The Pharma Innovation Journal 2023; SP-12(11): 163-171

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; SP-12(11): 163-171
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www.thepharmajournal.com
Received: 20-08-2023
Accepted: 24-09-2023

N Harika
MSc scholar,
Department of Entomology,
Agricultural College, Naira,
Andhra Pradesh, India

M Visalakshi
Principal Scientist,
Regional Agricultural Research
Station, Anakapalli,
Andhra Pradesh, India

D Anil Kumar
Assistant Professor,
Department of Entomology,
Agricultural College, Naira,
Srikakulam, Andhra Pradesh,
India

V Chandra Sekhar
Senior Scientist,
Department of Plant Pathology,
Regional Agricultural Research
Station, Anakapalli,
Andhra Pradesh, India

Corresponding Author:
N Harika
MSc scholar,
Department of Entomology,
Agricultural College, Naira,
Andhra Pradesh, India

Morphological and molecular characterization of entomopathogenic fungi isolated from soils of Eastern Ghats in Alluri Sitharama Raju district, Andhra Pradesh

N Harika, M Visalakshi, D Anil Kumar and V Chandra Sekhar

Abstract
Indigenous entomopathogenic fungi isolated from soils of Eastern Ghats in Alluri Sitharama Raju district, Andhra Pradesh during 2022-23 and characterized based on morphological and mplecular techniques. About 50 soil samples were collected from different habitats in (rice fields, plantations, orchards and vegetable fields) in five mandals Chinthapalli, Araku valley Vantlamamidi, Lambasingi and Paderu Isolated the entomopathogenic fungi from soil samples using serial dilution method and inoculated on Sabouraud dextrose agar yeast (SDAY) media. Morphological identification of grown fungal isolates were identified as *Beauveria bassiana* under microscope based on the hyphae branching, conidiophores, and colour of the sporulation. Molecular characterization was done through PCR specific ITS regions of the entomopathogenic fungi which confirmed the isolates as *Beauveria bassiana* by the NCBI-BLAST analysis of partial genome sequence amplified by ITS1 and ITS4. The ITS generated sequences were deposited in the NCBI Gene Bank database and accession numbers were obtained for five isolates of indigenous entomopathogenic fungi *Beauveria bassiana* as (Chinthapalli isolate-OR342367-Bb AKP-C Ia; Vantlamamidi isolate -OR35216- Bb AKP-V IIIa; Araku valley isolate-OR345217- Bb AKP-A IVb; Lambasingi isolate- OR345218- Bb AKP-L IIa and Paderu isolate-OR345219 -Bb AKP-P IIb). Using MEGA software (Version.10.0), a phylogenetic tree was constructed by considering most reference sequences from the NCBI with sequences of *Beauveria bassiana* isolates to study the evolutionary relationship between different isolates.

Keywords: Entomopathogenic fungi, *Beauveria bassiana*, identification, molecular characterization, phylogeny

1. Introduction
Entomopathogenic fungi are organisms that evolved to exploit insects. They comprise a wide range of morphologically, phylogenetically and ecologically diverse fungal species. Entomopathogenic fungi infect a wide range of insect hosts in all developmental stages: eggs, larvae, pupae, nymphs and adults. Such assortment of niches has resulted in these entomopathogenic fungi evolving a considerable morphological diversity, resulting in enormous diversity, the majority of which remains unknown.
Entomopathogenic fungi (EPF) belonging to the genera *Beauveria*, *Metarhizium* and *Paecilomyces* are widely used as biocontrol agents throughout the world (Khan *et al.*, 2016; Castro *et al.*, 2016; Rios Moreno *et al.*, 2016) [15, 3, 10]. Among these, the white muscardine fungus, *Beauveria bassiana* and green muscardine fungus, *Metarhizium anisopliae* are the most pronounced fungal entomopathogens for the control of sucking and chewing agricultural insect pests and play a vital role in the integrated pest management strategies (Malekan *et al.*, 2015) [8]. The *B. bassiana* was reported to infect 707 species of insect hosts (Imoulan *et al.*, 2016), whereas *Metarhizium anisopliae* infecting over two hundred species of insect pests (Jitendra *et al.*, 2012) [5].
The isolation and characterisation of native entomopathogenic fungi are essential for gaining insight into the naturally occurring fungal biodiversity of a specific region and to provide a pool of potential biological control agents for pest control purposes (Quesada *et al.*, 2007) [9]. Moreover, exotic strains of entomopathogenic fungi that have been developed for use as pest control agents in a different country could be ineffective due to strain and environmental differences (Lockwood, 1993) [7]. Therefore, investigating the occurrence and distribution of native EPF is critical for their use as pest control agents in a given location.

Previous studies revealed a marked variability among the isolates of various EPF collected from different geographical locations and their efficacy against insect pests (Vimaladevi *et al*. 2003) [20]. Therefore, it is important to exploit the native EPF and to evaluate their potential against target pests for developing a mycoinsecticide for specific geographic locations.

Molecular techniques have been used to identify genetic variation of entomopathogenic fungi. Determination of species could be done by analysis of electrophoretically separated restriction enzyme digested total genomic DNA with restriction fragment-length polymorphisms (RFLPs) for strain determination and with RAPD analysis for fungal isolates. (Kosir *et al.*, 1991 and Visalakshi *et al.*, 2020) [17, 21].

Thus, entomopathogenic fungi are a major component of integrated pest management techniques as biological control agent against insect pests and other arthropods in horticulture, forestry, and agriculture (Inglis *et al.*, 2000) [4].

2. Materials and Methods

2.1 Collection of soil samples

A total of 50 soil samples were collected from different habitats in Alluri Sitharama Raju district from five mandals: Chinthapalli, Vantlamamidi, Araku, Lambasingi and Paderu. From each habitat 3 soil samples were collected (vegetable fields, rice fields, ochards, planatations). Soil samples were collected at the root zone of about 5–10 cm with the help of shovel or auger. About 50 g of soil sample was collected and placed in plastic covers, labelled properly with date, place of collection and crop name.

The isolation of entomopathogenic fungi from soil samples was done using serial dilution method. Among 50 soil samples collected, 5 entomopathogenic fungi (EPF) were isolated. For each entomopathogenic fungi code was given for discriminating the fungal isolates obtained from soils of 5 mandals. Chinthapalli as C; Vantlamamidi as V; Araku valley as A; Lambasingi as L and Paderu as P. Habitats were represented as, I, II, III and IV for rice fields, plantations, orchards and vegetable fields respectively. Isolated entomopathogenic fungi (EPF) were represented as Bb (*Beauveria bassiana*) and F (*Fusarium sps*) which are the plant pathogenic fungi. The alphabets a, b and c represent the crops from different habitats (Table 1).

Example

Bb-I-AKP-C-a

Bb–*Beauveria bassiana*; I–Habitat (Rice fields); AKP–Anakapalle centre; C-Chinthapalli mandal; and a–crops.

Table 1: Soil sample codes and designation of fungal isolates from different locations of Alluri Sitharama Raju district, Andhra Pradesh

Location	Habitat (Crops)	Fungal isolate	Code
Chinthapalli (C)	I. Rice fields	*Beauveria bassiana*	Bb-1-AKP-C-a
	a) Rice		
	II. Plantations	-	-
	Coconut		
	Bamboo		
	Banana		
	III. Orchards	*Fusarium oxysporum*	F-III-AKP-C-c
	Sapota		
	Papaya		
	Guava		
	IV. Vegetable fields	*Fusarium solani*	F-IV-AKP-C-c
	Tomato		
	Brinjal		
	Ginger		
Vantlamamidi (V)	I. Rice fields	-	-
	Rice		
	II. Plantations	-	-
	Bamboo		
	Coconut		
	Tamarind		
	III. Orchards	*Beauveria bassiana*	Bb-III-AKP-V-a
	Custard apple		
	Papaya	*Fusarium solani*	F-III-AKP-V-c
	Ramphal		
	IV. Vegetable fields	*Fusarium oxysporum*	F-IV-AKP-V-a
	Brinjal		
	Green beans		
	Okra		
Araku valley (A)	I. Rice fields	-	-
	Rice		
	II. Plantations	*Fusarium solani*	F-II-AKP-A-a
	Bamboo		
	Coffee		
	Pepper		
	III. Orchards	-	-
	Mango		
	Guava		
	Sapota		
	IV. Vegetable fields	*Beauveria bassiana*	Bb-IV-AKP-A-b

	Cabbage		
	Tomato		
	Cauliflower		
Lambasingi (L)	I. Rice fields	-	-
	Rice		
	II. Plantations	*Beauveria bassiana*	Bb-II-AKP-L-a
	Pepper		
	Silver Oak		
	Bamboo		
	III. Orchards	-	-
	Jackfruit		
	Mango		
	Guava		
	IV. Vegetable fields	*Fusarium oxysporum*	F-IV-AKP-L-b
	Okra		
	Carrot		
	Bottle gourd		
Paderu (P)	I. Rice fields	-	-
	Rice		
	II. Plantations	*Beauveria bassiana*	Bb-II-AKP-P-b
	Bamboo		
	Cashew		
	Pepper		
	III. Orchards	-	-
	Papaya		
	Guava		
	Mango		
	IV. Vegetable fields	*Fusarium solani*	F-IV-AKP-P-c
	Tomato		
	Brinjal		
	Bottle gourd		

2.2 Isolation of soil samples by serial dilution method

Serial dilution is a laboratory technique used to reduce the concentration of a sample such as the culture of entomopathogenic fungi by a specific factor at each step. This technique is often used to determine the concentration of viable fungal spores or propagules in the sample.

Isolation of entomopathogenic fungi from soil using the serial dilution method involves diluting a soil sample in a series of tubes with sterile distilled water. About 0.2 g of soil sample was taken in a 1.5ml microcentrifuge tube containing 0.02% Tween-80 solution and vortexed for 15 min. The diluent present in the tubes were sterilized by autoclaving at 121 ºC for 15 min at 15 Pa. In a sterile test tube, a known volume of the soil sample (0.1 g) was added to a known volume of the diluent (in 9 ml). The solution was thoroughly mixed and then, using a micropipette, 1 ml of the diluted sample was carefully transferred into a new tube already containing 9 ml of diluent, resulting in the creation of a 10-fold dilution.

After preparing the serial dilution series with the soil sample, a small volume (e.g., 100 µl) of each dilution was aliquoted on to a petri dish containing the Sabouraud dextrose agar yeast (SDAY) medium. Each diluent concentration (10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8}) was replicated three times in the petri plates containing the SDAY media. The sample was spread evenly over the media using sterile L-spreader. The petri plates were incubated at an appropriate temperature of 25 °C and humidity of 45% for the growth of entomopathogenic fungi.

2.3 Morphological identification of entomopathogenic fungal cultures

Slide preparation was done for observing the entomopathogenic fungal spores under scanning electron microscope during which a drop of saline solution or water was added onto a glass slide and the fungal sample was transferred onto the drop of saline solution or water using a sterilized loop or needle. Coverslip was placed over the fungal sample duely taking care to avoid water bubbles. The slide was examined in the microscope under 10x power and then under 40x power using suitable magnification and focus settings.

The morphological features of the isolated fungi were studied using compound microscope (NIKON Eclipse E200) at 40x maginification and the images were captured digitally using V-image 2013 software. The macroscopic characters of the colonies observed were the growth pattern, colour, shape, surface texture, colony elevation. The microscopic characters of fungal culture observed under compound microscope were shape, size, colour of spores, length and width ratio of spores.

2.4 Molecular identification of entomopathogenic fungi

2.4.1 Preparation of SDB (Sabouraud's Dextrose Broth)

The SDB (Sabouraud's Dextrose Broth) medium was prepared using the following composition consisting of glucose, peptone and yeast extract, except agar. Then the medium was transferred to 500 ml conical flasks at 100 ml/flask and plugged with non- absorbent cotton. Finally medium was sterilized in an autoclave at 121 °C for 15 min at 15 Pa.

The mycelial mat of 5 mm disc of the isolated fungal cultures grown on SDAY media were inoculated aseptically into conical flasks containing the Sabouraud Dextrose Broth and incubated at 25 °C for 2weeks. Within two weeks the mycelia mat was developed in the conical flasks. The mycelia mat was separated by filtering the broth through filter paper. The separated mycelia mat was cleaned with distilled water and dried on tissue paper. The dried mat was wrapped in aluminium foil and stored at -20 °C. After one day, the fungal mat of the isolates was used for DNA extraction

2.4.2 DNA extraction
Fungal DNA was isolated by using CTAB method. The mycelial mat of 100 g fungal isolates was taken in a sterilized pre chilled mortar and pestle and ground to a fine powder using liquid nitrogen. Isolation buffer of 1 ml was mixed with powder and dispensed into a centrifuge tube. The samples were incubated in water bath at 65 °C for 1 hr. The samples were cooled to room temperature, added with equal volume of chloroform: isoamyl alcohol (24:1) mixture and centrifuged (Eppendorf refrigerated centrifuge, India) at 10,000 rpm for 10 min at 24 °C.
Aqueous phase was carefully collected and the samples were centrifuged again at 10,000 rpm for 10 min after adding equal volume of chloroform: isoamyl alcohol (24:1) mixture. Finally, the aqueous layer was collected into another centrifuge tube, added with 0.6 volumes of isopropanol and mixed gently by tilting the tube. DNA in the samples was allowed to precipitate by incubating at -20 °C. An hour after incubation, the samples were spun in refrigerated centrifuge at 10,000 rpm for 10 min. The supernatant was discarded and the pellet left in the centrifuge tube was washed with 70% ethanol thrice to purify the DNA from other metabolites and was air dried. Ethanol free pellets were dissolved in 20 µl of molecular grade water and stored at -20 °C for further use.

2.4.3 PCR amplification
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) [24]. The ITS region of the isolated fungus was amplified in a PCR, using ITS primers. Thermocycling conditions included initial denaturation at 94 °C for 5 min, followed by 38 amplification cycles of 94 °C for 1 min, 55 °C for 1 min, 72 °C for 1 min followed by final extension at 72 °C for 10 min.

2.4.4 Agarose gel electrophoresis
Amplified products were separated on 1 per cent agarose gel and stained with Ethidium bromide (10 ng/µl). TAE buffer at 1x was used as tank buffer for electrophoresis. After adding 2 µl of loading dye to 5 µl of PCR products, electrophoresis was conducted at 100 V for 90 min, and the gel was photographed under UV light using a gel doc system. The ladder used for running and separation of products was a 100 bp molecular marker (White *et al.*, 1990) [22]. The amplified products will be excised and outsourced (sequencing service company) for partial sequencing. Similarity of ITS region will be aligned using BLAST program of Gene Bank database (NCBI) and the sequences obtained from the NCBI database will be mentioned.
Sequences obtained from the service company will be compared with other fungi using the BLAST search tool from the NCBI database along with sequence data of entomopathogenic fungi obtained in the investigation. Phylogenetic tree will be prepared with respect to each fungal group i.e., *Beauveria*, *Metarhizium*, *Isaria* and *Verticillium* using MEGA software.

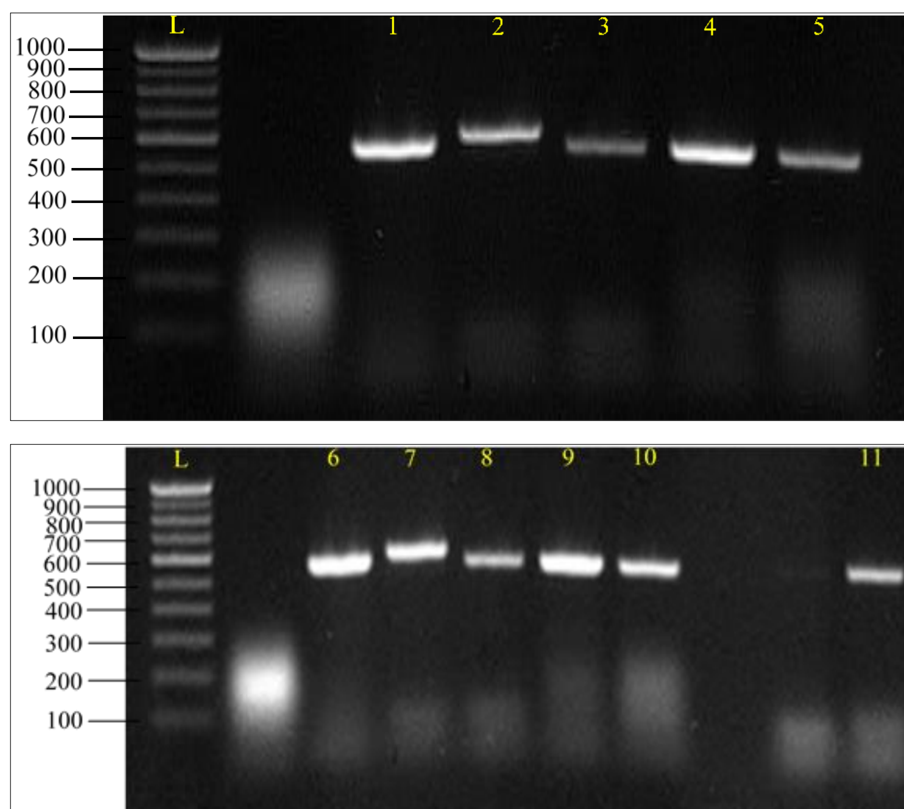

Fig 1: Gel image for identification of base pairs with genomic DNA of fungal isolates (1-11 numbers represent the band formation for the different fungal isolates, L- ladder DNA)

3. Results and Discussion
3.1. Morphological identification of entomopathogenic fungi
The soil samples collected from different crop habitats rice fields, plantations, vegetable fields, orchards located in Eastern ghats of Alluri Sitharama Raju district. Five fungal isolates showed typical characteristics of entomopathogenic fungi. All the five isolates corresponded to the

entomopathogenic fungi *Beauveria bassiana*. When observed under the compound microscope at 40x maginification.

The morphological and growth characteristics provide basis for comparing and differentiating between different entomopathogenic fungi and their potential as a biocontrol agent through laboratory bioassay studies. The five fungal isolates showed variations in morphological characteristics. The macroscopic characters of the colonies observed were the growth pattern, colour, shape, surface texture, colony elevation. The microscopic characters of fungal culture observed under compound microscope were shape, size, colour of spores, length and width ratio of spores (Table 2, 3 and Plate 1, 2).

The entomopathogenic fungi isolated by serial dilution method was identified as *Beauveria bassiana* when observed under compound microscope. Gebremariam *et al.* (2021) [23], studied the characters of *Beauveria bassiana* showed dispersed growth patterns, white to yellowish colony colour, smooth powdery to cotton texture, raise to flat elevation and round shape and globose to sub globose conidia with hyaline hyphae.

Table 2: Morphological characteristics of *Beauveria bassiana* fungal isolates collected from Eastern ghats of Alluri Sitharama Raju district

Isolates	Colony colour	Colony shape	Elevation	Colour of the spores	Shape of spores	Spore size (µm) (40X)		L/W ratio
						Length	Width	
Beauveria bassiana Bb AKP- C Ia	White	Round	Flat	White	Globose	12	8	1.5
Beauveria bassiana Bb AKP -A IVb	White	Round	Flat	White	Globose	15	5	3
Beauveria bassiana Bb AKP -L IIa	White	Round	Flat	White	Sub-globose	12	7	1.71
Beauveria bassiana Bb AKP- P IIb	White	Round	Flat	White	Globose	14	8	1.75
Beauveria bassiana Bb AKP -V IIIa	Yellow	Round	Flat	White	Globose	8	10	0.8

L/W – Length to width ratio

Table 3: Growth parameters of different *Beauveria bassiana isolates* collected from Eastern ghats of Alluri Sitharama Raju district

Fungal isolate	Radial growth	Concentric rings	Colony surface layer	Growth pattern	Colony diameter (cm)
Beauveria bassiana Bb- I-AKP C-a	Moderate	Circular rings	Smooth	Circular colonies	7.0
Beauveria bassiana Bb -IV-AKP A-b	Fast	Circular rings	Fluffy	Circular colonies	5.0
Beauveria bassiana Bb-II-AKP L-a	Slow	Circular rings	Smooth	Circular colonies	8.0
Beauveria bassiana Bb-II-AKP P-b	Fast	Circular rings	Powdery	Circular colonies	6.0
Beauveria bassiana Bb-III-AKP V-a	Moderate	Circular rings	Smooth	Circular colonies	4.0

a b c

d e

Plate 1: Radial growth of *Beauveria bassiana* native isolates. (a) Bb AKP-C Ia (Chinthapalli); (b) Bb AKP- A IVb (Araku valley); (c) Bb AKP-L IIa (Lambasingi); (d) Paderu Bb AKP-P IIb; (e) Bb AKP-V IIIa (Vantlamamidi)

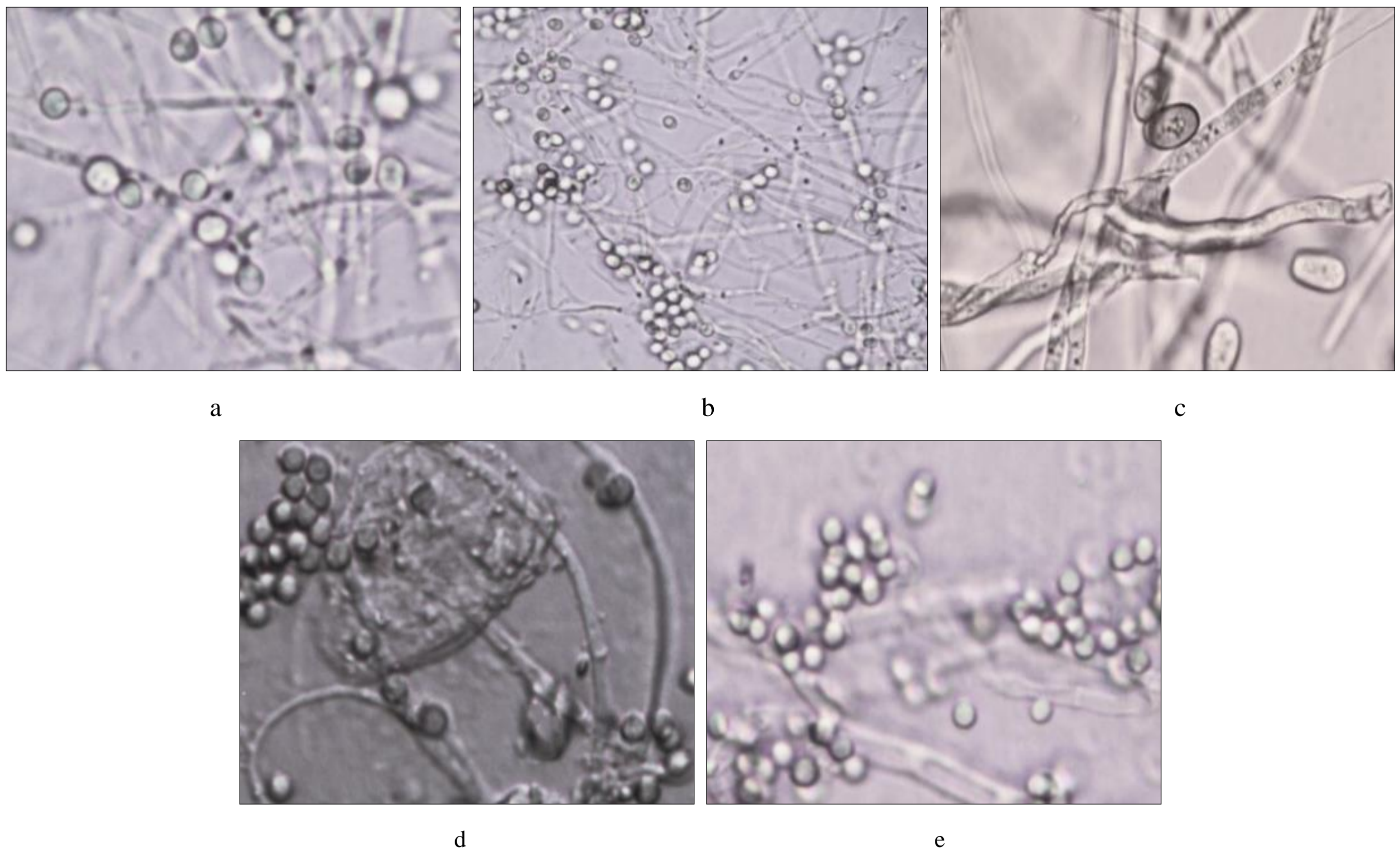

Plate 2: Morphological characteristics of *Beauveria bassiana* isolates observed under compound microscope at 40x magnification. (a) Bb AKP-C Ia (Chinthapalli); (b) Bb AKP- A IVb (Araku valley); (c) Bb AKP-L IIa (Lambasingi); (d) Paderu Bb AKP-P IIb; (e) Bb AKP-V IIIa (Vantlamamidi)

Beauveria spp. is characterized with dense clusters of conidiophores, flask shaped conidiogenous cells with apical denticulate rachis giving a distinct zig-zag appearance (Samson *et al*., 1988) [13].

Similar morphological identification studies were done by Varela and Morales (1997) [19]. They characterized some *B. bassiana* isolates and reported that the colony colour of different isolates showed wide variations between white and yellow. In conidial morphology, they observed three sizes and two types of conidial shapes: ≤ 2.5 µm (globose), 2.6–3.75 µm (globose) and 4.0-5.0×2.5-3.0 µm (ellipsoidal).

Ramarethinam *et al*. (2001) [1], reported that *B. bassiana* colonies are moderately rapid growing, spreading woolly, powdery or mealy in texture, white to yellowish white or occasionally pinkish. Conidiogenous cells are hyaline, flask shaped with a long zigzag appearing rachis bearing lateral conidia. Conidia are hyaline, single-celled and globose to ovoid shape.

The morphological studies of the present study were consistent with the observations made by Kirubhadharsini *et al.* (2017) [16], regarding the macroscopic characters of *Beauveria* isolates, which exhibited a white colony colour, powdery to cottony texture and a round shape. Microscopic examination of *Beauveria* isolates revealed globose to sub-globose conidia with hyaline hyphae.

Ramanujam *et al.* (2020) [12] identified the entomopathogenic fungi *B. bassiana* based on colony morphology, structure of conidia and conidiophores.

3.2 Molecular characterization of native strains of entomopathogenic fungi isolated from Eastern Ghats of Alluri Sitharama Raju, Andhra Pradesh

The molecular characterization was done for the 11 fungal isolates using the universal ITS primers. It was done by PCR based DNA sequencing analysis using ITS1 and ITS4 primers. The amplified DNA sequences of the 11 isolates were made to run in the gel electrophoresis equipment. The ladder (L) DNA containing 100 base pairs was taken as the reference for the gel run. These fungal isolates formed the bands at above 500 base pairs size after observing them under gel documentation kit using UV light.

After the band formation, five isolates among the eleven were confirmed as entomopathogenic fungi *Beauveria bassiana* based on nucleotide sequencing analysis results. The remaining isolates were identified as *Fusarium* spp. which are the plant pathogenic fungi (Fig.1).

The ITS generated sequences were deposited in the NCBI Gen Bank database and an accession number was obtained for the five *Beauveria bassiana* isolates: (Chinthapalli isolate-OR342367-Bb AKP-C Ia; Vantlamamidi isolate -OR35216-Bb AKP-V IIIa; Araku valley isolate-OR345217- Bb AKP-A IVb; Lambasingi isolate-OR345218- Bb AKP-L IIa and Paderu isolate-OR345219-Bb AKP-P IIb). Using MEGA software (Version 10.0), a phylogenetic tree was constructed by considering most reference sequences from the NCBI to study the evolutionary relationship between different isolates of entomopathogenic fungi B. *bassiana*.

Similar molecular identification using the primers ITS1 and ITS4 was done by Alvarez *et al.* (2018) [1] who isolated N. *Rileyi* DNA by using the CTAB method and internal transcribed spacer (ITS1, ITS4). The amplified products of 1335 bp were purified and sequenced at CINVESTAV-IPN in both directions using the above primers. A consensus sequence was obtained by alignment of the forward and reverse sequences for this region and deposited in GenBank

(MG637450). Blast analysis revealed that the sequence identity values were 99 percent identical to sequences of N. *rileyi*.
Brancini *et al.* (2018) [2] conducted molecular identification by amplifying the internal transcribed spacer (ITS1-5.8S-ITS4) rDNA regions of the 12 selected isolates showed DNA with an approximate size of 545 bp. DNA sequencing and phylogenic analysis tentatively positioned these isolates into *M. anisopliae* species, in which the topology was similar to that described by other authors.
Sayed *et al.* (2018) [14] constructed the molecular phylogenetic tree from ITS-rDNA sequence of isolates with the neighbour-joining method by using MEGA-X-10.1.8 software at 1000 bootstraps replication. The phylogenetic tree verified that isolates of *B. bassiana* were strongly supported clade with a bootstrap value of 90%.
Visalakshi *et al.* (2020) [21] characterized the *M. rileyi* isolated from *S. frugiperda* based on morphology and PCR. The NCBI-BLAST analysis of partial genome sequence amplified by ITS1 and ITS2 has confirmed the EPF as *M. rileyi*. The ITS generated sequence was deposited in the NCBI Gene Bank database and obtained with accession number (MN960559). Using the maximum parsimony method in MEGA software (ver. 10.0.5), a phylogenetic tree was constructed by considering the most relevant sequences from the NCBI database.
The phylogenetic tree was constructed by considering most relevant sequences from the NCBI database along with sequence data of *Beauveria bassiana* obtained in the investigation.

3.3 Phylogenetic tree analysis of entomopathogenic fungi using MEGA Software

The phylogenetic tree constructed by using Neighbor Joining (NJ) method, Gebremariam *et al.* (2021) [23] with the close reference of *Beauveria bassiana* isolates from different places. The phylogenetic tree analysis grouped into five major clusters. The isolates belonging to Mysore, USA and Vantlamamidi formed into separate clusters. The rest of the five formed clusters which included the native *Beauveria bassiana* isolates: Bb AKP-C Ia (Chinthapalli), Bb AKP-V IIIa (Vantlamamidi), Bb AKP-A IVb (Araku valley), Bb AKP-L IIa (Lambasingi) and Bb AKP-P IIb (Paderu). The isolates of Paderu, Chinthapalli, RARS, Tirupati formed into one sub-group with a boot strap value 96% indicates that they are strongly supported. The isolates of Tamil Nadu; Bagalkote, Karnataka; CICR, Coimbatore; PAU, Ludhiana; GKVK, Bangalore; Araku valley formed one sub-group with boot strap value 60–85% indicates that they are well supported. The isolates of Lambasingi; Pusa, New Delhi formed into one sub-group with boot strap value of 87% that they are strongly supported, the other isolates UAS, Raichur, Karnataka; Chandigarh; Chikkaballapur, Karnataka formed into separate sub-group with a boot strap value of 75% indicates that they are well supported (Fig.2, Table 4 and Table 5).

Table 4: Details of accession number for *Beauveria bassiana* isolates collected from soil samples of Eastern Ghats of Alluri Sitharama Raju district

Date of soil sample collection	Location	Latitude and longitude	Crop	Entomopathogenic fungi	Accession number
17-11-2022	Chinthapalli	17.72° N, 82.68° E	Paddy	*Beauveria bassiana* Bb AKP-C Ia	OR342367
07-12-2022	Vantlamamidi	18.22° N, 82.70° E	Custard apple	*Beauveria bassiana* Bb AKP-V IIIa	OR345216
12-12-2022	Araku valley	18.32° N, 82.85° E	Tomato	*Beauveria bassiana* Bb AKP -A IVb	OR345217
21-02-2023	Lambasingi	18.30° N, 82.62° E	Pepper	*Beauveria bassiana* Bb AKP-L IIa	OR345218
23-02-2023	Paderu	18.01° N, 82.66° E	Cashew	*Beauveria bassiana* Bb AKP- P IIb	OR345219

Table 5: NCBI accession numbers of fungal isolates in phylogenetic tree construction

NCBI accession number	Location of the fungal isolate	Entomopathogenic Fungal isolate
MT586632.1	Coimbatore, Tamil Nadu	*Beauveria bassiana*
OP103739.1	Mysore, Karnataka	*Beauveria bassiana*
JX313064.1	RARS, Tirupati	*Beauveria bassiana*
MKO49987.1	Coimbatore, Tamil Nadu	*Beauveria bassiana*
OM131742.1	Bagalkote, Karnataka	*Beauveria bassiana*
MK346250.1	Chikkaballapur, Karnataka	*Beauveria bassiana*
MK049987.1	CICR, Coimbatore, Tamil Nadu	*Beauveria bassiana*
MG670098.1	PAU, Punjab	*Beauveria bassiana*
MW425870.1	GKVK, Bangalore	*Beauveria bassiana*
ON259754.1	Pusa, New Delhi	*Beauveria bassiana*
JQ266099.1	Chandigarh	*Beauveria bassiana*
MT635020.1	UAS, Raichur, Karnataka	*Beauveria bassiana*
KU729042.1	USA	*Beauveria bassiana*
OR342367	Chinthapalli	*Beauveria bassiana* Bb AKP-C IIa
OR345216	Vantlamamidi	*Beauveria bassiana* Bb AKP-V IIIa
OR345217	Araku valley	*Beauveria bassiana* Bb AKP- A IVb
OR345218	Lambasingi	*Beauveria bassiana* Bb AKP-L IIa
OR345219	Paderu	*Beauveria bassiana* Bb AKP-P IIb

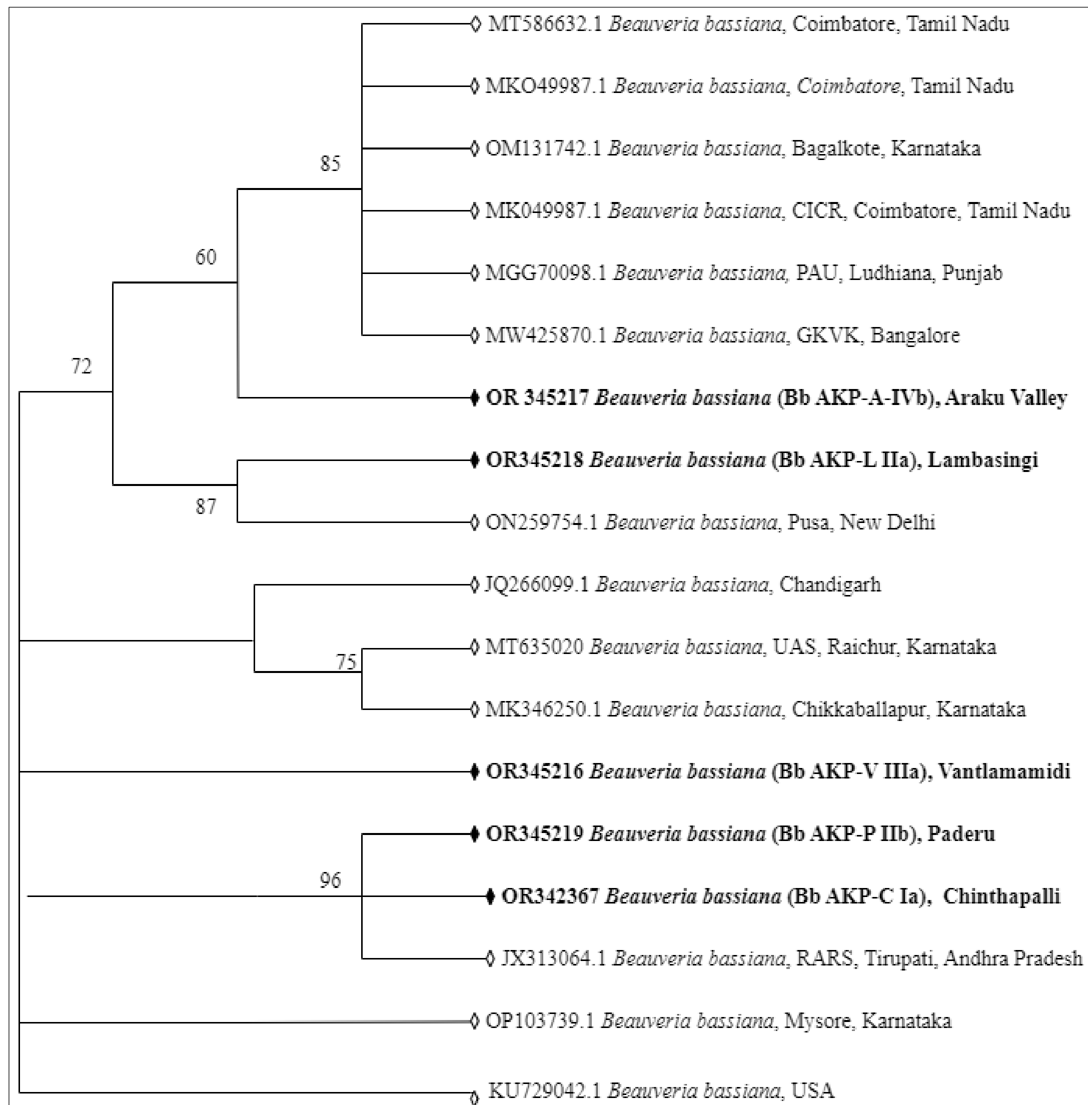

Fig 2: Phylogenetic tree based on the nucleotide sequences of ITS1-5.8S-ITS-4 r-DNA region of isolated *Beauveria bassiana* EPF and other relevant sequences from NCBI database

4. Conclusion

The present work provides information of indigenous isolates of entomopathogenic fungi associated with soils from different habitats: rice fields, plantations, vegetable fields, orchards located in Eastern ghats of Alluri Sitharama Raju district. The isolation process revealed a wide distribution of entomopathogenic fungi *Beauveria bassiana* isolates in different soil samples, suggesting their natural abundance and adaptability. Moreover, the morphological examination allowed for the characterization of distinct features and structures of the isolated *Beauveria bassiana* fungal strains, aiding in their classification and identification. Molecular techniques such as DNA sequencing and phylogenetic analysis, offered a more accurate and reliable means of species identification.

The phylogenetic analysis conducted in this study shed light on the evolutionary relationships among the entomopathogenic fungi and their classification within the broader fungal kingdom. Such insights contribute to a better understanding of their ecological roles and potential applications in integrated pest management strategies. In future, the *Beauveria bassiana* isolates can be further used for the lab and field studies based on their efficacy in order to promote the sustainable integrated pest management practices.

5. References

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