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Effect of seed inoculation with liquid and carrier based *Rhizobium* cultures and phosphorus levels on rhizobia population and yield of soybean (*Glycine max*)

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ABSTRACT

The present investigation was carried out at J.N.K.V.V. Jabalpur during kharif season of 2015 to evaluate the effect of Rhizobium cultures with supplementation of inorganic phosphorus (Pi) on growth, yield of soybean (Glycine max), rhizobial population and uptake of plant nutrients in a medium black soil. The experiment was laid out in randomized block design with 3 replications. Tteatments comprised of 12 treatments including two type soybean rhizobial cultures (liquid formulation and carrier based) in combination with three levels of Pi (@ 40, 80 and 120 kg P_2O_5 ha^{-1}) and one control (unfertilized + uninoculated). The results revealed that the growth characters increased after 45 days of sowing and attained the maximum values at harvest. The rhizobial population, irrespective of treatments, was maximum at 45 days after sowing followed by a reduction at harvest. The treatment P_{80}+LRh (80 kg P_2O_5 + liquid inoculums of Rhizobium) enhanced the plant height (45.7cm/plant), total chlorophyll content (3.05 mg g^{-1} leaf), plant biomass (fresh and dry weight 44.7 and 8.7g/plant), over the control (33.2 7cm/plant, 2.02 mg g^{-1} leaf, 30.17 and 5.16 g/plant, respectively) at 45 days after sowing. The maximum nitrogen (226.6 kg ha^{-1}) and phosphorus (20.1 kg ha^{-1}), uptake was noticed with 120 kg P_2O_5 + LRh and minimum in control. The same treatment also increased seed and stover yields of soybean by 75.4 and 88.8% over the control (1025 and 2206 kg ha^{-1}), respectively. Population of soybean rhizobia in rhizospheric soil was also found more with 80 kg P_2O_5 + LRh by 1.69, 1.55 and 1.57 log folds, respectively over the control (3.297 at 21, 5.499 at 45 DAS and 4.619 log cfu g^{-1} soil at harvest). While the treatment P_{120}+LRh exhibited numerically higher values of rhizobia population but was statistically at par to P_{80}+LRh.

Keyword: Soybean, carrier and liquid-based *Rhizobium* cultures, nodulation cfu counts and yield

INTRODUCTION

Soybean (*Glycine max* L.), a leguminous crop, is one of the most important and extensively grown crop. Since soybean is rich in oils (17-20%) and proteins (38-42%), it is suitable as food and feed for human and animals. Hence, the crop of soybean is one of the most economic legume crops of the world (Ibrahim and Kandil, 2007). In fact, soybean is estimated to fix 80% of its nitrogen (N) needs (Smaling *et al.,* 2008) from atmosphere. The fixation of N by soybean as much as 300 kg of N ha^{-1} in addition to the release in the soil of 20-30 kg N ha^{-1} for the following crop had been estimated (Hungria *et al.,* 2006). To improve soybean yield, biological N_2 fixation, contribution to soil fertility restoration, inoculation with efficient strains of Bradyrhizobia has already been tested in several countries (Tairo and Ndakidemi, 2014). Apart of N, phosphorus (P) is the second major plant growth-limiting nutrients in most agriculture soils. It plays an important role in the plant's energy transfer system since its deficiency retards growth (Shahid *et al.,* 2009). Symbiotic N_2 fixation needed high P as large amounts of energy being consumed during the process of photosynthesis and nodule development, production of protein, phospholipids and phytin in grains legume (Rahman *et al.*, 2008). Inadequate P restricts root growth, the process of photosynthesis, translocation of sugars and other such functions which directly influence N fixation by legume plants. Phosphorus supplementation can enhance plant growth by increasing the efficiency of biological N_2 fixation, enhancing the availability of other macronutrients in legumes (Makoi *et al.*, 2013). Hence, it can be attributed to have a positive interaction between Rhizobia inoculation and P supply (Akpalu *et al.,* 2014 and Tairo and Ndakidemi, 2014).

Liquid formulation biofertilizer is the promising and updated technology over the conventional carrier based production technology which has many advantages surmounting the hurdles over the later. The liquid inoculants developed were known to have

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population of *Rhizobium* sp., *Azotobacter* sp., *Azospirillum* sp. and PSB up to the levels of 10^8 cells ml^{-1} (Velineni *et al.,* 2011). Keeping the above facts in view, the present investigation was carried out to study the effect of rhizobial cultures and phosphorus levels on yield of soybean and rhizobial population.

MATERIALS AND METHODS

Plant materials

Both the types of inoculants of *Rhizobium*, the isolate R_{33} were obtained from the project AINP on Soil Biodiversity & Biofertilizers (ICAR), JNKVV, Jabalpur. All the technical efforts were endeavoured to maintain the soybean-rhizobial population up to the standard 10^8 to 10^{-9} cfu g^{-1} or ml for both liquid and carrier inoculants. The liquid inoculant was directly used for the experiment, whereas for carrier based inoculants lignite was used. 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 superphosphate and muriate of potash, respectively. Nitrogen and Kwere supplemented as basal applications to each plot as per recommendation and P was applied as per scheduled dose of treatments.

Seed inoculation, treatment and sowing

Soybean seeds in polythene bags were slightly moistened and then treated with carbendazim fungicide @ 2 g kg^{-1} seed. Seeds were allowed to air dry under shade. Then the seeds were inoculated individually with the bioinoculant *Rhizobium* liquid and carrier based cultures at double the recommended dose 20 ml or g kg^{-1} of seed, respectively using sterilized gum acacia (2%) as adhesive. The field experiment was carried out at research farm JNKVV Jabalpur during kharif season of 2015. The seeds of soybean (cv.JS 97-52) were sown in the respective plot @ 60 kg ha^{-1}. Recommended package of practices was followed to maintain plant population, protection and growth.Three plants from each plot were taken to measure plant heights at 30, 45 DAS and at harvest. Total leaf chlorophyll was estimated by acetone extraction method (Yoshida *et al.*, 1972) using the equation. Total chlorophyll (mg g^{-1}) = CH (a) + CH (b). At 45 DAS, the root portion of three plants was cut off and fresh plant biomass was recorded in g $plant^{-1}$, then plants was dried in hot air oven at 60 ^{0}C for 3 - 4 days (till constant weight) to record the dried plant biomass in g $plant^{-1}$. The crop was harvested plot wise and yields of seed and stover were recorded. Nitrogen contents was determined by Kjeldahl method and phosphorus was determined in digest (HNO_3: $HClO_4$) by vanadomolybdate yellow colour method (Jackon 1973). Uptake of the nutrients was calculated by multiplying yield data with concentration of nutrients.

Soybean rhizobia population counts in soil sample

Samples of rhizospheric soil were used as fresh as possible without grinding, sieving or any modifications. The collected samples in low density polyethylene bags could be stored in refrigerator at 4^{o}C for further study. Estimation of soybean rhizobia in rhizosheric soils periodically as influenced by the different treatments was carried out using 10 fold serial dilution method (10^{-1} to 10^{-9}). The microbial growth was obtained on YEMA media (Mannitol 10 g, K_2HPO_4 0.5 g, $MgSO_4.7H_2O$ 0.2 g, NaCl 0.1 g, $CaCO_3$ 1.0 g, Yeast Extract 1.0 g, Congo red (1:400) 2 ml, Agar-agar 15-18 g and distilled water 1000 ml) in Petri plates.

RESULTS AND DISCUSSION

Plant height

Response of the treatments (except P_{40}+UI, UF+LRh, UF+CRh and P_{40}+CRh) varied significantly over the control of unfertilized + uninoculated (UFUI). The plants attained nominal at 30 days after sowing. Thereafter, there was a steady rise in their character, which continued till harvest. Among all the treatments, P_{120}+LRh treatment increased the plant height at the maximum by 54.7%, at 30 DAS and P_{80}+LRh 39.0 and 40.6%, at 45 DAS and at harvest over the control (19.0, 33.2 and 53.7 cm, respectively). But the effect of P_{120}+LRh was at par to that of P_{80}+LRh. Results proved that *Rhizobium* inoculation with increased phosphorus levels had positive effects on plant height. The increased plant height in the inoculated plots might be due to symbiotic relationship between legume roots and *Rhizobium* inoculation supplemented with Pi (Tairo and Ndakidemi, 2013 and Singh and Singh, 2017).

Table 1: Effect of liquid formulation and carrier based *Rhizobium* inoculants and different levels of phosphorus on plant height, chlorophyll content and plant biomass of soybean

Treatment	Plant height (cm plant^{-1})			Total chlorophyll content (mg g^{-1} leaf)			Plant biomass at 45 DAS (g plant^{-1})	
	30 DAS	45 DAS	At harvest	15 DAS	30 DAS	45 DAS	Fresh weight	Dry weight
UFUI	19.0	33.2	53.7	1.27	2.05	2.58	30.17	5.16
P_{40}+UI	20.5	34.0	54.7	1.30	2.11	2.64	31.45	5.40
P_{80}+UI	23.8	37.1	65.7	1.65	2.57	3.30	37.02	7.57
P_{120}+UI	24.9	38.8	65.0	1.68	2.54	3.32	37.25	7.73
UF+LRh	20.4	36.2	60.2	1.56	2.55	3.17	38.57	7.03
P_{40}+LRh	24.2	39.9	65.3	1.59	2.76	3.35	38.60	7.19
P_{80}+LRh	29.2	45.7	76.6	2.06	3.05	3.97	44.70	8.86
P_{120}+LRh	29.4	44.3	76.3	1.94	3.02	3.99	44.32	9.63
UF+CRh	21.3	35.8	57.9	1.52	2.47	3.13	36.86	6.26
P_{40}+CRh	23.1	38.1	58.1	1.53	2.74	3.20	36.99	6.31
P_{80}+CRh	28.5	41.2	69.4	1.91	2.94	3.77	42.63	8.19
P_{120}+CRh	28.3	43.2	70.7	1.85	2.96	3.84	43.27	8.82
$SE_m\pm$	1.45	1.74	3.55	0.13	0.16	0.22	1.94	0.62
$CD_{5\%}$	4.16	4.98	10.19	0.37	0.45	0.63	5.58	1.78

Chlorophyll content

The chlorophyll content increased up to 45 days after sowing followed by reduction at maturity. The treatment P_{80}+LRh, increased leaf chlorophyll content (Table 1) by 62.2 and 48.8% at 15 and 30 DAS and P_{120}+LRh 54.7 % at 45 DAS, over the control (1.27, 2.05 and 2.58 mg g^{-1} leaf). However, the increment with P_{80}+LRh was at par to that of P_{120}+LRh. The increase might be ascribed to phosphorus as it stimulates the plant to manufacture its own food through photosynthesis process including energy transfer. The probable reason might be ascribed to phosphorus as it is the major component of the leaf chlorophyll, which stimulates the plant to manufacture its own food through photosynthesis process; Phosphorus plays a very important function in almost every plant process that involves energy transfer. High-energy phosphate, detained as a part of the chemical structures of adenosine diphosphate (ADP) and adenosine triphosphate (ATP), is the source of energy that drives the huge number of chemical reactions within the plant. The most important chemical reaction in nature is photosynthesis. It utilizes light energy in the presence of chlorophyll to combine carbon dioxide and water into simple sugars, with the energy being captured in ATP. The result goes in line with Tairo and Ndakidemi (2013) who reported that phosphorus had significantly increased the leaf chlorophyll content of soybean when inoculated with *B. japonicum*.

Biomass (fresh and dry weight)

The fresh and dry weight of plant ranged from 30.2 to 44.7 and 5.2 to 9.6 g plant^{-1} with the mean value of 38.4 and 7.4 g plant^{-1}, respectively (Table 1). It was apparent from the data that all the treatments (except P_{40}+UI) significantly increased fresh weight over UFUI. Among all the treatments, P_{80}+LRh increased the fresh weight by 48.2 % and P_{120}+LRh increased the dry weight by 84.6%, respectively over UFUI (30.17 and 5.16 g^{-1} plant). However, the response of P_{80}+LRh was at par to that of P_{120}+LRh. These results are in the line of findings reported by Tahir *et al.* (2009) and Chauhan and Raghav (2017).

***Rhizobium* population**

Rhizobium population increased up to 45 days after sowing. Thereafter, rhizobial population declined at harvest. The *Rhizobium* population in rhizospheric soil at 21 DAS varied from 3.297 log cfu (1.98×10^3 cfu g^{-1} soil) to 5.646 log cfu (4.42×10^5 cfu g^{-1} soil) with the mean value of 4.541 log cfu (3.49×10^4 cfu g^{-1} soil). The effects of all the treatments varied significantly in respect of *Rhizobium* population. Among all the treatments, P_{120}+LRh responded maximum by 1.71 log fold increase followed by P_{80}+LRh, P_{120}+CRh, P_{80}+CRh, P_{40}+LRh, and P_{40}+CRh by 1.69, 1.65, 1.63, 1.62, and 1.34 log fold increase over UFUI (3.297 log cfu =1.98×10^3 cfu g^{-1} soil).

Table 2: Effect of liquid formulation and carrier based *Rhizobium* inoculants and different levels of phosphorus on *Rhizobium* population in soil

Treatment	Population (cfu g^{-1} soil)		
	21 DAS	45 DAS	At harvest
UFUI	3.297* (1.98×10^3)	5.499 (3.15×10^5)	4.619 (4.15×10^4)
P_{40}+UI	3.364(2.31×10^3)	5.540 (3.39×10^5)	4.629 (4.25×10^4)
P_{80}+UI	3.741(5.51×10^3)	5.578 (3.78×10^5)	4.639 (4.35×10^4)
P_{120}+UI	3.794(6.21×10^3)	5.624 (4.20×10^5)	4.698 (4.98×10^4)
UF+LRh	4.221(1.66×10^4)	6.578 (3.78×10^6)	5.672 (6.69×10^5)
P_{40}+LRh	5.372 (2.40×10^5)	7.529 (3.38×10^7)	6.402 (2.52×10^6)
P_{80}+LRh	5.578 (3.78×10^5)	8.578 (3.78×10^8)	7.297 (1.98×10^7)
P_{120}+LRh	5.646 (4.42×10^5)	8.619 (4.15×10^8)	7.407 (2.55×10^7)
UF+CRh	4.192 (1.56×10^4)	6.379 (2.39×10^6)	5.579 (3.79×10^5)
P_{40}+CRh	4.445 (2.78×10^4)	6.642 (4.38×10^6)	5.762 (5.78×10^5)
P_{80}+CRh	5.384 (2.42×10^5)	7.652 (4.48×10^7)	6.639 (4.35×10^6)
P_{120}+CRh	5.458 (2.87×10^5)	7.698 (4.98×10^7)	6.680 (4.78×10^6)
$SE_m\pm$	0.368	0.492	0.458
$CD_{5\%}$	1.056	1.414	1.314

The *Rhizobium* population in rhizospheric soil at 45 DAS ranged from 5.499 log cfu (3.15×10^5 cfu g^{-1} soil) to 8.619 log cfu (4.15×10^8 cfu g^{-1} soil) with the mean value of 6.826 log cfu (6.68×10^6 cfu g^{-1} soil). The maximum population of 1.56 log fold was recorded with P_{120} + LRh, followed by P_{80}+LRh, P_{120}+CRh, P_{80}+CRh, and P_{40}+LRh by 1.55, 1.39, 1.38 and1.36 log fold, respectively over the control (5.499 log cfu = 3.15×10^5 cfu g^{-1} soil).At harvest, the population of *Rhizobium* in rhizospheric soil ranged from 4.619 log cfu (4.15×10^4 cfu g^{-1} soil) to 7.407 log cfu (2.55×10^7 cfu/g soil) with the mean value of 5.835 log cfu (2.41×10^5 cfu g^{-1} soil). The treatment P_{120}+LRh increased the population by 1.60 log fold, followed by P_{80}+LRh, P_{120}+CRh, P_{80}+CRh, and P_{40}+LRh by 1.57, 1.44, 1.43 and 1.38 log fold, respectively over UFUI (4.619 log cfu = 4.15×10^4 cfu g^{-1}). This can be inferred that healthy bio-inoculants with sound bacterial population of *Rhizobium* nurtured at the most optimum conditions induce further multiplication of the isolate under the pedological condition.

Table 3: Effect of liquid formulation and carrier based *Rhizobium* inoculants and levels of phosphorus on N and P uptake ((kg ha^{-1}) by seed, stover of soybean

Treatment	Nitrogen		Phosphorus		Total uptake	
	Seed	Stover	Seed	Stover	N	P
UFUI	55.4	34.8	3.1	4.3	90.2	7.4
P_{40}+UI	59.7	35.1	3.3	4.6	94.8	8.0
P_{80}+UI	89.1	63.7	5.4	7.3	152.8	12.7
P_{120}+UI	92.7	67.7	5.6	7.7	160.4	13.3
UF+LRh	73.6	55.9	4.8	6.2	129.5	10.9
P_{40}+LRh	80.6	59.9	5.3	6.6	140.6	11.9
P_{80}+LRh	114.7	104.9	7.7	11.4	219.7	19.1
P_{120}+LRh	119.5	107.1	8.1	11.9	226.6	20.1
UF+CRh	70.5	52.8	4.3	5.8	123.4	10.1
P_{40}+CRh	73.4	57.1	4.6	6.2	130.5	10.8
P_{80}+CRh	108.9	91.4	6.9	9.3	200.2	16.2
P_{120}+CRh	111.2	94.5	7.4	10.1	205.7	17.5
$SE_m\pm$	7.20	6.51	0.55	0.80	9.63	0.90
$CD_{5\%}$	20.68	9.20	1.57	2.31	27.64	2.58

Uptake of nutrients

The total N uptake by the crop varied from 90.0 to 226.6 kg N ha^{-1}. Most of the treatments increased significantly the total N uptake by the crop (except P_{40}+UI) over the control. The treatment P_{120}+LRh responded the best by 151.2% increase, followed by P_{80}+LRh,

P_{120}+CRh, P_{80}+CRh, P_{120}+UI, P_{80}+UI, P_{40}+LRh, P_{40}+CRh, UF+LRh and UF+CRh by 143.6, 128.0, 122.0, 77.8, 69.4, 55.9, 44.7, 43.6 and 36.8% increase, respectively over the control (90.2 kg ha^{-1}). The total P uptake by the crop ranged from 7.4 to 20.1 kg ha^{-1} and maximum values were noted with 120 kg P_2O_5+LRh treatment. On the other hand, maximum values of P uptake by soybean crop were recorded with control treatment. The treatment P120 +LRh increased the uptake by 171.6% over control. Similar increase in P uptake by 158.1% was noted with P80 +LRh treatment. Solaiman and Hossain (2006) studied the effectiveness of *Bradyrhizobium(B) japonicum* strains on soybean and had a significant positive effect on N content in shoot, N uptake by shoot by seed with inoculation by *B. japonucum* or the mixed culture of strains *B. japonucum.*

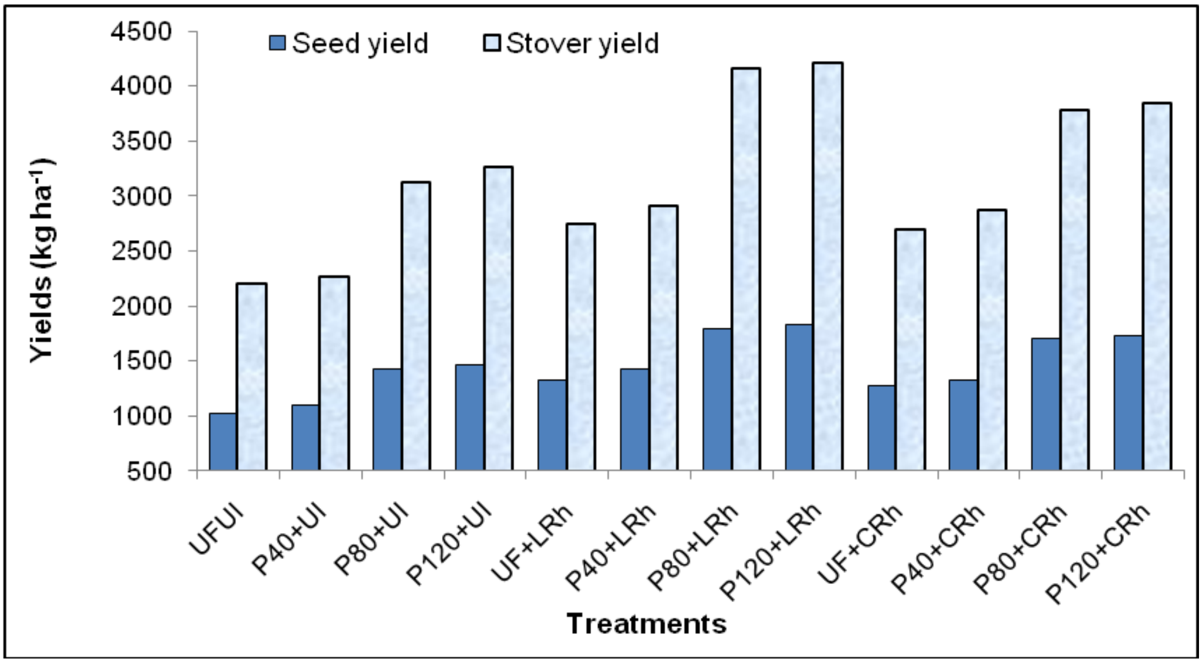

Fig. 1: Effect of liquid formulation and carrier based *Rhizobium* inoculants and different levels of phosphorus on soybean yields

Yield

The effects of liquid and carrier based *Rhizobium* inoculants supplemented with different levels of P had beneficial effect on seed and stover yields of soybean over the UFUI (Fig.1). The maximum seed yield was recorded with P_{120}+LRh and P_{80}+LRh which increased the seed yield by 78.5 and 75.4%, respectively over the control (1025 kg ha^{-1}). But, the results were mutually at par. Likewise, with P_{120}+CRh and P_{120}+CRh the increase was 69.0 and 66.9%, respectively. The relatively better response was recorded from the treatment of P_{80}+LRh by 5.1% over P_{80}+CRh (1711 kg ha^{-1}). Similar to seed yield, the higher stover yield was recorded with P_{120}+LRh and P_{80}+LRh which increased the yield by 91.0 and 88.8%, respectively over the control (2206 kg ha^{-1}) and the respective results were mutually at par. Similar was the case with P_{120}+CRh and P_{120}+CRh (by 74.0 and 71.1% increase, respectively) over the control. The relative performance of P_{80}+LRh was 10.3% more over P_{80}+CRh (3775 kg ha^{-1}). Increase in yields with liquid inoculums of *Rhizobium* with inorganic P fertilizer might be attributed to better nodulation, N_2 fixation and crop growth as against uninoculated control (Brahmaprakash *et al.,* 2004 and Gupta, 2005).

On the basis of findings, it may be concluded that the inoculation with *Rhizobium* cultures strains and phosphorus supply improved productivity of soybean. The use of these effective strains of *Rhizobium* and phosphorus supplementation could be an effective way to enhance the plant height, chlorophyll content, plant biomass, N, P, uptake by crop, yield and population of *Rhizobium* in rhizospheric soil of soybean.

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