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Productivity and nutrient uptake of soybean (*Glycine max*) as influenced by bio-inoculants and farmyard manure under rainfed conditions

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ABSTRACT

A field experiment was conducted at Wadura in Jammu & Kashmir during the rainy (*kharif*) season of 2004 and 2005 to study the efficiency of *Rhizobium, Azotobacter*, phosphate-solubilizing bacteria (PSB) and farmyard manure (FYM) on performance of rainfed soybean [*Glycine max* (L.) Merr.] and soil fertility. Results revealed that sole or dual inoculation of soybean with biofertilizers, application of FYM and recommended dose of fertilizer (RDF) significantly increased the plant growth, nodulation, seed and straw yields as well as N and K uptake over the control. Application of *Rhizobium + Azotobacter* + PSB + FYM showed significantly higher number and weight of nodules, but it was on par with that of *Rhizobium* + PSB + FYM and RDF. The improvement in seed yield over the control was 46.6, 42.2 and 38.8% under RDF, *Rhizobium + Azotobacter* + PSB + FYM and *Rhizobium* + PSB + FYM respectively. Further, inoculation of soybean with *Rhizobium* and PSB registered respectively 19.8 and 11.2% increase in grain yield over uninoculated control. Maximum N and P uptake, and available P were recorded with RDF, followed by *Rhizobium + Azotobacter* +PSB +FYM and *Rhizobium* + PSB + FYM, whereas the highest K uptake, organic C, and the available N and K were observed with *Rhizobium* + *Azotobacter* + PSB + FYM. Application of *Rhizobium + Azotobacter* + PSB + FYM increased the available soil N by 31.2 kg/ha and available P by 2.05 kg/ha over the initial level.

Key words: Bio-inoculants, Nodulation, Soil fertility, Soybean, Uptake, Yield

Soybean [*Glycine max* (L.) Merr.] is generally grown in soils with low N and P status or with the application of inadequate quantity of organic and inorganic sources of plant nutrients. This has resulted in deterioration of soil health and productivity under temperate conditions of Kashmir valley. The productivity of soybean can be increased by inoculation with bio-agents such as *Rhizobium* and phosphate solubilizing micro-organisms. Co-inoculation with these bio-cultures has shown encouraging results in sustaining the crop productivity and improving soil fertility (Dubey, 1997). *Azotobacter* has also been tried by researchers mainly in cereals and vegetables, who reported these to be beneficial for increasing the grain yield by increasing the N availability through N_2 fixation in the soil (Upadhyay, 2003). *Azospirillum* has attracted the attention of agronomists and ecologists due to its ability to fix N_2 in association with *Rhizobium japonicum* in soybean (Mel'nikova *et al.*, 2002), but inoculation of *Azotobacter* with soybean has not been tried so far though it has the same ability to fix N_2. Organic manures provide a good substrate for the growth of many micro-organisms and maintains a favourable nutritional balance and soil-physical properties. However, information on the combined use of bio-agents along with FYM is lacking in many crops including soybean under temperate conditions. A field experiment was therefore conducted to study the single as well as combined effect of *Rhizobium, Azotobacter* and phosphate-solubilizing micro-organisms along with FYM on the performance of soybean and enrichment of the fertility status under rainfed temperate conditions of Kashmir valley.

MATERIALS AND METHODS

A field experiment was conducted at Faculty of Agriculture, SKUAST (K), Wadura, Sopore, Kashmir ($34^0 17$'N and $17^0 33$'E, 1524 m above mean sea level, annual rainfall 732 mm) under temperate conditions during the rainy (*kharif*) season of 2004 and 2005. The soil was silty-clay loam, neutral in reaction (pH 7.2), having organic C 1.01%, available N 360 kg/ha, available P 14.9 kg/ha and available K 212.8 kg/ha. The initial status of *Rhizobium, Azotobacter* and phosphate-solubilizing bacteria (PSB) was $1.05 x 10^2$, $1.2 x 10^3$ and $1.7 x 10^3$ cfu/g soils

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respectively. The experiment was laid out in randomized block design with 3 replications involving 17 treatments, viz. the T_1, control; T_2, *Rhizobium*; T_3, *Azotobacter;* T_4, *Pseudomonas striata* (PSB); T_5, FYM; T_6, *Rhizobium* + *Azotobacter;* T_7, *Rhizobium* + PSB*;* T_8, *Rhizobium* + FYM; T_9, *Azotobacter* + FYM; T_{10}, *Azotobacter* + PSB*;* T_{11}, PSB + FYM*;* T_{12}, *Rhizobium* + *Azotobacter* + PSB; T_{13}, *Rhizobium* + *Azotobacter* + FYM; T_{14}, *Rhizobium* + PSB + FYM; T_{15}, *Azotobacter* + PSB + FYM; T_{16}, *Rhizobium* + *Azotobacter* + PSB + FYM; and T_{17}, RDF. Farmyard manure was applied @ 5 t/ha as per treatment, which contained 0.65% N, 0.19% P and 0.68% K on dry-weight basis. A uniform recommended dose of N, P and K @ 20, 17.5 and 33.3 kg/ha, respectively was applied in the form of urea, diammonium phosphate and muriate of potash in RDF treatment. Nitrogen was applied in two equal splits, half as basal and the remaining as top-dressing at 30 days after sowing, and full dose of P and K was applied basal. Soybean (cv. 'VL Soya 1') seeds were inoculated with charcoal-based inoculants of *Bradyrhizobium japonicum, Azotobacter chroococcum and Pseudomonas striata* (PSB) @ 500 g/ha. The crop was sown on 16 June 2004 and 9 June 2005, and harvested on 21 October 2004 and 16 October 2005. The data were recorded on different parameters and pooled, as the differences between the years were not significant. Economics was worked out on the basis of prevailing market prices of inputs and outputs.

RESULTS AND DISCUSSION

Growth parameters

There was a progressive increase in plant height and dry weight/plant with the application of FYM, and the single or combined inoculation of biofertilizers with FYM (Table 1). Application of RDF resulted in maximum plant height and dry weight/plant, followed by combined inoculation of *Rhizobium* + *Azotobacter* + PSB + FYM and *Rhizobium* + PSB + FYM, which were on par but significantly higher than the rest of the treatments. Biological N_2 fixation by *Rhizobium*, greater release of P by phosphate-solubilizing bacteria (Dubey, 1997), and the synthesis of growth-promoting hormones and vitamins by *Azotobacter* (Baskar *et al.*, 2000) might have favoured the plant growth.

Nodulation

Beneficial effect of biofertilizer inoculation alone in improving the nodulation was noticed, and combined inoculation of *Rhizobium* + *Azotobacter* + PSB along with FYM showed significant positive effects (Fig. 1). Application of *Rhizobium* + *Azotobacter* + PSB + FYM, being on par with that of *Rhizobium* + PSB + FYM, resulted in markedly higher nodules/plant, and the fresh and dry

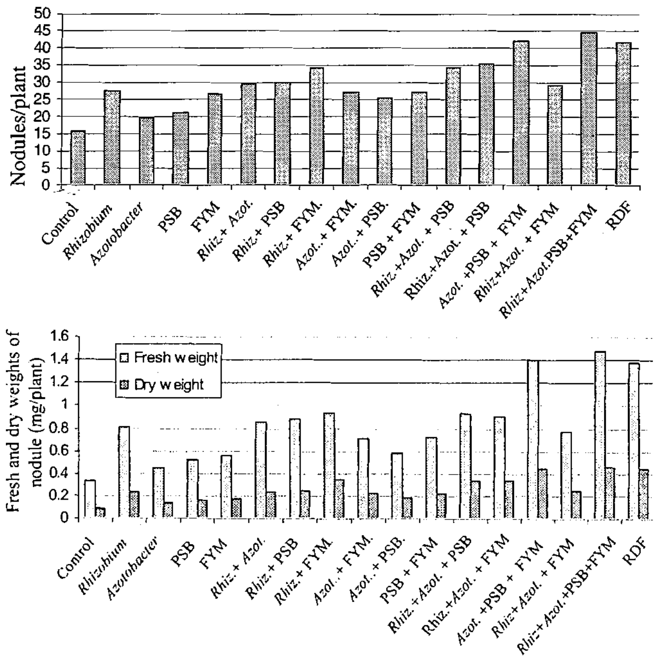

Fig. 1. Effect of bio-inoculants and FYM on nodulation at 70 DAS

weights of nodule over RDF and the rest of the treatments. The increase in number of nodules, and the fresh weight and dry weight of nodule in soybean when inoculated with *Rhizobium* + *Azotobacter* combined culture might be due to the production of growth-promoting hormones by *Azotobacter,* leading to curling and deformation of roots, which is a prerequisite for nodule formation (Mel'nikova *et al.*, 2002).

Yield attributes and yield

Biofertilizers inoculation, FYM and RDF significantly improved the pods/plant, seeds/plant and 100-seed weight over the control (Table 1). Maximum increase in yield attributes were noted under RDF, followed by co-inoculation of *Rhizobium* + *Azotobacter* + PSB + FYM and *Rhizobium* + PSB + FYM, which were significantly superior to other treatments. The beneficial effect of biofertilizers might be ascribed to biological N_2 fixation by *Rhizobium*, activation of amino acids for synthesis of carbohydrates and P-solubilization by phosphate-solubilizing bacteria. Co-inoculation of biofertilizers produced heavier seeds, perhaps due to better translocation of photosynthates (Kumrawat *et al.*, 1997).

The biofertilizers improved the seed and straw yields of soybean (Table 2), and the application of RDF, being on par with that of *Rhizobium* + *Azotobacter* + PSB + FYM and *Rhizobium* + PSB + FYM registered markedly higher value of seed and straw yields over the rest of the treatments. This was due to synergistic effect among *Rhizobium*, *Azotobacter* and phosphate-solubilizing bacteria

Table 1. Effect of bio-inoculants and FYM on growth (70 DAS), yield and economics of soybean (pooled data for 2 years)

Treatment	Plant height (cm)	Dry weight (g/plant)	Pods/ plant	Seeds/ plant	100-seed weight (g)	Yield (t/ha)		Cost of cultivation (Rs/ha)	Net returns (Rs/ha)	B : C ratio
						Seed	Straw			
T_1, Control	53.6	11.6	65.0	2.21	11.80	1.16	1.67	10,300	6,775	1.66
T_2, *Rhizobium*	65.2	15.8	88.8	2.37	12.85	1.39	1.93	10,400	10,025	1.96
T_3, *Azotobacter*	58.2	12.0	69.6	2.28	12.02	1.23	1.75	10,400	7,695	1.74
T_4, PSB	59.2	13.4	72.2	2.32	12.27	1.29	1.87	10,400	8,595	1.83
T_5, FYM	63.0	13.6	72.8	2.34	12.67	1.30	1.88	13,825	5,315	1.38
T_6, *Rhizobium* + *Azotobacter*	68.0	15.8	84.7	2.39	12.69	1.41	1.93	10,450	10,255	1.98
T_7, *Rhizobium* + PSB	68.7	16.3	85.0	2.40	12.80	1.46	2.05	10,450	11,015	2.05
T_8, *Rhizobium* + FYM	68.9	16.9	85.3	2.42	12.99	1.52	2.12	13,925	8,415	1.60
T_9, *Azotobacter* + FYM	63.7	15.4	75.0	2.36	12.74	1.31	1.94	13,925	5,385	1.39
T_{10}, *Azotobacter* + PSB	63.6	14.6	75.2	2.35	12.84	1.30	1.88	10,450	8,690	1.83
T_{11}, PSB + FYM	65.4	15.6	76.3	2.36	12.94	1.47	1.99	13,925	7,650	1.55
T_{12}, *Rhizobium* + *Azotobacter* + PSB	72.9	19.7	86.4	2.43	12.93	1.49	2.17	10,500	11,445	2.09
T_{13}, *Rhizobium* + *Azotobacter* + FYM	79.3	20.6	88.4	2.52	12.67	1.59	2.28	13,975	9,425	1.67
T_{14}, *Rhizobium* + PSB + FYM	82.2	22.5	92.5	2.57	13.26	1.61	2.37	13,975	9,750	1.70
T_{15}, *Azotobacter* + PSB + FYM	70.3	18.4	85.5	2.44	12.69	1.48	2.13	13,975	7,810	1.56
T_{16}, *Rhizobium* + *Azotobacter* + PSB + FYM	84.4	22.6	94.9	2.65	13.36	1.65	2.40	13,975	10,325	1.74
T_{17}, RDF	84.3	23.2	96.8	2.66	13.45	1.70	2.43	12,430	12,585	2.01
SEm±	1.72	0.60	2.20	0.03	0.10	0.40	0.63			
CD (P=0.05)	4.78	1.69	6.17	0.09	0.28	0.11	0.17			

(Mel'nikova *et al.*, 2002), which was more pronounced in the presence of FYM. The improvement in seed yield over untreated control was 46.6, 42.2 and 38.8% under RDF, *Rhizobium* + *Azotobacter* + PSB + FYM and *Rhizobium* + PSB + FYM respectively. Further, sole inoculation of *Rhizobium* and phosphate-solubilizing bacteria registered respectively 19.8 and 11.2% increase in grain yield over uninoculated control. *Rhizobium* proved superior to phosphate-solubilizing bacteria, indicating the significance of symbiosis in soybean (Dubey, 1997).

Nutrient uptake

Application of *Rhizobium* + *Azotobacter* + PSB + FYM (T_{16}), being on par with that of 100% RDF (T_{17}), recorded significantly higher uptake of N and P in both the years over the rest of the treatments (Table 2). Single inoculation of *Azotobacter* failed to improve the uptake of N and P, but sole inoculation of soybean with *Rhizobium*, PSB and FYM markedly improved their uptake. However, dual as well as multi-inoculation of biofertilizers with or without FYM statistically increased the uptake of N and P. This might be attributed to enhanced activity of nitrogenase and nitrate-reductase enzyme in the soil (Sarawgi *et al.*, 1998), leading to greater biological N_2 fixation by *Rhizobium* and *Azotobacter*. Increased availability of P in the soil was due to greater solubilization of phosphate compounds by phosphate-solubilizing bacteria and release of P with the application of FYM. Further, the uptake of K improved significantly with *Rhizobium* + *Azotobacter* + PSB + FYM over RDF and the control (T_1). Multi-inoculation of biofertilizer with or without FYM also improved the K uptake significantly over single inoculation and the control.

Nutrient availability

Organic C and available K in the soil after the harvest of soybean increased over the control significantly when FYM was applied alone or along with bio-inoculants (Table 2). The highest organic C (1.08%) and available K (222.3 kg/ha) were recorded with combined inoculation of *Rhizobium* + *Azotobacter* + PSB + FYM (T_{16}), followed by *Rhizobium* + PSB + FYM (T_{15}). In general, the available soil K status after soybean harvest was depleted from initial level under all the biofertilizer treatments (Sharma

Table 2. Effect of bio-inoculants and FYM on nutrient uptake, organic C, available nutrients, and nutrient balance of N, P and K in soil after soybean harvest (pooled data for 2 years)

Treatment	Nutrient uptake (kg/ha)			Organic	Available soil nutrients (kg/ha)			Nutrient balance (kg/ha)		
	N	P	K	C (%)	N	P	K	N	P	K
T_1	104.1	11.5	61.0	0.98	342.4	14.3	193.5	86.4	10.8	41.7
T_2	119.4	13.1	75.8	0.98	370.0	14.6	198.8	129.4	12.7	13.7
T_3	108.6	11.6	67.0	0.98	344.5	14.4	194.5	93.1	11.0	15.4
T_4	112.4	13.4	68.2	0.98	343.2	15.2	196.3	95.6	13.6	18.4
T_5	116.6	13.3	70.8	1.05	368.5	15.2	215.2	125.1	13.5	39.9
T_6	125.0	13.4	77.0	0.99	373.0	15.5	197.0	138.0	13.9	27.9
T_7	129.0	14.5	80.7	0.99	368.0	15.4	195.7	137.0	14.9	30.3
T_8	134.9	14.9	83.9	1.06	384.0	15.3	216.9	158.0	15.2	54.7
T_9	122.5	13.5	74.1	1.05	370.8	15.2	217.1	133.3	18.7	45.1
T_{10}	113.0	14.8	71.1	0.98	345.5	15.3	203.1	98.5	15.1	28.1
T_{11}	123.4	15.3	72.9	1.05	366.0	15.7	216.3	129.4	16.0	43.1
T_{12}	126.7	15.7	80.0	1.00	376.7	15.2	202.3	143.4	15.9	36.2
T_{13}	138.8	15.6	88.7	1.07	388.0	15.2	218.6	166.8	15.8	61.2
T_{14}	144.7	16.3	90.6	1.07	389.0	17.1	219.5	173.7	18.4	64.0
T_{15}	130.3	14.1	81.6	1.06	374.0	15.8	216.8	144.3	14.9	52.3
T_{16}	146.7	16.3	93.5	1.08	391.2	17.0	222.3	177.9	18.3	69.7
T_{17}	148.6	16.4	89.5	1.02	352.0	17.8	208.4	120.6	1.73	51.8
SEm±	2.7	0.7	1.4	0.02	7.3	0.3	4.0			
CD (P=0.05)	7.4	1.8	3.9	0.05	20.8	0.8	11.2			

*Treatment details given in Table 1

and Namdeo, 1999). Inoculation of biofertilizers along with FYM significantly increased the soil-available N and P, indicating their build-up in post-harvest soil. Combined inoculation of *Rhizobium* + *Azotobacter* + PSB + FYM registered higher soil-available N, and the increase was 48.8 kg/ha over the uninoculated control. However, maximum available P was recorded with RDF (T_{17}), followed by *Rhizobium*+ PSB+FYM (T_{17}) and *Rhizobium* + *Azotobacter* + PSB + FYM (T_{16}). Compared with the initial level, combined inoculation of *Rhizobium* + *Azotobacter* + PSB + FYM increased the available soil N by 31.2 kg/ha and available soil P by 2.05 kg/ha. The effect of *Rhizobium* was more pronounced in increasing the post-harvest soil available N and phosphate solubilizing bacteria in increasing the post-harvest available soil P compared with the uninoculated control. Improved soil N and P status at harvest was perhaps owing to the improved nodulation, nitrogenase and nitrate-reductase enzyme activity, and N_2 fixation (Sarawgi *et al.*,1998) by *Rhizobium* as well as improved solubilization of P through secretion of organic acids and also the activity of phosphatase enzyme by PSB (Pal, 1997) and their combined influence with FYM.

Nutrient balance

The highest net gain of N (177.9 kg/ha), P (18.3 kg/ha) and K (69.7 kg/ha) was observed under multi-inoculation of *Rhizobium* + *Azotobacter* + PSB + FYM (T_{16}) followed by *Rhizobium* + PSB + FYM (T_{15}) over the control where the lowest gain of N (86.4 kg/ha) and K (41.7 kg/ha) was recorded (Table 2). However, lowest gain of P was noted with RDF. An increase of N, P and K balance in the soil with *Rhizobium* + *Azotobacter* + PSB + FYM might be due to soybean, which makes less demand of N from the soil due to fixation of N_2 in their root nodules. These results support to the findings of Sarawgi *et al.* (1998). The improvement in net balance of P might be due to solubilization of inorganic P through the secretion of soil organic acid by inoculation of phosphate-solubilizing bacteria and addition of FYM, whereas maximum gain of K was due to the direct addition of K and FYM, which increased the available K pool of the soil besides reducing the K fixation and releasing K on interaction of FYM and organic matter with clay (Sharma and Namdeo, 1999).

Economics

The cost of cultivation was marginally higher with the application of *Rhizobium* + *Azotobacter* + PSB + FYM (Rs 13,975/ha) compared with RDF (Rs 12,430/ha) on application of FYM. The highest net monetary returns (Rs 12,585/ha) were recorded with the application of RDF (Table 1), whereas, maximum benefit: cost ratio was observed with co-inoculation of *Rhizobium* + *Azotobacter* + PSB (2.09), followed by *Rhizobium* + PSB (2.05) and RDF (2.01). This was attributed to higher grain and straw yields of soybean with these treatments than with FYM application.

Thus, the RDF and co-inoculation of *Rhizobium* + *Azotobacter* + PSB+FYM were found effective for improving productivity of soybean in Kashmir valley.

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