Insect Killer's: The Entomofungal Pathogens

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Indiscriminate use of pesticides by farmers to control economically important pest for several years led to severe ecological changes such as reduction in the population of beneficial soil microbes and insect as well as development of resistance in to the insect pest. It has also affected the water quality by groundwater contamination and accumulation of toxic chemical residues on food crops have deteriorating effects on human health. Therefore, these problems have forced the industry and scientists to ponder over the situation and to focus the development of alternative control measures. One of them is biopesticide prepared from microbial inoculants such as entomofungal pathogens which can be a parasite to quick killer causes secondary infection to insect pest, substantially develops mycosis and helps in control the insect population in nature. This enomopathogens are host specific and has minimal risk of attack to non-target organism. Entomopathogens becoming popular because of their specific nature and ecologically sustainable option to regulate insect pest. Entomopathogens have different approach to cause disease as compared to other microorganisms as they do not require ingestion rather, they infect through insect cuticle. The range of insect infection includes lepidoptera, diptera, hemiptera, coleopteran, hymenoptera which is a great concern in agriculture worldwide. It is important that entomopathogen should be virulent in nature. The virulence of fungal entomopathogens involves four steps: adhesion, germination, differentiation and penetration. Fungal spore or conidia disseminated through environment cause infection in the host insect. These propagules deposited on insect cuticle a multilayer structure which is the first barrier and its composition affects the penetration process. When attached to suitable host, recognition signal (lectins) are exchanged from both host and pathogen for successful colonization. A cascade of recognition and enzyme secretion initiates the germination process on suitable host. These layers are degraded by mechanical force and various enzymes such as proteases, chitinases and lipases act in order to the substrate encounter in their path. When this pathogen crosses the external barrier it forms germ tube or appresorium and gets entry to insect where it starts invading the insect body and hemolymph. The fungus utilize nutrients from host body and cause subsequent tissue damage. Low molecular weight secondary toxic metabolites are secreted in the insect body and death of the host insect takes place. After multiplying in to the host tissues it emerges and develops infection over the insect body (Mycosis) in suitable environmental conditions. Insects become hard and paralyzed by the action of entomofungal pathogens. The whole process depends on several factors which include temperature, humidity, carbon and nitrogen source, composition of insect cuticle.

Reports have shown various entemopathogens used for control of economically important insect pest. First report of controlling insect pest with fungus given by Agostino Bassi in mid 17th century by *Beauveria bassiana* for silkworm causing a white muscardine disease. The fungus named in 1912 by the French physician Jean Paul Vuillemin in the honor of French scientist Jean Beauverie. Fungus produces dry, powdery conidia in distinctive white spore balls. The conidiogenous cells of *B. bassiana* are short and ovoid, and terminate in a narrow apical extension called a rachis. Latter Elie Metchnikoff in mid-18th century demonstrated that green muscardine disease was caused by *Entomopthora anisopliae* against wheat cockchafers (*Anisoplia austriaca*). Later this fungus recognized as *Metarhizium* Greek word meta means change combined with another Greek word rhiza means root and the mycelium termed as root part of

the fungus by Sorokin. Conidia are long cylindrical spore like budding like body usually in group or sometimes in dispersed manner. *Lecanicillium lecanii* are opportunistic fungus widely called as the "white holo" and it causes mycosis in a number of insects of orders Homoptera, Coleoptera and Lepidoptera. *Paecilomyces* described as diverse fruiting structure. The Greek word poikilos means diverse and Latin word mykes means myces however this genera has been transferred in to *Isaria*. *Nomuraea* named after the scientist H. Nomura from Japan. The fungus infected Pionea *forficalis*. Other entomopathogens used widely to control insect pest are listed in table 1.

The use of entomopathogens to control insect pest is agriculturally sustainable option for farmers and it will also help to produce the healthy food by eliminating application of dangerous pesticides. It will also help to maintain soil health and environmental balance. However, many researchers have been criticised entomopathogens as biocontrol agents because its action is too slow, another drawback reported is lack of persistence and widespread activity. Apart from that these entomopathogens fails to stand at high temperature and in dry area. Lack of proper delivery system is also an area of research.

Entmopathogens	**Effective against insect pest**	**Metabolite**
Beauveria bassiana, B. brogniartii	*Sitophilus oryzae, S. zeamais, Schistocerca gregaria, Ostrinia nubilalis, Helicoverpa Armigera,* Whiteflies/Aphids/Thrips	Bassiacridin, Bassianin, Bassianolide
Metarhizium anisopliae, M. flavo-viride	Grasshoppers, Thrips, Cockchafers, Spittlebugs, Grubs, Borers and Ticks	Destruxins, Cytochalasin
Nomuraea rileyi, N. atypicola	Spiders , *Anticarsia gemmatalis, Spodoptera litura, Helicoverpa armigera, Thysonoplusia orichalcea*	
Lecanicillium lecanii	Aphids, Whiteflies, Scales, Mealybugs, Thrips, Epilachna beetles	Dipcolonic acid, hydroxycarboxylic, acid, Vertilecanins, aphidicolin
Isaria fumosorosea	Whiteflies/Thrips, Mites	Leucinostatins , beauverolides, isariotins A–F
Hirsutella gigantean, H. thompsonii, H. cryptosclerotium	*Rastrococcus invadens*	Hirsutellin A, Hirsutellin B, Phomalatone, hirsutellic acid
Aschersonia aleyrodis	Coccidae and Aleyrodidae,	Ascherxanthone A
Conidiobolus coronatus	*Reticulitermes xavipes,* Aphids	
Coelomomyces sp.	Mosquitoes and Chironomids	
Entomophthora muscae	Houseflies	
Tolypocladium sp.	*Rhopalosiphum padi*	Cyclosporin, Efrapeptins

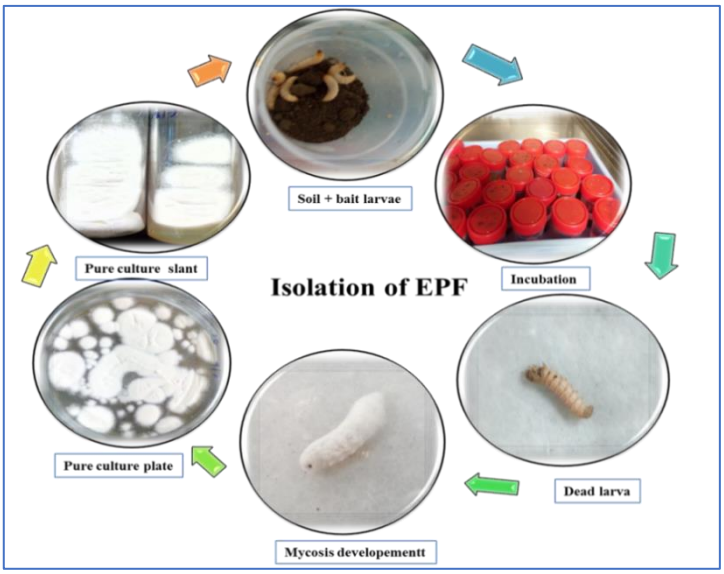

Fig. 1. Isolation and purification of entomopathogen

Isolation process

Isolation of entomopathogenic fungi can be done by insect bait method in which approximately 50 g of soil added into sterile container and then 5-10 larvae of *Galleria melonella* (Wax moth) or *Corcyra cephalonica* (Rice moth) are added in separate container. It is necessary to maintain moisture level according to prescribed standards, these containers

then incubated in growth chamber where temperature is maintained at 25°C and humidity as 70%. Ten days after incubation deformed and mummified larvae can be observed and separated.

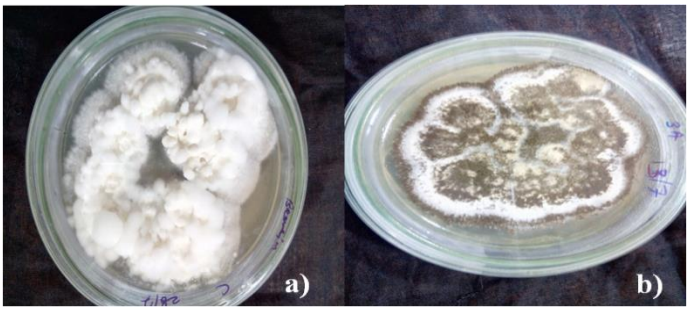

Fig 2. Colony morphology of *Beauveria* sp. and *Metarhizium* sp.

These dead larvae further kept on sterile moist filter paper in sterile Petri plate and sealed with parafilm and incubated for further mycosis development. When mycosis is developed after 2-3 days, insect cadavers need to be surface sterilized with 2% sodium hypochlorite and washed with distilled water twice. Small bits of this cadaver then streaked on Sabouraud dextrose agar plates fortified with 0.5 % yeast extract. Fig. 1 illustrates the isolation process and fig. 2 depicts colony morphology of the two different fungi after isolation.

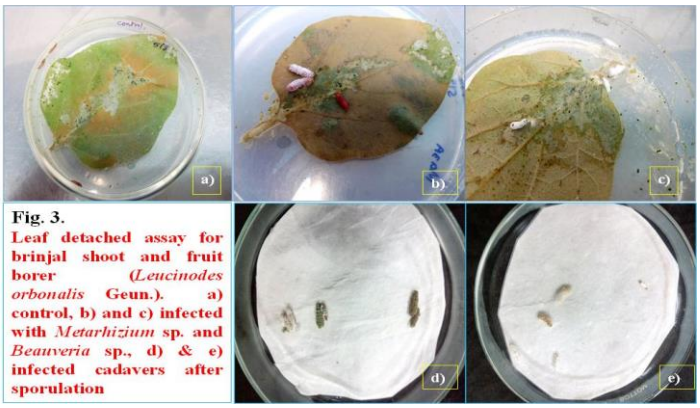

Fig. 3.
Leaf detached assay for brinjal shoot and fruit borer (*Leucinodes orbonalis* Geun.). a) control, b) and c) infected with *Metarhizium* sp. and *Beauveria* sp., d) & e) infected cadavers after sporulation

Bioassay

Leaf detached bioassay (Fig. 3)

In this bioassay clean, tender and fresh leaves of brinjal are rinsed with distilled water and then dipped in Sodium hypochlorite (1%) solution for two minutes, followed by two rinses in distilled water. After complete drying in aseptic condition, they are dipped in spore suspension for 30 seconds further dried in laminar air flow. After that they are placed individually in Petri plates containing sterilized agar medium (1%). Care should be taken that petiole of the leave is inserted deep into agar plate it will help to avoid water loss of the leave. To avoid bacterial contamination Chloramphenicol is added to the medium. Target insects are dipped in spore suspension for 2 seconds and excess solution is drained off and transferred to leaves placed over the agar medium. The petri plates are sealed with parafilm to avoid escape of aphids as well as to enhance the settlement of aphids on the leaf. The plates are maintained in an incubator at 25 ± 0.5°C. The newly hatched should be removed from the plates aseptically in laminar air flow before counting to avoid confusion in calculations.

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