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Application of Fluorescent Pseudomonads Inoculant Formulations on *Vigna mungo* through Field Trial

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Abstract**—**Vermiculite was used to develop inorganic carrier-based formulations of fluorescent pseudomonad strains R62 and R81. The effect of bio-inoculation of fluorescent pseudomonad strains R62 and R81 (plant growth promoting and biocontrol agent) on growth responses of *Vigna-mungo* under field condition was enumerated. The combined bio-inoculation of these two organisms in a formuation increased the pods yield by 300% in comparison to the control crop. There was also significant increment in the other plant growth responses such as dry root weight, dry shoot weight, shoot length and number of branches per plant.

Keywords**—**Bio-inoculants formulation, Fluorescent pseudomonad, Plant growth promotion activity.

I. INTRODUCTION

FLUORESCENT pseudomonads (plant growth promoting rhizobacteria) are a group of beneficial microorganisms, which rigorously colonize roots and provide beneficial effects to plant development. Microbial formulations are carrier-based preparations containing beneficial micro-organisms in a viable state intended for seed or soil application. They are designed to improve soil fertility and help plant growth by increasing their numbers and thus their biological activity in the root environment [2]. The PGPR have been known to directly enhance plant growth by a variety of mechanisms, namely, fixation of atmospheric nitrogen that is transferred to the plant, production of siderophores that chelate iron and mafe it available to the plant root, solubilization of minerals such as phosphorus and synthesis of phytohormones ([3], [20], [28]).

The inoculation of seeds with PGPRs is known to increase nodulation, nitrogen uptake, growth and yield response of crop plants ([9], [13], [16]). Phosphate solubilizing bacteria are also known to increase phosphorus uptake resulting in better growth and higher yield of crop plants ([1], [11], [12] [14]), Trichoderma and Gliocladium have long been known as effective antagonists against soil borne plant pathogenic fungi ([22], [29]). The combined inoculation of Rhizobium and phosphate solubilizing bacteria has increased nodulation, growth and yield parameters in chickpea ([1], [15], [17], [19]). However, little work has been done on the effect of combined inoculations of Rhizobium, phosphate solubilizing bacteria and Trichoderma spp. on plant growth, nutrient uptake and yield.

Several workers have attempted to develop inorganic carrier based (talc, vermiculite, perlite, ground rock phosphate, and calcium sulphate) and organic carrier based (charcoal, peat) powder formulation of fluorescent pseudomonads to promote plant growth and control their disease ([4], [5], [6], [7], [8], [10], [18], [23], [24], [27], [32], [35]). It has been suggested that combinations of biocontrol agents could be more effective in controlling soil-borne pathogens than a single agent [25], For example, a biological preparation termed LS213, which contains industrially formulated spores of *B. subtilis* strain GB03 as a growth promoting agent, B. amylo liquefaciens strain IN937a as an induced systemic resistance (ISR) agent and chitosan, was used to enhance the growth of several vegetable transplant systems and it also provided ISR activity to various foliar pathogens [18].

The present work was to test whether a combination of PGPR strains would enhance growth promotion activity on *Vigna mungo* under field conditions. Therefore, in this study, we decided to combine two different PGPR strains which have been shown to enhance plant growth activity of *Vigna mungo* as compare to individual and control. The study of combining these two organisms is of great potential value to organic agriculture in order to avoid fertilizers and pesticides. It is with this view that, in the present investigation the groups of microorganisms viz., fluorescent pseudomonad strains R62 and R81 (Siderophore, Antibiotic compound 2,4 DAPG, Iodole- 3-Actic Acid producer, phosphate solubilizing bacteria) were studied to their compatibility and their combined effects on the growth of *Vigna mungo* under the field conditions.

II. MATERIAL AND METRIALS

2.1 Bacterial culture: Fluorescent pseudomonad strains R62 & R81, isolated from the rhizosphere of wheat (variety UP 2338) from Budaun District, Uttar Pradesh ([11], [12], [28])

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were used in the study. These strains were characterized as positive for phosphate solubilization, indole-3-acetic acid, siderophores, 1-aminocyclopropane-1-carboxylate deaminase, and 2, 4 diacetyl phloroglucinol (DAPG), which makes them potential plant growth promoting rhizobacteria. Bacterial cultures were maintained as 50% glycerol stocks at -20^0 C in King's-B medium [10]. All chemicals (extra pure grade) used in this study were obtained from Merck. The fluorescent pseudomonad strains R62 and R81 were grown in shake flask using modified Schlegel's medium. The modified medium contained glycerol (10.00 g/l), succinic acid (0.5 g/ l), Na_2HPO_4 (4.20 g/l), KH_2PO_4 (1.30 g/l), NH_4Cl (0.32 g/l), urea (0.352 g/l), KCl (0.35 g/l), NaCl (0.65 g/l), $MgSO_4.7H_2O$ (0.50 g/l), ammonium ferric citrate(150 µg/ l), $CaCl_2.2H_2O$ (0.9 g/l), trace elements solution (0.9 ml/ l) of composition as in Schlegel's original medium. The final pH of the medium before sterilization was adjusted to 7.1. The cultivation was carried for 30 h at 28^0 C in an orbital shaker (Scigenics Biotech, India) at 240 rpm.

2.2 Estimation of cell growth and Siderophore: Two milliliter sample was centrifuged at 10,000 rpm for 10 min. The cell-optical density (OD) at 600 nm was estimated using cuvette of 1 cm path length in a spectrophotometer. The sample was diluted, if required, with 0.2% saline to get cell OD in range 0.1-0.4. For cell OD estimation, correspondingly diluted supernatant as blank was used. Dry cell weight (g/l) for the strains R62 and R81 was estimated by multiplying the optical density values by conversion factor 0.51 and 0.57 respectively. These factors were obtained from standard curve of dry cell weight (g/ l) versus OD. The estimation of siderophore was done according to [21]. The absorbance was measured at 400 nm against phosphate buffer (pH 7, 0.05M) as a blank. Siderophore concentration (mg/l) was calculated using the expression $(O.D)_{400nm}$ x Siderophore-MW/ε. The values of extinction coefficient, ε = 16,500 M^{-1} cm^{-1} and siderophore molecular weight (MW=1500 Da) were used.

2.3 Carrier powder sterilization: One hundred gram of powder with 20% moisture content was autoclaved once at 121^0 C and 0.1 MPa for 20 min. The sterilization was repeated on second & third consecutive days with an overnight incubation in between.

2.*4 Preparation of inorganic carrier based formulations:* A glycerol stock solution of PGPR strains was inoculated into the Schlegel's media and incubated in shaker at 240 rev min^{-1} for 30 h at 28^0 C. The fermented broth was then mixed uniformly in respective carriers of talc and aluminum silicate. The broth containing 15-20 x 10^9 cfu/ml was used for the preparation of formulation. To make 100 g of inorganic carrier based formulations, 80 g of 3-times sterilized carrier (sterilized at 121^0 C for 1 h each day for 3 days), 18 ml of bacterial suspension, 1ml of glycerol (50% w/v) and 1 ml of CMC solution (0.1 mg/ml) as adhesive, were mixed under sterile conditions, following the method of ([36], [37], [38]). The product was shade dried to reduce the moisture content to ~18% and then packed in UV sterilized polythene bags and sealed. The formulations contained 25x10^8 cfu/g and 35x10^8 cfu/g for R61 & R81 strains, respectively. Aseptic conditions were maintained throughout the process.

2.5 Soil preparation and sowing: The soil in the field was brought to a fine tilth by ploughing and 4.0 m x 3.5 m plots were laid out. The soil in each plot was mixed well and perfect leveling was ensured in each plot. Rows were 30 cm apart. The seeds, treated with fluorescent pseudomonad strains R62, R81 and *P. Indica* isolates, were sowed in each row with a distance of 10 cm between the seeds. PGPR and fungi were added to all the treatments except for the control and blank carriers control. Four replicates were set up for each treatment. The design used for the experiment was randomized complete block design (RCBD). The plots were irrigated time to time to maintain the moisture level in the field.

2.6 Seed surface sterilization and seed bacterization: Seeds of *V. mungo* was washed repeatedly with autoclaved distilled water, and soaked in distilled water for 10 min. Later, 50 g of seeds were treated with 0.5 g of inorganic based carrier formulations. For uniform treatment of seeds with formulation, the flasks were kept in an orbital shaker for 2 h at 500 rpm. Seeds were bio-inoculated (bacterized) with both moist heat sterilized carriers and non-sterile carriers. Later seeds of *Vigna mungo* were sown in the field. The average bacterial counts were about 1x10^4 cfu/seed for *V. mungo.*

2.7 Plant host: The plant growth responses of these microbes were checked under field trials for *Vigna mungo* (Urd). Seeds of *V. mungo* (Variety PU 35) was obtained from Department of Plant Breeding and Genetics, GB Pant University of Agriculture and Technology, Pantnagar, India.

2.8 Growth parameters assessment: Percentage germination was recorded on the 15^{th} day after sowing. Nodulation was recorded by carefully uprooting the plants at harvest and counting the number of nodules per root system. The crop was harvested on the 87^{th} day after sowing. The plants were air dried and observations on biomass dry root and shoot weight. The stems, leaves and roots of the plants were separated. The dry weight of stems and roots was estimated after drying in oven at 70^0 C for 48 h. Average number of pods were calculated from 1x1 square meter field of each treatment.

2.9 Statistical analysis: The experiments were carried out in a completely randomized design for *Vigna mungo* and randomized block design for wheat. Standard deviations for each treatment were calculated. The experimental data were analyzed statistically using ANOVA. Duncan's multiple range test (DMRT) was used to separate group mean values when ANOVA were significant at $P< 0.05^*$.

III. RESULTS AND DISCUSSION

Initial experiments were carried with two inorganic carrier based formulations namely talcum powder (TP) and vermiculite (Ver) (Figure 3A). Based on these results it has been adjudged that vermiculite being the better carrier for

Vigna mungo crop. Later on the experiments were performed on field scale to enumerate the efficacy of the bio-inoculant formulations. The results indicate that all the treatments worked significantly well in comparison to the control (Fig 1,2 & 3). In all the experiments two controls were used, one being absolute control (AB), the seed as such and second being the carrier control, sterile vermiculite powder (Ver) which does not carry any microorganism.

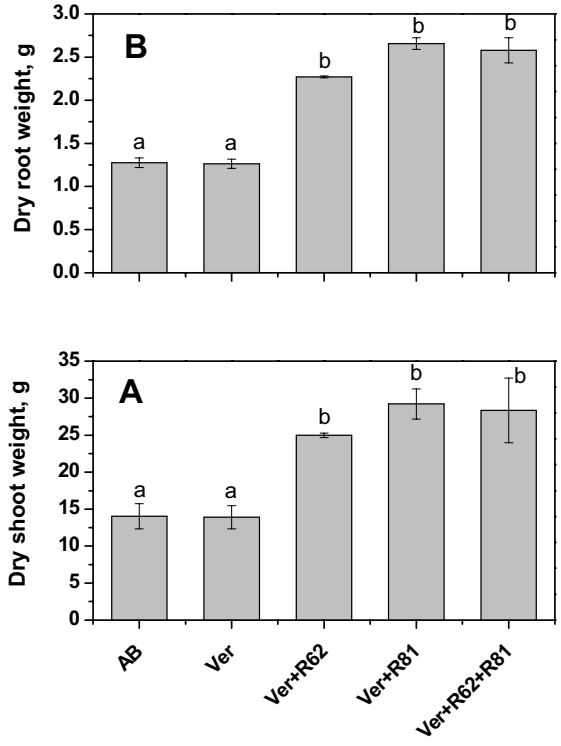

Fig. 1 Effect of vermiculite carrier (Ver) based formulations of fluorescent pseudomonad strains R62, R81, and/or R62+R81 on (A) dry shoot weight (g/plant), (B) dry root weight (g/plant). Columns labeled with the same letter represent not-significantly different means, according to Duncan's multiple range test ($P<0.05^{*}$), after ANOVA.

There was 110% and 102% increment in dry shoot weight when seeds were treated with Ver+R81 and Ver+R62+R81 respectively (Fig 1A). The individual treatment of fungus also increased the dry shoot weight by 103%. Thus it can be concluded that the bacterial strains performed well with respect to the control. In case of dry root weight, in all the treatments there was an average increment of 103% (Fig 1B).

The yield of Vigna mungo crop was estimated in terms of number of pods at the time of harvest. The Figure 2 indicates the variation of number of pods for different treatments. There was approximately 300% increment in the number of pods when the seeds were treated with the consortium of bacterial inoculants (Figure 2). Recent works suggest that the combination of fluorescent pseudomonad PGPRs could be more effective than individual strains ([26], [33]).

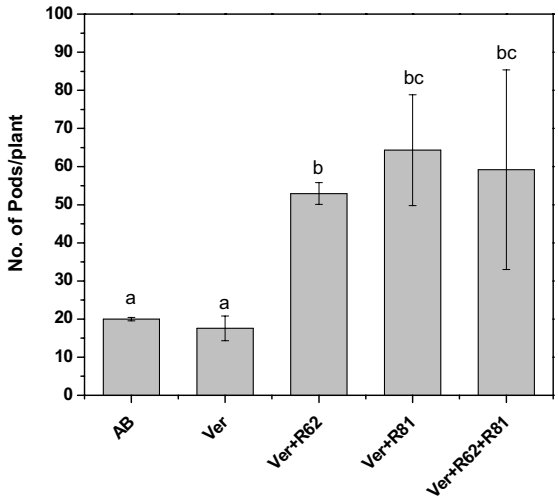

Fig. 2 Effect of vermiculite carrier (Ver) based formulations of fluorescent pseudomonad strains R62, R81, and/or R62+R81 on number of pods. Columns labeled with the same letter represent not-significantly different means, according to Duncan's multiple range test ($P<0.05^{*}$), after ANOVA.

The figure 3B shows the randomized field experimentation of *Vigna mungo*. There was significant variation in the number of plants in a square meter of the field for each treatment (Data not shown). This shows treatments had played effective role in germination of seeds sown.

Fig. 3A: The pot results of *Vigna mungo.* The treatments are (From right to left) Control, R81, R62 and R62+R81

There was also significant increase in the shoot length and number of branches per plant for all the treatments. All the treatments in an average increased the responses to same level (Figs. 4A, 4B).

Fig. 3B: The filed experimentation results of *Vigna mungo.*

IV. Conclusion

The fluorescent pseudomonads R62 and R81 can be potential bio-inoculants for agronomic interests.

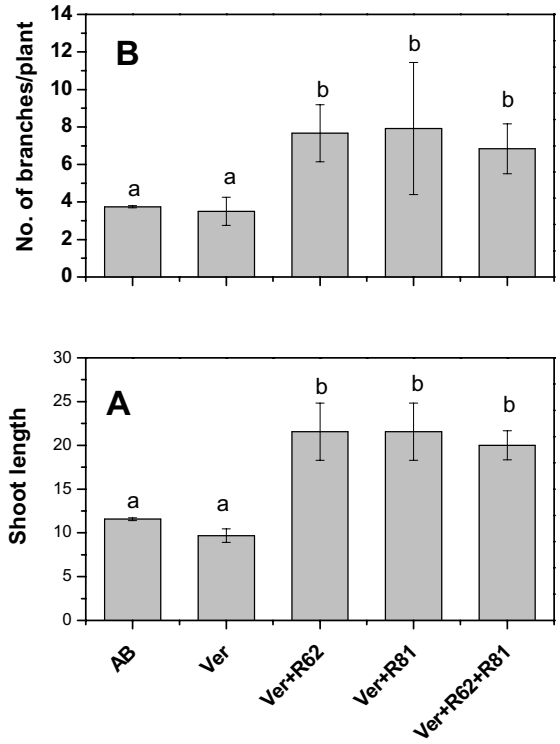

Fig. 4 Effect of vermiculite carrier (Ver) based formulations of fluorescent pseudomonad strains R62, R81, and/or R62+R81 on (A) Shoot length (cm/plant), (B) Number of branches (count/plant). Columns labeled with the same letter represent not-significantly different means, according to Duncan's multiple range test ($P<0.05^{*}$), after ANOVA.

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