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ISOLATION OF NATIVE ENTOMOPATHOGENIC FUNGI FROM NORTH COASTAL DISTRICTS OF ANDHRA PRADESH

K. PRIYANKADEVI*, M. VISALAKSHI , M.S.V.CHALAM , V. CHANDRASEKHAR AND M. RAJASRI

Department of Entomology, S.V. Agricultural College, ANGRAU, Tirupati-517 502.

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ABSTRACT

Entomopathogenic fungi (EPF) are a group of fungi that infect and kill insect pests making them valuable tools for biological pest control. These fungi occur naturally in the environment and can be used as biopesticides to manage insect pests in agriculture. Soil samples were collected from agricultural crops like Maize, Paddy, Sugarcane and wild tree soil samples like Cannon ball (Nagamalli), Peepal, Spanish cherry (*Mimusops elengi*), Anjeer, *Neolamarckia cadamba* from Srikakulam, Vizianagaram, Visakhapatnam and Anakapalle districts of Andhra Pradesh. The fungal isolates were isolated using soil serial dilution and inoculated on SDAY media. Microscopic observation of fungal isolates was done under NIKON Eclipse E200 at 40x magnification and images were captured using V-image 2013 software. Based on morphological features like white colony colour, branched conidiophore, hyaline oval conidia, one of the isolates was identified as Beauveria. Whereas the other isolate with olive green colony colour, whorls of round conidia was identified as *Metarhizium*.

KEYWORDS: Native entomopathogenic fungi, Isolation, Identification, *Beauveria*, *Metarhizium*.

INTRODUCTION

Biological control offers an environmentally sustainable approach for controlling insect pests. Among the key agents used are entomopathogenic fungi, which play a vital role in reducing pest populations and minimizing crop damage. The success of these fungi as biocontrol agents depends largely on the insect's susceptibility and the fungal virulence. Unlike other biological control organisms, entomopathogenic fungi do not require ingestion to infect; they penetrate the host directly through the cuticle. This unique infection pathway allows them to be used for regulating a broad range of insect pests. As a result, these fungi were widely recognized as integral components of integrated pest management strategies (Inglis *et al*., 2000). EPF are employed worldwide to combat several agricultural insect pests and are considered a promising strategy in insect pest management (Kumar *et al*., 2019; Liu *et al*., 2021).

Isolation of native entomopathogenic fungi is essential to identify locally adapted strains that can serve as effective, eco-friendly biocontrol agents against insect pests, particularly under the specific environmental conditions of the region.

Therefore, the present study is focused on the isolation and characterization of native entomopathogenic fungi from soils in the North Coastal districts of Andhra Pradesh, aiming to explore their potential for sustainable pest management.

MATERIAL AND METHODS

Collection of soil samples

A total of thirty-two soil samples were collected from agricultural ecosystems (Paddy, Sugarcane, Maize) and natural ecosystems like wild trees (Peepal, Spanish cherry (*Mimusops elengi*), *Neolamarckia cadamba*, Cannon ball (Nagamalli), Anjeer) from North coastal districts of Andhra Pradesh: Srikakulam, Vizianagaram, Visakhapatnam and Anakapalle. Soil samples were collected at the root zone of 5-10 cm depth with the help of shovel. About 400 g of soil sample was collected, cleaned and mixed homogenously and placed in a sterilized plastic polythene bags and labelled the soil samples with date, place of collection, crop or tree name. The isolation of native EPF was done using soil serial dilution method and from insect cadavers. Among the soil samples collected two entomofungal isolates were identified.

Isolation of soil samples by serial dilution method

Serial dilution is a fundamental microbiological technique employed to progressively reduce the

*Corresponding author, E-mail: priyankadevikonkipudi@gmail.com

concentration of microorganisms in a sample by a specific factor, typically tenfold at each step. This method is particularly useful for estimating the population of viable fungal spores or propagules present in a given sample. Serial dilution process involves a known quantity (0.1 g) of the soil suspension added to 9 ml of sterile diluent in a test tube and mixed thoroughly. Using a micropipette, 1 ml of this mixture is transferred to another tube containing 9 ml of fresh diluent, achieving a 10-fold dilution (10^{-1}). This step is repeated to obtain a series of dilutions (e.g.,10^{-2} to 10^{-8}) for the isolation of individual fungal colonies in subsequent steps. An aliquot of 100 µl from each dilution (10^{-4} to 10^{-8}) was aseptically spread onto petri dishes containing Sabouraud Dextrose Agar supplemented with Yeast Extract (SDAY). The distribution of entomopathogenic fungi in Central Brazilian soils and reported Metarhizium isolates from soil samples that were isolated through serial dilution method (Rocha *et al*., 2012). Each dilution is plated in triplicate to ensure accuracy and reproducibility. The inoculum is uniformly spread across the agar surface using a sterile L-shaped glass spreader.

Isolation of fungi by natural infection of insect cadaver

Insect cadaver collected from the agricultural ecosystem (Maize crop) was surface sterilized with 4% solution of sodium hypochlorite for one minute followed by rinsing in sterile distilled water to remove the external contaminants. Larva was placed on SDAY media and incubated under controlled conditions. Fungal growth was monitored from the larvae and sub culturing was done to obtain pure fungal isolates at regular interval. The fungal spores were serially diluted from 104 to 108 and inoculated on to the SDAY media.

Morphological identification of entomopathogenic fungi

Slide was prepared by placing a drop of sterile distilled water using a sterilized loop and fungal spore was transferred onto the drop of water and coverslip was placed. The slide prepared was examined under the microscope at 10x and 40x magnification using compound microscope (NIKON Eclipse E200) and at 40x magnification the images were captured digitally using V-image 2013 software. The morphological features like shape of the conidia, size, colour of spores, length and width ratio of spores were observed under compound microscope.

RESULTS AND DISCUSSION

The soil samples were collected from agricultural and natural ecosystems from North coastal districts of Andhra Pradesh. The native isolates of Beauveria bassiana and *Metarhizium rileyi* were identified in the present study. The isolate which appeared white in colour was identified as B. bassiana and white cadaver collected from maize crop showed white colony colour initially and upon incubation, turned to olive green colour was identified as M. rileyi (Norjmaa *et al*., 2019).

The fungal isolates were designated with a code AKP Bb-cd for B. *bassiana* and AKP Mr-2m for M. *rileyi*.

The native fungal isolates AKP Bb-cd and AKP Mr-2m showed round shaped colonies at 12 days after incubation on the petri plates that indicates uniform growth on the surface of SDAY media (Table 2). The growth pattern was circular for both the isolates. The colony diameter for AKP Bb-cd isolate was 6 cm and for AKP Mr-2m isolate the diameter was recorded as 4 cm. Fast radial growth was observed for B. *bassiana* AKP Bb-cd isolate and slow radial growth was observed for M. *rileyi* AKP Mr-2m isolate. The B. *bassiana* AKP Bb-cd isolate showed raised elevation initially and gradually become flatten (Figure 1) and M. rileyi AKP Mr-2m showed flat elevation (Figure 2). The shape of the conidia of AKP Bb-cd was globose and AKP Mr-2m showed whorls of round spores when identified under the microscope. The length and width ratio of the isolate AKP Bb-cd strain is 2.0 µm and AKP Mr-2m strain is 2.34 µm (Table 1).

Vimala *et al*. (2003) isolated M. *rileyi* from infected larvae of Helicoverpa armigera and *Spodoptera litura* collected from different geographic locations of South India. During the investigations it was found that a relative humidity of 90.8% and rainfall of 84 mm and a temperature of 23.67°C were found conducive for the natural mycosis of M. *rileyi*. Similar studies were conducted by Manjula and Krishna Murthy (2005) on H. *armigera* and S. *litura*.

Spore shape can vary within the same fungal strain depending on the type of substrate as demonstrated by Townsend *et al*. (1995). In their study, *Beauveria* isolates produced ellipsoidal spores when grown on insect hosts,

Table 1. Morphological characteristics of native fungal isolates

Isolates	Colony Colour	Colony elevation	Shape of the conidia	Spore size (µm) (40X)		L/W ratio
				Length	Width	
B. bassiana AKP Bb-cd	White	Initially raised and then flat	Globose	7.26	3.63	2.0
M. rileyi AKP Mr-2m	Olive green	Flat	Round to oval conidia	6.83	2.91	2.34

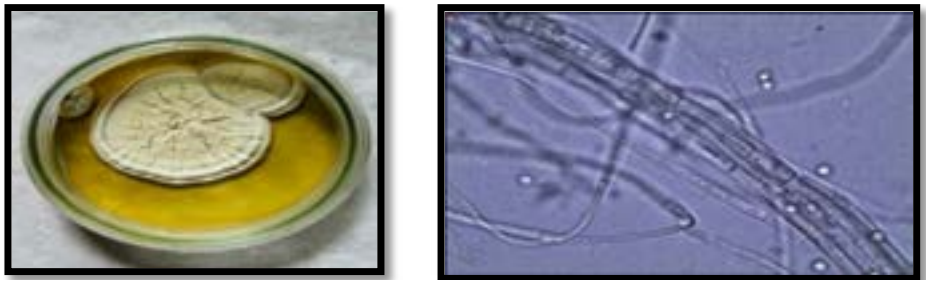

Figure 1. Morphological identification of B. bassiana AKP Bb-cd

Table 2. Growth characters of the isolated native entomopathogenic fungal isolates

Fungal isolate	Colony shape	Radial growth	Growth pattern	Colony diameter (cm)
AKP Bb-cd	Round shaped colony	Fast	Circular in nature	6 cm
AKP Mr-2m	Round shaped colony	slow	Circular	4 cm

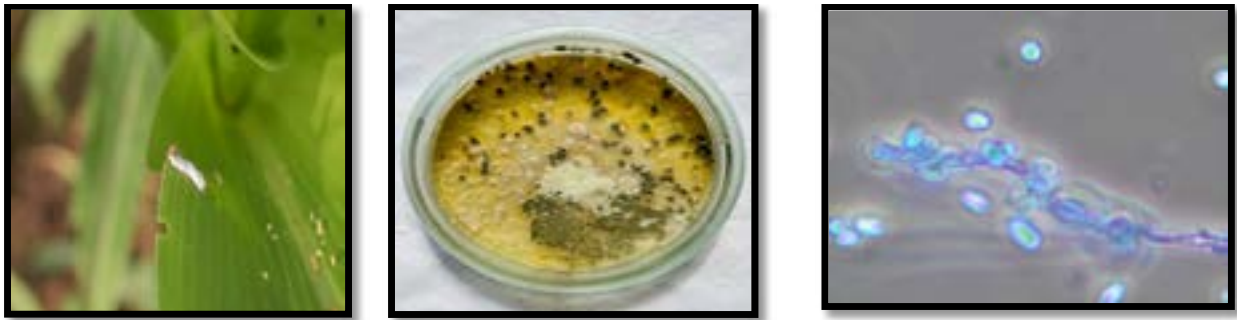

Figure 2. Insect cadaver collected from maize crop and Morphological identification of M. rileyi AKP Mr-2m under compound microscope

whereas spherical spores were observed when cultured on artificial media.

Visalakshi *et al*. (2020) isolated an entomopathogenic fungus *Nomuraea rileyi* from the infected larval instars. The fungus 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/ synnematous conidiophore.

Two entomofungal isolates B. *bassiana* and M. *rileyi* were obtained from the isolation process. Morphological characteristics played a significant role for distinguishing variations for both entomofungal isolates. Differences were noticed in colony morphology, along with distinct variations in conidial size and shape, aiding in the identification and comparison of the entomofungal isolates and furthermore, molecular characterization will help in understanding genetic diversity between the two isolates and assessment of phylogenetic relationships, which is critical for selecting effective biocontrol agents and understanding their ecological adaptability and host specificity (Santos *et al*., 2022). Integrating morphological and molecular data ensures robust identification, enhancing the efficacy and safety of using entomopathogenic fungi in biological control programs.

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