The fungi secrete a suite of cuticle-degrading enzymes such as chitinases, proteases, and lipases, which break down the structural components of the cuticle, enabling the fungal hyphae to invade the insect's body cavity (hemocoel). Inside the hemocoel, the fungal hyphae proliferate rapidly, absorbing nutrients from the insect's hemolymph (blood equivalent) and releasing toxic secondary metabolites and enzymes. These toxins disrupt the insect's physiological processes, suppress its immune response, and cause systemic infection leading to septicemia. The infected insect gradually weakens and dies, usually within several days to a week, depending on fungal strain, insect species, and environmental factors. After the host's death, the fungus emerges from the cadaver and produces new conidia on the insect's surface, which then disperse to infect other susceptible insects, perpetuating the infection cycle.This natural infection cycle allows EPF to act as an efficient and environmentally friendly biocontrol agent, targeting specific insect pests without harming non-target organisms, plants, or humans. Moreover, because EPF rely on active infection rather than ingestion, they are effective against a wide range of insect life stages, including those that are resistant to chemical insecticides. The ability of entomopathogenic fungi to survive in soil and plant surfaces further enhances their persistence in nursery and forest ecosystems, providing long-term pest suppression (Figure 3).

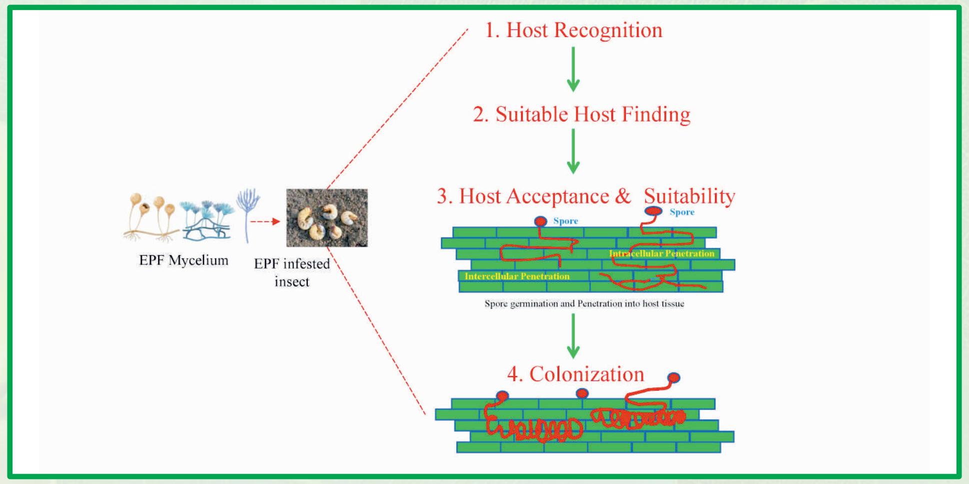

Figure 3. Host identification and establishment by entomopathogenic fungal endophytes involves diverse steps i.e. host recognition, suitable host finding, host acceptance and suitability followed by spore germination and penetration into host tissue leading to fungal colonization.

Application of EPF

The application of entomopathogenic fungi in forest nurseries serves as an eco-friendly and sustainable approach for managing insect pests that threaten seedling health and survival. Effective field application requires careful consideration of the fungal dose, formulation, delivery method, and environmental conditions to maximize infection rates and pest mortality. Typically, *M. anisopliae* and *B. bassiana* are applied in the form of conidial suspensions or formulated products such as wettable powders, granules, or oil-based emulsions. For seedling protection and soil treatment in nurseries, conidial concentrations ranging from 1×10^7 to 1×10^9 conidia per milliliter (conidia/ml) are generally recommended, depending on the target pest species and infestation severity.

Target insect

Entomopathogenic fungi are effective against a wide range of harmful insect pests commonly found in forest nurseries and plantations. These include termites, which attack roots and stems of young seedlings; mealybugs and scale insects, which suck sap and weaken plant vigor; aphids and whiteflies, which transmit plant viruses and cause stunted growth; caterpillars and shoot borers, which defoliate seedlings and damage growing shoots; bark beetles that bore into tree bark and disrupt nutrient flow; and root weevils that infest and damage plant root systems. By infecting and killing these pests, entomopathogenic fungi serve as valuable biological control agents, reducing the need for chemical insecticides and promoting healthier nursery stock.

Method of application

Spray application is the most common method, where a suspension of fungal conidia is evenly sprayed on the foliage, stems, and soil surface using hand-held or motorized sprayers. This ensures direct contact of spores with insect pests such as aphids, whiteflies, mealybugs, and termite larvae. Soil drenching with fungal suspensions is also practiced to target subterranean pests like root weevils and termites by allowing spores to persist in the rhizosphere and insect pests dwelling in the soil. Additionally, seed treatment with fungal formulations can protect seedlings during early growth stages by inhibiting pest colonization.

Timing of application

To improve efficacy, applications should be timed during early pest infestation stages and preferably under favorable environmental conditions (evening hours after 5.00 P.M.) cooler temperatures (20–28°C), high relative humidity (>70%), and low UV radiationto enhance fungal germination and penetration into the host insect. Multiple applications at intervals of 10 to 15 days may be necessary for sustained pest suppression, especially in areas with heavy infestations or extended pest pressure.

Dose of application

Proper dose calibration is essential to balance cost-effectiveness with biological control efficiency. For foliar sprays, a typical dose involves preparing a conidial suspension containing 1×10^8 conidia/ml. Soil applications usually use similar spore concentrations but may require lower volumes depending on soil type and pest density.

Precaution to be taken

- Use protective gear (gloves, masks, goggles) during handling and application to avoid inhalation or skin contact.
- Store fungal formulations in cool, dry, and dark conditions to maintain spore viability.
- Avoid exposing fungal spores to direct sunlight and high temperatures to prevent loss of efficacy.
- Apply EPF during favorable environmental conditionsmoderate temperature (20-28°C) and high humidity (>70%).
- Do not apply during heavy rain or drought, as spores can be washed away or fail to infect pests.
- Maintain hygienic conditions during culture and formulation to prevent contamination.
- Use recommended doses and avoid mixing with chemical pesticides that may kill the fungi.

Published by

DIRECTOR

ICFRE-Arid Forest Research Institute

Post – Krishi Mandi, New Pali Road, Jodhpur

Web: http://afri.icfre.org email: dir_afri@icfre.org

Phone +91-0291-2722549

Prepared by

DR. TANMAYA KUMAR BHOI

Scientist-C

Forest Protection Divison

Mobile : +91 7065079155

(Funded by Extension CAMPA 2025-26)

(Designed by Smt. Kusum Lata Parihar, ACTO, Extension Division)

Mass multiplication of Entomopathogenic Fungi and their Application in Forest Nurseries for Insect-pest Control

ICFRE-ARID FOREST RESEARCH INSTITUTE

JODHPUR

INDIAN COUNCIL OF FORESTRY RESEARCH & EDUCATION, DEHRADUN

(AN AUTONOMOUS BODY OF MOEF&CC, GOVT. OF INDIA)

Background

Forest nurseries play a critical role in the conservation, afforestation, and restoration of forest ecosystems. These nurseries provide healthy seedlings essential for successful plantation and reforestation programs. However, the productivity and quality of seedlings in forest nurseries are often compromised by various insect pests that cause significant damage by feeding on foliage, stems, and roots. The infestation by pests such as termites, aphids, leaf miners, and borers can lead to reduced growth rates, seedling mortality, and compromised seedling vigor, ultimately impacting forest regeneration efforts.Traditionally, chemical insecticides have been the primary tool for managing insect pests in nurseries. While effective, the indiscriminate use of synthetic pesticides raises concerns related to environmental pollution, human health risks, development of insect resistance, and negative impacts on non-target organisms including beneficial insects. Moreover, in fragile ecosystems such as those found in arid and semi-arid regions like Rajasthan, the ecological balance is delicate, and the introduction of chemical agents can further degrade soil health and biodiversity. These challenges necessitate the exploration of alternative pest management strategies that are eco-friendly, sustainable, and effective over the long term.

Biological control using entomopathogenic fungi (EPF) has emerged as a promising alternative for managing insect pests in forest nurseries. Entomopathogenic fungi are a group of naturally occurring fungi that infect and kill insects, acting as natural regulators of insect populations. Among the most studied EPF are *Metarhizium anisopliae* and *Beauveria bassiana*, both of which are known to infect a wide range of insect pests. These fungi penetrate the insect cuticle, proliferate inside the host body, and cause death through mechanical damage, nutrient depletion, and production of toxic metabolites. The use of EPF in pest management offers several advantages. They are environmentally safe, target-specific, and pose minimal risk to humans, animals, and non-target beneficial organisms. EPF can be integrated into integrated pest management (IPM) programs, reducing reliance on chemical pesticides and enhancing the sustainability of nursery operations. Additionally, EPF can be applied in various formulations and methods such as soil treatment, seed coating, and foliar sprays, offering flexibility to nursery managers.

Despite these advantages, the successful deployment of EPF in forest nurseries requires efficient mass multiplication techniques to produce large quantities of viable fungal spores. The efficacy of EPF depends on the quality and quantity of fungal propagules, their viability, and ability to infect target pests under nursery conditions. Various substrates and fermentation methods have been developed to upscale EPF production from laboratory to commercial levels. The choice of native fungal isolates is also crucial, as fungi adapted to local environmental conditions often show better performance against indigenous pest populations.In the arid and semi-arid conditions of Rajasthan, the research and development of native entomopathogenic fungi hold particular significance. Indigenous isolates of *M. anisopliae* and *B. bassiana* have been reported to exhibit enhanced adaptability and efficacy under harsh climatic conditions. This makes them ideal candidates for sustainable pest management in forest nurseries located in Rajasthan and similar agro-ecological zones.Given this background, the ICFRE- Arid Forest Research Institute (ICFRE-AFRI) has isolated and identified two native entomopathogenic fungi, M. anisopliae and B. bassiana, from Rajasthan soils and insect populations. These isolates are being studied for mass multiplication and application strategies to control insect pests in forest nurseries effectively (Figure 1).

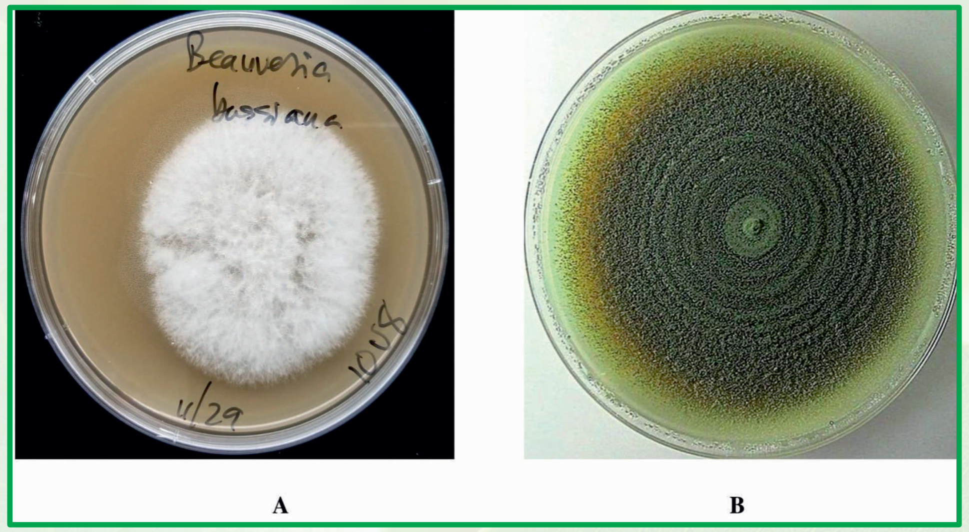

Figure 1. Fungal culture plates showing entomopathogenic fungi. A) *Beauveria bassiana* with white mycelium (white muscardine fungus). B) *Metarhizium anisopliae* with green mycelium (green muscardine fungus).

Mass Multiplication of *Metarhizium anisopliae* and *Beauveria bassiana*

The mass multiplication of entomopathogenic fungi (*M. anisopliae* and *B. bassiana*) requires precise control of environmental and nutritional conditions to achieve optimal fungal growth, sporulation, and production of highly virulent conidia suitable for biological control applications in forest nurseries. Both fungi can be propagated effectively using solid-state fermentation (SSF) and liquid fermentation methods, each demanding specific parameters for maximum yield and quality.

Temperature and Relative Humidity

Temperature and relative humidity are critical abiotic factors influencing fungal metabolism, mycelial growth, and sporulation. For both *M. anisopliae* and *B. bassiana*, the optimal temperature range for mass multiplication lies between 25°C to 28°C. Within this range, enzymatic activities are maximized, supporting rapid fungal biomass accumulation and prolific spore production. Temperatures above 30°C may inhibit growth and reduce conidial viability, while temperatures below 20°C slow fungal metabolism and delay sporulation.

Relative humidity is equally important, especially during solid-state fermentation and drying stages. Maintaining 65% to 80% relative humidity during fungal incubation promotes optimal moisture content in the substrate, which is essential for fungal colonization and conidia development. Insufficient humidity leads to desiccation stress, reducing sporulation, while excessive moisture may foster contamination by unwanted microorganisms and hamper aeration. During spore drying, humidity is reduced (below 10%) to ensure long-term viability and stability of fungal conidia in storage and formulation.

Liquid fermentation: media and process

Liquid fermentation is widely used to rapidly produce fungal biomass under controlled conditions. Two standard media commonly used for *M. anisopliae* and *B. bassiana* are Potato Dextrose Broth (PDB) and Sabouraud Dextrose Broth (SDB or SDA in liquid form) (Figure 2).

- Potato Dextrose Broth (PDB) is a nutrient-rich medium prepared by boiling peeled potatoes and adding dextrose (glucose) to provide carbon sources. It supports robust fungal growth by supplying essential carbohydrates, vitamins, and minerals. PDB favors the production of both mycelial biomass and conidia but is especially conducive to high-density fungal growth in liquid culture.
- Sabouraud Dextrose Broth (SDB), derived from Sabouraud Dextrose Agar (SDA), contains peptones and dextrose, creating an acidic environment (pH ~5.6) favorable for fungal proliferation while inhibiting bacterial contamination. This medium supports rapid fungal growth and conidial development, making it suitable for submerged fermentation processes.

During liquid fermentation, fungal cultures are maintained at 25°C to 28°C with continuous shaking at 100 to 150 revolutions per minute (rpm) to ensure adequate oxygenation and prevent clumping of mycelium. Aeration is provided either by agitation or sparging air through fermenters to sustain aerobic metabolism essential for fungal growth. Liquid fermentation cycles generally last 5 to 7 days, after which the fungal biomass is harvested.Liquid cultures usually produce more mycelial biomass than free conidia, necessitating downstream processing such as centrifugation and drying to isolate and concentrate spores for formulation. The conidia harvested are then formulated into biopesticides such as wettable powders, emulsifiable concentrates, or granules

Solid-state fermentation and environmental control

In solid-state fermentation, sterilized substrates such as polished rice, wheat bran, or sorghum grains are inoculated with fungal spores or mycelia and incubated at 25-28°C with 65-75% relative humidity. The substrate moisture content is carefully maintained around 40-60% to support fungal colonization without promoting bacterial growth. Aeration is critical; trays or fermentation bags are arranged to allow airflow and prevent heat buildup, as fungal metabolism generates heat that could otherwise kill the culture. The fungal mycelium colonizes the substrate fully within 10 to 14 days, producing high-density conidia. Spores are then harvested, dried to moisture content below 10%, and subjected to quality control for viability and pathogenicity (Figure 2).

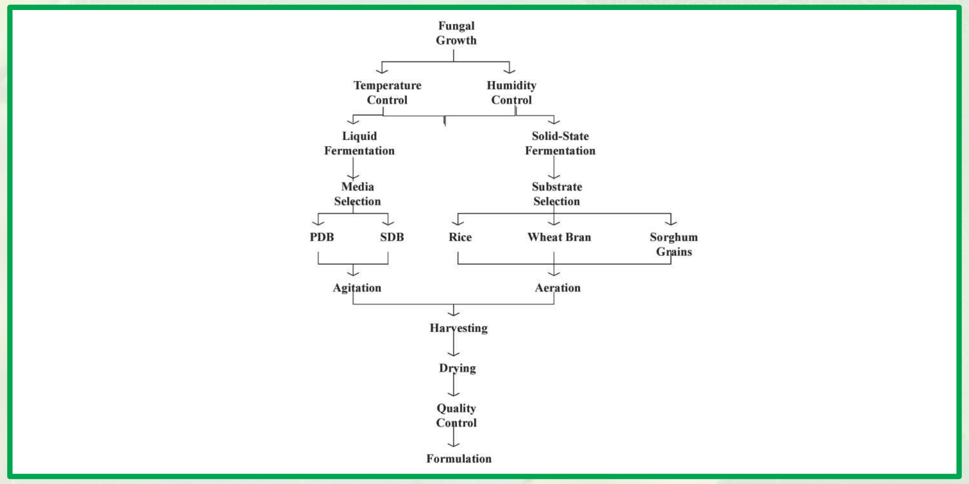

Figure 2. Stepwise process of mass multiplication of entomopathogenic fungi (*Metarhizium anisopliae* and *Beauveria bassiana*) including isolation, culture maintenance, inoculum preparation, fermentation (liquid and solid-state), harvesting of conidia, drying, formulation, and packaging for application in pest management.

Mode of action

Entomopathogenic fungi (EPF), control insect pests through a unique and complex infection process that distinguishes them from chemical insecticides and other biological agents. The mode of action begins when fungal spores, called conidia, come into contact with the insect's cuticlethe outer protective layer of the insect's exoskeleton. The spores adhere firmly to the insect surface, aided by hydrophobic interactions and specific adhesion proteins. Once attached, the conidia germinate under favorable environmental conditions, producing a germ tube that penetrates the insect cuticle by mechanical pressure combined with enzymatic degradation.