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DIVERSITY OF SOIL BORNE ENTOMOPATHOGENIC FUNGI IN DIFFERENT LAND USES OF NORTH EAST INDIA

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ABSTRACT
Fungal pathogens which cause diseases in insects through the insect cuticle are called as entomopathogens. These entomopathogens are mostly soil borne in nature. It needs high humidity level to develop, and the conditions are well suited in North East India, an attempt has been made to study the diversity of soil borne entomopathogenic fungi in different land uses of Assam, Meghalaya and Nagaland States. Soil samples collected from different land use systems were subjected to insect bait by using *Galleria mellonella* for trapping the soil borne entomopathogens. A total of 17 isolates of 12 species of entomopathogens have been isolated from 97 soil samples collected. *Beauveria bassiana* and *Metarhizium anisopliae* are the entomopathogenic fungi which dominated in different forest ecosystem. Other important fungi isolated which includes *Verticillium lecanii* and *Pacilomyces* sp.

KEY WORDS: *Galleria mellonella*, entomopathogenic fungi, *Beauveria bassiana, Verticillium lecanii* and *Metarhizium anisopliae.*

INTRODUCTION

Microbial control is one among the method in pest control which employs microorganisms, including insect fungi, to reduce pest populations to acceptable levels. The entomopathogenic fungi play a major role in regulation of pest population (Ferron, 1981). A suitable physical environment, such as the moist and warm tropics, enhances the success of fungal infection. Entomopathogenic fungi are useful additions to integrated pest management (IPM) systems. It can be mass produced at low technology levels, and are safe to natural enemies, humans and the environment. The fungus *Metarhizium anisopliae* (Deuteromycotina: Hyphomycetes) is one of the most common entomopathogen with worldwide distribution as a component of natural soil flora (Scholte *et al*., 2004). *Metarhizium* has been widely researched and has been reported to have great potential for use as a biological control agent for the management of various insect pests (Inglis *et al*., 2001, Liu *et al*., 2003, Khashaveh *et al*. 2008).The influence of three cultivars of *Sorghum bicolor* (L.) on the activity of the fungus *M. anisopliae* on the stem borer *Chilo partellus* was investigated in the field and laboratory (Maniania et al. 1998). Mythili *et al*., 2010 reported dark herbage green or yellowish green or olivaceous colonies for surface culture of *M. anisopliae* after 7 days of incubation and the spore shape was cylindrical to oval measuring 7.5 mm. Senthilkumar and Murugesan (2010) earlier reported the biocontrol efficacy of *B. bassiana* against early developmental stages of *C. leayana* on nursery and young plantations of *G. arborea.* Moreover, *B. bassiana* was also found to infect the larvae of *Indarbela quadrinotata,* a serious pest of *Casuarina* (Balu *et al*., 1998). Prior *et al.* (1995) developed an inoculation technique to assay isolates of EPFs, mainly *Metarhizium* spp. against *Schistocerca gregaria*. Selvasundaram and Muraleedhran (2000) reported the occurrence of the *Beauveria bassiana* on the shot hole borer of tea. Taking into account of the substantial information available on the effectiveness and potential use of the entomopathogenic fungi in control of many insect species and also most of the entomopathogenic fungi are soil borne in nature, an attempt was made to collect the soil samples from various land use systems and the entomopathogenic fungi were isolated from the infected cadavers of *G. mellonella* through insect bait method.

MATERIAL & METHODS

Study area and period

Field surveys were carried out in different locations and different land use systems as well the natural forest ecosystems in the states of Assam, Nagaland and Meghalaya for the collection of soil samples during the period from 2013-2015.

Collection of soil samples

The soil samples were collected from the top soil down to 10-15cm depth with the help of a scoop. The soil samples collected from 3 to 4 spots of same location or each site were mixed and one sample of approximately half a kilogram was drawn. The samples were secured in clean polythene bags separately along with collection details and brought to the laboratory and stored in refrigerated condition at 5˚C**.** The samples were subjected to insect bait method within five to seven days.

Isolation of entomopathogenic fungi from the soil

The wax moth larvae *Galleria mellonella* were used to trap the fungi from soil samples. The soil samples collected and kept in polythene covers were moistened and filled in small 100 ml plastic containers. Grown up third instar larvae of *G. mellonella were* released into the soil

filled plastic containers for incubation for 14 days. Every day the soil was agitated to ensure that the larvae remain exposed to the soil. Diseased and mummified larvae were collected from the container for isolation of the entomopathogenic fungi. If no external fungal sporulation was seen, the dead larvae were surface sterilized by submersion in 1% sodium hypochlorite, followed by rinsing with sterilized water and allowed for sporulation in moistened Petri plates. The fungi isolated from the cadavers were subcultured in Sabouraud dextrose agar medium (SDA). The fungi isolated from the artificial media were again subcultured until the pure cultures were obtained and then the pure cultures were stored for further studies.

Identification of entomopathogenic fungi:

Microslides of the pure cultured fungi were prepared and identified by referring manual Atlas of Entomopathogenic fungi (Samson, 1988), Entomopathogenic fungal identification (Richarad, 2005) and A Manual of Soil Fungi (Gilman, 1998).

RESULTS & DISCUSSION

In Assam, soil samples were collected from 26 locations of different land use systems. In Nagaland, soil samples were collected from 17 places of different land use systems and in Meghalaya 13 locations of different land use systems were selected and 97 soil samples were collected.

Through insect bait method by using *G. mellonella*, 17 fungi were isolated from the infected insect cadavers. 17 isolates of 12 species of entomopathogens isolated from the infected cadavers were identified on the basis of colony characteristics and microscopic studies. *Fusarium oxysporum*, *Fusarium* sp. (2 isolates.) *Mucor* sp. (2 isolates) *Aspergillus ochraceus, A. flavus, Trichoderma hamatum, Trichophyton* sp.(2 isolates), *Beauveria bassiana* (2 isolates), *Penicillium chrysogenum, Verticillium lecanii*, *Paecilomyces* sp. and *Metarhizium anisopliae* (2 isolates) were recovered from the cadavers of *G. mellonella* (PLATE.1). Occurrence of EPFs was observed to be high in natural forest ecosystems as compared to the other plantations and land use systems (Table 1).

TABLE 1: Details of Entomopathogenic fungi trapped from different locations and land uses

S.No.	State	Location	Land use	EPF trapped	GPS reading Latitude/longitude
1.	ASSAM	Rani	Medicinal plants	*Fusarium* sp.	N 25º 58'47.8" E 091º 30'47.8"
2.		Rani	RF Mixed vegetation	*Beauveria bassiana*	N 25º 58'44.6" E 091º 30'48.3"
3.		Salna (Salbari)	Agar plantation	*Trichophyton* sp.	N 26º 26'37.2" E 092º 59'48.7"
4.		Lakla Namphay	Jhum land	*Fusarium* sp.	N 27º 24'42.0" E 095º 56'20.7"
5.		Ledo	Coal mine	*Mucor* sp.	N 27º 17'55.2" E 095º 46'27.4"
6.		Dehing Patkai (Wildlife sanctuary)	Mixed vegetation	*Trichoderma hamatum*	N 27º 14'46.4" E 095º 34'06.6"
7.		Kholabhyan/ Morigaon	Grassland	*Trichophyton* sp.	N 26º 14'09.5" E 092º 05'15.2"
8.		Pabitora	Natural forest	*Metarhizium anisopliae*	N 26º 14'35.3" E 092º 02'57.9"
9.	MEGHALAYA	Barapani	Pine plantation	*Aspergillus flavus*	N 25º 38'41.5" E 091º 53'29.2"
10.		Cheerapunji	Mixed vegetation	*Aspergillus ochraceus*	N 25º 16'57.0" E 091º 43'47.8"
11.		Umkhuti	Pitcher plantation, Aromatic plants	*Penicillium chrysogenum*	N 25º 40'46.7" E 091º 54'28.0"
12.		Bhoirymbong	Agriculture land	*Fusarium oxysporum*	N 25º 42'24.5" E 092º 00'41.4"
13.	NAGALAND	Chenuky	Mixed vegetation	*Verticillium lecanii*	N 26º 25'17.4" E 094º 22'42.8"
14.		Mankoi	Jhum land	*Pacilomyces* sp.	N 26º 22'05.3" E 094º 33'36.1"
15.		Puliebadge	RF Mixed vegetation	*Beauveria bassiana*	N 25º 39'08.8" E 094º 04'11.4"
16.		Kukidolong	Teak plantation	*Metarhizium anisopliae*	N 25º 46'31.6" E 093º 49'11.4"
17.		Puliebadge	Jhum land	*Mucor* sp.	N 25º 39'21.7" E 094º 04'02.1"

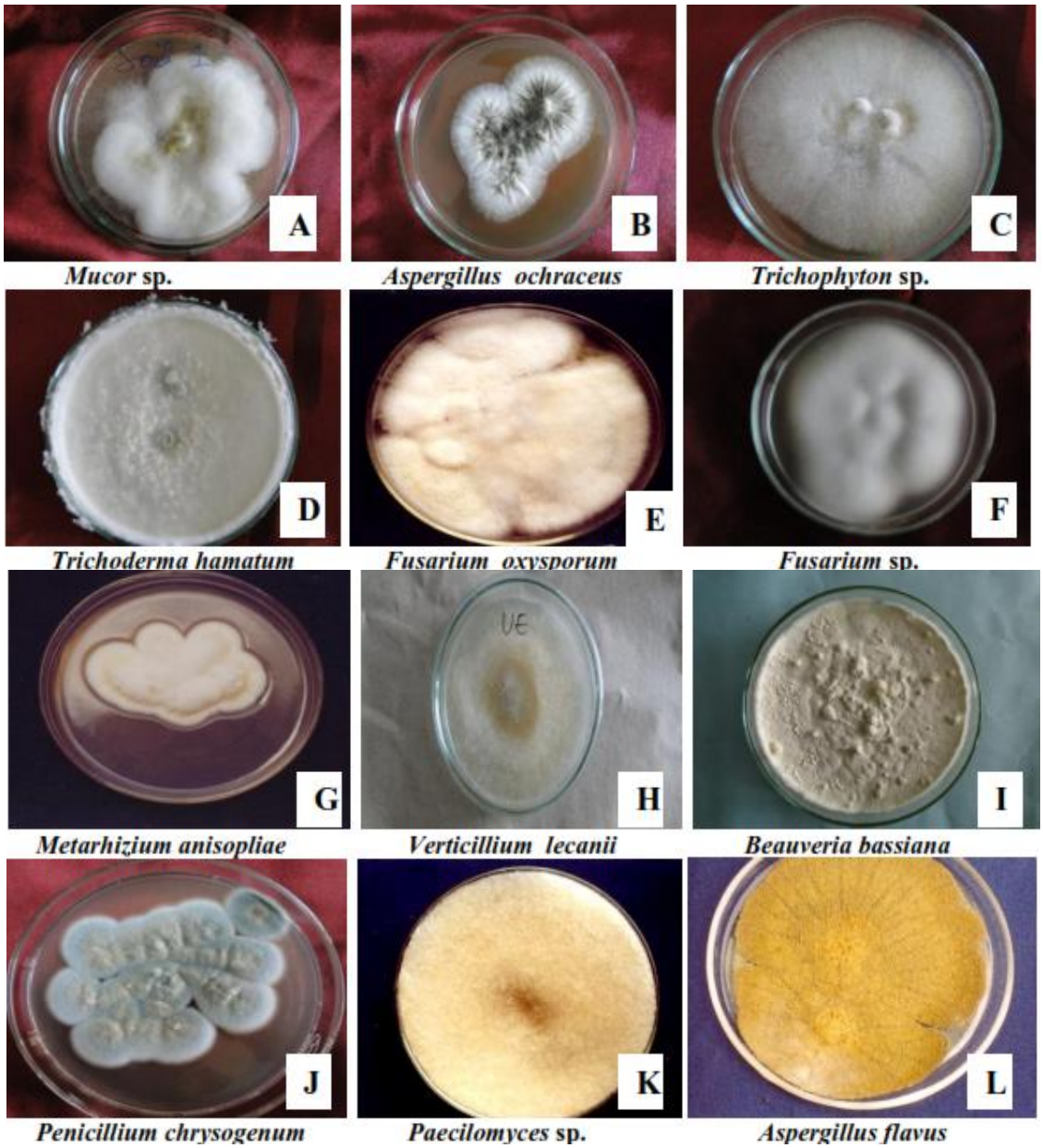

PLATE. 1 Entomopathogenic fungal cultures isolated from different soils through insect bait method

A. *Mucor* sp. B. *Aspergillus ochraceus* C. *Trichophyton* sp. D. *Trichoderma hamatum*
E. *Fusarium oxysporum* F. *Fusarium* sp. G. *Metarhizium anisopliae* H. *Verticillium lecanii*
I. *Beauveria bassiana* J. *Penicillium chrysogenum* K. *Paecilomyces* sp. L. *Aspergillus flavus*

Characteristics of Entomopathogenic fungi
Beauveria bassiana (Bals.-Criv.)Vuill.(1912)

Class : Sordariomycetes
Order : Hypocreales
Family : Clavicipitaceae

Beauveria bassiana, pale to yellow colour fungus has a wide host range and also attacks the larvae and adult stages. It grows in the soils naturally and acts as a parasite on various arthropods causing white muscardine disease. The conidia are white to cream in colour. The conidiophores are smooth and colourless, conidial chains often long and conidial heads diffuse. The shape of conidia is globose or in globose clusters. The length of the conidia is 1.5 -3.5µm. The spores are produced sympodially. Whenever a new spore was produced, the hyphal tip was used up as a new growth point. In this way a succession of spores were produced with the youngest spore at the tip and the spore head grow longer. When all the spores were dislodged the spore-bearing tip of the conidiogenous cell, had a zig zag appearance and was referred to as a rachis.

Metarhizium anisopliae (Metchnikoff) Sorokin (1883)
Class : Sordariomycetes
Order : Hypocreales
Family : Clavicipitaceae

Metarhizium anisopliae the yellow-green or green colour fungus has a wide host range and attacks the larval and adult stages. This is a soil borne fungus grows naturally in soils. It is being used as a microbial insecticide to control the insect pests such as termites, grasshoppers, larvae of moths and butterflies, beetles etc. The disease caused by the fungus is called green muscardine disease because of the green or pale green color of its spores. When these spores or conidia come into contact with the insect body, thereby it germinates and the hyphae that emerge penetrate the cuticle. The conidia are cylindrical, 7-9 µm long. The mycelium often wholly covers affected hosts, conidiophores in compact patches and individual conidiophores broadly branched densely intertwined. It forms chains usually aggregated into prismatic or cylindrical columns or a solid mass of parallel chains.

***Aspergillus flavus* Link (1809)**
Class : Eurotiomycetes
Order : Eurotiales
Family : Trichocomaceae

Aspergillus flavus is a saprophyte fungus found in soils. This fungus normally causes disease on some plants, sometimes infecting the lepidoptern insects, particularly in larval stages. The conidia are generally globose with smooth or decorated surfaces in chains on flask-shaped phialides with a short, broad neck on one or two layers of sterile cells. Over the phialides are the round conidia (2-5 µm in diameter) forming radial chains. Hyphae are septate and hyaline. The conidiophores originate from the basal of the supporting hyphae and terminate in to a vesicle. Vesicle is the typical identification for the genus Aspergillus. The morphology and color of the conidiophores varied from one species to another.

***Aspergillus ochraceus* Wilhelm, 1877**
Class : Eurotiomycetes
Order : Eurotiales
Family : Trichocomaceae

Aspergillus ochraceus is a saprophytic filamentous fungus that grows in soils. It has characteristic biseriate conidiophores. Its airborne spores are harmful to human beings causing asthma and lung diseases. The colonies grow very fast in SDA media. The conidiophores appear as a powdery mass. Smooth or finely roughened phialides are attached to the intermediate cells metulae (attached to the vesicle). The conidial heads are globose in initial stage, and then gradually the conidial chains adhere and develop into three to four divergent columns. The conidiophores are chalky yellow to pale yellow-brown in colour and are granular with pale yellow-brown wall attached abruptly to a globose vesicle. The diameter of the conidia is 2.5 – 3.5 µm.

***Fusarium oxysporum* Schlecht.**
Class : Sordariomycetes
Order : Hypocreales
Family : Nectriaceae

The mycelium of *Fusarium oxysporum* first appears white in colour but later changes to different colour variations and becomes purple, with discrete orange sporodochia. If sporodochia are abundant, the culture appears cream or orange in color. *F. oxysporum* produces three types of asexual spores: microconidia, macroconidia, and chlamydospores. Microconidia are one or two celled, abundant, mostly non-septate, ellipsoidal or cylindrical, straight or curved 2.5 – 3.5 µm. Macroconidia are three to five celled, gradually pointed and curved toward the ends 3-4.5 µm. These spores are commonly found on the surface of plants killed by this pathogen as well as in sporodochia like groups. Chlamydospores are round, thick-walled spores, terminally or intercalary produced on older mycelium. The spores are either one or two celled.

***Fusarium* sp.**
Class : Sordariomycetes
Order : Hypocreales
Family : Nectriaceae

Fusarium species are soil fungi and some are plant pathogens and sometimes infect the insects as opportunistic pathogens, particularly the lepidopterans. The pale or bright colonies are usually fast growing with a cottony aerial mycelium. The thallus varies the colour from whitish to yellow or pink shades. Macro and microconidia are produced from slender phialides. Macroconidia are three to several celled, gradually pointed, fusiform and curved toward the ends 3-4.5 µm. Microconidia are one or two celled, abundant, hyaline, smaller than macroconidia, fusiform to ovoid, straight or curved. Chlamydospores are round, thick-walled spores may be present or absent.

***Paecilomyces* sp.**
Class : Eurotiomycetes
Order : Eurotiales
Family : Trichocomaceae

Paecilomyces species is widespread in composts, soils and food products. Conidiophores erect, mononematous, but species on insects often synnematous, verticillate, bearing whorls of divergent branches and phialides; phialides flask-shaped or with swollen basal part, abruptly tapering into a distinct neck; conidia one celled, hyaline to slightly pigmented in mass 2.5 -3.0 µm diameter, produced in dry divergent chains, smooth-walled or occasionally spiny.

Trichoderma hamatum (Bonord.) Bainier
Class : Sordariomycetes
Order : Hypocreales
Family : Hypocreaceae

Trichoderma hamatum is a soil borne fungus with the conidiophores in pustules comprising a sterile elongation part arising near the base. The phialides arise from the short lateral branches at the base of the elongation. Lateral branches are comprised of one or a few broad cells with phialides arising at the tip and along the length, or sometimes the secondary branches arising from lateral branches also as single cells from which phialides arise. Conidiophore apical with elongations, sterile,

conspicuously extending beyond the surface of the pustules. The conidia are smooth, green, ellipsoidal with 2.7 – 3.0 µm dia.

Trichophyton sp**.**
Class : Eurotiomycetes
Order : Onygenales
Family : Arthrodermataceae

Trichophyton sp. is a soil borne fungus, and the molds characterized by the development of both micro and macroconidia. The microcondia are spherical in shape, pyriform to clavate, otherwise irregular in shape. The microcondia size range from 2 to 3 by 2 to 4 µm where as the macroconidia range from 4 to 8 by 8 to 50 µm in size. Macroconidia arise on the hyphae or on short pedicels, thin or thick walled, mostly fusiform.

Mucor sp**.**
Class : Mucormycotina
Order : Mucorales
Family : Mucoraceae

Mucor sp. is a soil borne fungus which grows very fast in SDA media and is typically coloured white to beige or grey. The mature colonies become brown in colour due to the spores. The sporangiophores are erect, hyaline, grey or brownish, simple or branched, forming large (60-300 µm in diameter), terminal, globose to spherical, multispored sporangia. A conspicuous collarette is usually visible at the base of the columellae after sporangiospore dispersal.

Verticillium lecanii
New name: ***Lecanicillium lecanii*** (Zimmerman) Zare & Gams.
Class : Sordariomycetes
Order : Hypocreales
Family : Clavicipitaceae

Lecanicillium lecanii is considered as entomopathogenic fungi infecting mainly the sap sucking pest like whiteflies, thrips and aphids. Therefore it is used as a biological pesticide. The conidiophores are differentiated from the vegetative hyphae, conidiogenous cells (phialides) in whorls (verticils) of 2-6, paired or solitary on hyphae. Conidia hyaline, aseptate, borne in slime droplets or dry chains, usually uniform in shape (2.2 – 3.5 x 1-1.5 µm).

Penicillium chrysogenum Thom (1910)
Class : Eurotiomycetes
Order : Eurotiales
Family : Trichocomaceae

The conidiophores are prominent, erect, bearing brush-like clusters of flask-shaped, short necked or neckless phialides forming long chains of globose (to ovoid) conidia with smooth or rough surfaces. The conidia are blue to blue-green in colors, and the mold exudes a yellow pigment. Earlier investigations using the insect bait method have confirmed the occurrence of EPFs in soil, of which the most important were *B. bassiana*, *M. anisopliae*, *P. farinosus* and *P. fumosoroseus* (Zimmermann, 1986; Vanninen *et al*., 1989; Kleespies *et al*., 1989). *Beauveria bassiana*, *P. farinosus*, *P. fumosoroseus* and *Metarhizium anisopliae* were isolated from the forest litter and soil using the Insect baits method. *B. bassiana* was the dominant species in all the localities. The practice of use of fungal pathogens for insect and mite control was reviewed by Burges (1981). Study conducted by Thungrabeab and Tongma (2007) suggests that *B. bassiana* was non-pathogenic to natural enemies like *Coccinella septempunctata*, *Chrysoperla carnea* and *Dicyphus tamaninii* as well as beneficial soil insect *Heteromurus nitidus.* The study of Kleespies *et al*. (1989) on the spectrum of EPFs in different kinds of soils in Germany confirmed the high frequency of *B. bassiana* in the forest soil.

CONCLUSION

The results of the present study confirm the occurrence of different entomopathogens in the soil of various landuse systems in Assam, Meghalaya and Nagaland states of North East India. A total of 17 isolates of 12 species of entomopathogenic fungi have been isolated from 97 soil samples collected. It was also found that the richness of diversity of the entomopathogenic fungi is more in the forest soil. Further studies is due to focus on the utilization of the virulent fungal pathogens among the isolates trapped sofar to be mass multiplied in cheeper media and to be evaluated on the important forestry pests.

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