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Effect of Bio-fertilizer Inoculations on Growth and Yield of Dwarf Field Pea (*Pisum sativum* L.) in Conjunction with Different Doses of Chemical Fertilizers

A. Mishra, K. Prasad and Geeta Rai

College of Agriculture, Chandra Shekhar Azad University of Agriculture and Technology,
Kanpur, U.P-208002, India

Abstract: A field experiment was conducted during two consecutive Rabi seasons of 2007 and 2008 to study the effect of bio-fertilizers in conjunction with inorganic fertilizers on growth and yield of dwarf field pea (cv. Jai) at Oil Seed Research Farm, Kalyanpur in C.S.A. University of Agriculture and Technology, Kanpur. The experiment was laid out in split plot design with three replications in sandy loam soil. The experiment comprised 32 treatment combinations of four levels of fertility (Control, 50, 75 and 100% RDF) and eight bio-fertilizer treatments (Control, *Rhizobium*, PSB, PGPR, *Rhizobium*+PSB, *Rhizobium*+PGPR, PSB+PGPR and *Rhizobium*+PSB+PGPR). Results indicated that the combined application of 100% RDF and seed inoculation with *Rhizobium*+PSB+PGPR improved all the growth; yield attributes and yields of field pea. Fresh and dry weight plant⁻¹, nodules number and dry weight plant⁻¹ were found significantly maximum. Number of grains pod⁻¹, number and weight of pods plant⁻¹ at maturity attributed significantly in increasing the grain yield of field pea up to 31.00 q ha⁻¹ and net return up to Rs.26187 ha⁻¹ with the application of 100% RDF and seed inoculation of *Rhizobium* + PSB + PGPR, yield was 10.96 and 11.93% higher over co-inoculation of *Rhizobium* + PSB + PGPR (27.60 q ha⁻¹) and 100% RDF (27.30 q ha⁻¹) application. Thus, it can be recommended that to obtain the maximum grain yield and net profit from dwarf field pea, seed should be inoculated with *Rhizobium* + PSB + PGPR and crop should be fertilized with 100% recommended dose of fertilizer.

Key words: *Rhizobium*, PSB, PGPR, RDF, inoculation, field pea

INTRODUCTION

Field pea (*Pisum sativum*) derives from the Middle East and was first cultivated roughly 10,000 years ago (Mithen, 2003). Field pea is a cool-season legume crop that is grown on over 25 million acres worldwide. Field pea or dry pea is marketed as a dry, shelled product for either human or livestock food. It is commonly used throughout the world in human diets and has high levels of amino acids, lysine and tryptophan, which are relatively low in cereal grains and contains approximately 21-25% protein. Being a legume crop and has the inherent ability to obtain much of its nitrogen requirement from the atmosphere by forming a symbiotic relationship with *Rhizobium* bacteria in the soil (Schatz and Endres, 2009). Pulse crops ability to use the atmospheric nitrogen through Biological Nitrogen Fixation (BNF) is economically sound and environmentally acceptable (Saikia and Jain, 2007). India is a largest pulse producing county, pulses accounts for one fifth cultivated area and one twelfth of the total food grain production. The area under pulse crops at present is around 23.31 million

hectare, the production is around 14.50 million tones and productivity is around 622 kg ha⁻¹ during 2007-08. Country would needs at least 30 million tones of pulses by 2020 A.D. Among pulses field pea is an important crop in India having about 7.93 lakh hectare area and 7.10 metric tones with 895 kg ha⁻¹ of productivity. (Banergee and Palke, 2010).

The term bio-fertilizer or microbial inoculants can be generally defined as preparation containing live or latent cells of efficient strains of nitrogen fixing and phosphate Solubilizing micro organism used for treatment of seed or soil. Biofertilizers are organic products containing living cells of different types of microorganisms, which have the ability to convert nutritionally important elements from unavailable to available form through biological processes (Vessey, 2003). They are composting the area with the objective of increasing the number of such micro organisms and accelerate microbial process to augment the extant of the availability of the nutrient in a form which can easily assimilated by plant (Subba-Rao, 1986). The *Rhizobium* as fertilizer in pulses could fix 50-200 kg of N/ha/season and is able to meet 80-90% of the crop

requirement for nitrogen. Inoculation in these crops was found to increase the crop yield by about 10-15% under on farm conditions (Khurana and Dudeja, 1997). In many situations this association also leaves substantial residual nitrogen for subsequent crops. The Biological Nitrogen Fixation (BNF) has been estimated to contribute more than 175 million tones of nitrogen out of which legume N_2 fixation accounts for almost 40% (Burns and Hardy, 1975). Nitrogen fixation by different annual legumes has been reported to vary from 35-270 kg N/ha/Year (Nutman, 1969).

Nutrients affected all most growth and yield attributing characters and yields through its doses as well as sources. Keeping the facts in consideration, the present investigation was under taken to estimate the effect of different bio-fertilizers (*Rhizobium*, PSB and PGPR) alone and in combination with fertility levels on growth and yield attributes and grain yield of field pea.

MATERIALS AND METHODS

The present experiment was conducted during Rabi Season of 2007-08 and 2008-09, with a view to find out the effect of different bio-fertilizers on growth and yield of field pea under different fertility levels at Oil seed research farm of C.S.A. University of Agriculture and Technology, Kanpur (INDIA). The soil of experimental field was sandy loam having 0.03% O.C., 196.00 kg ha⁻¹ available N, 25.20 kg ha⁻¹ available P₂O₅ and 175.00 kg ha⁻¹ available K₂O with 7.98 pH. This was not observed favorable to make the nutrient availability, because pH range between 6-7 seems to promote the availability of nutrients to the plants (Brady, 1988). Crop during experimental period received 4.6 mm rains spread over three days in first year and 5.5 mm rains spread over one day in second year. Maximum mean temperature rose up to 35.1 and 34.1°C, while minimum mean temperature gone up to 5.6 and 5.3°C during first and second year of crop, respectively. Relative humidity, wind speed and evaporation rate were also remained more or less similar during both years.

The experiment was laid out in split plot design with three replications. The treatments comprised 32 treatment combinations of four levels of fertility in main plots i.e., Control, 50, 75 and 100% RDF(40-60-40) and eight bio-fertilizer seed inoculation treatments in sub-plots (control, *Rhizobium*, PSB, PGPR, *Rhizobium*+PSB, *Rhizobium*+PGPR, PSB+PGPR and *Rhizobium*+PSB+PGPR). Sowing of inoculated seed as per treatment of fieldpea (*Pisum sativum* L.) variety Jai (KPMR-522) was done in furrows with the help of country plough at 22.5 cm apart using seed at 120 kg ha⁻¹. Full dose of fertilizer as per treatments was incorporated into the field at the time of sowing with the help of seeding spout

attached with country plough at below the seed. Experimental crop was grown under irrigated condition as per the recommended agronomic practices. The effect of treatments was evaluated on pooled basis on growth and yield attributes; yields and economics.

RESULTS AND DISCUSSION

Effect of fertility levels

On growth and yield attributes: Pooled data presented in Table 1 indicates that the plant height at 30 and 60 DAS was recorded significant, the significant increase in plant height was due to increasing doses of fertilizers similar findings was also reported by Bisen *et al.* (1985). Fresh weight per plant at 90 DAS was recorded significantly higher with 100% RDF (140.77 g) against 75% RDF (132.49 g), 50% RDF (125.64 g) and control (112.59 g). Dry weight per plant at 90 DAS was recorded significantly higher with 100% RDF (25.83 g) against 75% RDF (21.65 g), 50% RDF (20.97 g) and control (20.99 g). Number of nodules per plant at 60 DAS was recorded significantly maximum of 32.78 at 100% RDF against 26.01, 22.41 and 20.45 at 75% RDF, 50% RDF and control, respectively. Dry weight of nodules plant⁻¹ at 60 DAS was recorded significantly maximum of 33.13 at 100% RDF against 26.09, 22.41 and 20.54 mg at 75% RDF, 50% RDF and control, respectively. The effect of increasing fertility levels on number and dry weight of nodules per plant also corroborated the results of Solaiman and Rabbani (2006) and Maurya and Prasad (1998).

Number of pods per plant at harvest was recorded significantly maximum of 21.27 at 100% RDF against 17.31, 15.23 and 10.67 at 75% RDF, 50% RDF and control, respectively. Number of grains per pod at maturity was recorded significantly maximum of 5.02 at 100% RDF against 4.75, 4.50 and 3.92 at 75% RDF, 50% RDF and control, respectively. Length of pods at 30, 45 and 60 DAS was recorded significantly higher with 100% RDF (7.50 cm) against 75% RDF (6.94 cm), 50% RDF (6.81 cm) and control (6.43 cm). Weight of pod per plant was recorded significantly maximum of at 100% RDF (9.46 g) against 75% RDF (8.59 g), 50% RDF (7.88 g) and 7.33 g control, respectively. These results are in harmony with those reported by Negi *et al.* (2007). Hundred grain weight was also recorded significantly maximum of 19.89 g at application of 100% RDF against 18.93, 18.84 and 18.70 g at 75% RDF, 50% RDF and control, respectively (Table 1).

On yields: Significantly maximum grain yield of 27.30 q ha⁻¹ was obtained under 100% RDF against 26.20, 24.40 and 20.90 q ha⁻¹ at 75% RDF, 50% RDF and control, respectively. Straw yield was recorded significantly

Table 1: Effect different fertility levels and bio-fertilizer treatments on growth and yield attributes on field pea

Treatments	Plant height (cm) at 90 DAS	Fresh weight/ plant (g) at 90 DAS	Dry weight/ plant (g) at 90 DAS	Nodule		No. of pods/plant	No. of grains/pod	Length of pod (cm)	Pod weight /plant (g)	100 grain weight (g)
				No. of nodules at 60 DAS	dry weight/ plant (mg) at 60 DAS					
Fertility levels										
Control	43.82	112.59	20.99	20.45	20.54	10.67	3.92	6.43	7.33	18.70
50% RDF	45.49	125.64	20.97	22.41	22.41	15.23	4.50	6.81	7.88	18.84
75% RDF	47.56	132.49	21.65	26.01	26.09	17.31	4.75	6.94	8.59	18.93
100% RDF	50.57	140.77	25.83	32.78	33.13	21.27	5.02	7.50	9.46	19.89
SE+	2.30	1.75	0.39	0.61	0.60	0.40	0.26	0.10	0.36	0.05
CD (p = 0.05)	NS	3.82	0.84	1.33	1.32	0.881	0.565	0.211	0.785	0.099
Bio-fertilizers										
Uninoculated	43.90	87.48	15.46	11.84	11.85	12.50	3.58	5.64	5.84	18.35
<i>Rhizobium</i>	45.81	112.92	18.48	23.43	23.52	14.13	4.17	6.28	7.70	19.22
PSB	46.72	103.67	17.15	20.24	20.38	15.21	4.54	6.92	8.12	18.52
PGPR	46.56	112.38	18.04	22.03	22.12	16.00	4.25	6.86	8.17	19.23
<i>Rhizobium</i> +PSB	47.19	143.42	25.27	29.46	29.55	16.75	4.38	6.84	8.15	18.98
PSB+PGPR	47.31	140.81	23.81	27.77	27.87	18.13	4.88	7.46	8.99	19.51
<i>Rhizobium</i> +PSB	46.54	152.98	27.42	31.91	32.08	17.42	4.92	7.40	9.41	19.13
<i>Rhizobium</i> +PSB+PGPR	50.85	169.33	31.25	36.63	37.00	18.83	5.67	7.95	10.13	19.79

Table 2: Effect different fertility levels and bio-fertilizer treatments on yields, harvest and economics on field pea

Treatments	Pooled						B:C ratio
	Grain yield	Straw yield	Biological yield	Harvest index	Gross Income	Net return	
	----- (q ha ⁻¹) -----						
Fertility levels							
Control	20.90	22.70	43.50	46.87	29876.00	13798.00	1.86
50% RDF	24.40	25.90	50.30	50.27	34961.00	17859.00	2.04
75% RDF	26.20	27.30	53.50	49.40	37496.00	19934.00	2.13
100% RDF	27.30	28.30	55.60	46.07	39085.00	21064.00	2.17
SE+	0.10	0.30	0.04	0.37	195.92	195.92	0.01
CD (p = 0.05)	0.30	0.69	0.77	0.79	426.91	426.91	0.025
Bio-fertilizers							
Control	21.20	22.60	43.80	48.36	30340.00	13327.00	1.78
<i>Rhizobium</i>	24.20	25.10	49.30	48.69	34645.00	17503.00	2.02
PSB	23.10	24.40	47.50	48.80	33135.00	15993.00	1.93
PGPR	23.80	25.40	49.20	48.61	34099.00	16957.00	1.99
<i>Rhizobium</i> +PSB	25.70	27.00	52.60	47.32	36743.00	19497.00	2.12
<i>Rhizobium</i> +PGPR	25.50	26.70	52.20	47.64	36443.00	19197.00	2.11
PSB+PGPR	26.50	28.10	54.50	48.13	37913.00	20667.00	2.19
<i>Rhizobium</i> +PSB+PGPR	27.60	29.10	56.70	47.67	39518.00	22169.00	2.27
SE+	0.30	0.40	0.50	0.76	363.01	363.01	0.02
CD (p = 0.05)	0.51	0.70	0.95	NS	719.49	719.49	0.042

maximum with the application of 100% RDF (28.30 q ha⁻¹) against at 75% RDF, 50% RDF and control (27.30, 25.90 and 22.70 q ha⁻¹), respectively and Biological yield was recorded significantly maximum of 55.60 q ha⁻¹ at 100% RDF against 53.50, 50.30 and 43.50 q ha⁻¹ at 75% RDF, 50% RDF and control, respectively (Rizk and Shafeek, 2000). Nitrogen, phosphorus and potassium content in grain and straw and uptake by grain and straw (Sharma *et al.*, 2006) were recorded significantly maximum with 100% RDF. Harvest index was recorded significantly maximum with 50% RDF (50.27%). It might be due to comparatively higher increase of grain yield than that of respective vegetative yield of crop. Similar finding was also reported by Chanda *et al.* (2002) (Table 2).

On economics: Application of 100% RDF gave maximum gross income, which was Rs.39085 ha⁻¹ followed 75%

RDF (Rs.37496 ha⁻¹), 50% RDF (Rs.34961 ha⁻¹) and control (Rs.29876 ha⁻¹), respectively. Net return was noted significantly higher by a margin of Rs.21061 ha⁻¹ with 100% RDF over Rs.19934, 17859 and 13798 ha⁻¹ at 75% RDF, 50% RDF and control, respectively. The highest B:C ratio of 2.17 was calculated under 100% RDF followed by 2.13, 2.04 and 1.86 at 75% RDF, 50% RDF and at control, respectively (Table 2).

How bio-fertilizers are working and benefited the crop?

Rhizobium: Atmospheric nitrogen must be processed, or fixed, in order to be used by plants. Some fixation occurs in lightning strikes, but most fixation is done by free-living or symbiotic bacteria. These bacteria have the nitrogenase enzyme that combines gaseous nitrogen with hydrogen to produce ammonia, which is then further converted by the bacteria to make their own organic compounds. Nitrogen

fixing bacteria, such as *Rhizobium*, live in the root nodules of legumes. Here they form a mutualistic relationship with the plant, producing ammonia in exchange for carbohydrates.

Phosphate solubilizing bacteria: Phosphate Solubilizing Bacteria (PSB) are a group of beneficial bacteria capable of hydrolysing organic and inorganic phosphorus from insoluble compounds. P-solubilization ability of the microorganisms is considered to be one of the most important traits associated with plant phosphate nutrition. It is generally accepted that the mechanism of mineral phosphate solubilization by PSB strains is associated with the release of low molecular weight organic acids, through which their hydroxyl and carboxyl groups chelate the cations bound to phosphate, thereby converting it into soluble forms. In addition, some PSB produce phosphatase like phytase that hydrolyse organic forms of phosphate compounds efficiently. One or both types of PSB have been introduced to agricultural community as phosphate Biofertilizer. They are composting the area with the objective of increasing the number of such microorganisms and accelerate microbial process to augment the extent of the availability of the nutrient in a form which can easily assimilated by plant (Subba-Rao, 1986). However, a large portion of soluble inorganic phosphate which is applied to the soil as chemical fertilizer is immobilized rapidly and becomes unavailable to plants. Currently, the main purpose in managing soil phosphorus is to optimize crop production and minimize P loss from soils. PSB have attracted the attention of agriculturists as soil inoculums to improve the plant growth and yield.

Plant growth promoting rhizobacteria: Rhizobacteria are root-colonizing bacteria that form a symbiotic relationship with many legumes. Though parasitic varieties of rhizobacteria exist, the term usually refers to bacteria that form a relationship beneficial for both parties (mutualism). Such bacteria are often referred to as plant growth promoting rhizobacteria, or PGPRs.

PGPR enhance plant growth by direct and indirect means, but the specific mechanisms involved have not all been well-characterized (Glick, 1995; Kloepper, 1993). Direct mechanisms of plant growth promotion by PGPR can be demonstrated in the absence of plant pathogens or other rhizosphere microorganisms, while indirect mechanisms involve the ability of PGPR to reduce the deleterious effects of plant pathogens on crop yield. PGPR have been reported to directly enhance plant growth by a variety of mechanisms: fixation of atmospheric nitrogen that is transferred to the plant,

production of siderophores that chelate iron and make it available to the plant root, solubilization of minerals such as phosphorus and synthesis of phytohormones (Glick, 1995).

Effect of bio-fertilizers

On growth and yield attributes: Plant height at 90 DAS affected significantly with the different bio-fertilizers treatments. Lowest plant height (43.90 cm) was recorded at control and highest plant height (50.85 cm) was recorded at 90 DAS with combined application of *Rhizobium* + PSB + PGPR on pooled basis. The significant increase in plant height due to increasing doses of bio-fertilizers was also reported by Balachandran and Nagrajan (2002). Fresh and dry weight of plant at 90 DAS was significantly varied with the varying bio-fertilizers inoculations provided to the crop. Highest fresh (169.33 g) and dry weight (31.25 g) was recorded with combined inoculation of all the bio-fertilizers inoculation. These results are also close to confirm with Singh *et al.* (1997). Significant highest number (36.63) and dry weight (37.00 mg) of nodules plant⁻¹ at 60 DAS was found on pooled basis (Table 1), the effect of different bio-inoculations on number and dry weight of nodules plant⁻¹ also corroborated the results of Sudhansu (1997) and Balachandran and Nagrajan (2002).

Number of pods per plant at harvest was recorded significantly maximum of 18.83 at combined inoculation of (*Rhizobium* + PSB + PGPR) followed by 18.13 in dual (PSB + PGPR) and 16.00 in single (PGPR) inoculation against 12.50 over control. Number of grains per pod was recorded highest 5.67 at combined inoculation of (*Rhizobium*+PSB+PGPR) followed by 4.92 dual (*Rhizobium*+PSB) and 4.54 single (PSB) inoculation and 3.58 in control. Results also confirmed by the findings of Mehta *et al.* (1995).

Pod length at 60 DAS was recorded significantly higher with *Rhizobium*+PSB+PGPR (7.95 cm) followed by PSB+PGPR (7.46 cm), *Rhizobium*+PSB (7.40 cm) against control (5.64 cm). Weight of pod per plant at maturity was recorded significantly maximum of 10.13 g at *Rhizobium*+PSB+PGPR followed by dual 9.41 g (*Rhizobium*+PSB) and single 8.17g (PGPR) against 5.84 g at control. Hundred grain weight (Seed Index) was recorded significantly maximum of 19.89 g at inoculation of *Rhizobium*+PSB+PGPR followed by 19.51, 19.23 and 18.35 g in dual (PSB+ PGPR), PGPR alone and at control, respectively (Table 1).

On yields: Significantly maximum grain yield of 27.60 q ha⁻¹ was obtained under *Rhizobium*+PSB+PGPR

Table 3: Interaction effect of fertility levels and bio-fertilizers on grain, straw and biological yield; and net return

Factors	B1	B2	B3	B4	B5	B6	B7	B8
Grain yield (q ha⁻¹)								
F1	17.50	20.92	19.33	20.17	22.00	21.42	22.42	23.08
F2	21.42	24.00	22.92	23.92	25.08	25.00	25.92	27.08
F3	22.58	25.42	25.00	25.17	27.17	27.08	27.92	29.25
F4	23.25	26.50	25.33	26.00	28.42	28.33	29.67	31.00
SE+for (FxB) = 0.50 CD for (FxB) at 5% = 1.10								
Straw yield (q ha⁻¹)								
F1	18.75	22.17	21.50	22.83	23.08	23.00	24.83	25.33
F2	23.67	25.00	24.25	25.42	26.58	26.42	27.58	28.33
F3	24.00	25.42	26.08	26.42	28.58	28.50	28.83	30.42
F4	23.92	27.83	25.58	26.92	29.67	28.92	31.00	32.25
SE+for (FxB) 0.70 CD for (FxB) at 5% = 1.40								
Biological Yield (q ha⁻¹)								
F1	36.30	43.10	40.80	43.00	45.10	44.40	47.30	48.40
F2	45.10	49.00	47.20	49.30	51.70	51.40	53.50	55.40
F3	46.60	50.80	51.10	51.60	55.80	55.60	56.80	59.70
F4	47.20	54.30	50.90	52.90	58.10	57.30	60.70	63.30
SE+for (FxB) = 1.00 CD for (FxB) at 5% = 1.90								
Net return (Rs. ha⁻¹)								
F1	9163	13919	11682	12889	15359	14540	15995	16839
F2	13770	17297	15758	17193	18757	18636	19954	21506
F3	14953	18833	18269	18513	21274	21155	22332	24142
F4	15424	19962	18261	19235	22597	22457	24387	26187
SE+for (FxB) = 726 CD for (FxB) at 5% = NS								

inoculation followed by PSB+PGPR (26.50 q ha⁻¹) in dual and *Rhizobium* alone (24.20 q ha⁻¹) inoculation. Straw yield was recorded significantly maximum (29.10 q ha⁻¹) with the *Rhizobium*+PSB+PGPR inoculation followed by PSB+PGPR (28.10 q ha⁻¹) in case of dual inoculation. PGPR alone produced (25.40 q ha⁻¹) highest straw yield among single inoculations. Biological yield was recorded significantly maximum of 56.70 q ha⁻¹ at inoculation of *Rhizobium*+PSB+PGPR followed by 54.50 q ha⁻¹ at dual (PSB+PGPR) inoculation and *Rhizobium* alone produced 49.30 q ha⁻¹ among single inoculations (Table 2).

On economics: Application of *Rhizobium*+PSB+PGPR gave maximum gross income, which was Rs.39518 ha⁻¹ followed PSB+PGPR (Rs. 37913 ha⁻¹), *Rhizobium*+PSB (Rs.36743 ha⁻¹) and *Rhizobium*+PGPR (Rs.36443 ha⁻¹), respectively. Net return was noted significantly higher by a margin of Rs. 22169 ha⁻¹ with *Rhizobium*+PSB+PGPR over Rs. 20667.00, 19497.00 and 19197 ha⁻¹ at PSB+PGPR, *Rhizobium*+PSB and *Rhizobium*+PGPR, respectively. The highest B:C ratio of 2.27 was calculated under *Rhizobium* + PSB + PGPR followed by 2.19, 2.12 and 2.11 at PSB + PGPR, *Rhizobium* + PSB and *Rhizobium* + PGPR, respectively (Table 2).

The application of 100% RDF and co-inoculation of *Rhizobium*+PSB+PGPR either individually or in combination proved economically feasible for field pea cultivation. The maximum grain yield (31.00 q ha⁻¹) and

net profit of Rs.26187 ha⁻¹ was achieved with the use of 100% RDF and inoculation of *Rhizobium*+PSB+PGPR (Table 3).

CONCLUSION

Thus, it can be said that for obtaining maximum grain yield as well as profit from dwarf field pea (cv. Jai), seed should be inoculated with *Rhizobium*+PSB+PGPR along with application of 100% RDF.

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