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Integrated Effect of *Rhizobium* and *Azotobacter* Cultures on the Leguminous Crop Black Gram (*Vigna mungo*)

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Abstract

A pot experiment was performed to evaluate the integrated effect of *Rhizobium* and *Azotobacter sp.* on the plant growth, nodule appearance, no of leaf, qaO'osed sinsenXTgram during 2016 growing period at the Department of Microbiology, Dr. Ram Manc Faizabad, UP, India. Different treatments viz., T_1: Control (Sterile soil+Seeds without culture treatment), T_2: Sterile Soil and Seeds both are treated with *Azotobacter sp*., T_3: Sterile Soil and Seeds both are treated with *Rhizobium sp*., T_4: Sterile Soil and Seeds both are treated with mixed culture of *Azotobacter sp*. and *Rhizobium sp*., T_5: Sterile Soil+Seeds treated with *Azotobacter sp*., T_6: Sterile Soil + Seeds treated with *Rhizobium sp*., T_7: Sterile Soil+Seeds treated with mixed culture of *Azotobacter sp*. and *Rhizobium sp*. All experiments were carried out in triplicate set. The T_4 treatment showed maximum shoot length (51.6 cm), root length (17.3 cm), fresh and dry shoot biomass (12.99 and 3.21 g), fresh and dry root biomass (3.54 and 0.99 g), no. of leafs (20.4), root nodules per plant (18.2) and chlorophyll content (1.3 mg/g) and reducing (867.4 µg/g) and non-reducing sugar (1905.5 µg/g) content per plant biomass respectively. The *Azotobacter* and *Rhizobium sp*. have friendly associations and they have different physiology and habitat. Therefore, they help plant growth promotion by them own system. Therefore, such combination can be recommended for field application for sustainable agriculture. Excessive application of chemical fertilizers causes environmental and economic problems; hence the use of PGPR and *Rhizobium* bacteria can be acceptable due to cut contribution expenditure, increase in grain yield and environmental friendly.

Keywords: *Azotobacter*; Black gram; Co-culture; *Rhizobium*; Biofertilizers; *Vigna mungo*; Germination

Introduction

Black gram is one of the important pulse crops in India. It is also generally grown in other tropical/subtropical countries. Black gram is extremely nutritious due to having higher protein contents (24-26%) along with higher content of potassium, phosphorus, calcium, sodium and vitamins (retinoic acid, thiamine, ribofavin) [1]. It has several therapeutic properties, like curing diabetes, sexual dysfunction, nervous, hair, and digestive system disorders and rheumatic a f ictions [2]. Black gram seeds have shown anti-anthrogenic activity in guinea pigs.

Chemical fertilizers are frequently used to achieve maximum crop production in agricultural feld. T ese cost efective chemicals, however, when used roughly, have resulted in loss of soil fertility and consequently,

Azotobacter, *Klebsiella*, *Enterobacter*, *Alcaligenes*, *Arthrobacter*, *Burkholderia*, *Rhizobium*, *Flavobacteriaum*, *Bacillus* and *Serratia*, *phosphobacteria* and VAM fungi have been used as biofertilizers supplement of nitrogen and phosphorus fertilizers for improved crop production [7-16].

Rhizobium bio-fertilizer approx fx 50-200 kg of N/ha/season and increase the crop yield about 10-15% agriculture feld [17]. Bio-fertilizers comprised mostly the nitrogen fxing, phosphate solubilizing and plant growth-promoting microorganisms [18]. T e main agents of biofertilizers are *Azotobacter*, *Azospirillum*, blue green algae, Azolla,

was isolated from methi plants and wheat rhizosphere respectively.

Materials and Methods

Isolation, screening and identi fcation of *Rhizobia* and *Azotobacter sp.*

For the isolation of *Rhizobia*, healthy plants root nodules from methi was used. All selected root nodules were washed with water and then immerse the nodules in $HgCl_2$ (0.1%) or H_2O_2 (3-5%) for fve

minutes to surface sterilization. After that, nodules were washed in sterile water for 3-4 times. All nodules sticking to the root system were removed and surface sterilized by treating with 70% alcohol for 1 minute, after which they were treated by chloramine-T solution (1%) for 3 minutes and washed thoroughly by with sterile water. Now, nodules were crushed in 1000 µl of water with a sterile rod and make a suspension of *Rhizobia* with sterile water. Then suspension of *Rhizobium* (0.1 ml) was spread on the yeast extract agar medium plate which contain (g/l): Yeast extract: 1.0, K_2HPO_4: 0.5, $K_2SO_4.7H_2O$: 0.2, NaCl: 0.1, Mannitol: 10.0, Agar: 20 and 2.5 ml congo red solution (1%) with pH 6.9. The inoculated plates were incubated for 5-6 days at 26°C

Dry root biomass: After measuring the dry root biomass of the plants, it was dried in a hot air oven at 60°C, for 48 hours. After that, dry weights of the roots were calculated in grams by electrical balance.

Physiological parameters

10% + H_2

Effect of *Rhizobium* and *Azotobacter sp.* on black gram growth parameters

Both strains showed positive results in the experiments carried out on "Effect of *Azotobacter*, *Rhizobium* and mixed culture of both (*Azotobacter* and *Rhizobium sp.*) on the growth and biochemical aspects of black gram" were discussed in this section. To enhance a significant plant growth response, it is necessary to recognize the prominent strains of PGPRs for the sowing condition. It was in this context that an efforts were made to study the PGPRs of black gram with special reference to *Rhizobium* and *Azotobacter* and their mixed culture. The effects of enriched microbial inoculants in soil and on plant growth, biomass and biochemical characteristics were studied in polybag culture under natural condition. Soil which augmented with mix microbial inoculants was found to significantly increase shoot length, root length, number of leaf, number of nodules and fresh and dry weight of shoot and root, total fresh and dry weight of the plant. The microbial inoculants provide high-quality of plant nutrients has supported plant growth.

Effect on shoot length: In this experiment, the result showed that shoot length was greatly increased with the mixed culture of *Rhizobium* and *Azotobacter sp.* when compared with single culture of *Azotobacter sp.*, and *Rhizobium sp.*, at 30th psŋIn s ŨAwhs u mhe n № ! r

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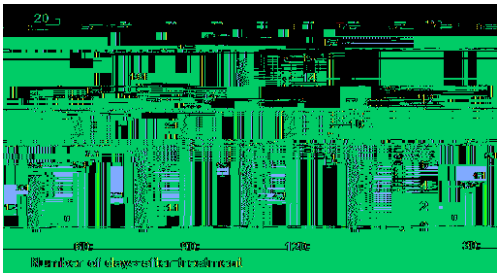

Figure 5: Effect of *Azotobacter sp.*, *Rhizobium sp.* and mixed culture of *Azotobacter sp.*, and *Rhizobium sp.* on root nodules number of Black Pea.

Fresh and dry shoot and root biomass: Accordingly, the root and shoot growth, the fresh and dry content in root and shoot as well as total dry contents of black gram were also increased due to the combined action of both strain. The maximum root and shoot fresh and dry weight was achieved in the T_4 treatment. The T_4 Treatment enhanced the root fresh and dry content of 3.54 and 0.99 g per plant and shoot fresh and dry content of 12.99 and 3.21 g per plant over the control (Tables 2 and 3). Mix-culture could increase the total root and shoot fresh and dry biomass of 16.5 and 4.2 g per plant, respectively (Tables 2 and 3).

Treatments	Shoot fresh biomass				Root fresh biomass				Total fresh biomass			
	Days after treatment											
6.29	30	60	90	120	30	60	90	120	30	60	90	120
T_1	0.85	1.46	2.11	4.81	0.32	0.77	1.01	1.6	1.17	2.23	3.12	6.1
T_2	1.33	2.73	4.27	7.55	0.45	1.18	1.31	2.1	1.78	4.53	5.58	9.65
T_3	1.68	3.98	5.41	9.01	0.51	1.23	1.51	2.40	2.19	6.91	6.92	11.41
T_4	2.87	6.95	9.53	12.9	0.67	1.48	1.73	3.54	2.58	8.45	10.26	16.53
T_5	1.21	2.38	3.87	5.90	0.40	1.0	1.28	2.0	1.61	3.30	5.69	7.9
T_6	1.42	2.77	4.91	7.01	0.5	1.08	1.38	2.2	1.92	3.85	6.29	9.21
T_7	1.58	3.75	6.72	10.1	0.62	1.28	1.41	2.38	2.2	5.03	8.13	12.43

Azotobacter sp. and *Rhizobium sp.* consortium, where more root hairs become liable for rhizo-microbial infection and also might be due to better condition for P-availability by P-solubilizers.

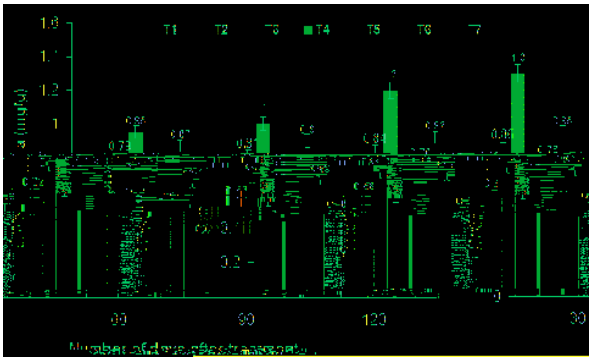

Figure 6: Effect of *Azotobacter sp., Rhizobium sp.* and mixed culture of *Azotobacter sp.*, and *Rhizobium sp.* on Chlorophyll a content of Black Pea.

Effect on non-reducing and reducing sugar content: In the present study, mixed culture of *Azotobacter* and *Rhizobium sp.* treatment (T_4) increased reducing (867.8 mg/g) and non-reducing (1509.5 mg/g) sugars quantity (Figures 7 and 8). Non-reducing sugar contents were increased due to the possible reasons to enhance in carbon fixation, activation of enzymes and improved photosynthetic rate.

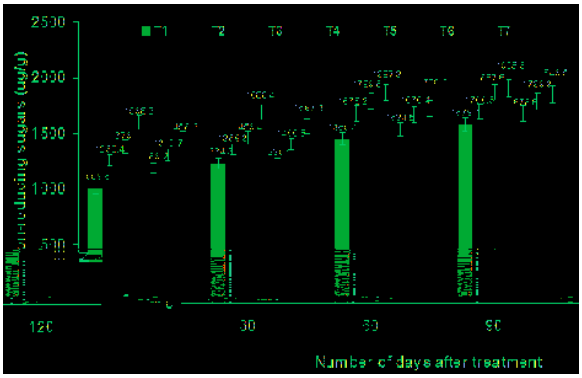

Figure 7: Effect of *Azotobacter sp., Rhizobium sp.* and mixed culture of *Azotobacter sp.*, and *Rhizobium sp.* on non-reducing sugar contents of Black Pea.

is due to improved carbon fxation, activation of enzymes and improved photosynthetic rate [34,35]. Growth parameters improved due to the mixed culture treatments.

Several methods have been recommended to elucidate the fact of plant growth enhancement by *Azotobacter* is due to increase in the nitrogen fxation, production of diferent hormones (auxins, gibberellins, cytokinin, and ethylene), phosphorus solubilization, sulfur oxidation, accessibility of nitrate, production of antibiotics, lytic enzyme, hydrocyanic acid, increase in root permeability, frm antagonism for the existing and root spot, inhibition of harmful rhizobacteria and improvement in the uptake of fundamental plant nutrients etc. [36-39].

It clearly indicate that Rhizobium nodulation (number and size) might have due to the presence was *Azotobacter* which could fx atmospheric N_2 and supported plant growth from initial growth of seedlings. In the early stage, plant roots might have supported the *Azotobacter* population. Such

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