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Exploration of Native Isolates of *Metarhizium rileyi* (Farlow) Samson (Ascomycetes: Hypocreales) in Maize

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ABSTRACT: *Metarhizium rileyi* (Farlow) Samson is an important entomopathogenic fungus of more than 30 species of Lepidoptera larvae. In India, the commercial biopesticides have been suggested against different insect pests on different crops, but their application shown only a decreased efficiency credited to differences in susceptibility of target pests or non-adaptability to Indian agro-climatic conditions, and another challenging issue is non availability of region specific entomopathogenic strains. Therefore, there is a need to isolate location-specific biopesticide strains to increase the efficacy of such biopesticides for insect pest management. This study aimed to explore the new isolates of the entomopathogenic fungus, *M. rileyi* species across the major cropping ecosystems of Raichur and Koppal districts of kalyana Karnataka and we found consistant growth of *M. rileyi* throughout the year.

From the survey data in Raichur district the highest number of fall armyworm (15.33/m^2) cadavers were recorded at UAS, campus Raichur with 51.11 per cent incidence and in Koppal district at Gondbal village 49.25 per cent natural incidence have been recorded. Pearson's correlation studies showed significantly positive correlation with relative humidity, minimum temperature and maximum wind speed. These isolates (UASRBC Mr-3 and UASRBC Mr-15) and its concentrations were evaluated on third instar larvae of *S. frugiperda* with the standard reference strain NrSf-1 from NBAIR. NrSf-1 recorded significantly highest per cent mortality (87.78 %) at 1 × 10^{10} spores per ml of concentration which was on par with Mr-3 (86.67 %) whereas, Mr-15 recorded 80 per cent mortality at 1 × 10^{10} concentration per ml.

Survey data indicated that natural incidence was highest from July second fortnight to December first fortnight. Later the infection rate gradually reduced with the decrease of precipitation, relative humidity and increase in temperature. Isolate UASRBC Mr-3 was more virulent with 86.67 per cent mortality which is on par with NBAIR isolate NrSf-1 with 80.66 per cent mortality against *S. frugiperda,* These isolate should be formulated as a myco-insecticide and tested under field conditions in further studies.

Keywords: *Metarhizium rileyi*, Exploration, *Spodoptera frugiperda*, virulent.

INTRODUCTION

Insect pests, despite constituting the second most important biotic stress in different cropping ecosystems after diseases, cause quality and considerable yield losses. Pest profiles in tropical and subtropical India display both overlap and distinctiveness. While in subtropical India the extreme climatic conditions support moderate crop growth but high pest level. Though synthetic chemicals are very effective in containing the pest but they have inherent problems as development of insecticide resistance, pest resurgence and residual toxicity and deleterious effects on natural enemies (Ahmad *et al*. 2007; FAO, 2019). To overcome the ill effects of pesticides an alternative method is to explore integrated pest management (IPM) (Singh and Joshi 2020) which employs the use of bioagents *viz*., parasitoids, predators, entomopathogenic bacteria, viruses and fungi.

The unique features of crop-pest-natural enemies system involves crop duration and continues host accessibility for the pests, pest multitude, staggered planting which support natural enemy spectrum, higher economic thresholds and low pesticide usage make the crop a semi-perennial habitat favourable for both applied and natural biological control.

The significance of surveys as natural enemies so identified could be part of a backup plan which will be utilized if the pest population assumed serious proportions (Srikanth *et al*., 2015) for example the parasitoid complex of the maize fall armyworm identified in recent surveys at Chikkaballapur, Hassan,

Shivamogga, Davanagere and Chitradurga during July-August 2018 (Shylesh *et al*., 2018), where the pest was observed for the first time (Sharanabasappa *et al*., 2018). The natural enemies spectrum documented from different parts of the world, including India (CABI 2018), furnishes a choice to select candidate biocontrol agents for augmentative or classical mode of control. Hence identification and elaborative study of potential entomopathogenic fungi (EPF) should be a continuous process as this would help to find candidate biocontrol agents for introduction and colonization against introduced resident pests. In this context, deployment of *Metarhizium rileyi* (Farlow) Samson against lepidopteran pests represents the classical mode in a restricted sense even though established yet less explored, associations deserve emphasis while identifying potential biocontrol agents.

Among various entomopathogenic fungi (EPF), *M. rileyi* caused a natural death to several lepidopteran pests and found to be highly efficacious against *Spodoptera litura* (Fab.), *Helicoverpa armigera* (Hub.), *Trichoplusia ni* (Hub.) (Namasivayam *et al*., 2013) and *Anticarsia gemmatalis* (Hub.) also on *Spodoptera frugiperda* (Shylesha *et al*., 2018). *M. rileyi* is a dimorphic, yeast-like hyphal bodies ubiquitous fungus with true mycelial filaments and earlier named as *Botrytis rileyi* (Farlow) and then as *Spicaria rileyi* (Farlow) Charles. The fungus was re-described and placed in the genus, *Nomuraea* by Kish *et al*. (1974) and named as *Nomuraea rileyi* Farlow (Kepler *et al*., 2014).

Morphologically *M. rileyi* is an imperfect and dimorphic fungus in its development and the color of the colony ranges from white-malachite green intense as colony progresses (Boucias *et al*., 2000 and Nandish *et al*., 2016). The hyphae measure between 2 and 3 µm in diameter, septate, hyaline to slightly pigmented and conidiophores are erect and septate; the conidia form divergent chains are smooth, ellipsoidal, and sometimes cylindrical with a pale green color (Ignoffo, 1981).

On molecular characters *viz.*, RAPD, amplified length polymorphism (AFLP), internal transcribed spacer (ITS) sequence analysis and telomeric finger printing methods found that *N. rileyi* isolates were more closely associated to *Metarhizium anisopliae* and *M. flavoviride* than to *N. atypicola* and *N. anemonoides* (Boucias *et al*., 2000). Recently, *Nomuraea rileyi* changed to *Metarhizium rileyi* on the basis of its morphological and molecular characterization by Kepler *et al*. (2014).

METHODS

Collection of cadavers in major crops of North Eastern Karnataka. Roving survey was carried out during cropping period in North Eastern region of Karnataka such as Raichur and Koppal districts for the natural incidence of *M. rileyi* infected cadavers. To record the number of Lepidopteran larvae one square meter area was marked and it was considered as a unit and such five units were randomly selected in maize crop. The total number of larvae in each unit was critically observed and the cadavers on plants were collected and brought to the laboratory for further studies.

The cadavers (Plate 1) were identified based on the following characters. The larvae became hard, mummified and adhered to the leaves and other plant parts with their prolegs with raised head and anterior part of the body. White mycelial growth covered the entire body of the infected caterpillar except for the head capsule. The fungus on infected caterpillars sporulated profusely in the field itself and such cadavers were seen covered with fine light green spores all over the body.

The cadavers were collected in sterilized vials and kept in a petri plate on moistened filter paper for the development of fungus. Similarly, the prevailing weather conditions across the survey were recorded. The incidence of *M. rileyi* calculated by using the following formulae (Mallapur *et al*., 2018).

Per cent natural incidence of *M. rileyi* =

$$\frac{\text{Number of infected larvae by } M.rileyi}{\text{Total number of larvae observed}} \times 100$$

Confirmation test for *M. rileyi.* Cadavers collected from the different cropping ecosystem during the survey were kept for incubation for profuse sporulation in BOD, then a small quantity of spores were taken out from the sporulated cadavers for each isolates for confirmation test of *M. rileyi* by preparing semi-permanent slide. A drop of lactophenol cotton blue was placed on a microscopic slide and a small amount of fungal mycelium was transferred on the slide. Cover slip was slowly kept on the drop of lactophenol cotton blue containing mycelium without air bubbles and it was sealed with fingernail polish. The fungal pathogen identified based on morphological characters (Kish, 1975) for morphological features like the color, media pigmentation, size and shape of the conidia. The spores were observed at 40X magnification using a phase contrast microscope (Bail *et al*., 2015) and after confirmation of *M. rileyi*, pathogenicity tests were conducted according to Koch's postulates for the fungi collected from cadavers for testing their virulance.

Isolation of *M. rileyi* from infected cadavers. Dead cadavers surface sterilized by immersing in 0.1 per cent sodium hypochlorite solution followed by rinsing in three changes of sterilized distilled water. The surface sterilized diseased specimens were cut in a sterile watch glass and a small portion of the infected tissue was transferred to a sterile culture plate containing Sabouraud Maltose Yeast Extract Agar Medium (SMAY) method followed by Bell (1975). The plates were incubated at room temperature 25 ± 2 °C with 80 to 85 per cent relative humidity for 9-14 days and the colonies were further purified by repeated subculture on SMAY medium and pure cultures were maintained in slants.

Fungal spore suspension preparation. For fungal spore suspension preparation, each fungal isolate was grown on broken rice with one per cent yeast for laboratory bioassay and incubated for 18 to 21 days. The spore suspension was prepared by taking one gram of 19 days old conidiated rice in nine milliliters of

sterile distilled water containing 0.01 per cent Tween 80. The suspension was filtered through three layers of muslin cloth to get hyphal-free spore suspension, and the spore concentration was adjusted to various concentrations such as 1×10^2, 1×10^3, 1×10^4, 1×10^5, 1×10^6, 1×10^7, 1×10^8 and 1×10^{10} spores/ml using Neubauer's improved haemocytometer (Kulkarni, 1999).

***In vitro* screening.** The pre starved third instar larvae (30 larvae/treatment-3 replications) of *S. frugiperda* were dipped in the suspension containing 1×10^2, 1×10^3, 1×10^4, 1×10^5, 1×10^6, 1×10^7, 1×10^8 and 1×10^{10} spores/ml for 15 seconds. The treated larvae were transferred into plastic containers and supplied with fresh detached maize leaves. Then the larvae were maintained under controlled conditions of 25 ± 2 °C temperature and 70 ± 5 per cent relative humidity for ten days. Fresh maize leaves were provided as food to the larvae in 24 hours interval. Control was treated with sterile water containing 0.01 per cent Tween 80. Observation on mortality due to fungal infection was carefully noted and recorded. Per cent mortality of the larvae for each isolate were calculated using Abbott's formula (Abbott, 1925). The data were subjected to one-way analysis of variance (ANOVA) using statistical software SPSS windows version 20.0. Further studies were carried out using promising isolates.

$$\text{Per cent mortality} = \frac{\text{No. of larvae dead}}{\text{No. of larvae treated}} \times 100$$

RESULTS

Collection and isolation of native isolates of *Metarhizium rileyi* on Lepidopteran pests

Description of *M. rileyi* and its symptoms. The key role of knowing the description was to identify the fungus infected cadavers collected during the survey, hence symptoms on the cadavers are of great use in collection of the cadavers. The characteristic symptoms of *M. rileyi* on larvae initially are the presence of small yellow to brown spots on the integument. Later reduced feeding, pale color of the body, lethargy and slight swelling of the posterior abdominal segments encountered after 2-5 days of exposure. Due to growth of the fungus after it reached the haemocoel is by budding which produces discrete yeast like hyphal bodies called blastospores and eventually transported throughout the haemocoel and which develops into localized concentrations of mycelia. Heavy growth of intertwining mycelia with fusiform cells developed in the haemocoel during five days after exposure. Death occurs about six to eight days after initial symptoms, depending upon the larval stage, dose and temperature. After death, the larval body becomes mummified and covered with dense white mycelial mat, conidiophores arise close together and produce a pile of pale green conidia after 9-10 days. In nature, the dead cadavers were found fastened to the eaten-up leaves with head and abdominal region in a raised position.

Collection of cadavers in Raichur. The roving survey was carried out in Raichur district of Karnataka (Fig. 1) in different cropping ecosystems during 2020-21.

Table 1: Incidence of *Metarhizium rileyi* on maize Lepidopteran pests in Raichur district.

Sr. No.	Village	Crop	Host	No. of larvae per meter square area	No. of cadavers per meter square area	Per cent natural incidence
1	UAS, campus, Raichur	Maize	*S. frugiperda*	30.00 (5.52)	15.33 (3.97)	51.11 (45.67)
2	Udamgal, Raichur	Maize	*S. frugiperda*	17.67 (4.24)	2.00 (1.52)	11.32 (19.61)
3	Askhal, Raichur	Maize	*S. frugiperda*	9.66 (3.15)	0.33 (0.88)	3.45 (7.59)
4	Kallur, Raichur	Maize	*S. frugiperda*	17.00 (4.16)	3.00 (1.85)	17.64 (25.27)
5	Kalmal, Raichur	Maize	*S. frugiperda*	23.33 (4.87)	2.33 (1.67)	10.00 (18.40)
6	Manvi, Raichur	Maize	*S. frugiperda*	30.00 (4.63)	2.33 (1.67)	7.78 (16.48)
7	Kardchalami, Sindhnoor, Raichur	Maize	*S. frugiperda*	02.33 (1.68)	0.00 (0.71)	0.00 (0.29)
8	Udamgal, Raichur	Maize	*S. litura*	03.33 (2.64)	2.33 (1.67)	1.43 (1.22)
SEm±				**2.78**	**0.68**	**3.49**
CD @ 5%				**6.75**	**1.70**	**8.73**

Figures in the parenthesis are arcsine values for per cent natural incidence and $\sqrt{x+0.5}$ transformed values for no. of larvae per meter square area and no. of cadavers per meter square area

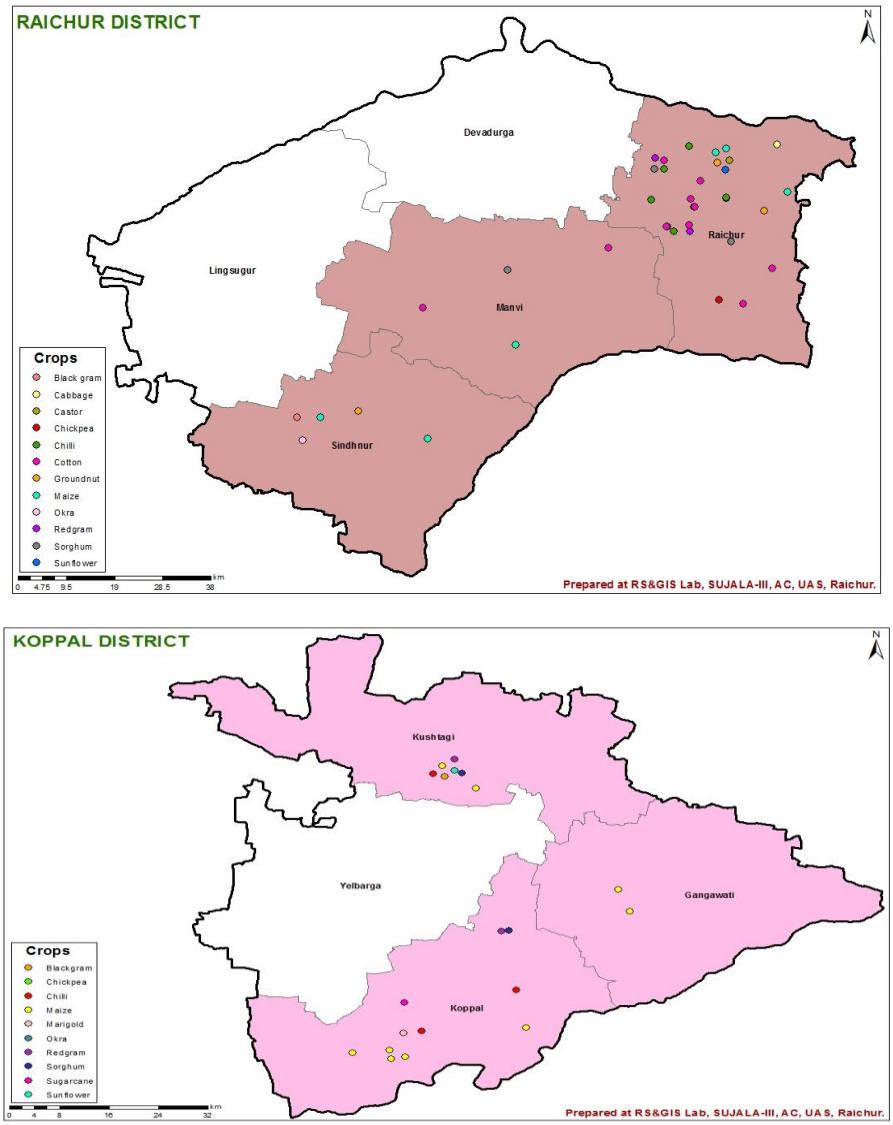

Fig. 1. Natural epizootics of *Metarhizium rileyi* on major lepidopteran pests in North Eastern region of Karnataka (Raichur and Koppal) district during 2020-21.

Natural incidence of *M. rileyi* in Raichur. In Raichur district the survey was conducted (Fig. 1) to record the natural incidence of *M. rileyi* in maize crop. Highest number of fall armyworm (15.33/m^2) cadavers were recorded at UAS, campus Raichur with 51.11 per cent incidence and it was followed by Kallur, Udamgal, Kalmal, Manvi and where as the number of fall armyworm collected were 15.33, 3.00, 2.00 and 2.33 per m^2 area in maize ecosystem (Fig. 2).

Table 2: Incidence of *M. rileyi* on lepidopteran pests of major crops in Koppal district.

Sr. No.	Taluk	Village	Crop	Host	No. of larvae per meter square area	No. of cadavers per meter square area	Per cent natural incidence
1.	Koppal	Gondbal	Maize	*S. frugiperda*	22.33 (4.73)	11.00 (3.38)	49.25 (44.56)
		Chukkankal	Maize	*S. frugiperda*	10.33 (3.26)	2.00 (1.58)	19.36 (26.88)
		Muddaballi	Maize	*S. frugipera*	26 (5.03)	2.00 (1.56)	7.69 (16.19)
		Tavargere	Maize	*S. frugipera*	6.00 (2.42)	0.00 (0. 71)	0.00 (0.28)
2.	Kustigi	Kandakur	Maize	*S. frugiperda*	5.66 (2.32)	0.00 (0.71)	0.00 (0.28)
		Kustigi	Maize	*S. frugiperda*	13.66 (3.70)	0.00 (0. 71)	0.00 (0.28)
3.	Gangavathi	Hiresindhogi	Maize	*S. frugiperda*	22.33 (4.78)	0.00 (0. 71)	0.00 (0.28)
		Advibhavi	Maize	*S. frugiperda*	11.00 (3.38)	0.00 (0. 71)	0.00 (0.28)
	SEm±				**2.37**	**0.54**	**2.44**
	CD@ 5%				**5.92**	**1.35**	**6.10**

Figures in the parenthesis are arcsine values for per cent natural incidence and $\sqrt{x + 0.5}$ transformed values for no. of larvae per meter square area and no. of cadavers per meter

Natural incidence of *M. rileyi* in Koppal. Highest *S. frugiperda* cadavers (11.0/m^2) were noticed in Gondbal village of Koppal (Fig. 3) taluka with 49.25 per cent natural incidence which was followed by Chukkankal, Muddaballi (2.00/m^2) village of Koppal taluka with 19.36 and 7.69 per cent natural incidence respectively, (Table 3). Pearson's correlation studies showed that

there was significantly positive correlation with relative humidity, minimum temperature, maximum wind speed and only positive correlation with rainfall and minimum wind speed during 2019-20 in Raichur and Koppal (Fig. 2 and 3) whereas, during 2020-21 the natural infection was significantly positive for minimum temperature, rainfall and maximum wind speed and there was negative correlation with maximum temperature for natural infection

Coding of cadavers collected from North Eastern Karnataka (Raichur and Koppal). The fungal infected cadavers collected from different location in different cropping ecosystems were sorted out in laboratory and naming was done (Table 1 and 2). Two *M. rileyi* isolates were successfully subcultured (Table 5) and remaining isolates were degenerated (Table 4).

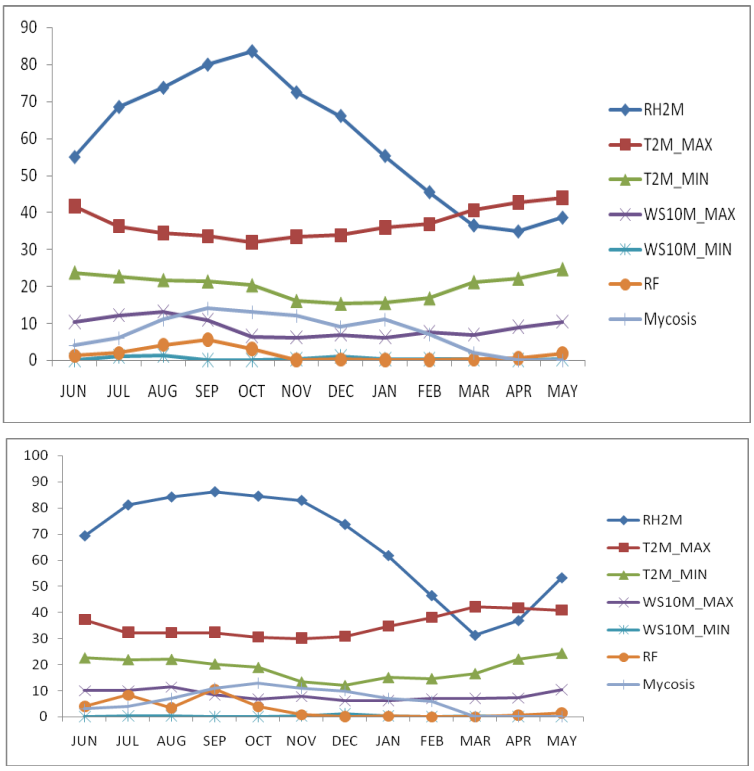

Fig. 2. Weather data on collection of entomopathogenic fungi infected cadavers in Raichur.

Table 3: Correlation of weather parameters with natural incidence of *M. rileyi* in Raichur and Koppal districts of North Eastern region of Karnataka during 2019-20 and 2020-21.

District	Mycosis	Relative humidity	Maximum temperature	Minimum temperature	Maximum wind speed	Minimum wind speed	Rainfall	Mycosis
Raichur	2019-20	0.392**	-0.367	0.116*	0.228*	0.455	0.056	1
	2020-21	0.391	-0.429	0.174**	0.167	0.446	0.194**	1
Ballari	2019-20	0.588*	-0.468	0.098*	0.045**	0.458	0.221	1
	2020-21	0.152	-0.207	0.110*	0.084**	0.187	-0.185	1

**. Correlation is significant at the 0.05 level (2-tailed); *. Correlation is significant at the 0.05 level (2-tailed)

Table 4: Culturing and isolation of *M. rilyei* infected cadavers collected from different locations and crops during 2020-21.

Sr. No.	Crop	Host	Location	Isolate number
1.	Maize	*S. frugiperda*	UAS, campus Raichur	UASRBC Mr- 1
2.	Maize	*S. frugiperda*	Askhal, Raichur	UASRBC Mr -3
3.	Maize	*S. frugiperda*	Udamgal, Raichur	UASRBC Mr -5
4.	Maize	*S. litura*	Udamgal, Raichur	UASRBC Mr-6
5.	Maize	*S. frugiperda*	Kallur, Raichur	UASRBC Mr-9
6.	Maize	*S. frugiperda*	Kalmal, Raichur	UASRBC Mr -11
7.	Maize	*S. frugiperda*	Gondbal, Koppal	UASRBC Mr -15
8.	Maize	*S. frugiperda*	Chukkankal, Koppal	UASRBC Mr -16
9.	Maize	*S. frugiperda*	Muddaballi, Koppal	UASRBC Mr -17
10.	Maize	*S. frugiperda*	Manvi, Raichur	UASRBC Mr -25

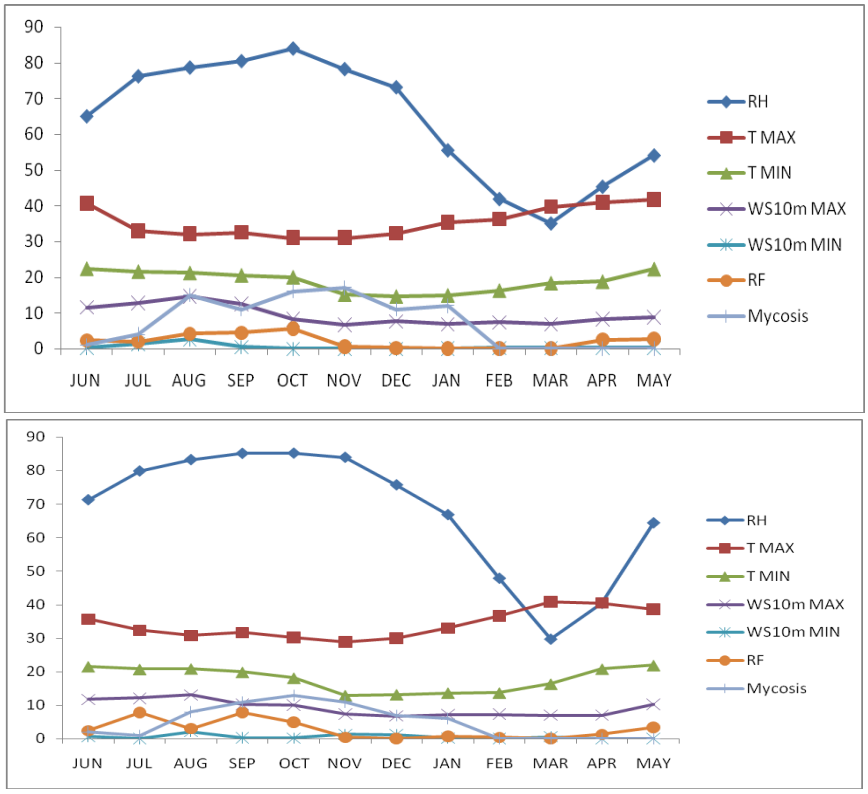

Fig. 3. Weather data on collection of entomopathogenic fungi infected cadavers in Koppal.

Table 5: Subcultures of *M. rileyi* on SMAY media.

Sr. No.	Crop	Host	Location	Isolate number
1	Maize	*S. frugiperda*	Askhal, Raichur	UASRBC Mr -3
2	Maize	*S. frugiperda*	Gondbal, Koppal	UASRBC Mr -15

Plate 1. Mycosed cadavers of *S. frugiperda* collected in maize ecosystem.

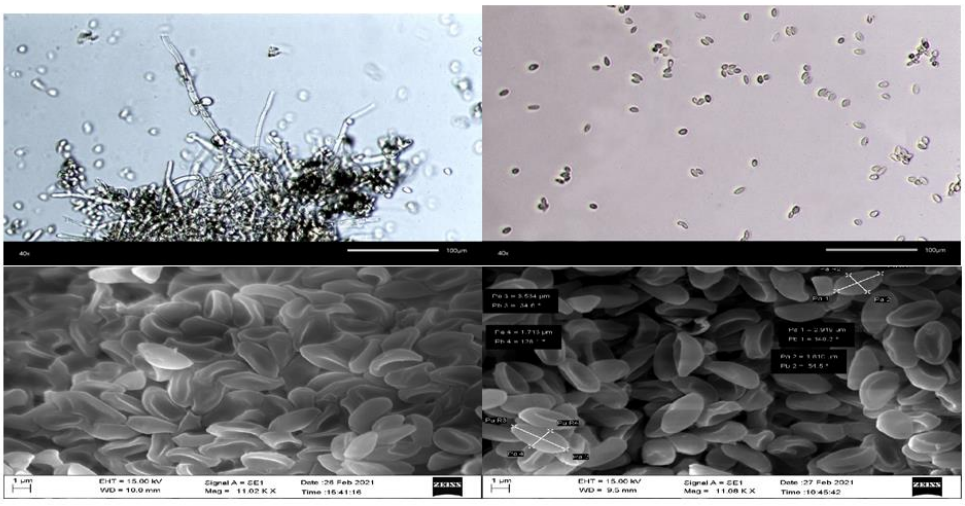

Plate 2. Morphometry of conidium of *M. rileyi* isolates E) UASRBC Mr-3 F) UASRBC Mr 15 under i) Phase contrast microscope (40X) ii) Scanning Electron Microscope (10X).

From the morphommetric studies of *M. rileyi* under Phase contrast microscope at 40X showed that spores are ellipsoidal to oval in shape, with pale green colour whereas hyphae and condiophores septate, smooth and hyaline with pale green colour. The colony produces yellow pigmentation with white coloured vegetative stage at nineth day after inoculation whereas on sporulation malachite green colonies were observed after 14 days after inoculation.

UASRBC Mr-3. The mortality per cent on *S. frugiperda* after nine days of treatment recorded 86.67 per cent, 8.3×10^{10} spores per ml, whereas the lowest mortality of 10 per cent recorded at 8.3×10^{2} spores per ml of concentration. There was a significant difference in per cent mortality from 22.22 to 75.56 per cent at different concentrations from 1×10^{3} to 1×10^{8} spores per ml in increasing order. The lowest dose of 8.3×10^{2} spores per ml recorded 10 per cent mortality and was on par with control (3.33 %) (Table 6).

The corrected mortality was 25.25 per cent which was on par with 21.89 per cent at 1×10^{10} and 1×10^{8} spores per ml. The remaining concentrations ranged from 2.02 to 21.89 per cent at 1×10^{2} to 1×10^{8} spores per ml.

UASRBC Mr- 15. Among the various concentrations evaluated the highest mortality of 80 per cent was recorded at 9 days after treatment and significantly differed with mortality per cent 61.11 and of 51.11 per cent at 1.8×10^{8} and 1.8×10^{7} conidia per ml concentration. The lowest concentration of 1.8×10^{2} recorded 14.44 per cent on the ninth day after treatment which was significantly lower to the rest of the treatments. Per cent mortality of 43.33, 37.78, 35.56 and 25.56 recorded at 1×10^{6}, 1×10^{5}, 1×10^{4} and 1×10^{3} spores per ml, respectively (Table 7).

From the data it was clear that 21.65 corrected per cent mortality was the highest with 24 larval death out of 30 larvae treated at 1×10^{10} spores per ml of concentration, hence there was a significant difference in mortality with respect to control. The remaining concentrations recorded 1.37 to 15.81 per cent corrected mortality from 1×10^{2} to 1×10^{8} spores per ml.

UASRBC NrSf-1. The standard reference strain NrSf-1 recorded significantly highest per cent mortality (87.78 %) at 1×10^{10} spores per ml of concentration followed by 82.22 per cent at 1×10^{8} spores per ml. Remaining concentrations recorded 74.44, 64.44, 57.78, 42.22, 31.11, 20, 3.33 per cent mortality from 1×10^{7} to 1×10^{2} spores per ml, respectively. The corrected mortality per cent also showed similar results. The highest and lowest per cent mortality was 25.32 and 4.99 per cent at 1×10^{10} and 1×10^{2} spores per ml conidial concentrations, respectively (Table 8).

Table 6: Bioassay of UASRBC Mr-3 isolate of *M. rileyi* on *S. frugiperda.*

Treatments (spores per ml)	No. of larvae treated	No. of larvae dead	Per cent mortality	Corrected per cent mortality
1×10^{10}	30.00	26.00 (5.14)	86.67 (68.57)^{a}	25.25 (30.16)^{a}
1×10^{8}	30.00	22.67 (4.81)	75.56 (60.36)^{a}	21.89 (27.87)^{a}
1×10^{7}	30.00	19.00 (4.41)	63.33 (86.13)^{b}	18.18 (25.22)^{b}
1×10^{6}	30.00	15.67 (4.02)	52.22 (46.26)^{c}	14.81 (22.61)^{c}
1×10^{5}	30.00	13.00 (3.67)	43.33 (70.44)^{c}	12.12 (20.34)^{c}
1×10^{4}	30.00	10.00 (3.24)	33.33 (35.256)^{d}	9.09 (17.53)^{d}
1×10^{3}	30.00	6.67 (2.67)	22.22 (28.12)^{e}	5.72 (13.75)^{e}
1×10^{2}	30.00	3.00 (1.87)	10.00 (18.43)^{f}	2.02 (7.98)^{f}
Control	30.00	1.00 (1.22)	3.33 (8.28)^{h}	0.00 (0.52)^{g}
SEm ±		**2.88**	**1.21**	**1.30**
CD @ P= 0.01 at 1%		**7.20**	**3.28**	**3.27**

Figures in the parenthesis indicate $\sqrt{x+0.5}$ transformed values for number of dead larvae

Figures in the parenthesis are arcsine values for corrected per cent mortality
CD @ P= 0.01 significant at (0.01) per cent level of significance
Means followed by same letters in a column not significantly different by DMRT

Table 7: Bioassay of UASRBC Mr-15 isolate of *M. rileyi* on *S. frugiperda*

Treatments (spores per ml)	No. of larvae treated	No. of larvae dead	Per cent mortality	Corrected per cent mortality
1×10^{10}	30.00	24.00 (4.94)	80.00 (63.43)^{a}	21.65 (27.69)^{a}
1×10^{8}	30.00	18.33 (4.34)	61.11 (51.41)^{b}	15.81 (23.39)^{b}
1×10^{7}	30.00	15.33 (3.97)	51. 11 (45.63)^{bc}	12.71 (20.86)^{bc}
1×10^{6}	30.00	13.00 (3.67)	43.33 (41.16)^{cd}	10.31 (18.71)^{cd}
1×10^{5}	30.00	11.33 (3.43)	37.78 (37.92)^{d}	8.59 (16.92)^{d}
1×10^{4}	30.00	10.67 (3.34)	35.56 (36.59)^{d}	7.90 (16.30)^{d}
1×10^{3}	30.00	7.67 (2.85)	25.56 (30.36)^{e}	4.81 (12.55)^{e}
1×10^{2}	30.00	4.33 (2.19)	14.44 (22.31)^{f}	1.37 (6.63)^{f}
Control	30.00	3.00 (1.87)	10.00 (18.43)^{g}	0.00 (0.52)^{g}
SEm ±		**2.23**	**1.29**	**1.55**
CD @ P= 0.01 at 1%		**5.55**	**3.49**	**3.89**

Figures in the parenthesis indicate $\sqrt{x+0.5}$ transformed values for number of dead larvae

Figures in the parenthesis are arcsine values for corrected per cent mortality
CD @ P= 0.01 significant at (0.01) per cent level of significance
Means followed by same letters in a column not significantly different by DMRT

Table 8: Bioassay of NrSf-1 isolate of *M. rileyi* on *S.frugiperda.*

Treatments (spores per ml)	No. of larvae treated	No. of larvae dead	Per cent mortality	Corrected per cent mortality
1×10^{10}	30.00	26.00 (5.14)	80.66 (63.90)[a]	26.26 (30.81)[a]
1×10^{8}	30.00	24.00 (4.94)	79.92 (63.37)[a]	24.24 (29.49)[ab]
1×10^{7}	30.00	23.33 (4.88)	77.68 (61.80)[ab]	22.22 (28.09)[bc]
1×10^{6}	30.00	18.66 (4.37)	62.14 (52.02)[bc]	18.86 (25.71)[cd]
1×10^{5}	30.00	17.33 (4.22)	57.71 (49.43)[cd]	16.84 (24.21)[d]
1×10^{4}	30.00	14.77 (3.91)	49.18 (44.53)[d]	12.12 (20.37)[e]
1×10^{3}	30.00	11.55 (3.47)	38.46 (38.32)[e]	8.08 (16.35)[f]
1×10^{2}	30.00	7.66 (2.85)	25.51 (30.33)[f]	4.04 (11.53)[g]
Control	30.00	1.00 (1.22)	3.33 (8.28)[g]	0.00 (0.52)[h]
SEm ±		**2.74**	**3.08**	**1.37**
CD @ P= 0.01 at 1%		**6.85**	**7.70**	**3.44**

Figures in the parenthesis indicate $\sqrt{x+0.5}$ transformed values for number of dead larvae

Figures in the parenthesis are arcsine values for corrected per cent mortality
CD @ P= 0.01 significant at (0.01) per cent level of significance
Means followed by same letters in a column not significantly different by DMRT

DISCUSSION

The correlation studies on natural incidence of *M. rileyi* on various Lepidopteran pests in different districts with weather parameters indicated that relative humidity had positive and significant correlation in Koppal (0.39), Raichur (0.23) with the incidence of *M. rileyi* and negative correlation with maximum temperature. The present findings on influence of weather parameters on *M. rileyi* are in line with those of Vimaladevi *et al.* (1996) who noticed epizootics of *M. rileyi* on *S. litura* and *Helicoverpa* sp. in *kharif* groundnut at high relative humidity. The maximum cadavers of *S. frugieprda* were noticed in maize growing districts *viz.*, Raichur and Koppal which indicated that in traditional maize growing areas the incidence of *M. rileyi* was high and the present findings are in line with Mallapur *et al.* (2018) who also observed that traditional maize growing areas (Dharwad) had highest incidence (18.30 %) while non traditional areas like Vijaypur had low incidence of *M. rileyi*. The present findings are also corroborates with Sharanabasappa *et al*. (2018) who opined that natural incidence of *M. rileyi* mainly depends on the availability of the host for the perpetuation of the disease. Similarly, Shylesh *et al.* (2018) stated that the *M. rileyi* was predominant in maize growing areas and also Visalakshi *et al.* (2020) opined that natural incidence of *M. rileyi* also depended on the availability of on *S. frugiperda* varied with the cropping pattern in Andhra Pradesh. The prevalence of *M. rileyi* at Raichur and Sindhnoor villages in maize ecosystem may be imputed to congenial weather factors and canopy coverage which might have supported the inoculum development. Non-occurrence of the fungus in traditional areas might be due to indiscriminate use of pesticides in pigeon pea and cotton ecosystems which might have affected the perpetuation and fungus growth which is supported by Sreenivas (1996); Kulkarni (1999) who observed the growth inhibition of *N. rileyi* by pesticides application at recommended dose under laboratory conditions. Survey data indicated that natural incidence was highest from July second fortnight to December first fortnight. Later the infection rate gradually reduced with the decrease of precipitation, relative humidity and increase in temperature. Present results are in line with those of Visalakshi *et al*. (2020) who noticed that *M. rileyi* infection initiated from first fortnight of October (5.61 %) and reached maximum (38.02 %) during November first fortnight and reduced from December second fortnight onwards. High rainfall received during the second fortnight of September (239.7 mm) and second fortnight of October (236.7 mm) with a high relative humidity was positively correlated and found congenial for initiation of mycosis and further progress. The study on per cent mortality are in line with Goh *et al*. (1992) who recorded 100 per cent mortality in fifth and sixth instars of *S. frugiperda* at 1×10^{8} spores per ml and also Kulkarni and Lingappa (2002a) recorded 86.50 per cent mortality of *S. litura* after 10 days of the exposure period. Similarly, It was also confirmed with the studies done by Manjula and Krishnamurthy (2005) who reported the highest larval mortality of 91.2 per cent was recorded in the first instar *S. litura* larvae and 95 per cent in the second instar of *H. armigera* with the highest concentration of 1×10^{9} of *M. rileyi* spores per ml and Hazarika *et al*. (2016) found 78.89 per cent mortality at 1×10^{8} spores per ml and 85.92 per cent mortality at 1×10^{8} spores per ml on *H. armigera.* Mortality due to each isolate was positively correlated with conidial concentrations hence it was increased with an increase in conidial concentration.

A variation in mortality among the isolates of *M. rileyi* could be due to the process of infection and development of disease in larvae which is being directly related to the specificity of fungal isolates along with the tolerance possessed by the host species (Fronza *et al*. 2017). The invasion of host by pathogen and germination success could be affected by the absence of certain characteristics in the fungal isolates pertaining to penetration mechanisms as well as the characteristics of host integument (Ignoffo and Garcia 1985). The main barrier for the penetration of a fungus into the host insect is created by its cuticle. To dissolve this barrier, fungi produce different cuticle-degrading enzymes like chitinase, protease and lipase (de Moraes *et al*. 2003) hence there is a variation with respect to isolate.

CONCLUSIONS

In Raichur district the survey was conducted to record the natural incidence of *M. rileyi* in maize crop. Highest number of fall armyworm (15.33/m^2) cadavers were recorded at UAS, campus Raichur with 51.11 per cent incidence. Whereas in Koppal highest *S. frugiperda* cadavers (11.0/m^2) were noticed in Gondbal village with 49.25 per cent natural incidence.

Pearson's correlation studies showed that during 2020-21 the natural infection was significantly positive for minimum temperature, rainfall and maximum wind speed and there was negative correlation with maximum temperature for natural infection in Raichur and Koppal. Isolate UASRBC Mr-3 was more virulent with 86.67 per cent mortality which is on par with NBAIR isolate NrSf-1 with 80.66 per cent mortality against *S. frugiperda* UASRBC Mr-3 isolate should be formulated as a myco-insecticide and tested under field conditions in further studies. From results showed that the EPF *M. rileyi* isolates were the most pathogenic to *S. frugiperda* larvae. The results presented in this study increase the knowledge about natural zoonosis of *M. rileyi* on different lepidopteran pests and open new avenues for studies regarding the virulence against *S. frugiperda* larvae.

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Conflict of Interest. None.

List of abbreviations

BOD	:	Biological Oxygen Demand
CD	:	Coefficient of Deviation
CABI	:	Centre for Agriculture and Bioscience International
DMRT	:	Duncan's Multiple Range Test
EPF	:	Entomopathogenic fungi
FAO	:	Food and Agriculture Organisation
IPM	:	Integrated Pest Management
NrSf-1	:	*Nomuraea rileyi*, *Spodoptera frugiperda*
NBAIR	:	National Bureau of Agricultural Insect Resources
SE	:	Standard Error of Mean
SMAY	:	Sabouraud Maltose Yeast Extract Agar Medium
UASRBC Mr	:	University of Agricultural Sciences Raichur Biocontrol lab *Metarhizium rileyi*

REFERENCES

Abbott, W. S. (1925). A Method of Computing The Effectiveness of an Insecticide. *Journal of Economic Entomology*, *18*, 265-267.

Ahmad, M., Arif, M., and Ahmad, M. (2007). Occurrence of Insecticide Resistance in Field Populations of *Spodoptera litura* (Lepidoptera: Noctuidae) in Pakistan. *Crop Protection*, *26*(6), 809-817.

Anonymous (2019). Food and Agricultural Organisations, Government of India.

Bail, N. S., Sasidharan, T. O., Remadevi, O. K., Dharmarajan, P., Pandian, K. S. and Balaji, K. (2015). Morphology and RAPD analysis of certain potentially entomopathogenic isolates of *Metarhizium anisopliae* Metsch. (Deuteromycotina: Hypocreales). *Journal of Microbiology and Biotechnology Resources*, *5* (1), 34-40.

Bell, J. V. (1975). Production and pathogenicity of the fungus, *Spicaria rileyi* from solid and liquid media. *Journal of Invertebrate Pathology*, *26*, 129-130.

Boucias, D. G., Tigano, M. S., Sosa-Gomez, D. R., Glare, T. R. and Inglis, P. W. (2000). Genotypic properties of the entomopathogenic fungus *N. rileyi. Journal of Biological Control*, *19*, 124-138.

de Moraes, C. K., Schrank, A. and Vainstein, M. H. (2003). Regulation of extracellular chitinase and proteases in the entomopathogen and acaricide *Metarhizium anisopliae*. *Current Microbiology*, *46*(3), 205–210.

Fronza, E., Specht, A., Heinzen, H. and Monteiro de Barros, N. (2017). *Metarhizium* (*Nomuraea*) as biological control agent. *Biocontrol Science and Technology*, *27*(11), 1243-1264.

Goh, H. G., park, J. D., Choi, K. M. and Lee, S. G. (1992). Effectiveness of selective insecticides and *Nomuraea rileyi* against beet armyworm *Spodoptera exigua* Hubner, *Journal of Crop Proection.*, *34*, 96-100.

Hazarika, S., Patgiri, P., Dutta, P., Borkataki, S. and Das, K. (2016). Efficacy of local isolate of *Nomuraea rileyi* (Farlow) Sampson against *Helicoverpa armigera* (Hubner). *Journal of Entomology and Zoological Studies*, *4*, 167-169.

Ignoffo, C. M. (1981). The fungus *Nomuraea rileyi* as a microbial insecticide. In: Burges HD (ed) Microbial control of pest and plant diseases. Academic Press, Londres, 513–538.

Ignoffo, C. M. and Garcia, C. (1985). Host spectrum and relative virulence of an Ecuadoran and a Mississippian biotype of *Nomuraea rileyi*. *Journal of Invertebrate Pathology*, *45*(3), 346–352.

Kepler, R. M., Humber, R. A., Bischoff, J. F. and Rehner, S. A. (2014). Clarification of generic and species boundaries for *Metarhizium* and related fungi through multigene phylogenetics. *Mycologia*, *106*(4), 811-829.

Kish, L. P., Samson, R. A. and Allen, G. E. (1974). The genus, *Nomuraea* Maublanc. *Journal of. Invertebrate Pathology*, *24*, 154-158.

Kish, L. P. (1975). The biology and ecology of *Nomuraea rileyi* doctoral dissertation, *Ph.D. thesis* dissertation, University of Florida, Gainesville, Florida.

Kulkarni, N. S. (1999). Utilization of fungal pathogen *Nomuraea rileyi* (Farlow) Samson in the management of lepidopterous insect pests. *Ph.D. Thesis*, University Of Agricultural Sciences, Dharwad, Karnataka.

Mallapur, C. P., Naik, A. K., Hagari, S., Praveen, T., Patil, R. K. and Lingappa, S. (2018). Potentiality of *Nomuraea rileyi* (Farlow) Samson against the fall armyworm, *Spodoptera frugiperda* (JE Smith) infesting maize. *Journal of Entomology and Zoological Studies*, *6*(6), 1062-1067.

Manjula, K. and Krishnamurthy, K. V. M. (2005). Efficacy of *Nomuraea rileyi* against different instars of *Spodoptera litura* and *Helicoverpa armigera*. *Annals of Plant Protection Sciences*, *13*(2), 347 - 350.

Nandish, M. S., Suchitha, Y. and Shivaprakash, M. K. (2016). Collection, characterization and screening of entomopathogenic fungi against field bean pod borer (*Helicoverpa armigera*, Hubner). *International journal of recent scientific research, 7*, 10713-10717.

Namasivayam, K. R., Bharani, R. S. A. and Ansari, M. R. (2013). Natural occurrence of potential fungal biopesticide *Nomuraea rileyi* (Farlow) Samson associated with agriculture fields of Tamil Nadu, India and its compatibility with metallic nanoparticles. *Journal of biofertilizers & biopesticides*, *4*, 132.

Singh, H. and Joshi, N. (2020). Management of the aphid, *Myzus persicae* (Sulzer) and the whitefly, *Bemisia tabaci* (Gennadius) using biorational on capsicum under protected cultivation in India. *Egyptian Journal of Biological Pest Control, 30*(1), 67.

Sharanabasappa, Kalleshwaraswamy, C. M., Asokan, R., Swamy, H. M., Maruthi, M. S., Pavithra, H. B., Hegbe, K., Navi, S., Prabhu, S. T. and Goergen, G. E. (2018). First report of the fall armyworm, *Spodoptera frugiperda* (J E Smith) (Lepidoptera: Noctuidae), an alien invasive pest on maize in India. *Pest management in horticultural ecosystems*, *24*(1), 23-29.

Shylesha, A. N., Jalali, S. K., Gupta, A., Varshney, R., Venkatesan, T., Shetty, P., Ojha, R., Ganiger, P. C., Navik, O., Subaharan, K., Bakthavatsalam, N. and Ballal, C. R. (2018). Studies on new invasive pest *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) and its natural enemies. *Journal Biological Control*, *32*(3), 1-7.

Sreenivas, A. G. (1996). Biological methods of managing boll worm, *Helicoverpa armigera* (Hubner) on cotton. *PhD. Thesis*, University Of Agricultural Sciences, Dharwad, Karnataka.

Srikanth, J., Mahesh, P., Salin, K. P. and Poorani, J. (2015). Occurrence of the hispa *Asamangulia cuspidata* and its parasitoids in southern India. *Current Science, 109*(12), 2288-2295.

Vimaladevi, V. P. S., Prasad, Y. G., Rajeswari, B. and Vijaya Bhaskar, L. (1996). Epizootics of the entomofungal pathogen, *Nomuraea rileyi* on lepidopterous pests of oil seed crops. *Journal of Oilseeds Research*, *13*, 144-148.

Visalakshi, M., Varma, P. K., Sekhar, V. C., Bharathalaxmi, M., Manisha, B. L. and Upendhar, S. (2020). Studies on mycosis of *Metarhizium* (*Nomuraea*) *rileyi* on *Spodoptera frugiperda* infesting maize in Andhra Pradesh, India. *Egyptian Journal of Biological Pest Control*, *30*(1), 1-10.

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