AATCC Review

14 June 2024: Received
17 July 2024: Revised
20 August 2024: Accepted
22 September 2024: Available Online

www.aatcc.peerjournals.net

Original Research Article

Open Access

Evaluation of laboratory-prepared water dispersible granules of *Metarhizium rileyi* (Farlow) Kepler, S.A. Rehner, and Humber against major soil pupating insect pests

Raja Reddy Gundreddy, Jayanth B. V, Shashikala M, Udaykumar E, Gouranga Saw and Bishwajeet Paul*

Biological Control Laboratory, Division of Entomology, ICAR-Indian Agricultural Research Institute, New Delhi-110012.

ABSTRACT

Entomopathogenic fungi (EPF) exhibit formidable effectiveness that, under specific conditions, can offset the shortcomings of chemical insecticides within pest control programs. Among these EPFs, Metarhizium rileyi stands as one of the most extensively studied agents for global pest control. The study delves into the assessment of the relative virulence of M. rileyi water-dispersible granules (WDG) when deployed against distinct soil-pupating stages of lepidopteran and dipteran insects. M. rileyi WDG formulations were subjected to evaluation against various soil-pupating stages of insects, including lepidopteran and dipteran species. The results, based on dose-response relationships and LC_{50} values, revealed distinct levels of virulence exhibited by M. rileyi. Among the lepidopteran insects tested, M. rileyi demonstrated the highest level of virulence against H. armigera, followed by A. albistriga, A. flava, S. frugiperda, A. ipsilon, S. litura, S. obliqua, and A. janata. The LC_{50} values of these treatments, arranged in ascending order, were 2.01×10^7, 7.17×10^7, 1.13×10^8, 1.41×10^8, 1.54×10^8, 3.35×10^8, 3.62×10^8, and 1.00×10^9, respectively. In contrast, M. rileyi exhibited the least virulence against S. exigua, with an LC_{50} value of 1.35×10^9. Notably, the efficacy of M. rileyi WDG formulations was significantly lower when targeting dipteran pupae compared to lepidopteran pupae. For B. cucurbitae and B. dorsalis, the LC_{50} values were 7.26×10^9 and 1.29×10^{10}, respectively. M. rileyi WDG demonstrated remarkable virulence when tested against the pupae of H. armigera, marking the highest level of efficacy observed. In contrast, these WDG exhibited limited efficacy when targeting dipteran pupae, such as those of B. dorsalis and B. Cucurbitae.

Keywords: *Entomopathogenic fungi, Metarhizium rileyi, water dispersible granules, relative virulence, Lepidopteran larvae, Dipteran pupae*

Introduction

Food security and sustainable crop output are seriously threatened by insect pests, particularly in the absence of efficient management techniques. Current control measures often rely on heavy and indiscriminate application of broad-spectrum insecticides, which negatively affect human health, the environment, and natural enemies [40,32]. The neonicotinoids and pyrethroids used in the management of defoliators are also known to be highly toxic to parasitoids and predators. The use of these broad-spectrum insecticides often results in inconsistent insect control [45,25]. Many insects spend a substantial part of their life cycle below ground, such as the American bollworm, tobacco caterpillar, jute hairy caterpillar, cutworm, cotton semi-looper, castor semi-looper, beet armyworm, fall armyworm, and fruit fly. Targeting the most destructive active stage of the insect is always considered, often inactive stages are ignored. However, neglecting the soil-dwelling pre-pupae and pupae can create a critical gap in pest management strategies [29]. Addressing these life stages is essential for achieving effective and sustainable pest control. Without causing harmful environmental residues or developing insect host resistance, microbial pesticides - like mycoinsecticide - have a potential future in the biological control of insect pests. Microbial pesticides, such as mycoinsecticide, play a promising role in the biological management of insect pests without leaving toxic residues in the surroundings or inducing resistance in their insect hosts [39]. The efficacy of entomopathogenic fungi (EPF) always stands out when compared with other conventional biopesticides. These EPFs can target all the growth stages of insect development, including non-feeding stages like eggs, pupae of insects, and immature and adult stages of many insect species, within both natural and artificial ecosystems [7]. Moreover, their ability to invade non-feeding stages was due to the specific mechanism of invading the host directly through the cuticle [38]. *Metarhizium rileyi*, erstwhile known as *Nomuraea rileyi*, has been preferred for managing lepidopteran pests. The growth of *M. rileyi* under field conditions requires optimum temperature and high relative humidity of approximately (25°C, and 70–75% respectively) [2,10,19,22]. Wind speed is mainly responsible for the dispersal of conidia [18]. Usually, these conditions are not present in the field always. However, such congenial conditions favorable for the growth and spread of the EPFs are usually prevalent in tropical countries e.g., sub-Saharan Africa, India, and Brazil. It is highly effective against Noctuiid defoliators such as *Spodoptera litura* (Fabricius), *Helicoverpa armigera* (Hubner), *Anticarsia gemmatalis* (Hubner), and *Trichoplusia ni* Hubner for eco-friendly pest management. A review of the literature indicates that approximately 60 lepidopteran species are susceptible to this pathogen, with half of them belonging to the Noctuiidae family [17].

*Corresponding Author: ***Bishwajeet Paul***

DOI: https://doi.org/10.58321/AATCCReview.2024.12.03.318

Foliar damage is correlated to monetary losses, which can lead to yield losses of approximately 50% [42]. Phytophagous Dipterans including fruit flies are the most destructive insect pest of fruits and vegetables around the [47]. The last instar larvae exit the fruit and drop down and burrow in the soil and pupate. Larvae generally pupate in the upper four cm of the soil. These lepidopteran and dipteran species are more destructive in agriculture. As a result, alternative, more sustainable management techniques against these insect pests are needed of the hour. To deal with such situations, alternative use of microbial insecticides becomes a more relevant option. Mycoinsecticides play an important role in the biocontrol of insect pests, serving as alternatives to chemical control, without leaving toxic residues in the environment or inducing resistance in their insect hosts [13].

Various foliar sprayable EPF formulations are present in the market to be used against larval stages of insect pests; their effectiveness has been reported to be inconsistent. Synthetic chemical pesticides persist in the environment for a longer period and contaminate the soil, groundwater, water bodies, and aquatic organisms. This leads to biomagnifications of the metabolites of insecticides [5,48]. A formulation specifically designed to target the subterranean stage of the insect could significantly enhance its efficacy. [3] demonstrated that under laboratory conditions soil treated with EPF conidia has shown promising results against *S. litura* pupae. Fruit flies pupate in the soil, suggesting that applying fungi in this environment has the potential for fruit fly management [11]. Granules made from EPF have an advantage over conventional insecticides when managing soil-dwelling insect pests [44]. When soil application of EPF-based granular formulations is carried out, the conidia can be maintained under conditions of relatively high humidity, which enhances conidial survival, germination, and virulence. This study aims to assess the management effectiveness of *M. rileyi* water dispersible granule (WDG) formulation against the soil-dwelling stages of insect pests.

Material and Methods

Insect rearing

All test species' eggs were gathered from the fields of ICAR-Indian Agricultural Research Institute, New Delhi, India. The rearing of *Agrotis ipsilon* (Hufnagel) (Noctuiidae: Lepidoptera), *Spodoptera litura* (Fabricius) (Noctuiidae: Lepidoptera), *Spilarctia obliqua* (Walker) (Arctiidae: Lepidoptera), and *Spodoptera exigua* (Hübner) (Noctuiidae: Lepidoptera) was completed in compliance with the guidelines given by [21]. *Spodoptera frugiperda* (Smith) (Noctuiidae: Lepidoptera) was reared following the protocols given by [27].

The rearing of *Achaea janata* (Linn.) (Lepidoptera: Noctuiidae) was carried out following a method described by [4]. A laboratory colony of *Amsacta albistriga* (Walker) (Lepidoptera: Noctuiidae), was developed as per [37] and *Anomis flava* (Fab.) (Lepidoptera: Erebidae) laboratory stock culture was developed and maintained by following [43].

Rearing of *H. armigera*: *H. armigera* was reared on overnight soaked seeds of chickpea. Plastic vials (5 cm x 3 cm size) were used to rear the larvae individually. The larvae were reared individually in plastic vials. The larvae were fed with freshly sopped seeds every day. The fully developed last instar caterpillars were gathered and released in plastic trays (30 cm x 45 cm size) containing two kilograms of sterilized soil. A muslin cloth was used to cover the plastic trays for proper aeration. The moths emerged after seven days and were gathered and maintained in glass jars (30 cm x 25 cm size) for mating and oviposition, and fed with 10% sucrose solution provided in a cotton wick.

Rearing of *B. cucurbitae*: *B. cucurbitae* was reared on bitter gourd (*Momordica charantia* Linn.) fruits. An oviposition cage was prepared using glass jars of 30 cm x 45 cm. Fresh tender bitter gourd fruits were provided for oviposition. Twenty-four hours after oviposition the fruits containing newly deposited eggs were taken out and placed in rearing glass jars (30 cm x 45 cm). Two kilograms of germ-free soil was added to each rearing jar to facilitate the pupation of the fully developed maggots at a later stage.

All the stock cultures of the test insects were maintained at a temperature of 27°C ± 2°C, with a photoperiod of 12:12 (dark: light) and a relative humidity of 65 ± 5%. The stock culture of these pests was maintained at the Biological Control Laboratory, Division of Entomology, ICAR-IARI, New Delhi. Germ-free soil was provided for pupation to all the test insect larvae irrespective of species.

Mass production of *Metarhizium rileyi*

At first, *M. rileyi* was isolated from its native environment from naturally infected larvae of *H. armigera* collected from an infested red gram field in Bilakalagudur village, Nandyal, Andhra Pradesh, India, in November 2022. The pathogen was isolated on Sabouraud's maltose yeast extract agar (SMYA) and mass-produced on rice grains following the protocols described by [9].

Preparation of water-dispersible granules of *Metarhizium rileyi*

Water-dispersible granules of *M. rileyi* were prepared by using attapulgite (75 g) as carrier carboxy methyl cellulose (1.0 g) as binder and sodium lignosulfonate (1.0 g) as dispersing agent. Glycerol (1 ml) was added as a moisturizer. After mixing all these components, one gram of mass-produced *M. rileyi* (containing media residues and *M. rileyi* conidia) was added. Viable conidia population was estimated by taking one gram of this mixture and after serial dilutions, the plate counts were obtained. The final adjustments in the ingredient mixture were made to obtain a population count of 10^6 conidia/g of the mixture. The dough was made by adding 20 ml of distilled water to this mixture. After running this dough through a granulator, granules were produced. After being air dried in the shade, the granules were sieved through 18 mesh to achieve a consistent one-millimetre size. The viable conidia population was estimated by a plate-count method.

Bioassay of *Metarhizium rileyi* granular formulation

Covered plastic trays (30 cm x 45 cm size) containing two kilograms of germ-free soil were used for all bioassays. To achieve a population of 10^4, 10^5, 10^6, 10^7, 10^8, 10^9, and 10^{10} *M. rileyi* conidia per gram of soil, the necessary number of granules was added to each tray individually. Care was taken to mix the granules only on the upper one centimeter of the soil. Twenty fully grown last instar larvae of the test insects were released in each replication. For every conidia concentration, three replications were kept. The control treatment trays were treated with the same number of blank granules (i.e., granules without *M. rileyi*). Three days after the larvae were released, the observations were made. Observations were recorded for dead/moribund insects every 24 hours up to 8 days. The dead/moribund insects collected every day were surface sterilized with 70% ethanol washed with sterile double distilled water placed in Petri dishes containing sterile filter paper and

incubated (27±2°C and 75±5% relative humidity) for 7 days. Cadavers showing mycosis were only considered dead because of fungal infection.

Statistical analysis

In none of the replications in any treatment, the mortality in control treatments ranged between 5-10%. To adjust the mortality percentage, Abbott's formula was applied [1]. Mortality data were subjected to probit analysis as per [16]. We performed a probit analysis on the mortality data, and LC_{50} values were calculated by using SPSS 16.0 statistical software

Results

Efficacy of laboratory-prepared water-dispersible granules of Metarhizium rileyi against some lepidopteran and dipteran pupae

In the present investigation, we studied the effects of *M. rileyi* WDG on various lepidopteran soil-pupating insects. The responses of different soil pupating insects to the respective EPF granular formulations are fitted in the log (dose)/probit (mortality) model at $p < 0.05$ (Table 1). The slope coefficients of the granular formulations ranged between 0.33 and 0.63. The estimated LC_{50} values of the respective treatments differed significantly, as indicated by the 95% confidence intervals ($p < 0.05$) (Table 1). This finding is exciting because entomopathogenic fungi (EPFs) often exhibit low efficacy against dipteran pupae compared to lepidopteran pupae. For *B. cucurbitae* and *B. dorsalis* the LC_{50} values were 7.26×10^9 and 1.29×10^{10}, respectively. Based on the LC_{50} values, *M. rileyi* was found to be more virulent against lepidopteran insects, such as *H. armigera*, followed by *A. albistriga, A. flava, S. frugiperda, A. ipsilon, S. litura, S. obliqua*, and *A. janata* and the LC_{50} values of the corresponding treatments arranged in ascending order are 2.01×10^7, 7.17×10^7, 1.13×10^8, 1.41×10^8, 1.54×10^8, 3.35×10^8, 3.62×10^8, and 1.00×10^9 respectively. *M. rileyi* was found to be least virulent against *S. exigua*, with an LC_{50} value of 1.35×10^9. The effect of *M. rileyi* WDG exhibited more virulence towards *H armiger* and did not vary significantly with LC_{50} values of *A. albistriga, A. flava, S. frugiperda, A. ipsilon, S. litura*, and *S. obliqua*. However, significantly varied with *A. janata, S. exigua, B. cucurbitae,* and *B. dorsalis*.

Relative virulence of laboratory prepared water dispersible granules of Metarhizium rileyi against some lepidopteran and dipteran pupae

In the present investigation, the relative virulence against different soil-pupating insects was compared using LC_{50} values of *S. litura* as a reference because this entomopathogenic fungus is generally recommended for controlling *S. litura*. During this investigation, *M. rileyi* WDG were assessed against various soil-pupating lepidopteran and dipteran insects in their early pupal stage (Table 1). Upon examining the data, it was revealed that *H. armigera* late larval instar was 16.67 times relatively more susceptible to *M. rileyi* WDG. In contrast, *B. dorsalis* was found to be relatively least susceptible (0.26 times) as compared to the LC_{50} value of *S. litura*. The overall relative efficiency of the test WDG presented in descending order, was as follows *H. armigera* (16.7) > *A. albistriga* (4.7) >*A. flava* (2.96) > *S. frugiperda* (2.37) > *A. ipsilon* (2.17) > *S. litura,* (1.0) > *S. obliqua* (0.92) > *A. janata* (0.333) > *S. exigua* (0.24) >*B. Cucurbitae* (0.046) > *B. dorsalis* (0.026).

Discussion

The pupal stage of the insect is non-motile. The habits of the above-tested insect pests to pupate under subterranean conditions prevent their exposure to insecticides. This leads to the repeated application of insecticides, which in turn pollutes the soil, water bodies, and groundwater [5,48]. The use of granules containing entomopathogenic fungi provides an attractive alternative that is safe for to environment and human health. *M. rileyi* often cause epizootics in nature and the ability of its aerial conidia to penetrate the insect integument and cause pathogenesis makes it more suitable to be used as contact insecticide [33]. In the present study, we used nine lepidopteran and two dipteran polyphagous species because they cause extensive damage to crops all over India. Foliar sprays using fungal formulation have several limitations viz. the conidia get dried up and die or high temperature coupled with low relative humidity adversely affects conidial germination in addition to UV inactivation of the conidia [8,15,26,36,41,46]. On the other hand, conidia applied in the soil as granular formulations would be protected from abiotic factors [33]. EPF formulations have been proven to be better than conventional sprays to manage insect pests with soil-dwelling life stages [28,49]. In India, no EPF granular formulation is available in the market.

In the present investigations, we evaluated laboratory-prepared *M. rileyi* WDG against nine species of lepidopteran caterpillars and maggots of two dipteran species. We showed that this isolate is highly virulent to the *H. armigera* last instar larvae and pupae and kills them in laboratory experimental conditions. The habits of the above-tested insect pests to pupate under subterranean conditions prevent their exposure to insecticides. Our results showed that the application of fungal WDG is highly effective against the insect larvae.

The dose-response relationships recorded in the present study are consistent with those obtained by [34]. *M. rileyi* was found to cause pupal mortalities (with a maximum of 25.42%) and produced malformed adults (ranging from 60% to 100%) when used against one-day-old pupae of *S. litura* and *H. armigera* at spore concentrations ranging from 10^5 to 10^9 spores per milliliter. Various types of morphological changes were observed in the larvae and pupae treated with entomopathogenic fungi. Morphogenetic abnormalities observed amongst the pre-pupae and pupae of lepidopteran insects caused by the pathogenic fungal infection have been well documented including shorter body length, crumpled wing lobes, reduced antenna size, and significantly shorter abdomen length, the pupae displayed dark coloration with black ends, or they had brown or dark brown-colored markings on the entire pupa, with dark endpoints [14,23,35]. Notably, these effects were observed in one-day-old pupae, which have just formed and possess thin and soft cuticles.

[11] indicated that the entomopathogenic fungi, particularly the granular formulation, outperformed diazinon. The granular formulation remained effective for up to 668 days after soil inoculation and resulted in a 54% reduction in some fruit fly species. However, in our study, we were able to induce high levels of infection in pupae through soil inoculation before introducing the pupae into the medium. This suggests that soil treatments targeting *H. armigera* pupae may provide a viable method for population suppression. Previous studies on the effects of other entomopathogenic fungi against pupae have shown a reduction in adult emergence rates [12,24], an extension of the pupal stage duration accompanied by a decrease in adult longevity [29,31].

Additionally, malformation and reduced fecundity were reported in adults emerging from fungal-treated pupae [29].

This is an intriguing finding, as entomopathogenic fungi (EPFs) often exhibit low efficacy against dipteran pupae. This is primarily because the dipteran puparium acts as a barrier, hindering the penetration and outgrowth of EPFs [6,30]. Lepidopteran pupae have obtect characteristics, with appendages closely attached to the body and surrounded by a cocoon. In contrast, Dipteran pupae have coarctate features, being enclosed within the cuticle of the third-stage larvae [20]. These differences have a negative impact on fungal penetration and explain the challenges faced when targeting dipteran pupae with EPFs. Another major reason for poor efficacy against dipteran larvae/pupae could be that the last instar maggots just drop down and immediately enter the soil and pupate. Whereas, the last instar lepidopteran larvae wander on the soil for some time and then burrow into it to make cocoon and pupation. This activity exposes the last instar caterpillars for a relatively longer period of exposure to the soil containing EPF conidia.

Conclusions

Our studies also revealed that the fungus could successfully grow from the WDG, colonize, and spread in the soil leading to mortality among resting larvae and pupae. Therefore, we suggest an application of *M. rileyi* formulated WDG in *the Kharif* season when the above-mentioned insect pests are predominant in the field. The longer persistence of EPF in the soil is an important factor for the management of soil-dwelling stages of insect pests because the conidia are protected from high temperatures and direct UV rays from the sun. Indeed, the application of granular formulation in soil Favors germination of conidia and further survival due to relatively congenial temperature and humidity.

Geolocation information: New Delhi, India.

Conflict of interest: The authors declares no competing interests.

Acknowledgments: The authors are thankful to the Director, ICAR-IARI for providing facilities to carry out the research work. The fellowship received by Rajareddy, G. from ICAR is duly acknowledged.

Disclosure statement: No potential conflict of interest was reported by the author(s).

Funding: This work was not supported by any external funding agency other than IARI in-house funding.

Statements and Declarations: RG and BP planned and designed the research experiments. RG has conducted all the bioassays and wrote the research article, JBV, SM, GS, and UE maintained the test insect cultures and helped in analyzing the bioassay data, all the authors have read and approved the manuscript.

Data availability statement: The data that support the findings of this study are available from the corresponding author, BP, upon reasonable request.

Table 1. Virulence of laboratory prepared water dispersible granules of M. rileyi against some lepidopteran and dipteran pupae.

Sl No	Insect species	df	Regression equation	Heterogeneity	LC_{50}	Fiducial Limits		Relative* virulence
						Minimum	Maximum	
1	*Helicoverpa armigera*	5	Y=0.393302216+0.630646875x	4.3967	$2.01x10^{7}$	$1.22 x10^{7}$	$33.0 x10^{7}$	16.66667
2	*Amsacta albistriga*	5	Y=0.454285233+0.5786515x	0.8264	$7.17 x10^{7}$	$4.38 x10^{7}$	$1.17 x10^{8}$	4.672245
3	*Anomis flava*	5	Y=0.851654643+0.514930477x	3.3525	$1.13 x10^{8}$	$6.63 x10^{6}$	$1.95 x10^{8}$	2.964602
4	*Spodoptera frugiperda*	5	Y=1.253917569+0.459543925x	0.3508	$1.41 x10^{8}$	$7.80 x10^{7}$	$2.57 x10^{8}$	2.375887
5	*Agrotis ipsilon*	5	Y=0.877302033+0.503463524x	0.5771	$1.54 x10^{8}$	$8.89 x10^{7}$	$2.67 x10^{8}$	2.175325
6	*Spodoptera litura*	5	Y=1.121709358+0.454871607x	0.4477	$3.35 x10^{8}$	$1.79 x10^{8}$	$6.29 x10^{8}$	1.00000
7	*Spilarctia obliqua*	5	Y=0.681331937+0.504583084x	1.1105	$3.62 x10^{8}$	$2.04 x10^{8}$	$6.42 x10^{8}$	0.925414
8	*Achaea janata*	5	Y=0.367551837+0.514652598x	1.199	$1.00 x10^{9}$	$5.37 x10^{8}$	$1.87 x10^{9}$	0.335
9	*Spodoptera exigua*	5	Y=0.726179045+0.467979546x	0.6535	$1.35 x10^{9}$	$6.66 x10^{8}$	$2.76 x10^{9}$	0.248148
10	*Bactrocera cucurbitae*	5	Y=0.446232635+0.461778528x	7.5161	$7.26 x10^{9}$	$3.88 x10^{9}$	$1.35 x10^{10}$	0.046143
11	*Bactrocera dorsalis*	5	Y=1.661176459+0.330204696x	0.8534	$1.29 x10^{10}$	$5.57 x10^{9}$	$2.99 x10^{10}$	0.025969

**Relative virulence = LC_{50} of Spodoptera litura / LC_{50} of respective insects where LC_{50} value of Spodoptera litura is considered as unit*

References

1. Abbott, W. S. (1925). A method of computing the effectiveness of an insecticide. *Journal of Economic Entomology*, 18, 265–267. https://doi.org/10.1093/jee/18.2.265a

2. Allen, G. E., Greene, G. L., & Whitcomb, W. H. (1971). An epizootic of *Spicaria rileyi* on the velvetbean caterpillar, *Anticarsia gemmatalis*, in Florida. *Florida Entomologist*, 54(2), 189-191. https://doi.org/10.2307/3493569

3. Anand, R., Prasad, B., & Tiwary, B. N. (2009). Relative susceptibility of *Spodoptera litura* pupae to selected entomopathogenic fungi. *BioControl*, 54, 85–92. https://doi.org/10.1007/s10526-008-9157-x

4. Chauhan, V. K., Dhania, N. K., Chaitanya, R. K., & Senthilkumaran, B. (2017). Larval mid-gut responses to a sub-lethal dose of Cry toxin in lepidopteran pest *Achaea janata*. *Frontiers in physiology*, 8, 287-282. https://doi.org/10.3389/fphys.2017.00662

5. Cimino, A. M., Boyles, A. L., Thayer, K. A., & Perry, M. J. (2017). Effects of neonicotinoid pesticide exposure on human health: a systematic review.- *Environmental Health Perspectives*, 125, 155–162. http://dx.doi.org/10.1289/EHP515

6. Cossentine, J., Thistlewood, H., Goettel, M., & Jaronski, S. (2010). Susceptibility of preimaginal western cherry fruit fly, *Rhagoletis indifferen*t (Diptera: Tephritidae) to *Beauveria bassiana* (Balsamo) Vuillemin Clavicipitaceae (Hypocreales). *Journal of Invertebrate Pathology*, 104(2), 105-109. https://doi.org/10.1016/j.jip.2010.02.006

7. Cuthbertson, A. G. S., & Audsley, N. (2016). Further screening of entomopathogenic fungi and nematodes as control agents for *Drosophila suzukii*. *Insects*, 7(24), 1–9. https://doi.org/10.3390/insects7020024

8. Da Silva, R. Z., & Neves, P. M. (2005). Techniques and parameters used in compatibility tests between *Beauveria bassiana* (Bals) Vuill and in vitro phytosanitary products. *Pest Management Science*, 61(7), 667-674. https://doi.org/10.1002/ps.1035

9. Devi, P. S. V., & Prasad, Y. G. (2001). *Nomuraea rileyi* — A potential mycoinsecticide. In: Upadhyay, R. K, Mukerji, K. G., & Chamola, B. P. (eds) *Biocontrol Potential and its Exploitation in Sustainable Agriculture. Springer*, Boston, MA. https://doi.org/10.1007/978-1-4615-1377-3_3

10. Edelstein, J. D., Trumper, E. V., & Lecuona, R. E., (2005). Temperature-dependent development of the entomopathogenic fungus *Nomuraea rileyi* (Farlow) Samson in *Anticarsia gemmatalis* (Hübner) larvae (Lepidoptera: Noctuidae). *Neotropical Entomology*, 34, 593-599. https://doi.org/10.1590/S1519-566X2005000400009

11. Ekesi, S., Dimbi, S., & Maniania, N. K. (2007). The role of entomopathogenic fungi in the integrated management of tephritid fruit flies (Diptera: Tephritidae) with emphasis on species occurring in Africa. *Use of Entomopathogenic Fungi in Biological Pest Management* (ed. by S. Ekesi & N. K. Maniania), pp. 239–274. Research Signpost, Kerala, India.

12. Ekesi, S., Maniania, N. K., & Lux, S. A. (2002). Mortality in three African tephritid fruit fly puparia and adults caused by the entomopathogenic fungi, *Metarhizium anisopliae*, and *Beauveria bassiana*. *Biocontrol Science and Technology*, 12(1), 7-17. https://doi.org/10.1080/09583150120093077

13. Evans, H. C. (1999). Biological control of weed and insect pests using fungal pathogens, with reference to Sri Lanka.- *Biological Conservation News Inform*, 20(2), 63N-68N.

14. Fergani, Y. A., & Refaei, E. A. E. (2021). Pathogenicity induced by indigenous *Beauveria bassiana* isolate in different life stages of the cotton leafworm, *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) under laboratory conditions. *The Egyptian Journal of Biological Pest Control*, 31(1), 1-7. https://doi.org/10.1186/s41938-021-00411-8

15. Fernandes, E. K., Rangel, D. E., Moraes, A. M., Bittencourt, V. R., & Roberts, D.W. (2008). Cold activity of *Beauveria* and *Metarhizium*, and thermotolerance of *Beauveria*. *Journal of Invertebrate Pathology*, 98(1), 69-78. https://doi.org/10.1016/j.jip.2007.10.011

16. Finney, D. J. (1971). Statistical logic in the monitoring of reactions to therapeutic drugs. *Methods of Information in Medicine*, 10(04), 237-245. https://doi.org/10.1055/s-0038-1636052

17. Fronza, E., Specht, A., Heinzen, H., & de Barros, N. M. (2017). *Metarhizium (Nomuraea) rileyi* as a biological control agent. *Biocontrol Science and Technology*, 27(11), 1243-1264. https://doi.org/10.1080/09583157.2017.1391175

18. Garcia, C., & Ignoffo, C. M. (1977). Dislodgment of conidia of *Nomuraea rileyi* from cadavers of the cabbage looper, *Trichoplusia ni*. *Journal of Invertebrate Pathology*, 30(1), 114-116. https://doi.org/10.1016/0022-2011(77)90050-7

19. Gardner, W. A. (1985). Effects of temperature on the susceptibility of *Heliothis zea* larvae to *Nomuraea rileyi*. *Journal of Invertebrate Pathology*, 46(3), 348-349. https://doi.org/10.1016/0022-2011(85)90082-5

20. Garrido-Jurado, I., Resquín-Romero, G., Yousef-Naef, M., Ríos-Moreno, A., & Quesada-Moraga, E. (2020). Soil drenching with entomopathogenic fungi for control of the soil-dwelling life stages and adults of the same generation of *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae). *Bulletin of Entomological Research*, 110(2), 242-248. https://doi.org/10.1017/S0007485319000

21. Gautam , R.D. (2008). Biological Pest Suppression , New Delhi : *Westville Publishing House* ,80-107.

22. Getzin, W. (1961). *Spicaria rileyi* (Farlow) Charles, an entomogenous fungus of *Trichoplusia ni* (Hubner). *Journal of Insect Pathology*, 3, 2-10. https://doi.org/10.1303/aez.34.399

23. Gul, H. T., Freed, S., Akmal M., & Malik, M. N. (2015). Vulnerability of different life stages of *Bactrocera zonata* (Tephritidae: Diptera) against entomogenous fungi. *Pakistan Journal of Zoology*, 47(2), 307-317.

24. Hafez, M., Zaki, F. N., Moursy, A., & Sabbour, M. (1997). Biological effects of the entomopathogenic fungus, *Beauveria bassiana* on the potato tuber moth *Phthorimaea operculella* (Seller). *Anzeiger für Schädlingskunde, Pflanzenschutz, Umweltschutz*, 70, 158-159. https://doi.org/10.1007/BF01907353

25. Harrison, R. D., Thierfelder, C., Baudron, F., Chinwada, P., Midega, C., Schaffner, U., & van den Berg, J. (2019). Agro-ecological options for fall armyworm (*Spodoptera frugiperda* JE Smith) management: providing low-cost, smallholder friendly solutions to an invasive pest. *Journal of Environmental Management*, 243, 318–330. https://doi.org/10.1016/j.jenvman.2019.05.011

26. Hu, G., & St Leger, R. J. (2002). Field studies using a recombinant mycoinsecticide (*Metarhizium anisopliae*) reveal that it is rhizosphere competent. *Applied and Environmental Microbiology*, 68(12), 6383-6387. https://doi.org/10.1128/AEM.68.12.6383-6387.2002

27. Idrees, A., Afzal, A., Qadir, Z. A., & Li, J. (2023). Virulence of entomopathogenic fungi against fall armyworm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae) under laboratory conditions. *Frontiers in physiology*, 14, 1107434. https://doi.org/10.3389/fphys.2023.1107434

28. Jackson, M. A., Dunlap, C. A., & Jaronski, S. T. (2010). Ecological considerations in producing and formulating fungal entomopathogens for use in insect biocontrol. *BioControl*, 55, 129-145. https://doi.org/10.1007/s10526-009-9240-y

29. Jaronski, S. T. (2010). Ecological factors in the inundative use of fungal entomopathogens. *BioControl*, 55, 159–185. https://doi.org/10.1007/s10526-009-9248-3

30. Kaaya, G. P., & Munyinyi, D. M. (1995). Biocontrol potential of the entomogenous fungi *Beauveria bassiana* and *Metarhizium anisopliae* for tsetse flies (*Glossina* spp.) at developmental sites. *Journal of Invertebrate Pathology*, 66(3), 237-241. https://doi.org/10.1006/jipa.1995.1095

31. Kaaya, G. P., & Okech, M. A. (1990). Horizontal transmission of mycotic infection in adult tsetse, *Glossina morsitans*. *Entomophaga*, 35, 589-600. https://doi.org/10.1007/BF02375093

32. Kim, S., Kim, J. C., Lee, S. J., Lee, M. R., Park, S. E., Li, D., & Kim, J. S. (2020). Soil application of *Metarhizium anisopliae* JEF-314 granules to control, flower chafer beetle, *Protaetia brevitarsis seulensis*. *Microbiology*, 48(2), 139-147. https://doi.org/10.1080/12298093.2020.1735765

33. Lee, S. J., Kim, S., Kim, J. C., Lee, M. R., Hossain, M. S., Shin, T. S., & Kim, J. S. (2017). Entomopathogenic *Beauveria bassiana* granules to control soil-dwelling stage of western flower thrips, *Frankliniella occidentalis* (Thysanoptera: Thripidae). *BioControl*, 62, 639-648. https://doi.org/10.1007/s10526-017-9818-8

34. Manjula, K. (2002) Studies on the entomogenous fungus *Nomuraea rileyi* (Farlow) Samson with special reference to its pathogenicity to *Spodoptera lirura* Fabricius and *Helicoverpa armigera* Hubner. Thesis submitted to Acharya NG Ranga Agricultural University, Andhra Pradesh 205p.

35. Montecalvo, M. P., Navasero, M. M., & Navasero, M. V. (2022). Lethal effect of native *Metarhizium rileyi* (Farlow) Samson isolate to invasive fall armyworm, *Spodoptera frugiperda*(J.E. Smith), infesting corn in the Philippines. *International Journal of Agricultural Technology*, 18(1), 257-270.

36. Morley-Davies, J., Moore, D., & Prior, C. (1996). Screening of *Metarhizium* and *Beauveria* spp. conidia with exposure to simulated sunlight and a range of temperatures. *Mycological Research*, 100(1), 31-38. https://doi.org/10.1016/S0953-7562(96)80097-9

37. Narayanan, M., Ranganathan, M., Subramanian, S. M., Kumarasamy, S., & Kandasamy, S. (2020). Toxicity of cypermethrin and enzyme inhibitor synergists in red hairy caterpillar *Amsacta albistriga* (Lepidoptera: Arctiidae). *The Journal of Basic and Applied Zoology*, 81(1), 1-8. https://doi.org/10.1186/s41936-020-00185-9

38. Nguyen, N. C., Borgemeister, H. P., & Zimmermann, G. (2007). Laboratory investigations on the potential of entomopathogenic fungi for biocontrol of *Helicoverpa armigera* (Lepidoptera: Noctuidae) larvae and pupae. *Biocontrol Science and Technology*, 17, 853–864. https://doi.org/10.1080/09583150701546375

39. Pawar, V. M., & Borikar, P. S. (2005). Microbial options for the management of *Helicoverpa armigera* (Hubner) In: "Recent Advances in *Helicoverpa* Management" Eds. Hem Saxena, AB Rai, R Ahmad, and Sanjeev Gupta. Indian Society of Pulses Research and Development, IIPR, Kanpur 193-231.

40. Peng, G., Xie, J., Gu, R., Keyhani, N. O., Zeng, D., Yang, P., & Xia, Y. (2021). Long-term field evaluation and large-scale application of a *Metarhizium anisopliae* strain for controlling major rice pests. *The Journal of Pesticide Science*, 94, 969–980. https://doi.org/10.1007/s10340-020-01313-8

41. Rangel, D. E., Alston, D. G., & Roberts, D. W. (2008). Effects of physical and nutritional stress conditions during mycelial growth on conidial germination speed, adhesion to host cuticle, and virulence of *Metarhizium anisopliae*, an entomopathogenic fungus. *Mycological Research*, 112(11), 1355-1361. https://doi.org/10.1016/j.mycres.2008.04.011

42. Russell, D. A., Radwan, S. M., Irving, N. S., Jones, K. A., & Downham, M. C. A. (1993). Experimental assessment of the impact of defoliation by *Spodoptera littoralis* on the growth and yield of Giza'75 cotton. *Crop Protection*, 12, 303–309. https://doi.org/10.1016/0261-2194(93)90051-J

43. Santos, R. L. D., Torres, J. B., Pontes, V., Barros, E. M., & Bastos, C. S. (2012). The lesser cotton leafworm, *Anomis impasta* (Guenée) (Lepidoptera, Noctuidae), in cotton. *Revista Brasileira de Entomologia*, 56, 492-498. https://doi.org/10.1590/S0085-56262012000400015

44. Skinner, M., Gouli, S., Frank, C. E., Parker, B. L., & Kim, J. S. (2012). Management of *Frankliniella occidentalis* (Thysanoptera: Thripidae) with granular formulations of entomopathogenic fungi. *Biol Control*, 63(3), 246-252. https://doi.org/10.1016/j.biocontrol.2012.08.004

45. Stokstad, E. (2017). New crop pest takes Africa at lightning speed.- *Science*, 356, 473–474. https://doi.org/10.1126/science.356.6337.473

46. Ugine, T. A., Wraight, S. P., & Sanderson, J. P. (2007). Effects of manipulating spray-application parameters on efficacy of the entomopathogenic fungus *Beauveria bassiana* against western flower thrips, *Frankliniella occidentalis*, infesting greenhouse impatiens crops. *Biocontrol Science and Technology*, 17(2), 193-219. https://doi.org/10.1080/09583150600937618

47. White, I. M., & Elson-Harris, M. M. (1992). Fruit Flies of Economic Significance: Their Identification and Bionomics. CAB international.

48. Wood, T. J., & Goulson, D. (2017). The environmental risks of neonicotinoid pesticides: a review of the evidence post-2013. *Environmental Science and Pollution Research*, 24, 17285-17325. https://doi.org/10.1007/s11356-017-9240-x

49. Zhang, X., Lei, Z., Reitz, S. R., Wu, S., & Gao, Y. (2019). Laboratory and greenhouse evaluation of a granular formulation of *Beauveria bassiana* for control of western flower thrips *Frankliniella occidentalis*. *Insects*, 10 (2), 58. DOI: https://doi.org/10.3390/insects10020058