SURVIVAL OF BENEFICIAL MICROBES IN LIQUID BIOFERTILIZER AMENDED WITH DIFFERENT CARRIER MATERIALS

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Abstract - Salinity has emerged as one of the most severe environmental stresses on agricultural plants. It has become an obstacle to meeting the world's increasing food demand, which causes a global economic loss of up to US$ 27.3 billion. Soil salinity in rice cultivation areas is considered a severely limiting factor that adversely affects the quantity and quality of wetland rice production. Recently, the alternative use of salt-tolerant plant growth-promoting rhizobacteria (PGPR) inhabiting extremesaline conditions has gained remarkable attention and has positive impacts on soil and crops. These microbes invade plant roots, promote plant growth, and improve crop yield production. Multi-strain salt-tolerant rhizobacteria such as Bacillus tequilensis and Bacillus aryabhattai strains is a type of PGPR with a short shelf-life due to its structural and cellular components, with a non-producing resistance structure (spores). Therefore, optimum formulations must be developed to prolong the bacterial shelf-life by adding liquid carrier materials as an amendment that could benefit the microbes by providing shelter and carbon sources as food for bacteria. Therefore, a study has been undertaken to develop a liquid biofertilizer formulation from locally isolated multi-strain salt tolerant PGPR, using glycerol, trehalose and polyvinylpyrrolidone (PVP) as additives. The liquid carrier formulation was validated using an optimum amount of the cell protectants or chemical amendments, namely Glycerol (5 mM), Trehalose (10 mM), and polyvinyl pyrrolidone (PVP) at (1 %). The selected amendments were added in the tryptic soy broth (TSB) growth media and inoculated with Bacillus tequilensis(UPMRB9) and Bacillus aryabhattai (UPMRE6)and the combination of both strains. The shelf-life was recorded, and viable cells count, optical density and bacterial dry mass were used to determine the bacterial population and growth trend at monthly intervals. Determination of the optimal formulation of organic fertilizer to achieve maximum cell density of the strains were performed through the two-level factorial design and central composite design using Response Surface Methodology (RSM) concept. After eight months of incubation, the initial population were highest in the mixed strain amended with trehalose at 85.67 cfu g-1, followed by UPMRE6 and UPMRB9 at 72.67cfu g-1, and 70.34cfu g-1.The mixed strain also significantly stimulated theoptical density and recorded the highest value at 1.80, followed by UPMRB9 and UPMRE6 at 1.76 and1.69 respectively.After six-month, the population were started to decrease, and the maximum population and density was recorded with mixed strain amended with trehalose additive at 76.34 cfu g-1 and 1.84 respectively.Results showed that anoptimal concentration of trehalose has successfully prolonged the bacterial shelf-life with minimal cell loss.Validation of the quadratic optimization through response surface methodology wasrevealed that the cell density of the missed strainwas 42.78 log CFU/mL after 24 h which was 99.7% accuracy towards the predicted valuein minimized medium formulation: 0.267 g/ml Trehalose, 1% Glycerol, 120 rpm agitation.

Keywords - PGPR, Additives, Shelf-life, Bio formulation, RSM