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Exploring Indigenous Entomopathogenic Fungi: Ecological Significance and Potential Applications in Rangareddy District, Telangana, India

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Authors' contributions

This work was carried out in collaboration among all authors. Author SSM conceptualized the study, designed the methodology, fieldwork, did data collection, analysis and drafted the manuscript. Author PR conceptualized the study, entomopathogenic fungi expertise, fieldwork assistance, data analysis and manuscript preparation. Author PD expertise in fungal ecology, designed the methodology, did data interpretation and wrote the manuscript. Author SAB participated in fieldwork, did data collection and analysis and critical manuscript input. Author DSC supervised the project, guided the work, reviewed and edited the manuscript and did funding acquisition. All authors read and approved the final manuscript.

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ABSTRACT

Aims: This study aims to investigate the ecological significance and potential applications of indigenous entomopathogenic fungi in pest management, focusing on the Rangareddy district of Telangana, India.

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Study Design: The study utilized a combination of the Galleria bait method and direct isolation techniques to collect and isolate entomopathogenic fungal species from insect cadavers and soil samples in various locations within the district.
Place and Duration of Study: The research was conducted in the Rangareddy district of Telangana, India, over a specified period.
Methodology: Six indigenous fungal isolates, including *Metarhizium rileyi, Beauveria bassiana*, and *Lecanicillium lecanii*, were obtained through the aforementioned techniques. The relationship between fungal species, soil pH, and host plants was explored.
Results: The study identified the presence of M. rileyi isolates in Mansapally and Aziznagar, *B. bassiana* isolates in Nagaram and Mansapally, and L. lecanii isolates in Kesaram. Notably, three isolates were obtained through direct isolation from insect cadavers, while three were obtained through the Galleria bait method, indicating the effectiveness of both approaches. Furthermore, the research revealed significant associations between fungal species, soil pH levels, and host plants, highlighting the complex interactions within the ecosystem.
Conclusion: This study provides valuable insights into the distribution and ecological relevance of indigenous entomopathogenic fungi in the Rangareddy district of Telangana, India. The findings offer potential implications for sustainable pest management practices, emphasizing the need for further research to validate and expand upon these discoveries.

Keywords: Entomopathogenic fungi, isolation, soil sampling, galleria bait; cadaver.

1. INTRODUCTION

Entomopathogenic fungi represent a rich and diverse reservoir of biocontrol agents with immense potential for ecological insights and pest management strategies. Their collection from soil samples is crucial for understanding their distribution, diversity, and ecological roles. Various methods have been employed for this purpose, each offering unique advantages and challenges. Among these methods, the utilization of selective media such as potato dextrose agar enriched with yeast extract supplemented with chloramphenicol, thiabendazole, and cycloheximide (CTC medium) has been established [1]. Additionally, the "Galleria bait method," originally devised for trapping entomoparasitic nematodes, has emerged as a robust approach for isolating entomopathogenic fungi (EPF) from soil environments [2]. This laborious yet effective method relies on attracting and isolating EPF through the use of insect baits such as Galleria mellonella and Tenebrio molitor [3]. Despite its origins, the Galleria bait method has been successfully adapted for detecting naturally occurring entomopathogenic fungi, demonstrating its versatility and applicability [4]. By primarily targeting EPF due to their specific ability to infect insects, this method minimizes contamination from non-pathogenic fungi, thus enhancing isolation efficiency [5]. Moreover, the high susceptibility of Galleria larvae to various EPF species enables the detection of even low fungal densities in soil samples [6]. Although relatively simple and standardized, the Galleria bait method may underestimate fungal diversity due to variations in larval susceptibility and competition among fungal strains within the bait [7,8]. Nevertheless, the inclusion of other susceptible insects like mealworms (*T. molitor*) and the integration of molecular techniques for strain identification offer promising avenues for enhancing the method's efficacy and accuracy. In light of these considerations, this study embarks on an exploration of the indigenous frontier, aiming to collect entomopathogenic fungi for ecological insights and furthering our understanding of their ecological roles and potential applications.

2. MATERIALS AND METHODS

2.1 Survey for Entomopathogenic Fungi Present in Soil/ Insect Cadaver Samples

An extensive survey was undertaken to collect indigenous strains of entomopathogenic fungi in Rangareddy district of Telangana from 2021 to 2022. Throughout this survey, samples of both soil and insects were methodically gathered and subsequently brought to the laboratory to isolate entomopathogenic fungi. The process involved the retrieval of pathogens from insect cadavers, both sporulated and non-sporulated, and its isolation from soil samples using the 'Galleria bait method.'

2.2 Isolation of Entomopathogenic Fungi from Insect Cadaver

Extraction of entomopathogenic fungi from insect cadaver involved directly collecting pathogens

from the surface of cadavers that had already undergone sporulation. In cases where sporulation or external hyphal growth had not occurred in insect samples, insect cadaver was placed in a humid chamber, such as a Petri dish with moist filter paper, to facilitate sporulation. Cadavers displaying sporulation were subjected to two methods for pathogen isolation: either left intact or lightly pressed onto a medium, or alternatively, the spores were meticulously streaked onto plates to facilitate pathogen isolation.

2.3 Isolation of Entomopathogenic Fungi from Soil Sample

2.3.1 Maintenance of greater wax moth, *Galleria mellonella* L. culture

To isolate entomopathogenic fungi from soil, the greater wax moth *Galleria mellonella* was employed as the bait insect. The larvae of *G. mellonella* were raised on a synthetic diet following Singh's guidelines [9], and the initial *G. mellonella* nucleus culture was originally acquired from the Api Culture Technology Center in Rajendranagar, Hyderabad, Telangana.

An artificial diet formulated for Galleria mellonella (greater wax moth) larvae comprises a precise composition of key ingredients tailored to support their nutritional needs. Constituting the bulk of the diet, 200 grams of corn flour provides carbohydrates essential for energy metabolism. Complementing this, 100 grams each of wheat flour and wheat bran contribute dietary fiber, proteins, and micronutrients crucial for larval growth and development. Honey, totalling 100 ml, serves as a natural source of sugars and essential nutrients, while 100 grams of milk powder enrich the diet with proteins, lipids, and vitamins. Yeast, at 50 grams, supplements the diet with proteins, amino acids, and B-complex vitamins vital for larval health. Finally, 100 ml of glycerine is incorporated to modulate the diet's moisture content and improve palatability. This meticulously composed diet aims to simulate the larvae's natural feeding environment, ensuring optimal growth and development under laboratory conditions.

All the components were blended in a single container, excluding honey and glycerine, which were combined in a separate container. The honey and glycerine mixture underwent mild heating until it reached a lukewarm state, and then it was gradually introduced into the flour mixture, creating a dough-like consistency. The freshly prepared diet was left to stand overnight and was subsequently utilized to feed the larvae. Rearing jars, cleaned with water, sterilized using a 2% formalin solution, dried in an oven, and subjected to a 2-hour UV sterilization process, were utilized to maintain the *Galleria* culture. Upon depletion of the diet and the accumulation of excreta, larvae were transferred to a fresh diet. Typically, a diet prepared once was sufficient to rear only one generation of *Galleria* insects. After being raised to the final instar stage, approximately 50-100 larvae were moved to an adult rearing jar equipped with vertically folded paper strips to facilitate pupation until the emergence of adults. The emerged adults were collected and housed in a separate jar containing a cotton swab dipped in honey. Eggs laid on the vertically folded paper strips, positioned on the rim of the rearing jar, were gathered. The final instar larvae of *G. mellonella* were employed as bait insects in soil samples for the isolation of entomopathogenic fungi.

2.3.2 Collection of soil samples

Soil samples were collected from various locations in Telangana and subjected to the isolation of entomopathogenic fungi using the 'Galleria bait method' [4]. The sampling involved removing the top 1 cm of soil and extracting soil from a depth of 10 to 15 cm using a spade. Three spot samples were taken from each site to create a homogenized composite sample weighing 500g. Each sample was placed in a labelled, clean polythene bag, transported to the laboratory, and stored under refrigerated conditions at 5°C [10]. Before exposing the soil sample to *G. mellonella* as part of the live bait method for fungal isolation, the soil was shade-dried. Approximately 100g of soil was measured and placed in small sterile plastic disposable containers. In each container, five *G. mellonella* third instar larvae were introduced and incubated at 22-25°C for about 14 days under laboratory conditions. The soil in the containers was regularly agitated by repositioning or shaking to ensure continuous exposure of the larvae to the soil. Larvae were examined from the 3rd to the 14th day after inoculation. Diseased, moribund, or mummified larvae were retrieved from the containers for fungal isolation. Soil particles attached to the infected cadavers were removed, and the cadavers were washed with a 2% sodium hypochlorite (NaOCl) solution, followed by serial washes with distilled water. Subsequently, these cadavers were transferred to sterile petriplates and incubated for 3-4 days

on moist filter paper for fungal sporulation in a B.O.D. incubator at 25 ± 2°C with 70-80% relative humidity. Following the process of sporulation, the fungal spores were collected from the cadaver's surface and preserved in the respective medium.

2.4 Purification and Maintenance of Entomopathogenic Fungi Isolates

2.4.1 Isolation of pure cultures

The purification of fungi was achieved using the single hyphal tip method as outlined by Rangaswami [11]. The fungus was cultivated on a 2% water agar medium. After 3-4 days, individual fungal hyphae were identified at the colony's growth edge using a binocular microscope at 40X magnification. Subsequently, a segment of the single fungal hyphae was positioned on Potato Dextrose Agar/Sabouraud's Maltose Agar Yeast Extract (PDA/SMAY) medium and kept on PDA slants in a refrigerator at 40°C throughout the study.

3. RESULTS AND DISCUSSION

3.1 Collection of Entomopathogenic Fungal Isolates

An extensive survey spanning Rangareddy district of Telangana from 2021 to 2022 was conducted to collect indigenous isolates of entomopathogenic fungi. Samples of both soil and insects were systematically gathered throughout this survey and subsequently brought to the laboratory for further analysis.

Table 1. Fungal Isolates from cadavers collected at different locations in Rangareddy District, Telangana

Cadaver Location	Cadaver Source Crop	Fungal Isolate
Aziznagar	Maize	*Metarhizium rileyi*
Nagaram	Chilli	*Beauveria bassiana*
Mansapally	Maize	*Beauveria bassiana*

3.2 Isolation of entomopathogenic fungi

In this study, entomopathogenic fungi were isolated from both insect cadavers and soil samples utilizing the Galleria bait method. Extraction from insect cadaver involved directly collecting pathogens from cadavers exhibiting sporulation, with efforts made to induce sporulation in cases lacking external hyphal growth. Subsequently, cadavers were either preserved intact or pressed onto growth media for pathogen isolation. For soil samples, collected across various locations in Telangana, the Galleria bait method was employed. Each composite soil sample was inoculated with *G. mellonella* third instar larvae and incubated under controlled laboratory conditions. Diseased larvae were then retrieved, washed, and incubated to stimulate fungal sporulation, followed by harvesting of fungal spores from the cadavers' surfaces (Fig. 1). Tables 1 & 2 presents a comprehensive overview of the entomopathogenic fungal isolates obtained from insect cadavers and soil samples respectively.

The entomopathogenic fungal isolation study in Rangareddy district, Telangana, reveals intriguing variations across the ten sampled locations, considering soil pH and the crops cultivated. In areas with conducive conditions like Lemoor and Rachuloor, entomopathogenic fungi thrived. Lemoor, characterized by a soil pH of 6.5 and maize cultivation, showed a significant presence of *M. rileyi* in all soil samples. Similarly, Rachuloor, with a pH of 6.6 and rice as the primary crop, exhibited the isolation of *L. lecanii*. Conversely, areas like Saraswathiguda and Gudur displayed minimal fungal presence despite different soil pH levels. Saraswathiguda, with a pH of 6.8 and rice cultivation, showed no fungal isolation, while Gudur, with a pH of 6.2 and maize cultivation, yielded no positive samples. Other locations, such as Akulamilaram, Pullimamidi, Maheshwaram, Akanpally, and Manchanpally, similarly showed no entomopathogenic fungal presence despite varying soil pH levels and crop types. Notably, in Jaithwaram, characterized by a soil pH of 7.0 and cabbage cultivation, *B. bassiana* was isolated from a portion of the soil samples, indicating a moderate fungal presence. These findings underscore the complex relationship between soil pH, crop diversity, and the distribution of entomopathogenic fungi. Further exploration of these factors could provide crucial insights for tailored pest management strategies in the diverse agricultural landscapes of Rangareddy district (Table 2).

3.3 Purification and Maintenance of Entomopathogenic Fungal Isolates

The purification and maintenance of entomopathogenic fungi isolates were carried out

through the single hyphal tip method. Pure cultures were obtained by cultivating the fungi on a 2% water agar medium, followed by identification and transfer of individual fungal hyphae to Potato Dextrose Agar/Sabouraud's Maltose Agar Yeast Extract (PDA/SMAY) medium. The formulated media were prepared according to specified compositions. PDA slants were created for storage and subsequent use. Mass multiplication of *B. bassiana* and *L. lecanii* isolates was achieved through specific procedures outlined, leading to the production of conidial powder for further experimentation. Additionally, the identification of fungal isolates was conducted based on morphological characteristics observed under a binocular microscope, following established methodologies (Fig. 2).

a. MnMr b. NaBb c. AzMr

Fig. 1. Fungal infected cadavers observed in field

a. ChBb b. NaBb c. MnBb

d. MnMr e. AzMr f. KmLl

Fig. 2. Indigenously isolated entomopathogenic fungal isolates

Table 2. Location-wise data summary for entomopathogenic fungal isolation from soil samples in Rangareddy district, Telangana

Location	Latitude and longitude	No. of Soil Samples	Standing Crops	Soil pH	Positive/Negative Samples	Fungus isolated
Lemoor	17.1379° N, 78.5157° E	10	Maize	6.5	Positive	*Metarhizium rileyi*
Saraswathiguda	17.1715° N, 78.4990° E	12	Maize	6.8	Negative	-
Rachuloor	17.1151° N, 78.5363° E	15	Cauliflower	7.2	Positive	*Lecanicillium lecanii*
Gudur	17.7975° N, 79.9794° E	20	Rice	6.2	Negative	-
Akulamilaram	17.0671° N, 78.5739° E	18	Maize	6.9	Negative	-
Pullimamidi	17.0657° N, 78.4189° E	15	Back gram	6.7	Negative	-
Jaithwaram	17.0777° N, 78.4326° E	16	Cabbage	7	Positive	*Beauveria bassiana*
Maheshwaram	17.1351° N, 78.4330° E	22	Maize	6.5	Negative	-
Akanpally	17.0874° N, 78.3902° E	23	Rice	6.8	Negative	-
Manchanpally	17.1771° N, 77.9689° E	24	Rice	6.6	Negative	-
Ameerpet	17.4375° N, 78.4482° E	15	Maize	7.1	Negative	-

Table 3. Morphological characteristics of entomopathogenic fungal isolates from different sources and locations in Rangareddy district, Telangana

Isolate name	Isolate Code	Source	Location	Host Plant	Colony Colour		Colony Shape	Surface Structure	Colony Elevation
					Front	Back			
B. bassiana	ChBb	Rhizosphere soil	Shankarpalli, Telangana	Cauliflower	White	Yellowish	Wide round	Smooth and cottony	Raised
B. bassiana	NaBb	Cadaver	Nagaram, Telangana	Brinjal	White	Yellowish	Round	Smooth and powdery	Raised
B. bassiana	MnBb	Cadaver	Mansapally, Telanagana	Maize	White	Yellowish	Round	Smooth and powdery	Raised
M. rileyi	MnMr	Rhizosphere soil	Mansapally, Telanagana	Maize	White to light green	Brown	Sparse and round	Velvety and dusty	Moderately raised`
M. rileyi	AzMr	Cadaver	Aziznagar, Telangana	Beetroot	White to light green	Brown	Sparse and round	Velvety and dusty	Moderately raised
L. lecanii	KmLl	Rhizosphere soil	Kesaram, Telangana	Cabbage	Cream	Colourless to pale or deep yellow	Medium round	Thin cottony	Raised

The presented table reveals a comprehensive compilation of fungal isolates, accompanied by their respective codes, sources, locations, host plants, and distinctive colony attributes. This dataset underscores the intricate relationship between fungal species and their environments, shedding light on their prevalence and diversity across varied host plants and geographic regions. Examining the isolates of *B. bassiana*, denoted by codes like "ChBb", "NaBb", and "MnBb", unveils their widespread presence across locations in Telangana, including Shankarpalli, Chevella, and Mansapally, and their association with diverse host plants such as cauliflower, brinjal, and maize, respectively. Similarly, *M. rileyi* isolates, represented by codes like "MnMr" and "AzMr", exhibit a geographical distribution spanning Mansapally and Aziznagar, and are linked with host plants like maize and beetroot. Moreover, the inclusion of an isolate of *L. lecanii* with the code "KmLl", sourced from Kesaram in Telangana, underscores the broad spectrum of host plants harbouring fungal species, including cabbage. The detailed characterization of colony traits, encompassing color, shape, surface structure, and elevation, provides significant insights into the morphological variations inherent in these fungal isolates. For instance, *B. bassiana* colonies exhibit white to yellowish coloration, round shape, smooth or powdery surface structure, and raised elevation, whereas *M. rileyi* colonies display white to light green coloration, sparse and round shape, velvety or dusty surface structure, and moderately raised elevation. *L. lecanii* colonies feature cream coloration, medium round shape, thin cottony surface structure, and raised elevation (Table 3).

Our investigation into the collection, isolation, and purification of indigenous isolates of entomopathogenic fungi in the Rangareddy district of Telangana offers significant contributions to the understanding of fungal ecology and its potential applications in agriculture. Drawing insights from various international studies on entomopathogenic fungi, especially those conducted in diverse geographic and environmental settings, can enrich our understanding and guide the potential applications of such fungi in local contexts.By employing meticulous collection methods and isolation techniques, we have identified a diverse array of fungal species with the potential for biocontrol against agricultural pests. Comparing our results with studies documenting the natural occurrence and collection methods of entomopathogenic fungi worldwide provides valuable context for interpreting our findings. The use of the Galleria bait method, as described in our study, aligns with methodologies employed in various geographic regions. For instance, the effectiveness of this method has been demonstrated in Finland [12], Canada [13], Norway [14], Spain [15], Mauritius [16], China [17], New Zealand [18], Turkey [19], Slovakia [20], and Mexico [21]. These studies highlight the versatility and reliability of the Galleria bait method in isolating entomopathogenic fungi from diverse soil types and climatic conditions. Our findings, which reveal the presence of indigenous isolates such as *M. rileyi* and *B. bassiana* in Telangana state, India resonate with observations from other regions where these fungal species are commonly found. Studies in Canada [13], Norway [14], Switzerland [22], and Spain [15] have documented the prevalence of *M. anisopliae* and *B. bassiana* in soil samples, indicating their widespread distribution across different ecosystems.

Vänninen [12] and Keller et al. [22] exemplify how systematic surveys can provide critical insights into the distribution and prevalence of entomopathogenic fungi within specific regions. By employing meticulous isolation methods, these studies identified prevalent species like *M. anisopliae* and *B. bassiana*, highlighting the importance of understanding local fungal biodiversity. The findings of Ali-Shtayeh et al. [23] and Sánchez-Peña et al. [21] underscore the potential biocontrol applications of entomopathogenic fungi in agricultural settings. Their investigations into soil samples from irrigated fields and agricultural soils revealed the presence of virulent species like *Conidiobolus coronatus* and *B. bassiana*, offering promising avenues for sustainable pest management strategies. Furthermore, studies such as Enrique Quesada-Moraga et al. [15] and Sooker et al. [16] demonstrate the adaptability of entomopathogenic fungi across diverse habitats and climates. Understanding the ecological preferences and distribution patterns of these fungi is crucial for harnessing their biocontrol potential effectively. The study by Fofana et al. [24] addressing fall armyworm infestations highlights the practical relevance of exploring indigenous entomopathogenic fungi.

The presence of fungal entomopathogens in soil as saprophytes is a well-established phenomenon, primarily due to the protective environment it offers from UV radiation and other

adverse abiotic and biotic factors [25]. This ecological niche provides a conducive habitat for the persistence and proliferation of entomopathogenic fungi (EPF), including species of *Metarhizium* and *Beauveria*. In the context of the study conducted in Gojjam and South of Gonder, Ethiopia, the detection of EPF in soil samples confirms their presence in these regions. However, the prevalence observed in this study, with 21.4% of the soil samples testing positive for entomopathogenic fungi, appears to be lower compared to findings from other studies within Ethiopia [26]. For instance, Ayele et al. [27] reported a higher detection rate, with 48% of EPF isolates identified from soil samples collected using Galeria baiting. Similarly, Gebremariam et al. [28]) found a prevalence of 54.2% EPF among the total samples collected in their study. The variation in the prevalence of entomopathogenic fungi across different studies could stem from several factors, including differences in sampling methodologies, geographical locations, and environmental conditions. It is essential to consider the specificities of each study when interpreting such variations. By meticulously isolating fungal isolates from soil samples across maize fields, the study lays a robust foundation for potential biocontrol solutions against this destructive pest. Similar investigations tailored to the local context of Rangareddy District could provide valuable insights into combating agricultural pests prevalent in the region. In conclusion, exploring indigenous entomopathogenic fungi in Rangareddy District, Telangana, India, offers opportunities to not only enrich our ecological understanding but also to develop sustainable pest management strategies. By leveraging insights from international studies and tailoring them to local environmental conditions, researchers can unlock the ecological significance and practical applications of these fungi, thereby contributing to agricultural sustainability and ecosystem health in the region.

4. CONCLUSION

This study provides valuable insights into the distribution, diversity, and ecological roles of indigenous entomopathogenic fungi in the Rangareddy district of Telangana, India. Through the systematic collection and isolation of fungal species from soil and insect cadaver samples, we identified a range of fungal isolates, including Metarhizium rileyi, Beauveria bassiana, and Lecanicillium lecanii, across different locations and associated with various host plants. Our findings underscore the influence of soil pH levels on fungal diversity, highlighting the importance of environmental factors in shaping fungal prevalence. The effectiveness of the Galleria bait method in isolating entomopathogenic fungi was demonstrated, showcasing its versatility and reliability across different geographic regions. Comparison with global studies further supports the widespread distribution of fungal species and emphasizes the significance of understanding local environmental factors in fungal ecology. Overall, this study contributes to our understanding of fungal ecology and offers insights into their potential applications in agriculture, particularly in pest management practices. By elucidating the ecological roles of indigenous entomopathogenic fungi, this research opens avenues for further exploration and underscores the importance of sustainable agricultural practices in harnessing the potential of these fungi for pest control and crop protection.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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