Int.J.Curr.Microbiol.App.Sci (2018) *7*(6): 1807-1814

EXCELLENT PUBLISHERS

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 7 Number 06 (2018)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2018.706.215

Effect of Soybean (*Glycine max*) Seed Inoculation with Liquid and Carrier Based *Rhizobium* cultures and Phosphorus Levels on Productivity and Physico-Chemical Properties of Soil

Shish Ram Jakhar[1], Vinod Kumar[1], N. G. Mitra[1] and Ompal Singh[2*]

[1]*Department of Soil Science and Agricultural Chemistry, J.N. Krishi Vishwa Vidyalaya, Jabalpur - 482 004 (M.P.), India*
[2]*Department of Plant Physiology, J.N. Krishi Vishwa Vidyalaya, Jabalpur - 482 004 (M.P.), India*

**Corresponding author*

Keywords

Soybean, carrier and liquid-based *Rhizobium* cultures, nodulation and yield

Article Info

Accepted:
18 May 2018
Available Online:
10 June 2018

A B S T R A C T

A field experiment was conducted at JNKVV, Jabalpur during kharif 2015, to evaluate the effect of *Rhizobium* cultures with supplementation of inorganic phosphorus (Pi) onthe performance of soybean and physico-chemical properties of soil in a Vertisol soil. The experiment was laid out in Randomized Block Design with three replications 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 result reveled that the nodulation higher at 45 days after sowing (DAS) and followed by a reduction at maturity. Treatment P_{80}+LRh (80 kg P_2O_5 + liquid inoculum of *Rhizobium*) for enhanced the nodulation attributes, over the control at 45 DAS. The treatment P_{80}+LRh gave maximum nodules number (81.1%), weight (89.1% fresh and 78.5% dry) and N content (64.4%) over control (6.9 number/plant, 103 mg/plant, 65 mg/plant and 2.03%), respectively. Similar treatment increased available N andPin soil at harvest by 42.8 and 92.8%, respectively over control (189 and 14 kg/ha, respectively) and statistically no difference was notice in case of K and it was maximum by P_{80}+LRh (295 kg/ha). The treatment P_{80}+LRh also increased seed and stover yields of soybeanby 75.4 and 88.8% over the control (1025 and 2206 kg/ha), respectively. While the treatment P_{120}+LRh exhibited numerically higher values of P_{80}+LRh but statistically at par to P_{80}+LRh.

Introduction

Soybean (*Glycine max*), is an important legume crop in the world in terms of total production and international trade (Simmond *et al.*, 1999). Soybean contains about 20% oil on dry matter basis with 30-50% of protein (Kwarteng and Towler, 1994). It also has superior amino acid profile and its protein has great potential as a major source of dietary protein and can play an important role to solve malnutrition problems (Ruhul *et al.*, 2009).

In fact, soybean is estimated to fix 80% of its nitrogen (N) needs (Smaling *et al.*, 2008) from atmosphere. The fixation of 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).

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 (Tairo and Ndakidemi, 2014). There were several reports on the interaction between Rhizobia inoculation and P supply. Akpalu *et al.*, (2014) had reported that combination of beneficial bacteria of soil and phosphorus in legume plants significantly increased nodulation, pod formation and development, and a subsequent grain yield comparatively to the single use of phosphorus or beneficial bacteria.

The inoculation with *Rhizobium* and phosphorus supplementation improved the macronutrient uptake (N, Pand K) in different organs of the whole plant of soybean (Tairo and Ndakidemi, 2014). Highly effective and competitive *Rhizobium* strains and a supply of appropriate amount of phosphorus (Scherer *et al.*, 2008) could markedly increase legume growth and N fixation.

Phosphorus (P) and N play specific role in symbiotic N_2-fixation through their effects on nodulation and N_2-fixation process (O' Hara *et al.*, 2002). N_2-fixation is very sensitive to P deficiency due to reduce nodule mass and decreased ureide production. Symbiotic N-fixation has a high P demand because the process consumes large amounts of energy and energy generating metabolism strongly depends upon the availability of P (Plaxton, 2004).

Materials and Methods

A field experiment was conducted at research field of the Department of Soil Science & Agricultural Chemistry, JNKVV, on soybean and were inoculated with both the types of *Rhizobium* inoculants (liquid formulation and carrier based), the isolate R_{33}wereobtained from the project AINP on Soil Biodiversity & Biofertilizers (ICAR), JNKVV, Jabalpur. All the technical efforts were endeavored to maintain the soybean-rhizobial population to up the standard 10^8 to 10^{-9} cfug^{-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 super phosphate (SSP) and muriate of potash (MOP). Urea and MOP were supplemented as basal applications to each plot as per recommendation and SSP was applied as per scheduled dose of treatments.

Seed Inoculation 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 dose20 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 of2015.The seeds were sown in the respective plot @ 60 kg ha^{-1}. Recommended package of practices was followed to maintain plant population, protection and growth.

Nodulation

From each replication were carrying out nodulation with three plants per plot. Nodulation studies were done at 21 and 45 DAS by irrigating the plots and uprooting plants carefully taking care to avoid any losses or damage of nodules. The rhizosphere soil was washed in the running tap water. After proper washing, nodules per plant were counted manually. After counting, the nodules were detached from the roots and its fresh weight was recorded in number of nodules/plant. These nodules were kept in small paper bags

The nodules were oven dried in hot air oven at 60 ^{0}C for 3-4 days (till constant weight) to record their oven dried weight (mg/plant).The estimation of nitrogen in nodules was performed as done with the plant samples. Contents of N in nodule were analyzed on dry weight basis as per standard procedures (Amma, 1989).

Soil Properties

The surface (0-15 cm) soil samples were collected from the experimental site before sowing of soybean crop and after harvest. The soil samples were air dried and crushed with wooden pestle and mortar and sieved through 2 mm sieve. The material passed through the sieve was used for determination of various characters. Soil pH was determined in 1:2.5 soils-water suspensions (Piper, 1950) using Systronics pH meter. Determination of organic carbon was done by Walkley and Black's rapid titration method (1934) as described by Piper (1950).A suitable quantity of the soil was digested with chromic acid and sulphuric acid making the use of heat of dilution of sulphuric acid. Excess of chromic acid left over unreduced by the organic matter of the soil was determined by a titration with Ferrous Ammonium Sulphate solution using diphenylamine indicator. Available N in soil was determined by using alkaline permanganate method (Subbiah and Asija, 1956). A known weight of soil is mixed with excess of alkaline permanganate and distilled. The phosphorus content of soil was estimated by extraction procedure as described by Olsen *et al.,* (1954). Soil available phosphorus was extracted using 0.5 M $NaHCO_3$ (pH 8.5) and determination was done by ascorbic acid method as described by Miller and Keeney (1982). The available potassium was extracted by neutral 1N ammonium acetate and it was estimated using flame photometer (Mohr *et al.,* 1963).

Results and Discussion

Nodulation Studies

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, table 1).The treatmentP_{120}+LRhsignificantly increased the number of nodules plant^{-1}by 88.4 and 103.2% response, at 21 and 45DAS over the control (6.9 and 21.3 nodules plant^{-1}, 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 number of nodules plant^{-1}.

Fresh weight of nodules at 21 and 45 DAS among all the treatment combinations, P_{80}+LRh responded the best by 84.4 and 93.7%, over UFUI (103 and 240 mg plant^{-1}), respectively. But, the response from P_{80}+LRh was at par to that from P_{120}+LRh. Dry weight of nodules at 21 DAS increase the dry weight of nodules with the treatment combination of P_{120}+LRh by 69.2%, over UFUI (65 mg plant^{-1}). However, the response of the treatment combination of P_{120}+LRh was at par to that of P_{80}+LRh.While at 45 DAS nodule dry weight the treatment combination of P_{120}+LRh increased dry weight of nodules by 96.6%, over UFUI (120 mg plant^{-1}). However, the effect of P_{80}+LRh was statistically at par to that of P_{120}+LRh.

N content of nodules at 21 and 45 DAS the treatment combination of P_{80}+LRh, among all, increased significantly the highest N content in nodules by 77.3 and 47.5%, over UFUI (2.03 and 3.0% N) respectively. But, the performance of P_{80}+LRh was at par to that of P_{120}+LRh.

The positive results obtained might be due to are contributed by essential role of phosphorus in legumes which support early root formation and development of lateral, fibrous and healthy roots. Furthermore, phosphorus is an essential component in seed formation as it plays huge role in protein synthesis, phospholipids and phytin. These results are in agreement with the findings of (Son *et al.,* 2007) where results showed that the application of bradyrhizobia (*Bradyrhizobium japonicum)* and phosphate solubilizing bacteria (*Pseudomonas* sp.) liquid inoculants on soybean seed before sowing plus 20 kg N/ha enhanced the nodule number, fresh weight, dry weight of nodules.

Dhami and Prasad (2009) also reported that the increase in nitrogen content as well as number of nodules per plant due to the application of inoculation in combination with nitrogen and phosphorus fertilizer.

pH and Organic Carbon

Table-2 presents the data on soil pH and content of organic carbon in soil at harvest. Soil reactions (soil pH) and organic carbon were statistically unaffected due to different levels of phosphorous and *Rhizobium* inoculation. However, maximum organic carbon was recorded by treatment P_{120}+LRh (0.58%). Gattani *et al.*, (1976) reported that there was no noticeable change in soil pH due to continuous use of chemical fertilizer and biofertilizer.

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

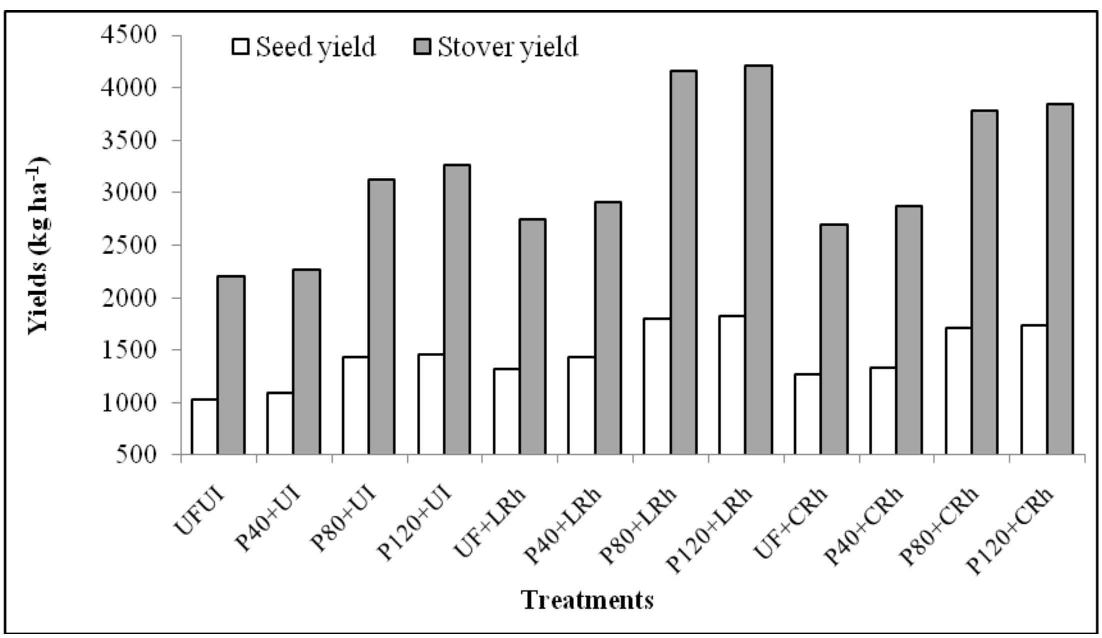

Table.1 Effect of liquid formulation and carrier based *Rhizobium* inoculants and different levels of phosphorus on nodulation attributes of soybean at different growth stages of crop

Treatments	21 DAS				45 DAS			
	Number / plant	Fresh wt. (mg/ plant)	Dry wt. (mg/ plant)	N content (%)	Number/ plant	Fresh wt. (mg/ plant)	Dry wt. (mg/ plant)	N content (%)
UFUI	6.9	103	65	2.03	21.3	240	120	3.03
P_{40}+UI	6.9	93	67	2.13	23.5	260	130	3.06
P_{80}+UI	9.3	140	90	2.60	31.7	353	183	3.68
P_{120}+UI	9.7	127	84	2.77	33.8	357	187	3.75
UF+LRh	7.5	114	81	2.80	28.4	277	120	3.45
P_{40}+LRh	9.7	123	83	3.00	33.7	350	180	3.69
P_{80}+LRh	11.3	190	107	3.60	42.3	465	231	4.47
P_{120}+LRh	13.0	185	110	3.53	43.3	450	236	4.28
UF+CRh	8.1	107	75	2.70	26.7	260	153	3.35
P_{40}+CRh	8.2	111	80	2.90	31.6	283	157	3.45
P_{80}+CRh	10.8	147	93	3.43	36.8	387	207	4.23
P_{120}+CRh	11.0	143	98	3.30	40.6	373	200	4.20
$SE_m\pm$	0.80	11.05	5.88	0.17	2.83	29.88	14.07	0.22
$CD_{5\%}$	2.29	31.72	16.89	0.49	8.13	85.77	40.38	0.64

Table.2 Effect of liquid and carrier based *Rhizobium* inoculants at different levels of phosphorus on soil pH, organic carbon and available NPK in soil after harvest of crop

Treatments	pH	OC (%)	Available NPK (kg ha^{-1})		
			N	P	K
UFUI	7.42	0.52	189	14	236
P_{40}+UI	7.35	0.52	197	16	239
P_{80}+UI	7.31	0.53	235	21	243
P_{120}+UI	7.29	0.55	231	22	245
UF+LRh	7.34	0.54	224	18	263
P_{40}+LRh	7.30	0.55	237	22	264
P_{80}+LRh	7.26	0.57	278	27	295
P_{120}+LRh	7.14	0.58	270	29	286
UF+CRh	7.22	0.53	216	16	260
P_{40}+CRh	7.23	0.54	217	19	268
P_{80}+CRh	7.13	0.55	260	23	284
P_{120}+CRh	7.17	0.56	251	25	275
SE_m±	7.32	0.02	11.47	1.50	22.33
$CD_{5\%}$	0.20	0.06	32.94	4.30	64.11

Available N, P and K

The data regarding available N, P and K content in soil (0-15 cm) after harvest of the crop are presented in table 2. Available N in soil varied from 189 to 278 kg N ha^{-1}. All the treatments (except P_{40}+CRh, UF+CRh and P_{40}+UI) significantly increased the available N content in soil over the control UFUI plot. Among all the treatments, P_{80}+LRh responded the best by 47.1% increase, followed by P_{120}+LRh and P_{80}+CRh, by 42.9 and 37.6 % increase, respectively over UFUI (189 kg N ha^{-1}). But, the performance of P_{80}+LRh was at par to that of P_{120}+LRh.

Available P in soil varied from 14 to 29 kg ha^{-1}. All the treatments (except P_{40}+CRh, UF+LRh, UF+CRh and P_{40}+UI) performed significantly over the control. Among all the treatments, P_{120}+LRh responded the best by 107.1% increase, followed by P_{80}+LRhand P_{120}+CRh, by 92.9 and 78.6% increase, respectively over the control (14.0 kg P ha^{-1}). The response of P_{80}+LRh was at par with P_{120}+LRh.The data on available K in soil varied from 236 to 295 kg ha^{-1}. All the treatments varied numerically but non-significantly over the control of UFUI. Among all the treatments, P_{80}+LRh responded the best by 25.0% increase and this was followed by P_{120}+LRh over the control of UFUI.

However, the performance of P_{80}+LRh was at par to that of P_{120}+LRh.Pandey *et al.*, (2015) also reported that combined inoculation of Rhizobium, PSB and PGRR improved the nutrient status of soil through their synergistic effect on nitrogen fixation and solubilisation of native soil phosphorus which increased availability and uptake of these nutrients by the crop plant (Urd bean) resulted in enhanced yield indices. Higher NPK uptake in combined inoculation of Rhizobium + PSB + PGRR is due to their additive effects (Prasad *et al.,* 2002). Seed inoculation with biofertilizers reduced the bulk density and enhanced organic carbon and available N, P and K contents of the soil.

Crop Yields

The effects of liquid and carrier based *Rhizobium* inoculants supplemented with different levels of Phadbeneficial 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 by78.5 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% increase, 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 91.0and 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). In 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 thenodulation attributes, available soil N, P and K in soil and as well as yield of soybean.

References

Akpalu, M. M., Siewobr, H., Oppong-Sekyere, D. and Akpalu, S. E. 2014. Phosphorus application and Rhizobia inoculation on growth and yield of soybean (*Glycine max* L. Merrill).*Am. J. Exp. Agr*. 4(6): 674-685.

Amma, M. K. 1989. Plant and soil analysis. Rubber res. ins. Rubber Board, Kottayam Kerala.

Brahmaprakash, G. P., Girisha, H. C., NaviVithal and Hedge, S. V. 2004. Biological nitrogen fixation in pulse crops. Pulses in New Perspective (Masood Ali, B.B Singh, Shiv Kumar and Vishwa Dhar, eds.), *Indian Society of Pulses Research and Development, IIPR, Kanpur, India*. 271-286.

Dhami N. and Prasad, B. N. 2009. Increase in root nodulation and crop yield of soybean by native *Bradyrhizobium japonicum*strains. *Botanica Orientalis–Journal of Plant Science*. 6: 1-3.

Gattani, P. D., Jain, S. V., Seth, S. P. 1976. Effect of continuous use of chemical fertilizer and manures on soil physical and chemical properties. *J. Indian Society Soil Science*. 24: 284-289.

Gupta, S. C. 2005. Evaluation of liquid and carrier based *Rhizobium* inoculants in chickpea. *Indian Journal Pulses Research*. 18(1): 40-42.

Hungria, M. R. J., Campo, I. C. Mendes and Graham, P. H. 2006. Contribution of biological nitrogen fixation to the N nutrition of grain crops in the tropics: the success of soybean (*Glycine max* L. Merr.) in South America. In: Nitrogen nutrition and sustainable plant productivity. (Eds) Singh R.P., N. Shankar and P.K. Jaiwa. Stadium Press, Houston. 43-93.

Kwarteng, J. A., Towler and M. J. 1994. West Africa Agriculture. A textbook for schools and colleges, by Macmillan Publication Press Limited, p. 144-116.

Makoi, J. H. J., Bambara, R. S. and Ndakidemi, P. A.2013. *Rhizobium* Inoculation and the Supply of Molybdenum and Lime Affect the Uptake of Macronutrients in Common Bean (*P. vulgaris* L.). *Plants. Aust. J. Crop Sci*. 7(6): 784-793.

Miller, R. H., and Keeney, D. R. 1982. Estimation of available phosphorus in soil by extraction with sodium bicarbonate. *Circ. U.S Department Agriculture*. 939: 1-20.

Mohr, G. R., Daija, N. P., Subbaramany, H. S., Leleyand, V. K. and Danalrue, R.L. 1963. Soil testing in India Asian Press, New Delhi.

O'Hara, G., Yates, R. and Howieson, J. 2002. Selection of strains of root nodule bacteria to improve inoculant performance and increase legume productivity in stress full environments. In: Herridge D (eds) Inoculants and Nitrogen fixation of legumes in Vietnam. *ACIAR Proceedings*. 109: p. 75-80.

Olsen, S. R., Cole, C. V., Vatanabe, F. S. and Dean, L. A. 1954. Estimation of available phosphorus in soil by extraction with sodium bicarbonate. *Circ. U.S. Department. Agriculture*. 939: 1-19.

Pandey, I.B., Pandey, R. K. and Kumar, R. 2015. Integrated nutrient management for enhancing productivity and profitability of long duration pigeon-pea (*Cajanus cajan*) under rainfed condition. *India nJ. Agron*. 60(3): 436-442.

Piper, C. S. 1950.Soil and Plant Analysis. Intel Science Publishers. Inc. New York.

Plaxton, W. C. 2004. Plant response to stress: biochemical adaptations to phosphate deficiency. In: Goodman R (eds) Encyclopedia of Plant and Crop Science. Marcel Dekker, New York. p. 976-980.

Prasad, H., Chandra, R., Pareek, R.P. and Kumar, N. 2002. Synergism among phosphate solubilizing bacteria, rhizo bacteria and Rhizobium with urdbean. Indian J. Pulse Res., 15(2): 131-135.

Ruhul, A. A. K. M., Jahan, S. R. A., Karim, M. F., Hasanuzzaman, M. 2009. Growth dynamics of soybean (*Glycine max* L.) as affected by varieties and timing of irrigation. *American Eurasian J of Agronomy*. 2: 95-103.

Scherer, H.W., Pacyna, S. Spoth, K. R. and Schulz, M. 2008. Low levels of ferredoxin, ATP and leghemoglobin contribute to limited N_2 fixation of peas (*Pisum sativum* L.) and alfalfa (*Medicago sativa* L.) under S deficiency conditions. *Biol. Fert. Soils*.44: 909-916.

Simmonds, N. W., Smartt, J., Millen, S. and Spoor, W. 1999. Principles of crop improvement, 2^{nd} edition. *Published Longman Group Ltd*. p 252-256.

Smaling, E. M. A, Roscoe, R., Lesschen, J. P., Bouwman, A. F. and Comunello, F. 2008. From forest to waste: Assessment of the Brazilian soybean chain, using nitrogen as a marker, *Agric. Ecosyst. Environ*. 128: 185-197.

Son, T. T. N., Diep, C. N., Giang, T. T. M. and Thu, T. T. A. 2007. Effect of co-inoculants *(*Bradyrhizobia and Phosphate solublizing Bacteria) Liquid on soybean under rice based cropping system in the Mekong delta. *Omonrice* 15: 135-143.

Subbiah, B. V. and Asija, E.C. 1956. A rapid procedure for estimation of available nitrogen in soil. *Current Sciences*25: 259-260.

Subbiah, B.V., and Asija, E.C. 1956. A rapid procedure for estimation of available nitrogen in soil. *Current Sciences*.25: 259-260.

Tairo, E. V. and Ndakidemi, P.A. 2014. Macronutrients uptake in soybean as affected by *Bradyrhizobium japonicum* inoculation and phosphorus (P) supplements. *Am. J. Plant Science*.5: 488-496.

Walkley, A. and I. A. Black. 1934. An examination of Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. *Soil Sci*. 37: 29-37.

How to cite this article:

Shish Ram Jakhar, Vinod Kumar, N. G. Mitra and Ompal Singh. 2018. Effect of Soybean (*Glycine max*) Seed Inoculation with Liquid and Carrier Based *Rhizobium* cultures and Phosphorus Levels on Productivity and Physico-Chemical Properties of Soil. *Int.J.Curr.Microbiol.App.Sci.* 7(06): 1807-1814. doi: https://doi.org/10.20546/ijcmas.2018.706.215