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Microbial Isolation and Household Waste Recycling for sustainable Biofertilizer Formation

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Abstract: *This study develops a sustainable biofertilizer using specific soil microorganism (Azotobacter, Rhizobium, Phosphate solubilizing bacteria) to decompose household kitchen waste. The isolated microbes perform key function like nitrogen fixation and organic breakdown. The process effectively converts vegetable and fruit waste into a nutrient-rich, value-added product. The leaves of Neem, Tulsi and Gokarna improve soil fertility and produces antifungal, antioxidant agent to prevent fungal growth. The bacillus thuringiensis produces cry toxin control fungal growth. Evolution showed the biofertilizer enhances soil fertility and maintains microbial viability and also control fungal growth. This integrated approach offers a cost-efficient and eco-friendly path for waste reduction and sustainable crop production.*

Keywords: Biofertilizers, Plant growth promotion, Eco-friendly fertilizer, soil microorganism.

I. INTRODUCTION

Biofertilizers are eco-friendly and sustainable alternatives to chemical fertilizers that improve soil fertility and plant growth. Beneficial microorganisms such as *Azotobacter, Rhizobium* and phosphate-solubilizing bacteria (PSB) play an important role in agriculture by fixing atmospheric nitrogen and converting insoluble phosphorus into plant-available forms. *Azotobacter* is a free-living, aerobic, gram-negative bacterium commonly found in soil that helps in nitrogen fixation, production of growth-promoting hormones, and improvement of soil health. *Rhizobium* is a symbiotic nitrogen-fixing bacterium that forms nodules on the roots of leguminous plants. Inside these nodules, the bacteria coverts atmospheric nitrogen into ammonia, which can be easily utilized by plants for their growth and development. These symbiotic relationships increase soil fertility. Similarly, Phosphate-solubilizing bacteria (PSB) also play important role by converting insoluble form of phosphorus into soluble forms that plants can easily absorb. which supports root development, seed germination, and crop productivity. Plant-based materials such as Neem (*Azadirachta indica*), Tulsi (*Ocimum sanctum*) and Gokarna (*Clitoria ternatea*) possess strong antimicrobial and antifungal properties due to the presence of various phytochemicals. These medicinal plants contain compounds like eugenol, azadirachtin, flavonoids and phenolic compounds. These compounds help inhibit the growth of harmful microorganisms and protect plant from diseases. In addition, biocontrol agents such as *Bacillus thuringiensis* produce insecticidal proteins that are effective against specific insect pests while remaining safe for humans and beneficial organisms. The combination of beneficial microbes and plant-derived bioactive compounds provides an eco-friendly strategy for improving plant growth, protecting crops from pathogens and promoting sustainable agriculture. Such integrated biofertilizer formulations can enhance crop productivity while reducing the dependence on chemical fertilizers and pesticides.

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II. MATERIALS AND METHODS

Material used:

Rhizosphere soil [for *Azotobacter* & PSB]
Root nodules of leguminous plant [for *Rhizobium*]
CRYMA, Ashby's & Pikovskaya's media & broth
Carrier [Household waste]
Medicinal plants (Neem, Tulsi, Gokarna)
Bacillus thuringiensis

Sample Collection:

Samples were collected from a farm, where root nodules were taken for the isolation of *Rhizobium* and Rhizosphere soil was collected for the isolation of *Azotobacter* and phosphate-solubilizing bacteria (PSB).

Culture preparation:

Culture is prepared by following methods:

Sampling and enrichment:

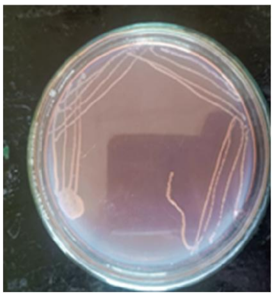
Samples are collected from the rhizosphere soil and root nodules of leguminous plants.
The collected samples are then enriched in suitable culture media (CRYMA, Ashby's, Pikovskaya's medium broth) to increase the growth of useful microorganism.

Isolation and Identification:

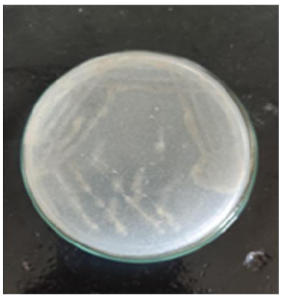
The enriched sample are streaked on selective media like CRYMA, Ashby's medium, Pikovskaya's medium.

They are incubated at 37∘C for 24-48 hrs.

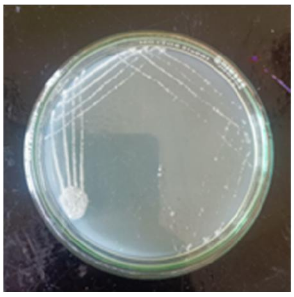
After incubation, individual colonies are observed and isolated to obtain pure cultures.
These cultures are then identified based on their characters.



Rhizobium



Azotobacter



PSB

Table no.1 Culture characters of Rhizobium

Size	Shape	Colour	Margin	Elevation	Opacity	Consistency
1mm	Round	White	Entire	Convex	Opaque	Sticky

Table no.2 Culture characters of Azotobacter

Size	Shape	Colour	Margin	Elevation	Opacity	Consistency
1mm	Round	White	Entire	Convex	Opaque	Sticky

Table no.3 Culture characters of Phosphate Solubilizing Bacteria (PSB)

Size	Shape	Colour	Margin	Elevation	Opacity	Consistency
1mm	Round	White	Entire	Flat	Opaque	Moist

Preparation of a starter culture:

Isolated colonies transferred to flask containing selective Broths. Then flask is kept on a rotary shaker system in a constant temperature (28±2∘C). Then broth enriched within 5-6 days and this how starter culture is prepared.

Table no.4 Bacteria and Broth used

Sr.no	Bacteria used	Broth used
1.	*Rhizobium*	Yeast Extract mannitol Broth
2.	*Azotobacter*	Ashby's Broth
3.	Phosphate Solubilizing Bacteria (PSB)	Pikovskaya's Broth

Preparation of mass culture:

Yeast extract mannitol broth is used for mass culture production.
Starter culture is used as the Mass culture.
1 litre starter culture required for production of 100 litre mass media broth.
Ratio 1:100

Household waste and medicinal plant processing:

Household organic waste like vegetable waste and fruit waste and medicinal plant like Neem (*Azadirachta indica*), Tulsi (*Ocimum tenuiflorum*), Gokarna (*Clitoria ternatea*) are added to prevent fungal and bacterial contamination. Dry the all material and make the fine powder after that use this fine powder as carrier.

Making the carrier-based inoculant

A carrier is neutral medium used to mix with a cultured broth for handling it easily. The powdered form carrier is mixed with Egg shell Powder. After that autoclave the carrier and then allow to remove the moisture in sunlight. After the drying broth culture and the carrier are mixed properly in trays. Carrier is mixed into broth till it receives 40-50% of moisture. Inoculant and Carrier mix properly and keep it for 2-7 days for multiplication of organism. This all-overall process is called Curing / Stabilization.

Packaging:

After Stabilization, the inoculant is packed in paper bags. The product is properly labelled with respect to name of the biofertilizer, names of the recommended crops, price, weight, date of manufacture and expiry and instruction for storage and precautions. Paper bags are store in a cool & dark place.

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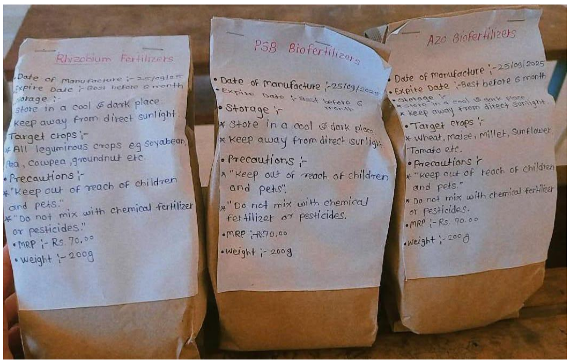

Packaging of Biofertilizers

Pot trials:

The formulated biofertilizer is applied to plant grown in pots to observe its effect on plant growth.

This helps to evaluate the effectiveness of the biofertilizer in promoting plant growth and improving soil fertility and crop productivity.

Crop Response:

For *Rhizobium*: Inoculation increases root nodulation, nitrogen fixation, plant growth and grain yield in legume crops such as chickpea. It also improves protein content and nutrient uptake (N and P) in plants yield increase up to 10-35%.

For *Azotobacter*: Enhances plant growth, biomass and nutrient absorption. Studies reported increased plant dry weight and improved nutrient uptake compared with uninoculated control and together with 75% of recommended dosage of nitrogen fertilizers boosts yield in many crop plants.

For Phosphate Solubilizing Bacteria (PSB): Improves phosphorus availability in soil leading to higher seed germination, root length, shoot growth and crop productivity. Experiments showed 40–80% increase in seed germination and improved plant growth parameters.

Observations:

Without fertilizer

With fertilizer

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III. RESULT

Azotobacter, Rhizobium, Phosphate solubilizing bacteria with *Bacillus thuringiensis* successfully produced and characterized.

Household waste supported high microbial viability.

IV. CONCLUSION AND FUTURE DIRECTION

The present study confirmed successful production of *Azotobacter, Rhizobium*, Phosphate solubilizing bacteria as biofertilizers. These microorganisms demonstrated significant abilities in nitrogen fixation, nodulation and phosphate solubilization, thereby enhancing soil fertility. Biofertilizers are thus and eco-friendly, cost-effective and sustainable alternative to chemical fertilizers for improving crop productivity and maintaining soil health.

Future research should focus on the isolation and characterization of efficient microbial strains from diverse environments to enhance biofertilizer production. The utilization of household organic waste as a substrate for microbial growth and nutrient recycling can provide a sustainable and low-cost approach for biofertilizer formulation. Further studies are required to optimize microbial consortia, carrier materials and storage conditions to improve the stability and effectiveness of these biofertilizers. In addition, large-scale field evaluations should be conducted to assess their long-term impact on soil fertility, crop productivity and environmental sustainability.

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