Legume Research, 42(2) 2019: 205-210
Print ISSN:0250-5371 / Online ISSN:0976-0571

AGRICULTURAL RESEARCH COMMUNICATION CENTRE
www.arccjournals.com

Seed bio-priming of green gram with *Rhizobium* and levels of nitrogen and sulphur fertilization under sustainable agriculture

Mahipal Choudhary, B.A. Patel, Vijay Singh Meena*[1], R.P. Yadav[1] and Prakash Chand Ghasal[2]

B.A. College of Agriculture,
Anand Agricultural University, Anand-388 110, Gujarat, India.
Received: 21-01-2017 Accepted: 17-07-2017 DOI: 10.18805/LR-3837

ABSTRACT

The experiment was laid out in a factorial randomized block design comprising eighteen treatment combinations of three levels of N (0, 10 and 20 kg ha^{-1}), three levels of S (0, 10 and 20 kg ha^{-1}) and two levels of biofertilizer (seed bio-priming and without *Rhizobium*) which were replicated three times. The results indicated that plot with the application of N and S @ 20 kg ha^{-1} gave significantly ($p< 0.05$) higher seed yield (~ 32 and 21%) and stover yield (~ 16 and 18%) as compared to control plot, respectively. Bio-primed observed significantly ($p < 0.05$) higher seed yield (~ 996 kg ha^{-1}) and stover yield (~ 1829 kg ha^{-1}) as compared to un-inoculated treatments. Meanwhile, plot with the application of N and S @ 20 kg ha^{-1} + bio-priming @ 25 g kg^{-1} seeds on loamy sand soil resulted the increased the seed yield ~ 32, 21 and 7%, respectively and nutrients availability besides organic carbon (5, 2 and 2.3%), available N (5, 4.5 and 4.6%), P_2O_5 (7.5, 2 and 0.5%), and S (12, 22 and 11%, respectively) concentration increased in soil after harvest of green gram under bio-primed treatments.

Key words: Green gram, Nitrogen, Productivity, *Rhizobium*, Sulphur.

INTRODUCTION

Green gram (*Vigna radiata* L.) commonly known as "Mung" or "Mungbean" is one of the most important pulse crop in India ranking fourth in respect of cultivated area next to chickpea ~ 40%, pigeon pea ~ 20%and black gram ~ 12% (Singh, 2013). In India, total cultivated area of pulses is ~ 29 Mha with total production ~ 19 Mt, and average productivity of ~ 750 kg ha^{-1} (Annonymous, 2016). In India, green gram is grown ~ 3.38 Mha with an annual production of ~ 1.61 Mt with a productivity of ~ 474 kg ha^{-1} (Annonymous, 2016). The green gram being a leguminous crop, meets its nitrogen requirement through symbiotic N-fixation. Nitrogen is the chief promoter of plant growth, however, in case of green gram, nitrogen does not usually show its effect, except that the vegetative growth is increased. However, the starter dose is required for initial growth (Sipai *et al.*, 2015). Nitrogen is one of the most important primary nutrients among non-metal element which require in large quantity for the plant growth and nutrition (Lowenfels *et al.*, 2011).

The effect of sulphur reported as a synergistic effect on productivity of crops, it is ~ 90% present in plants in the form of amino acids. Sulphur is essential for chlorophyll formation, synthesis of protein, vitamins, thiourea, plant hormones, thiamin, biotin, glutathione and S-containing essential amino acids viz. methionine, cystine and cysteine (Kokani *et al.*, 2015). One possible way to mitigate the nutrients requirement of plant by the efficient utilization of biofertilizers as an ecofriendly technologies and based on renewable energy sources has gained momentum in recent years to supplement the parts of chemical fertilizers (Meena *et al.*, 2017). In leguminous crops the *Rhizobium* plays a significant role in maintaining and improving the soil fertility and sustainability through its ability to fix atmospheric nitrogen in the soil through root nodules (Patel *et al.*, 2016). An efficient use of microbes increased yield reduces the soil borne pathogens and improves soil health (Haque *et al.*, 2012; Kumar *et al.*, 2017). Therefore, the present investigation was conducted to evaluate the effect of different nutrient with bio-priming on productivity and soil nutrient status after harvest the crops with following objectives (i) to study the effect of N, S and bio-priming on yield and yield attributes; (ii) to study the effect of N, S and bio-priming on nutrient uptake and soil fertility status.

MATERIALS AND METHODS

A field experiment was conducted on loamy sand soil (*Typic Ustochrept*), during the *summer* season of 2012 at College Agronomy Farm, B.A. College of Agriculture, Anand Agricultural University, Anand, Gujarat, situated at 22° -35' N latitude, 72° -55' E longitude with an elevation of 45.1 m above sea level. The average annual rainfall of this region was ~ 850 mm. The weekly mean minimum temperature ranged between 13 to 28° C and mean maximum temperature ranged between 30 to 44p C during the crop season. The initial properties of experimental soil presented in the Table 1. There were total 18 numbers of treatments

*Corresponding author's e-mail: vijay.meena@icar.gov.in
[1]ICAR- Vivekananda Parvatika Krishi Anusanthan Sansthan, Almora-263 601, Uttarakhand, India.
[2]ICAR- Indian Institute of Farming System Research (IIFSR), Modipuram, Meerut-250 110, Uttar Pradesh, India.

Table 1: Physical and chemical properties of the experimental site.

Properties	Values	Adopted Methodology	Adopted Reference
Physical properties			
Mechanical Composition (g 100^{-1} g)			
Coarse sand	00.65		
Fine sand	81.10	International pipette method	Piper (1966)
Silt	12.21		
Clay	06.04		
Texture	Loamy sand	Traingle	
Bulk density (Mg m^{-3})	1.34	Brass Cup Method	Jalota *et al.* (1998)
W.H.C. (g 100g $soil^{-1}$)	35.5	Brass Cup Method	Chopra and Kanwar(1976)
Chemical properties			
pH (1:2.5)	7.90	Potentiometry	Jackson (1973)
EC (1:2.5) dSm^{-1}	0.22	Conductometery	Jackson (1973)
Organic carbon (g kg^{-1})	2.30	Wet oxidation method	Walkley and Black, (1934)
Available N (kg ha^{-1})	184.67	Alkaline $KMnO_4$ method	Subbiah and Asija (1956)
Available P_2O_5 (kg ha^{-1})	41.58	Colorimetric Method (0.5 M $NaHCO_3$, pH 8.5, Ascorbic acid)	Olsen *et al.* (1954)
Available K_2O (kg ha^{-1})	210.00	Flame photometry method (Neutral N NH_4OAc)	Jackson (1973)
Available S (ppm)	7.27	Turbidimetric method (0.15 % $CaCl_2$ extractable S)	Chesnin and Yien (1950)
Available Fe (ppm)	6.12	Atomic absorption spectroscopy (0.005 M DTPA, pH 7.3)	Lindsay and Norvell (1978)

with three times replicated in FRBD. Treatments comprising combinations of nitrogen (0, 10 and 20 kg ha^{-1}), Sulphur (0, 10 and 20 kg ha^{-1}) and seed bio-priming (uninoculated and inoculated with *Rhizobium*). Green gram variety *Meha* was raised on first week of June with a spacing of 45 × 10 cm in gross plot area of 3.6 × 5.4 m. It matures in ~ 70 days during summer season. To assess fertility status of soil, representative composite soil samples from each net plot area were collected from harvested field and following the standards procedure to analysis of soil and plant samples (Table 1). The uptake of these nutrients was computed by using the following formula.

$$\text{For major and secondary nutrient uptake (kg ha}^{-1}) = \frac{\text{Nutrient content (\%)} \times \text{Yield (kg ha}^{-1})}{100}$$

$$\text{For micronutrient uptake (g ha}^{-1}) = \frac{\text{Nutrient content (ppm)} \times \text{Yield (kg ha}^{-1})}{1000}$$

$$\text{Protein content (\%)} = \text{Total nitrogen} \times 6.25$$

Statistical analysis of the data was done by using analysis of variance (ANOVA), as described by (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

Yield and yield attributes: Data presented in Table 2, revealed that application of 20 kg N ha^{-1} (N_2) gave significantly highest number of pods (~ 27), root nodules per plant (~ 26), dry weight of root nodules (~ 44 mg $plant^{-1}$), test weight (~ 35 g) but it remained at par with application of 10 kg N ha^{-1},seed yield (1100 kg ha^{-1}), stover yield (1925 kg ha^{-1}) and protein content in seed (~ 24%) were observed, which were ~ 42, 21, 22, 11, 32, 16, 11% significantly higher as compared to control (N_0), respectively. This increase might be owing to better vegetative growth, metabolism and chlorophyll formation thereby, increased photosynthetic efficiency and thus increased the amount of photosynthates and larger translocation and partitioning of assimilates towards yield attributes (Sipai *et al.*, 2016).

Application of 20 kg S ha^{-1} (S_2) gave significantly highest number of pods per plant (23.83), root nodules per plant (23.94), dry weight of root nodules (41.49 mg $plant^{-1}$), test weight (33.84 g) but it remained at par with application of 10 kg N ha^{-1},were observed, over control (N_0). Significantly highest seed yield (1056 kg ha^{-1}), stover yield (1889 kg ha^{-1}), and protein content in seed (~ 24%) were obtained under the treatment of 20 kg S ha^{-1} over the rest of the treatments (Table 2). This might be due to the fact that increasing levels of S application improved over all nutritional environment of the rhizosphere as well as plant system which could be more advantageous for profuse vegetative and root growth which activated higher absorption of nutrients (Singh and Yadav, 2004).

Data showed that the bio-priming with *Rhizobium* obtained significantly higher seed (996 kg ha^{-1}), stover yield (1829 kg ha^{-1}), and protein content (~ 23%) which was significantly superior over control (Table 2). *Rhizobium* inoculation generally initiated the early nodules formation in the crown root system this might be probably due to effect of proliferation of nodule forming bacteria in the root system and enhance the N-fixation from atmosphere and results in better growth and development of plant (Nandan *et al.*, 2012).

Table 2: Effect of different fertilizer levels and biofertilizer on yield attributes, yield, and nodulation of green gram.

Treatment	Pods	Root nodules	Dry weight of root nodules (mg plant^{-1})	Test weight (g)	Seed	Stover	Protein content in seed (%)
	------Plant^{-1}------				-------- Yield kg ha^{-1}--------		
Nitrogen (N) (kg ha^{-1})							
N_0-0	18.66	21.00	36.13	31.91	833	1659	21.39
N_1-10	22.12	22.61	39.84	33.75	963	1791	22.57
N_2-20	26.50	25.44	44.25	35.34	1100	1925	23.69
SEm±	0.60	0.58	0.62	0.60	13	17	0.14
C.D. (P=0.05)	1.72	1.67	1.77	1.71	39	49	0.39
Sulphur (S) (kg ha^{-1})							
S_0-0	21.04	22.22	38.58	32.69	877	1595	22.05
S_1-10	22.44	22.28	40.15	33.46	964	1792	22.49
S_2-20	23.83	23.94	41.49	33.84	1056	1889	23.70
SEm±	0.60	0.58	0.62	0.60	13	17	0.14
C.D. (P=0.05)	1.72	1.67	1.77	1.71	39	49	0.39
Biofertilizer (B)							
B_0-No bio-priming	19.49	21.40	39.24	32.62	935	1755	21.85
B_1-Seed bio-priming	25.37	24.62	40.91	34.71	996	1829	23.25
SEm±	0.49	0.47	0.50	0.49	11	14	0.11
C.D. (P=0.05)	1.40	1.37	1.45	1.40	32	40	0.32

Nutrient uptake: Plot with the application of 20 kg N ha^{-1} (N_2) recorded significantly the highest N, P, K and S uptake by seed (~ 42, 4.39, 8.33 and 1.41 kg ha^{-1}) and stover (~ 17, 3.33, 31.27 and 1.70 kg ha^{-1}) as compared to rest of the treatments, which were ~ 46, 57, 71 and 48% in seed and ~ 32, 35, 36 and 38% higher in stover as compared to control (N_0). Results revealed that highest micronutrient uptake in seed and stover were application of 20 kg N ha^{-1} as compared to 10 and 0 kg ha^{-1} (Figs. 1a and b). This might be due to that the higher nutrient content helps in higher carbohydrates metabolism, synthesis of amino acids, protein and chlorophyll resulted higher nutrient uptake (Morshed *et al.*, 2008).

Results showed that N, P, K and S uptake amongst different S levels ranged from ~ 31 to 39, 3.16 to 4.03, 5.76 to 7.48 kg ha^{-1} in seed and 13.32 to 15.53, 2.66 to 3.11, 24.85 to 29.33 and 1.23 to 1.73 kg ha^{-1} in stover. It was observed that the plot with the application of S showed that the increase in nutrient uptake (Chaurasia *et al.,* 2009). Meanwhile, the significantly higher nutrients uptake of Fe, Mn, Zn and Cu by seed (69.13, 17.29, 10.93 and 13.91 g ha^{-1}) and stover (634.4, 137.8, 31.1 and 38.7 g ha^{-1}) were recorded with application 20 kg S ha^{-1} (S_2) than the two lower treatments (Table 3). Increased in the Zn removal may be due to increased root surface area resulting from better growth due to S supply (Islam *et al.,* 2009). Data showed that the significantly higher NPK and S uptake of ~ 37, 4, 7, 1.26 kg ha^{-1} in seed and the uptake of in stover ~ 15, 3, 28, 1.57 kg ha^{-1} of NPK and S, respectively. It may be due to the effect of symbiotic rhizobacteria augment nodulation in root and increased N-fixation and improve the availability of N and others nutrient in soil (Sipai *et al.,* 2015).

Available soil nutrients: After harvesting of crop, soil was analyzed for available soil nutrient and data was analyzed and tabulated in (Table 4). Application of 20 kg N ha^{-1} gave significantly higher N and S content in soil over control and 10 kg N ha^{-1} but it was statistically at par with application of 10 kg N ha^{-1}. Similar results were also observed by Yadav *et al.* (2010). Plot with the application of increasing level of S recorded lower values of soil reactionand higher values of EC, organic carbon (OC), available P, K and Fe but they were non-significant. Meanwhile in case of OC, available NP and S content increased (2, 4.5, 2 and 22%), respectively by application of 20 kg S as compared to control plot (Table 4). Available N and S content increased with increasing doses of S (Sipai *et al.,* 2015). Bio-priming also influenced the available soil nutrients (Meena et al., 2016). Available N and S status of soil was significantly increased ~ 5 and 11% by *Rhizobium* inoculation (Table 4). Available N and S level slightly increased in comparison to other treatments application. The levels of these nutrients were low in control treatment which was expected due to uptake of nutrient from the soil source only.

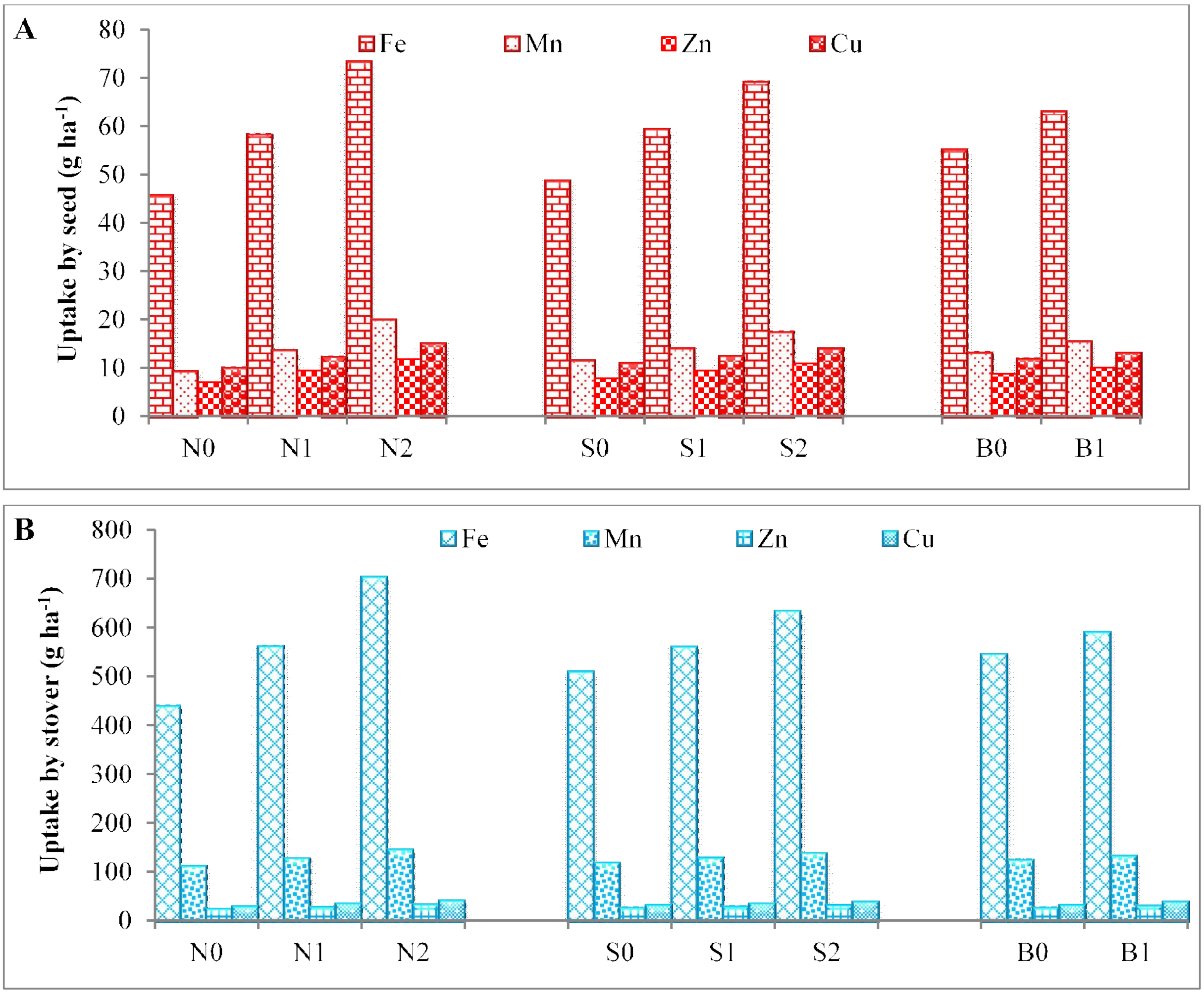

Fig 1: Effect of fertilizers levels and seed priming through biofertilizers on (A) micronutrient uptake in seed and, (B) micronutrient uptake in stover of green gram.

Table 3: Effect of N, S and biofertilizer on nutrient uptake by seed and stover of green gram.

Treatment	Nutrient uptake by seed (kg ha^{-1})				Nutrient uptake by stover (kg ha^{-1})			
	N	P	K	S	N	P	K	S
Nitrogen (N) (kg ha^{-1})								
N_0-0	28.57	2.80	4.88	0.95	12.48	2.47	23.01	1.23
N_1-10	39.41	3.55	6.58	1.19	14.20	2.87	27.01	1.49
N_2-20	41.84	4.39	8.33	1.41	16.53	3.33	31.27	1.70
SEm ±	0.58	0.05	0.12	0.02	0.22	0.05	0.48	0.03
C.D. (P=0.05)	1.68	0.16	0.36	0.07	0.63	0.13	1.40	0.10
Sulphur (S) (kg ha^{-1})								
S_0-0	31.16	3.16	5.76	0.99	13.32	2.66	24.85	1.23
S_1-10	34.87	3.54	6.56	1.17	14.37	2.89	27.11	1.46
S_2-20	39.28	4.03	7.48	1.40	15.53	3.11	29.33	1.73
SEm ±	0.58	0.05	0.12	0.02	0.22	0.05	0.48	0.03
C.D. (P=0.05)	1.68	0.16	0.36	0.07	0.63	0.13	1.40	0.10
Biofertilizer (B)								
B_0-No bio-priming	32.86	3.33	6.12	1.11	13.75	2.74	25.72	1.37
B_1-Seed bio-priming	37.35	3.82	7.08	1.26	15.06	3.03	28.48	1.57
SEm ±	0.47	0.04	0.10	0.02	0.18	0.04	0.40	0.02
C.D. (P=0.05)	1.37	0.13	0.29	0.06	0.52	0.11	1.14	0.08

Table 4: Effect of nitrogen, sulphur and biofertilizer on chemical properties of soil after harvest of green gram.

Treatment	pH (1:2.5)	EC (dS m^{-1})	OC (%)	Soil available nutrient status				
				Av. N	Av. P_2O_5	Av. K_2O	Av. S	Av. Fe
				------kg ha^{-1}------			--------ppm--------	
Nitrogen (N) (kg ha^{-1})								
N_0-0	8.26	0.195	0.369	200.10	39.31	293.48	9.72	6.33
N_1-10	8.27	0.195	0.377	205.10	40.67	296.90	10.51	6.36
N_2-20	8.27	0.194	0.387	209.78	42.27	300.14	10.86	6.40
SEm ±	0.08	0.003	0.003	1.80	0.83	4.26	0.29	0.08
C.D. (P=0.05)	NS	NS	0.011	5.19	NS	NS	0.82	NS
Sulphur (S) (kg ha^{-1})								
S_0-0	8.26	0.195	0.373	201.07	40.38	295.91	9.25	6.34
S_1-10	8.29	0.193	0.378	205.31	40.67	296.67	10.52	6.36
S_2-20	8.24	0.196	0.381	208.71	41.19	297.94	11.31	6.38
SEm ±	0.08	0.003	0.003	1.80	0.83	4.26	0.29	0.08
C.D. (P=0.05)	NS	NS	NS	5.19	NS	NS	0.82	NS
Biofertilizer (B)								
B_0-No bio-priming	8.26	0.195	0.373	201.96	40.66	294.57	9.76	6.34
B_1-Seed bio-priming	8.27	0.195	0.382	208.15	40.84	299.11	10.96	6.38
SEm ±	0.07	0.003	0.003	1.47	0.67	3.48	0.23	0.07
C.D. (P=0.05)	NS	NS	NS	4.23	NS	NS	0.67	NS

CONCLUSION

The present investigation results showed that the plot receiving the 20 kg ha^{-1} N, S and bio-priming with *Rhizobium* @ 25 g kg^{-1} seeds on loamy sand soil enhance the productivity, nutrients uptake and soil nutrient availability under nitrogen and sulphur deficiency soils for sustainable food production of green gram.

ACKNOWLEDGEMENT

Authors express sincere thanks to Editor and two anonymous reviewers for much help in improving this manuscript.

REFERENCES

Annonymous (2016). Area, Production and Productivity of Major Pulses (http://www.iipr.res.in/).

Chaurasia, A.K., Richharia, G.P. and Chaurasia, S. (2009). Response of soybean (*Glysine max*) to doses and sources of sulphur. *Indian Journal of Agricultural Sciences*,**79**(5): 356-358.

Chesnin, L. and Yien, C.H. (1950). Turbidimetric determination of available sulphates. *Soil Science Society American Proc*., **15**:149-51.

Chopra, S.L. and Kanwar, J.S. (1976). Analytical Agricultural Chemistry, Kalyani publications, New Delhi.

Gomez, K.A. and Gomez, A.A. (1984). Statistical Procedures for Agricultural Research (2nd Edn.). John Wiley and Sons, New York. pp. 680.

Haque, M., Ilias, G.N.M., Molla, A.H. (2012). Impact of *Trichoderma*-enriched biofertilizer on the growth and yield of mustard (*Brassica rapa* L.) and tomato (*Solanum lycopersicon* mill.). *Agriculturists*, **10**(2):109-119.

Islam, M., Ali, S. and Hayat, R. (2009). Effect of integrated application of phosphorus and sulphur on yield and micronutrient uptake by chickpea (*Cicer arientinum* L.). *International Journal Agricultural Biology*, **11**: 33-38.

Jackson, M.L. (1973). Soil Chemical Analysis. Prentice Hall of India Pvt. Ltd., New Delhi.

Jalota, S.K., Khera, R. and Ghuman, B.S. (1998). Method of Soil Analysis. Narosa Publishing House, New Delhi.

Kokani, J.M., Shah, K.A., Tandel, B.M. and Bhimani, G.J. (2015) Effect of FYM, phosphorus and sulphur on yield of summer black gram and post-harvest nutrient status of soil. *The Bioscan*, **10**(1):379-383.

Kumar, A., Maurya, B.R., Raghuwanshi, R., Meena, V.S., Islam, M.T. (2017). Co-inoculation with *Enterobacter* and *Rhizobacteria* on yield and nutrient uptake by wheat (*Triticum aestivum* L.) in the alluvial soil under Indo-Gangetic Plain of India. *Journal of Plant Growth Regulation*, http://dx.doi.org/10.1007/s00344-016-9663-5.

Lindsay, W.L. and Norvell, W.A. (1978). Development of a DTPA soil test for zinc, iron, manganese and copper. *Soil Science Society of American Proc*, **42** (3): 421-428.

Lowenfels, Lewis, Jeff, Wayne (2011).*Teaming with microbes*. pp. 49, 110. ISBN 978-1-60469-113-9.

Meena, S.K., Rakshit, A., Meena, V.S. (2016). Effect of seed bio-priming and N doses under varied soil type on nitrogen use efficiency (NUE) of wheat (*Triticum aestivum* L.) under greenhouse conditions. *Biocatalysis and Agricultural Biotechnology*, **6**: 68–75.

Meena, V.S., Meena, S.K., Verma, J.P., Kumar, A., Aeron, A., Mishra, P.K., Bisht, J.K., Pattanayak, A., Naveed, M., Dotaniya, M.L. (2017). Plant beneficial rhizospheric microorganism (PBRM) strategies to improve nutrients use efficiency: A review. *Ecological Engineering*, DOI: 10.1016/j.ecoleng.2017.06.058.

Morshed, R.M., Rahman, M.A. and Rahman, M.M. (2008). Effect of nitrogen on seed yield, protein content and nutrient uptake of soybean. *Journal of Agricultural Rural Development*, **6**(1):13-17.

Nandan, R., Mohd, Kaleem and Moinuddin (2012). *Rhizobium* inoculation, phosphorus and sulphur fertilization and their effect on green gram (*Vigna radiata*). Supplement: 3rd *International Agronomy Congress*, New Delhi

Olsen, S.R., Cole V.C., Watenable P.S. and Dean L.A. (1954). Estimation of available phosphorus in soil by extraction with sodium bicarbonates *Chemical Abstract*, **48**(16): Ab.1598.

Patel, A.R., Patel, D.D., Patel, T.U. and Patel, H.M. (2016). Nutrient management in summer green gram (*Vigna radiata*). *International Journal of Applied and Pure Science and Agriculture,* **2**(2):133-142.

Piper, (1966). Soil and Plant Analysis. Reprint for Asia Hans. Publishers, Bombay (India).

Singh, U. and Yadav, D.S. (2004). Response of greengram to sulphur and zinc. *Annals of Agricultural Research,* **25**:463-64.

Singh, A., Srivastava, V.K., Meena, R.S., Kumar, S. and Kumar, S. (2013). Effect of biofertilizers and NP levels on growth parameters of green gram under custard apple based agri-horti System. *Agriculture for Sustainable Development,* **1**:61-63.

Sipai, A.H., Jat, H.S., Rathore, B.S., Sevak, K. and Jodha, J.S. (2015). Effect of phosphorus, sulphur and biofertilizer on productivity and soil fertility after harvest of moongbean grown on light textured soil of Kachchh. *An Asian Journal of Soil Science,* **10**(2): 228-236.

Sipai, A.H., Jat, J.R. and Rathore, B.S. (2016). Effect of phosphorus, sulphur and biofertilizer on growth, yield and nodulation in mungbean on loamy sand soils of Kutch. *Crop Research,* **51**:1-10.

Subbiah, B.V. and Asija, G.C. (1956). A rapid procedure for the estimation of available nitrogen in soil. *Current Science,* **25**:259-260.

Walkley, A. and Black, I.A. (1934). An extraction of the Degtjareff method for determination of soil organic matter and a proposed modification of the chromic acid titration method. *Soil Science,* **34**:29-38.

Yadav, H.K., Thomas, T. and Khajuria, V. (2010). Effect of different levels of sulphur and biofertilizer on the yield of Indian mustard (*Brassica juncea* L.). *Journal of Agricultural Physics,* **10**:61-65.