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Original Research Article

Role of microbial inoculants in liquid fertilizers for next generation farming

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ABSTRACT

Background: Microbial inoculants are an important part of the integrated nutrient supply systems as they are a cost-effective and renewable source of plant nutrients, which complements chemical fertilizers and support sustainable farming. Liquid formulations of microbial inoculants typically are broth based, oil, or polymer-based products. Biofertilizer in form of liquid broth is a special formulation containing large number of desired microorganisms with maximum shelf life and minimum contamination.
Methods: For present study, Soil survey and isolation of microbes was done. Results were showing that the growth of microbial inoculants on yeast extract MMannitol Agar (YEMA), Jensen's Media, Mannitol Ashby agar medium, Pikovskaya's (PVK) medium, NBRIP National Botanical Research Institutes's medium, and *Azospirillum* media, Bunt and Rovira agar medium prominently very effective for carrier based liquid biofertilizers.
Results: Isolation of *Rhizobium, Azotobacter, Azospirillum,* phosphate solubilizing bacteria (PSB) and different formulation are prepared at laboratory scale and carrier based microbial inoculants were also prepared and having very good shelf life.
Conclusions: Different formulation of liquid inoculants are recomended for farming. Nitrogen fixer, phosphate solubilizer, potassium solubiliser, zinc solubiliser play important role in next generation farming.

Keywords: Soil, Farming, *Rhizobium sp.,* PSB*, Azotobacter*

INTRODUCTION

In India, 80% of the rural population depends agricultural and related activities. To meet the food needs of a constantly growing population it is essential to maximize the productivity of each unit of agricultural land. For more than half a century the world has focused on increasing crop yields to fulfill the rising demand for food. Therefore, the development of food production systems is crucial. Enhancing the productivity of agricultural land requires understanding the role of different crop nutrients in increasing yield. India is an important global market in the production and the use of biofertilizers. Microbial inoculants are an important part of the integrated nutrient supply systems as they are a cost-effective and renewable source of plant nutrients, which complements chemical fertilizers and support sustainable farming. India is one of the key countries in the production and consumption of both chemical and biofertilizers. To promote organic farming, the use of biofertilizers such as *Rhizobium, Azotobacter, Azospirillum,* PSB and VAM, *Mycorrhizal* colonies has been included in the FCO, 1985. Biofertilizers are also known as microbial inoculants or bioinoculants.[1] The issue related with solid carriers can be addressed by using liquid inoculants. Liquid inoculants are made from low-cost materials and perform well in the field.[2] A good liquid inoculant should be non-lethal, cost-effective, easily available, uniform, and adaptable to normal cell culture conditions, allow for nutrient supplementation, release microorganisms quickly in the soil, support their growth and survival, and be easy to handle during mixing and packaging.[2] Research studies have been conducted to compare the effectiveness of liquid biofertilizers with solid carrier-based products. Solid fertilizers require high cost for crushing, deactivation and sterilization, among other processes. Liquid biofertilizers are aqueous, oil-based, or polymer-based products. These contains the desired organisms, nutrients, cell protectants and chemicals that helps to maintain cell survival during storage and application to seeds or soil field at farming location.[3] In liquid formulations sprayable propagules are suspended in suitable liquid medium and their biological activity is enhanced at target site. As per the reports of liquid biofertilizers should be easy to deliver to the field, it should tolerate adverse environmental factor at target site, it should increase viability, reproduction, contact and interaction with the target crops. Polysaccharide such as gums, carboxymethylcellulose and polyalcohol derivatives alter fluid properties of liquid formulation.[4] Rhizobia can endure in liquid medium with the support of cell protectants like trehalose, polyvinylpyrrolidone for nearly six months. *Rhizobium* liquid biofertilizer are eco-friendly, sustainable, cost-effective for Indian farmers and also increase soil fertility with respect to nutrients (N, P and K).[5] *Rhizobium* sp. Found to be appropriate to the same region as liquid biofertilizer formulation to make the farmers and agricultural performs economically and socially sustainable. *Azotobacter* liquid biofertilizer also enhances the growth of plant and it is free living nitrogen fixer. It is unobtainable to plants because in the soil it is mostly present in the fixed form or in inorganic form PSB convert it into available form.

In present investigation, attention was directed toward developing biologically driven nutrient management approaches through the integration of microbial inoculants into liquid fertilizer systems. The research involved the selection and detailed characterization of efficient microbial strains possessing important plant-beneficial functions, including atmospheric nitrogen fixation, phosphate solubilization, and plant growth-promoting activity. In addition, liquid biofertilizer formulations were designed and optimized to enhance microbial persistence, stability, and metabolic performance during storage and application. The role of formulation constituents, such as nutrient substrates and protective compounds, was analyzed to determine their contribution to maintaining microbial viability and extending shelf life under controlled conditions. The functional capability of these liquid formulations was further assessed by examining their effectiveness in supporting nutrient transformation processes, particularly nitrogen fixation and phosphorus mobilization.

Moreover, the stability, efficiency, and practical applicability of liquid biofertilizers were evaluated in comparison with conventional carrier-based inoculant systems. The outcomes of this investigation are expected to support the development of advanced microbial formulations that contribute to sustainable agriculture and environmentally responsible farming practices.

METHODS

Sample collection

Collection of rhizospheric soil samples from different fields using sterilize hand auger and push probe. Rhizospheric soil samples were taken to laboratory for isolation of bacteria and fungus.

Screening and cultural characteristics

Selective media like YEMA, Jensens Media, Mannitol Ashby agar medium, PVK agar medium were used for isolation of *Rhizobium spp, Azotobacter spp and Phosphate* solubilizing microorganisms respectively. Isolation and screening was carried at 28°C for 2 to 7 days. Cultivation conditions are maintained for all the isolates.

Identification of isolates

The selected isolates of bacteria and fungi were characterized on the basis of morphological, biochemical and cultural characteristic for comparative studies and confirmation. Pure cultures of selected isolates were also maintained for further studies and analysis.

Formulation of liquid microbial inoculants

Selection of isolated microbial inoculants and incubation at 25-28°C.

Liquid broth formulation-Liquid broth formulation was prepared in Yeast extract Mannitol broth, Jensen broth, nutrient broth for *Rhizobium spp, Azotobacter spp* and PSB respectively. Cell count was maintained at 10^8 CFU/ml. Potato dextrose broth was used for biofertilizer formulation with fungal cultures. Addition of cell protectant to increase shelf life.[12]

Carrier addition the grown culture were mixed with the carrier to confirm constancy and equal distribution all through use in the field.

Quality check by calculated total viable count of the prepared liquid formulated broth (CFU/mL) and shelf life.

RESULTS

Soil samples were inoculated as per standard protocol of SPC and the organism were inoculated on selective media like YEMA, Jensen Media, Mannitol Ashby agar medium, PVK medium, PDA to observe growth and their characteristics on the pertiplates and noted the result found.

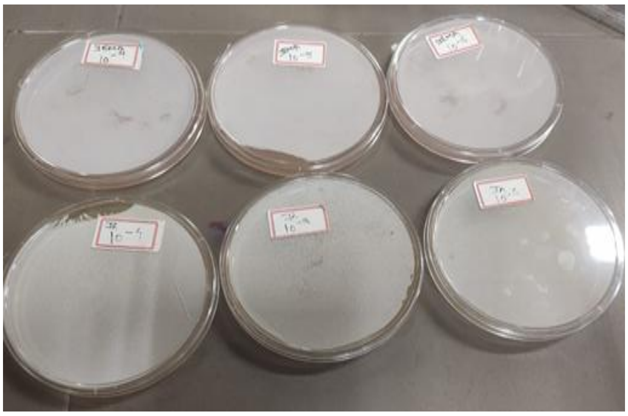

Figure 1: SPC of soil sample on selected media for isolation of *Rhizobium, Azotobacter*, etc.

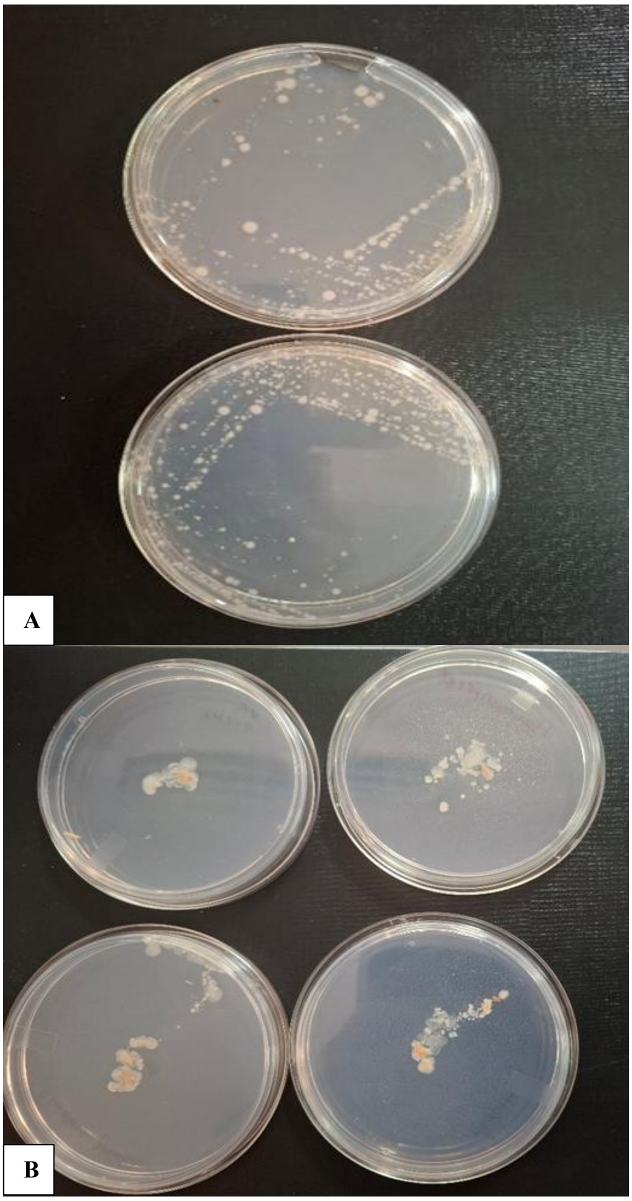

Figure 2 (A and B): A) Colonies on YEMA plates and B) Isolation of *Rhizobium* from root nodules on YEMA.

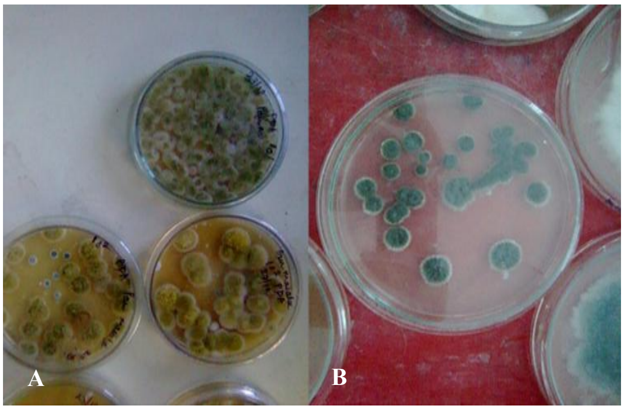

Figure 3 (A and B): Isolated fungal colonies on PDA agar.

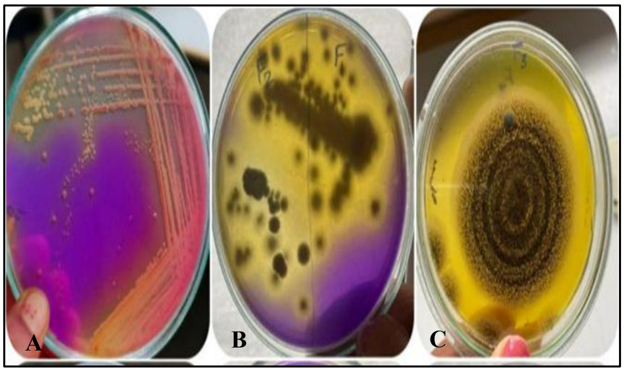

Figure 4 (A-C): Isolation of phosphate solubilizing fungus on NBRIP medium.

The isolated colonies of *Rhizobium* were milky, smooth, irregular and raised (Figure 2) while the colonies of PSB (Figure 4 and 5) was found to be white colored, smooth colonies on PVK medium.

All the isolated colonies were identified on the basis of Gram staining, confirmatory biochemical test and characteristics of cultured colonies on selected agar medium.

In the present study, microorganisms capable of solubilizing mineral nutrients were successfully isolated using selective culture media. Yeast isolates grown on PVK agar exhibited distinct transparent zones surrounding the colonies, indicating their ability to mobilize insoluble phosphate (Figure 4 and 5). This phenomenon is primarily associated with the release of low-molecular-weight organic acids, which facilitate the conversion of complex phosphate into soluble forms.

Similarly, bacterial isolates obtained on Bunt and Rovira medium demonstrated clear zone formation in the presence of insoluble zinc compounds. The appearance of these zones confirms the capacity of the isolates to transform unavailable zinc into forms accessible for biological uptake (Figure 6). Variability in halo diameter among isolates suggests differences in their metabolic efficiency and solubilization potential.

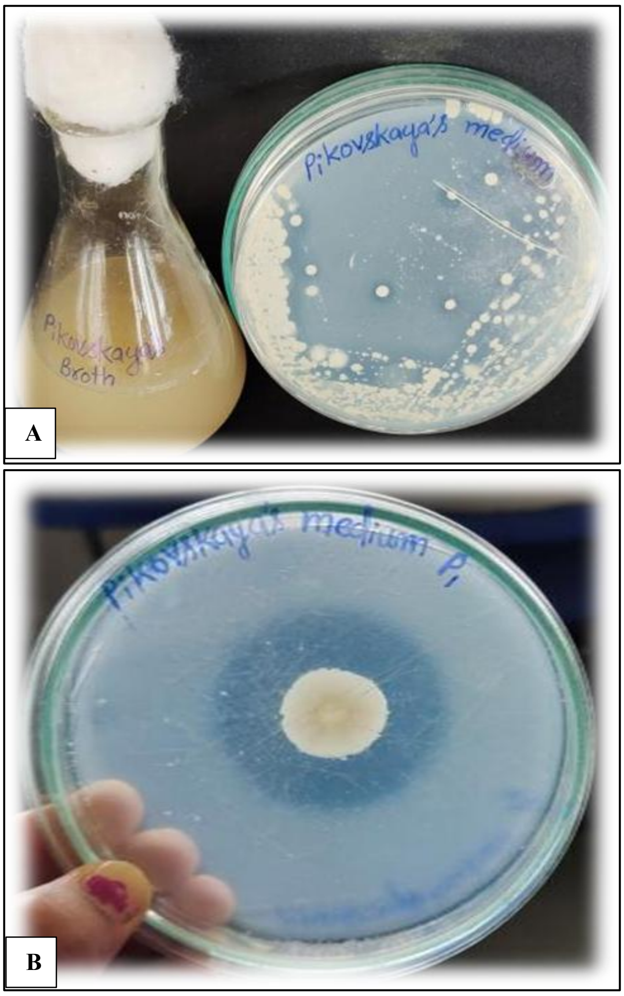

Figure 5 (A and B): Isolation of phosphate solubilizing yeast on PVK medium.

Figure 6: Isolation of zinc solubilizing bacteria of Bunt and Rovira agar medium.

A series of carrier-based liquid biofertilizer formulations were developed under laboratory conditions by incorporating selected microbial inoculants into sterile carrier matrices supplemented with liquid stabilizing agents. The prepared formulations appeared homogeneous, with no signs of contamination during the observation period, confirming the effectiveness of aseptic preparation procedures. Despite these differences, all formulations-maintained conditions supportive of microbial viability.

Evaluation of microbial survival indicated that viable cell counts were sustained throughout the storage period, although the extent of survival varied among formulations. This enhanced stability may be attributed to the availability of nutrients and reduced exposure to environmental stress factors.

In addition, functional assessment demonstrated that microbial activity, particularly nutrient solubilization capacity, was preserved over time in most formulations. Preparations containing supplementary organic components exhibited relatively improved performance, indicating their role in maintaining metabolic activity and prolonging shelf life.

Physical stability analysis showed that optimized formulations remained well-dispersed without visible sedimentation or phase separation. In contrast, a few formulations exhibited minor settling upon storage, which may influence uniform application.

Figure 7: Different formulation of carrier based liquid biofertilizes having microbial inoculants at laboratory scale.

Collectively, these observations indicate that formulation composition significantly influences microbial survival, activity, and stability. Selected formulations (Figure 7) showing consistent viability and functional efficiency under laboratory conditions may be considered suitable candidates for further evaluation.

DISCUSSION

Liquid formulation for *Azospirillum* by using various cell protectants (trehalose, polyvinylpyrrolidone and glycerol) and found rise in shelf life by maintaining population level at 10^8 CFU/ml.[6] Liquid formulation of Rhizobium by adding various additives in the yeast extract mannitol media and claimed cell numbers of 1×10^{10} cells/ml in the liquid inoculant.[2]

A field experiment was carried out to evaluate and compare the response of pigeon pea to liquid and solid carrier-based *Rhizobium* inoculant. The results showed the presence of higher number of nodules in case of liquid-based inoculant treatments as compared to the solid carrier-based inoculants.[7]

In an experiment conducted with *Rhizobium* inoculants, liquid inoculant was found equally effective in comparison to solid carrier-based inoculant.[8] Cell protectants in liquid formulation ensure long shelf life, better adaptive capacity and ability survive in harsh conditions.[6] Cell protectants in liquid formulation protect PGPR cells from desiccation, osmotic pressure, temperature, maintain proper enzyme activity and support cell membrane.[6]

Rhizobium

The study on the effect of inoculation with culture of Rhizobium on nodulation, N_2 fixation and yields of gram (*Cicer arietiuum L.*) was undertaken under laboratory and field condition. In the field, addition of seed through an active and viable various strain of *Rhizobium* gives a significant growth in nodulation formation in plants, N_2 content in different parts of plant and grain yield over uninoculated control. *Rhizobium* liquid biofertilizer treated pots showed more efficiency with compare to the other uninoculated control. These results are in agreement with the results obtained and observed that inoculated common vetch cultivars give higher biological yield (8.5%), seed yield (7.6%), straw yield (70.4%), pod yield (25.5%), number of seed per pod (16.2%), number of pods (28.4%) compared to non-inoculated cultivars.[9]

Azotobacter

Significant variation were observed in plant height and number of leaves due to application of liquid biofertilizers in Tamil Nadu region. Increase in plant height, number of leaves, shoot length, Root length, number of roots, number of nodules was confirmed with statistical method. It is mentioned that the soil sample was subjected to Tamil Nadu Government Agriculture Department, Mobile Test Centre, Thiruvarur.[10]

Phosphate solubilizing organisms

The presence of phosphate microorganisms increases the crop productivity as phosphorus is one of the important nutrients for plants and crops. PSB like *Bacillus spp., Pseudomonas spp., Enterobacter* spp. etc. are actual useful for growing the plant available phosphorus in soil as well as the progress and harvest of crop yield. So, exploitation of PSB through biofertilizer has enormous potential for making use of ever-increasing fixed P in the soil, and natural reserves of phosphate rocks.[11]

Azospirillum

Efficiency of *Azospirillum* as liquid biofertilizer. The effects of nature and concentration of additives on the performance of liquid inoculum. The formulation of liquid inoculants of *Rhizobium* sp., *Azotobacter* sp., *Azospirillum* sp. and PSB create maintaining population up to the growth of 10^8 cells per ml.[2,3,13-18] Inoculants of *Azotobacter sp., Azospirillum sp., Acinetobacter sp., Bacillus sp*. and *Pseudomonas* sp. tested with different osmolytes in different concentration to optimize it for liquid inoculant preparation

A liquid inoculant formulation with good field performance characteristics that uses low-cost materials and are easily attainable by small producers, could reduce many problems associated with processing charcoal.[6] Liquid formulation of *Azospirillum* by using various cell protectants (trehalose, polyvinylpyrrolidone and glycerol) which resulted in increased shelf life by maintaining population level at 10^8 CFU/ml. Liquid inoculants of *Rhizobium* by adding various additives in the yeast extract mannitol media and claimed cell numbers of 1×10^{10} cells/ml in the liquid inoculant.[2]

Yadav et al studied effect of solid based and liquid based rhizobium fertilizer on growth of pigeon pea. They carried out a field experiment to evaluate and compare the account of pigeon pea to liquid and solid binder-based *Rhizobium* inoculant, the results showed the presence of higher number of nodules in case of liquid-based inoculant treatments as compared to the solid carrier-based inoculants and experiment found that liquid bioinoculants yielded equal or better nodulation than that observed with peat inoculant in pea plants.[15-17] In an experiment conducted with *Rhizobium* inoculants, liquid inoculant was found equally effective in comparison to solid carrier-based inoculant.[8]

CONCLUSION

India's agricultural sector supports a substantial proportion of the population, with a large segment relying on farming as their primary livelihood. Despite this dependence, farmers frequently encounter challenges related to soil fertility, input costs, and environmental sustainability. In this context, the application of beneficial microorganisms such as *Rhizobium*, *Azotobacter*, *Azospirillum*, and PSB has emerged as an effective strategy to enhance crop productivity in an eco-friendly manner.

The continuous and excessive application of chemical fertilizers has raised serious environmental concerns, including soil acidification, depletion of organic matter, and contamination of water bodies through runoff. These issues highlight the need for sustainable alternatives that maintain soil health while supporting crop yield.

Biological nitrogen fixation plays a crucial role in sustainable agriculture. *Rhizobium* forms symbiotic associations with leguminous plants and converts atmospheric nitrogen into plant-available forms. In contrast, *Azotobacter* functions as a free-living nitrogen fixer, contributing to soil nitrogen without requiring a host plant. *Azospirillum*, a plant growth-promoting rhizobacterium (PGPR), enhances plant development through multiple mechanisms, including phytohormone production, improved root architecture, and increased efficiency in water and nutrient uptake. Additionally, it produces protective compounds that help plants tolerate environmental stresses such as drought and flooding.

Phosphate-solubilizing microorganisms are naturally present in soil ecosystems and play a vital role in mobilizing insoluble phosphate. These microorganisms secrete organic acids containing functional groups such as hydroxyl and carboxyl moieties, which facilitate the release of phosphate ions by chelating bound cations, thereby increasing nutrient availability to plants.

The use of plant growth-promoting rhizobacteria as biofertilizers represents a significant step toward sustainable agricultural practices. Traditionally, carrier-based biofertilizers have been widely used; however, they are often associated with limitations such as reduced shelf life, contamination risks, and decreased microbial viability due to desiccation during storage and transportation. These constraints have led to the development of liquid biofertilizer formulations as a more efficient alternative. Liquid biofertilizers incorporate protective additives, including compounds such as trehalose, glycerol, and polyvinylpyrrolidone, which enhance microbial survival by minimizing stress and prolonging shelf life. Unlike solid carrier systems, liquid formulations maintain higher viability and metabolic activity of microbial inoculants over extended periods.

In the present study, conventional carrier-based inoculants were modified into liquid formulations to improve their stability and functional performance. The observed increase in shelf life and maintenance of viable populations of nitrogen-fixing and phosphate-solubilizing microorganisms demonstrate their potential for agricultural application. Such advancements support the adoption of biologically based inputs and contribute to the development of sustainable and resource-efficient farming systems.

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