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Natural occurrence of entomopathogenic fungus *Beauveria felina* (DC.) J.W. Carmich on fall armyworm, *Spodoptera frugiperda* (J. E. Smith)

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Abstract

Fall army worm, *Spodoptera frugiperda* was reported as an invasive pest on maize in India during May 2018 at Karnataka, India. Surveys were conducted for occurrence of pathogens on *S. frugiperda* in affected maize fields in Karnataka, India. Mycosed larvae of FAW with abundant external white fungal growth and synnemata were observed in maize fields. About 20-30% of 4 and 5th instar larvae of FAW were affected by this fungus. Isolations from the infected cadavers on Sabourauds Dextrose Yeast extract Agar yielded creamish white, round, flat colonies initially, which later produced erect synnematus structures with abundant long erect clusters of conidiogenous cells. The conidia were oval-ellipsoid shape with the size of 1.5-2.5 X 3- 3.5 µm. Further molecular characterization of the fungus using ITS region identified it as *Beauveria felina*. To best of our knowledge, this is the first report of occurrence of *B. felina* on maize fall armyworm.

Keywords: fall armyworm, *Spodoptera frugiperda*, maize, entomopathogenic fungi, *Beauveria felina*

Introduction

Fall armyworm *Spodoptera frugiperda*, is an invasive pest which has been reported to cause serious damage to the maize crop in India from 2018 onwards ^[1]. In a very short time, it has become a serious pest on maize causing 6.00 to 100 percent infestation ^[2] in the affected fields. Some of the entomopathogenic fungi reported to cause epizootics during favorable environmental conditions as provide natural pest control ^[3]. A few of these fungi have been developed worldwide as biopesticides against many pests, predominantly the strains of *M. anisopliae*, *Metarhizium robertsii* and *B. bassiana* ^[4]. In Mexico, natural infection of *Metarhizium rileyi*, *B. bassiana*, and *M. anisopliae* have been observed on fall armyworm populations ^[5]. *Nomuraea rileyi* (= *Metarhizium rileyi*) infections were observed on *S. frugiperda* larvae in maize fields in some parts of Karnataka, India ^[1, 2]. Further surveys were conducted to record occurrence of other fungal pathogens on *S. frugiperda* in FAW affected maize fields in Karnataka, India.

Materials and Methods

Surveys were conducted during July-December 2019 on natural enemies of Fall armyworm (FAW) on affected maize fields in Chikkaballapura and Bangalore Rural districts of Karnataka, India. Fungal infected larvae of FAW observed in the maize fields were collected and used for culture of the fungal pathogen and identification. Mycosed larvae were surface sterilized using 4% Sodium hypochlorite and rinsed three times with sterile water. The haemolymph from the cadavers was streaked on Sabouraud's Dextrose Yeast extract Agar (SDYA; Dextrose 20 g, Mycological peptone 10 g, yeast extract 5 g, in 1L distilled water medium) Petri plates and incubated at 26 ± 1 °C for one week and observed appearance of fungal colonies. The fungal colonies observed on the Petri plates were purified on SDYA plates and used for further studies on the morphological and molecular characteristics. Morphological studies on colony characters and light microscopic studies on asexual reproductive structures were carried out to characterize the fungal pathogen.

Molecular Characterization of entomopathogenic fungus

Fungal DNA was extracted using CTAB method and subjected to Polymerized Chain Reaction (PCR) for amplification of ribosomal DNA of internal transcribed spacer (ITS) region. The ITS primers ITS1 (5' TCC GTA GGT GAA CCT TGC GG 3') and ITS4 (5' TCC TCC GCT TAT TGA TAT GC 3') were used for PCR study. The master mix 50 µl consists of 50ng DNA, 10x Tag buffer with 2.5mM MgCl₂, 10 pmol of Forward and Reverse primer each, 1.25 mM of each dATP, dGTP, dTTP, dCTP, 3units of *Taq* DNA polymerase and sterile de-ionized water. The DNA amplification was carried out in a thermocycler (BioRad) with the steps of initial denaturation at 95 °C for 5 min and 35 cycles of denaturation at 94 °C for 1 min, annealing at 52 °C for 1 min, extension at 72 °C for 1 min and final extension at 72 °C for 10 min and stored at 4 °C. The amplified PCR product was visualized on 1.42% agarose gel to observe the molecular weight of the fragment. The amplified PCR product was given for sequencing at Eurofins, Bangalore, India. The obtained forward and reverse sequences were checked and aligned in BioEdit^[6] and blasted for identification in NCBI GenBank database. Further the ITS sequence obtained was compared with similar sequences using NCBI BLAST Programme.

Results and Discussion

Mycosed larvae of FAW with abundant external white fungal growth and erect synnematus structures were observed in maize fields in the villages near Devanahalli (Bangalore rural district) and Gowribidanur (Chikkaballapura district) of Karnataka, India (Fig 1). About 20-30% of 4 and 5th instar larvae of FAW were affected by this fungus.

This is the first report of natural infection of *Beauveria felina* on maize Fall armyworm. Earlier, Mallapur *et al.*^[1] reported 1-65% of infection of *Nomuraea rileyi* (= *Metarhizium rileyi*) on *S. frugiperda* in Dharwad District of Karnataka, India. Temperature, humidity and rainfall play a major role in the incidence, distribution, prevalence of entomopathogenic fungi on various crop pests^[7]. In Karnataka, so far only *N. rileyi* (= *M. rileyi*) epizootics were reported on maize fall armyworm and this is the first report of natural infection of *B. felina* on FAW. Further studies on its usage as biocontrol agent against maize fall army worm have to be carried out. In India, *B. felina* MTCC 6294 was isolated from the Beetles by R.D. University, Jabalpur, India and *B. felina* MTCC 2499 was isolated from mouse by CSIR-NCL, Pune, India.

The fungal colonies on SDYA initially appeared as creamish white, round flat colonies and later after 7 days, the aerial hyphae from the colonies aggregated and produced erect synnematus structures with abundant long erect clusters of conidiogenous cells arising from conidiophores. The reverse side of the plate showed yellow to light brown colour after 7 days (Fig 2). Single oval-ellipsoid shaped hyaline conidium appeared on the tip of each conidiogenous cell. The conidia are in the size range of 1.5-2.5 X 3- 3.5 µm (Fig 3).

The purified DNA was amplified by PCR and obtained partial rDNA ITS sequence with molecular weight of 580bp (Fig 4). The nucleotide sequence was deposited in NCBI GenBank

and the accession number, MN833071 was obtained. The rDNA ITS nucleotide sequence was analyzed with the NCBI GenBank database using BLAST program showed 98% homology with different strains of *B. felina* (Table 1).

Beauveria can be identified easily at the genus level using morphological characters. In the cultures, species of *Beauveria* generally grow slowly, rarely forming synnemata. Later, it becomes powdery due to production of large number of conidia on surface^[8, 9]. In present study, *B. felina* found as a slow growing fungus forming synnemata after 7 days and conidia after 10 days of inoculation. Internal transcribed spacer (ITS) region of rDNA consisting of ITS1 and ITS2 regions have been extensively used in fungal identification and in phylogenetic studies because it shows a low level of intraspecific variation and a high level of interspecific variation^[10, 11]. Similarly, in our study, ITS primers were used for identification of the fungus isolated from FAW and identified it as *B. felina* using NCBI GenBank database. The Phylogenetic tree determined the close relationship between our strain of *B. felina* with the strains from other countries of India and also with Asian continental strains like China strains.



Fig 1: Natural infection of *Beauveria felina* on *Spodoptera frugiperda*



Fig 2: Morphological characterisation of *Beauveria felina*, plate morphology on SDYA medium at 26°C after 10 days: A: front; B: reverse

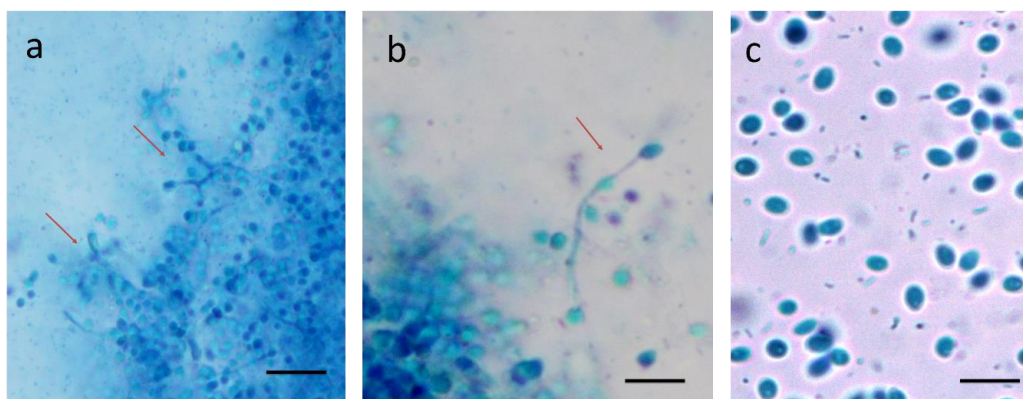


Fig 3: Microscopic structures (Scale bar-10μm) a. Conidiogenous cells with clusters. b. Conidiogenous cell with conidia on the tip c. Conidia

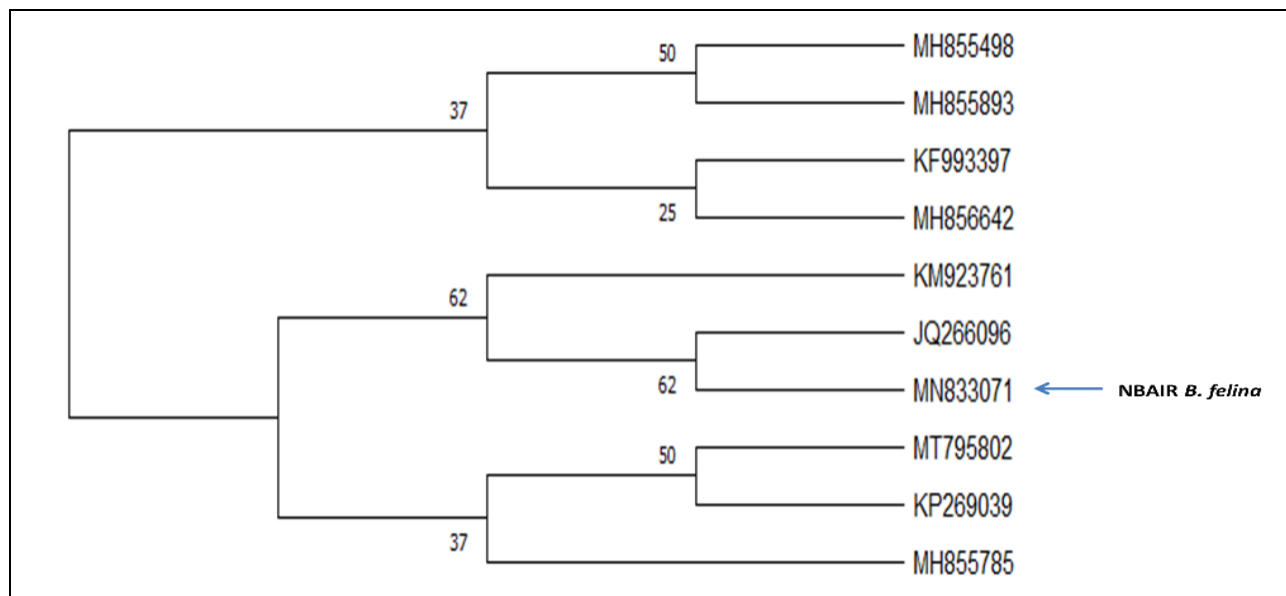


Fig 4: PCR Amplification of ITS region of *Beauveria felina* 1. 100bp ladder; 2. *B. felina*

Table 1: Details of Phylogenetic tree of *Beauveria felina*

| S. No | NCBI Accession number | Location |
|-------|-----------------------|------------------------------------|
| 1 | MN833071 | Bangalore, Karnataka, India |
| 2 | MH856642 | Upsalalaan, Utrecht, Netherlands |
| 3 | MT795802 | Beijing, China |
| 4 | JQ266096 | Chandigarh, Punjab, India |
| 5 | KM923761 | Vallabh Vidyanagar, Gujarat, India |
| 6 | KP269039 | coast of Qingdao, China |
| 7 | MH855893 | Upsalalaan, Utrecht, Netherlands |
| 8 | MH855498 | Upsalalaan, Utrecht, Netherlands |
| 9 | MH855785 | Upsalalaan, Utrecht, Netherlands |
| 10 | KF993397 | Pavia, Italy |

Conclusion

Beauveria felina has been reported to infect fall armyworm in the affected maize fields for the first time in India. This entomopathogenic fungus has to be further studied for its use as a biocontrol agent in the management of maize FAW.

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