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Morphological Characterization of Native Isolates of Hypocreale Fungi *Metarhizium rileyi* (Farl.) Samson from Maize Fall Armyworm

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Authors' contributions

This work was carried out in collaboration among all authors. Authors MM, AH, GP, B, HSG, VNG and ADS conceptualized and designed the experiments. Authors GP and B designed the research, conducted the study and performed statistical analysis. Author AH contributed in morphological identification of M. rileyi. Author VNG contributed in molecular characterization and phylogenetic tree construction. Authors HSG and AYS contributed in conducting the research. All the authors read and approved the manuscript.

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ABSTRACT

The Present studies indicates the collection of entomopathogenic fungi from the roving survey in Northeastern region of Karnataka, India to know the diversity of *M. rileyi* distribution and also its growth pattern. Different morphological parameters used for describing the growth pattern which indicates diversity and uniqueness of *M*. *rileyi i.e.,* colony colour which was white during initial stage and then during sporulating stage the colour changed into malachite green colour. The conidiophores were in compact patches. Conidia were aseptate, hyaline, round or ovoid in shape and elongate by forming chains. Conidiophores were short, which bears round to ovoid aseptate conidia in different isolates.

The conidia size on its ratio found that, the highest size ratio was recorded in UASRBC Mr-20 2.19 then remaining isolates which has shown less than 2.0. The radial growth found only in Mr-20 and Mr-22nd Isolates, colony diameter ranged from 0.3 to 2.23 cm, concentric rings of colonies found in all the isolates except Mr-19 and Mr-20th isolates. The colony surface layer was white cottony growth to pale yellow color was found in all the isolates, the colony pigmentation in most of the isolates was light brown colored pigmentation. The growth appearance was irregular patchy growth was observed in most of the isolates. Among six different isolates the highest width was recorded in isolate UASRBC Mr-20 (6.46) followed by UASRBC Mr-22 (5.73) which was significantly superior than remaining isolates. The highest colony diameter was recorded in UASRBC Mr-18 (19 mm).

Keywords: Entomopathogenic fungi; chemical pesticides; beneficial insects; parasitize; virulence diversity; morphological; molecular identification; fungal species.

1. INTRODUCTION

Modern agriculture uses large amounts of chemical pesticides to control pests, which can act against the environment, beneficial insects and even human health [1]. These drawbacks are currently generating a shift towards a sustainable agriculture, with a gradual reduction in the application of synthetic chemical compounds. Currently, research in the area of sustainable agriculture seeks the development of pesticides using organisms with the natural ability to parasitize and kill plant pests [2,3]. These organisms are commonly called biological control agents among them one is Entomopathogenic agents which includes bacteria, fungi, viruses, nematodes and protozoa, and among the fungi the most studied are microorganisms of the genera *Beauveria*, *Metarhizium* and *Paecilomyces* [4]. In this context, the biotechnological use of entomopathogenic fungi as biocontrol agents is presented as a sustainable alternative to the limited number of available chemical pesticides. The characterizations of strains of entomopathogenic fungi, as well as the knowledge of the biological aspects related to the behavior of these strains against susceptible pests, are prerequisites for effective integrated pest management practices [5].

The climate characteristics and conditions promote the development of a wide variety of plant and fungal species, turning the province of Northeastern region of Karnataka into one of the most favorable regions for the search and isolation of fungal strains. Traditional morphological identification of fungi is based on the study of macroscopic and microscopic structures [6,7]. Especially, the shape and size of the conidia are the most used traits for the identification of *Beauveria* and *Metarhizium* species [8,9].

In addition to biotic stress, the effectiveness of EPF in controlling insect pests is influenced by the diversity of varieties or strains or isolates of them. The pathogenicity, virulence, enzymatic characteristics, and DNA also varied among different isolates of different insects, hence the origin of the isolate affects the virulence diversity of the fungus against the host insect. Therefore morphological and molecular identification of fungi is considered an essential step in the selection of biocontrol agents [10,11]. In this work the objectives proposed were to isolate and identify morphologically the native isolates of *M. rileyi* from maize ecosystem in the province of Northeastern region of Karnataka.

2. MATERIALS AND METHODS

2.1 Collection of Cadavers in North Eastern Karnataka

The roving survey was carried out in different districts of North Eastern region of

Table 1. Subcultures of *M. rileyi* on SMAY media

Sl. No.	Crop	Host	Location	Co-ordinates	Isolate number
1	Maize	*S. frugiperda*	Askhal,Raichur	16.1963977.32708	UASRBC Mr -3
2	Maize	*S. frugiperda*	Gondbal, Koppal	15.2855176.14853	UASRBC Mr -15
3	Maize	*S. frugiperda*	Konchigeri, Ballari	15.41996476.875882	UASRBC Mr -18
4	Maize	*S. frugiperda*	ARS,Siruguppa	15.617576.9006	UASRBC Mr – 19
5	Maize	*S. frugiperda*	Tekkalakote, Ballari	15.5248,76.8793	UASRBC Mr -20
6	Maize	*S. frugiperda*	Halekota, Ballari	15.57175,76.88616	UASRBC Mr- 22

Karnataka, India in different cropping ecosystems during 2020-21.

2.2 Isolation of *M. rileyi* Collected from Different North Eastern Karnataka

In maize ecosystem around 1-12 fungal infected fall armyworm cadavers were colleted from the cob, leaf sheath, tassels, and on soil surface and these were white mummified cadavers, the white hyphae was covered entire body and were brought to the laboratory and kept for sporulation in incubation chambers and later surface sterilized by immersing in 0.5 per cent $HgCl_2$ for two minutes. Infected tissue of the fungi was transferred on SMAY medium [12]. The fungi inoculated plates were incubated at room temperature 25 ± 2 °C for a week and the sporulated colonies were further purified by repeated subculture on SMAY medium. Initially, the colour of the colony was pale green to pale turtle green, then changing into olive to malachite green.

2.3 Taxonomic Position of the Test Mycoagent, *M. rileyi*

Kingdom : Fungi
Division : Ascomycota
Class : Sordariomycetes
Order : Hyphocreales
Family : Clavicipitaceae
Genus : Metarhizium
Species : rileyi

2.4 Morphological Characterization of *Metarhizium rileyi*

The pure isolates of *M. rileyi* were grown on SMAY media. The inoculated Petri plates were incubated at 25 ± 2 °C for 14 days. The growth pattern of *M. rileyi* with other entomofungal isolates were observed on morphological and growth characteristics of individual isolates of *M. rileyi* and were recorded after 14 days of incubation at 25 ± 2 °C. The following observations of individual isolates of *M. rileyi* were recorded [13]. The radial growth of each isolate, the presence or absence of radial growth was recorded. For each of the isolates, five mm circular plugs were cut from non-sporulating mycelia on 8-9 days old culture dishes using a cork-borer from the colony growth edge. Radial growth of the colony using scale was recorded daily for up to 18 days [14].Colony colour was recorded on 16th day after inoculation, colony diameter recorded by taking average of the radial growth of the colony in two directions on the 12th, 14th and 16th day after inoculation and the data were analyzed statistically, the presence or absence of concentric rings/circles were recorded on 16th day by visual observation, colony surface layer as a secondary growth on colonies such as presence or absence of white cottony and pale yellow to cottony growth was observed on the 19th day by visual observation, colony pigmentation was recorded in each *M. rileyi* isolate during the growth from 21 days old culture, the appearance of growth *i.e*. circular, irregular or patchy, thin or thick, *etc*., was recorded at 21 Days After Inoculation (DAI) by visual observation, shape of ten spores and the colour of ten spores were observed under the microscope for each isolate, to measure the size of spores the ocular micrometer was calibrated with a stage micrometer for microscope (40X), the length and width of ten spores per isolate was measured and calculated as follows.

$$\text{Length and width ratio} = \frac{\text{Length of spore}}{\text{Width of spore}}$$

3. RESULT AND DISCUSSION

3.1 Morphology and Growth Characteristics of *M. rileyi* isolates

Selected morphological characters of each isolate of *M. rileyi* on SMAY

medium were studied and observations were recorded (Fig. 1B).

The colony colour of each isolate was recorded at 18th DAI on SMAY medium by visual observation. The results presented in Table 3 showed that all the 6 isolates were visually differentiated into three main colour categories *viz*., pale green, dark to pale green, pale yellow to pale green. This is in accordance with the Vimaladevi et al. [15] who reported that the fungus was slow-growing with white to dull white colony was produced on SMAY medium which subsequently transformed into olive green to malachite green in colour with onset of sporulation. and the radial growth was present in only two isolates *viz*., UASRBC Mr-20 and UASRBC Mr- 22 with 1.2 cm and 1.6 cm respectively after 12 DAI but there was no radial growth in remaining isolates. The studies are in confirmation with Ganeshrao [16] who recorded 38.72 mm of radial growth of *M. rileyi* on SMAY than SDAY and PDA media.

The concentric rings of each isolate were recorded at 16th DAI. The results found that all 6 isolates showed concentric rings except UASRBC Mr-19 and UASRBC Mr-2.

The colony surface layer for all the isolates at 19th DAI showed variation. Among all the isolates, the isolate UASRBC Mr-3, UASRBC Mr-15, UASRBC Mr-19, UASRBC Mr-22 showed white cottony growth.

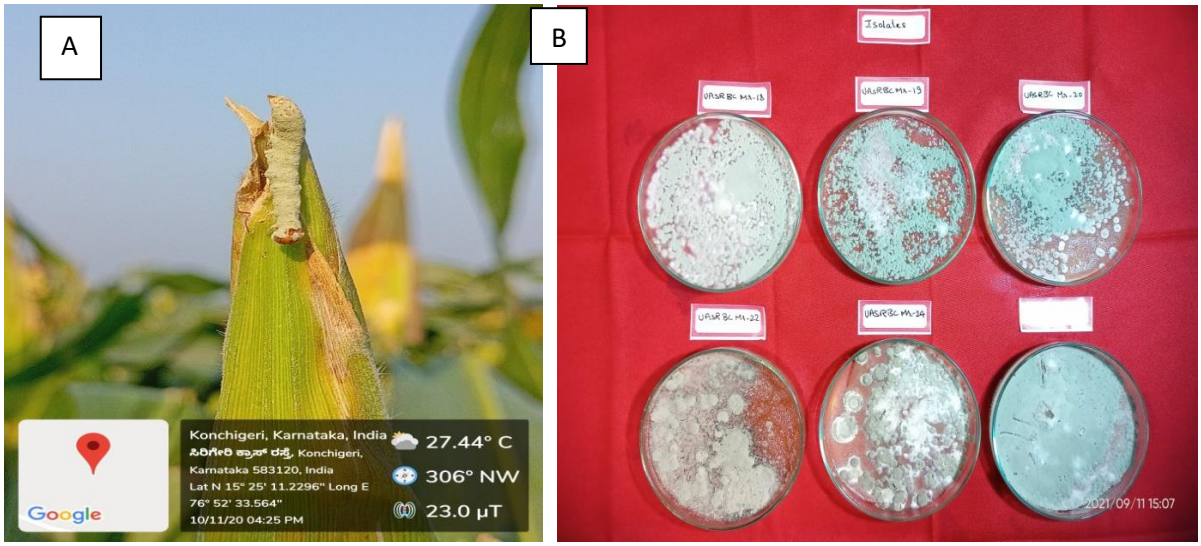

Fig. 1. A) Natural infestation of Entomopathogenic fungi on fall armyworm B) Pure cultures of *Metarhizium rileyi*

Table 2. Morphological characteristics of subcultured (colony diameter) isolates of *M. rileyi* collected from different locations of North Eastern Karnataka

Sl. No.	Isolates	Colony diameter (mm) at different time intervals		
		12th DAI	14th DAI	16th DAI
1	UASRBC Mr-3	3.50(2.00)	4.50(2.24)	5.00(2.35)^{cd}
2	UASRBC Mr-15	13.5(3.74)	14.00(3.81)	15.00(3.94)^{b}
3	UASRBC Mr-18	15.00(3.94)	17.5(4.24)	19.00(4.42)^{a}
4	UASRBC Mr-19	1.00(1.22)	2.00(1.58)	3.00(1.87)^{g}
5	UASRBC Mr-20	8.00(2.92)	10.00(3.24)	11.00(3.39)^{bc}
6	UASRBC Mr-22	2.00(1.58)	3.00(1.87)	4.00(2.12)^{f}
SEm±		0.95	1.2	1.23
CD @ 0.01		4.18	4.42	4.13

Figures in the parenthesis indicate $\sqrt{(x+0.5)}$transformed values and means followed by same letters in a column not significantly different by DMRT

Colony pigmentation was recorded on 19 days old cultures showed that an isolates showed light to dark brown pigmentation, while two isolates produced light brown pigmentation and where as dark brown pigmentation found in UASRBC Mr-18. Whereas, the only isolate UASRBC Mr-19 recorded yellow to light brown pigmentation. The variation in pigmentation of *M. aniospliae* was due to the isolates able to produce specific secondary metabolites that can change the colour of some culture media [17].

The appearance of growth was recorded at 19 DAI and presented in Table 3 showed the variation in the appearance of growth. The isolates showed irregular growth and irregular patchy growth. Few isolates showed thin covering type of appearance and also thin to thin flaky covering, thick irregular and irregular to patchy appearance in different isolates (Table 3 and Fig 2B).

Since there was no work carried on concentric rings, colony surface layer, colony pigmentation and appearance of growth particularly on *M. rileyi*. The variation in growth parameters for different isolates, may be due to the fact that the collections were made on varied climatic conditions which might have resulted in the variation.

3.2 Colony Diameter at 12th, 14th and 16th DAI

At 12th DAI all the 6 isolates showed diameter from 1mm to 17 mm. At 14th DAI showed diameter from 2mm to 19 mm in Table 2. At 16th DAI all the 6 isolates showed 3mm to 22.5 mm (Fig. 2B). The results showed statistically significant variation in colony diameter on SMAY medium. Which is in line with Ganeshrao [16] recorded the highest colony diameter of 26 mm of *M. rileyi* and also opined that the temperature influenced the diameter of the colony was found at 25 ^{o}C and lowest at (13.50 mm) 40 ^{o}C temperature (Table 3 & Fig. 2A).

3.3 Shape of Spores

After incubation for up to seven days, showed that the shape of spores varies from elliptical to globose and cylindrical to oval shape (Table 4, Fig. 3) which is in line with Brayford [18] where conidia appears dusty pale green and are aseptate, smooth, hyaline, elliptical to sometimes cylindrical and 3-4 x 2-2.5 µm in size at 10X.

3.4 Length and Width Ratio of Spores

Among all the six isolates, the highest length/width ratio of spores was observed in isolate UASRBC Mr-20 (2.19) and UASRBC Mr-15 (2.09) (Table 4 & Fig. 3).

The remaining isolates showed 1.48-1.96 ratio. The study is in accordance with Sampson (1994), as conidia aseptate, smooth, round to ovoid or elongate or single smooth ellipsoidal and slightly curved, in short, divergent chains, pale to dark green.

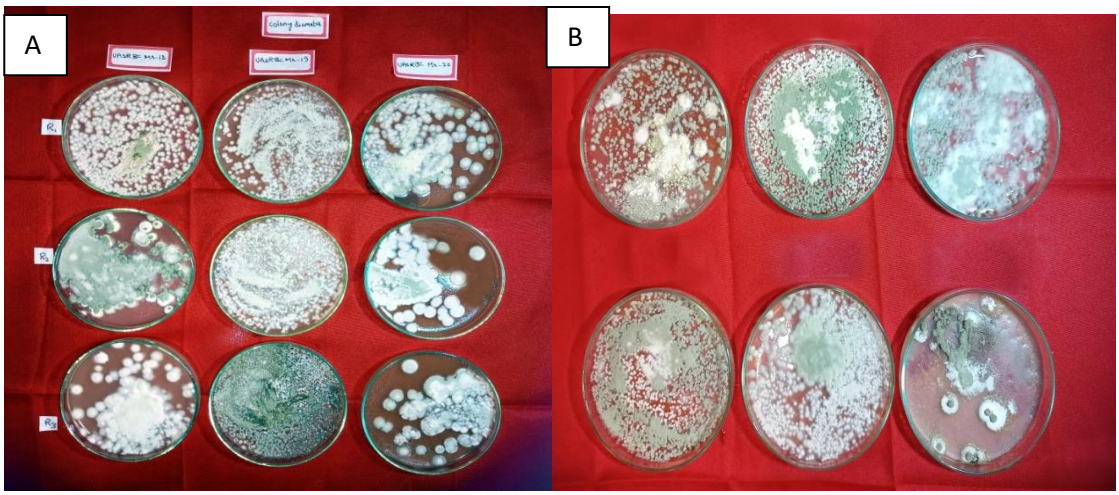

Fig. 2. A) Colony diameter of native isolates of *Metarhizium rileyi*B) Colony surface layer of native isolates of *Metarhizium rileyi*

Table 3. Morphological characteristics of subcultured (colonies) isolates of *M.rileyi* collected from different locations of North Eastern Karnataka

Sl. No.	Isolate	Colony colour	Radial growth	Concentric rings	Colony surface layer	Colony pigmentation	Appearance of growth
1	UASRBCMr-3	Pale green	Absent	Present	White cottony	Light to dark brown	Irregular
2	UASRBCMr-15	Pale green	Absent	Present	White cottony	Light brown	Thick irregular
3	UASRBCMr-18	Pale green	Absent	Present	No cottony growth	Dark brown	Irregular to patchy
4	UASRBC Mr-19	Pale yellow to pale green	Absent	Absent	White cottony growth	Yellow to light brown	Thin covering
5	UASRBC Mr-20	Dark to pale green	Present(1.2cm)	Absent	No white cottony growth	Light to dark brown	Thin covering
6	UASRBC Mr-22	Dark to pale green	Present(1.6cm)	Present	Pale yellow to cottony white	Light brown	Thin flakycovering

Table 4. Morphological characteristics of subcultured (conidia) isolates of *Metarhizium rileyi* collected from different locations of North Eastern Karnataka

Sl. No.	Isolate	Shape	Colour of the Spores	Length (µm) (40X)	Width (µm) (40X)	Ratio	Mycelial width (µm)
1	UASRBC Mr-3	Oval to globose	Pale green	8.09 ± 0.05	5.48 ± 0.04	1.48(1.41)^{ab}	4.36(2.20)^{c}
2	UASRBC Mr-15	Oval to cylindrical	Pale green	9.57 ± 0.09	4.57 ± 0.02	2.09(1.61)^{a}	2.84(1.83)^{d}
3	UASRBC Mr-18	Cylindrical	Pale green	7.44 ± 0.05	3.79 ± 0.09	1.96(1.57)^{ab}	4.41(2.23)^{c}
4	UASRBC Mr-19	Elliptical to oval	Pale green	9.82 ± 0.17	6.05 ± 0.48	1.62(1.46)^{bc}	1.94(1.56)^{e}
5	UASRBC Mr-20	Oval to cylindrical	Pale green	9.55 ± 0.17	4.36 ± 0.50	2.19(1.64)^{a}	6.46(2.64)^{a}
6	UASRBC Mr-22	Cylindrical	Pale green	8.14 ± 0.42	4.33 ± 0.20	1.87(1.54)^{ab}	5.73(2.49)^{ab}

Figures in the parenthesis indicate $\sqrt{x+0.5}$ transformed values means followed by same letters in a column not significantly different by DMRT

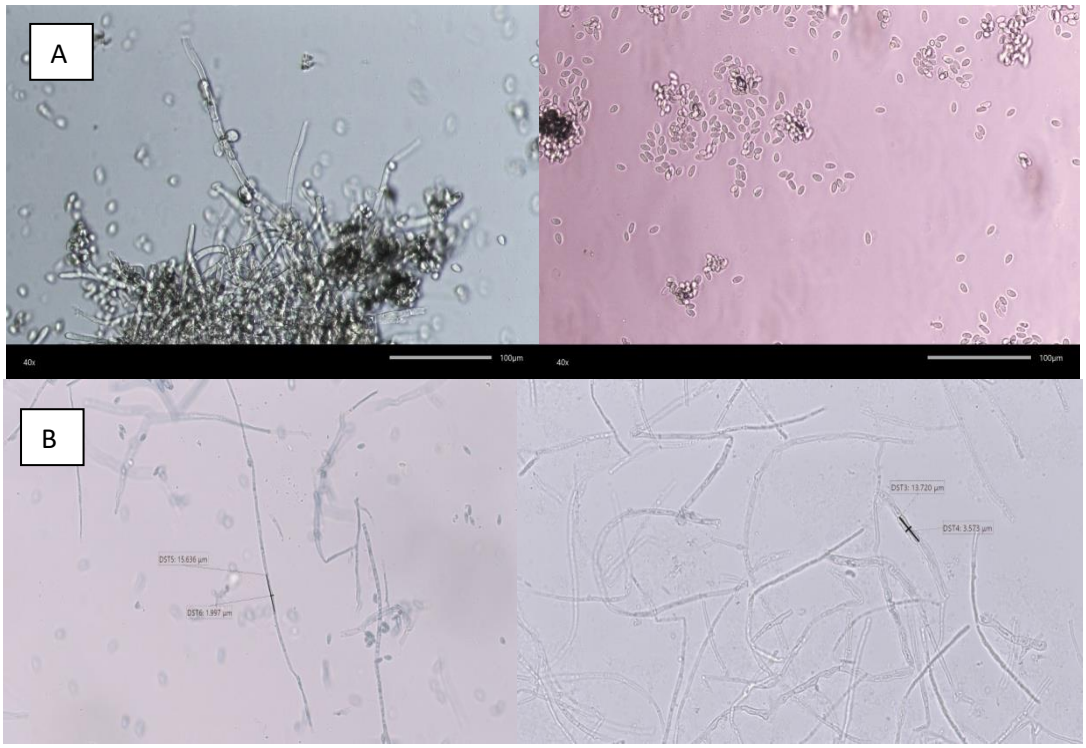

Fig. 3. A) Conidia and mycelial characters of native isolates of *M. rileyi*, B) Mycelial characters of native isolates of *M. rileyi*

3.5 Mycelial Width

The highest width of 6.46 µm was noticed in UASRBA Mr-20 and it was followed by UASRBC Mr-22 which recorded 5.73 µm mycelia width. The remaining isolates recorded 1.94- 4.41 µm mycelial width (Table 2 & Fig. 3).

Overall, the vegetative hyphae was hyaline, septate, smooth walled, sparse growth showed variation in mycelial width ranged from 1.94 to 5.73 µm. The present results are in line with the earlier studies of Brayford [18-19] reported that the diameter of hyphae was 2-3 µm.

4. CONCLUSION

Highest cadavers of *S. frugiperda* was recorded in maize ecosystem at Ballari, Koppal and Raichur district. The present study is important to know the variation or diversity among the different isolates of *M. rileyi* and it also provides as base study for the future isolates of *M. rileyi.*

ETHICAL APPROVAL

All experimental works were approved by University of Agricultural Sciences, Raichur.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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