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Interactive effect of bio–fertilizers *viz*. rhizobium, PSB and VAM on nitrogen, phosphorus and protein content in lentil (*Lens culinaris* L.) crop

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Abstract: This study was conducted to evaluate the effect of biofertilizers viz. *Rhizobium* , PSB and VAM in lentil (*Lens culinaris* L.) crop, in the department of microbiology . In this experiment five treatment were taken as Un-inoculated (T1), Rhizobium (T2), PSB + Rhizobium (T3), VAM + Rhizobium (T4) and Rhizobium + PSB + VAM (T5). The experiment was carried out during Rabi 2010 in 2.0 m^2 plot size at pot culture house. The result of this study revealed that the least nitrogen content in grain and straw was found in uninoculated micro plat (T1) whereas maximum nitrogen content was noticed in Rhizobium + PSB + VAM treated field (T5). Lowest phosphorous content in grain and straw was found in uninoculated micro plat (T1) whereas maximum nitrogen content was noticed in Rhizobium + PSB + VAM treated field (T5). Least nitrogen uptake in grain and straw was found in uninoculated micro plat (T1) whereas maximum nitrogen uptake was noticed in Rhizobium + PSB + VAM treated field (T5). The lowest phosphorus uptake in grain and straw was found in uninoculated micro plat (T1) whereas maximum phosphorus uptake was noticed in Rhizobium + PSB + VAM treated field (T5). Least protein content in grain and straw was found in uninoculated micro plat (T1) whereas maximum protein content was noticed in Rhizobium + PSB + VAM treated field (T5). Therefore it was recommended that the interactive use of Rhizobium , PSB and VAM in the field was more efficient, productive and accessible to marginal and small farmers.

Key words: Nitrogen, Phosphorus, Protein and Rhizobium

Introduction

India, the second largest populous country, mostly depends on agriculture for living. The population explosion has created a tremendous pressure on agriculture. The use of agricultural land for various industrial purposes reduces the area for production. Most of Indian agricultural lands are deprived of some of the essential nutrients for growth and development of crop plants. One of the major essential elements for growth of plants is nitrogen. Nitrogen is required in large quantities for plants to grow, since it is the basic constituent of proteins, and nucleic acids. Nitrogen is provided in the form of synthetic chemical fertilizer (urea). Such chemical fertilizers pose a health hazard and microbial population problem in soil besides beings quite expensive and making the cost of production high. In such a situation the biofertilizers play a major role (Tiwary *et al*., 1998). Biofertilizers are the formulation of living microorganisms, which are able to fix atmospheric nitrogen in the available form for plants either by living freely in the soil or being associated symbiotically with plants (Subba Rao, 1993). Biofertilizers are inputs containing microorganisms which are capable of mobilizing nutritive elements from non-usable form to usable form through biological processes (Tien *et al*., 1979). Biological nitrogen fixation is carried out by both symbiotic and free living bacteria and blue green algae. Symbiotic nitrogen fixation provides 80% of the biologically fixed nitrogen on land. Nitrogen fixing bacteria are very selective in choosing roots of particular legumes species to infect, invade and form root nodules (Subba Rao, 1993). A unique blend of organic manure using micro nutrients and some beneficial microorganisms with sugarcane press mud as base materials has been reported as useful (Arangarasan *et al*., 2000). Field trials conducted in India showed that depending on the legume, soil and agro climatic conditions nearly 50% of nitrogenous fertilizer could be saved through rhizobial inoculations with considerable increase in yield (Rewari, 198 and Tilak, 193). Rhizobium, when co-inoculated with Phosphate Solubilising Bacteria (PSB) has revealed synergistic effect on symbiotic parameters and grain yield. Phosphate solubilzing bacteria improve the competive ability and symbiotic effectiveness of inoculated Rhizobium sp. in lentil under field conditions (Kumar *et al*., 208). Indiscriminate use of synthetic fertilizers has led to the pollution and contamination of the soil, has polluted water basins, destroyed micro-organisms and friendly insects, making the crop more prone to diseases and reduced soil fertility. Depleting feedstock/fossil fuels (energy crisis) and increasing cost of fertilizers. This is becoming unaffordable by small and marginal farmers, depleting soil fertility due to widening gap between nutrient removal and supplies, growing concern about environmental hazards, increasing threat to sustainable agriculture. Besides above facts, the long term use of biofertilizers is economical, eco-friendly, more efficient, productive and accessible to marginal and small farmers over chemical fertilizers.

Materials and Methods

The experiment was carried out during Rabi 2010 in 2.0 m^2 plot size at pot culture house of the department of soil science and agricultural chemistry, C.S.A.U.A.&T., situated in sub tropical and semi arid zone falling between latitude of 25^0 28' to 26^0 58' North and longitude of 79^0 31' to 80^0 34' east with an elevation of 129 metres from the sea mean level.

Characteristics of soil of experimental field: The samples of soil were collected before starting of the experiment from different places of experimental site after removing the undesirable materials *i.e.* glass pieces, stone, plant roots etc. A composite sample of about

Table-1: Physio-chemical characteristics of soil sample

Particulars	Value	Mechanical analysis	Value
pH (1:2)	8.0	Coarse sand (%)	1.45
EC (1:2) (dSm^{-1} at 25^0C)	2.5	Fine sand (%)	57.10
CEC (Cmols kg^{-1} soil)	7.0	Silt (%)	23.50
Organic carbon (%)	0.44	Clay (%)	18.0
Available N (kg ha^{-1})	40.50	Texture	Sandy loam
Available P_2O_5 (kg ha^{-1})	18.20		
Available K_2O (kg ha^{-1})	128.00		

500 g was prepared. The soil sample was analysed for physio-chemical characteristics. The results are reported in the table-1.

Five treatments were tested in randomized block design with three replications. The slurry prepared from microbial cultures (*Rhizobium*, PSB and VAM) was mixed with seeds homogenously and then dried in shade, and thereafter were sown. The cultivar 'LL 699' was shown on 22 November 2008 in rows 22.5 cm apart using a seed rate of 35 kg/ha. Weeds were managed manually by hand weeding at 30 days after sowing (DAS) and 60 DAS. No infestation of any insect pests or disease was observed and therefore no chemicals were sprayed. The phosphorus was applied through Single super phosphate (SSP) as per treatment at the time of sowing. Nitrogen was applied as basal dressing @ 15 kg per ha. Numbers of root nodules were recorded at 30, 60 and 90 days after sowing. The crop was harvested at maturity and threshed out. The treatment wise grain and straw yields were recorded separately.

Soil Analysis: In the Mechanical analysis, the International pipette method as out lined by Piper (1966), Electrical conductivity meter as described by Jackson (1967). pH was measured in 1:2 soil water suspension by Digital pH meter. Cation Exchange capacity was determined accordingly to the method out lined by Piper (1966).

Chemical Analysis: The organic carbon was determined by Walkley and Black's rapid titration method as described by Jackson (1967). Available nitrogen content in soil samples was estimated by alkaline permanganate method as described by Subbiah and Asija (1956). The seeds and plant samples were finely ground with stainless steel grinding machine .The well ground sample digested in diacid mixture of KNO_3 and $HClO_4$ (4:3), Olsen *et al.* (1954).

Statistical Methods: The statistical procedure was adopted as described by Snedcor and Cochron (1994).

Results and Discussion

Nitrogen content in grain and in straw (%): The result showed that inoculation of rhizobium, PSB and VAM either alone or in combination increased significantly the nitrogen content as compared to uninoculation (control). Maximum nitrogen content of 3.88% was found of T5 fallowed by 3.80, 3.85, 3.84 and 3.75% in T3, T2, T4, and T1 respectively. Least content was found in uninoculated micro plat whereas maximum nitrogen content was noticed in Rhizobium + PSB + VAM treated field. Nitrogen uptake, which is a calculated product of nitrogen content and yield also significantly increased by microbial inoculants. The results obtained agree with the work done by Shah *et al.* (2000). A perusal of the data revealed that the nitrogen content in straw also significantly increased due to bio inoculants over control. The result showed that inoculation of rhizobium, PSB and VAM either alone or in combination increased significantly the nitrogen content as compared to uninoculation (control). Maximum nitrogen content of 0.98% was found of T5 and T3 fallowed by 0.97, 0.96 and 0.94 % in T4, T2, and T1 respectively. Least content was found in uninoculated micro plat whereas maximum nitrogen content was noticed in Rhizobium + PSB + VAM treated field. The results obtained agree with the work done by Shah *et al.* (2000) and Singh *et al.* (2005).

Phosphorus content in grain and in straw (%): The result showed that inoculation of rhizobium, PSB and VAM either alone or in combination increased significantly the Phosphorus content as compared to uninoculation (control). Maximum Phosphorus content of 0.69% was found of T5 and T3 fallowed by 0.68, 0.66 and 0.65% in T4, T2, and T1 respectively. Least Phosphorus content was found in uninoculated micro plat whereas maximum Phosphorus content was noticed in Rhizobium + PSB + VAM treated field. Phosphorus uptake both in grain and straw was significantly affected by bioinoculants and phosphorus application in case of grain. The result showed that inoculation of rhizobium, PSB and VAM either alone or in combination increased significantly the Phosphorus in straw content as compared to uninoculation (control). Maximum Phosphorus content of 0.269% was found of T5 fallowed by 0.268, 0.265, 0.263 and 0.260% in T3, T4, T2, and T1 respectively. Least Phosphorus content was found in uninoculated micro plat whereas maximum Phosphorus content was noticed in Rhizobium + PSB + VAM treated field. The higher uptake in view of higher grain and straw yield as the result of microbial inoculation is well explained (Shah *et al.,* 2000).

Nitrogen uptake in grain and in straw (g/micro plot): The result showed that inoculation of rhizobium, PSB and VAM either alone or in combination increased significantly the Nitrogen uptake

Table-2: Effect of Rhizobium, PSB and VAM on N P and Protein of lentil crop

Treatment	N_2 content in grain (%)	N_2 content in straw (%)	P content in grain (%)	P content in straw (%)	N_2 uptake in grain (g)	N_2 uptake in straw (g)	P uptake in grain (g)	P uptake inStraw (g)	Protein in grain (%)	Protein in straw (%)
Uninoculated	3.75	0.94	0.65	0.260	60.75	17.67	10.53	4.88	23.43	5.87
Rhizobium	3.85	0.96	0.66	0.263	71.22	19.39	12.21	5.31	24.06	6.00
PSB+Rhizobium	3.84	0.98	0.69	0.268	82.17	23.42	14.76	6.40	24.00	6.12
VAM+Rhizobium	3.80	0.97	0.68	0.265	74.49	20.75	13.32	5.67	23.75	6.06
Rhizobium+PSB+VAM	3.88	0.98	0.69	0.269	92.3	26.67	16.42	7.04	24.25	6.12
Mean	3.82	0.96	0.67	0.265	76.195	21.38	13.45	5.96	23.89	6.03
SE (D)	0.013	0.006	0.004	0.00163	1.146	0.363	0.30	0.10	0.038	0.012
CD	0.030	0.01	0.008	0.0030	2.32	0.735	0.62	0.22	0.077	0.024

in grain (g/micro plot) in straw content as compared to uninoculation (control). Maximum Nitrogen uptake in grain (g/micro plot) content of 92.3 g / plot was found of T5 fallowed by 82.17, 74.49, 71.22and 60.75 g / plot in T3, T4, T2, and T1 respectively. Least Nitrogen uptake in grain (g/micro plot) content was found in uninoculated micro plat whereas maximum Nitrogen uptake in grain (g/micro plot) content was noticed in Rhizobium + PSB + VAM treated field. The result showed that inoculation of rhizobium, PSB and VAM either alone or in combination increased significantly the Nitrogen uptake in straw (g/micro plot) in straw content as compared to uninoculation (control). Maximum Nitrogen uptake in straw (g/micro plot) content of 26.67 g / plot was found of T5 fallowed by 23.42, 20.75, 19.39 and 17.67 g / plot in T3, T4, T2, and T1 respectively. Least Nitrogen uptake in straw (g/micro plot) content was found in uninoculated micro plat whereas maximum Nitrogen uptake in straw (g/micro plot) content was noticed in Rhizobium + PSB + VAM treated field. The results obtained agree with the work done by Sharma *et al.* (1989) and Singh *et al.* (2007).

Phosphorus uptake in grain and straw (g/micro plot): The result showed that inoculation of rhizobium, PSB and VAM either alone or in combination increased significantly the Phosphorus uptake in grain (g/micro plot) in straw content as compared to uninoculation (control). Maximum Phosphorus uptake in grain (g/micro plot) content of 16.42 g / plot was found of T5 fallowed by 14.76, 13.32, 12.21and 10.53 g / plot in T3, T4, T2, and T1 respectively. Least Phosphorus uptake in grain (g/micro plot) content was found in uninoculated micro plat whereas maximum Phosphorus uptake in grain (g/micro plot) content was noticed in Rhizobium + PSB + VAM treated field. The result showed that inoculation of rhizobium, PSB and VAM either alone or in combination increased significantly the Phosphorus uptake in straw (g/micro plot) in straw content as compared to uninoculation (control). Maximum Phosphorus uptake in straw (g/micro plot) content of 7.04 g / plot was found of T5 fallowed by 6.40, 5.67, 5.31and 4.88% g / plot in T3, T4, T2, and T1 respectively. Least Phosphorus uptake in straw (g/micro plot) content was found in uninoculated micro plat whereas maximum Phosphorus uptake in straw (g/micro plot) content was noticed in Rhizobium + PSB + VAM treated field. This result was agreed with the work done by Singh *et al*. (2005) and Sharma *et al.* (1989).

Protein content in grain and straw (%): The result showed that inoculation of rhizobium, PSB and VAM either alone or in combination increased significantly the Protein content in grain content as compared to uninoculation (control). Maximum Protein content in grain content of 24.25% was found of T5 fallowed by 24.06, 24.00, 23.75and 23.43% in T2, T3, T4, and T1 respectively. Least Protein content in grain content was found in uninoculated micro plat whereas maximum Protein content in grain content was noticed in Rhizobium + PSB + VAM treated field. There was significant increase in protein content in grain and straw due to use of microbial inoculants. The result showed that inoculation of rhizobium, PSB and VAM either alone or in combination increased significantly the Protein percent content in straw content as compared to uninoculation (control). Maximum Protein percent content in straw content of 6.12% was found of T5 and T3 fallowed by 6.06, 6.06 and 5.87% in T4, T2 and T1 respectively. Least Protein percent content in straw content was found in uninoculated micro plat whereas maximum Protein percent content in straw content was noticed in Rhizobium + PSB + VAM treated field. The results obtained agree with the work done by Sharma *et al.* (1989) and Singh *et al*. (2007).

The result of this study revealed that the least nitrogen content in grain and straw was found in uninoculated micro plat (T1) whereas maximum nitrogen content was noticed in Rhizobium + PSB + VAM treated field (T5). Lowest phosphorous content in grain and straw was found in uninoculated micro plat (T1) whereas maximum nitrogen content was noticed in Rhizobium + PSB + VAM treated field (T5). Least nitrogen uptake in grain and straw was found in uninoculated micro plat (T1) whereas maximum nitrogen uptake was noticed in Rhizobium + PSB + VAM treated field (T5). The lowest phosphorus uptake in grain and straw was found in uninoculated micro plat (T1) whereas maximum phosphorus uptake was noticed in Rhizobium + PSB + VAM treated field (T5). Least protein content in grain and straw was found in uninoculated micro plat (T1) whereas maximum protein content was noticed in Rhizobium + PSB + VAM treated field (T5). Therefore it was recommended that the interactive use of Rhizobium , PSB and VAM in the field was more efficient, productive and accessible to marginal and small farmers.

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