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Efficacy of actinomycetes, *Rhizobium* and plant growth promoting rhizobacteria consortium inoculants on symbiotic traits, nodule leghemoglobin and yield of soybean in central India

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Abstract
A field experiment was carried out research field, JNKVV, Jabalpur (M.P.) during the kharif season of 2016 to find out the efficacy of Actinomycetes, *Rhizobium* and PGPR consortium inoculants on symbiotic traits, nodule leghemoglobin and yield of soybean in a Vertisol of Central India. The said experiment was the field testing part of research which was conducted during 2002-2007 to 2007-2012. The efficacy of two isolates of each microbial inoculants as mono-inoculation and their combinations as co-inoculation of Actinomycetes, *Rhizobium* and PGPR were tested along with fertilized uninoculated control (FUI) and unfertilized uninoculated control (UFUI) were also evaluated. Results revealed that the among microbial inoculants, the Actinomycetes + consortia of *Rhizobium* and PGPR (CRP) was found most effective in respect of symbiotic parameters including nodule number (30.8 and 30.0 nodules plant^{-1}), nodule dry weight (42.1 and 61.7 mg/plant) at 35 and 55 DAS respectively, shoot and root dry weight (2.78 and 0.56 g plant^{-1}) at 45 DAS respectively, leghemoglobin content (1.88 and 2.15 mg/g of fresh nodule) at 35 and 55 DAS respectively, followed by CRP nodule number (26.0 and 28.3 nodules plant^{-1}), nodule dry weight (37.5 and 56.2 mg plant^{-1}), shoot and root dry weight (2.52 and 0.53 g plant^{-1}), leghemoglobin content (1.68 and 1.92 mg g^{-1} of fresh nodule) and also showed its positive effect in enhancing all the yield attributing parameters, seed and stover yields.

Keywords: Efficacy, consortium, soybean, leghemoglobin, crop yield

Introduction
Soybean [*Glycine max* (L.)] is native to East Asia, and is grown for oil and protein around the world cultivated primarily in warm and hot climate. Soybean is one of the most important kharif oilseed crop of Madhya Pradesh. Besides high yield potential (25-30 q/ha), in terms of food components, soybean contains 35-40% protein, 19% oil, 35% carbohydrate (17% of which is a dietary fibre), 5% minerals and several other components including vitamins (Liu, 1997) [8]. Soybean has played a significant contribution to the yellow revolution in India. In Madhya Pradesh the area under soybean cultivation during kharif 2014 was55.46 lac ha with 1086 kg/ha yield and 60.25 lakh MT production (SOPA 2014) [10].

Soil is a habitat for a vast community of soil microorganisms, whose activities largely determine the biological condition of the soil and influence the plant growth right from seed germination to maturity. Majority of plant associated soil microorganisms inhabit the rhizosphere and found in great numbers and diversity compared to non-rhizosphere soil. Number of interactions exists between plant roots and rhizosphere microorganisms like synergism, mutualism, parasitism etc., which will have a profound influence on plant growth.

Although it is the plant which overwhelmingly determines the composition of the rhizosphere microbial community, yet the rhizosphere could be temporarily manipulated by application of specific microbes to seeds/soil or to the roots to get benefits in crop production like biological nitrogen fixation, P solubilization or mobilization, potash solubilization *etc.* Manipulation of microflora could be done to control disease causing micro organisms to increase the economic yields. Plant growth promoting rhizobacteria (PGPR) improve plant growth directly or by indirect mechanisms. The actinomycetes area versatile group of microorganism widely distributed in arable dry soils (Wellington *et al.*, 1967) [13]. They are abundant in all cultivated and uncultivated soils (Waksman and Curtis, 1916) [12]. The number and type of actinomycetes present in soil are greatly influenced by soil temperature, soil type, soil pH, organic matter content, cultivation practices, aeration and moisture content.Actinomycetes perform important activities in soil like production of growth promoting substances, phosphorus solubilization, decomposition of organic matter, antibiotic production, suppression of soil borne plant pathogens. Actinomycetes are of particular interest as they posses relatively more antagonistic activity than others and serve as an important biocontrol agent. Many soil borne plant pathogens which cause serious diseases in plants can be controlled by actinomycetes by their antibiotic activity. The mode of action of actinomycetes in controlling plant diseases include antibiosis, parasitism, production of extracellular hydrolytic enzymes and competition for iron (Getha *et al.*, 2005; Errakhi *et al*., 2007) [5, 4]. Microbial inoculants are cost effective, ecofriendly and renewable sources of plant nutrients (Khan *et al*., 2007) [7]. *Rhizobium*, PGPR and Actinomycetes isolates individually are found beneficial but their consortium could be more valuable resource to augment the supply of nutrients through solubilization or mobilization therefore, an experiment was designed to evaluation of Actinomycetes, *Rhizobium* and PGPR consortium on soybean in Kymore Plateau & Satpura Hills Region of Madhya Pradesh.

Material and Methods

The field experiment was conducted at the research field Department of Soil Science & Agricultural Chemistry, JNKVV, Jabalpur, Madhya Pradesh (23^0 12’ N, 79^0 56’ E and 428 m above mean sea level).The efficacy of 2 isolates of each microbial inoculants as mono-inoculation and their combinations as co-inoculation of Actinomycetes (A_6 and A_{10}), *Rhizobium* (R_{33} and R_{34}) and PGPR (P_3 and P_{10}) were tested along with Fertilized un inoculated control (FUI) and Unfertilized un inoculated control (UFUI) were also evaluated. The treatments were laid out in Randomized Block Design (RBD) with four replications. The recommended dose of fertilizer N: P_2O_5: K_2O was applied @ 20:80:20 kg ha^{-1} for soybean crop in the form of urea, single super phosphate (SSP) and muriate of potash (MOP). Urea and MOP were supplemented as basal applications to each plot as per recommendation and SSP was applied as per scheduled dose of treatments.

All the technical efforts were endeavoured to maintain the microbial population to up the standard 10^7 to 10^{-8} cfu/ml both. Soybean seeds in polythene bags were slightly moistened and then treated with carbendazim fungicide @ 2 g/ kg seed. Seeds were allowed to air dry under shade. Then the seeds were inoculated as per treatment combination using sterilized gum acacia (2%) as adhesive. The seeds were sown in the respective plot @ 60 kg/ha. Recommended package of practices was followed to maintain plant population, protection and growth.

Three plants from each plot were taken to estimate soybean nodulation (number of nodule, nodule dry weight and leghemoglobin content) and shoot and root weight was recorded in g/plant, dried in hot air oven at 60 ^{0}C for 3 - 4 days (till constant weight) at 35 and 55 days of sowing (DAS). The crop was harvested plot wise and yields of seed and stover were recorded.

Results and Discussion

Soybean nodulation

Nodule number, nodule dry weight and leghemoglobin content of soybean responded significantly to inoculation of different inoculants (Table 1). All the treatments produced significantly nodule number, nodule dry weight and leghemoglobin content of soybean. The nodule number ranged from 15.5 to 30.8/14.6 to 30.0 per plant, nodule dry weight 22.7 to 42.1/43.8 to 61.7 mg/plant and leghemoglobin content 0.93 to 1.88 mg/g of fresh nodule at 35 and 55 DAS, respectively due to seed inoculation with different inoculants. The highest nodule number (30.8/30 per plant), nodule dry weight (42.1/61.7 mg/plant) and leghemoglobin content (1.88/2.15 mg/g of fresh nodule) at 35 and 55 DAS, respectively was found in the treatment receiving CRP+A6+A10 which was significantly higher than all other treatments. The percentage increase in nodule number 70.2/77.5, nodule dry weight 61.9/34.7% and leghemoglobin content 66.4/59.3 over fertilized uninoculated (FUI) due to seed inoculation with consortium. Dashti *et al.* (1998) [3] reported that co-inoculation of soybeans with *B. japonicum* and PGPR increased soybean nodulation and hastened the onset of N fixation. Similar results found by Yuming, *et al. (*2002) [14]. The increase in nodule number, nodule dry weight and leghemoglobin content with application of recommended dose of fertilizers may be attributed to adequate supply of nutrient and optimum number *Rhizobium* in soil of that might have increased the nodulation efficiency and leghemoglobin content in nodule resulting in improvement in metabolic activities and also due to the effect of inoculation on the proliferation of roots.

Table 1: Effect of Actinobacterial, *Rhizobium* and PGPR isolates and their consortium on nodulation status of soybean at 35 and 55 DAS.

Treatments	Nodulation at 35 DAS			Nodulation study at 55 DAS		
	Nodule of number (per plant)	Dry weight of nodule (mg/plant)	Leghemoglobin content (mg/g of fresh nodule)	Nodule of number (per plant)	Dry weight of nodule (mg/plant)	Leghemoglobin content (mg/g of fresh nodule)
Actonomycetes	22.8	29.6	1.33	21.7	49.3	1.50
	22.1	29.9	1.30	21.8	49.9	1.47
Rhizobium	26.9	36.2	1.55	24.4	54.5	1.78
	26.5	35.9	1.53	24.4	54.6	1.81
PGPR	25.9	30.7	1.42	22.5	49.1	1.61
	23.1	31.7	1.42	23.1	48.8	1.63
CRP	26.0	37.5	1.68	28.3	56.2	1.92
CRP+A6	29.0	38.5	1.75	29.2	57.8	2.00
CRP+A10	29.2	39.8	1.77	29.6	58.5	2.08
CRP+A6+A10	30.8	42.1	1.88	30.0	61.7	2.15
FUI	18.1	26.0	1.13	16.9	45.8	1.35
UFUI	15.5	22.7	0.93	14.6	43.8	1.15
Sem±	2.47	3.07	0.19	2.96	3.90	0.20
CD (P=0.05)	7.06	8.78	0.54	8.45	11.13	0.57

Shoot and root dry weight

The shoot and root dry weight (Table 2) ranged from 2.78 to 1.78 and 0.56 to 0.30 g / plant, respectively. Among all the treatments, CRP+A6+A10 responded the best by 50.0 and 47.4%, respectively over FUI (1.89 and 0.30 g/plant). However, the response of CRP+A6+A10 was at par to that of *Rhizobium*, PGPR, CRP, CRP+A6 and CRP+A10. These results are in the line of findings presented by Tahir *et al*. (2009) [11] who examined the effects of *Rhizobium* inoculation and NP fertilization on shoot and root biomass of soybean and found increase in fresh and dry weight of shoot and roots. Alam *et al.* (2015) [1] and Zahran (2001) [15] found higher shoot and root biomass, by inoculation with *Rhizobium* sp. than non-inoculated.

Table 2: Effect of Actinobacteria, *Rhizobium* and PGPR isolates and their consortium on shoot and root dry weight at 45 DAS and seed and stover yield of soybean.

Treatments	Shoot and root dry weight (g/plant)		Yields (kg/ha)	
	Shoot	Root	Seed	Stover
Actonomycetes	2.33	0.42	1896	1923
	2.39	0.42	1885	1914
Rhizobium	2.45	0.47	2229	2122
	2.48	0.48	2235	2140
PGPR	2.41	0.45	2085	2010
	2.43	0.45	2046	1995
CRP	2.52	0.53	2244	2177
CRP+A6	2.66	0.53	2245	2214
CRP+A10	2.69	0.55	2309	2229
CRP+A6+A10	2.78	0.56	2347	2520
FUI	1.89	0.38	1741	1585
UFUI	1.78	0.30	1629	1504
SEm±	0.13	0.04	130.95	128.11
CD (P=0.05)	0.38	0.12	374.26	366.15

Yield

The data in table 2 on yields of seed and stover of soybean at harvest expressed that the effects of seed inoculation with different inoculants supplemented with recommended dose of chemical fertilizer (N, P and K) were significant over the fertilized uninoculated (FUI) and UFUI. The maximum seed and stover yields were recorded with the treatment combinations of CRP+A6+A10 by 34.8 and 59.0% increase, respectively over the FUI control (1741 and 1585 kg/ha). But, the results were mutually at par among all the treatments (except Actomycetes and UFUI). While minimum seed and stover yields (1629 and 1504 kg/ha) were recorded under the treatment unfertilized uninoculated (UFUI). Increase in yields with the treatments of inoculation with different inoculants and inorganic fertilization might be attributed to better nodulation, N_2 fixation and crop growth as against uninoculated control (Brahmaprakash *et al.*, 2004 and Gupta, 2005) [2, 6].

Correlation

Figure 1 reveals about the correlation between shoot dry weight (at 45 DAS) and seed yield of soybean. Since the value of R^2 is found to be 0.86 which suggests that 86% variability in seed yield by crop can be explained due to oven dried weight of shoot while remaining 14% variability may be due to other factors. Similar result also reported by Okereke *et al*. (2001) [9].

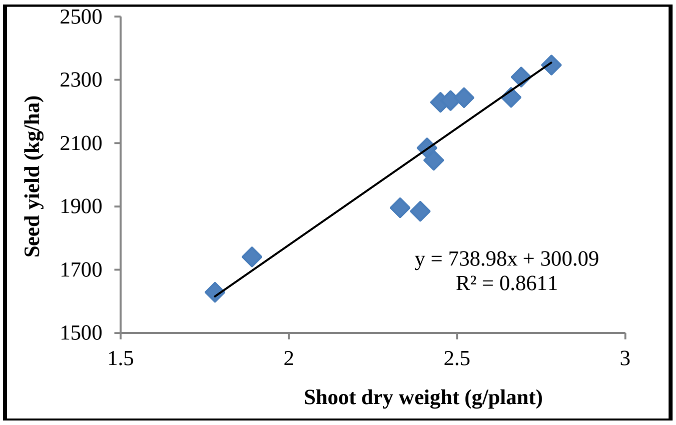

Fig 1: Correlation between shoot dry weight (45 DAS) and seed yield of soybean

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