

AMF Biofertilizer: Pot Culture Production and Application Protocol

Arbuscular Mycorrhizal Fungi (AMF) are beneficial soil microbes that form symbiotic associations with the roots of most agricultural, horticultural, and forest plant species. They colonize the root cortex and extend an extensive network of external hyphae into the soil, greatly enhancing the plant's ability to absorb water and essential nutrients. The improvement in nutrient uptake by AMF, reduce the requirement of chemical fertilizers and also contribute to organic crop production. They contribute significantly in nutrient-deficient, degraded, or disturbed soils where natural mycorrhizal populations are limited. Mass production of AMF biofertilizer is commonly achieved through pot or trap culture using suitable host plants such as wheat, barley, sorghum, maize, or selected grasses. Once adequate root colonization of host plant and sporulation in rhizosphere achieved, the rhizosphere soil containing infected roots, spores, and hyphae is harvested and used as AMF biofertilizer. The Institute has isolated a consortium of native AMF strains from the Northwestern Himalaya, and their mass multiplication has been successfully carried out using the pot culture method following the steps outlined below.

Production Protocol

- i. Fill the pots to three-fourths with a sterilized soil-sand mixture (1:1). Mix the AMF mother culture with the remaining soil and fill the pot completely. Sow seeds of a suitable seasonal host plant.
- ii. Irrigate the pots as needed to ensure healthy growth of the host plants.
- iii. At plant maturity, gradually reduce irrigation to induce mild water stress conditions. This promotes rapid multiplication of AMF already established in the root system.
- iv. Remove the aerial parts of the host plants and harvest the roots along with the potting soil.
- v. Prepare the biofertilizer by chopping the colonized roots into small pieces and thoroughly mixing them with the pot soil enriched with AMF propagules.
- vi. Use the root-soil mixture as AMF biofertilizer. This will also serve as the mother culture for initiating the next production cycle.



Method of Application

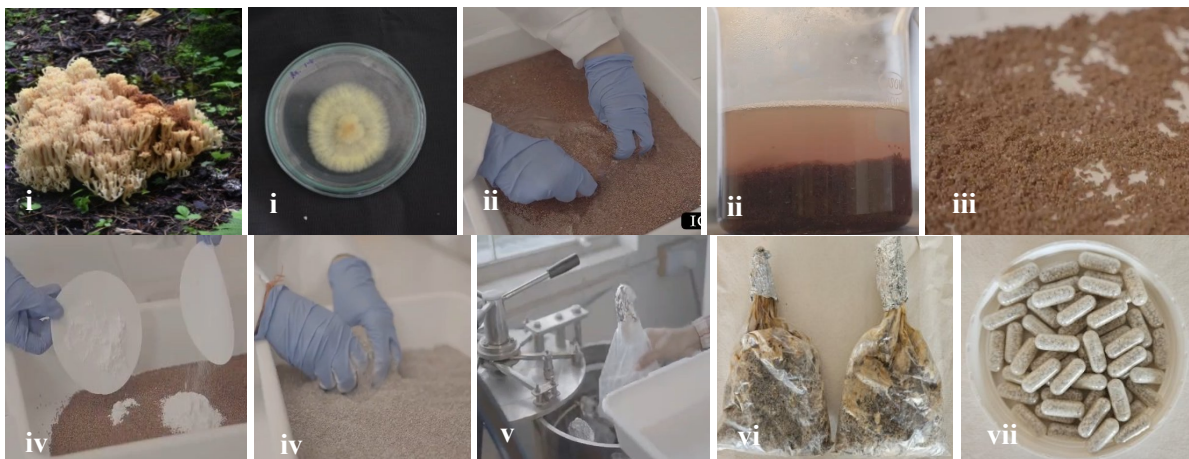
- **Pot Crops:** Add 5–10 g of biofertilizer into the potting soil at the time of seed sowing or apply around the rhizosphere of established plants.
- **Polybag seedlings:** Mix 3–5 g of biofertilizer with the polybag soil during seed sowing or apply around the rhizosphere of established plants.
- **Nursery Beds:** Apply 2–3 kg of biofertilizer per acre of nursery beds at the time of seed sowing or mixing with FYM to the established crop.
- **Field Application:** Apply 3–4 kg of biofertilizer per acre. For uniform distribution and improved effectiveness, mix the biofertilizer with vermicompost or FYM before application.

Ectomycorrhizal Biofertilizer for Conifers: Production on Ragi Grains and Application

Conifers play a crucial role in Himalayan forest ecosystems by providing vital habitats for wildlife and contributing significantly to carbon sequestration. Mycorrhizal associations are essential for the proper growth and establishment of conifer seedlings, and it is well established that artificial inoculation with suitable mycorrhizal fungi enhance seedling vigor and reduce the nursery period. At the institute, cultures of selected ectomycorrhizal fungi have been mass-produced on ragi (finger millet) grains and subsequently encapsulated using talc-based carriers, following the standardized steps described below. These formulations demonstrated excellent performance when applied to seedlings of conifers, improving root colonization, nutrient uptake, and overall seedling development.

Production Protocol

- i. Isolate pure cultures of ectomycorrhizal fungi from freshly collected sporocarps.
- ii. Soak ragi grains in water for 30 minutes, boil for 20 minutes, allow to cool, boil again for 20 minutes, and allow to soak for 30 minutes.
- iii. Drain excess water and shade-dry the grains for about 4 hours.
- iv. Mix the dried grains thoroughly with 2% CaSO_4 and 0.5% CaCO_3 to prevent clumping and maintain suitable pH.
- v. Fill the treated grains into polypropylene bags and autoclave at 121 °C and 15 psi for 2 hours.
- vi. After cooling, inoculate the sterilized grains with the EM fungal mother culture and incubate at 25 °C for 3–4 weeks, or until complete colonization is observed.
- vii. Blend the colonized grains with sterilized talc powder and pack in airtight bags, or fill into capsules, for use as an EM biofertilizer for conifer seedlings.



Method of Application

- At the time of seed sowing, add 1–2 g of EM biofertilizer or one capsule containing EM fungi to the soil in each polybag.
- During outplanting, apply 4–5 g of EM biofertilizer or 2–3 capsules around the rhizosphere of each seedling.