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Current status and future prospects of Entomopathogenic fungi in North East India

Pranab Dutta[1], Joyarani Pegu[2], and Puzari, K.C.[3]
Department of Plant Pathology
Assam Agricultural University, Jorhat-785 013, Assam, India.
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ABSTRACT

North East India is recognised as the paradise for several microflora and fauna because of high (80-90%) relative humidity (RH) and other topographical factors. The region is bestowed with a rich biodiversity of entomopathogenic fungi and exploitation of these natural and renewable resources are essential for successful bio control strategy which can be use as an important component of IPM. The most significant progress in recent years in the region has come from studies of entomopathogenic fungi by a team of Scientists. They have isolated and characterized the entomopathogens like *Beauveria bassiana, Aspergillus* spp*, Fusarium* spp*, Pecicillium* spp*, Geotrichum* spp*, Mucor* spp, *Conidiobulous thromboides, Metarhizium anisopliae, Verticillium lecanii, Arthrinium urticae, Aschersonia aleyrodis, Cordyceps unilateralis, Nomurea reliyii* from the agro ecosystem of North East India. Pathogenicity and bio-efficacy test on the target pests showed their potentiality to use as an alternative to chemical pesticides. Molecular characterization of twelve indigenous isolates of *M. anisopliae* were done using polymerase chain reaction (PCR) amplification of polymorphic DNA using random primers (RAPD1 to RAPD12) and found highest similarity of 54.3 per cent and lowest similarity of 8.1%. *In vitro* efficacy of *B. bassiana* and *M. anisopliae* was tested with commonly applied agrochemicals at two different concentrations (lethal and sub lethal dose) and the insecticides were found to be less detrimental. Multilocational field performance of the standardized bioformulation of *B. bassiana* was found superior over the conventional practices. This has paved the way for further exploitation of other entomopathogenic fungi reported from the region.

Keywords: Entomopathogenic fungi, status, prospects

INTRODUCTION

The North Eastern region of India comprising the eight states of Arunachal Pradesh, Assam, Meghalaya, Manipur, Tripura, Mizoram, Nagaland and Sikkim has been in focus for its high biodiversity and this region has been a priority for leading conservation agencies of the world.

Recognition that fungi have potential for biological control of insect pests dates back to Louis Pasteur. During 1880s *Metarhizium* species was used to control wheat chafer, *Anisoplia austriaca* and the sugar beet curculio, *Cleonis punctiventris.* The genera such as *Metarhizium, Beauveria, Verticilium, Nomurea, Entomopthora, Neozygites* etc are commonly encountered in nature. More than 400 species of entomopathogenic (EP) fungi are recognized as entomogenous in nature. These fungi belong to five subdivisions: Mastigomycotina, Zygomycotina, Ascomycotina, Basidiomycotina and Deuteromycotina Fungal epidemics occur periodically and can cause high levels of mortality in affected arthropod populations. Only a few species of fungi are reported from the rice ecosystems. Most terrestrial species of fungi depend on high RH at several points of their life cycle. The species diversity and total number of natural enemies in tropical rice are impressive, but usually a few species dominate the complex. It is difficult to generalize about the precise role and relative importance of individual species.

Likewise, several fungal pathogens infect rice leafhoppers and plant hoppers. The fungus infects the body of the hopper and when the insect is dead, fungus emerges from the body through the joints. Common pathogens are (Metchnikoff) Sorokin (Moniliales: Moniliaceae), *Metarhizium flavoviride* Gams and Roszypal, *Beauveria bassiana* (Balsamo) Vuillemin, *Hirsutella citriformis* Speare (Moniliales: Stilbaceae),*Erynia delphacis* (Hori.) (Entomophthoraceae), *Entomophthora coronata*

*Corresponding Author. Email: pranabdutta74@gmail.com

(Constantin) Kevorkian (Entomophthoraceae) and *Fusarium* sp. From 15 years of research work conducted at Assam Agricultural University, Jorhat, Assam, technology has been generated to mass production, formulation and field application of *B. bassiana,* for management of *D. armigera* in Assam (Hazarika *et al.*, 2005; Puzari *et al.,* 2006).

B. bassiana has a wide host range and is reported from all over the world; however, it is found to be more predominant in the tropics and sub-tropics under moist and wet conditions. Nevertheless, it was also reported from the temperate regions. The rice ecosystem, being moist and wet, serves as a favourable environment for exploitation of mycoinsecticides including *B. bassiana* and *Pandora delphacis*.

M. anisopliae, the green muscardine fungus, has been the subject of experiments at intervals extending over 80 years, but from the forgoing account it is evident that in no case has any result been demonstrated as sufficiently successful to warrant its adoptability as a means of destroying insects. In Brazil a number of semi commercial preparations are in vogue in the various regions against diverse pests. Another pathogen, *Hirsutela* sp., is specific to rice mite, *Oligonychus indicus*. The cadaver is usually attached to topmost leaves of the rice plant. The whole body of the mite is engulfed in mycelial filaments radiating in all directions. The locomotory appendages are left uninfected. Early symptoms of the disease are difficult to diagnose due to small size of mites. The first sign of infection is the sluggish movement of mites and later, change their body colour from normal lemon yellow to deep yellow brown.

Baruah *et al.,* (2003) reported many opportunistic fungi attacking different rice pests. For example, diseased nymphs and adults of grasshopper, *Hierglyphus banian* infected by *Aspergillus flavipes* exhibited grayish brown fungal outgrowths on their lateroventral sides. Hyphae sometimes develop conidiophores and emerge out even from the live hosts. It is of significance that most records of premortem emergence or sporulation by Hyphomycetes concern species of *Aspergillus*. The entomogenous fungus, *Conidobolus thromboides,* causes infection naturally on the rice bug, *Leptocorisa acuta* and appear as white cottony mycelial growth on meso and meta thoracic eusternites and abdominal pleurites. *Nomuraea rileyi*, infects the larval stages of the rice cut worm. Coppery green fungal spores are deposited all over the body except the head capsule. Thus severely infected larvae are mummified and the body becomes brittle after exposure to sunlight. The mycelia are pinkish white in colour, which upon sporulation, become typical malachite green.

The genus *Cordyceps* is a cosmopolitan Ascomycete confined almost entirely to insects. About 200 species are recognized attacking a great variety of immature and adult insects. *Ophiocordyceps* is a closely related genus. *Cordyceps* is characterized by the growth of long fruiting stems, sometimes branched, arising from a sclerotium within the body of the insect. Certain genera of Fungi Imperfecta as *Isaria*, *Botrytis* and *Hirsutella* in part are considered as conidial stages of *Cordyceps*. When a *Cordyceps* spore under favourable conditions strikes an insect, the germ tube penetrates the integument. On reaching the haemocoele, they soon fill the cavity; absorb most of the blood and the host dies. The mycelium attacks and absorbs most of the tissue distending the insect integument to near normal size. The sclerotium thus formed may remain dormant for months or in favourable situations develops the perfect conspicuous fruiting stems.

RECORD OF ENTOMOPATHOGENIC FUNGI OF NORTH EAST INDIA

Except a few earlier works, little efforts have been made to study entomopathogenic fungi from North East region of India (Roy and Puzari, 1979) especially on rice ecosystem, though the area is recognised as the paradise for fungi (Saikia,1986) because of high (80-90%) relative humidity (RH) and other topographical factors.

Natural occurrence of entomopathogenic fungi and its pathogenecity on target host of North East India:

During April-May 1977, a large number of banana leaf beetle (*Nodostoma subcostatum*) infesting banana in the horticultural orchard, Assam Agricultural University (AAU), Jorhat, were found to be infected by white muscardine fungus causing death of the pests (Roy and Puzari., 1979). They noticed that white frosty growth of the fungus first emerges through the suture of elytra, thoracic segment and ventral side of abdomen of the death insect which ultimately covered the whole body. They also found that in most of the cases the infected beetles were fixed to the banana leaves by the fungal mass. Later on they identified the fungus as *B. bassiana* (Bals.)Vuillemin (IMI215268) which have spore of single celled, hyaline, ellipsoidal or oval, 2.5-4.5x1.5-2.4μ in size. *In vitro* assays against the beetle showed a mortality of 71 % per cent. The fungus could also be reisolated on Potato dextrose agar (PD). During the *in vitro* assay they observed that artificial inoculation cause infection after 4 to 5 days when room temperature varied from 29 - 34p C.

Among the major insect pests of rice in North East India, rice hispa *Dicladispa armigera* (Olivier) (Coleoptera : Chrysomelidae) causes extensive damage to the vegetative stages of the crops grown in Boro, Ahu, Bao and Sali seasons covering 8.5 lakh ha. In Assam the post flooded rice suffers 100% loss due to its attack. Extent of damage may range from 35-65 % loss in yield. Besides rice, the rice hispa attacks other crops like wheat, maize and sugarcane and several grasses and sedges belonging to the Poaceae, Cyperaceae and Commelinaceae.

Control of rice hispa has been a major problem for farmers who depend primarily on rice as subsistence crop. Chemical control may sometimes proves to be detrimental by creating additional problems, such as environmental hazards, development of insect resistance and resurgence, killing non target organisms especially aquatic fauna and so on. Hence, a search for alternative to these agents which are compatible with a range of control tactics for *D. armigera* has resulted in identifying an entomopathogenic fungus *B. bassiana* (Hazarika and Puzari 1990). Subsequently, a field survey in rice ecosystem on rain fed rice was conducted by Puzari *et. al.,* 1992 and recovered 240 cadavers of rice hispa, *D. armigera* (Olivier) (Coleoptera: Chrysomelidae). The entomogenous association formed/established by the fungi on the insects were *B. bassiana, A. flavus, Fusarium hetetoporum* Nees ex Fr., *Penicillium cyclopium* Westling, *Geotrichum* sp. and *Mucor* sp. The former three were found as entomopathogenic while the remaining two were of opportunistic in association. *B. bassiana* and *A. flavus* showed their potentiality as bio control agents (Table 1).

Penicillium cyclopium was characterized by granular colonies of dull blue-green colour, finely roughened conidiophores (on Czapek agar) and pre-dominantly sub-globose spores.

The mole cricket, *Gryllotalpa africana* P.de B. is reported as a minor pest of tea seedlings in the nursery (TRA, 1994) and was recorded as one of the soil macro fauna in the tea ecosystem (Kakoty, 1982). Soil treatment with endosulfan and chloropyriphos is recommended for its control (TRA, 1994). During a routine search for an alternative, two entomogenous fungi have been identified (Hazarika *et. al.,* 1994) to be associated with the insect which cause epizootics in the population. This report further describes the disease symptoms, taxonomic accounts of the species and their pathogenecity on the mole cricket. Two fungi, *Paecilomyces carneus* (Duche et Helm) Brown and *Scopulariopsis* sp. acted as naturally occurring entomopathogens on the mole cricket, *Gryllotalpa africana* P.de B., a minor pest of tea. The former caused 37% mortality on the tested population. However, the latter was an opportunistic fungus causing 40% mortality in combination with *P. carneus.*

Table 1. Entomogenous fungi identified from *D. armigera*, their occurrence and pathogenecity under field and laboratory conditions

Fungi	Nature of association	Percentage of insects infested with fungi under field condition	Percentage of insects killed on laboratory inoculation
Beauveria bassiana	*EP	2	>90
Aspergillus flavus	EP	18	50
Fusarium heterosporum	EP	16	7
Penicillium cyclopium	**EO	38	0
Geotrichum sp.	EO	32	0
Mucor sp.	EO	20	0

*EP= Entomopathogenic; **EO=Entomoopportunistic. Source: Puzari *et. al.,* 1992

B. bassiana occurred in field conditions of Sensua Pathar, Sivsagar at low levels in comparison with other fungi. On adults of rice hispa, white frosty growth of *B. bassiana* first emerged through the intersegment sutures and lateroventral sides of the thorax and abdomen which ultimately covered the whole body and appendages (Hazarika and Puzari, 1990). The fungus was confirmed and deposited on IMI335352.

A. flavus Link were isolated both from the adults and eggs of rice hispa. The identification was confirmed and deposited on IMI335354.

F. heterosporum develops extensive red staining over adults as well as on larvae. Although, the association was also observed on dead eggs of the rice hispa in field condition, the fungi could not be isolated from eggs. The fungus was more prevalent on glutinous rice varieties than the non glutinous varieties.

Hazarika and Puzari, 1989, 1990 and Puzari and Hazarika, 1992 have identified and isolated few entomopathogenic fungi from *D. armigera* under rain fed crops and tested their efficacy. Baruah *et. al.,* (2003) reported association of four different entomogenous fungi with six species of insect pests belonging to Hemiptera, Orthoptera and Lepidoptera in the rice field of Assam. The entomopathogenic fungi so obtained were *Conidiobulus thromboides, Fusarium oxysporum, Aspergillus flaviceps* and *Beauveria bassiana.* The pathogenicity test of the fungi on the respective insect pests confirmed their pathogenic relationship with a varying degree of infectivity. Based on the mortality percentage, *B. bassiana* and *F. oxysporum* with more than 50% mortality were found to be potential for exploitation as bio control agents (Table 2).

Five different species of entomogenous fungi were isolated from six species of insect's pests belonging to Hemiptera, Orthoptera, and Lepidoptera. Out of these *B. bassiana* was found to be highly pathogenic followed by *F. oxysporum*. *F. oxysporum* occurred in field condition of ICR farm, Jorhat at very low level (6.65%). The initial site of infection was observed to be inter segmental surfaces between the metasternum and the first abdominal sternum.

Table 2. Entomogenous fungi identified from different insect pests of rice, their occurrence and pathogenecity under field and laboratory conditions

Insect pests species	Place of collection	Fungi associated with cadavers	Field occurance (%)	Mortality (%) under laboratory condition
Southern green stink bug, *Nazara viridula* (Linn.) (Hemiptera:Pentatomidae)	Instructional Cum Research (ICR Farm, AAU, Jorhat	*Fusarium oxysporum*	6.65	51.96
Rice case worm ,*Nymphula depunctalis* (Guenee) (Lepidoptera: Pyralidae)	ICR Farm, AAU, Jorhat	*Beauveria bassiana*	36.69	88.43
Paddy stem borer, *Scripophaga incertulus* (Walk) (Lepidoptera:Pyralidae)	Sensua Pathar, Sivsagar and ICR Farm, AAU, Jorhat	*Arthrinium urticae*	1.50	7.00
Rice ear bug, *Leptocorisa acuta* (Thub.) (Hemiptera:Alydiadae)	Sensua Pathar, Sivsagar	*Conidiobulus thromboides*	3.60	24.31
Short horned grasshopper, *Heiroglyhus banian* (F) (Othoptera: Arctidae)	Jakai Chuck, Sivsagar	*Aspergillus flavipes*	0.12	36.21
Tiger mot, *Creatonotos gangis* (Linn.) (Lepidoptera: Arctidae)	Lepai Pathar, Sivsagar	*Beauveria bassiana*	0.25	74.66

Source: Boruah *et. al.*, 2003

B .bassiana was isolated from larvae of *N. depunctalis* collected from ICR farm, Jorhat which causes an infection of 36.69 per cent. The cadavers were found to cover with white mycelia growth over the entire body surface.

A very low per cent (1.50) of field occurrence of the fungus *Arthrinium urticae* was found associated with the rice stem borer collected from Sensua Pathar, Sivasagar. White fluffy mycelia growth was observed on the larval body surfaces. Mycelia were visible inside the tunnels. Mycelium are branched, septate, and hyaline to pale brown, smooth walled walled, 1.5-2µm thick; conidia spherical to sub-sperical5-6.5 (-7µm diameter. Laboratory inoculation of the fungus was found to be low pathogenic and kills 7.00 per cent of host after 8 days of inoculation.

The fungus *Conidobolus thromboides* was isolated from cadavers collected from Sensua Pathar, Sivasagar with having 3.60 per cent of field occurrence. The fungal mycelia growth was prominent on Meso and Meta coax. White cottony mycelia growth was also found localized on the ventral surface. Mycelia septate, ramifying light brown in colour forming coenocytes segment separated by the decay of intervening length of evacuated hyphae, measuring 3.32µm in diameter. Conidiophores single celled, short terminated with bulbous tip bearing conidia. Conidia spherical to subspherical with a smooth and rounded apex, thick walled, scattered dark brown in colour measuring 13.28-18.36µm in diam. An infection of 24.31 per cent was recorded in the laboratory condition.

Diseased nymphs and adults were collected from rice fields of Jakaichuk, Sivasagar where a very low per cent (0.12%) of natural occurrence of the fungi was recorded.

In the rice field of Lepai Pathar, Sivasagar 0.25 per cent of natural infection of the fungus *B.bassiana* on the tiger moth was observed.

Six fungal species were found associated with 14 different insects' species of the order Lepidoptera, Hemiptera, Coleoptera, Diptera and Hymenoptera (Dutta *et. al.,* 2012). Pathogenecity test confirms their pathogenic relationship with a varying degree of infectivity. The pathogenic fungus were characterised and identified as *Metarhizium anisopliae*, *Beauveria bassiana, Aschersonia aleyrodis, Nomuraea rileyi, Cordyceps unilateralis* and *Aspergillus* spp.

Pathogenecity test for the isolated microorganism on the respective insect host confirms their pathogenic relationship with varying degrees of infectivity. The pathogenic fungus were characterised and identified as *M. anisopliae, B. bassiana, Nomurea rileyi, Aschersonia aleyrodis, Cordyceps unilateralis* and *Aspergillus* spp. out of which *A. aleyrodis* was found to be highly pathogenic (81.28%) to mulberry aphid followed by *N. rileyi* (73.32%) and *B. bassiana* (72.67%) respectively on cabbage looper and cabbage butterfly mortality per cent reduced under laboratory conditions due to *B. bassiana* on white fly, tea mosquito bug, black ant, dung beetle and white grub was recorded as 68.77, 65.55, 48.76, 43.45 and 43.33 per cent respectively. Similarly 63.46 per cent was recorded in citrus whitefly due to *A. aleyrodis*. Lowest mortality per cent (23.45) was recorded in spider due to *Aspergillus* spp. Exploitation of the entomopathogenic fungi like *M. anisiopliae, B. bassiana, A. aleyrodis, N. rileyii* and *Aspergillus* spp may act as significant factors in long lasting, stable ecosystems, were they exert a steady (enzootic) control of arthropod population (Table 3).

Cutworm or greasy surface caterpillar, *Agrotis ipsilon* is an important pest of potato especially in transplanted potato seeds. The seedlings mortality may be as high as 25-30 per cent within 10-20 days of germination. A naturally occurring entomopathogenic fungus growing on adult cadavers of *A. ipsilon* was noticed during an extensive survey of cutworm conducted on potato fields of Instructional-cum-Research Farm, Assam Agricultural University, Jorhat during 2004-06 (Bhattacharyya *et al.,* 2008). The cadavers infected with the fungus were collected from the potato fields and the fungus was isolated, purified on Czapek-Dox agar medium and identified as *M. anisopliae var. anisopliae* (Metsch.)

Table 3. Entomopathogenic fungi identified from different insect pests, their occurrence and pathogenecity test under laboratory condition

Insect spp.	Order	Family	Stage of infection	Fungal spp	Mortality (%) under laboratory condition
Cabbage butter fly, *Pieris brasicae* (L.)	Lepidoptera	Pieridae	Larvae	*Metarizium anisopliae*	72.67
White grub, *Anomela* spp. *Apogonia* spp	Coleoptera	Scarabaeidae	Grub	*M. anisopliae*	43.33
Spider, *Oxypes* spp.	Acaradiae		Adult	*Aspergillus* spp.	23.45
Sugarcane shoot borer, *Chilo infuscatellus* (Snellen)	Lepidoptera	Pieridae	Larvae	*B. bassiana*	21.22
White fly, *Bemisia tabaci*	Hemiptera	Aelyrodidae	Adult	*B. bassiana*	68.77
Tea mosquito bug, *Helopeltis theivora* (Water)	Hemiptera	Miridae	Adult	*B. bassiana*	65.65
Dung beetle, *Catharsius molossun* (L)	Coleoptera	Scarabaeidae	Adult	*B. bassiana*	43.45
Black ant, *Diacamma rugosum* (Rogor)	Hymenoptera	Poneri	Adult	*B. bassiana*	48.76
Cabbage looper, *Spodoptera litura* (Fabricius)	Lepidoptera	Noctuidae	Larvae	*Nomurea rileyi*	73.32
Mulberry aphid	Hemiptera	Aphididae	Adult	*Aschersonia aleyrodis*	81.28
Cutworm, *Agrotis ipsilon* (Hunfnagel)	Lepidoptera	Noctuidae	Grub	*M. anisopliae*	42.34
Citrus white fly, *Dialeurodes citri* (Ashmead)	Hemiptera	Aelyrodidae	Adult	*Aschersonia aleyrodis*	63.46
Muga silkworm, *Antheraea assamensis* (Helfer)	Lepidoptera	Saturniidae	Larvae	*Cordyceps unilateralis*	9.23
Rice Weevil, *Sitophilus oryzae*	Coleoptera	Curculionidae	Adult	*B. bassiana*	70.59

Source: Dutta *et. al.,* 2012

Sorok, var.*anisopliae.*

The cowpea aphid, *Aphis craccivora* Koch (Homoptera: Aphididae) is ubiquitous and is also responsible for transmission of cowpea aphid borne mosaic virus in persistent and non persistent manner resulting in substantial damage to the crop especially early in the season. Four different entomopathogenic fungi were also isolated from seven different insects' species belonging to the order Lepidoptera, Hemiptera, Isoptera, Homoptera and Orthoptera from vegetable growing areas of Majuli (the largest river island of the world) under the Jorhat. The pathogenic fungi were characterised and identified as *M. anisopliae, B. bassiana, N. rileyi and Fusarium* sp. (Pegu et al., 2012; Pegu *et al.,* 2013) (Table 4).

Termites are an important pest of agricultural, horticultural and plantation crops, forest trees, structural timbers and various wood and textile products. Tea (*Camellia sinensis* L (O) Kuntze), being a perennial crop attracts insects and mites that thrive and flourish on tea. In the tea growing areas of Barak Valley, termites are the major and predominant among the pest causing considerable damage to tea (Das, 1962; Barbora, 1994). Termites are considered as good candidate for control with the entomopathogenic organisms because they live in conducive environment-humid, minimal diurnal temperature fluctuations, crowded and with considerable social interaction. The pathogenecity of *M. anisopliae* (Metschnikoff) Sorokin and *B. bassiana* (Balls.) Vuill. were evaluated against the workers of termite *Microtermes obesis* Holmgren in the laboratory (Singha *et. al.*, 2010). The treated insects showed changes in their normal behavior and in morphology. With the help of scanning electron microscopy the minutiae deatails of morphological changes in the cuticles and cuticular sensilla present in various locations of the body were revealed. The observations suggested that the ventral cuticle of the abdomen have been totally distorted along with the deformation in sensilla trichoidae. Fungal colonies were also clearly visible throughout the ventral portion of the body, which suggest that fungal growth can cause serious

Table 4. Entomopathogens isolated from insect pests of North East India:

Insect pest species	Host crop	Order	Family	Stage of infection	Fungal spp.
Brinjal aphid, *Myzus persicae*	Brinjal	Hemiptera	Aphididae	Nymph and adult.	*Metarhizium anisopliae , Beauveria bassiana*
Citrus aphid, *Toxoptera aurientii*	Citrus	Hemiptera	Aphididae	Nymph and adult.	*Fusarium* spp.
Cowpea aphid, *Aphis craccivora*	Cowpea	Hemiptera	Aphididae	Nymph and adult.	*Metarhizium anisopliae*
Short horned grasshopper, *Heiroglypus banian*	Rice	Orthoptera	Acrididae	Nymph and adult.	*Fusarium* spp.
Termite, *Odontotermes obesus*	Tea	Isoptera	Termitidae	All stages	*Metarhizium anisopliae*
Tobacco caterpillar, *Spodoptera litura*	Gerbera	Lepidoptera	Noctuidae	Larvae	*Nomuraea rileyi*
Whitefly, *Bemisia tabaci*	Guava	Homoptera	Aleyoridade	Maggot	*Beauveria bassiana*
Chiili aphid, *Aphis gossypii*	Chilli	Hemiptera	Aphididae	Nymph and adult.	*Metarhizium anisopliae*

Source: Pegu *et al.,* 2013

damage to the pest disturbing its major physiological activities resulting in its death.

BIOEFFICACY OF ENTOMOPATHOGENIC FUNGI UNDER LABORATORY CONDITIONS; PATHOGENICITY OF *B. BASSIANA* TO RICE HISPA

The white muscardine fungus *(B. bassiana*) occurs naturally and is found as an effective bio control agent on rice hispa (*D. armigera*) (Hazarika and Puzari 1990, Puzari and Hazarika 1991, 1992, Puzari *et al.,* 1994), a serious insect pest of rice (Hazarika and Dutta 1991, Puzari and Hazarika, 1992). It has been utilised for the control of many insect pests of rice and recorded as the most versatile fungus capable of attacking various stages of the host at different maturity stages. Thus an experiment was conducted (Puzari *et. al.,* 1994) during summer, autumn and spring seasons of 1990, 1991 and 1992 to study the infection of the white muscardine fungus on eggs and adults of rice hispa in rice fields of Sivasagar district.

The field and laboratory tests confirmed its pathogenicity on eggs, larvae, pupae and adults. In the field, infection on eggs was 16.95-45.15%, depending on the seasons, being more than that during other stages. Low (1.67%) to very high (40.63%) level of infection of adults was observed in summer, autumn and spring seasons (Hazarika and Puzari, 1995).

Entomopathogenecity of *B. bassiana* against *Odontotermes*:

Tamuli *et. al.,* 2011 conducted pathogenecity of *B. bassiana* (Bals.) Vuill. (BBFF-135) by, a strain isolated from bark beetle (*Ips trpographus*), against the worker termites of *Odontotermes obesus* (Rambur) under laboratory conditions. The fungus was applied as conidia and significant mortality rate of *O. obesus* was obtained. The conidial count and their respective concentrations varied significantly (P=0.05) in direct proportion up to 28 days in terms of conidial dilution and incubation period of the fungal culture. Higher mortality rate was observed with increasing conidial concentration of *B. bassiana* which significantly differed in relation to incubation period and conidial concentration of the fungus. The days of exposure of termites to *B. bassiana* for obtaining the observed mortality was inversely proportional to the incubation period as well as the conidial concentration. *B. bassiana* (BPFF-135) required higher conidial concentration and shorter exposure period to kill 50 percent of the termite workers. Ninety percentage mortality was not achieved after seven days exposure except at conidial concentration $1x10^6$ conidia per ml^{-1} of *B. bassiana* incubated for 28 days.

Bio-efficacy of *Metarhizium anisopliae* (Metchnikoff) Sorokin against cowpea aphid, Aphis craccivora Koch

Nine different conidial concentrations of *M. anisopliae* ranging from $1x10^3$ to $1x10^{11}$ conidia/ml were tested to study the bio-efficcay of *M. anisopliae* against 150 cow pea aphids, *Aphis craccivora* Koch under *in vitro* condition. After the adult aphids have been released to the cowpea twigs raised in 250 ml Erlenmeyer flasks filled with distilled water, the respective concentrations of all the fungal spore suspensions were sprayed on the twigs using a Holm Sprayer. Aphids After spraying, the cowpea twigs were kept under lantern chimney to avoid the escape of aphid population and to maintain the humidity. White paper (JK copier, Size: A4) was placed under the conical flask for the easy collection of dead aphids. Mortality of aphids was recorded separately at 24 h interval up to five days of treatment. Dead aphids were collected daily, and placed in petri dish containing a sterilized moist Whatman No-1 filter paper and kept in humid/ growth chamber (ORBITEK, Scigenics Biotech) at 25 ± 1^0 C, RH: 85± 1 per cent with 12 hr light and dark period. The dead aphids which produced mycelia growth were considered for the mortality count The data on bio-efficacy of the fungus *M. anisopliae* on cowpea aphid, *A. craccivora* at 1day after treatment (DAT) revealed that there was 2% mortality of the pest at the conidial concentration of 1×10^3 conidia/ ml. However, the highest mortality (8%) of aphids was recorded at $1x10^{11}$ conidia/ml followed by $1x10^{10}$conidia/ ml with mortality of 7.33 %. This was further followed by $1x10^9$conidia/ml with mortality of 6.67 %. At 2 DAT, conidial concentration of $1x10^{11}$conidia/ml resulted significantly highest mortality (16.44%). Mortality rate recorded for conidial concentration of $1x10^{10}$ and $1x10^9$ conidia/ml with 12.93 and 11.69 % respectively were statistically at par with each other. Similarly, at 4 DAT, the aphid mortality was significantly highest (55.35%) in $1x10^{11}$conidia/ml. Among the different treatment the significantly least aphid mortality was noticed in $1x10^3$ conidia/ml (5.56%). Similarly at 5 days after spraying the highest mortality rate of aphid (83.83%) was recorded in $1x10^{11}$ conidia/ml followed by $1x10^{10}$conidia/ml (64.72%). This was followed by $1x10^9$conidia/ml and $1x10^8$conidia/ml with a mortality rate of 52.96 and 46.35 per cent. Over all, $1x10^{11}$conidia/ml has recorded highest mortality rate (83.83%) as compared to other treatment after five days of treatment. (Pegu, 2013, Unpublished Thesis Work)

Bio-efficacy of *Metarhizium anisopliae* against *Odontotermes obesus* Rambur (termitorium soil based medium)

Bio-efficacy of *M. anisopliae* against *O. obessus* was tested against adult's *O. obesus* of tea ecosystem of Assam (Pegu, 2013 , Unpublished Thesis Work) The mortality of termites at 1 DAT ranged from 0.67 to 6.67 per cent. Conidial concentration of $1x10^{11}$ conidia/ml showed highest mortality (6.67%). Similarly, at 2 DAT, conidial concentration of $1x10^{11}$ conidia/ml recorded highest mortality (16.00%).At 3 DAT the mortality of termites varied from 4.00 - 26.67 % among the treatments after exposure to *M. anisopliae* as compared to no mortality in control. The highest mortality (26.67%) was recorded in case of $1x10^{11}$conidia/ml. Similarly on 5 DAT 47.15%, on 6 DAT, 61.89% while on 7 DAT, the highest mortality was recorded in $1x10^{11}$ conidia/ml treatments with 83.88 %.

EFFECT OF ENTOMOPATHOGENIC FUNGI ON HOST PHYSIOLOGY

Morphology of haemocytes of healthy and *B. bassiana* infected *D. armigera* adults was examined in a phase contrast microscope to characterize them into different types (Phukan *et. al.,*2008). The total haemocyte count (THC) and the differential haemocyte count (DHC) were also estimated. Four types of haemocytes were identified: prohaemocyte, plasmatocyte, granulocyte and spherulocyte. In the healthy adults, the THC varied between 5055 and 5950/mm3, out of which 85% were composed of granulocytes and plasmotocytes. As B. bassiana infection time increased, THC decreased and was associated with significant concomitant increase in granulocytes and a decrease in prohaemocytes. Granulocytes reacted in various ways, including disintegration of the plasma membrane, formation of fine pseudopod-like cytoplasmic extensions and finally, clumping of cells. Granulocytes and plasmatocytes were the predominant haemocytes involved in all the cell mediated defence reactions.

ULTRASTRUCTURAL DETAILS OF MORPHOLOGICAL CHANGES DUE TO ENTOMOPATHOGENIC FUNGAL INFECTIONS

In termite (Microtermes obesi Holmgren) pest of tea exposed to entomopathogenic fungi in vitro

Singha *et al.,* 2010 evaluated the pathogenecity of *Metarhizium anisopliae* and *B. bassiana* were evaluated against the workers of termite *Microtermes obesi* in the laboratory. The treated insects showed changes in their normal behaviour and in morphology. With the help of scanning electron microscopy the minutiae details of morphological changes in the cuticles and cuticular sensilla present in various locations of the body were studied. The observation suggested that the ventral cuticle of the abdomen had been totally distorted along with the deformation in sensilla trichoidea. Sensilla trichoidea are known to function as mechanoreceptor or contact chemo receptors and are distinguished from other sensilla in being or relatively greater in length having thicker walls. Deformation of both these sensory structures may disturb the insect in sensing its micro habit leading to abnormal behavior. Fungal colonies were also clearly visible throughout the ventral portion of the body, which suggested that fungal growth could cause serious damage to the pest disturbing its major physiological activities resulting in its death. The adverse effect of both the fungus appears to be relevant because the success of insect as terrestrial animals in resisting desiccation, breathing, and adapting to adverse environmental conditions is directly related to the proper functioning of the cuticle. The abnormal ultra structural features of the cuticle and sensilla of the pest insect *M. obesi* suggest that the fungal growth could cause serious damage to the insect, disturbing its physiological function ultimately leading to death. The abnormalities in the cuticle and sensory system of the insect indicated that the fungus *M. anisopliae* and *B. bassiana* changed the physiology of the insect leading to unusual behavior like sluggishness in movement, decreased feeding, shrinkage of the body surface, colour change, brittleness of the appendages, and ultimately death. However, further biochemical studies are needed to confirm the exact nature of the toxicant.

MASS PRODUCTION

One of the greatest obstacles for biological control by introduced agents has been lack or scarcity of methods for mass culturing and delivering the bio agents. The unique problem in developing bioformulation is that it represents a living system, which must be able to stand the process of formulation and should remain sufficiently viable for a period until it reaches the end users. Despite the limited progress, scientists are engaged in developing effective experimental system for growth and delivery of bio agents. After a continuous effort made by the team working on entomopathogens, we have standardize the mass production technique of *B. bassiana.* The solid – state artificial media contains rice husk: saw dust: rice bran (1:1:4) + 2% dextrose + 2% chitin which has ability to yield 39.33×10^7 conidia/ml water (Puzari *et al.,*1997) with high pathogenecity (LC_{50} 90.16 conidia/ml), ability to penetrate through elytral punctuations (Puzari *et al.*1994), and found superior performance in the field to that of the recommended insecticides (Puzari and Hazarika, 1994)

Presently the group is engaged in standardizing the protocol for liquid formulation of entomopathogens like *B. bassiana* and *M. anisopliae.* Besides, the team also concentrating on developing drying technique, which may allow retention of maximum number of viable propagules in dried product.

SHELF LIFE AND VIRULENCE OF ENTOMOPATHOGENIC FUNGI

Loss of viability over time is one of the critical obstacles for commercialization of a bio pesticides preparation. Several attempts have been made to determine the viability of entomopathogens in their preparations when stored at room temperature and in refrigerator.

An attempt was made to study the virulence of *B. bassiana* under 3 storage conditions i.e., Room temperature, refrigerated condition and deep freeze. A technique for overcoming the problem for loss of the virulence in the shelves was also tried (Puzari et. al., 2003). Result of the experiment showed that shelf life of *B.bassiana* varied on differential shelved conditions and where the temperature plays an important role. Its conidial density, virulence and viability were found decreased at their different rates in different shelf conditions, i.e., at room temperature 24±1p C, in refrigerated conditions (4p C) and in deep-freeze condition. At room temperature its virulence (90.97%) and conidial density (39.41x10^7conidia/ml) did not differ up to 90 days of storage. After 90 days they declined significantly to 82.00% and 30.27x10^7conidia/ml, respectively, with concomitant increase of percentage of dried conidia. There was considerable reduction of its viability and infectivity on the basis of the percentage of conidia dried. With increase in age, the viability and infectivity of the culture declined significantly. Desiccation of conidia in other way can be prevented to a considerable extend by adding certain osmoticum like mannitol, silica powder, sucrose, sodium glutamate, anti oxidizing agents like sodium ascorbate etc. Rich carbon source with a minimum compactness of the medium for space and aeration of fungal growth, the rice husk: sawdust: ricebran medium produced maximum numbers of propagules (Puzari et al.,1997) in which temperature played a major role in the extension of shelf life.

In refrigerated and deep-freezed condition a significant reduction in virulence occurred from 92.27% on 120 days and 86.7% on 150 days, respectively. While complete loss of virulence was observed after 270 and 300 days, respectively. The loss of virulence in storage could be improved by passaging the fungus through the host few times.

The temperature had significant effect on spore germination of *B.bassiana*. Significantly higher spore germination was observed at temperature range of 25-28p C. The highest germination was observed in case of AAU-09 strain (99.72%) followed by MTCC-4500 (99.38%) and MTCC-4506 (98.91%) at temperature 25p C. The biomass production of *B.bassiana* strains showed that the fungus could grow well at temperature 20-25ºC. The biomass production among the strains had significant effect in respect of temperature; the highest biomass production (283.67 mg) was recorded in AAU-09 strain followed by MTCC-4497 (273.67mg) strain at temperature 25ºC.

COMPATIBILITY OF *B. BASSIANA* WITH DIFFERENT INSECTICIDES COMMONLY USED IN RICE ECOSYSTEM OF NORTH EAST INDIA

Quite often microbial may not bring about desired control of the targeted pests, under such situations combination of microbial with chemical pesticides may prove to be useful for which compatibility of pesticides and entomopathogens must be tested (Puzari *et al.*, 2006).

Integrated management of insect pests is an important way in reducing the severe impact of chemicals pesticides on ecosystem. Bio control agents have been studied inadequately as component of integrated pest management system. *B.bassiana* a potential bio control agent occurred naturally and it readily attacks a large number of insect pests. It is an effective bio control agent of rice hispa in Assam, India (Hazarika and Puzari 1990, Puzari and Hazarika,

1991, 1992). Insecticides may have antagonistic or synergistic effect on the potentiality of *B.bassiana,* and may disrupt natural epizootics. Under such epizootic conditions it is expected to enhance its effectiveness through joint action of pathogen and compatible insecticide, which would reduced not only the cause of protection but also reduce the contamination of the environment. Puzari *et. al.,* 2006 have also shown that sub lethal dose of an insecticide would make the insect more susceptible to the attack of the entomopathogens. Earlier Puzari and Hazarika, 1991, observed that conidia of *B.bassiana* at a concentration of 10^7/ml, when mixed with Sandovit, Triton-AE, Teepol-80 and Hamam toilet soap (all at 0.025%) caused 81, 74, 81, 96 and 92% mortality to the adult hispa, respectively. Growth inhibition of entomopathogenic fungi is a useful criterion for initial testing of its compatibility.

Compatibility of *Metarhizium anisopliae* with pesticides used in tea cultivation

Compatibility of *M. anisopliae* with 17 pesticides including 10 insecticides, 5 fungicides and 2 herbicides was assessed under *in vitro* by poisoned food technique. The formulations of pesticides were tested at two different concentrations (lethal dose and sub lethal dose). All the fungicides viz., Captaf (Captan 50% WP) at 0.25%, Turf (Carbendazim 12% + Mancozeb 63%), Bavistin (Carbendazim 50% WP), Ridomil Gold (Metalaxyl- M 4% + Mancozeb 64%), Indofil M-45 (Mancozeb 75% WP) at both the concentrations were found to be highly toxic to the fungus. Herbcides like Roundup (Glyphosate 41% SL) at 0.3% and Gramoxone (Paraquate Dichloride 24% SL) 0.15% and 0.3% were also found to be detrimental inhibiting the fungal growth. Insecticides, Decis (Deltamethrin 2.8% EC) at 0.014%, inhibited the growth of the fungus to an extent of 85.40%. Insecticides like Actara (Thiamethoxam 25% WG), Dunet (Methomyl 40%W/W) at 0.04%, Pegasus (Diafenthiuron 50%) at 0.04%) and Imidacel (Imidacloprid 17.8% at 0.2% and 0.1% were safe insecticides showing higher mycelia growth. Similarly, Omite (Propargite 57% EC) at 0.2% was found to be compatible with less detrimental effects (35.62%).

Varying effects of chemicals on the fungi, their actual effects at cellular and field level need to be investigated to understand if the effects are permanent or temporary. In case of temporary arrest of fungus activity, it may recover after degradation of toxicant and such chemicals can be employed in combination with the fungus under field conditions. Field studies to evaluate the compatibility of these pesticides with fungal isolates, applied either as combinations or incorporated singly with the isolate, should generate additional information on how *M. anisopliae* can be successfully incorporated in the integrated pest, management systems together with pesticides.

FIELD EFFICACY

Pilot field trial on efficacy of *B. bassiana* was conducted in farmers' field of hispa endemic area to study the efficacy of *B. bassiana* against the pests. The result showed that **c**ombine application of Chloropyriphos (at sub lethal dose) + *B. bassiana* showed a sharp decrease of population of all the embryonic and post embryonic stages of rice hispa in both Sali and Ahu season in all the different localities. A significant increase in the population of egg/leaf, larva/leaf, pupa/leaf and adult/hill was observed in the control plot of all the localities. In Sali season, highest percentage of the decrease in the population of egg/leaf, larva/leaf and adult/hill was observed in Konwarpur and highest reduction of the pupa/leaf was recorded in Badulipar whereas in Ahu season highest percentage of the decrease in population of larva/leaf and adult/hill was observed in Banmukh while egg/leaf and pupa/leaf were recorded in Deesangmukh. Best results demonstrated were from combined application of chloropyriphos (at sub-lethal dose) + *B. bassiana.* Earlier Puzari and Hazarika, 1991, observed *B. bassiana* (1X10^7conidia /ml) in combination with 0.023% aqueous solution of Sandovit, Triton-AE, Teepol 80 and Hamam toilet soap caused 81%, 74%, 81%, 96% and 92% mortality to the adult rice hispa respectively.

Yield data (q/ha) of rice recorded at the various localities showed that the Chloropyriphos at sub lethal dose + *B. bassiana* was significantly higher than with those of untreated control. Highest yield (58.12 q/ha and 59.25 q/ha) was recorded in the treated plot of Badulipar, Jorhat district and Banmukh, Sibsagar district respectively in *Sali* and *Ahu* season. Yield recorded in Sali season in the treated plot of Dikhowmukh and Konwarpur was 56.66 q/ha and 54.83 q/ha respectively. Whereas in Ahu season it was 56.00 q/ha and 50.05 q/ha in Deesangmukh and Lepaipathar, respectively. Higher yield recorded in chloropyriphos (sub lethal dose) + *B. bassiana* treated field might bre enhanced action of *B. basiiana* in presence chemical thereby causing a quick death of the host resulting in reduced damage of the plant surface.

MOLECULAR CHARACTERIZATION

At AAU, Jorhat, molecular characterization of twelve indigenous isolates of *M. anisopliae* were done using polymerase chain reaction (PCR) amplification of polymorphic DNA using random primers (RAPD1 to RAPD12). Each primer tested generated a specific pattern of bands displaying 8-27 bands per isolate. RAPD analysis of the twelve isolates of *M. anisopliae* showed highest similarity of 54.3 per cent between isolate 4 and 5 followed by 43.2 per cent between isolate 9 and 10 and 29.6 per cent between the isolate 11 and 12 (Figs. 1 and 2) (Pegu et al.,2013). However, the lowest similarity (8.1%) was showed by the isolate 7 and 11. The cluster analysis of

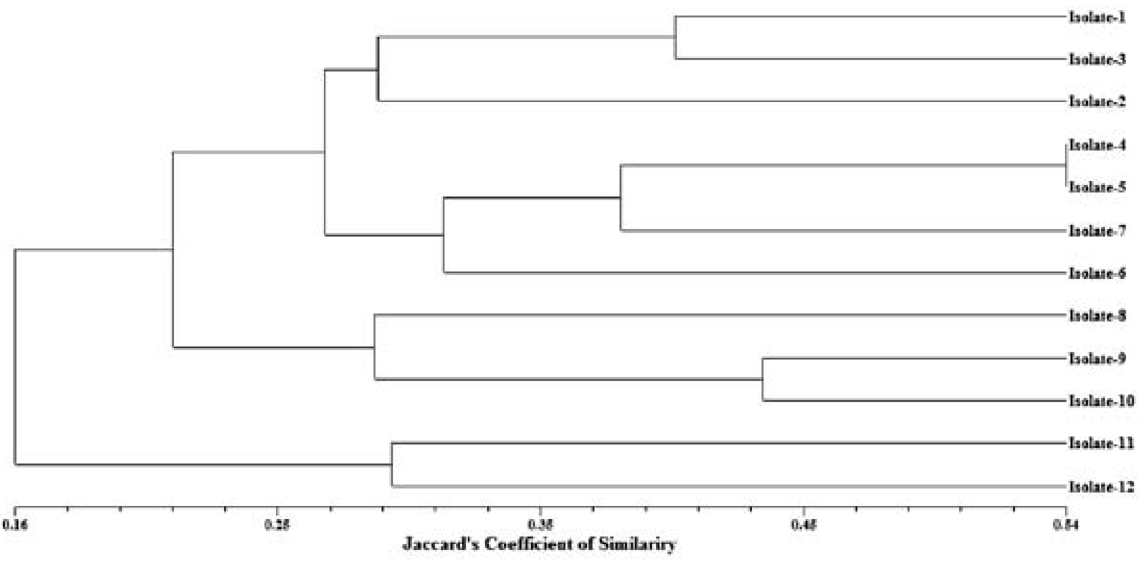

Fig. 1. Dendogram showing clustering of the different isolates of *Metarhizium anisopliae* (Metchnikoff) Sorokin isolated from vegetable ecosystem of Assam and used in the present experiment

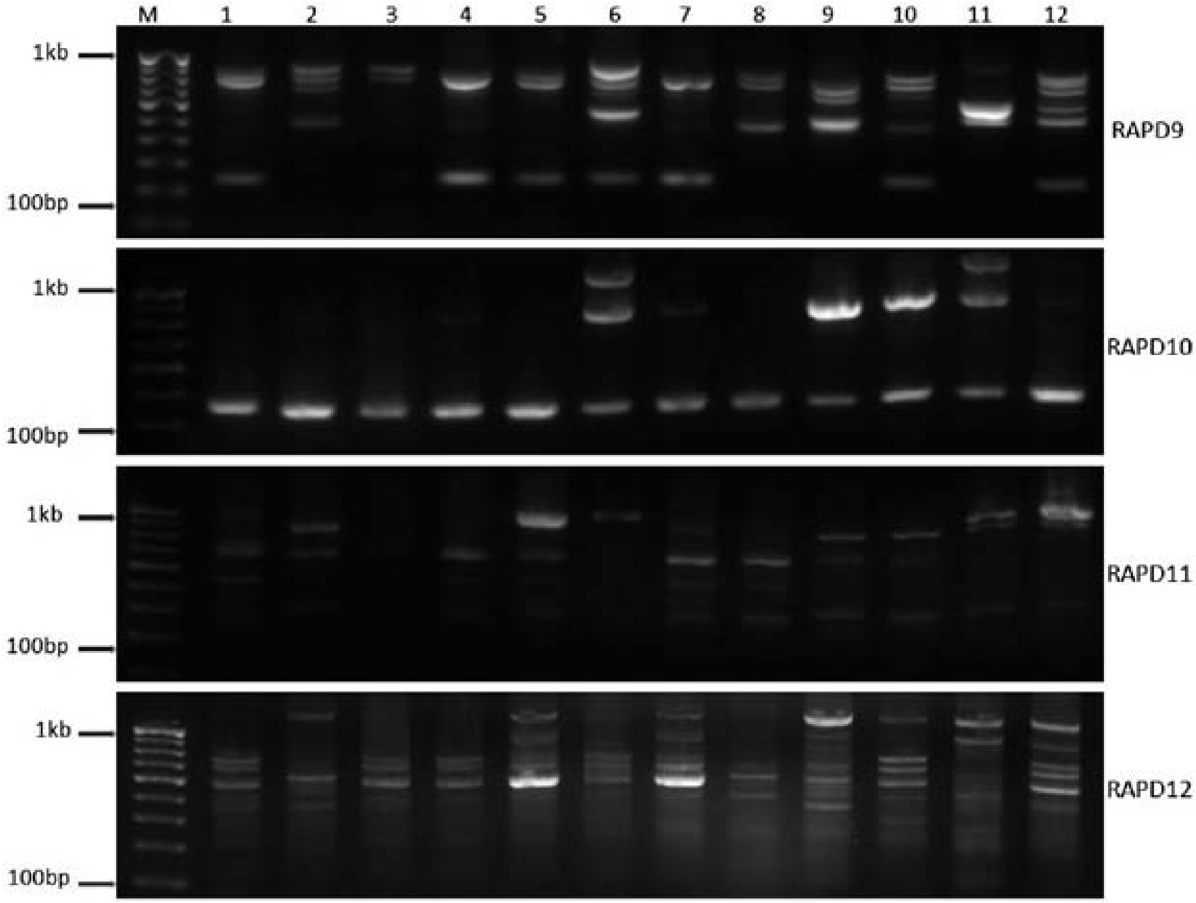

Fig. 2. RAPD profile obtained using RAPD9-12; "M"-100bp DNA marker; Lane 1-12 *"Metarhizium anisopliae* isolates"

the twelve isolates of *M. anisopliae* classified the samples into four subgroups: 1:1-3 (Majuli and AAU, Jorhat), 2: 4-7 (Lakhimpur and Sibsagar), 3: 8-10 (Nalbari) and 4: 11-12 (Kamrup). Sub groups 1, 2 and 3 showed 22.00 per cent similarities and among sub groups 1 and 2 have 27.00 per cent similarities. Within the same species collected from different host and places, the genetic variability was high. Even though some isolates were from the same host they were hyper variable indicating the polymorphism percentage of 47.52

FUTURE PROSPECTS

In reality biological management of agricultural pests more particularly by using entomopathogens may not totally replace the chemical pesticides in immediate future. But, judicious use of entomopathogenic fungi can significantly reduce the use of pesticides and thereby contribute to sustainable development of agriculture. Though the research on entomopathogen is well established in many countries but it has yet become an integral part of protection technology. Areas awaiting the attention of research scholars, agricultural scientist and policy makers include,

- Biodiversity of entomopathogenes.
- Genetic manipulation (mutation, protoplast fusion) of entomopathogens for enhanced efficacy, virulence and higher shelf life.
- Integration of entomopathogens with other management tactics.
- Establishment of tritrophic interaction of Entomopathogen, Target pest and Environment.
- Compatibility with commonly used agrochemicals.
- Selection of indegineous strains which are not only good entomopathogen but also growth promoters and plant defence inducers.
- Development of nanobioformulation with UV protectant, enhanced shelf life, long term and enhanced effectiveness.
- Long term preservation of strains with improved quality

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