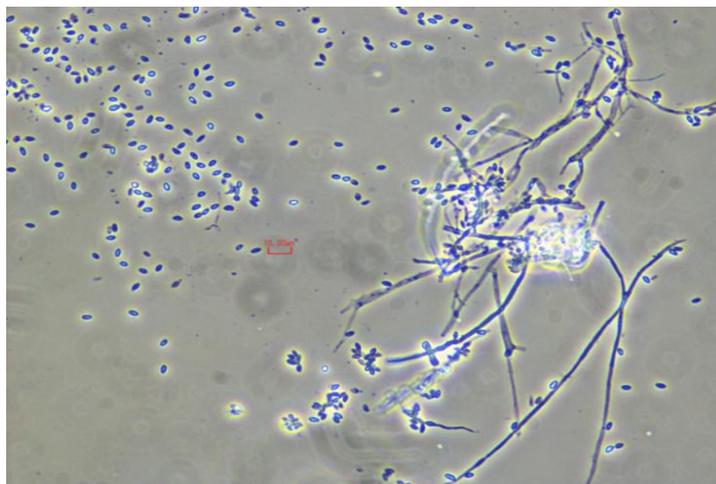


Fig. 1. Microscopic examination of *Metharhizium rileyi* isolates: vegetative hyphae and conidiophores of *M. rileyi* isolate (400× magnification);

#### F. Bioassays

Bioassay with isolates of *M. rileyi* Infectivity of isolates to *Spodoptera litura* larvae was studied through larval bioassays at the effective dose of  $5,2 \times 10^8$  conidia  $\text{ml}^{-1}$ . For carrying out bioassays against *S. litura*, *M. rileyi* spore suspension was applied in the tegument in the insect. How larvae were embedded in the solution of spores with 0.02% Tween-80 solution. Controls were maintained by dipping larvae on solution sprayed with 0.02% Tween-80 solution.

For carrying out bioassays against *S. litura*, the larvae were immersed (soaked) in the spore solution for ten seconds, and then placed on a blotting paper to drain out the excess spore solution, after this, was maintained the feeding of larvae with artificial diet. Three replicates of 35 larvae were tested.

#### G. Statistical Analysis

LC<sub>50</sub>, LT<sub>50</sub> values and levels of significance (ANOVA,  $P < 0.05$ ) were measured with SPSS 22.0, and the graph was drawn with Microsoft Excel.

### III. RESULTS

Bioassays demonstrated an instar (age) resistance to infection by topical exposure. The LC<sub>50</sub> values increased from  $6.35 \times 10^6$  conidia/ml to as high as  $3.95 \times 10^7$  conidia/ml from the 3<sup>rd</sup> and 5<sup>th</sup> instar larvae (Table 2). Furthermore, to compare the pathogenicity of *M. rileyi* conidia against different instar larvae of *S. litura*,

virulence index was adopted by defining the LC<sub>50</sub> value of 3<sup>rd</sup> instar larva as standard as 1, calculated as: virulence index = LC<sub>50</sub> value of 3<sup>rd</sup> instar/LC<sub>50</sub> value of 5<sup>th</sup> instar. The results showed that the virulence of *M. rileyi* decreased along with the development of larvae, especially in this study of 5<sup>th</sup> instar which was around 10 times lower than the 3<sup>rd</sup> instar (Tables 3 and 4, Figure 2).

Table 2. Lethal concentration (LC<sub>50</sub>) of *Metarhizium. rileyi* against larvae of *Spodoptera litura*.

Instar	Regression	LC <sub>50</sub> Conidia/ml	95% Confidence Limits	Virulence Index
3 <sup>rd</sup>	Y= -4.547 + 0.639 x	6.35 x 10 <sup>6</sup>	1.64 x 10 <sup>6</sup> ~ 1.90 x 10 <sup>7</sup>	1
5 <sup>th</sup>	Y = -4.848 + 0.543 x	3.95 x 10 <sup>7</sup>	1.45 x 10 <sup>6</sup> ~ 8.45 x 10 <sup>7</sup>	0.160

Analyzed with PROBIT model: PROBIT (Y) = Intercept +Bx (Covariates x are transformed using the base 10.000 logarithm).

Table 3. Effect of *Metarhizium. rileyi* on growth of *Spodoptera litura* larvae (3<sup>rd</sup> instar)

Treatments	Head capsule (mm)	Width (mm)	Length (mm)	Weight (mg)
control	0.99 ± 0.12 (0.9 - 1.00) c	1.54 ± 0.04 (1.50 - 1.56) b	11.38 ± 2.3 (9.00 - 13.00) c	370.8 c
5 x 10 <sup>5</sup>	0.95 ± 0.11 (0.9 - 1.00) b	1.55 ± 0.05 (1.50 - 1.60) b	11.30 ± 3.1 (9.00 - 13.00) c	360.8 b
5 x 10 <sup>6</sup>	0.95 ± 0.12 (0.9 - 1.00) b	1.55 ± 0.02 (1.50 - 1.56) b	11.21 ± 2.4 (9.00 - 13.00) b	360.2b
5 x 10 <sup>7</sup>	0.90 ± 0.14 (0.9 - 1.00) a	1.40 ± 0.04 (1.50 - 1.58) a	10.01 ± 2.3 (9.00 - 13.00) a	351.7a
5 x 10 <sup>8</sup>	0.90 ± 0.11 (0.9 - 1.00) a	1.39 ± 0.07 (1.50 - 1.56) a	10.05 ± 2.2 (9.00 - 13.00) a	351.3a
CD	0.52	4.15	10.12	43.6

Each value means of triplicate. Different letters in each column differ significantly (5%) by LSD.

Table 4. Effect of *Metarhizium. rileyi* on growth of *Spodoptera litura* larvae (5<sup>th</sup> instar)

Treatments	Head capsule (mm)	Width (mm)	Length (mm)	Weight (mg)
control	2.75 ± 0.50 (2.50 - 3.25) a	5.28 ± 0.70 (5.00 - 7.00) b	37.40 ± 2.30 (35.00 - 40.00) b	396.5 c
5 x 10 <sup>5</sup>	2.74 ± 0.10 (2.70 - 3.00) a	5.31 ± 0.95 (5.00 - 6.00) b	37.30 ± 3.1 (36.00 - 40.00) b	395.8 c
5 x 10 <sup>6</sup>	2.75 ± 0.12 (2.50 - 3.0) a	5.29 ± 0.12 (5.00 - 6.00) b	37.25 ± 2.3 (35.00 - 40.00) b	394.2c
5 x 10 <sup>7</sup>	2.76 ± 0.14 (2.60 - 3.00) a	4.35 ± 0.25 (4.00 - 5.00) a	31.13 ± 4.2 (30.00 - 40.00) a	380.7b
5 x 10 <sup>8</sup>	2.75 ± 0.11 (2.50 - 3.00) a	4.21 ± 0.45 (4.00 - 5.00) a	30.89 ± 2.4 (30.00 - 40.00) a	370.3a
CD	0.50	5.27	8.22	23.3

Each value means of triplicate. Different letters in each column differ significantly (5%) by LSD.

## IV. DISCUSSION

Insects have several defense strategies and mechanisms that can be used to combat biological agents, such as entomopathogenic fungi. These defense mechanisms can be of a chemical or physical nature (or both).

Probably, that cuticle is the primary barrier to fungal infection [20], therefore, fast and direct penetration of the cuticle, which can result in a shorter lethal time against insects, is of vital importance for the pathogenicity of the fungi [21]. Many researches have been made to interpret the infection mechanisms of entomopathogenic fungi [22] and interaction with the insect body, especially the cuticle degrading enzymes. Some mechanisms to defend the cuticle penetration, include production of cuticular antimicrobial compounds, shedding of the cuticle

during development, and environmental conditions [23]. It was inferred that development of cuticle is involved in conidia adhesion and fungal growth, because the  $LT_{50}$  of 5<sup>th</sup> instar is bigger than 3<sup>rd</sup> instar (Fig. 1).

Studies revealed that when exposed to  $10^8$  conidia/ml suspension of *M. rileyi*, the median lethal time ( $LT_{50}$ ) of the 3<sup>rd</sup> instar *S. litura* (Fabricius) (Lepidoptera: Noctuidae) larvae ranged from 5.5 to 6.6 days [24]. In this study it was showed 4.84 days, the inferred value is in accordance with Liu et al. [25] which obtained 4.35 days for  $LT_{50}$  of the 3<sup>rd</sup> instar. It is very interesting to notice that the  $LT_{50}$  increased the number of days when it comes to the age (instar) of the larvae, probably because the insect has better body and nutritional development parameters. Furthermore, environmental conditions could reduce the pathogenicity from application. It was possible to inferred that the pathogenicity reduction effect caused from comparison of the lethal time in different instar.

After death, the observation of total cadaver mummification was inferred (Fig. 2) in seven days after application, and it was possible it was possible to monitor the development of infection in the period (30 days).

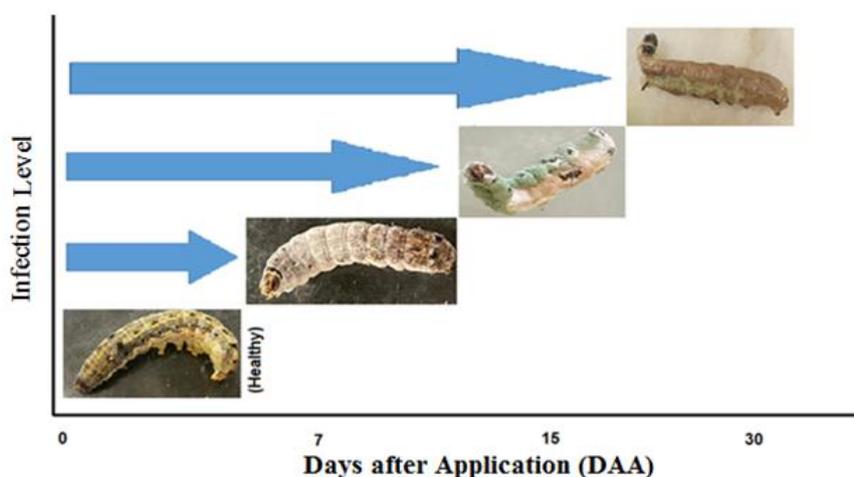


Fig 2. Days After Application.

## V. CONCLUSION

It can be inferred from this study that *Metharhizium rileyi* isolate has great potential for use in the control of *Spodoptera litura* at the recommended dose of  $5.2 \times 10^8$  causing the mortality around 60-80%. Factors such as virulence and spore viability must also be considered for commercial production of the biological product.

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## REFERENCES

- [1] T.S. Rajan, and N. Muthukrishnan. Pathogenicity of *Nomuraea rileyi* (Farlow) Samson isolates against *Spodoptera litura* (Fabricius). *Journal Biological Control*, v. 23, n. 1, p. 17-20, 2009.
- [2] N. Mallikarjuna, K.R. Kranthi, D.R. Jadhav, S. Kranthi, and S. Chandra. Influence of foliar chemical compounds on the development of *Spodoptera litura* (Fab.) in interspecific derivatives of groundnut. *Journal of Applied Entomology*, v. 128, n. 5, p. 321-328, 2004.
- [3] J. Yao, Y. Liu, Y. Tuo, J. Zhu, X. Qin, J. Dong, S. Qu, and Z. Yu. Studies on the growth metabolism of *Bacillus thuringiensis* and its vegetative insecticidal protein engineered strains by microcalorimetry. *Applied Biochemistry and Microbiology*, v. 42, n. 3, p. 274-277, 2006.
- [4] B.C. Dhir, H.K. Mohapatra, and B. Senapati. Assessment of crop loss in groundnut due to tobacco caterpillar, *Spodoptera litura* (F.). *Indian Journal of Plant Protection*, v. 20, n. 2, p. 215-217, 1992.
- [5] G.S. Dhaliwal, V. Jindal, and A.K. Dhawan. Insect pest problems and crop losses: changing trends. *Indian Journal of Ecology*, v. 37, n. 1, p. 1-7, 2010.
- [6] S.P. Singh, and S.K. Jalali. Management of *Spodoptera litura* (Lepidoptera: Noctuidae) In India. Proc. National Scientists Forum *Spodoptera litura*, 2-4, April, 1996, ICRISAT Asia Centre, ICRISAT, Patancheru, Andhra Pradesh, India, 27-65, 1997.
- [7] K.N.H. Joshi. *Characterization of groundnut (Arachis hypogaea L.) germplasm in relation to major foliar pests and diseases*. Ph. D. Thesis, Saurashtra University, Rajkot, India, 2005.
- [8] M. Ahmad, M.A. Saleem, and A.H. Sayyed. Efficacy of insecticide mixtures against pyrethroid-and organophosphate-resistant populations of *Spodoptera litura* (Lepidoptera: Noctuidae). *Pest Management Science: formerly Pesticide Science*, v. 65, n. 3, p. 266-274, 2009.
- [9] A.H. Sayyed, M. Ahmad, and M.A. Saleem. Cross-resistance and genetics of resistance to imidacloprid in *Spodoptera litura* (Lepidoptera: Noctuidae). *Journal of Economic Entomology*, v. 101, n. 2, p. 472-479, 2014.
- [10] M.A. Saleem, G.K. Naidu, P.S. Tippannavar, and H.L. Nadaf. Biophysical and biochemical mechanism of resistance to *Spodoptera litura* in groundnut (*Arachis hypogaea L.*). *Journal of Entomology and Zoology Studies*, v. 7, n. 4, p. 86-96, 2019.
- [11] R.M. Kepler, R.A. Humber, J.F. Bischoff, and S.A. Rehner. Clarification of generic and species boundaries for *Metarhizium* and related fungi through multigene phylogenetics. *Mycologia*, v. 106, n. 4, p. 811-829, 2014.
- [12] S.B. Alves. Controle microbiano de insetos. 1998.
- [13] C.M. Ignoffo. The fungus *Nomuraea rileyi* as a microbial insecticide. In: H.D. Burges (ed). *Microbial Control of Pests and Plant Diseases 1970-1980*. New York: Academic Press, p. 513-538, 1981.
- [14] J. Takatsuka, S. Okuno, M. Nakai, and Y. Kunimi. Genetic and phenotypic comparisons of viral genotypes from two nucleopolyhedroviruses interacting with a common host species, *Spodoptera litura* (Lepidoptera: Noctuidae). *Journal of invertebrate pathology*, v. 139, p. 42-49, 2016.
- [15] M.S. Goettel, and G.D. Inglis. Fungi: Hyphomycetes. In: L.A. Lacey (ed). *Manual of techniques in insect pathology*. London: Academic Press. pp 213-249, 1997.
- [16] M.C. ROMBACH, R.M. Aguda, B.M. Shepard, and D. Roberts. Infection of rice brown planthopper, *Nilaparvata lugens* (Homoptera: Delphacidae), by field application of entomopathogenic *Hyphomycetes* (Deuteromycotina). *Environmental Entomology*, v. 15, n. 5, p. 1070-1073, 1986.
- [17] K.R. Sasidharan, and R.V. Varma. Laboratory evaluation of *Beauveria bassiana* (Balsamo) Vuillemin against *Indarbela quadrinotata* Walker (Lepidoptera: Metarbelidae)-a key pest of *Casuarina equisetifolia L.* in Tamil Nadu. *Journal of Biological Control*, v. 19, n. 2, p. 197-199, 2005.
- [18] R.A. Samson, H.C. Evans, J.P. and Latge. Atlas of Entomopathogenic Fungi. Springer Verlag, Berlin, 1988.
- [19] J. Aoki. *A key to Insect Pathogenic Fungi*. Zenkoku Nosen Kyoiku Kyokai (Sonkyo Sonkyo), Tokyo, Japan. p. 280, 1989.
- [20] N.O. Keyhani. Lipid biology in fungal stress and virulence: entomopathogenic fungi. *Fungal biology*, v. 122, n. 6, p. 420-429, 2018.
- [21] S.A. Dar. B.A. Rather, and A.A. Kandoo. Insect pest management by entomopathogenic fungi. *J. Entomol. Zool. Stud.*, v. 5, p. 1185-1190, 2017.
- [22] K.M.S. Aw, and S.M. Hue. Mode of infection of *Metarhizium* spp. fungus and their potential as biological control agents. *Journal of fungi*, v. 3, n. 2, p. 30, 2017.
- [23] A. Ortiz-Urquiza, N.O. Keyhani. Action on the surface: entomopathogenic fungi versus the insect cuticle. *Insects*, v. 4, n. 3, p. 357-374, 2013.
- [24] M.S. Padanad, and P.U. Krishnaraj. Pathogenicity of native entomopathogenic fungus *Nomuraea rileyi* against *Spodoptera litura*. *Plant Health Progress*, v. 10, n. 1, p. 11, 2009.
- [25] S. Liu, Z. Xu, X. Wang, L. Zhao, G. Wang, X. Li, and L. Zhang. Pathogenicity and in vivo Development of *Metarhizium rileyi* Against *Spodoptera litura* (Lepidoptera: Noctuidae) Larvae. *Journal of economic entomology*, v. 112, n. 4, p. 1598-1603, 2019.

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