

Assessment of Multifunctional Liquid Biofertilizer On Seed Vigour and Growth of Rice Seedlings (ADT - 37)

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ABSTRACT

Single and combined inoculation of liquid PGPR inoculants on rice seed germination and growth of seedlings under green house trial were investigated. The selected plant growth promoting rhizobacteria namely *Azospirillum sp*, *Azotobacter sp* and *Pseudomonas* were mixed with protective substances (Trehalos 1%) base, for individual bacteria and on rice ADT-37. These formulated inoculants were used for seed treatment, seedling treatment and soil application, in rice under in vitro germination conditions. Higher percentage of seed germination was recorded in T5 treatment (*Azospirillum sp* + *Pseudomonas* + *Bacillus sp* - 89.25) followed by (T4 – *Azotobacter sp* + *Pseudomonas* + *Bacillus sp* - 88.50) than the single inoculation. In all the treatments seed vigour index was higher (T5 - 7348 ± 97.2) than control. The other growth parameters such as plant height, number of tillers, chlorophyll content and number of leaves were enhanced by the combination of both of the bacteria when compared to individual formulations. The results revealed that the multifactorial mixed inoculants can be used as better alternative to reduce the use of chemical fertilizer in rice cultivation for sustainable agriculture and soil conservation.

Key words: *Azospirillum sp*, *Azotobacter sp*, Seed germination, Rice.

INTRODUCTION

Contemporary soil management approach is mainly dependent on chemical-based fertilizers, which caused a hazardous effect to human health and soil fertility. The exploitation of agriculturally important microbes as biofertilizer has become greatest significance in agriculture sector for their beneficial role in food safety and supporting long term ecological balance. The green revolution practices incite as in broad spectrum of application of plant growth promoting rhizobacteria (PGPRs), and many other useful soil microflora help to better nutrient uptake, enhance plant growth and tolerate to various environmental stress (Bhardwaj *et al.*, 2014).

Plant Growth Promoting Rhizobacteria (PGPR) is a group of beneficial bacteria that interact with plant roots, promote the plant growth and improve the soil nutrient availability by variety of mechanisms (Wu *et al* 2004). They acted as rhizoremediators by degrading organic pollutants and enrich the soil fertility (Somers *et al.*, 2004), and also act as biopesticides by production of siderophores, the synthesis of antibiotics, enzymes and/or fungicidal compounds (Ahmed *et al* 2004; Bharathi *et al.*,2006; Jeun *et al.*, 2004). Interactions of these PGPR determine soil health in natural agroecosystem by providing numerous services to soil viz., organic matter decomposition, nutrient acquisition, water absorption, nutrient recycling and weed control.

PGPR significantly influence the growth and yield of various agronomical important crop have been reported. (Asghar *et al.*, 2002; Bashan *et al.*, 2004 and Biswas *et al.*, 2000). *Azospirillum*, *Pseudomonas* and *Azotobacter* strains could enhance seed germination and seedling growth (Shaukat *et al.*, 2006). Kloepper *et al* (1992) have been reported that wheat yield increased up to 30% with *Azotobacter* inoculation and up to 43% with *Bacillus* inoculation. Strains of *Pseudomonas putida* and *Pseudomonas fluorescens* could increase root and shoot elongation in canola (Glick *et al.*, 1997).

Bioinoculants can increase the nutrient uptake of plants by fixing atmospheric nitrogen, solublize phosphorous with the production of organic acids and promote the plant growth by

secreting growth hormone, speed up seed germination, seed emergence and increase the volume of root.

The application of bio-fertilizers that contain living microorganisms, is one of the mainframe practices that can help to maintain or increase the content of organic matter and improve soil fertility in cultivable soils. Despite the fact that bio inoculants have been known for many years, relatively little research has been done to document their effects (or noneffects) on crop production or to provide evidence of their potential effects on soil properties and processes, especially in outstanding peer reviewed scientific journals (Dinesh *et al.*, 2010; Khaliq *et al.*, 2006; Mayer *et al.*, 2010; Piotrowska *et al.*, 2012; Wu *et al.*, 2005; Zhao *et al.* 2005).

Increase plant nutrient availability of P, K through chelation, exchange reaction and production of organic acids promote plant growth by producing hormone to increase plant growth, speed up seed germination, improve seedling growth without infection.

Microbial inoculants applied as seed treatments imparts microorganisms directly to the plant rhizosphere—the root surrounding soil zone where plants interact directly with microorganisms (Philippot *et al.*, 2013). It is a zone of potent microbial activity, with growth of plants and microorganisms dependent on corresponding supply of nutrients and a wide range of other compounds including plant growth hormones and antagonistic substances. Many beneficial microorganisms of agricultural importance are rhizosphere colonising species, with ability to increase plant growth via a range of mechanisms (Babalola, 2010)

There is evidence that co-inoculation with multiple microorganisms can improve plant yield compared to the use of a single inoculant. Use of PGPR with microbial pest control agents is considered to be an innovative approach in soil health management and for the improvement crop yield and quality (Janisiewicz, 1996; Marimuthu *et al.*, 2002). Co-inoculation may also broaden the environmental range over which the seed treatment is effective, for example by supplying strains active at a range of different soil temperatures. Single and dual inoculations of field-grown wheat with two isolates of P-solubilising microorganisms (*Pseudomonas sp.* and *Aspergillus awamori*) resulted in significant gains in yield and P uptake (Babana and Antoun

2006). Hence the present article deals with the assessment of efficacy of co inoculation liquid inoculants of PGPR on seed germination and seedlings growth on rice plants.

MATERIALS AND METHODS

PREPARATION OF LIQUID INOCULANTS

The plant growth promoting rhizobacteria were isolated from rice rhizosphere and their growth promoting abilities were determined. The selected PGPR namely *Azotobacter sp*, *Azospirillum sp*, *pseudomonas* and *Bacillus sp* were formulated with protective substances trehalose (1%) and stored at 25°C to maintained their viability. (Data Published)

PREPERATION OF RICE SEEDS

Rice seeds of the variety ADT – 37 were purchased from local market Mannargudi. Rice seeds were subjected to surface sterilized with 90 % ethanol, followed by 5 % sodium hypochlorite and washed with sterile distilled water.

RICE SEED GERMINATION TEST

100 rice seeds were selected for each treatment. Each set of rice seeds were tightly covered by cotton cloth and dipped in clean glass vessels filled with distilled water and 1 ml of liquid PGPR inoculants namely *Azotobacter sp*, *Azospirillum sp* *Pseudomonas* and *Bacillus sp* with sufficient population density of 10^6 to 10^7 cfu/ml and shaken overnight in 150 rpm. Seeds were dipped in sterile distilled water considered as control. For each liquid inoculants, 100 seeds in 3 replications were allocated and in each replication, 100 seeds were put on sterile filter paper in Petri dishes. They were irrigated with sterile distilled water during the incubation period and temperature was maintained at 25° C.

Germination rate or seedling emergence was calculated using the following formula proposed by International Rice Research Institute (2011) on last day of experiment:

Percentage of seed germination was calculated by

$$\frac{\text{No of seeds germinated}}{\text{No of seeds on the tray}} \times 100$$

Vigor index of seedlings were measured on last day of experiment (day 7) according to the formula proposed by Abu-Bakr and Anderson (1973):

$$\text{Seed vigour index} = \% \text{ of seed germination} \times \text{seedling length}$$

Seedling trays were sterilized with 70 % ethanol and washed thoroughly with sterile distilled water. They were filled with autoclaved soil and surface sterilized rice seed (Variety Co - 43) were sown for nursery raising. Seedling trays were irrigated with sterile distilled water until the stage of unfolded 3 leaves. Height and root length of the seedlings were measured. For weight measurements seedlings were uprooted and dry weight was taken after the seedlings kept at hot air oven at 40 c for 72 hours. Weight measurements was made by digital magnetic balance and noted in gram.

EFFICACY ASSESSMENT OF LIQUID PGPR INOCULANTS IN GREEN HOUSE TRIAL

Liquid inoculants of PGPR were tested for their efficacy to promote the growth parameters of rice cultivar ADT -37 in green house condition. This experiment contains five treatment includes

T1 – Control

T2 – *Azotobacter sp*

T3 – *Azospirillum sp*

T4 – *Azotobacter sp*+ *Pseudomonas sp*+ *Bacillus sp*

T5 – *Azospirillum sp* + *Pseudomonas sp*+ *Bacillus sp*

Each treatment was replicated 4 (four) times, thus there were 20 experimental units in this experiment. Each experimental unit consisting of 10 plastic pots of 30 cm diameter. Soil was taken from rice field at 20 cm depth mixed with vermicompost (3:1 w/w). Application of liquid inoculants was done through seedlings treatment, where the seedlings were soaked in liquid suspension (10^9 (CFU/ml) for 2 h before transplanting to pot. Treatment with rhizobacteria was also done at the time of transplanting by incorporating 10 ml of each liquid inoculants(10^7 CFU/ml) per pot in the respective treatments. Several plant parameters were observed during this experiment, such as plant height, number of tillers and content of chlorophyll to determined their efficacy.

ESTIMATION OF CHLOROPHYLL (ARNON, 1949):

The chlorophyll content of the leaf was determined by the method of Arnon (1949). 1 gram of leaf sample was taken and cut in to small pieces, homogenized in a pre-cooled mortar and pestle using 80% (V/V) acetone. A pinch of calcium carbonate was added while grinding. The extracts were centrifuged at 3000 rpm for 15 min and made up to 25 ml with 80% (V/V) acetone. The clear solution was transferred to a special glass tube and the optical density was measured at 645 nm and 663 nm, against an 80% acetone blank in UV visible spectrophotometer (UV 240).The levels of chlorophyll 'a' and chlorophyll 'b' were estimated by using the equation given below:

$$\text{Chlorophyll 'a' } (\mu\text{g/ml}) = (12.7 \times \text{O.D. at 663 nm}) - (2.69 \times \text{O.D. at 645 nm})$$

$$\text{Chlorophyll 'b' } (\mu\text{g/ml}) = (22.9 \times \text{O.D. at 645 nm}) - (4.08 \times \text{O.D. at 663 nm})$$

$$\text{Total chlorophyll } (\mu\text{g/ml}) = (20.2 \times \text{O.D. at 645 nm}) + (8.02 \times \text{O.D. at 663 nm})$$

The chlorophyll content was expressed as mg chlorophyll per gram fresh weight of the leaf.

STATISTICAL ANALYSIS:

Collected Data were subjected to analysis of variance (ANOVA) in SPSS 16.0 followed by Duncan's multiple range test at 5 % level (Duncan, 1955).

TABLE 1 : EFFECT OF FORMULATED LIQUID INOCULANTS OF PGPR ON GROWTH ENHANCEMENT IN RICE PLANT (ADT -37) AT 15 DAYS (DAS)

Treatments	Seed germination (%)	Seed vigour Index	Root Length	Fresh weight (g/seedling)	Dry weight (g/seedling)
T1 – Control	87.00 ± 0.40 ^a	43262±3.51 ^a	6.77±0.09 ^a	5.65±0.15 ^a	1.85±0.0.23 ^a
T2 – Azotobacter sp	87.75± 0.47 ^{ab}	57330±1.54 ^b	6.78±0.12 ^a	5.61±0.12 ^a	1.82±0.08 ^a
T3 – Azospirillum sp	88.25 ± 0.62 ^{ab}	66872±1.16 ^c	7.48±0.29 ^b	6.69±0.09 ^b	2.27±0.19 ^{ab}
T4 – Azotobacter sp + Pseudomonas + Bacillus sp	88.50 ± 0.64 ^{ab}	72265±1.37 ^{cd}	7.58±0.12 ^b	7.22±0.13 ^c	2.89±0.36 ^b
T5 – Azospirillum sp +Pseudomonas+Bacillus sp	89.25± 0.47 ^b	7348±97.2 ^d	7.45±0.09 ^b	6.87±0.17 ^{bc}	2.86±0.36 ^b

Values are represented as Mean±S.E

Value in the same column same letters are not significantly different (P ≥ 0.05) according to Duncan’s Multiple Range test at 5% level

TABLE 2 : EFFECT OF FORMULATED LIQUID INOCULANTS OF PGPR ON GROWTH ENHANCEMENT IN RICE PLANT (ADT - 37) AT 30 DAYS (DAT)

Treatments	Plant height (cm/seedling)	No of leaves	No of roots	No of tillers	Total Chlorophyll (mg /g of
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					FWL)
T1 – Control	18.60 ± 0.22 ^a	14±0.40 ^a	12±0.40 ^a	11.5±0.64 ^a	3.35±0.08 ^a
T2 – <i>Azotobacter sp</i>	19.59 ± 0.07 ^b	16.75±0.47 ^b	12.70±0.10 ^b	12.00±0.40 ^{ab}	3.46±0.07 ^a
T3 – <i>Azospirillum sp</i>	19.47± 0.08 ^b	17.25±0.85 ^b	12.72±0. 06 ^b	12.75±0.47 ^{abc}	3.93±0.20 ^b
T4 – <i>Azotobacter sp</i> + <i>Pseudomonas</i> + <i>Bacillus sp</i>	19.83 ± 0.10 ^b	16.75±0.47 ^b	12.85±0.11 ^b	14.25±0.47 ^c	4.39±0.14 ^c
T5–<i>Azospirillum sp</i> +<i>Pseudomonas</i> + <i>Bacillus sp</i>	19.41±0.18 ^b	15.5±0.64 ^a	12.82±0.10 ^b	13.5±0.64 ^{bc}	4.49±0.20 ^c

Values are represented as Mean ± S.E

Value in the same column the same letters are not significantly different (P ≥ 0.05) according to Duncan’s Multiple Range test at 5% level

RESULTS AND DISCUSSION

RICE SEED GERMINATION

Seven days after seed treatment with liquid inoculants of PGPR and sterile distilled water as control the seed germination percentage and seed vigour index were calculated. Seed treatment with combined liquid bioinoculants resulted in increased seed germination, seed vigour index when compared to control and single inoculation. Higher percentage of seed germination (89.25) was recorded in T5 treatment (*Azospirillum sp* + *Pseudomonas*+ *Bacillus sp*) followed by T4 –*Azotobacter sp* +*Pseudomonas* + *Bacillus sp* (88.50) .In all the treatments seed germination and seed vigour index were higher (T5 - 7348±97.2) than control. After 15 days of seed treatment root length and the fresh and dry weight of the seedlings were calculated.

The present study revealed that valuable effects of the PGPR on rice. This improvement of the germination percentage observed in our study, could be due to the production of vitamins and growth substances which enhance the seed germination (Neeru Narula, 2000). Production of IAA and other auxins i.e gibberellins, cytokinins (Martinez –Toledo *et al.*, 1988) which help in enhancing the root growth and help the plant in better nutrient adsorption.

All treatments with PGPR in the study showed increased vigor index of rice seeds 7 days after germination (Table 1). The seeds inoculated with the combination of *Azotobacter sp* and *Pseudomonas* showed the highest vigor index , which was higher than the control.

The one way ANOVA at 15 days showed that biomass of plants treated with single and combined PGPR liquid inoculants resulted significantly higher in comparison to uninoculated control.

EFFICACY ASSESSMENT ON POT CULTURE

The effect of liquid inoculants above the ground at 30 days in the pot culture under green house conditions, on the growth parameters is presented in table -2. The highest root length also recorded in single and combined inoculation of *Azospirillum sp* (7.48, 7.58 and 7.45). The highest value of fresh and dry weight of the seedlings was recorded in combined inoculation of PGPR treated seeds when compared to the uninoculated one (7.22, 2.86). The lowest dry weight of the seedlings was recorded in control (5.65 and 1.85). *Azospirillum* is considered the most effective plant hormone producer among soil bacteria (Fallik *et al.*, 1996; Horemans *et al.*, 1986) In liquid culture the major hormones biosynthesized by *Azospirillum* are the auxins IAA and related indoles (Baca *et al.*, 1994; Bashan and Levanony, 1990; Crozier *et al.*, 1988; Tien *et al.*, 1979). Hence the early growth promotion could be contributed by *Azospirillum sp*.

Highest number of tillers were observed in co inoculation of *Azotobacter sp* (14.25 ± 0.47^c) with other two PGPR . The response of rice to inoculation with *Azotobacter* was studied by several investigators. Several parameters like plant height, number of tillers, panicle length, number of panicles and grain yields with biofertilizers along with graded levels of

nitrogen were studied in rice (Govedarica,1993). Co inoculation of *B. subtilis* and *A. brasilense* increased the root length and plant height in *Lycopersicon esculentum* (Marco Nuti *et al.*, 2008).

ESTIMATION OF CHLOROPHYLL

The highest content of total chlorophyll was recorded in T5 – 4.49 (mg/g fr.wt) and T 4 – 4.39 (mg/g fr.wt) than control T1 – 3.35 (mg/g fr.wt). The increased chlorophyll content might be linked with the high level of photosynthesis due to uptake of more nitrogen from the soil, which is fixed by this bacteria. The increased protein content could be due to the presence of kinetin which promotes the amino acid content which in turn helps in active protein synthesis (Tien *et al.*, 1979).

Besides plant-growth effects due to co-inoculation, another aspect taken into account was the impact of these two microorganisms on the indigenous soil microflora, which naturally colonizes rhizosphere and is composed of microbes that could have a crucial role on plant growth and health, and on maintenance of soil quality e.g. nutrient recycling, decomposition of organic matter, plant response to pathogens, plant-growth promotion (Miethling *et al.*, 2000).

CONCLUSION

The multifunctional liquid being essential components of organic farming play a vital role in conserving long term soil fertility and maintaining ecological balance. The liquid bioinoculants would be the feasible option for farmers to increase agricultural productivity and create green and clean environment, and thus this could form one of the strategies for the sustainable agricultural practices.

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