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REPORT OF ENTOMOPATHOGENIC FUNGI, *BEAUVERIA BASSIANA*, AND *METARHIZIUM RILEYI* FROM CHOTANAGPUR PLATEAU, INDIA

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ABSTRACT
The occurrence of entomopathogenic fungi has been reported from different parts of India, but very few from Jharkhand. Therefore, keeping the above fact in mind, a study on the survey and collection of entomopathogenic fungi from different areas of Ranchi district was done. On the basis of the macroscopic and microscopic examination of spore attributes under the phase contrast microscope, the isolated entomopathogenic fungal genera were determined to be *Beauveria bassiana* and *Metarhizium rileyi* . *B.bassiana mycelium* was septate, white hyaline, smooth walled, with round conidia measuring 3-5μ in diameter; culture showed a dispersed growth pattern, round with raised elevation, and a white smooth powdery texture. M.rileyi showed septate, hyaline mycelium, short conidiogenous cells, cylindrical conidia measuring 5m-9μm long, a radial colony, smooth texture, and whitish to greenish colour.
The natural occurrence of entomopathogenic fungi during a particular period of time explains their dependency upon factors such as climate, habitat, *etc.*, as well as the outbreak of pests that can cause a heavy infestation of fungi. Keeping in mind their ability to parasitize and kill their hosts, these pathogens can be utilised as agents in the biocontrol of insect pests.

INTRODUCTION

Entomopathogenic fungi are a group of phylogenetically diverse, heterotrophic, eukaryotic, opportunistic pathogens of insects, and because of their wide host range, unique mode of action, good survival skills even in adverse conditions, and zero resistance; they have proven to be a promising agent for bio-control of insect pests (Firake and Behere, 2020). They infect their hosts by penetrating their bodies, eventually killing them and feeding on them (Dara, 2017, Brunner-Mendoza *et al*., 2019), whereas other microbes enter through ingestion and then cause diseases. They are classified into three divisions: Ascomycota, Zygomycota, and Deuteromycota (Esparza-Mora *et al*., 2017). The most widely used genera/ species for the preparation of mycoinsecticides are *Beauveria bassiana and Metarhizium anisopliae* (Maina *et al*., 2018). Several reports indicated that weather parameters, *viz*., temperature, humidity, and rainfall, play a crucial role in the distribution, prevalence, and antagonistic efficacy of entomopathogenic fungi (Maurya *et al*., 2013, Choudhary *et al*., 2012). They are found in nature, yet their epizootics are observed impacting pest populations.

The occurrence of Entomopathogenic fungi naturally in an environment or agro-ecosystem indicates their potential role as biotic factors in controlling insect pest populations (Meyling and Eilenberg, 2007; Donga *et al*., 2021; Ramdani *et al*., 2022). Among the various eco-friendly techniques of pest management, an entomopathogenic fungus has been reported to be of greater advantage as it won't pollute the water around it, enter our food chain, or impact biodiversity (Butt *et al*., 2001). High mortality from fungal infections occurs rarely in nature until it is utilised as a bio-pesticide, which has a tremendous effect on reducing the pest population. Vanlalkiki *et al*., 2013 isolated and tested the efficacy of *Beauveria bassiana* for the management of diamondback moths infecting cabbage and reported it to be effective in controlling the pest population. Fronza *et al*., 2017, Verma *et al*., 2020, studied the mycosis of *Metarhizium rileyi* on *Spodoptera frugiperda* infecting maize, Ullah *et al*., 2022 isolated and identified the virulence of two fungal strains, *Beauveria Bassiana* and *Metarhizium anisopliae*, against M. persicae and *S. Frugiperda*. Looking at the benefits of entomopathogenic fungi, the basic idea for this study was to perform a survey of the natural occurrence of various entomopathogenic fungi in Ranchi district. The present study is aimed at doing a survey of EPF and isolating and identifying indigenous entomopathogenic strains.

MATERIALS AND METHODS

Survey and collection

During the survey, insects were found dead with a white or green covering over them, adhering to the bark of trees as well as the abaxial surface of leaves. Samples were analysed using a method of fungal isolation on PDA media using standard laboratory measures (Humber 1997). All the research work was carried out in the plant pathology laboratory of the ICAR-Research Complex for Eastern Region, Research Centre,

Ranchi.

In September 2018, white mummified bodies of caterpillars were found adjoined to the bark of Sal trees (*Shorea robusta*) in Bhagwan Birsa Biological Park, situated north-east of Ormanjhi in Ranchi, Jharkhand, India (23.2702°N latitude, 85.3061° E longitude, covering a total area of 104 hectares bounded partially on two sides of the Sapahi River) (Figure 1). A similar finding in December 2018 was seen in which many insect cadavers were collected from the Okra experiment field of the ICAR Research Complex for Eastern Region Research Centre, Ranchi, Jharkhand, India (23° 452 N latitude, 85° 302° E longitude, elevation 620 m AMSL) (Figure 2) and from Birsa Agricultural University, Ranchi (23.4259° N longitude and 85.3164° E latitude at an altitude of 615 m (MSL) (Figure 3). These sites were selected because there was no insecticide or pesticide application and because of the knowledge of insect pest activities in those areas. Insect cadavers were placed in Petri dishes and brought to the laboratory for the isolation of entomopathogenic fungi.

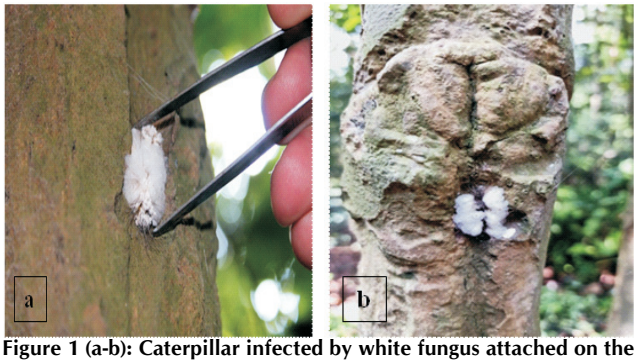

Figure 1 (a-b): Caterpillar infected by white fungus attached on the bark of Sal tree (*Shorea robusta*)

Isolation

Identification of the entomopathogenic fungi was done by the standard laboratory measure (Humber 1997; Herlinda *et al.*,

Figure 2 : (a-c) Bihar Red hairy caterpillar covered with white fungus was found in the Okra experiment field (d) Infected caterpillar collected

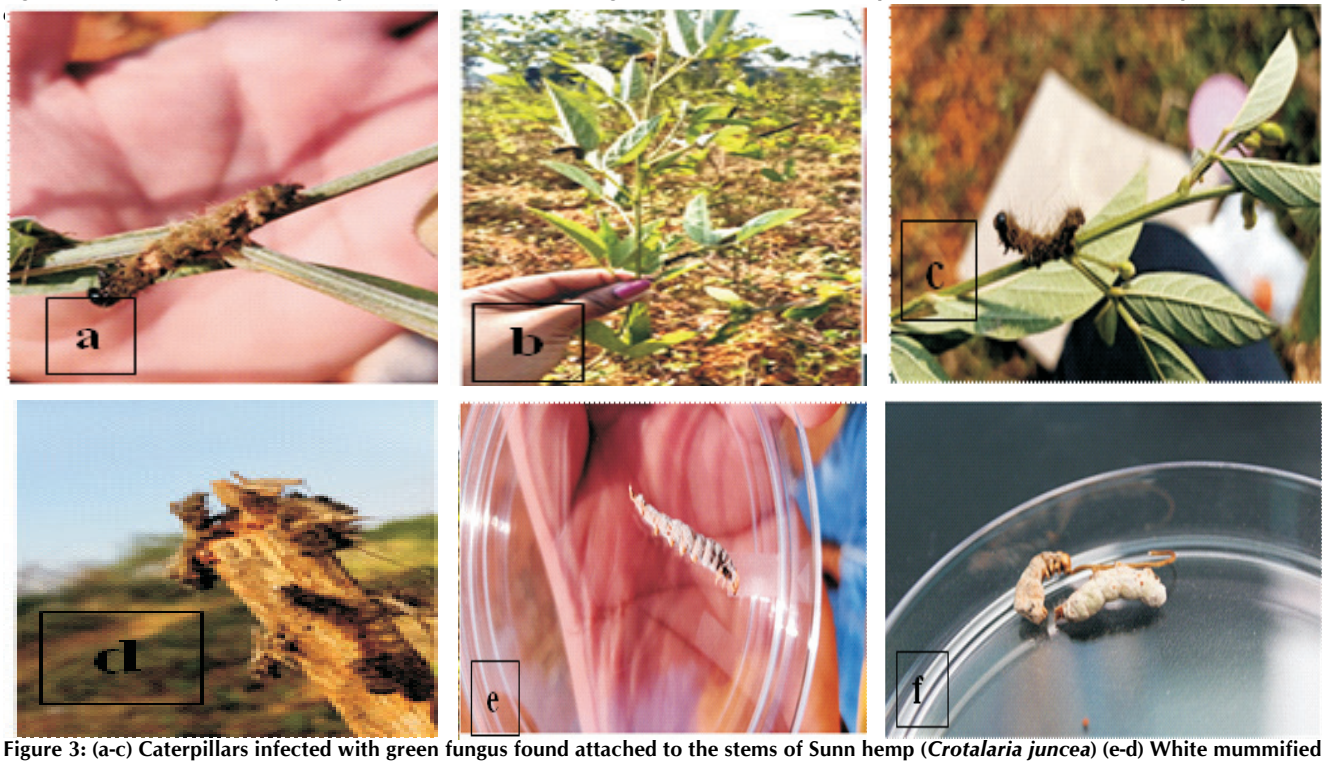

Figure 3: (a-c) Caterpillars infected with green fungus found attached to the stems of Sunn hemp (*Crotalaria juncea*) (e-d) White mummified caterpillar collected on a Petri dish

Figure 4 : (a-d) samples from different areas were collected and placed in separate Petri dishes lined with moist cotton

2008). Samples were kept in moist conditions by using wet cotton before transferring into media, as they sporulate readily in moist conditions (Figure 4). Samples were rinsed three times with distilled water and dried. Subsequently, these larvae were placed in glass petriplates lined with moistened sterile filter paper discs and incubated at 25°C to stimulate conidial germination. The conidia were transferred to a potato dextrose agar plate with the help of a sterile inoculation needle under the laminar air flow chamber. Incubated at 25°C inside a B.O.D. incubator. The entomopathogenic fungus was purified by the hyphal tip isolation technique

Morphological identification of fungi

Identification was based on morphological features such as growth pattern, colony formation of the fungal culture. Clean temporary microscopic slides were prepared; with the help of a sterilised inoculating needle, some portion of the growth was teased and placed on the slide, to which a drop of lactophenol blue was added to examine it under the microscope. The slide was covered with a clean glass cover slip and examined under a phase contrast microscope equipped with a photomicrograph camera (Moanaro *et al*., 2017), and diagnostic features were recorded and confirmed on the basis of available literature (Choudhary *et al*., 2012, Maurya *et al*., 2013).

RESULTS AND DISCUSSION

On the basis of studying their morphological characters under the microscope, two genera of entomopathogenic fungi were identified. Macroscopic examination of *Beauveria bassiana* showed a dispersed growth pattern, a round shape, a raised elevation, an intact edge with a white smooth powdery texture, and apical growth in all directions (Figure 5). The microscopic examination (Figure 6) of *Beauveria bassiana* revealed septate hyphae, white, hyaline, smooth walled, flask-like conidiogenous cells; each conidium was round or ovoid in shape, $<3.5\mu$m in diameter, attached on the tip. This result

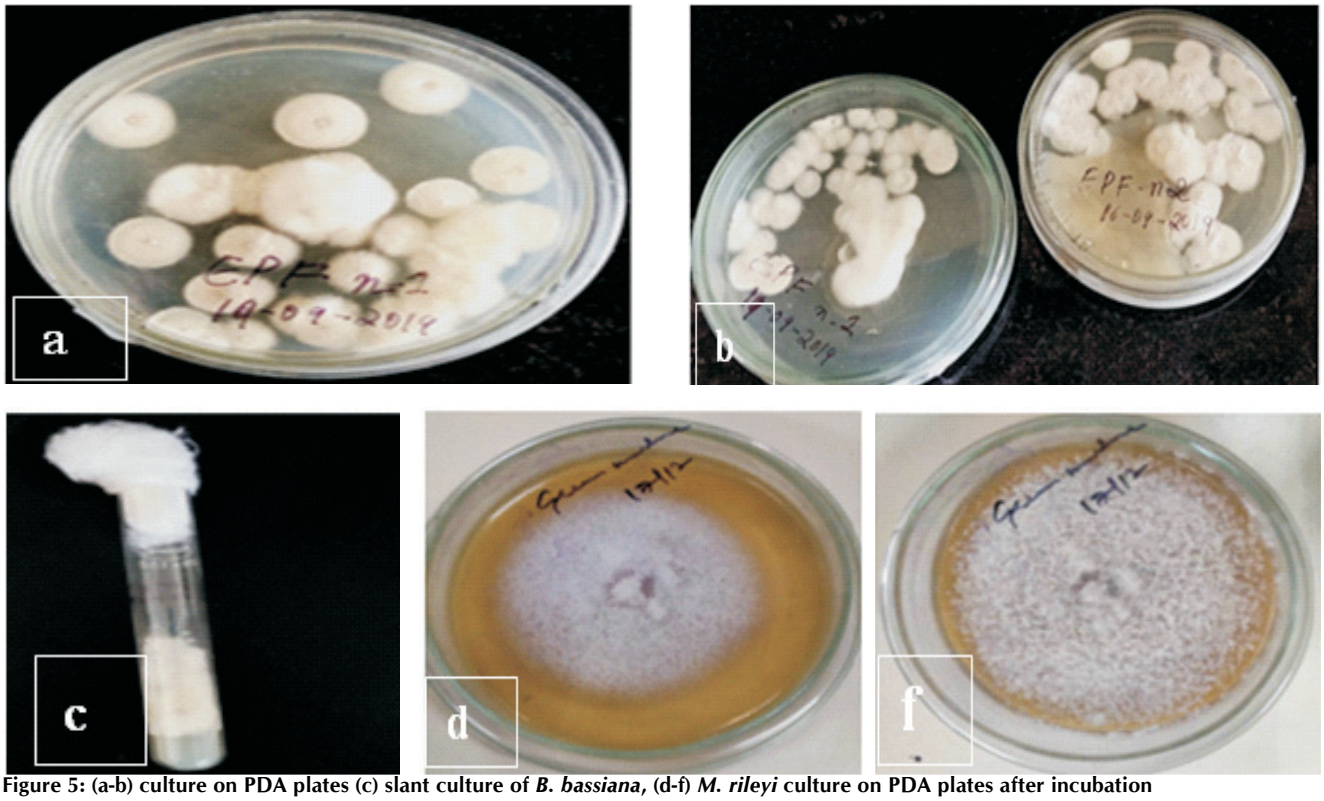

Figure 5: (a-b) culture on PDA plates (c) slant culture of *B. bassiana*, (d-f) *M. rileyi* culture on PDA plates after incubation

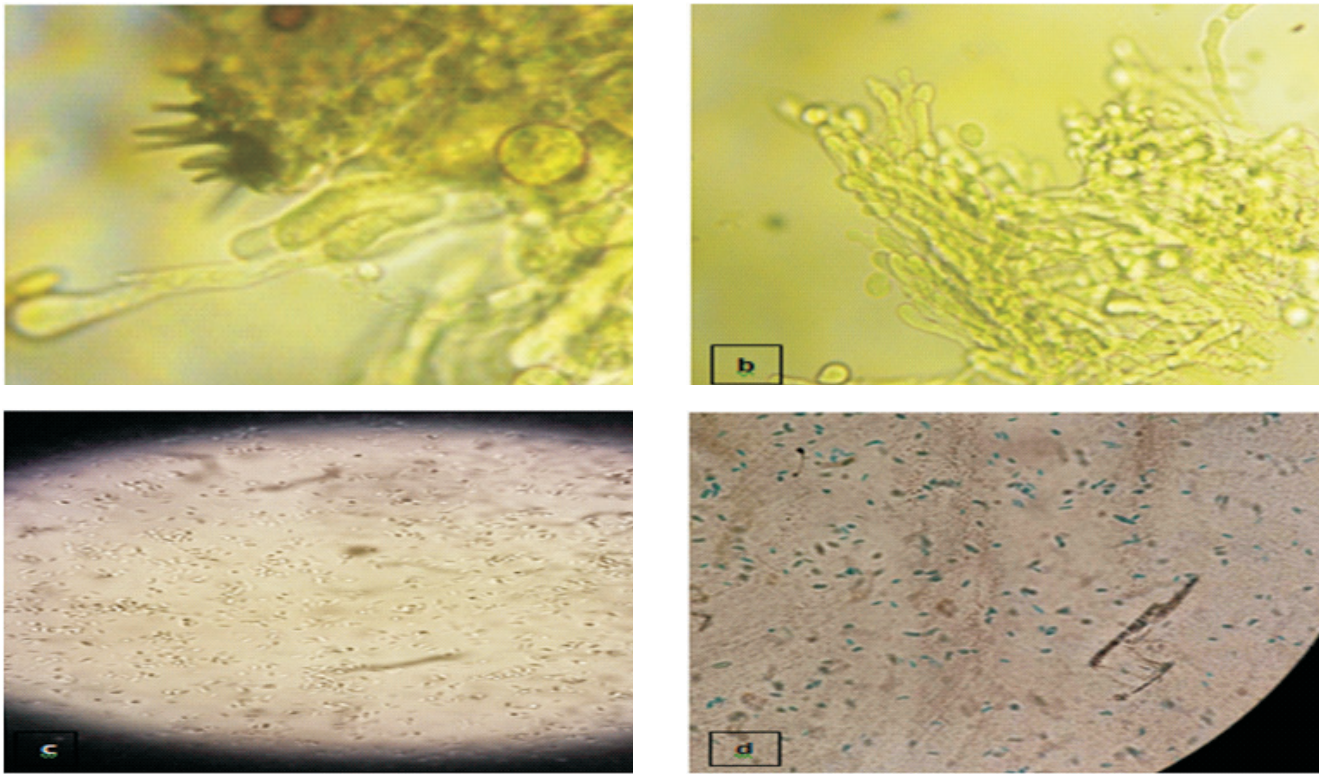

Figure 6: (a–b) Microphotograph of *B. Bassiana* with an oval conidium (c-d) cylindrical conidium of *M. rileyi*

was in accordance with Oliveira *et al*., 2010, Fernandes *et al*., 2006. Macroscopic examination (Figure 6) of Metarhizium rileyi showed a radial growth pattern, no raised elevation, and a smooth texture. Microscopic examination showed septate hyphae, hyaline mycelium, short conidiogenous cells with erect conidiophores, and conidia cylindrically 5-9μm. Similar results were reported in previous reports on *S. litura* (Dutta *et al*., 2014), H. armigera (Nandish *et al*., 2016), and *S. frugiperda* (Alvarez *et al*., 2018).

Further investigations, such as pathogenicity tests and field experiments, are needed for both *Beauveria bassiana* and *Metarhizium rileyi* so that they can be used in novel research approaches like developing a commercial and technically viable formulation.

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