

**ENHANCING SHELF LIFE OF CARRIER BASED  
MICROBIAL PLANT GROWTH STIMULANTS DURING  
STORAGE AND ITS APPLICATION IN CULTIVATION  
OF SOME ECONOMICALLY IMPORTANT PLANTS**

**SUMMARY**

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Agriculture is one of the oldest and major occupation of the human settlements that fulfills the basic food needs of the people and their domesticated animals. To meet the food needs of the growing population during the last several decades there has been overuse of chemical fertilizers, pesticides and other agrochemicals support the plant growth, but by leaching into surface water and diffusion as well as emission in the air it causes pollution and climate change. The health and flora of the soil and water deteriorate alarmingly when synthetic fertilizers and other agrochemicals are used excessively without appropriate management of their residues.

The persistence of these agrochemicals has a consistent negative impact on the environment and causes many severe disorders on human health, necessitating an urgent need for safer alternatives. Presently, a call for nature-based agriculture is given by the United Nations and governments of developing countries including our Indian government and the farmers are interested in integrating agroecological practices that can enhance the production systems for transition to organic agriculture with good crop yield. It helps in the efficient use of water and nutrients, and the food and forage products are non-contaminated and of high nutritional quality within the normative standards of good agricultural practices. Farmyard manure (FYM), Zero-Budget Natural Farming (ZBNF), Vermicompost, no-tillage conservation farming, and application of plant growth-promoting microbes (PGPMs) are seen as different possible alternatives to synthetic fertilizer working on the situation provided. The use of PGPMs is one of the alluring and more technologically defined strategies to increase crop productivity and net yield of nature-based agricultural inputs and enhance soil biodiversity in a cost-effective and is scientific environment-friendly manner. Plant growth-promoting microbes can successfully colonize the surface of the roots, improve nutrient bioavailability to the plants and can improve bio-assimilation. In addition,

PGPMs aid in the biodegradation of toxic agrochemicals and shield soil infections of crop plants. They can act as an effective alternative to chemical fertilizers, synthetic pesticides and gene transfer through genetically modified crops.

Beneficial soil microbes can assist in fulfilling the United Nations' 2nd Sustainable Development Goal, which aims to boost agricultural output and incomes by 2030 in order to end poverty and malnutrition while also ensuring sustainable food production systems based on ecological rejuvenation. Beneficial soil microorganisms derived from various ecological niches have been treated exogenously to boost plant output in the agricultural fields. However, inoculation of single microbial inoculates under field conditions could not show promising benefits due to the poor adaptability of microbial inoculants to the changing environmental conditions and its ill competition potential with the native microbes in many cases. Therefore, a strategy is emerging to use them in the form of compatible communities called microbial consortia. Microbial consortia can perform better in different soils, as they can produce multiple benefits to the plants and soil and can adapt in a diverse range of climatic conditions with multiple inputs of root colonization, disease resistance, metabolite production, nutrient solubilization and antibiotic production etc. In this ever-changing soil environment, sometimes the population of PGPMs starts declining after its application in the rhizospheric niche as their density and population dynamics in the rhizosphere depend on various factors like soil moisture, soil temperature, soil pH, salinity, availability of pesticide residue and other toxic residues etc. The short shelf life of inoculated microbes and contaminations of soil with agrochemicals are possibly the major bottlenecks in the commercialization of microbial bioformulations. In order to commercialize the technology, inoculae viability in an appropriate formulation for a longer duration is essential. Therefore, there must be some medium or materials which can provide a niche

to the selected inoculates as a single microbe or in consortia form which can help in increasing its viability and shelf life during storage and application. These Carrier materials support the growth and delivery of microbes to the rhizosphere and have the potential to protect its competitive strength with other endemic microbes, soil pH and availability of organic carbon in the soil.

Sixteen climate-resilient agriculturally beneficial, isolated and characterized PGPMs (bacteria and fungi) were selected from the depository of the research group of Prof Rana Pratap Singh. The microbes stored at 0-4°C were recharacterized for their plant growth promotion activities and vibrancy through the cfu count. Based on the retention of PGP activities and their compatibility, seven microbial strains (*Bacillus filamentosus* RS3B, *B. pseudomycooides* RS6B, *B. paramycooides* RPB3, *Alcaligenes faecalis* RS10B, *Aspergillus tamarisii* RS8F, *A. luchuensis* RS6F and *Trichoderma lixxi* TvR1) have been selected for this study. The selected bioinoculants were applied to the earthen pots of Tomato (*Lycopersicon esculantum* Mill.) as a single microbe and in the consortia of compatible communities to evaluate their efficacy in vivo. The microbial bioinoculants developed by us have improved soil fertility and increased the growth and productivity of the plants significantly even in pot conditions. Most of the microbial strains selected for this study perform better in the mixed cultures of microbial strains than the monocultures. The present study aimed to assess the shelf-life of the microbial inoculants as single species or in consortia of the compatible communities in storage conditions and room temperature and to design bio-stimulants with suitable carriers to increase its in vitro and in vivo viability and effectivity during storage and application to the crop plants.

Our question was to assess the role of carrier material in enhancing the shelf life of bioformulations and their possible effect on plant growth and productivity under the

natural conditions of crop cultivation. We hypothesize that increased microbial diversity and carrier-based support systems in the formulations can enhance the shelf life of microbial bio-stimulants and can help in the cultivation of plants can improve plant growth, productivity and restoration the soil fertility without any agrochemical-based external inputs. The major objectives of the present study were reassessment of viability and efficacy of the stored beneficial soil microbes and to develop a novel formulation to increase its efficacy during the storage and application to the crops. Our results indicate that carrier materials and microbial diversity in the bioformulations could help in enhancing the shelf life of microbial consortia stored at different temperatures. The maximum viability of microbial consortia in terms of cfu/g of bioformulation was reported in bioformulation immobilized in talc+gluten carrier-based bioformulation (BC(I)4) followed by BC(II)4, BC(I)1 and BC(I)8. The selected formulations were applied to pot cultivation of Spinach (*Spinacea oleracea* L.) and Tomato (*Lycopersicon esculantum* Mill.) could increase their yield and productivity even using the microbes isolated and characterized before 2-3.5 years. The results of the pot study with Spinach and Tomato indicate that enhancing microbial diversity in consortia significantly enhances shoot length, shoot biomass, root length, root biomass, number of fruit and fruit biomass per plant. It seems that a better understanding of plant-microbe interactions and the ecological succession of these microbial inoculates will pave the way for environment-friendly agriculture and crop production in the future.

After storage of 1 year at 0-4°C and room temperature, the most viable and effective bioformulation BC(I)4 was selected and evaluated for its efficacy in promoting plant growth, productivity and enhancing soil fertility and root colonization in pot culture with four commercial crops (Spinach, Tomato, Basil and Chilli) for two consecutive years. The results of the study indicate that this bioformulation proves to

be the most efficient in enhancing plant growth and productivity in terms of root length, shoot length, plant biomass, leaf area, pigments and protein in crop plants even after storage of one year at 0-4°C and room temperature. Apart from increasing plant growth and productivity, microbial bioformulation also improves soil health by solubilizing insoluble nutrients like phosphorus, nitrogen and potassium and enhancing the availability of these essential nutrients to the plant for their growth and productivity. In addition, microbial bioformulation also enhances soil fertility by increasing the soil microbial biomass carbon, alkaline phosphatase and population of soil microbes in the rhizosphere. Thus, the outcome of the study reflected that the formulation of microbes in carrier material is an alternative to conventional unsustainable approaches for agriculture. The results of the bioformulation application on Basil and Chilli in pot condition indicate that this bioformulation is also proved to be very effective in promoting the growth and productivity of other crops besides Tomato and Spinach.

Overall, the findings of the study showed that bioformulation of competent microbes (*Bacillus filamentosus* RS3B, *B. pseudomycooides* RS6B, *B. paramycooides* RPB3, *Alcaligenes faecalis* RS10B, *Aspergillus tamarisii* RS8F, *A. luchuensis* RS6F and *Trichoderma lixxi* TvR1) in carrier material (talc+gluten) is a viable alternative to traditional, non-sustainable synthetic chemical-based methods of crop production. Therefore, in order to maintain soil fertility and plant growth, it can be inferred that plants seeded with microbial bioformulation can readily reduce the input cost of crop production, reliance on chemical-based fertilizers and eventually enhance the soil health and nutritional quality of the crop. Therefore, the application of microbial consortia along with these carrier materials could be the competent bioformulations that not only improve the soil health but enhance the yield and nutrient status of the crops in an economical and eco-friendly manner. Further studies in the natural field

conditions are suggested with the selected bioinoculant to investigate their effectiveness in field cultivation.

### **Key findings of the study**

- ✓ The question was, whether microbial consortia and carriers could help in enhancing the shelf-life and efficacy of the amended soil microbes has been addressed positively.
- ✓ The study indicates that beneficial soil microbes, stored at 0-4°C can be revived after 2-3.5 years as it shows viability and plant growth promoting activities after 2 - 3.5 years on revival in fresh culture media.
- ✓ In our study certain microbes like *B. filamentosus* RS3B, *Aspergillus luchuensis* RS6F, *A. tamaris* RS8F and *Trichoderma. lixi* TvR1 showed additional PGP activities e.g. Nitrogen fixation, Phosphate solubilization, siderophore production and ammonia production after 2-3.5 years of storage and revival.
- ✓ Our data indicate that increase in species diversity and number of strains in the consortia increase the shelf-life and efficacy of the microbial formulation for plant growth, productivity as well as soil properties.
- ✓ The carrier material talc+gluten could immobilize the microbial cells and helps in maintaining its viability and efficacy even after 2 years of storage at 0-4°C and room temperature, which is evident with its effect on soil fertility, plant growth, productivity and microbial colonization in the rhizosphere.
- ✓ The study provides evidence that the shelf-life and efficacy of microbial inoculants can be increased significantly by developing novel formulations in consortium form and its immobilization in carrier like talc+gluten.