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INNOVATIVE LIQUID FORMULATION OF MICROBIAL STRAINS FOR PRODUCING A BIO-FERTILIZER: FIELD TESTING ON *EMBLICA OFFICINALIS*

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Abstract: Liquid bio-fertilizer is increasingly available in the market as one o f the alternatives to chemical and organic fertilizers as well as solid substrate-based Bio-fertilizers. One of the benefits from Bio-fertilizer is the contribution of micro-organisms. These micro-organisms may enhance the plant growth and create healthy rhizosphere. The advantage of a liquid Bio-fertilizer is that no solid carrier is needed. These products are also developed for potential application in modem agriculture such as soil less farming systems such as hydroponics. Traditionally, liquid Bio-fertilizer is produced from fermentation of effective microorganisms which was recommended to be used within three months. Three inoculums (phosphate solubilizing bacteria and plant growth promoting bacteria) were developed and formulated as liquid Bio-fertilizer. The liquid Bio-fertilizers were kept at low temperatures (9 + 2 °C) and room temperatures (28 ± 2°C) for shelf-life study.

Index Terms **- Micro-organisms, Liquid Bio-Fertilizer, Growth Effect, Activation media.**

I. INTRODUCTION

Bio-fertilizer is a substance containing living microorganisms which, when applied to seeds, plant surfaces, or soil, colonizes the rhizosphere or the interior of the plant and promotes growth by increasing the supply or availability of primary nutrients to the host plants (Vessey, 2003). Bio-fertilizers usually need a carrier as medium for the microbial inoculants. A suitable carrier material needs to be inexpensive, easily available, and high in organic matter content, and should have a high water-holding capacity and a favorable H^+ concentration (Gaind and Gaur, 1990). Furthermore, a good quality carrier should be free from microbial contamination, and can optimize the growth of the Bio-fertilizer microorganisms (Phua *et al*., 2009a). However, it is not easy to get a carrier that meets the desired qualities. Liquid Bio-fertilizer is the solution to the problems where no solid carrier is needed. ThamizhVendan and Thangaraju (2007) reported liquid and cyst formulation of *Azospirillum* exhibited better adherence and survival on seeds, seedling roots and in the rhizosphere than the carrier-based forms. This shows that liquid Bio-fertilizer has greater potential than carrier-based Bio-fertilizer.

II. MATERIALS AND METHOD

In this study, four isolates, namely *Rhizobium* sp., *Azospirillum* sp., *Bacillus* sp. and *Pseudomonas* sp. (Fig.1) were isolated from the nursery soil samples of State Forest Research Institute, Kolapakkam, Chennai by using the ten-fold serial dilutions technique. The bacterial colonies were characterized by different biochemical methods in a nutrient agar media. These isolates were grown in their specific medium (*Rhizobium* – Yeast Extact Mannitol Broth, *Azospirillum*– Azospirillum broth, *Bacillus* – Nutrient broth, *Pseudomonas* – Pikovskaya broth). These liquid Bio-fertilizer organisms were incubated for 48 hours on an incubator at 28°C.

2.1 Production in Activation Medium

The consortium of Biofertilizer organism in the Liquid Bio-fertilizer was fermented by activated medium for seven days. The activation media requires 1 kg Jaggery 18 Litres of chlorine free water and 1 Litre of Liquid Biofertilizer. Mix all the components in a plastic can and leave to ferment for 7-8 days. Add a handful of black salt to hasten the fermentation process and mix it daily in clockwise within 3-5 seconds to accelerate the growth of the organisms. Samples were drawn at regular intervals for microbial analysis. The shelf life of all isolates in the Activated Liquid Biofertilizer (ALB) was studied by dilution plating technique.

Through pilot study (seed germination), the percentage was standardized by eight treatments such as Treatment 1 – Vermicompost enriched with 5% ALB, Treatment 2 – Vermicompost enriched with 15% ALB, Treatment 3 – Vermicompost enriched with 25% ALB, Treatment 4 – Vermicompost enriched with 50% ALB, Treatment 5 – Vermicompost enriched with 75% ALB, Treatment 6 – Vermicompost enriched with 100% ALB, Treatment 7 – Vermicompost only (Positive control) and Treatment 8 – Soil (Negative control). The morphological characters such as germination period, germination index, percentage of germination and shoot height were observed. The germination percentage and the germination rate index were calculated according to the following formulae:

$$\text{Germination \%} = \frac{\text{No. of Seeds Germinated}}{\text{Total no. of seeds sowed}} * 100$$

$$\text{Germination Rate Index} = \frac{\text{Germination \% on Day 1}}{\text{No. of days taken for full growth}} * 100 + n$$

The percentage standardized bio-fertilizer was further analyzed some chemical test parameters, macro and micro nutrients in Greenlink Analytical and Research Laboratory (India) Private Ltd, Coimbatore, Tamilnadu.

III. RESULTS AND DISCUSSION

The liquid formulated bio-fertilizer cells are sheltered by 4% Glycerol as protectant to longer its shelf life. The effect of different treatment of Activated Liquid Biofertilizer (ALB) on seed germination of *Emblica officinalis* was notably higher than the control (Vermicompost and soil). A significant variation in seed germination period and shoot length due to application of bio-fertilizer was found. In this study, the mean germination period of seeds in Treatment 1 take minimum time (8 days) followed by Treatment 2 (9 days), Treatment 3 (9.25 days) and Treatment 8 takes the maximum time (12.5 days). Treatment 1, 2 and 3 show maximum percent of germination (80%) followed by other treatments (Treatment 4, 5, 6 & 7) but Treatment 8 was minimum in germination percent (Fig 1). The germination growth index (% per day) (Fig 2) and the mean value of shoot height were maximum in Treatment 1 and minimum in Treatment 8.

Besides microbial quality of the liquid bio-fertilizer, chemical properties such as p^H(5.6), density (1.05g/cm^3) and acidity(2.5%) and physical character like colour (Slightly yellow) and odour (Strong)of liquid bio-fertilizer were observed. Major (Nitrogen, Phosphorus, Potassium, Magnesium, Calcium and Sulphur) and minor (Boron, Iron and Zinc)plant nutritional compounds were also analyzed. Based on the Test Report, the microbial strains in the bio-fertilizer have the sufficient amount of nutrients which can fulfill the need of the plant.

Fig. 1: Biofertilizer organism

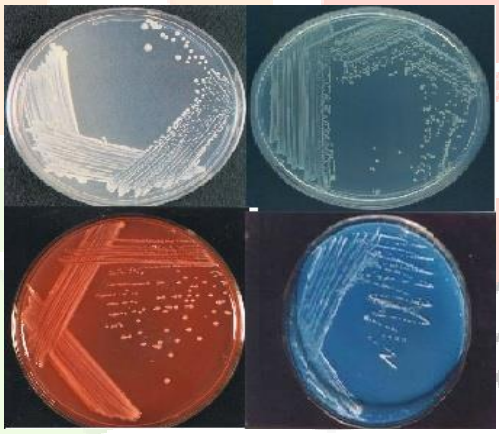


Fig. 2: Germination percentage (%)

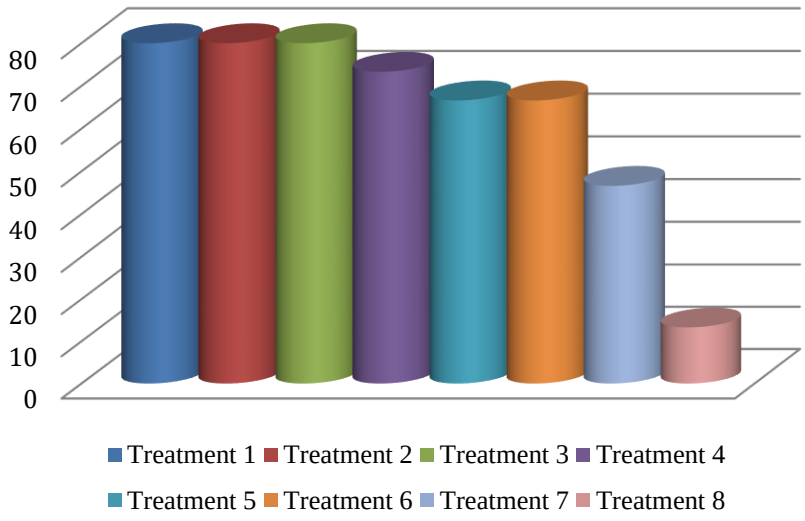
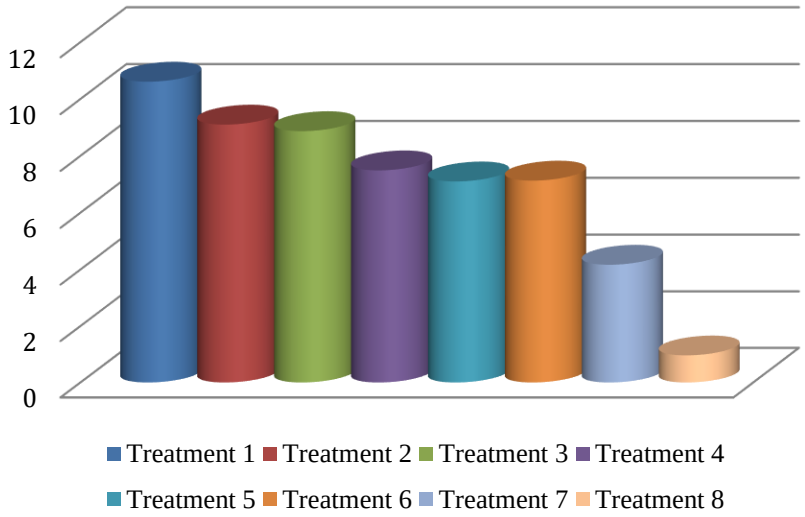


Fig. 3: Germination Rate Index



IV. SUMMARY

Hence our study clearly highlighted that combined inoculation of bio-fertilizers such as *Rhizobium* sp, *Azospirillum* sp, *Bacillus* sp and *Pseudomonas* sp (5%) with Vermicompost could fasten seed germination, enhance plant growth, protect plants from pathogenic micro-organisms and increasing environmental concern.

V. ACKNOWLEDGMENT

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