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Effect of biofertilizer on protein content in grain of summer black gram and soil nutrient status after harvest

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Abstract
Production of the pulses is not adequate to meet the national demand in India. Along with extremely limited use of biofertilizers farmers are still facing a diverse technological gap in the cultivation of black gram. The present investigation is carried to study the effect of different treatments for summer black gram on the quality of grain and post-harvest soil fertility status of the soil. Field experiment is conducted during the summer season of the years 2020 and 2021 on Black gram variety Gujarat 1 in fruit zone area of Marwadi University, Gauridad, Rajkot (Gujarat). Six treatments comprised of alone and combined application of chemical fertilizers (50% and 100% RDF) and biofertilizers (Seed treatment with Rhizobium and application PSM on the soil) following randomized block design (RBD) with four replications. The results of the experiment revealed that treatment T6 (Rhizobium inoculation + PSM +100% RDF) produced significantly the maximum protein content 25.18% which was superior over of other treatments in the pooled results. Treatment T6 (Rhizobium inoculation + PSM +100% RDF) increased significantly the available nutrients such N and P in the soil after harvest of the crop. However, available nitrogen in the soil after harvest remained statistically on par with treatments T2, T5 and T3 in the pooled results. The data indicated that treatment effect was found non-significant in both the years as well as in pooled results, which reveals that the treatments did not exert any adverse effect on available potassium in the soil after harvest of summer black gram.

Keywords: Protein content, soil nutrient status, biofertilizers, RDF, summer black gram

Introduction

Legumes grain provides about one-third of human dietary protein. (Htwe *et al*., 2019) [7] In general in developing countries, grain legumes are an important protein source to a large sector of the vegans' population and in special in India. (Shroti *et al*., 2018). During 2017-18 with an 841 kg/ha productivity rate, the pulses cultivation area with more than 29 Million hectares produced the highest ever production of 25.23 million tonnes (Keifer & Effenberger, 2018) [12].
In India the per capita net availability of pulses has reduced from 60.7 gm day -1 in 1951 to 47.9 gm day –1 in
2019 (Agricultural Statistics at a glance 2019, 2020). As against FAO recommended For Indian adults an intake of 60 – 120 grams of pulses per day if the diet includes plant- based food and dairy products and 30 – 60 grams per day if the diet includes a portion of an egg, meat, or fish. (FAO, 2019) [6]. So in fact, the production of the pulses is not adequate to meet the national demand.
Black gram known as “urd” or “urd bean” (Shekhawat *et al*., 2018). The cultivated black gram belongs to the Leguminosae family, sub-family Papilionaceae (Sathees *et al*., 2019) [22]. A black gram contains approximately 26% protein and is rich in sodium, phosphorus, calcium, potassium and vitamin B1, B3, and A all of which contribute to its high nutritive value (Dineshkumar *et al*., 2020) [5]. The proteins of these beans are valued for their high digestibility and freedom from flatulence. In addition, this crop has a lot of medicinal benefits (Hussain *et al*., 2014) [8]. The crop has an important role to play as well in maintaining and improving the soil fertility by symbiotic fixation of atmospheric nitrogen through root nodules which take possession of Rhizobium bacteria (Jangir *et al*., 2016) [10]. In India, foodgrains occupy 65% of the total gross cropped area comprising pulses in about 15%. within pulses, black gram occupies the second position 3% after gram 5% of the gross cropped area. Black gram occupies the third position with a 13.40% production share in total pulses after gram (46 percent), and tur (17%) under the individual crop category. In India, the Black gram yield is around 352 kg ha-1 during 2017-2018 (Keifer & Effenberger, 2018) [12].

Poor soil fertility is the main factor contributing to black gram's low productivity. It is estimated that Indian soils are medium to poor in terms of both nitrogen and phosphorus availability (Shekhawat *et al*., 2018).

It is necessary to develop new and different methods to reduce environmental pollution and to increase crop production due to increased demand for agricultural products, as well as the use of chemical fertilisers has become more restricted (Celador-lera *et al*., 2018) [4]. There are many important advantages of using bio-fertilizers where it reduces environmental pollution, It doesn't leave any residues behind in the soil, is cost-effective and environmentally friendly, improves nutrient absorption up to 25%, improves crop yields up to 15%, improves product quality and reduces fertilizer use up to 25%. Rhizobium is commonly used in biofertilizers, and P- solubilizers are commonly used (Kumar, 2018) [13]. Effective, efficient rhizobial N-fixing bacteria is used as a substitute for nitrogen fertilizer, either individually or in combination with it (Htwe *et al*., 2019) [7]. A bacterium called Rhizobium fixes nitrogen from the atmosphere as it colonizes legume roots (Baliah *et al*., 2017) [3]. By harbor nitrogenase, the enzyme complex that stimulates the converting nitrogen from the atmosphere to ammonia so it can be used by plants (Celador-lera *et al*., 2018) [4]. It is distributed widely in tropical soil areas that have the capacity to nitrogen fixation gas in symbiotic association and the population of it in soil determines the success of symbiotic biological nitrogen fixation. The quantity of nitrogen that is fixed relies on the plant species, as well as environmental conditions and Rhizobium strain (Kant *et al*., 2017) [11]. Rhizobium is found in low to medium amounts in about 40% of pulse-growing regions. So grain inoculation with bio-fertilizer (Rhizobium and PSB) are low-cost inputs and are known to increase pulse productivity by 10-12% (Keifer & Effenberger, 2018) [12]. Furthermore, Phosphate Solubilizing microorganisms provide an important means for the solubilization of phosphorous that isn't readily supplied to plants in an organic form and then make it available for their uptake once soluble. In recent years, bio-fertilizers have been integrated into nutrient supply systems in farming on a large scale as a promising component (Palaniraja, 2018) [19]. The present investigation is carried to study the effect of different treatments for summer black gram on the quality of grain and post- harvest soil fertility status of the soil.

Materials and Methods

The experiment was conducted in the fruit zone area of Marwadi University, Gauridad, Rajkot (Gujarat) during the summer season of the years 2020 and 2021. Before fertilizer application and sowing, soil samples from 0-15 cm profile were drawn randomly from each plot and a composite sample was prepared and analyzed for physico- chemical properties of soil. The average values of soil analysis along with methods followed are furnished in Table-1. Also, Soil samples were drawn from 0 to 15 cm depth by screw augar after harvesting from each plot and determined NPK content in the soil. The black gram variety Gujarat.1 was selected for this study. Randomized Block Design (RBD) was used with six treatments and four replications and each consisted of a T1= Absolute control, T2= Recommended dose of fertilizer (RDF) 20-40-0 Kg NPK/ha, T3= Rhizobium inoculation + 50% RDF, T4=
Phosphate Solubilizing Microorganism (PSM) + 50% RDF, T5= Rhizobium inoculation + PSM + 50% RDF, T6= Rhizobium inoculation + PSM + 100% RDF. The crop was fertilized with farm yard manure at the rate @5 t ha-1 in all plots. Chemical fertilizer was applied as per treatments. PSM was applied at basal application along with irrigation water @1 L/ha. The seeds were treated with Rhizobium inoculation before sowing @10 ml/Kg seed as per treatments. The crop was sown on 18 th March. 2020 and 1st March 2021 at a depth of 5 cm keeping inter row spacing of 45 cm using recommended seed rate of 20 kg ha-1. The protein content of black gram grains was worked out by multiplying nitrogen content in grain (%) with the factor of 6.25 as reported by Angelo and Mann (1973) [2]. Statistical analysis of the data for the growth parameters and yield attributing characters, yield were carried out through the procedure appropriate to the design RBD of the experiment.

Results and Discussion

Protein content

The data regarding the effect of different treatments on the Protein content of black gram are presented in Table-2 and their analysis of variance is furnished in Appendix-I. Critical examination of data in Table -2 revealed that the different treatments exhibited their significant effect on protein content in grains during 2020, 2021 and in pooled results. In the year 2020 treatment T6 (Rhizobium inoculation + PSM +100% RDF) recorded significantly higher protein content (25.88%) over rest of the treatments except treatment T2 (Recommended dose of fertilizer (RDF) 20-40-0 Kg NPK/ha) and T5 (Rhizobium inoculation + PSM + 50% RDF). Treatment T1 recorded significantly lower protein content (20.25%). In the second year significantly maximum protein content 24.49% was observed with treatment T6 (Rhizobium inoculation + PSM +100% RDF), which was at par with other treatments T2, T5, T3 and T4. Significantly the lowest protein content 19.75% was observed with treatment T1 (Absolute control). Significantly maximum protein content 25.18% was recorded with treatment T6 (Rhizobium inoculation + PSM +100% RDF) in pooled results.Treatments T2, T5, T3 and T4 were found at par with each other and inferior to treatment T6. Treatment T1 (Absolute control) was found significantly inferior by recording the lowest protein content 20.00% in pooled results.

After combination RDF and biofertilizers have been applied, the protein content may have increased because the availability of nitrogen and phosphorus was present in optimum quantities. It is a result of the mutually beneficial and synergistic roles played by each group of biofertilizers used that an additive effect has been observed. Where seed inoculation with Rhizobium may be attributed to the nitrogen consumption of plant increases due to the biological nitrogen fixation by Rhizobium. Also, PSM application helps in more fixation of atmospheric N in the soil. It is also possible that increased phosphorus availability may have contributed to increased nitrogen uptake by plants and eventually accumulated as protein in grains. The results are in accordance with Nandania

(2005) [16] on the effect of Rhizobium + PSM + 100% RDF in respect of protein content in grain of gram, Mutkule (2009) [15] on the effect of Rhizobium + 10:20 NP in respect of protein content in grain of black gram, Baliah *et al.* (2017) [3] on the effect of Rhizobium + urea 1% in respect of protein content in grain of black gram and Shekhawat *et al.* (2018) on the effect of Rhizobium + PSB + 40 kg P2o5 /ha in respect of protein content in grain of black gram.

Available nutrients in soil after harvest of the crop

The data on available nitrogen, phosphorus and potassium in the soil after harvest is given in Table-3. The analysis of variance is furnished in Appendix I. Data indicated that the effect of different treatments on NP available in the soil after harvest was found significant in both the years as well as in pooled results. Whereas in the case of K available in the soil after harvest was not significantly affected due to various treatments.

In the first year significantly higher available Nitrogen in the soil after harvest (245.25 kg/ ha) was recorded with treatment T6 (Rhizobium inoculation + PSM + 100% RDF), but it was statistically on par with treatment T5, T3 and T2. Lower N available in soil (204 kg/ ha) was reported in treatment T1 (Absolute control) which was statistically at par with treatment T4. In the Second year significantly higher available Nitrogen in the soil after harvest (241.25 kg/ ha) was recorded with treatment T6 (Rhizobium inoculation + PSM + 100% RDF), but it was statistically on par with other treatments T5, T3, T4 and T2. On the other hand, significantly, the minimum N available in soil (199.25 kg/ ha) was observed with treatment T1 (Absolute control). In the pooled results the treatment T6 (Rhizobium inoculation + PSM +100% RDF) produced significantly the highest available nitrogen in the soil after harvest (243.25 kg/ ha) which was statistically on par with treatments T5, T3 and T2. Significantly the lowest available nitrogen (201.63 kg/ ha) was recorded with treatment T1 (Absolute control) which was statistically on par with T4.

In the year 2020 significantly higher available phosphorus in the soil after harvest (43 kg/ ha) was recorded with treatment T6 (Rhizobium inoculation + PSM + 100% RDF), but it was statistically on par with treatment T2 (RDF 20-40-0 (NPK Kg /ha)). Treatments T5 (Rhizobium inoculation + PSM + 50% RDF), T3 (Rhizobium inoculation + 50% RDF), T4 (PSM + 50% RDF) found at par with each other and inferior to treatment T6 and T2.Lower available phosphorus in soil (30 kg/ ha) was reported in treatment T1 (Absolute control). In Second year significantly higher available phosphorus in soil after harvest (43.5 kg/ ha) was recorded with treatment T6 (Rhizobium inoculation + PSM + 100% RDF), but it was statistically on par with treatment T5 (Rhizobium inoculation + PSM + 50% RDF) and T2 (RDF 20-40-0 (NPK Kg /ha)). Significantly lower phosphorus available in soil (33 kg/ ha) was reported in treatment T1 (Absolute control) which was statistically at par with treatment T4 (PSM + 50% RDF), T3 (Rhizobium inoculation + 50% RDF). In the pooled results treatment T6 (Rhizobium inoculation + PSM +100% RDF) recorded significantly higher available phosphorus in the soil after harvest (43.25 kg/ ha) over rest of the treatments. Treatment T1 recorded significantly lower available phosphorus (31.5 kg/ ha).

The higher available potassium in soil after harvest (247.75 kg/ ha) (238.50 kg/ ha) (243.13 kg/ ha) was recorded with treatment T6 (Rhizobium inoculation + PSM + 100% RDF) in 2020 and 2021 and in pooled results, respectively. Lower potassium in soil after harvest (224.75 kg/ ha) (218.25 kg/ ha) (221.5 kg/ ha) in 2020 and 2021 and in pooled results, respectively was reported in treatment T1 (Absolute control).

The application of recommended doses of fertilizer and biofertilizers may increase the availability of nitrogen and phosphorus in the soil to the optimum quantities may be due to continuous symbiotic fixation of nitrogen by Rhizobium and solubilizing action of PSM on fixed soil phosphorus through the production of organic acids and bringing down the soil PH. Reduction in available potassium status of soil may be due to its increased uptake with microbial inoculums application. The results are in accordance with Nandania *et al.* (2005) [16] on the effect of Rhizobium + PSM + 100% RDF in respect to available NP status in the soil after harvest of gram, Kant *et al.* (2017) [11] on the effect of Rhizobium + PSB + 75 kg ha-1 in respect to available NP status in the soil after harvest of black gram, Nelwade *et al.* (2019) [17] on the effect of Rhizobium phaseoli + Pseudomonas striata + RDF in respect of NP status in the soil after harvest of black gram. And Mandale *et al.* (2021) [14] on the effect of Rhizobium + RDF in respect to available NP status in the soil after harvest of mung bean.

Table-1: Phsio-chemical properties of the experimental Field

Particulars	Value at soil depth 0-15 (cm)		Method followed
	2020	2021	
A. Physical properties			
1. Sand (%)	23.65	22.40	International pipette method (Piper, 1950) [20]
2. Silt (%)	12.25	13.10	
3. Clay (%)	64.10	64.50	
4. Textural class	Clayey	Clayey	
B. Chemical properties			
1. Available nitrogen (kg ha-1)	235.5	230.10	Alkaline permanganate method (Subbaiah and Asija, 1956)
2. Available P2O5 (kg ha-1)	34	36	Olsen's method (Olsen *et al.*, 1954) [18]
3. Available K2O (kg ha-1)	260	251	Flame photometric method (Jackson, 1974) [9]
4. Organic Carbon (%)	0.85	0.80	Walkey and Black method (Jackson, 1974) [9]
5. Soil pH (1:2.5 soil: water ratio)	7.9	7.8	PH meter (Richard, 1954) [21]
6. Electrical Conductivity (dSm-1 at 25°C)	0.50	0.45	EC meter (Jackson, 1974) [9]

Table 2: Effect of different treatments on Protein content of black gram (%)

Treatment	Protein content		
	2020	**2021**	**Pooled**
T1=Absolute control	20.25	19.75	20.00
T2= RDF 20-40-0 (NPK Kg /ha)	23.35	23.31	23.33
T3= Rhizobium inoculation + 50% RDF	23.03	23.13	23.08
T4= PSM + 50% RDF	22.50	21.80	22.15
T5= Rhizobium inoculation + PSM + 50% RDF	23.20	23.18	23.19
T6= Rhizobium inoculation + PSM + 100% RDF	25.88	24.49	25.18
SEm±	0.93	1.11	0.63
C.D. at 5%	2.81	3.34	1.81
C.V%	7.01	8.49	7.77

RDF: Recommended dose of fertilizers, PSM: Phosphate Solubilizing microorganisms, C.V: Coefficient of variation, C.D: Critical difference, NS- Non Significant at *P*>0.05

Table 3: Effect of different treatments on available nitrogen, phosphorus and potassium in soil after harvest of summer black gram (Kg ha-1)

Treatment	Available nitrogen (Kg/ ha)			Available phosphorus (Kg/ ha)			Available potassium (Kg/ ha)		
	2020	**2021**	**Pooled**	**2020**	**2021**	**Pooled**	**2020**	**2021**	**Pooled**
T1=Absolute control	204.00	199.25	201.63	30.00	33.00	31.50	224.75	218.25	221.50
T2= RDF 20-40-0 (NPK Kg /ha)	233.00	228.75	230.88	37.50	37.25	37.38	228.50	220.00	224.25
T3= Rhizobium inoculation + 50% RDF	234.25	237.00	235.63	35.50	35.25	35.38	236.75	237.00	236.88
T4= PSM + 50% RDF	220.25	233.50	226.88	36.00	36.75	36.38	238.25	236.00	237.13
T5= Rhizobium inoculation + PSM + 50% RDF	239.75	238.00	238.88	36.50	37.75	37.13	242.25	237.25	239.75
T6= Rhizobium inoculation + PSM + 100% RDF	245.25	241.25	243.25	43.00	43.50	43.25	247.75	238.50	243.13
SEm±	8.24	7.98	4.97	2.04	2.17	1.29	9.36	9.47	5.76
C.D. at 5%	24.84	24.05	14.34	6.16	6.56	3.73	NS	NS	NS
C.V%	6.22	6.02	6.12	9.73	10.11	9.93	6.85	7.09	6.97

RDF: Recommended dose of fertilizers, PSM: Phosphate Solubilizing microorganisms, C.V: Coefficient of variation, C.D:Critical difference, NS- Non Significant at *P*>0.05

Conclusion

For getting maximum protein content in grains of summer black gram crop it should be fertilized with the recommended dose of fertilizer ((RDF) 20-40-0 Kg NPK/ha) along with seed inoculation with rhizobium and soil application of Phosphate Solubilizing Microorganism.

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Appendix I: Analysis of variance (M.S.S.) of different characters

	Year 2020			**Year 2021**			**pooled**				
S V	**R**	**T**	**E**	**R**	**T**	**E**	**R/Y**	**Y**	**T**	**Y × T**	**E**
d. f.	3	5	15	3	5	15	6	1	5	5	30
Characters											
Protein content (%)	9.99	12.99	2.60	2.27	10.77	3.68	6.13	2.15	23.12	0.64	3.14
Available nitrogen (Kg ha-1)	207.61	898.97	203.74	111.82	958.48	190.92	159.72	0.52	1760.43	97.02	197.33
Available phosphorus (Kg ha-1)	28.28	69.37	12.54	11.72	49.30	14.19	20.00	8.33	115.73	2.93	13.57
Available potassium (Kg ha-1)	123.49	291.78	262.55	171.00	351.77	268.77	147.24	325.52	616.57	26.97	265.66