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# Studies on mycosis of *Metarhizium (Nomuraea) rileyi* on *Spodoptera frugiperda* infesting maize in Andhra Pradesh, India

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## Abstract

**Background:** Mycosis on the fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), infecting maize was observed in research farm of Regional Agricultural Research Station, Anakapalli from October 2019 to February 2020.

**Main body:** High relative humidity (94.87%), low temperature (24.11 °C), and high rainfall (376.1 mm) received during the month of September 2019 predisposed the larval instars for fungal infection and subsequent high relative humidity and low temperatures sustained the infection till February 2020. An entomopathogenic fungus (EPF) was isolated from the infected larval instars as per standard protocol on Sabouraud's maltose yeast extract agar and characterized based on morphological and molecular analysis. The fungus was identified as *Metarhizium (Nomuraea) rileyi* based on ITS sequence homology and the strain was designated as AKP-Nr-1. The pathogenicity of *M. rileyi* AKP-Nr-1 on *S. frugiperda* was visualized, using a light and electron microscopy at the host-pathogen interface. Microscopic studies revealed that all the body parts of larval instars were completely overgrown by white mycelial threads of *M. rileyi*, except the head capsule, thoracic shield, setae, and crotchets. The cadavers of larval instars of *S. frugiperda* turned green on sporulation and mummified with progress in infection. In vitro pathogenicity tests revealed the potential of AKP-Nr-1 strain of *M. rileyi* in management of *S. frugiperda*.

**Short conclusion:** The results indicated the potential of *M. rileyi* AKP-Nr-1 as biocontrol agent for management of the fall armyworm. This AKP-Nr-1 strain of *M. rileyi* needs further evaluation under field conditions to evaluate its efficacy against *S. frugiperda* and its effects on other hosts.

**Keywords:** *Metarhizium rileyi*, *Spodoptera frugiperda*, Maize, Efficacy, Microscopy

## Background

Fall armyworm (FAW), *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) is a serious polyphagous pest of voracious nature with a wide host range and causes significant losses to agricultural crops. Damage due to the pest larval instars was reported on foliage, leaf whorls, tassel, and cobs based on the stage of infection (Goergen et al. 2016). FAW attack on maize has the potential to cause yield losses in a range from 8.3 to 20.6 million tons per annum, in the absence of any control measure. This is a nightmare to

maize farmers and poses a major threat to food security and agricultural trade in Africa (Igyuve et al. 2018).

Various control approaches like cultural methods and use of chemical pesticides and natural plant products are in vogue for the management of the FAW in maize. However, overuse of chemical insecticides had witnessed deleterious effects on soil health and the environment as well as beneficial flora and fauna like earthworms, natural predators, and parasites (FAO 2018). Use of biocontrol agents in sustainable management of insect pests is "oflate" gaining importance in view of environmental safety. Fungal bioagents infecting insects can provide an effective alternative for ecofriendly management of insect pests.

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Mycosis of entomopathogenic fungus (EPF) on various insect pests was reported worldwide under favourable environmental conditions for fungal growth and multiplication (Vimala et al. 2003; Meyling and Eilenberg 2007; Ingle 2014; and Patil and Abhilash 2014). Prevalence, distribution, and mycosis of entomo-fungal pathogens were found influenced by weather parameters like temperature, rainfall, and humidity (Maurya et al. 2013). Some of these fungi were isolated in pure form, evaluated and identified as potential biocontrol agents for pest management owing to their specificity and ease of multiplication.

Among various EPF, *Metarhizium (Nomuraea) rileyi* (Farlow) Samson was reported to infect several lepidopteran pests and found highly effective against noctuid defoliators like *Spodoptera litura* Fabricius, *Helicoverpa armigera* (Hubner), *Anticarsia gemmatalis* (Hubner), and *Trichoplusia ni* Hubner (Keller and Zimmermann 1989) for their ecofriendly management. *M. rileyi* is a dimorphic, ubiquitous fungus with yeast-like hyphal bodies and true mycelial filaments and named initially as *Botrytis rileyi* (Farlow) and later as *Spicaria rileyi* (Farlow) Charles. The fungus was re-described and placed in the genus, *Nomuraea* by Kish et al. (1974). Based on molecular analysis, using RAPD, internal transcribed spacer (ITS) sequence analysis, amplified length polymorphism (AFLP), and telomeric finger printing methods, Boucias et al. (2000) stated that *N. rileyi* isolates were more closely related to *Metarhizium anisopliae* and *M. flavoviride* than to *N. atypicola* and *N. anemonoides*. More recently, *N. rileyi* has been changed to *M. rileyi* based on its morphological and molecular characterization by Kepler et al. (2014).

*M. rileyi* produces conidia on conidiophores, which are airborne and the infective propagules that will initiate the pathogenesis. The conidia adhere to the host surfaces, germinate and produce germ tube that penetrates the host cuticle, and colonizes the haemocoel of the insect leading to death of the insect (Srisukchayakul et al. 2005).

Previous studies revealed a marked variability among the isolates of various EPF, collected from different geographical locations and their efficacy against insect pests (Vimala et al. 2003). Therefore, it is important to exploit native EPF and to test their potential against target pests for developing a myco-insecticide for specific geographic locations.

Hence, the present study was taken up to characterize the EPF, naturally infecting *S. frugiperda* on maize and to visualize host colonization through light and electron microscopy. Further, pathogenicity tests were also conducted to study the efficacy of the native strain of the fungus on FAW in maize.

## Main text

### Materials and methods

The present study was carried out at the Regional Agricultural Research Station (RARS), Anakapalli (17° 40'

48" N, 83° 01' 12" E), Visakhapatnam, Andhra Pradesh, India, during 2019–2020 crop seasons. The maize hybrid, Syngenta S6668, was sown in the first fortnight of August 2019 in 6 blocks of 27 m<sup>2</sup> each. Maize cultivation was carried out as per the regular cultural practices and data on FAW incidence was recorded on first appearance of the pest.

### Correlation of weather parameters with mycosis on fall armyworm in maize

Observations on FAW incidence in maize were recorded at fortnightly intervals from September 2019 to February 2020. Data on weather parameters, viz., temperature, relative humidity, and rainfall, were recorded during this period and correlated with *M. rileyi* natural infection. The data were analysed statistically (Panse and Sukatme 1985). The data was subjected to stepdown regression by Efroymson (1960) was followed for generation a prediction system, using XLSTAT software version 2016.03.30882.

### Isolation of entomopathogenic fungus (EPF)

Larval instars of *S. frugiperda* infected with EPF were collected from the maize field in the research farm of Regional Agricultural Research Station (RARS), Anakapalli, during October 2019 and the pathogen was isolated on Sabouraud's maltose yeast extract agar (SMYA) by standard protocol of Vimala et al. (2002). The dead larvae collected from the field were surface sterilized by immersing in 4% sodium hypochlorite solution for 1 min, followed by rinsing in 3 changes of sterile 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's maltose agar media fortified with 2% yeast extract. The plates were incubated at 26 ± 1 °C for 8 days and the colonies formed were further purified by subculture on SMYA medium.

### Pathogenicity test

Pathogenicity of the isolated fungus was carried out as per the method of Dutta et al. (2014) with slight modification. Laboratory reared 3rd instar larvae of *S. frugiperda* ( $N = 30$  in 3 replications) were inoculated by spraying with conidial suspension of *M. rileyi* containing  $2 \times 10^8$  spores/ml. The inoculated larvae were kept in plastic beakers covered with muslin cloth. Thoroughly washed maize leaf bits were provided as food for FAW larvae in the beakers. Similarly, 30 individuals of 3rd instar larvae were sprayed by sterile distilled water, which served as a control. The progress of larval infection and mortality by the fungus was monitored at 24-h interval.

### Morphological and molecular characterization of EPF

Morphological features of the isolated fungus were studied using compound microscope (NIKON Eclipse E 200)

and the images captured digitally using V-image 2013 software. Molecular identity of the test fungus was established through homology analysis of internal transcribed spacer (ITS) region of ribosomal DNA (rDNA). The genomic DNA was isolated according to the standard protocol of Sambrook and Russell (2001). The ITS region of the test fungus was amplified in a PCR, using ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS2 (5'-GCTGCGTTCTTCATCGATGC-3') primers. Thermocyclic conditions included initial denaturation at 94 °C for 4 min, followed by 38 amplification cycles of 94 °C for 30 s, 55 °C for 30 s, 72 °C for 90 s, followed by final extension at 72 °C for 10 min. The PCR amplified products were resolved in 1% agarose gel in 1X Tris-acetate EDTA, run for 90 min at 100 V, and the amplified products were excised and outsourced (Bioserve Biotechnologies (India) Pvt. Ltd., Hyderabad) for partial sequencing. Similarity of ITS region was aligned using BLAST Program of GenBank database (NCBI) and phylogenetic tree was constructed, using MEGA software version 10.0. The sequences obtained from the NCBI database were mentioned in the Table 1.

#### Ultrastructure of mycosis on fall armyworm

Infected larvae, which are white in growth, were taken to the laboratory and placed in sterile Petri dishes containing moist filter papers and then incubated at 26 °C with 70% relative humidity (RH) for 3 days to induce sporulation of the fungus. The infected larvae with olive green spores and the sporulating cultures of EPF on SMYA were used to study the mycosis on *S. frugiperda*. The mycosis of FAW was captured, using a stereo binocular microscope (Magnus MSZ-TR).

Colonization of EPF on larvae of the fall armyworm was visualized by scanning electron microscopy (SEM). Samples were fixed in 2.5% glutaraldehyde in 0.1 M

phosphate buffer (pH 7.2) for 24 h at 4 °C and post fixed 1% in aqueous osmium tetroxide for 4 h. Dehydrated in series of graded alcohols and dried to critical point drying with critical point drying (CPD) (EMS 850) unit/vacuum desiccation for 35–45 min for complete drying of specimens. The dried samples were mounted over the stubs with double-sided carbon conductivity tape, and a thin layer of heavy metal (gold) was coated over the samples by using an automated sputter coater (Model JEOL JFC-1600) for 3 min and scanned under SEM (Model JOEL-JSM 5600) at required magnifications as per the standard procedures at RUSKA Lab's College of Veterinary Science, Rajendranagar, Hyderabad, India.

## Results and discussion

### Incidence of fall armyworm in maize crop and correlation studies

Initial infestation of FAW in maize was recorded during 1st week of September 2019 and sustained till harvest of the crop, i.e., up to February 2020 (Table 2). The FAW incidence ranged from 11.02 to 46.88%. High infestation of FAW was recorded from 2nd fortnight of October to December 1st fortnight with minimum temperatures of 19.63–24.39 °C and high RH (90–98%).

Mycosis of *M. rileyi* on *S. frugiperda* was noticed as insect cadavers on maize leaf, leaf sheath, silk, and cobs (Fig. 1). *M. rileyi* infection started from 1st fortnight of October (5.61%) and reached the maximum (38.02%) during November 1st fortnight and reduced from December 2nd fortnight onwards. High rainfall received during the 2nd fortnight of September (239.7 mm) and 2nd fortnight of October (236.7 mm) with a high RH (> 95%) was found supportive for mycosis initiation and further progress.

Similar findings by Vimala et al. (2002) were reported in a study where severe outbreak of *N. rileyi* infection on *Spodoptera litura* larvae was reported under favourable weather conditions. High relative humidity (90.8%) and high rainfall (84 mm) with a minimum temperature of 23.67 °C were found conducive for natural mycosis of *N. rileyi* on *S. litura*. The high humidity and rainfall favour primary infection of the insects by EPF and subsequent invasion of various tissues of the host. Further, these conditions also support growth and sporulation of EPF leading to dispersal of propagules onto plant surfaces, which initiate fresh infections and progress of mycoses. Allen and Buren (1974) discussed the role of biotic and abiotic factors in the occurrence and outbreaks of *M. rileyi* on insect pests. Temperatures of around 25 °C with high RH (70–75%) prevailing in tropical regions like India, Brazil, and Sub-Saharan Africa were found highly favourable for *M. rileyi* development (Edelstein et al. 2005).

Correlation studies revealed a high positive correlation between the incidence of FAW and natural incidence of *N. rileyi* (0.854) (Table 3). Further, the studies revealed

**Table 1** The sequences along with accession numbers used from the NCBI database for the purpose of phylogenetic tree construction

NCBI accession number	Location of the isolate
MN602591	Bangalore, India
MH860365	The Netherlands
KY436756	Chennai, India
HQ165711	Thailand
EU553337	Brazil
MN907775	Bengaluru, India
JQ686668	Chennai, India
JX276733	Jaipur, India
KJ728725	Tirupati, India
MK697304	Ludhiana, India
KJ728726	Tirupati, India

**Table 2** Natural incidence of *Metarhizium rileyi* infection on maize fall armyworm in relation to weather variables during 2019–2020

Observation period, week (fortnight)	FAW infestation (%)	<i>Metarhizium rileyi</i> infection (%)	Rainfall (mm)	Temp, °C, max	Temp, °C, min	RH%, FN	RH%, AN
Sept. I fortnight, 33 and 34 stdw	12.51	0.0	136.4	31.44	24.97	86.6	75.13
Sept. II fortnight, 35 and 36 stdw	19.64	0.0	239.7	31.99	25.06	99.47	72.0
Oct. I fortnight, 37 and 38 stdw	32.01	5.61	75.9	32.98	24.11	94.87	65.63
Oct. II fortnight, 39 and 40 stdw	41.24	11.39	236.7	30.61	24.39	95.30	50.75
Nov. I fortnight, 41 and 42 stdw	46.88	38.02	3.3	30.29	23.63	82.6	62.6
Nov. II fortnight, 43 and 44 stdw	46.44	22.93	0.0	31.18	20.63	90.6	48.6
Dec. I fortnight, 45 and 46 stdw	40.90	16.48	0.2	30.07	20.28	90.8	52.27
Dec. II fortnight, 47 and 48 stdw	27.11	14.12	0.0	27.23	19.63	98.0	54.87
Jan. I fortnight, 1 and 2 stdw	21.05	5.84	0.8	29.17	17.01	93.6	55.13
Jan. II fortnight 3, and 4 stdw	18.25	3.9	0.0	31.39	18.72	89.56	52.63
Feb. I fortnight, 5 and 6 stdw	14.7	3.1	1.11	30.49	19.88	91.73	58.46
Feb. II fortnight, 7 and 8 stdw	11.02	0.0	0.06	32.68	19.06	91.07	45.14

Temp temperature, RH relative humidity, FN forenoon, AN afternoon, stdw standard week

that minimum temperature ( $T_{min}$ ) had positive correlation with the incidence of *N. rileyi* as well as with FAW incidence but the influence is low as indicated by the values (0.075 and 0.233, respectively). All the other weather factors showed negative correlation with low values. Thus, the studies revealed that the factors which are favourable for the FAW incidence are also favourable for the natural incidence of *M. rileyi*. Further, FAW incidence will favour the natural incidence of *M. rileyi* under field conditions, which indicates the highest adoptability of the present strain of *M. rileyi*, which can be used as a potential bio-control agent against FAW under field conditions.

The data was subjected to step down regression and a model was developed for the prediction of *M. rileyi* natural incidence under field conditions as given below:

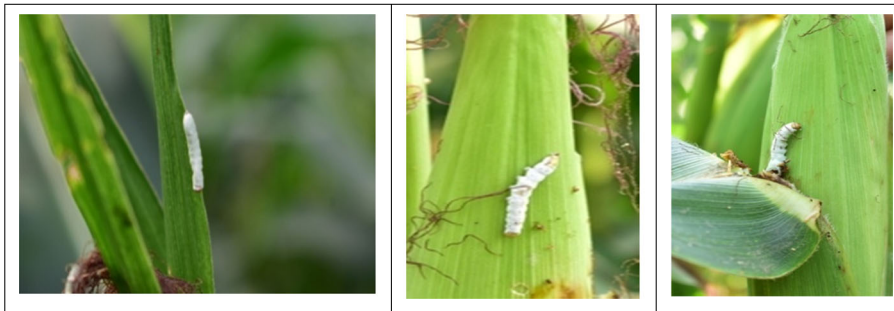
$$\begin{aligned}
 M.rileyi \text{ natural incidence (\%)} &= 91.8173 + 0.57 \times \text{FAW (\%)} \\
 &\quad - 3.99E^{-02} \times \text{Rainfall} - 2.03 \\
 &\quad \times T_{max} + 1.26 \times T_{min} - 0.62 \\
 &\quad \times \text{RH I} - 4.7E^{-02} \times \text{RH II}
 \end{aligned}$$

The prediction equation clearly suggests the natural incidence of *M. rileyi* was highly positive with the

incidence of FAW and can be effectively utilized for the prediction of the chance of natural incidence of *M. rileyi* under field conditions as suggested by its high adjusted  $R^2$  values of 0.923. The same can be ascertained from the graph (Fig. 2) as the predicted vs. actual natural incidence values of *M. rileyi* falling close to the axis. Thus, from the present study it can be deciphered that the developed prediction model based on step down regression method can be effectively utilized for assessing the natural incidence of *M. rileyi* under field conditions based on weather factors and FAW incidence after validation.

**Isolation of entomopathogenic fungus**

The fungus was isolated from infected larvae on SMYA. It is a slow-growing fungus with white to dull white colony was produced on SMYA which subsequently transformed into olive green to malachite green in colour with onset of speculation (Fig. 3). Sporulation was noticed 8 days after inoculation. Ingle (2014) also reported a high radial growth of *M. rileyi* on SMYA medium with sporulation period less than 9.53 days.



**Fig. 1** *Metarhizium rileyi* natural infection on fall armyworm, *Spodoptera frugiperda* larvae on Maize leaf, leaf sheath, silk, and cob

**Table 3** Correlation matrix of natural incidence of *Metarhizium rileyi* under field conditions during study period

Parameters	FAW (%)	Rainfall	$T_{max}$	$T_{min}$	RH I	RH II	MR (%)
FAW (%)	<b>1</b>	-0.028	-0.194	0.233	-0.168	-0.235	0.854
Rainfall	-0.028	<b>1</b>	0.305	0.772	0.414	0.490	-0.318
Temp. max	-0.194	0.305	<b>1</b>	0.400	-0.136	0.201	-0.358
Temp. min	0.233	0.772	0.400	<b>1</b>	-0.009	0.687	0.075
RH I	-0.168	0.414	-0.136	-0.009	<b>1</b>	-0.024	-0.466
RH II	-0.235	0.490	0.201	0.687	-0.024	<b>1</b>	-0.194
<b>MR (%)</b>	<b>0.854</b>	<b>-0.318</b>	<b>-0.358</b>	<b>0.075</b>	<b>-0.466</b>	<b>-0.194</b>	<b>1</b>

*Temp. max*, maximum temperature; *Temp. min*, minimum temperature; *RH I*, relative humidity forenoon session; *RH II*, relative humidity afternoon session; *MR*, *M. rileyi*% infestation

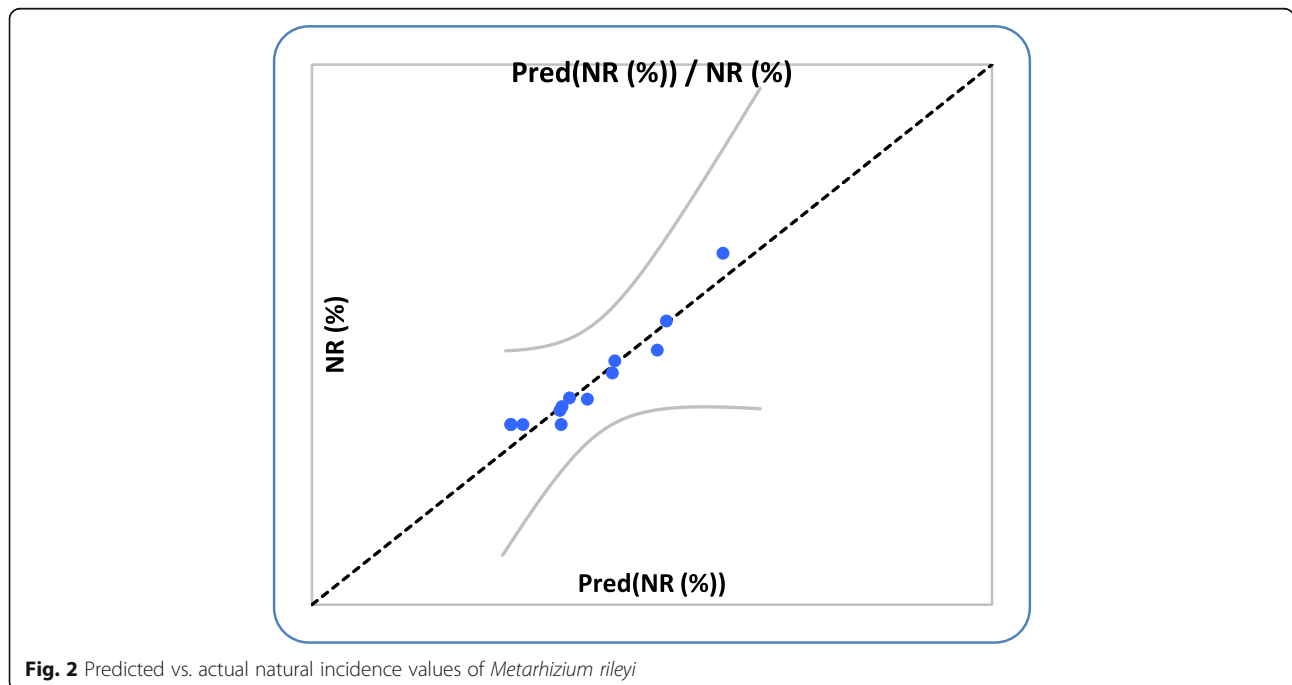
**Pathogenicity of *M. rileyi* on *S. frugiperda***

Inoculated larvae were found less active from the 3rd day of inoculation, feeding was stopped, body colour changed to straw colour, and gradually larvae remain immotile and died subsequently (Fig. 4). Ninety percent of the larvae inoculated were dead within 1 week of inoculation. Sparse growth of mycelium of the fungus was noticed on the surface of larval integument from fourth day and the white growth of the fungus was conspicuous from 8 day after inoculation. The whitish mycelial growth of the fungus turned olive green in colour with initiation of sporulation from the 10th day onwards. Similar observations were reported by Dutta et al. (2014) on *S. litura* invaded by *M. rileyi*. Grain formulation of *M. rileyi* was tested against 3rd instar larvae of *S. litura* by Krishnaveni et al. (2016) and observed 80% mortality.

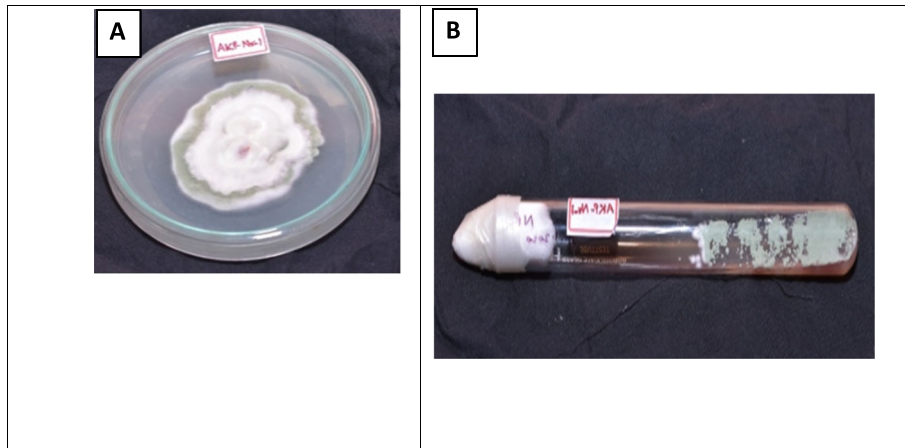
**Morphological and molecular characterization of entomopathogenic fungus**

The isolated fungus was identified as *Metarhizium rileyi* based on morphological characters. The fungus has produced septate hyaline mycelium with erect conidiophores produced on short conidiogenous cells. Whorls of aseptate round to ovoid conidia are formed on phialides produced on smooth, erect, single/synnematosus conidiophores (Fig. 5). Similar results were reported in previous reports on *S. litura* (Dutta et al. 2014), *H. armigera* (Nandish et al. 2016), and *S. frugiperda* (Alvarez et al. 2018).

The fungus provisionally identified as *Metarhizium* based on morphology was further characterized to species level by amplification of ITS region using PCR. The NCBI-BLAST analysis of partial genome sequence amplified by ITS1 and ITS2 has confirmed the EPF as *Metarhizium*



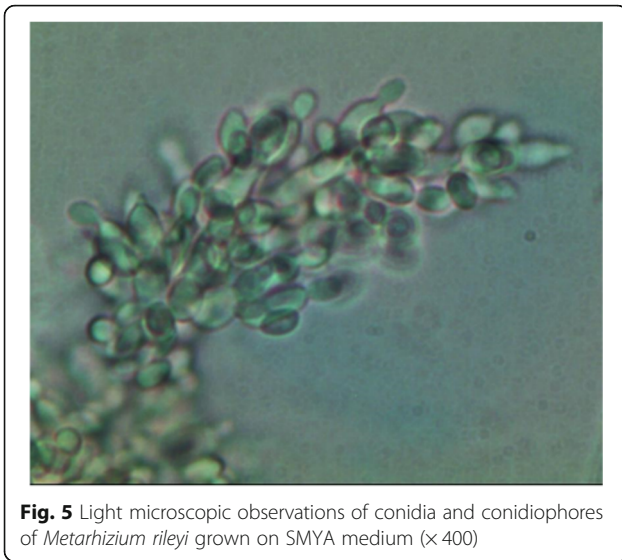
**Fig. 2** Predicted vs. actual natural incidence values of *Metarhizium rileyi*



**Fig. 3** In vitro growth of *Nomuraea rileyi* on SMYA medium. **a** Growth of fungal hyphae (white in colour) and **b** spore production (green in colour)



**Fig 4** Development of mycosis of *Metarhizium rileyi* on larval instars of *Spodoptera frugiperda*: colour of the larval instars turned straw in colour and were killed 4 days after topical application with *M. rileyi* (**a-c**), progress of infection (**d-f**), flocculent whitish growth of fungus on larval instars 8 days after inoculation (**g-i**), and sporulation of *M. rileyi* on *S. frugiperda* 10 days after inoculation (**j-l**)



*rileyi*. The ITS generated sequence was deposited in NCBI Gene Bank database and obtained with accession number (MN 960559). Using maximum parsimony method in MEGA software (Ver. 10.0.5), a phylogenetic tree was constructed by considering most relevant sequences from the NCBI database along with sequence data of *M. rileyi* obtained in this investigation. The phylogeny was depicted (Fig. 6).

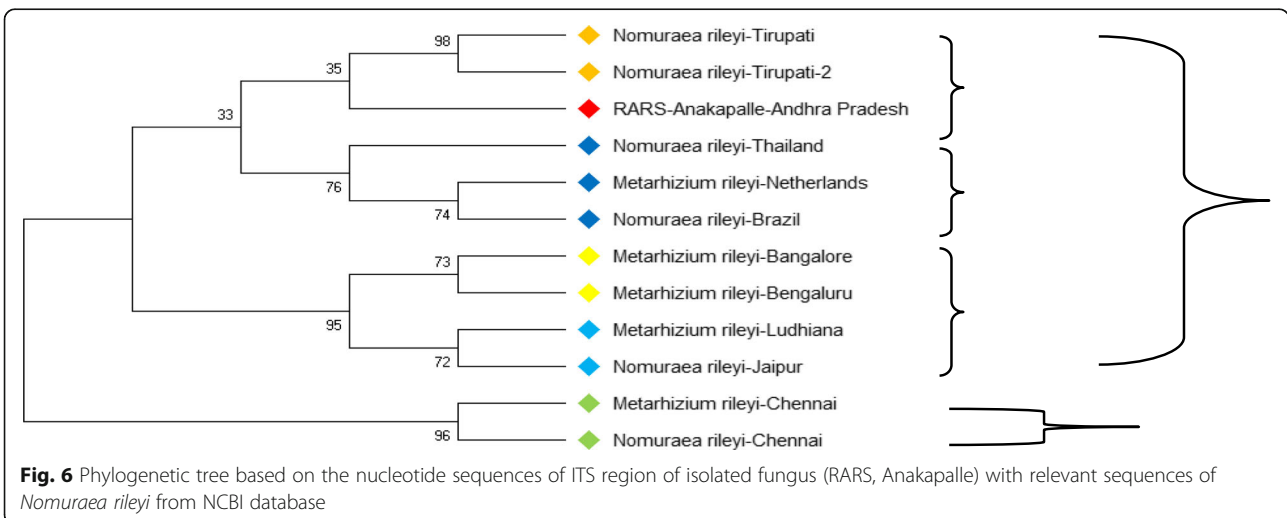
The phylogenetic tree analysis resulted into 2 major clusters. The species belonging to Chennai formed a separate cluster and rest of the species formed a cluster including the isolate in investigation. Within this major cluster, 3 subgroups were observed. All the species of Bengaluru, Ludhiana, and Jaipur formed into one subgroup. Similarly, isolates from Thailand, the Netherlands, and Brazil grouped into other subgroup, whereas Tirupati isolates and RARS-Anakapalle isolate (AKP-Nr-1) were grouped into other

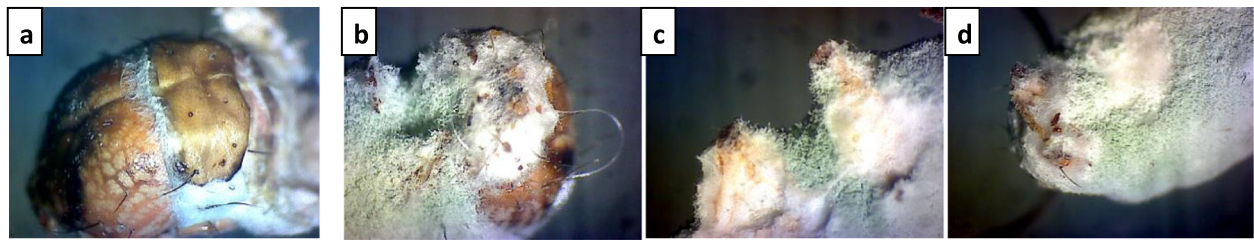
subgroup. This probably suggests that the isolate of *M. rileyi* from the RARS-Anakapalle in the study might have evolved from the same ecological niche as that of Tirupathi isolates.

Lee et al. (2012) used ITS region along with  $\beta$ -tubulin and EF-1 $\alpha$  for molecular identification of *N. rileyi*. Among the three regions, the ITS region have shown best resolution for the molecular identification of the *N. rileyi* in comparison to  $\beta$ -tubulin and EF-1 $\alpha$ . Further, to study the evolutionary pattern of *N. rileyi*, a phylogenetic tree was constructed using the most relevant sequences of NCBI of closely related genera using neighbour joining method. In their study, they found that all the *Nomuraea* grouped together. These results were highly concurrent and matched with the present study. In contrast to ITS region, analysis of the partial beta-tubulin gene based on neighbour joining and 1000 bootstrap, *N. atypicola* failed to form a monophyletic grouping with the other two species of *Nomuraea* (Han et al. 2002). This strongly suggests that ITS was the effective region for species identification and grouping as well as to study the evolutionary pattern of the *Nomuraea* spp.

**Description of mycosis on maize fall armyworm**

The fungus *Nomuraea* infection was observed on few larvae of *S. frugiperda* in early October and by November 1st fortnight; it had nearly infected 38% of the larval instars of *S. frugiperda*. Stereomicroscopic observations of mycosis have showed a complete colonization of the prothorax, mesothorax, metathorax, and all abdominal segments by whitish mycelial growth of the fungus, except at the head capsules, thoracic shield, setae, and crotchets (Fig. 7). Though head capsule and thoracic shield were not colonized, the mycelium and conidia were found emerging from the neck region between the head and the black thoracic shield. This reveals that in



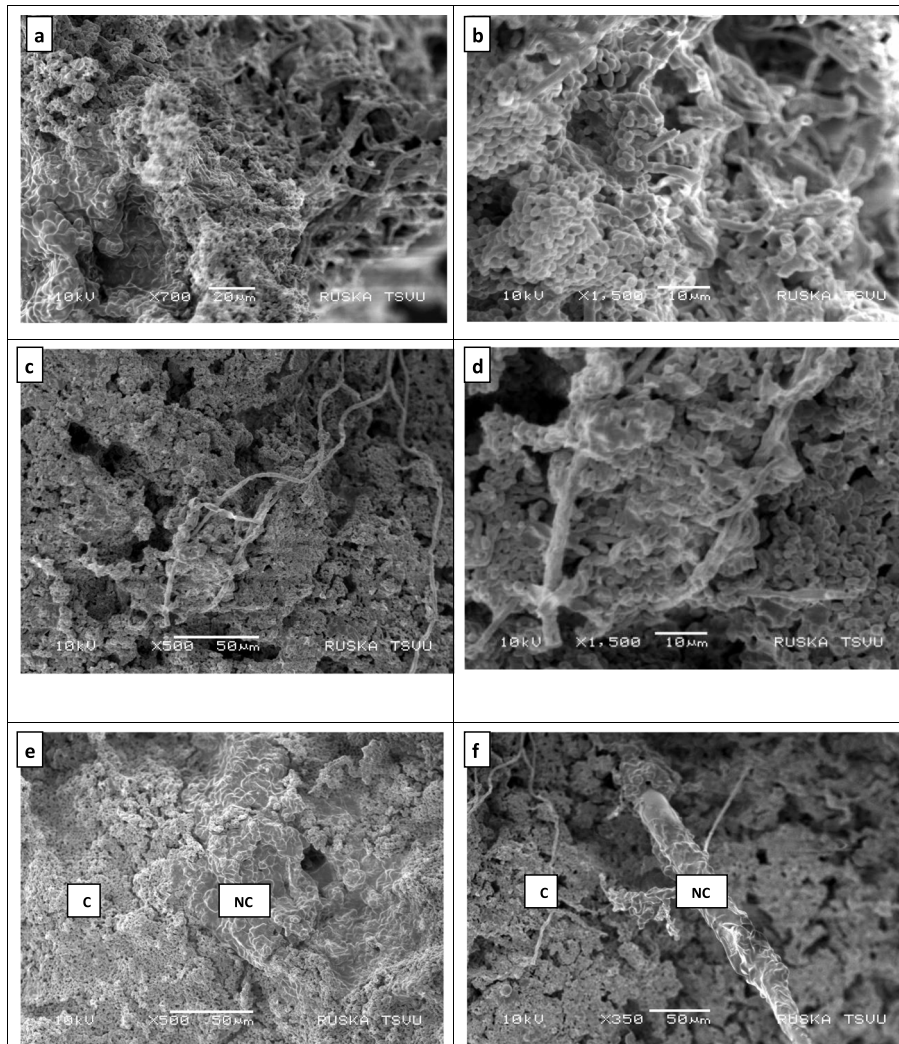


**Fig. 7** Mycosis of *Metarhizium rileyi* on various body parts of *Spodoptera frugiperda*

the initial stages, larvae of fall armyworm were found to be superficially colonized by the mycelial growth of the fungus and subsequently invaded the tissues of the insect and produced numerous conidia under humid conditions. The infected larvae were killed and often did not

reach adulthood. Various types of setae and proleg crotchets were found free of *M. rileyi*.

Scanning electron microscope (SEM) showed a dense network of *M. rileyi* with abundant conidia on surface of *S. frugiperda* larva (Fig. 8). The mycelium had further



**Fig. 8** Scanning electron microscopy of *Metarhizium rileyi* mycosis on larval instars of *Spodoptera frugiperda*. **a, b** Mycelium and conidia of *M. rileyi* on integument of *S. frugiperda*. **c, d** Hyphal penetration into the integument of *S. frugiperda*. **e, f** Non-colonization of *M. rileyi* on thoracic shield and setae



penetrated into the insect cuticle and other orifices and invaded all the tissues of the host. Most of the isolates of *M. rileyi* were reported to produce chitinolytic and proteolytic enzymes, which help in penetration of the cuticle and further invasion of internal tissues of the host (Vargas et al. 2003). In the present study, head capsules, thoracic shield, setae, and proleg crotchets were found not colonized by the fungus which could be clearly distinguished from the colonized areas. These observations agree with the SEM study of *M. anisopliae* on *Agrotis ipsilon* larva (Gabarty et al. 2014). Infection of insects by EPF requires adhesion, penetration into the insect (St. Leger 2008), and establishment of pathogen in the host (Charnley 1984). Further, proliferation of the fungus in the haemocoel or other tissues of the insect body collapse the insect immune system making the host sluggish and moribund finally leading to death.

## Conclusion

In the present study, the initiation and development of mycosis of *M. rileyi* was found to be influenced by host density, rainfall, humidity, and minimum temperatures. The weather from October 1st fortnight to November 2019 was found highly conducive for the growth, sporulation, and spread of *M. rileyi*. The native strain of *N. rileyi*, Akp-Nr-1, had a great potential in reducing the population and damage caused by FAW and hence might be utilized as one of the components of integrated pest management module in maize. However, the isolate needs to be tested for its efficacy against FAW at different concentrations on *S. frugiperda*, survival at various temperatures, humidity levels, time to colonize its host (incubation period) under controlled and field conditions, multiplication and survival in different substrates, effect on other organisms, etc., prior to commercial utilization.

## Abbreviations

RARS: Regional Agricultural Research Station; SMYA: Sabouraud's maltose yeast extract agar; EPF: Entomopathogenic fungus; CPD: Critical point drying; SEM: Scanning electron microscope; FAW: Fall armyworm; ITS: Internal transcribed spacer; AKP-Nr: Anapalli-Nomuraea rileyi; RAPD: Random amplified polymorphic DNA; AFLP: Amplified length Polymorphic DNA; XLSTAT: Excel Statistics; MEGA: Molecular Evolutionary Genetic Analysis; BLAST: Basic local alignment sequence tool; DNA: Deoxyribonucleic acid; PCR: Polymerase chain reaction; EDTA: Ethylenediaminetetraacetic acid; NCBI: National Centre for Biotechnology Information;  $T_{max}$ : Temperature maximum;  $T_{min}$ : Temperature minimum; RH: Relative humidity

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## Authors' contributions

The concept and design of the experiments were prepared by all authors. MV designed the research, conducted the study, and performed statistical analysis. PK contributed in morphological identification and ultrastructure of

mycosis by SEM. VC contributed in molecular characterization and phylogenetic tree construction. BL analysed the weather data correlation with mycosis. US contributed to the SEM studies. All the authors read and approved the manuscript.

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## Availability of data and materials

All data are available in the article and the materials used in this work are of high quality and grade.

## Ethics approval and consent to participate

All experimental works were approved by Acharya N G Ranga Agricultural University, Guntur.

## Consent for publication

Not applicable.

## Competing interests

The authors declare that they have no competing interests.

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