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Effect of rhizobium, PSB and fertility levels on nutrient contents and their removal and yield of blackgram (*Vigna mungo* L.) Under custard apple (*Annona squamosa* L.) Based agri-horti system in Vindhyan region of Uttar Pradesh

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Abstract
An experiment was conducted at the Agricultural Research Farm of RGSC, Banaras Hindu University, Brakachha, Mirzapur during the rainy (kharif) season 2011. Results showed that application of 36 kg N, 70 kg P_2O_5 and 30 kg S/ha gave the maximum values of grain yield as well as removal of N, P and S over lower fertility levels. Seed inoculation of Rhizobium and phosphate solubilizing bacteria (PSB) was also found to increased yield. Removal of N, P, and S was significantly increased with combination of Rhizobium and PSB inoculants as compared with single inoculation of either Rhizobium or PSB and the control (No biofertilizers).

Keywords: Agroforestry, Biofertilizers, Blackgram, Custard apple, Fertility levels

1. Introduction
Agri-horti system (fruit based agroforestry system) is an improved indigenous cropping system in India for full utilization of the growing season and markedly increasing the return per unit area per unit time. Tree based cropping system have proved to be very successful in areas receiving less than 1000 mm rainfall with nine months of dry season. Agroforestry system complement conservation agriculture systems in the provision of soil cover, animal feed, nutrients, household fuel, and hillside protection against soil erosion and wind erosion control through shelter belts. Custard apple (*Annona squamosa* L.) is distributed throughout the tropics and is pre eminently a desert fruit, normally eaten fresh. Blackgram (*Vigna mungo* L. Hepper) is an important pulse crop having high nutritive value, contains about 26% protein which is grown during rainy (*kharif*) as well as summer (*zaid*) seasons in arid and semi-arid regions of India. In total pulse production, black gram occupies an area of 3.25 million hectares with production of 1.45 million tones in India. In India pulse production is very low due to the several factors but most important factor is nutrient management. The nutrient requirement of crop is met by the chemical fertilizers. However, fertilizer alone cannot sustain productivity of land in modern farming. Similarly, nutrient supply through organic manures and biofertilizers can hardly fulfill the need of a crop. So an integration of organic and inorganic nutrient sources sustains the productivity and may improve the soil properties. Biofertilizers are natural and organic fertilizer that increase availability of nitrogen and phosphorus besides increase in biological fixation of atmospheric nitrogen and enhance phosphorus availability to crop. Inoculation of seeds with Rhizobium culture is a very low cost method of nitrogen fertilization in legume and has been found beneficial. Phosphorus has novel function of special importance in the process of energy storage, transfer, roots proliferation, nodules development, bacterial activity and nitrogen fixation. Sulphur is one of the essential plant nutrients and it contributes to yield and quality of crops. The legume crops are more susceptible for sulphur deficiency. Since blackgram is a legume crop it is quite likely that it may respond sulphur. Therefore, present research was carried out to study the effect of seed inoculations, various combinations of nitrogen, phosphorus and sulphur on growth, yield economics and nutrient removal by blackgram.

Materials and methods

The field experiment was conducted at the Agricultural Research Farm of Rajiv Gandhi South Campus, Banaras Hindu University, Brakachha, Mirzapur (25º 10' N and 82º 37' E and 427 m above mean sea level) during rainy (*kharif*) season 2011 under the 4-year old custard apple (*Annona squamosa* L.) cv. 'Mammoth' planted at a spacing of 5 m x 5 m. The following growth parameters of custard apple, grown at border of the plot, were recorded at the scheduled dates of blackgram. The soil was sandy loam having pH 6.45, electric conductivity 0.29 dS/ m, low in organic carbon (0.35%), and available nitrogen, phosphorus and sulphur were 167.7, 17.0 and 9.5 kg/ ha, respectively. The experiment was laid out in a randomized block design with three replication and a set of 12 treatments involving four levels of biofertilizers [No biofertilizer, Rhizobium (Rhizobium, MOR-1), PSB (Pseudomonas aeruginosa, PSBBHU01) and Rhizobium + PSB] with 3 levels of fertility (control, *i.e*. no N or P_2O_5, 12 kg N + 30 kg P_2O_5 + 10 kg S, 24 kg N + 50 kg P_2O_5 + 20 kg S and 36 kg N + 70 kg P_2O_5 + 30 kg S/ ha). Blackgram cv. 'Type-9' was sown manually on 1st August, 2011 in the row by kudal at a distance of 45 cm as per treatment in between two-row of custard apple as alley crop. Relatively a higher seed rate (20 kg/ha) was used for proper maintenance of plant population. A plant spacing of 10 cm within the row was maintained by thinning done about 15 days after sowing. The agronomic practices were followed as per recommendation and harvesting was done on 17th October. The total rainfall, mean maximum and minimum temperatures during the crop season was 458.6 mm, 30.9ºC and 18.2ºC, respectively. Grain and straw samples were dried, processed and analysed for their total N content by micro Kjeldahl's, P by Vanadomolybdo phosphoric acid-yellow colour method and K was estimated by flame-photometer. Nutrient removal was estimated by multiplying the content with the oven-dry weight of biological yield. The samples were oven dried at 700C ± 20C till constant weight was achieved. Economics of the treatments were computed on the basis of the prevailing market price of produce and agro inputs.

Result and discussion

Effect of NPS levels on contents (%)

Higher concentration of nitrogen, phosphorus and sulphur in grain and straw of blackgram were recorded under 36, 70, 30 kg NPS however in respect of phosphorus content in grain, it remained at par with 24, 50, 20 kg NPS ha^{-1}. As stated earlier, the adequate supply of nitrogen, phosphorus along with sulphur play a vital role in metabolic process of photosynthesis that result in increased flowering and fruiting thereby improving number of pods $plant^{-1}$, grains pod^{-1} and test weight. The significant increase in test weight due to the application medium level of fertility might be on account of better removal and translocation of nutrients, especially phosphorus, resulting in hold seed formation by increasing the size and weight of grains. The results are in close accordance with findings of Kumar *et al*. (2002) [3].

Effect of biofertilzers on NPS content (%)

Seed inoculation with either *rhizobium* or PSB and combined inoculation of the both significantly enhanced N, P and S contents and their removal by the crop over uninoculation. Dual inoculation of *rhizobium* and PSB recorded the maximized content and found superior to other. The inoculation of seed with *rhizobium* and PSB increased the nitrogen, phosphorus and sulphur contents in grain as well as straw. This might be due to more nitrogen fixation by the bacteria which in turn helped in better absorption and utilization of all the plant nutrients, thus resulting in more N and P contents in grain and straw. This beneficial influence might be due to better root establishment by nodulation, nitrogen fixation from the atmosphere.

Effect on NPS removal by grain and straw (kg/ha)

The nutrient removal is an integrated function of soil-crop environment, together with amounts and source of nutrient supply and cultivars of the crop. Higher nitrogen, phosphorus and sulphur removal by grain and straw of blackgram were recorded under 36, 70, 30 kg NPS however in respect of phosphorus content in grain, it remained at par with 24, 50, 20 kg NPS/ ha. This might be due to higher yield and nutrient concentration. In general, the system which produced higher dry matter, removed more nutrients than the one which produced less. The results are in close conformity with the findings of Shahi *et al*. (2002) [2], Singh and Pareek (2003) [5], and Yakadry (2004) [6].

Effect of biofertilzers on NPS removal by grain and straw (kg/ha)

The N-content in grain and straw, and N-removal by grain, straw and total N-removal by blackgram were also affected significantly by *Rhizobium* inoculation. It was due to higher dry matter accumulation and nitrogenase activity under *Rhizobium* inoculation which beneficially improved bacterial population in rhizosphere which in turn improved N-content in grain and straw and N-removal by grain, straw and total N-removal. *Rhizobium* inoculation fixes nitrogen through nodules of the plant whereas PSB solubilizes native P rendering more phosphorus to soil solution. Thus, combined inoculation of grain with *Rhizobium* and PSB improved N, P and S status of soil and ultimately increased N, P and S removal. Nitrogen, phosphorus and sulphur removal by the plant increased considerably with corresponding increase in nitrogen, phosphorus and sulphur levels. Similar finding were also reported by Beg and Singh (2009) [2] in respect of nitrogen and phosphorus.

Effect on yield

Effect on yield Distinct positive effect of NPS levels was noticed on yield viz. grain and straw yields and harvest index. All these parameters attained higher values with increasing NPS levels. The highest level 36, 70, 30 kg NPS/ ha produced maximum grain yield as a consequence of its favorable effect on number of pods/ plant, grains/ pod and test weight and this ultimately lead to maximum grain yield/ ha. Straw yield is a function of vegetative growth. Increasing levels of NPS up to 36, 70, 30 kg/ ha augment plant height, number of trifoliate leaves/ plants, branches/ plant and dry matter production which finally resulted in higher straw yield. The high yield associated with higher level of sulphur application under NPS level may be due to its greater uptake and active participation in all structure, carbon assimilation, photosynthesis, starch formation, translocation of protein and sugar, entry of water into plants root and development etc. The yield was obtained from the seed inoculation with Rhizobium increased due to higher number of pods/ plant, grains/ pod, test weight were the major parameters governing the yield. The significant increase in grain and straw yields appeared to be on account of the beneficial effects of Rhizobium and PSB on growth and yield attributes which finally reflected in higher yield of blackgram. The results are in accordance with the finding of Singh and Pareek (2003) [5].

Table 1: Effect of different levels of NPS and biofertilizers on content and removal of nitrogen at harvest of blackgram

Treatment	Nitrogen content (%)		Nitrogen removal (kg ha^{-1})		Total (Grain + Straw) removal of Nitrogen (kg ha^{-1})
	Grain	Straw	Grain	Straw	
NPS levels					
$N_{12}P_{30}S_{10}$*	3.48^{b}	1.57^{b}	23.74^{c}	30.63^{b}	54.37^{b}
$N_{24}P_{50}S_{20}$	3.68^{b}	1.79^{a}	29.77^{b}	42.79^{a}	72.56^{a}
$N_{36}P_{70}S_{30}$	3.76^{a}	1.88^{a}	32.08^{a}	44.63^{a}	76.71^{a}
SEm±	0.07	0.04	0.58	1.43	1.74
LSD (*P*=0.05)	0.21	0.10	1.70	4.20	5.10
Biofertilizers					
No biofertilizer**	3.51^{a}	1.69^{a}	25.45^{c}	36.86^{b}	62.31^{b}
Rhizobium	3.68^{a}	1.76^{a}	27.95^{b}	38.52^{b}	66.47^{b}
PSB	3.63^{a}	1.74^{a}	27.80^{b}	37.24^{b}	65.04^{b}
Rhizobium + PSB	3.74^{a}	1.78^{a}	32.93^{a}	44.77^{a}	77.70^{a}
SEm±	0.08	0.04	0.67	1.65	2.01
LSD (*P*=0.05)	NS	NS	1.96	4.85	5.89

*N_{12} P_{30} S_{10}: 12 kg N, 30 kg P_2O_5 and 10 kg S ha^{-1}
**No Bio: No biofertilizer of *Rhizobium* and phosphate solubilizing bacteria (PSB)

Table 2: Effect of different level of NPS and biofertilizers on content and removal of phosphorus at harvest of blackgram

Treatment	Phosphorus content (%)		Phosphorus removal (kg ha^{-1})		Total (Grain + Straw) removal of phosphorus (kg ha^{-1})
	Grain	Straw	Grain	Straw	
NPS levels					
$N_{12}P_{30}S_{10}$*	0.45^{b}	0.19^{a}	3.08^{c}	3.67^{b}	6.75^{b}
$N_{24}P_{50}S_{20}$	0.51^{a}	0.20^{a}	4.11^{b}	4.86^{a}	8.97^{a}
$N_{36}P_{70}S_{30}$	0.54^{a}	0.21^{a}	4.61^{a}	4.89^{a}	9.50^{a}
SEm±	0.02	0.01	0.1^{3}	0.15	0.24
LSD (*P*=0.05)	0.05	NS	0.39	0.45	0.71
Biofertilizers					
No biofertilizer**	0.46^{b}	0.19^{a}	3.35^{c}	4.22^{b}	7.57^{c}
Rhizobium	0.49^{a}	0.20^{a}	3.74b^{c}	4.29^{b}	8.03b^{c}
PSB	0.51^{a}	0.20^{a}	3.93^{b}	4.38^{b}	8.31^{b}
Rhizobium + PSB	0.53^{a}	0.20^{a}	4.71^{a}	5.00^{a}	9.71^{a}
SEm±	0.02	0.01	0.15	0.18	0.28
LSD (*P*=0.05)	0.05	NS	0.45	0.52	0.82

*N_{12} P_{30} S_{10}: 12 kg N, 30 kg P_2O_5 and 10 kg S ha^{-1}
**No Bio: No biofertilizer of *Rhizobium* and phosphate solubilizing bacteria (PSB)

Table 3: Effect of different levels of NPS and biofertilizers on content and removal of sulphur at harvest of blackgram

Treatment	Sulphur content (%)		Sulphur removal (kg ha^{-1})		Total (Grain + Straw) removal of sulphur (kg ha^{-1})
	Grain	Straw	Grain	Straw	
NPS levels					
$N_{12}P_{30}S_{10}$*	0.32^{b}	0.12^{a}	2.52^{c}	2.36^{b}	4.57^{b}
$N_{24}P_{50}S_{20}$	0.36^{a}	0.13^{a}	2.89^{b}	3.03^{a}	5.92^{a}
$N_{36}P_{70}S_{30}$	0.38^{a}	0.12^{a}	3.21^{a}	2.97^{a}	6.18^{a}
SEm±	0.01	0.00	0.08	0.09	0.12
LSD (*P*=0.05)	0.02	NS	0.22	0.27	0.34
Biofertilizers					
No biofertilizer**	0.35^{a}	0.13^{a}	2.52^{b}	2.74^{b}	5.26^{b}
Rhizobium	0.35^{a}	0.12^{a}	2.67^{b}	2.67^{b}	5.34^{b}
PSB	0.35^{a}	0.12^{a}	2.72^{b}	2.62^{b}	5.34^{b}
Rhizobium + PSB	0.36^{a}	0.12^{a}	3.18^{a}	3.11^{a}	6.29^{a}
SEm±	0.01	0.00	0.09	0.09	0.14
LSD (*P*=0.05)	NS	NS	0.26	0.27	0.40

*N_{12} P_{30} S_{10}: 12 kg N, 30 kg P_2O_5 and 10 kg S ha^{-1}
**Bio: No biofertilizer of *Rhizobium* and phosphate solubilizing bacteria (PSB)

Table 4: Effect of different levels of NPS and biofertilizers on grain and straw yields and harvest index of blackgarm

Treatment	Yield (kg ha^{-1})		Harvest index (%)
	Grain	Straw	
NPS levels			
$N_{12}P_{30}S_{10}$*	682.1^{c}	1947.1^{b}	25.94^{b}
$N_{24}P_{50}S_{20}$	806.2^{b}	2381.6^{a}	25.57^{b}
$N_{36}P_{70}S_{30}$	853.3^{a}	2384.4^{a}	26.45^{a}
SEm±	8.10	59.49	0.54
LSD (*P*=0.05)	23.76	174.47	1.59
Biofertilizers			
No biofertilizer**	722.7^{c}	2163.9^{b}	25.03^{a}
Rhizobium	758.7^{b}	2182.0^{b}	25.78^{a}
PSB	763.2^{b}	2128.6^{b}	26.45^{a}
Rhizobium + PSB	877.5^{a}	2476.4^{a}	26.55^{a}
SEm±	9.35	68.69	0.63
LSD (*P*=0.05)	27.43	201.47	NS

*N_{12} P_{30} S_{10}: 12 kg N, 30 kg P_2O_5 and 10 kg S ha^{-1}

**No Bio: No biofertilizer of *Rhizobium* and phosphate solubilizing bacteria (PSB)

Conclusion

From the present studies, it is concluded that the dual inoculation of blackgram variety 'Type-9' intercropped in alleys of custard apple with Rhizobium and phosphate solubilizing bacteria (Bacillus subtilis) along with 30 kg N, 70 kg P_2O_5 and 30 kg S/ha NPS level proved beneficial for boosting grain yield (853.3 kg/ ha) and thus it could be recommended for profitable production under the Vindhyan region.

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