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EFFECT OF INTEGRATED NUTRIENT MANAGEMENT ON SYMBIOTIC EFFICIENCY AND SOIL FERTILITY UNDER GREEN GRAM [*VIGNA RADIATA* (L.) WILCZEK] CULTIVATION

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ABSTRACT

A field experiment was conducted at Instructional Farm (Agronomy), RCA, Udaipur, (Rajasthan) during *Kharif*, 2014 on clay loam soil to study the effect of integrated nutrient management on symbiotic efficiency and fertility status of post-harvest soil under Green gram cultivation. The experiment was laid out according to a factorial randomized block design with three replications. The experiment comprised four fertility levels (75% RDF, 75% RDF+VC @ 2 t ha^{-1}, 100% RDF and 100% RDF+VC @ 2 t ha^{-1}) and four biofertilizers levels (control, *Rhizobium*, PSB and *Rhizobium* + PSB). The RDF was 20 kg N and 40 kg P_2O_5 per hectare. The soil of the experimental site was clay loam in texture, slightly alkaline in reaction, medium in available nitrogen and phosphorus, while high in potassium and DTPA extractable micronutrients sufficiently above the critical limits. The application of fertility level significantly increased the number of total and effective root nodules, fresh and dry weight of root nodules and leg haemoglobin content in root nodules upto 75% RDF+VC @ 2 t ha^{-1} however, their further increase with application of 100% RDF+VC @ 2 t ha^{-1} was found non-significant. Seed inoculation with *Rhizobium* + PSB significantly increased the number of total and effective root nodules, fresh and dry weight of root nodules and leg haemoglobin content in root nodules. The organic carbon, available nitrogen, phosphorus, potassium, copper, zinc, iron and manganese in soil significantly increased with inoculation of *Rhizobium* + PSB at the harvest stage of the crop, but EC and pH remain non-significant.

KEY WORDS : Green gram, Symbiotic efficiency, Soil fertility, RDF, Vermicompost, *Rhizobium, PSB.*

INTRODUCTION

Pulses are an important source of dietary protein and have a unique ability to maintain and restoring soil fertility through biological nitrogen fixation as well as the addition of an ample amount of residues to the soil. Pulse crops leave behind a reasonable quantity of nitrogen in the soil to the extent of 30 kg ha^{-1}. Green gram (*Vigna radiata* (L.) Wilczek] commonly known as *Mungbean and Goldengram,* is one of the important *Kharif* pulse crops. It ranks third among all pulses grown in India after chickpea and pigeonpea. It is a quite versatile crop grown for seeds, green manure and forage; as mixed or sole crop either on residual moisture of the previous crop or as a catch crop to make use of the land left fallow between two main season crops. It makes good manure is incorporated into the soil. Further, it enriches the soil by atmospheric nitrogen fixation through root nodules. The crop gives such a heavy vegetative growth and covers the ground so well that it checks the soil erosion in problem areas and can later be ploughed down for green manure. It has considerable promise as an alternative pulse crop in dry land farming. This crop is of great importance because of the availability of short duration (65-70 days), high-yielding and quick-growing varieties. In terms of significance, phosphorus is the most indispensable mineral nutrient for pulse crops as it helps in better root growth and development and thereby making them more efficient in biological nitrogen fixation. Phosphorus is an essential constituent of nucleic acids such as ribonucleic acid (RNA) and deoxyribonucleic acid (DNA), adenosine diphosphate (ADP) and adenosine triphosphate (ATP), nucleoproteins, amino acids, proteins, phosphatides, phytin, several co-enzymes *viz.* thiamine, pyrophosphate and pyrodoxyl phosphate.

Bio-fertilizers, an integrated nutrient management component in sustainable agriculture, are found as cost-effective, environment-friendly and renewable sources of a non-bulky plant nutrient complementary component. In the current context of the very high costs of chemical fertilizers, their role takes on special importance. Increasing the efficiency of fertilizer utilization may have greater importance. For available nitrogen and available phosphorus, Indian soils are low to medium in content. The rhizobium seeds are inoculated to increase the number of rhizosphere plants so that the number of microbiologically fixed nitrogen is significantly increased for plant development. The combination of rhizobium and pulse plants contributes to improved soil fertility and is a cost-effective nitrogen fertilization method in legumes. Therefore, there may be a substantial saving of applied nitrogen and phosphorus when seeds are inoculated with *Rhizobium* and phosphate solubilizing bacteria inoculants.

Vermicompost is an environmentally friendly, economical and efficient method of waste recycling. Vermicompost is a new technological innovation in composting. It is a mix of castings of earthworm, organic material, humus and other bodies. The vermicompost can be recycled with agricultural residues, waste animals, waste dairy and poultry, waste in the food industry, biogas sludge. Vermicompost has been recommended in recent years as an integrated nutrient management system for crops in the field (Shroff and Devathali, 1992). After collection of wheat, sorghum, maize, cotton, etc., vermicomposting can be efficiently utilized, large crop residues are left in Rajasthan. Keeping the above facts in view an experiment was conducted to find out the effect of integrated nutrient management on symbiotic efficiency and soil fertility under Green gram cultivation.

MATERIALS AND METHODS

A field experiment was conducted at Agronomy Farm, Rajasthan College of Agriculture, Udaipur, (Rajasthan) during the *Kharif* season of 2014 to find out the effect of integrated nutrient management on symbiotic efficiency and fertility status of post-harvest soil under Green gram. The experimental region falls under Agro-climatic Zone IVa (Subhumid Southern Plain and Aravalli Hills) of Rajasthan.The mean annual rainfall of Udaipur is 610.2 mm, most of which is contributed by the southwest monsoon from July to September. The soil of the experimental site was clay loam in texture, slightly alkaline in reaction. The soil was medium in available nitrogen, phosphorus while high in potassium, and sufficient in DTPA extractable micronutrients.The experiment was laid out in a factorial randomized block design with three replication. The treatment combinations were randomized with the help of a random number table (Fisher, 1950).

The seeds were inoculated with *Rhizobium* and PSB culture or both as per the plan of treatments, using the standard method and dried in shade. The total number of root nodules per plant was counted at the pre-flowering stage. Five plants randomly selected from the sample row of each plot were uprooted carefully, the soil mass embodying the roots of the plant was washed off by water and total root nodules were counted. The mean value was recorded as the total number of root nodules per plant. Numbers of effective root nodules were counted from the same plants as taken for the total number of nodules. Healthy pink coloured nodules were counted and the mean value was recorded as an effective number of root nodules per plant. Effective root nodules obtained from the five plants of each plot were weighed with the help of an electronic balance and the average was work out and recorded age fresh weight of effective root nodules per plant.

Total root nodules so obtained from the five plants from each plot were subjected to oven-dry at 70 ^{o}C till a constant weight was obtained and then the average was worked out. Leg haemoglobin content of root nodules was estimated as hemochrome as described by Appleby *et al.* (1986) and Bergerson (1982). Fresh nodules (100 mg) were macerated with 5 ml of 0.1 M potassium phosphate buffer (pH 7.4), filtered through chasse cloth and the extract was centrifuged at 10,000 rpm for 30 minutes. An equal volume of alkaline pyridine reagent (4.2 M pyridine in 0.2 M NaOH) was added and mixed. To half of the solution added few crystals of sodium dithionate to reduce the hemochrome and mixed taking care to avoid alteration and measured the colour intensity at 556 nm using reagent blank as control. To the other portion few crystals of potassium Hexacyanoferrate were added to oxidize the hemochrome and the intensity was recorded at 539 nm. The concentration of leg haemoglobin was calculated as below:

$$\text{Lb (nm)} = \frac{A_{556} - A_{539} \times 2D}{23.4}$$

Where, D is the initial dilution

To assess the fertility status of soil, the soil sample (0-15 cm depth), from each plot at harvest of the crop was taken. The samples were passed through a 2 mm plastic sieve to avoid metallic contamination. The soil sample was analyzed for EC, pH, & OC and available NPK and cationic micronutrients (Cu, Zn, Fe, Mn) content as per standard methods. To test the significance of variation in experimental data obtained for various treatment effects, the data were statistically analyzed as described by Fisher (1950). The critical differences were calculated to assess the significance of treatment mean wherever the 'F' test was found significant at a 5 per cent level of probability. To elucidate the nature and magnitude of treatment effects, summary tables along with SEm ± and CD (P=0.05) were prepared.

RESULTS AND DISCUSSION

Symbiotic Efficiency

Effect of fertility levels

Application of different levels of fertility significantly increased the total and effective root nodules, fresh and dry weight of root nodules and leg haemoglobin content at the flowering stage of Green gram (Table 1). The application of 100% RDF+VC @ 2 t ha^{-1} recorded a significant value of these parameters, which was at par with the 75%RDF+VC @ 2 t ha^{-1}. This may be attributed primarily to the beneficial effect of fertility levels on the overall physical condition of the soil (Aswal and Yadav, 2007). The reason for better growth and development in the above treatments might be due to increased availability of nitrogen and phosphorous to the plant initially through fertilizers and then through vermicompost in the cropping season (Yadav *et al.* 2021). The better growth in terms of total and effective root nodules, fresh and dry weight of root nodules at flowering stage due to application of 100% RDF+VC @ 2 t ha^{-1} as compared to other treatments, which was at par with the 75% RDF+VC @ 2 t ha^{-1}. Vermicompost plays an important role in root development and proliferation resulting in better nodule formation and nitrogen fixation by supplying assimilates to the roots, better environment in the rhizosphere for growth and development (Yadav and Yadav, 2003). Since, fertility being representative of almost all the plant nutrients required for the proper growth and development of plants, its addition in the soil enhanced the availability of these nutrients. A higher population of the desired organisms will always have greater possibilities of infection and consequently the formation of more healthy and effective root nodules having a higher amount of leg haemoglobin content. These results corroborate the finding of Tiwari and Kumar (2009), Choudhary and Yadav (2011) and Das *et al.* (2013).

Effect of biofertilizers

A significant increase in total and effective root nodules, fresh and dry weight of root nodules, leghaemoglobin content at the flowering stage observed due to seed inoculation with *Rhizobium* and PSB alone as well as their combined inoculation over control (Table 1). Inoculation of seed with *Rhizobium* might have increased the concentration of an efficient and healthy strain of *Rhizobium* in the rhizosphere, which in turn resulted in greater fixation of atmospheric nitrogen in the soil for use by the plants and consequently resulting in higher plant growth, which in term resulted into higher production of assimilates and their partitioning to different reproductive structures such as the result of increased plant growth in terms of total and effective root nodules, fresh and dry weight of root nodules, leg haemoglobin content at flowering stage due to overall better nutritional environments in the rhizosphere. Chattopadhyay and Dutta (2003), Singh and Pareek (2003) and Vikram and Hamzehzarghani (2008) also reported similar results for Green gram.

PSB increased the availability of phosphorus in the root zone, which in turn resulted in better growth and development of roots and shoots and also helped in better nodulation. The overall development of a plant in terms of root and shoot might have absorbed more nutrients and enhanced photosynthesis and production of assimilates, which in turn increased the total and effective root nodules, total fresh and dry weight of root nodules, leg haemoglobin content at the flowering stage in Green gram. The combined inoculation of *Rhizobium* + PSB proved significantly superior to PSB and control and slightly superior to *Rhizobium* alone in terms of symbiotic efficiency parameters *viz*., total and effective root nodules, fresh and dry weight of root nodules, leg haemoglobin content at flowering stage. *Rhizobium* + PSB might have improved both available nitrogen and phosphorus in the rhizosphere as they are symbiotic nitrogen fixers and phosphorus solubilizers, respectively. Thus, the increased availability of nitrogen due to *Rhizobium* coupled with phosphorus due to PSB might open the door for increased utilization of other nutrients also and have resulted in more increase in growth in comparison to *Rhizobium* and PSB inoculations. The result obtained in the present investigation is in line with the finding of Kushwaha (2007) and Mir *et al.* (2013).

Soil Fertility

Effect of fertility levels

The data presented in Tables 2 and 3 showed that the 100% RDF+VC @ 2 t ha^{-1} had a significant

Table 1. Effect of fertility and biofertilizer levels on the total number of root nodules and effective root nodules per plant at flowering stage of Green gram

Treatments	Total number of root nodules plant^{-1}	Effective root nodules plant^{-1}	Fresh weight (mg plant^{-1})	Dry weight (mg plant^{-1})	Leg haemoglobin in content (mg g^{-1})
Fertility levels					
F_1:75%RDF	47.11	28.60	70.59	51.08	2.35
F_2:75% RDF+VC (2 t ha^{-1})	55.49	32.33	82.44	65.21	2.58
F_3:100%RDF	49.37	29.49	73.79	53.90	2.46
F_4:100%RDF+ VC (2 t ha^{-1})	56.26	32.43	83.53	65.80	2.68
SEm±	0.60	0.24	0.85	0.75	0.03
CD (P= 0.05)	1.74	0.70	2.46	2.18	0.10
Biofertilizer levels					
B_0:Control	45.22	28.14	67.92	49.05	2.34
B_1:*Rhizobium*	53.85	31.26	80.12	60.99	2.54
B_2:PSB	53.16	31.17	79.14	60.21	2.49
B_3:*Rhizobium* + PSB	56.00	32.29	83.16	65.74	2.66
SEm±	0.60	0.24	0.85	0.75	0.03
CD (P= 0.05)	1.74	0.70	2.46	2.18	0.10

Table 2. Effect of fertility and bio fertilizer levels on EC and pH of soil after harvest of Green gram

Treatments	pH	EC (dSm-^{1})	Organic carbon (%)	Available nitrogen (kg ha^{-1})	Available P_2O_5 (kg ha^{-1})	Available K_2O (kg ha^{-1})
Fertility levels						
F_1:75%RDF	8.48	0.824	0.62	261.86	20.44	392.02
F_2:75% RDF+VC (2 t ha^{-1})	8.04	0.810	0.76	300.50	23.19	421.14
F_3:100%RDF	8.51	0.828	0.66	274.10	21.32	411.62
F_4:100%RDF+ VC (2 t ha^{-1})	8.27	0.817	0.78	305.92	23.96	422.80
SEm±	0.07	0.002	0.01	2.83	0.27	2.61
CD (P= 0.05)	0.21	NS	0.02	8.17	0.77	7.55
Biofertilizer levels						
B_0: Control	8.25	0.817	0.67	261.73	20.02	401.99
B_1: *Rhizobium*	8.31	0.820	0.71	289.63	22.41	412.56
B_2: PSB	8.32	0.820	0.71	288.48	22.57	411.19
B_3: *Rhizobium* + PSB	8.42	0.821	0.74	302.53	23.92	421.85
SEm±	0.07	0.002	0.01	2.83	0.27	2.61
CD (P= 0.05)	NS	NS	0.02	8.17	0.77	7.55

profound influence on the organic carbon, available N, P, K, Cu, Zn, Fe and Mn status of soil, which was at par with the 75% RDF+VC @ 2 t ha^{-1}. Application of 100% RDF+VC @ 2 t ha^{-1} in general recorded an increased organic carbon, available N, P, K, Cu, Zn, Fe and Mn under all treatment. The significant increase in organic carbon, available N, P, K, Cu, Zn, Fe and Mn content of the soil after harvesting of the crop may be ascribed to the beneficial role of vermicompost in the mineralization of native as well as its nutrient content which enhanced the available nutrient pool of the soil. All the available nutrients are not taken up by the plant and the rest remains in the soil which improves the available nutrient status of soil after harvesting of the crop. The favourable conditions for microbial as well as chemical activity due to the addition of vermicompost integrated with other nutrients augmented the mineralization of nutrients and ultimately increased the available nutrient status of the soil. There was no significant variation in pH and EC of the soil of the experimental field of Green gram due to different fertility levels (Table 2). The addition of organic manure to soil had a beneficial effect on nutrient availability (Yadav *et al*., 2005). All the treatments resulted in increasing available nutrients in soil over control (Tables 2 and 3). The application of vermicompost showed the highest increase in available nutrients. The use of organic manures being a storehouse of almost all the macro and micronutrients required for plant growth, improved the soil environment by way of improving the Physico-chemical properties of soil. In addition to organic manures, the available nutrients status of soil increased considerably due to the mineralization of native as well as applied nutrients through organics (Yadav and Chhipa, 2007). The increased availability is also due to the formation of organic chelates of higher stability with organic legends, which have lower susceptibility to adsorption, fixation and precipitation in soil. These results are in agreement with those of Zhao *et al.* (2009), Yadav and Kumar (2009), Ghanshyam *et al.* (2010) and Chesti and Ali (2012) in green gram.

Effect of biofertilizers

The result presented in Tables2 and 3 reveals that the seed inoculation with *Rhizobium*, PSB and *Rhizobium* + PSB significantly enhanced organic carbon, available N, P, K, Cu, Zn, Fe and Mn content of the soil after harvesting of the Green gram. There was no significant variation in pH and EC of the soil of the experimental field due to bio fertilizer inoculation (Table 2). Biofertilizers can make a significant contribution in maintaining soil health and balancing soil fertility and have an important role in improving nutrient supplies to soil fertility build-up and have long-term effects without any adverse effects. Biofertilizers can fix atmosphere nitrogen through the process of biological nitrogen fixation (BNF), solubilizing plant nutrients and synthesis of growth-promoting substances (Chandra *et al*., 2012). Inoculation of seed with N- fixers might have increased the concentration of an efficient and healthy strain of *Rhizobium* bacteria in the root nodules which in turn might have resulted in greater

Table 3. Effect of fertility and bio fertilizer levels on available copper, zinc, iron and manganese in the soil after harvest of Green gram

Treatments	Available Cu (mg kg^{-1})	Available Zn (mg kg^{-1})	Available Fe (mg kg^{-1})	Available Mn (mg kg^{-1})
Fertility levels				
F_1: 75% RDF	2.39	2.06	3.61	3.42
F_2: 75% RDF+VC (2 t ha^{-1})	2.62	2.30	4.48	4.16
F_3: 100% RDF	2.49	2.20	3.88	3.66
F_4: 100% RDF+ VC (2 t ha^{-1})	2.66	2.36	4.64	4.30
SEm±	0.02	0.02	0.06	0.05
CD (P= 0.05)	0.07	0.07	0.17	0.16
Biofertilizer levels				
B_0: Control	2.36	2.05	3.59	3.41
B_1: *Rhizobium*	2.58	2.27	4.26	3.99
B_2: PSB	2.51	2.21	4.10	3.84
B_3: *Rhizobium* + PSB	2.66	2.35	4.63	4.29
SEm±	0.02	0.02	0.06	0.05
CD (P= 0.05)	0.07	0.07	0.17	0.16

fixation of atmosphere N- consequently higher accumulation of N in the soil. *Rhizobium* is a genus of aerobic bacteria that form nodules in the root of leguminous plants and increase nodules mass, nodules number, leg haemoglobin, nitrogenase activity which positively correlated with N -fixation. Improved soil fertility status might be due to an increase in nodules masses (dry and fresh) with the fertilizers and bio inoculants. The micro-organism responsible for nutrient transformation besides providing favourable physical properties which help in the mineralization of soil N leading to higher available N and also solubilized the native P in the soil through the release of various organic acids. The beneficial effect of micro-organisms on K availability includes minimization of the losses from leaching through the action of organic acids, released during decomposition besides minimizing losses due to fixation. These findings are similar to the results obtained by Singh *et al.* (2009)

Increased organic carbon, available N, P, K, Cu, Zn, Fe and Mn in the soil might be due to the beneficial effect of *Rhizobium* on root growth, development and nodulation of Green gram which led to more N-fixation, Green gram being legume crop which absorbed more soil nutrients from subsurface layers to meet their requirement and part of which was left in surface soil with the root residues after harvest of the crop decomposed and improved the soil fertility. *Rhizobium* and PSB improve the N and P status of the soil. Combined inoculation of N- fixers and PSB benefit the plant than either group of organisms alone and may have added advantage in the degraded agro-ecosystem. Dual inoculation might have contributed something towards enhanced plant growth and increased the soluble P. Increased nodulation under *Rhizobium* + PSB inoculation might be due to the close association of both the microbial population and their activities resulting in improving soil fertility status. These findings are similar to the results obtained by Thenua *et al*. (2010), Singh *et al.* (2012) and Kumari *et al.* (2012).

Biological nitrogen fixation is of great practical importance since the potential environmental hazards of nitrogenous fertilizers have raised ecological concerns and also the fertilizers are becoming steadily less economic. Most nitrogen-fixing prokaryotes are free-living microorganisms found in the soil. A few forms of symbiotic associations with higher plants in which the prokaryote directly provides the host plant with fixed nitrogen in exchange for the nutrients and carbohydrates. Such symbiosis occurs in nodules that form on the roots of the plants and contain nitrogen-fixing bacteria. The most common type of symbiosis occurs between leguminous plants and soil bacteria called *Rhizobium* and the available P content of the soil with the application of phosphorus could be due to the utilization of native phosphorus with increasing rates of phosphorus which resulted in a build-upof higher soil P status. Further mungbean being legume crop absorbed more soil P from subsurface layers to meet their requirements and part of which was left in surface soil with the root residues. These findings are similar to the results obtained by Khandelwal (2012).

CONCLUSION

Based on one-year field experimentation, it can be concluded that under the agro-climatic condition of zone IV-a (Sub-humid Southern Plain and Aravali Hills) of Rajasthan, application of 100% RDF+VC @ 2 t ha^{-1} + *Rhizobium* + PSB (F_4B_3), is the better option for realizing higher symbiotic efficiency and is the better option for improved fertility status of the soil. The highest available nitrogen, phosphorus, potassium, copper, zinc, iron and manganese status of soil after harvesting of Green gram crop were observed under fertility level 100% RDF+VC @ 2 t ha^{-1} (F_4) and inoculation of *Rhizobium* + PSB (B_3) treatment as compared to 75% RDF (F_1) and control (B_0), respectively.

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