The process of preparing a biopesticide formulation for use against coffee berry borer (CBB)

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ABSTRACT

A process for preparing a biopesticide formulation comprising the steps of:
- Isolating the fungus from coffee berry borer (CBB) cadaver;
- Preparing a liquid medium;
- Culturing the said isolation fungus of B. bassiana in said liquid medium to form a mycelial mat;
- Inoculating mycelial agar plugs cut from actively growing mycelial mat into a medium broth;
- Incubating the broth;
- Harvesting the conidiated mycelial by decantation.

DESCRIPTION (OCR text may contain errors)

FIELD OF THE INVENTION

This invention relates to the process of preparing a biopesticide formulation for use against coffee berry borer (CBB).

BACKGROUND OF THE INVENTION

At present coffee berry borer is being controlled using the toxic organochlorine chemical Endosulfan 35 EC. Apart from the use of chemicals certain phytosanitary practices are also followed like clearing the canopy of plantations, plucking all the leftover berries, using plucking mats etc., in coffee plantations. This method of control is not only uneconomical but also hazardous to the environment destroying most of the non-target beneficial insect populations in the plantations like Honey bees, Ladybird beetle etc., and other beneficial microorganisms. Moreover coffee berry borer develops resistance to this chemical very fast in 2 to 3 generations. Once the CBB develops resistance, only the non-target insects are killed and CBB thrives more profusely. Endosulfan residue that remains in coffee beans has deleterious effects on humans.

Be uveri has been reported to cause infective mortality in many terrestrial and aquatic insect pests (Bidochka and Khachatourians, 1991) and also has been successfully used against European corn borer, migratory grass hopper, green house whitfly, pine caterpillar coding moth, mosquitoes and lygus bug (Bidochka and Khachatourians, 1991 and Khachatourians, 1992). Larval and adult stages of several insects are hosts to many Deuteromycetoous fungal species. These fungi cause mycosis to both Coleopterans and Lepidopterans. Among these pathogens, Beauveria, Metarhisium and Paecilomyces spp. have been proved to be the efficient biological control agents (Hegedus and Khachatourians, 1995). The selected natural enemies from the native range of a given pest are used to reduce the pest's negative effects (DeVault et al., 1996). B. bassiana has been successfully used as foliar applications by several researchers for suppressing populations of several insects, like Bemisia tabaci (Gennadius) (Wright, 1992; Carruthers et al., 1993), Ostrinia nubilalis (Hubner) (Lewis et al., 1996), and Leptinotarsa decemlineata (Say) (Poprawski et al., 1997). B. bassiana has been tried as early season applications to control

CLAIMS (OCR text may contain errors)

We Claim:

1. A process for preparing a biopesticide formulation comprising the steps of:
   - Isolating the fungus from coffee berry borer (CBB) cadaver;
   - Preparing a liquid medium;
   - Culturing the said isolation fungus of B. bassiana in said liquid medium to form a mycelial mat;
   - Inoculating mycelial agar plugs cut from actively growing mycelial mat into a medium broth;
   - Incubating the broth;
   - Harvesting the conidiated mycelial by decantation.

2. The process as claimed in claim 1, wherein the said step of isolation is performed by washing the coffee berry borer with sterile distilled water, homogenized using a pestle and mortar in sterile distilled water, serially diluted and plated on potato dextrose agar (PDA) plates and after incubation at 25±2°C it was isolated and identified.

3. The process as claimed in claim 1, wherein said liquid medium comprises:
   - 5 to 15 g/lit of peptone;
   - 5 to 15 g/lit of yeast extract;
   - 10 to 30 g/lit of glucose;
   - 10 to 30 g/lit of starch;
   - 2 to 8 g/lit of sodium chloride;
   - 2 to 8 g/lit of calcium carbonate and
   - 125 mg/lit of chloramphenicol.
Colorado potato beetle by Poprawski et al. (1997). B. bassiana has been shown as an important entomopathogen in controlling diamondback moth, Plutella xylostella (L.) by Vandenberg et al. (1998). -5. bassiana has been successfully used for control of P. xylostella with crucifer transplants by Shelton et al. (1998). The effect of ento ogenous fungus B. bassiana was tested on insect predators like Coccinella sp., C. septemtara, Menochilus sexmaculatus, Erysyrphus alternans and Ischiodon scuteUaris and was proved that all the predator species were susceptible to the infection of B. bassiana (Haseeb and Murad, 1998).

The most frequently recorded fungus on H. kampei is -5. bassiana, though others have also been recorded from many countries (Steyaert, 1935; Pascalet, 1939; Ticheler, 1961; Villacorta 1984). The levels of incidence of this fungus vary from country to country and it is believed that among the different strains of the fungus, some are being more virulent than others (Hawskworth, 1974 and Bridge et al., 1990). Schafer (1936) described the appearance of -5. bassiana on the leaf lous. In United States, Charles (1941) and Mook and Wolfenbarger (1943), frequently recorded the fungus infecting insect larvae and adults. Doane (1959) reported epizootic among larvae of the small elm bark beetle, Scolytus multistriatus in the United States, caused by -5. bassiana. The report suggested the possibility of employing entomogenous fungi for the biological control of the elm bark beetles, S. scolytus and S. multistriatus, the vectors of Dutch elm disease in Britain.

Marcandier and Khachatourians (1987) have reported the susceptibility of the migratory grasshopper, Melanoplus sanguinipes to B. bassiana. There are several records of B. bassiana infecting grasshoppers and it can cause natural epizootics in these insects (Goettel, 1992; Moore and Erlanson, 1988; Prior and Greathead, 1989). B. bassiana has been successfully used as foliar applications by several researchers for suppressing populations of several insects, like Bemisia tabaci (Gennadius) (Wright, 1992; Carruthers et al., 1993), Ostrinia nubilalis (Hubner) (Lewis et al., 1996), and Leptinotarsa decemlineata (Say) (Poprawski et al., 1997). Early season applications of B. bassiana has been tried to control Colorado potato beetle by Poprawski et al. (1997) and for control of P. xylostella with crucifer transplants by Shelton et al. (1998).

Haseeb and Murad (1998) have tested the effect of the entomogenous fungus B. bassiana on insect predators like Coccinella sp., C. septemtumata, Menochilus sexmaculatus, Erysyrphus alternans and Ischiodon scuteUaris and have concluded that all the predator species were susceptible to the infection of -5. bassiana. This entomopathogen -9. bassiana has been shown as an important organism in controlling diamondback moth, Plutella xylostella (L.) by Vandenberg et al. (1998). The role of entomopathogenic fungi in causing disease in insects was first recognized in the early part of the 19st Century from studies on silk worms. There are 700 species of entomopathogenic fungi (Robert et al., 1991). Of these Beauveria bassiana has been studied most extensively. -5. bassiana an hypomycetous fungus has no known sexual cycle. The asexual conidia attaches to the host cuticle and degrade pectin, chitin and iipids in the insect integument by various enzymatic activities (Khachatourians, 1991). The host insects are killed due to depletion of their haemolymph nutrients and or due to toxemia caused by fungal toxic metabolites (Roberts, 1981; Khachatourians, 1991).

To date, more than 200 insects species have recorded as hosts of -5. bassiana worldwide (Li, 1988). It has been used against Red Imported Fire Ant in the US (Siebeneicher et al., 1992), migratory grasshopper (Marcandies and Khachatourians, 1987), predatory insects (Donegan, 1989), Stem -borer (Maniania, 1993) and Rice hispa (Hazarik and Puzari, 1990). The virulence of the fungus and pathogenicity of isolates and temperature conditions to establish the virulence. Variation in virulence of B. bassiana may also be related to enzyme production and activities during the course of penetration of the host cuticle (Biodochka and Khachatourians, 1990). The B. bassiana production has recently been developed by Mycotech Corporation, USA (Bradley et al., 1992). A corn starch and corn starch oil formulations of B. bassiana mycelia as well as pure dry mycelial preparations was obtained by Feng et al. (1994). Biodochka et al. (1997) have found out that hydrophobic interactions, appressoria formation, and mucus production by the fungus are involved in adhesion to the cuticle. The technology of protoplast fusion is a valuable method for intra- and inter species hybridization. Through this a somatic hybrid of a cross between two strains of B. bassiana has been obtained (Viaud, 1998).

OBJECTS OF THE INVENTION

An object of this invention is to propose a process for preparing a biopesticide formulation against coffee berry borer.

Further, object of this invention is to propose a formulation containing entomopathogenic fungus which is eco-friendly.

Yet another object of this invention is to prepare a formulation which is economical and easy to deliver.

SUMMARY OF THE INVENTION

According to this invention there is provided a process for preparing a biopesticide formulation comprising the steps of:

Isolating the fungus from coffee berry borer (CBB) cadaver;
Preparing a liquid edium;
Culturing the said isolation fungus of B. bassiana in said liquid medium to form a mycelial mat;
Inoculating mycelial agar plugs cut from actively growing mycelial mat into a medium broth; Incubating the broth;
Harvesting the conidiated mycelial by decantation,

In accordance with a further embodiment of this invention there is also provided a process for preparing talcum powder formulation comprising: harvesting mycelial mat of B. bassiana from roux bottles; sterilizing purified talcum powder; mixing sterile talcum powder with the harvested mycelial mat; mixing sterilized carboxy m thyl cellulose; spreading out the formulation in trays for drying.

DESCRIPTION OF THE INVENTION

In accordance with this invention, the fungus is first isolated from coffee berry borer (CBB) cadavers.
CBB cadavers were washed with sterile distilled water, homogenized using a pestle and mortar in sterile distilled water, serially diluted and plated on Potato Dextrose Agar (PDA) plates. After incubation, fungi was isolated and identified as B. bassiana based,

PREPARATION OF MAT

The fungus was cultured on Dextrose-Yeast extract liquid medium supplemented with peptone (YPD). The fungus formed a white mycelial mat and sporulated after 12-14 days of incubation at 25±2°C.

Fresh culture in Liquid broth
Medium compoaitlilio (Yeast extract ept ne dextrose ---tedium [YPD]). The liquid medium was developed with the following composition- Peptone - 5 to 15g/lit; Yeast extract - 5 to 15g/lit; Glucose -10 to 30g/lit; Starch - 10 to 30g/lit, Sodium chloride 2 to 8g/lit and Calcium carbonate - 2 to 8g/lit. Chloramph nic l-1 5 mg/liter. 
A mycelial agar plug cut out from the actively growing seed culture on specific medium (YPD A) was inoculated into roux culture bottles containing sterile YPD broth.

Incubation conditions:
Temperature - 20±28°C
Light - 12/12 h NUV and dark conditions
Time - 10 to 20 days

Product recovery:
The broth was decanted without mixing. The mycelial mat was taken into a clean container and stored at 4°C for use within 24 hours. To prepare lyophilized culture, after the mycelial mat is taken from the Roux bottle it is put into round bottom coming flask frozen at -40°C for 6 hours and connected to a manifold assembly for lyophilisation. After lyophilisation the dry mycelia mat is stored under-40°C for use within 60 days.

EXAMPLES:
Formulation 1 - Fresh culture in liquid broth
Fresh culture is produced in specific YPD liquid medium in Roux bottles.
YPD broth is prepared and 100 ml is dispensed into Roux bottles. The bottles are autoclaved at 15 psi for 30 min. The cooled medium is inoculated with mycelial agar plugs cut from actively growing colonies on YPD agar medium under aseptic conditions. The bottles are incubated at 25±2°C under 12/12h alternate cycles of NUV and darkness for 12 to 14 days. The profusely conidiated mycelial mat is harvested by decantation, packed in polythene bags and kept at 4°C for use within 24 hours or processed for lyophilisation.

Formulation 2 - Talcum powder formulation
Formulation of B. bassiana was prepared using purified talcum powder.

Mycelial mat was harvested from roux bottles blot dried and mixed with talcum powder (1:10) to obtain a conidial concentration of approximately $10^8$ conidiospores/g. Carboxy methyl cellulose at the rate of 1% was added as sticker. The formulation was spread out in plastic trays and allowed to dry under shade in a fume hood for 24 hours and packed in polythene bags. The formulations thus prepared are stored in dark at 4°C to be used before 60 days.

**PATENT CITATIONS**

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**CLASSIFICATIONS**

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**LEGAL EVENTS**

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