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Short Comminucation

Effect of biofertilizers on nodulation, nutrient uptake, yield and energy use efficiency of field pea (*Pisum sativum* L.)

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The field pea (*Pisum sativum L.*) crop is a high yielding, input responsive and relatively stable pulse crop of *Rabi* season that contributes significantly to the total pulses production of the country. It has high level of amino acids, especially lysine and tryptophan. Inorganic fertilizers play a special role in crop production. Being a legume crop, it 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 by the process of biological nitrogen fixation, thereby required amount of fertilizers decrease proportionately. Many authors stated that yields could be increased by legume seed inoculation compared to nitrogen fertilizer variants (Dobereiner *et al.* 1995). It has been reported that low doses of applied nitrogen had favourable effects on nodulation and nitrogen fixation whereas higher doses of mineral nitrogen resulted in nodule mass reduction. This directly affected the value reduction of all yield elements (Ergorov 1985; Waterer *et al.* 1992). Seed inoculation with *biofertilizers* prior to sowing improve the productivity of grain legumes, as it influences the nodulation, biological nitrogen fixation, produces growth hormones and ultimately increases grain yield of field pea per degree day per gram. So heat use efficiency (HUE) is directly related to biofertilizers application.

The study was conducted at Research Farm Area of Pulses Section, Department of Genetics and Plant Breeding at CCS Haryana Agricultural University, Hisar, during *Rabi* season 2013-14 in sandy loam soils under irrigated conditions. Crop was sown on 24th Nov., 2013 and harvested on 14th April, 2014. The experiment was conducted in randomized block design with nine treatments *viz*., control, seed inoculation with *Rhizobium*, *Rhizobium* + PSB (Phosphate Solubilizing Bacteria) , *Rhizobium* + PSB + PGPR, Recommended dose of fertilizers (RDF), RDF + *Rhizobium*, RDF +*Rhizobium* + PSB, RDF + *Rhizobium* + PSB + PGPR (Plant Growth Promoting Rhizobacteria), RDF + $ZnSO_4$@ 25 kg ha^{-1}. The seed was wetted with a sugar solution, and 50 ml of bioinoculants viz. *Rhizobium, pseudomonas* and bacillus (Department of Microbiology, CCSHAU, Hisar) was used per 10 kg of seed. Sowing was done at 30 cm row spacing by *Pora* method. Recommended dose of nitrogen (20 kg ha^{-1}), phosphorus (40 kg ha^{-1}) and zinc sulphate (25 kg ha^{-1}) were applied as per treatments through urea, single super phosphate and zinc sulphate, respectively. The crop was planted with a pre-sowing irrigation. In addition, two more irrigations were applied according to the need of crop during the life span. Three plants were uprooted very carefully along with roots at 40 and 60 DAS. The roots were washed in clean water in the bucket in order to remove the soil particles adhered to the roots and then numbers of nodules were counted from all the three plants. Average was worked out and expressed as number of nodules plant^{-1}. After counting the nodules, they were removed from the roots and the average fresh weight of nodules plant^{-1} was taken out and expressed in g plant^{-1}. After taking the fresh weight, the nodules were sun dried for some time and then oven dried at 60°C for 48 hours and constant weight was recorded. The average dry weight of nodules plant^{-1} was taken and expressed in mg plant^{-1}. Nitrogen content (%) was determined by Nessler's Reagent method as described by Jackson (1973). Phosphorous content (%) by Vanadomolybdo Phosphoric Acid Yellow Colour method, potassium content (%) by Flame Photometric method and zinc content was determined by atomic absorption spectrophotometer. Agrimet data were collected from Department of Agricultural meteorology, CCS HAU Hisar to computation the agrometeorological indices viz., growing degree day (GDD), heliothermal unit (HTU), photothermal unit (PTU), hydrothermal unit (HYTU), heat use efficiency (HUE), hydrothermal use efficiency (HYTUE) utilization from sowing to harvesting (24 Nov, 2013 to 14 April, 2014) of the following Rao et al. (1999). The hydrothermal unit (HYTU) was determined by multiplying

Table 1: Influence of sole and combined application of inorganic and bio fertilizers on nodulation of field pea

Treatments	Number of nodules (Plant^{-1})		Nodules fresh weight (mg plant^{-1})		Nodules dry weight (mg plant^{-1})	
	40 DAS	60 DAS	40 DAS	60 DAS	40 DAS	60 DAS
Control	14.0	25.8	40	80	18	28
Rhizobium	21.0	35.7	130	160	42	48
Rhizobium + PSB	21.8	36.3	140	170	43	50
Rhizobium + PSB + PGPR	23.7	36.6	150	190	44	53
RDF	20.5	32.2	110	150	38	46
RDF + *Rhizobium*	25.5	37.7	170	200	48	55
RDF + *Rhizobium* +PSB	28.3	37.9	180	210	50	56
RDF + *Rhizobium* + PSB + PGPR	29.1	38.5	200	220	54	58
RDF + $ZnSO_4$@ 25 kg ha^{-1}	21.0	32.8	120	160	40	47
CD (P=0.05)	4.3	2.6	30	30	3	5

Table 2: Yield and nutrient uptake studies in field pea as affected by sole and combined application of inorganic and bio fertilizers

Treatment	N uptake (kg ha^{-1})		P uptake (kg ha^{-1})		K uptake (kg ha^{-1})		Zn uptake (g ha^{-1})		Yield (kg ha^{-1})	
	Seed	Straw	Seed	Straw	Seed	Straw	Seed	Straw	Seed	straw
Control	54.7	20.0	8.4	2.7	18.5	85.3	68.6	60.2	1595	2798
Rhizobium	62.1	24.2	10.0	3.8	20.4	94.8	75.6	66.3	1727	3012
Rhizobium + PSB	65.8	23.8	11.5	4.9	21.6	91.4	78.0	62.3	1802	2873
Rhizobium+PSB +PGPR	78.4	26.7	14.0	5.5	25.9	99.8	91.8	68.7	2116	3136
RDF	94.2	25.7	16.7	5.6	31.5	94.6	110.2	65.2	2499	2925
RDF + *Rhizobium*	100.6	28.7	17.6	6.1	32.9	101.1	115.4	70.8	2577	3119
RDF + *Rhizobium* + PSB	107.1	28.1	19.2	6.5	35.2	97.6	121.3	67.8	2731	3025
RDF+*Rhizobium* + PSB + PGPR	116.0	29.6	21.3	7.2	38.5	100.5	130.7	69.9	2931	3095
RDF + $ZnSO_4$@ 25 kg ha^{-1}	96.6	22.6	17.2	4.9	32.4	82.3	117.3	60.5	2549	3058
CD (P=0.05)	5.4	3.5	0.9	1.0	4.8	NS	8.5	NS	137	392

the GDD to the average relative humidity (RH_a).

Nodulation study

Development of effective root nodules (number and weight) is generally used as an index for measuring nitrogen fixation capacity of legumes. The maximum number of nodules, their fresh and dry weight per plant were recorded in RDF (Recommended dose of fertilizers) + *Rhizobium* +PSB + PGPR treatment being at par with treatments RDF + *Rhizobium* and RDF + *Rhizobium* + PSB at 40 and 60 DAS except nodule dry weight at 40 DAS (Table 1). Inoculation with *Rhizobium* alone produced significantly higher number and more weight (fresh and dry) of nodules over control at 40 and 60 DAS. The treatments *Rhizobium* alone, *Rhizobium* + PSB and *Rhizobium* + PSB + PGPR at 40 and 60 DAS, did not differ significantly with each other regarding number and weight (fresh and dry) of nodules, but additional inoculation of *Rhizobium, Rhizobium* + PSB and *Rhizobium* + PSB + PGPR alongwith RDF increased the number and weight (fresh and dry) of nodules over control and RDF, respectively. The lowest number of nodules, their fresh and dry weight was observed with absolute control (Table 1). An overall improvement in the growth of field pea crop due to application of chemical fertilizers in combination with biofertilizers was possibly through increase in nodulation,

Table 3: Heat, hydrothermal and energy efficiency utilized in seed yield (kg ha^{-1})

Treatments	RUE kg $ha^{-1}MJ^{-1}$	HUE kg ha^{-1} $^0C^{-1}day^{-1}$	HTUE kg $ha^{-10}C^{-1}day^{-1}$	PTUE kg ha^{-1} $^0C^{-1}day^{-1}$	HYTUE kg ha^{-1} $^0C^{-1}day^{-1}$%
Control	2.69	1.02	0.15	0.09	0.01
Rhizobium	2.91	1.11	0.16	0.10	0.02
Rhizobium + PSB	3.04	1.15	0.17	0.10	0.02
Rhizobium+PSB+PGPR	3.57	1.36	0.20	0.12	0.02
RDF	4.21	1.60	0.23	0.14	0.02
RDF + *Rhizobium*	4.35	1.65	0.24	0.14	0.02
RDF+*Rhizobium*+PSB	4.61	1.75	0.25	0.15	0.02
RDF+*Rhizobium*+PSB+ PGPR	4.94	1.88	0.27	0.16	0.03
RDF + $ZnSO_4$@ 25kg ha^{-1}	4.30	1.63	0.24	0.14	0.02

nitrogenase activity and leghaemoglobin content of nodules as these parameters contributes greatly towards atmospheric nitrogen fixation which is an essential nutrient for plant growth and development.

Nutrient uptake

The highest and the lowest N and P uptake in grain and straw were recorded in RDF + *Rhizobium* + PSB + PGPR and control treatments, respectively. The N uptake in straw was recorded significantly higher in RDF + *Rhizobium* + PSB + PGPR treatment and at par with treatments RDF + *Rhizobium* and RDF + *Rhizobium*+ PSB. The treatments RDF and RDF + *Rhizobium* was at with RDF + $ZnSO_4$ regarding phosphorus uptake in grain (Table 2). The K uptake in grain was also recorded significantly higher in RDF + *Rhizobium* + PSB + PGPR treatment than rest of treatments except RDF + *Rhizobium* + PSB, whereas lowest K uptake in grain was recorded in absolute control which was at par with treatments *Rhizobium* and *Rhizobium* + PSB. The differences in K uptake between other treatments were non-significant. The Zn uptake in grain was recorded significantly higher in RDF + *Rhizobium* + PSB + PGPR treatment and the lowest Zn uptake was recorded in control. Combined inoculation of *Rhizobium* + PSB + PGPR recorded significant higher Zn uptake compared to both (*Rhizobium* + PSB) and single (*Rhizobium*) inoculation. Various treatments did not result in any significant effect on Zn uptake in straw (Table 2).

Seed and straw yield

Application of RDF + *Rhizobium* + PSB + PGPR recorded the highest seed and straw yield, which differ significantly from rest of the treatments. Significantly the lowest seed yield was recorded in control as compared to rest of treatments except sole inoculation of *Rhizobium* (Table 2).

Energy use efficiency

The various energy use viz, GDD, HTU, PTU, HYTU and IPAR by the pea during it life period (sowing to harvest) was 1561 ^0C day, HTU 10764^0C day hour, PTU 17840^0C day hour, HYTU 112883^0C day percent and IPAR 593 MJ/m^2 respectively. The result revealed that among different treatments, the highest energy use efficiency were found in the treatment RDF+ *Rhizobium* + PSB+ PGPR and the were in control as compared to other treatments (Table 3).

An overall improvement in the growth of field pea crop due to application of chemical fertilizers in combination with biofertilizers was possibly through increase in nodulation.

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