Indian Journal of Ecology (2025) 52(6): 1555-1559
DOI: https://doi.org/10.55362/IJE/2025/4691

Manuscript Number: 4691
NAAS Rating: 5.38

Morphological and Molecular Characterization of Native Entomopathogenic Fungi *Beauveria bassiana* and *Metarhizium rileyi* from North Coastal Andhra Pradesh

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***Abstract*:** The investigation was undertaken on isolation and assessment of native entomopathogenic fungi from the North coastal districts of Andhra Pradesh at the Department of Entomology, Regional Agricultural Research Station, ANGRAU, Anakapalle, Andhra Pradesh during 2024-25. The soil samples were collected from both natural and agricultural habitats across Srikakulam, Vizianagaram, Visakhapatnam and Anakapalle districts. Two entomopathogenic fungal species were successfully isolated: *Beauveria bassiana* (AKP Bb-cd) from *Neolamarckia cadamba* soil and *Metarhizium rileyi* (AKP Mr-2m) from an insect cadaver collected from maize field. Morphological examination revealed white mycelial colonies in both isolates with *M. rileyi* transitioning to olive green upon sporulation. Molecular characterization using ITS1 and ITS4 primers produced 600 bp amplicons for both the isolates and sequences were deposited in NCBI GenBank. Phylogenetic analysis using Neighbour Joining method in MEGA 12 confirmed their genetic identity.

Keywords: Entomopathogenic fungi, Isolation, *B. bassiana*, *M. rileyi*, Biocontrol

Biological control offers an environmentally sustainable approach for managing insect pests. Among the key agents employed 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 fungus virulence. Unlike other biological control organisms, entomopathogenic fungi do not require ingestion to infect, they penetrate the host directly through the cuticle (Inglis et al., 2000). Entomopathogenic fungi have been widely explored as promising biological control agents due to their natural occurrence, mode of action through cuticular penetration and environmental safety. Different entomopathogens such as *Metarhizium anisopliae*, *M. rileyi*, *Beauveria bassiana*, *Isaria* and *Lecanicillium* are the most extensively studied EPFs for pest control applications (Chandel et al., 2018, Timmi and Joshi 2024). Several studies have emphasized their potential as alternatives to chemical insecticides, especially under integrated pest management strategies (Fang et al., 2014, Niu et al., 2019). The genus *Metarhizium* is known for its ecological diversity and infect a wide range of insect hosts (Wang et al., 2016, Moonjely and Bidochka 2019). Likewise, *Beauveria* is another extensively researched entomopathogen with proven efficacy in insect pest control. Furthermore, indigenous fungal isolates, adapted to local environmental conditions often outperform commercial strains in terms of persistence and efficacy (Abott 1925, Abid et al., 2022). Molecular identification using ITS regions has further enabled accurate characterization and differentiation of native strains (Rehner and Buckley 2005). Species identification can be achieved through the analysis of total genomic DNA digested with restriction enzymes and separated electrophoretically using restriction fragment length polymorphisms (RFLPs) for strain differentiation. Additionally, Random Amplified Polymorphic DNA (RAPD) analysis has also been used for characterizing fungal isolates as demonstrated by Kosir et al. (1991) and Visalakshi et al. (2020).

MATERIAL AND METHODS

Sampling and insect cadaver collection: A total of 32 soil samples were collected from natural and agricultural ecosystems and two entomofungal isolates were isolated, one from *Neolamarckia cadamba* tree soil collected from Devipuram, Anakapalle district located at 17.977°N, 83.329°E coordinates and another from an insect cadaver collected from Maize crop, Padmanabham, Visakhapatnam district located at 17.698°N, 83.003°E coordinates in Andhra Pradesh, India. The soil samples were collected from the rhizosphere zone between 5 to 10 cm from the soil surface and approximately 100 g of soil sample were collected at each location from different soil habitats in separate sterile polythene bags and labelled properly with name of the crop, date and place of collection and stored in a darker place until further testing. Insect cadaver collected from 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.

Isolation of entomopathogenic fungi: Entomopathogenic fungi were isolated from soil samples by using serial dilution

method, with dilutions ranging from 10^{-4} to 10^{-8}. The procedure was replicated three times and plated on SDAY media, leading to the successful recovery of fungal isolates. In addition, fungal inoculum scraped from the insect cadaver was also plated on SDAY media to initiate the growth of the fungus.

Morphological identification of entomopathogenic fungi: The morphological characteristics such as spore size, shape, colour, colony morphology, shape of the conidia, length and width ratio of the spore of fungal isolates were examined. Microscopic observations were conducted at 10x and 40x magnifications and digital images were captured at 40x magnification using V-image 2013 software.

Preparation of SDB (Sabouraud's dextrose broth): Sabouraud's Dextrose Broth (SDB) was prepared using glucose, peptone and yeast extract, excluding agar. The medium was sterilized in an autoclave at 121°C for 15 minutes under 15 psi pressure. Five mm disc of mycelial mat from fungal cultures grown on SDAY medium was aseptically transferred into conical flasks containing the sterilized SDB. These flasks were incubated at 25°C for two weeks. After incubation, the mycelial mat was separated by filtering the broth through filter paper, washed with distilled water and dried on tissue paper. The dried mat was wrapped in aluminum foil and stored at -20°C. After 24 hours, the preserved fungal mat was used for DNA extraction.

Molecular Identification of Entomopathogenic Fungi

Fungal DNA extraction: The DNA was extracted using Sambrook and Russell protocol (2001). Fungal mats of both the isolates were homogenized **in** liquid Nitrogen **using** a pre-sterilized mortar and pestle. The macerated fungus was transferred to sterile 1.5 ml eppendorf tubes followed by the addition of pre-heated (65°C) 2% CTAB buffer. These samples were incubated in a dry bath at 65°C for one hour. After incubation, an equal volume of Chloroform and Phenol (1:1 ratio) was added and the samples were centrifuged in a refrigerated centrifuge (Centrifuge 5418 R) at 10,000 rpm for 10 min at 24°C. The supernatant was transferred to 1.5 ml fresh eppendorf tubes. Centrifugation was repeated and supernatant again collected into fresh tubes. Equal volume of Chloroform and Isoamyl alcohol mixture was added and centrifuged at 10,000 rpm for 10 min. The supernatant was collected in a separate eppendorf tube and 0.6 volume of ice-cold isopropanol, 0.1volume of sodium acetate buffer (0.3M, pH 5.0) were added and then incubated for 24 hours at -20°C. After incubation, the tubes were taken out and centrifuged at 10,000 rpm for 10 min at 4°C. The supernatant was discarded and pellet was washed with 70 per cent ethanol and again centrifuged at 10,000 rpm for 10 min at 4°C. After discarding the supernatant, the pellets were air dried and dissolved in 100µl of molecular grade water. The DNA samples of fungal isolates were stored at -20°C.

PCR amplification of ITS region: The identification of the purified isolates based on morphological characteristics was complemented with the sequencing of the Internal transcribed region (ITS) sequences. The amplification of ITS region was carried out using Universal primers ITS 1 and ITS 4 primers. Thermocycling conditions included initial denaturation at 95°C for 5 min, followed by 35 amplification cycles of 94°C for 1 min, 55°C for 1min, 72°C for 1 min, followed by final extension at 72°C for 10 min.

Running of gel electrophoresis: Agarose gel electrophoresis of PCR amplified DNA was carried out using a 1% (w/v) agarose gel prepared by dissolving 1.0 g of MB-grade agarose in 100 ml of 1x TAE buffer. The solution was heated until fully melted, then cooled to 50-55°C before adding 4 µl of ethidium bromide (10 mg/ml). The gel was poured into a casting tray fitted with 0.5 mm combs, ensuring bubble free pouring. Once solidified, the comb was gently removed and the gel was placed in a tank containing 1xTAE buffer. DNA samples and a 1 kb ladder were loaded and electrophoresis was performed at 100 V for 45-60 minutes. DNA band migration was visualized and documented using a Vilber E-Box gel documentation system in auto exposure mode. The amplified PCR products were excised and sent to a sequencing service provider for partial sequencing. The Internal Transcribed Spacer (ITS) region sequences obtained were compared with reference sequences using the BLAST tool available in the NCBI GenBank database to identify similarity with known sequences. Phylogenetic trees for *Beauveria* and *Metarhizium* constructed using MEGA software (version 12.0) to analyze their evolutionary relationships.

RESULTS AND DISCUSSION

The soil sample and insect cadaver collected from natural and agricultural ecosystems from north coastal districts of Andhra Pradesh yielded two fungal isolates that showed typical characteristics of entomopathogenic fungi when observed under the microscope at 40x magnification.

Morphological identification of EPF: In this study, *Beauveria bassiana* (AKP Bb-cd) was isolated from rhizosphere of *Neolamarckia cadamba* tree while *Metarhizium rileyi* (AKP Mr-2m) was isolated from an insect cadaver collected from maize crop. The preliminary characterization showed that the isolate *B. bassiana* initially produced white cottony colony growth which later turned into powdery growth. In contrast, the isolate *M. rileyi* initially showed white colony growth and later turned into olive green colour with velvety spores upon sporulation (Table 1, 2).

Cokola et al., (2023) examined the morphological characteristics of fungal structures, including shape and size of the conidia, conidiophores and identified the isolates as belonging to the genus *Beauveria*. The genus *Metarhizium* is characterized by cylindrical to ellipsoid conidia in mature colonies, which were dark green in colour, formed chains of equal length in the clusters obtained. The conidia were typically oblong, elliptical in shape varying 9.0 µm in length and 4.3 µm in width (Mathulwe et al., 2021).

Molecular characterization of EPF: The molecular identification was done for both the isolates by using ITS Universal primers. The primers ITS 1 and ITS 4 were used for PCR amplification of DNA. Along with primers, DNA sample, Dream Taq (2x) master mix and nuclease free water were added in a PCR tube and PCR amplification was done and the ladder DNA of 1 kb was taken as reference for the gel run. A single discrete PCR amplicon band of 600 bp was observed for both the isolates when resolved under gel documentation system (Vilber E-box). Molecular analysis confirmed the identification of *Beauveria* and *Metarhizium* through amplication and sequencing of the ITS1-5.8S-ITS2 region of recombinant DNA followed by sequence comparison using databases such as NCBI and fungal barcoding resources demonstrated by Kearse et al. (2012).

DNA sequencing: PCR products were eluted and sequenced with an automated DNA sequencing facility. The obtained Sequences were identified by homology using Basic Local Alignment Search Tool (BLAST). The nucleotide sequences of *B. bassiana* and *M. rileyi* in the present study were compared with other *B. bassiana* and *M. rileyi* sequences available in the National center for Biotechnology Information (NCBI) GenBank database (http://www.ncbi.nlm.nih.org). Phylogenetic tree was constructed for both the isolates using the Neighbour joining method and 1000 bootstrap replicates using MEGA 12.0 version software (Kumar et al., 2024).

Phylogenetic tree analysis of EPF using MEGA software: The phylogenetic tree was constructed to elucidate the evolutionary relationships among various *B. bassiana* and *M. rileyi* isolates from different geographic regions using sequence data retrieved from NCBI database for comparison. The phylogenetic tree was constructed ~~by~~ using Neighbour joining method with bootstrap analysis to assess the robustness of each clade. Bootstrap values shown at the nodes indicate the percentage of 1000 bootstrap replicates in which the associated group of taxa clustered together. The image (Fig. 3) represents a phylogenetic tree constructed using sequence data from various isolates of *Beauveria bassiana* along with a single outgroup *Metarhizium rileyi*. The phylogenetic analysis of *Beauveria bassiana* isolates revealed distinct clustering patterns reflecting their geographic origins. Isolates from Kerala, Raichur, Gujarat and Puducherry formed a well-supported clade, whereas isolates from southern India, including Bangalore, Chennai, Tirupati and Devipuram grouped together with strong bootstrap support. The Devipuram isolate, AKP Bb-cd

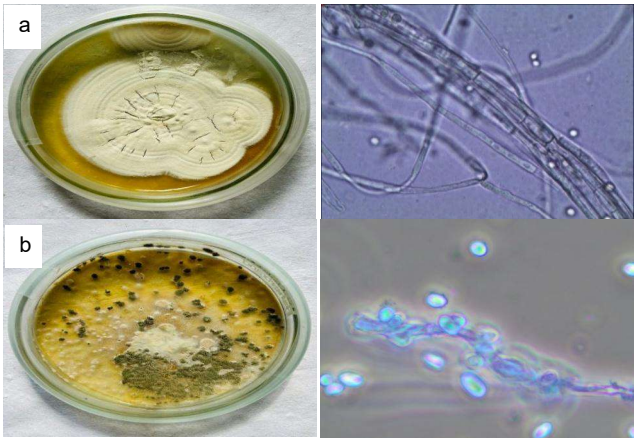

Fig. 1. a. Morphological identification of *B. bassiana* AKP Bb-cd; b. Morphological identification of *M. rileyi* AKP Mr-2m

Table 1. Morphological characteristics of entomopathogenic fungal isolates

Fungal isolate	Colony colour	Colony shape	Colony elevation	Radial growth	Shape of the conidia	Colony diameter (cm)
B. bassiana AKP Bb-cd	White	Round	Initially raised and then flat	Fast	Globose	6 cm
M. rileyi AKP Mr-2m	Olive green	Round	Flat	Slow	Round to oval	4 cm

Table 2. Growth characteristics of *B. bassiana* (AKP Bb-cd) and *M. rileyi* (AKP Mr-2m)

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

L/W – Length to width ratio

(PV791015.1) clustered closely with the Araku Valley isolate (OR345217.1) with high confidence (bootstrap value of 99) indicating a close evolutionary relationship. The use of *Metarhizium rileyi* as an outgroup confirmed the divergence of *B. bassiana* isolates, highlighting the genetic diversity within the species.

The tree represents the evolutionary relationships among *Metarhizium rileyi* isolates across different regions in India and other countries with one *Beauveria bassiana* isolate used as an outgroup (Fig. 4). The isolates formed multiple

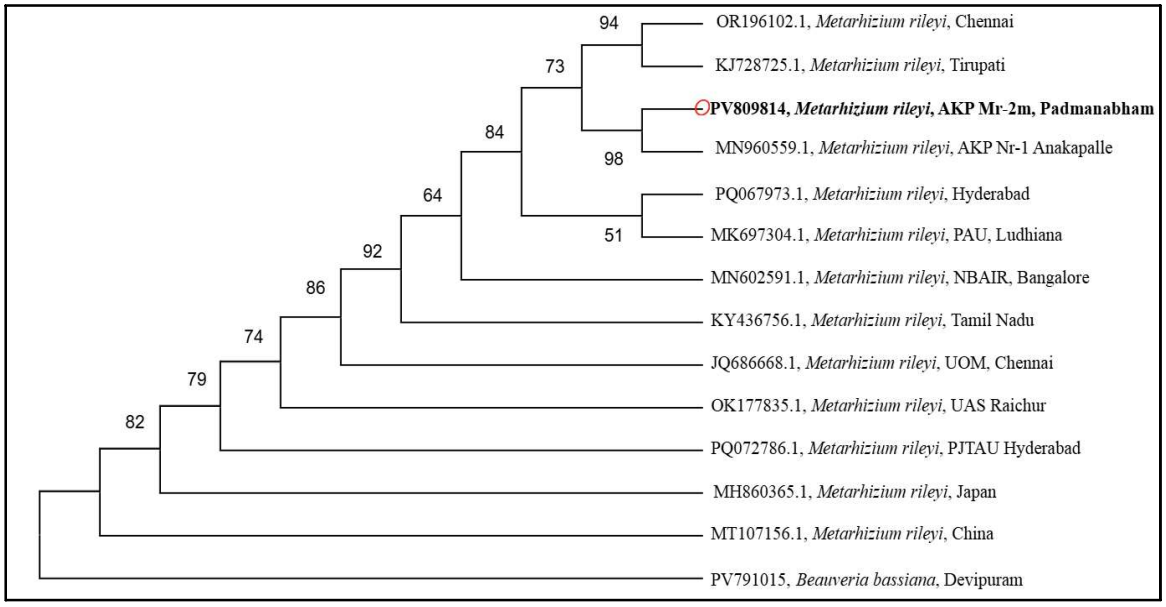

Fig. 3. Phylogenetic tree based on the nucleotide sequences of ITS 1-5.8S-ITS 4 r DNA region of isolated *B. bassiana* AKP Bb-cd along with closely related reference sequence retrieved from the NCBI database

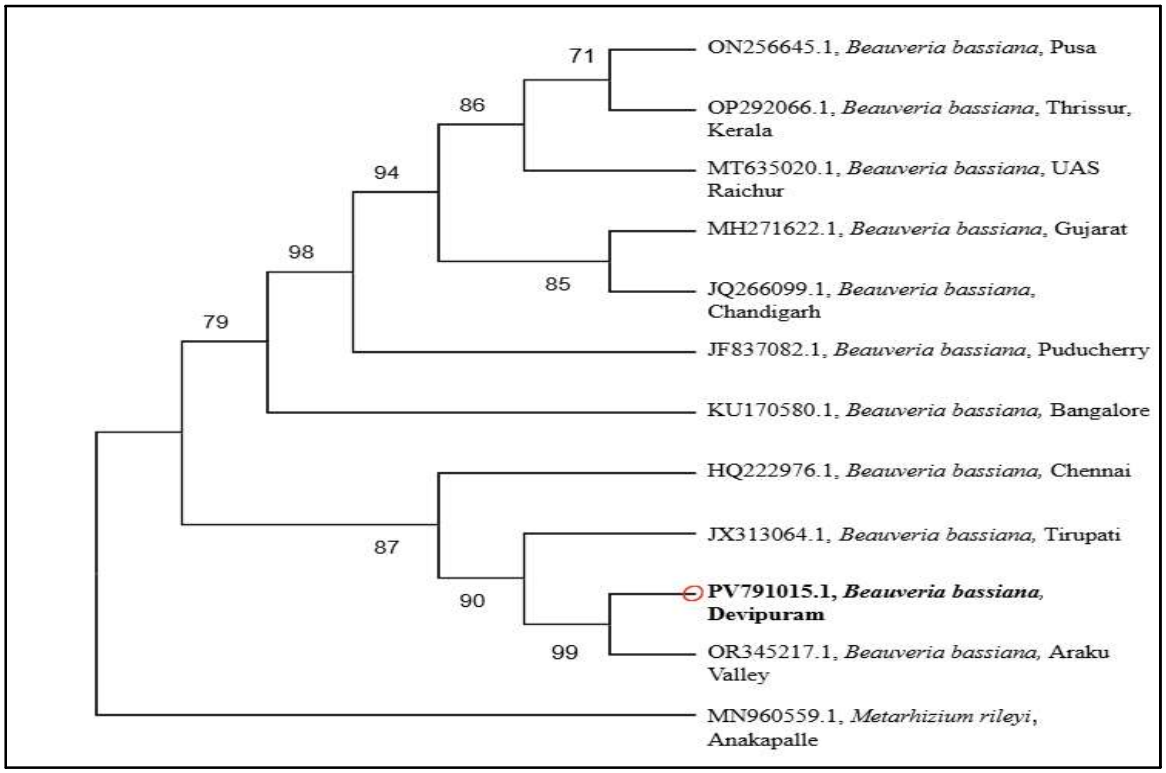

Fig. 4. Phylogenetic tree based on the nucleotide sequences of ITS 1-5.8S-ITS 4 r DNA region of isolated *M. rileyi* AKP Mr-2m along with closely related reference sequences retrieved from the NCBI database

clades, each supported by bootstrap values that indicated the reliability of the branching. The clade containing isolates from Chennai (OR196102.1), Tirupati (KJ728725.1) and Padmanabham (PV809814) showed strong bootstrap support (94-98). The native isolate AKP Mr-2m Padmanabham isolate (PV809814) clustered with the Anakapalle isolate (MN960559.1) and other closely related isolates from Hyderabad and Ludhiana, suggesting genetic relatedness across geographically distinct locations. The strong bootstrap value of 98% for this cluster confirmed a robust evolutionary relationship. Isolates from southern India (Chennai, Tirupati, Anakapalle, Hyderabad, Bangalore, Tamil Nadu and Raichur) were distributed across different clades, showing moderate to strong bootstrap support. Interestingly, isolates from Japan (MH860365.1) and China (MT107156.1) also clustered within the Indian lineages suggesting a broader evolutionary conservation of *M. rileyi*.

CONCLUSION

Two entomopathogenic fungal isolates were obtained in this study: *Beauveria bassiana* AKP Bb-cd, isolated from natural ecosystem using the soil serial dilution method and *Metarhizium rileyi* AKP Mr-2m, isolated from an agricultural ecosystem as an insect cadaver. Morphological characteristics played a crucial role in distinguishing between the two isolates. Notable differences were observed in colony morphology as well as in the size and shape of conidia, thereby facilitating the identification and comparison of the entomopathogenic fungi. Molecular techniques provided additional insights into the genetic identity and evolutionary relationships of the isolates.

REFERENCES

Abbott WS 1925. A method of computing the effectiveness of an insecticide. *Journal of Economic Entomology* **18**(2): 265-267.

Abid M, Ahmed S and Ahmad R 2022. Evaluation of native entomopathogenic fungi against *Spodoptera frugiperda*. *Egyptian Journal of Biological Pest Control* **32**(1): 89.

Chandel RS, Rana A, Sanjta S and Mehta PK 2018. Potential of entomopathogens in managing potato white grubs in Himachal Pradesh. *Indian Journal of Ecology* **45**(1): 210-213.

Received 10 October, 2025; Accepted 15 November, 2025

Cokola MC, Ben Fekih I, Bisimwa EB, Capparos Megido R, Delvigne F and Francis F 2023. Natural occurrence of *Beauveria bassiana* (Ascomycota: Hypocreales) infecting *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) and earwig in eastern DR Congo. *Egyptian Journal of Biological Pest Control* **33**(1): 54.

Fang W, Azimzadeh P and St Leger RJ 2014. strain improvement of fungal insecticides for controlling insect pests and vector-borne diseases. *Current Opinion in Microbiology* **15**: 232-238.

Inglis GD, Ivie TJ, Duke GM and Goettel S 2000. Influence of rain and conidial formation on persistence of *Beauveria bassiana* on potato leaves and Colorado potato beetle larva. *Journal of Biological Control* **18**(1): 55-64.

Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Meintjes P and Drummond A 2012. Geneious Basic: an integrated and extendable desktops of software platform for the organization and analysis of sequence data. *Bioinformatics* **28**(12): 1647-1649.

Kosir JM, Macpherson JM and Khachatourians GG 1991. Genomic analysis of a virulent and a less virulent strain of the entomopathogenic fungus *Beauveria bassiana* using RFLP. *Canadian Journal of Microbiology* **37**: 534-538.

Kumar S, Stecher G, Suleski M, Sanderford M, Sharma S and Tamura K 2024. MEGA12: Molecular Evolutionary Genetic Analysis version 12 for adaptive and green computing. *Molecular Biology and Evolution* **41**(12): 263.

Mathulwe LL, Jacobs K, Malan AP, Birkhofer K, Addison MF and Addison P 2021. Characterization of *Metarhizium majus* (Hypocreales: Clavicipitaceae) isolated from the Western Cape Province, South Africa. *Plos one* **16**(2): e0240955.

Moonjely S and Bidochka MJ 2019. Insect-pathogenic fungi: Ecology, evolution and mechanisms. *Microbial Control of Insect and Mite Pests.* Academic Press 51-71.

Niu X, Xie W, Zhang J and Hu Q 2019. Biodiversity of entomopathogenic fungi in the soils of South China. *Microorganisms* **7**(9): 311.

Rehner SA and Buckley E 2005. A molecular phylogeny of *Beauveria* and related fungi. *Mycologia* **97**(1): 84-98.

Sambrook J and Russell DW 2001. Detection of DNA in agarose gels. **In**: *Molecular Cloning, A Laboratory Manual (3rd Ed)*. Cold Spring Harbor Laboratory Press, New York 5-14.

Timmi and Joshi N 2024. In vitro interactions of biorationals, *Bacillus thuringiensis* and synthetic insecticides. *Indian Journal of Ecology* **51**(6): 1337-1340.

Visalakshi M, Varma PK, Sekhar VC, Bharathalaxmi M, Manisha BL and Upendhar S 2020. Studies on mycosis of *Metarhizium* (*Nomuraea*) *rileyi* on *Spodoptera frugiperda* infesting maize in Andhra Pradesh, India. *Egyptian Journal of Biological Pest Control* **30**: 1-10.

Wang JB, St Leger RJ and Wang C 2016. Advances in genomics of entomopathogenic fungi. **In**: Lovett B, St. Leger RJ (eds) *Advances in Genetics*. Academic Press p 67-105.