

Bioinoculant preparation simplified for farmers : 'produce and use' on-farm system

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A minimal and economically viable method for mass-multiplication of plant-beneficial bacterial and fungal inoculants (bioinoculants) has been standardized for the benefit of the farmers. The method utilizes locally available wastes such as rice gruel and mature coconut water synergistically blended with biochar, as a medium for on-farm production of contaminant-free bioinoculants. The method produces aqueous bioinoculant formulation that can be easily applied as seed treatment, seedling dip, soil drenching and foliar spray and is suitable for immediate field application. Decentralized on-farm bioinoculant production by farmers paves way for enhanced adoption of bioinoculant technology by farmers, helping them to improve their farm soil health and fertility and ultimately crop production capacity.

Microorganisms are becoming an important basis for sustainable and environmental-friendly agriculture. Several plant beneficial microorganisms such as nitrogen fixers, phosphate, potash, zinc and silicate solubilizers, plant growth promoting rhizobacteria, arbuscular mycorrhizae and microbial control agents of insect pests, diseases and weeds offer immense potential to increase the sustainability of agricultural system. To make available such plant beneficial microorganisms for farmers use, 'bioinoculant technology' has been in the forefront. This technology is based on mixing proven plant beneficial microbes with solid or aqueous carrier. The carriers used for harbouring the live microbes must be: easily available, inexpensive, non-toxic

to inoculants and plants, have sufficient moisture absorbing potential and good pH buffering capacity. The plant-beneficial bacteria or fungi are usually mixed with powder carriers such as peat, lignite, talc or appropriate aqueous medium containing ethylene glycol, xylene, methanol or the regular synthetic media broths.

To produce carrier based bioinoculant broadly following steps are involved viz. multiplication of proven bacterial or fungal isolates in known medium to a high population level, mixing the cells of the isolates in sterilized solid or aqueous carrier material, and packaging and marketing them to the farmers.

The carrier based inoculants are applied by the farmers in four ways: seed treatment, seedling dip, soil application and foliar spray. Production of bioinoculant formulations in India during 2012-13 was 65527 Mt as compared to 25005 Mt in 2008-09 (Indian Fertilizer Scenario 2014, Dept. of Fertilizers, GOI). The drive for safe and environment friendly agriculture will only see the need to significantly boost bioinoculant production in the coming years. This situation provides ample opportunities for developing bioinoculant production process that uses locally available liquid wastes of organic nature to be used as substrate for the mass-multiplication of the plant-beneficial bacteria and fungi.

Bioinoculant formulations currently available in market mostly depend upon peat, talc, lignite, and such others as carriers of the live inoculants. These carrier materials are normally mined from earth and then processed for use. Their availability is

not ubiquitous and unlimited requiring them to be transported to distant places for the bioinoculant preparation. Aqueous carriers such as ethylene glycol, xylene, methanol or regular growth media broths used are predominantly synthetic in nature and generally expensive. Under certain conditions the organic solvents are toxic to the inoculants and plants too. They also are hampered by limited pH buffering capacity during the multiplication of the inoculant organism. Therefore, a large carbon-footprint is left by the current bioinoculant production industry which adds to the escalating weather perturbations. Moreover, the technology also has to grapple with the issues of contamination and lower population of viable inoculant bacteria or fungi present in the carriers than the prescribed levels at the time of application by the farmers. With emphasis on safe and environmental friendly farming increasing each passing day, the need for producing large volumes of bioinoculants is only imperative.

A method for on-farm production of plant-beneficial bacterial and fungal inoculants is described here using aqueous by-products that otherwise go as waste, i.e., rice gruel and mature coconut water. This production system will be useful in places where large areas are under coconut cultivation and rice is the staple food for the population and therefore ensuring constant supply of rice gruel and mature coconut water. This method is expected to reduce the carbon-footprint and have a contaminant-free and decentralized bioinoculant production.

Description of the process

Pressure cooker, heater or gas stove with LPG cylinder, candle or bunsen burner, rice gruel which is by-product from parboiled rice cooked in traditional Indian method, mature coconut water which is obtained from mature coconut in homes using coconut for preparing daily food, powdered biochar or charcoal commonly available in farm households and bioinoculants, bacteria or fungi, in 5-10 ml plastic syringe or ink-filler are the materials required for the process.

The method

Mix filtered rice gruel and mature coconut water in equal proportions. Add appropriate volume of the fresh rice gruel + coconut water mixed in 1:1 ratio into the pressure cooker. Biochar or any other charcoal is added to the liquid medium @ 1g/L.

Close the lid of pressure cooker and sterilize the contents by heating on stove. After 3 whistle sounds,

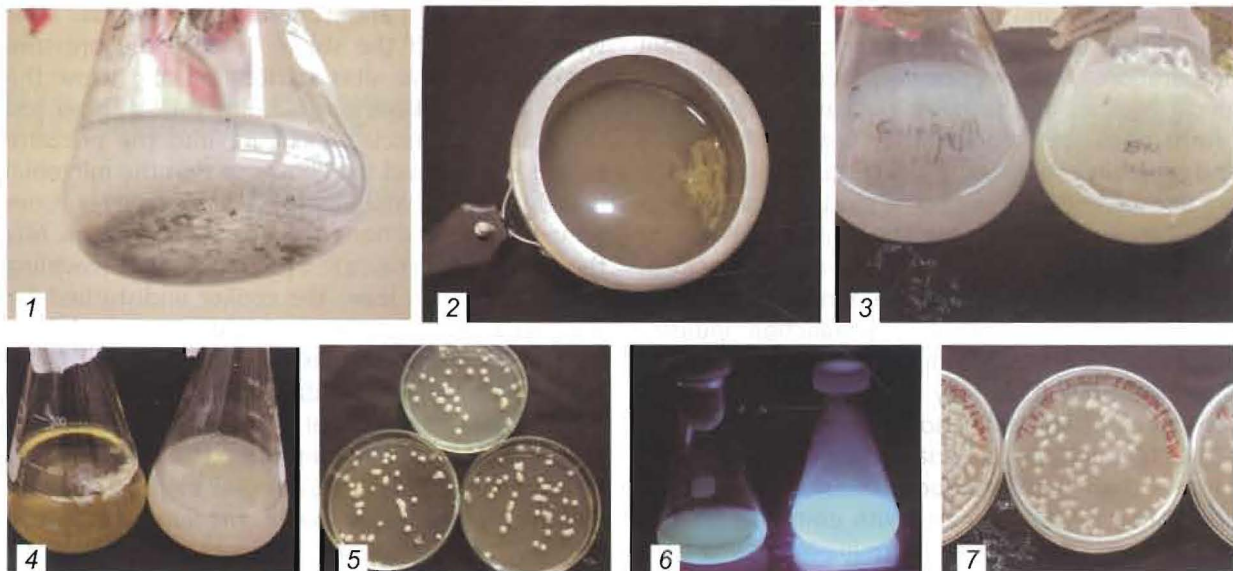
lower the flame and continue for 20 min. after which switch off the stove. Remove the pressure cooker from heat after sterilization and allow the contents to cool without opening the lid. Now add the starter bioinoculant culture into the pressure cooker. Slowly swirl the cooker so that the microbial inoculum mixes well. Keep the cooker under room temperature conditions in a clean and dry area. Mix the contents well twice in 4 to 5 hr interval by swirling the cooker. Then leave the cooker undisturbed for the rest of the incubation period. The incubation period for bacterial mass-multiplication is 24-72 hrs and for fungi is 6-7 days. After incubation, the mass-multiplied microbial inoculants will be ready for application. A minimum of $n \times 10^7$ to 10^9 colony forming units of the inoculants will be present in per ml of rice gruel-coconut water medium prepared in this way. It will be devoid of any contaminant bacteria or fungi. The aqueous bioinoculant can now be used for seed treatment, seedling root dip, soil application or foliar spray.

Advantages of the method

The simplicity of this method empowers small and marginal farmers to mass-multiply plant-beneficial bacteria or fungi themselves on their farm. Locally available substrates are used as medium for bioinoculant mass multiplication. Contamination-free bioinoculants containing BIS stipulated population of bacteria or fungi can be obtained.

This method adheres to BIS standards for aqueous bioinoculant preparation like, the pH of the rice-gruel-biochar medium ranges between 5.4-5.8. This range falls within the BIS stipulated range of pH 5-8 for aqueous media. The medium offers excellent suspensibility for the multiplication of the plant-beneficial microorganisms. The BIS stipulated minimum population of 10^7 to 10^8 cfu/ml of media for plant-beneficial bacteria or fungi is easily maintained by this method. No external bacterial or fungal contamination is observed in this method if the procedure is followed carefully. The method produces aqueous bioinoculant with adequate viable cells for seed treatment as well as field application. The method also circumvents the necessity for storage of the bioinoculants as they can be produced and used immediately. The method, if practiced by individual farmers or group of farmers, can greatly reduce the carbon-foot print for bioinoculant mass-multiplication because of the decentralized approach.

It offers scope for further innovation at farmers'



1. Sterilized coconut water-rice gruel medium with micronized biochar
2. *Bacillus megaterium* multiplied by this method in rice gruel+coconut water medium in a pressure cooker
3. Luxuriant growth of PGPR *Bacillus megaterium* in rice gruel+coconut water medium indicated by the turbidity (right). Medium without culture (left).
4. PGPR *Bacillus megaterium* multiplied in rice gruel+coconut water medium (right) in comparison to that multiplied in commonly used nutrient broth (left)
5. Colony forming units of more than 107 of PGPR *Bacillus megaterium* could be obtained in rice gruel+coconut water medium by this method, no external contaminant is seen other than the pure culture.
6. Bioinoculant *Pseudomonas putida* multiplied by this method showed higher level of fluorescence (right)
7. This method yielded colony forming units of more than 109 of PGPR *Bacillus licheniformis* without any external contamination

level itself as they can identify other appropriate locally available substrates for bioinoculant mass-multiplication.

Some important precautions

Once the bioinoculant is used, the cooker should be immediately cleaned with boiling water. The nozzle, weight and rubber gasket must be cleaned with more precaution. The clean dry cooker can be used for next round of mass-multiplication. A separate cooker for bacterial inoculants and fungal inoculants will help in preventing cross contamination. Care should be taken to keep the syringe and ink-filler out of reach of children. The cooker must NOT be used for preparation of food.

Summary

The method described here relates to

contamination free mass-multiplication of plant-beneficial bacteria and fungi in a synergistic mixture of rice gruel and mature coconut water (1:1 ratio) combined with minute quantities of biochar for immediate field application. The rice gruel-coconut water - biochar medium described here supports growth of all types of plant-beneficial bacterial inoculants, fungal antagonists and microbial entomopathogens. The bioinoculants thus multiplied can be easily applied as seed treatment, seedling dip, soil drenching and foliar spray. Owing to its minimal technical requirements, the bioinoculant can be produced at any time needed for the farmers, provided the starter inoculant cultures are made available. Reliable certified agencies can supply starter bioinoculants in plastic syringe or ink-filler, which is an important external requirement in this method. ■