Estimation of effect of *Rhizobium* inoculant, phosphorus and sulphur on the growth and yield of blackgram

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Abstract: An experiment was conducted at the experimental field of Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh during November 2013 to January 2014 to study the effect of P, S and *Bradyrhizobium* inoculant on nodulation, yield and yield contributing characters of blackgram. Eight treatments were: Control, Inoculum, P, P+ lnoculum, S, S + Inoculum, P + S, and P + S + Inoculum. The experiment was laid out in a Randomized Complete Block design with three replications. The rates of P, S and *Bradyrhizobium* inoculant were 40 kg P ha,$^{-1}$ 15 kg S ha^{-1} and 15 g inoculant kg^{-1} seed, respectively. TSP was used as a source of P and gypsum for S. Basal application of 40 kg K ha^{-1} and 2 kg Zn ha^{-1} was made to all plots. At 30 and 55 DAS, 5 plants were uprooted from each plot for recording data on nodulation and growth characteristics. The crop was harvested at maturity at 90 DAS. The results indicated that combined application of chemical and biofertilizer performed better than single application of fertilizer in respect of plant height (15.50 cm), dry matter plant^{-1} at 30 DAS (310.3 mg) and 55 DAS (4487mg); and number of nodules plant^{-1}at both 30 DAS (14.67) and 55 DAS (27.00). Yield and yield contributing characters like number of seed weight plant^{-1}, seed yield, stover yield were significantly influenced by biofertilizer application. Thus *Bradyrhizobjum* inoculation was found essential for blackgram cultivation at BINA Farm and an application of P and S further increased the yield.
Key words: *Rhizobium* inoculant, phosphorus, sulphur and blackgram.

Introduction

Blackgram *(Vigna mungo*) is one of the most important pulse crops grown in Bangladesh. The cultivated blackgram belongs to the family Leguminosae, sub-family Papilionaceae. It is mainly a day neutral warm season crop commonly grown in semi-arid to sub-humid low land tropics and sub-tropics. Pulse originated in Southeast Asia and widely grown in India, Pakistan, Bangladesh, Myanmar, Thailand, Philippines, China and Indonesia. Different kinds of pulses are grown and consumed by the people of Bangladesh. Among them blackgram locally known as maskalai, grows well in north or north-west part of Bangladesh especially in Rajshahi and Chapai Nowabganj districts. It is mainly grown for human consumption but also used as fodder for cattle and green manure for soil fertility. Seeds are mainly cooked, as 'Dal' in our country. Being a legume crop, blackgram has the ability to fix atmospheric nitrogen symbiotically with the nodule producing bacteria *Rhizobium* sp. Responses of blackgram to added fertilizers such as nitrogen and phosphorus have been found to vary with soil conditions.

Pulse crop covers the area of about 1042000 acres where blackgram occupies only 62000 acres (BBS, 2012). In Bangladesh, yield of blackgram is very lower than other pulses and average yield is around 1150 kg ha^{-1} (BBS, 2012). It is generally recognized that pulses offer the most practical means of solving protein deficiency in Bangladesh but there is an acute shortage of grain legumes in relation to its requirements. Increase of pulse production is urgently needed to meet up the demand of the people to reduce import, to save foreign currency and to increase pulse consumption for maintaining good health. Increase of pulse production can also minimize the scarcity of fodder because the whole plant or its by products can he used as good animal feed.

Considering the significance of blackgram in Bangladesh context, it is therefore of utmost necessity to improve this pulse crop both in terms of its quantitative and qualitative values. Various practices may help in to achieving this goal. Application of biofertilizer seems to be the most significant in view of convenience, cost and labor efficacy.

The successful growing of blackgram is dependent on the availability of its microsymbiont bacteria in soil. All effective Rhizobium strains are present in all soils of Bangladesh. In this situation inoculation can meet the challenge of providing superior strains in the soil, so that the most effective nodulation and nitrogen fixation are obtained. Thus, it is obvious that there is a scope for utilizing the effective Rhizobial strains for obtaining more yield of blackgram under field conditions which may also play a vital role in improving soil environment and agricultural sustainability. With this view in mind, a piece of earch work was conducted to evaluate the performance of *Rhizobium* inoculants on the growth and yield of black gram; and investigate the combined effect of *Rhizobium* inoculants with P and S fertilizer.

Materials and Methods

The experiment was conducted at farm of Bangladesh Institute of Nuclear Agriculture (BINA) head quarter farm, Mymensingh; under Old Brahmaputra Floodplain (AEZ 9) during November to January 2013-2014. The crop used in this study was Blackgram *(Vigna mungo*). There were eight treatments in different combinations of biofertilizer ,P,and S replicated thrice in randomized complete block design.Details of treatment combination were given in Table 1.

Table 1. Treatment combinations of different doses of P, S and biofertilizer for Blackgram

Treatment	Combination
T_1	Control(without any fertilizer)
T_2	Inoculum
T_3	P_{40}
T4	P + Inoculums
T_5	S_{15}
T_6	S+ Inoculum
T_7	P+S
T_8	P + S + Inoculum

The *Rhizobium* strain was inoculated into yeast mannitol broth medium containing 250 ml Erlenmeyer flask under laminar flow cabinet. The inoculated flask was then placed on an electric shaker for seven days for optimal growth of

Rhizobial cell in the broth culture. After seven days, the bacterial culture was tested its purity and growth. The Rhizobial cell was counted through plate count method. The population was maintained above 10 cells per ml broth culture. The population of *Rhizobium* cells were $1.9x10^9$, $2.0x10^9$ and $2.1x10^9$ per ml broth culture respectively. All the chemical fertilizers except nitrogenous fertilizer were mixed with soil.The rates of P, S and *Bradyrhizobium* inoculant were 40 kg P ha,$^{-1}$ 15 kg S ha^{-1} and 15 g inoculant kg^{-1} seed, respectively. TSP was used as a source of P and gypsum for S. Basal application of 40 kg K ha^{-1} and 2 kg Zn ha^{-1} was made to all plots.

Five plant samples were collected from the field at 35 and 55 days of sowing and at harvest, from each plot, at random and were carefully uprooted with the help of a nirani cuttin into a soil block in the f so that no nodules were left in the soil. The soil blocks were kept for sometime in water buckets to soak before gently washingout the soil from the roots. The roots were then finally washed carefully with water. The nodules from root of each plant were collected. The length of the roots and shoots of each plant were recorded. Initial soil samples were collected and analyzed at soil science laboratory BINA,following standard procedure.(Table 2).

Table 2. Soil characteristics of the experimental site at BINA head office farm.

Properties	Value	Interpretation
Sand(%)	27.06	
Silt(%)	63.6	Silt loam
Clay(%)	9.64	
pH	6.5	Slightly acidic
OM(%)	1.57	Medium
Total N(%)	0.098	Low
Available P(mg kg-1)	13	Low
Exchangeable K(meq.100g-1 soil)	0.10	Low

Results and Discussion

The results obtained from the experiment on the growth and yield parameters of blackgram showed that the maximum numbers of nodules were recorded for the use of biofertilizer in combination with chemical fertilizers at both 30 and 55 DAS.These results revealed that use of biofertilizer increased the nodule number of blackgram. Podder (1999*),* Bhuiyan and Rahman *(*1998) and Rao and Sharma (1980) found similar findings who reported that biofertilizer increased the nodule numbers. The highest dry shoot weights (310.3 and 4487 mg $plant^{-1}$) were found for the treatment of T_8 (P + S + *Rhizobium*) at 30 and 55 days after sowing (Table 3).

Table 3. Effects of phosphorus, Sulphur and Rhizobium on Plant height, Shoot dry weight,Number of nodule and Nodule dry weight at 30 and 55 days after sowing (DAS).

Treatments	Plant height (cm) after DAS		Shoot dry weight(mg)		Nodule no. $plant^{-1}$		Nodule dry weight (mg)	
	30	55	30	55	30	55	30	55
T_1	11.40 c	19.97 c	252.3 d	4000 b	4.333 e	8.667 f	15.33 g	90.30 e
T_2	14.60 ab	23.87 ab	297.2 ab	4451 a	9.667 c	18.33 c	32.00 cd	120.3 bc
T_3	14.20 ab	23.07 ab	287.0 bc	4437 a	7.000 d	13.00 e	25.13 e	110.4 cd
T_4	15.23 ab	24.77 ab	302.7 ab	4463 a	12.00 b	21.67 b	42.37 b	127.3 b
T_5	13.20 bc	22.33 b	272.7 c	4208 ab	5.333 e	9.333 f	20.20 f	100.5 de
T_6	15.10 ab	24.27 ab	301.3 ab	4451 a	10.33 c	20.33 bc	35.43 c	125.2 b
T_7	14.23 ab	23.43 ab	290.3 abc	4443 a	7.333 d	15.67 d	28.33 de	115.2 bc
T_8	15.50 a	25.0 a	310.3 a	4487 a	14.67 a	27.00 a	47.67 a	140.3 a
$LSD_{0.05}$	1.85	2.28	20.04	311.60	1.55	2.34	3.82	12.64
Level of sign.	**	**	**	*	**	**	**	**
SE (±)	0.611	0.750	6.60	102.72	0.510	0.771	1.26	4.17
CV (%)	7.46	5.57	3.96	4.07	10.00	7.98	7.08	6.21

T_1=control; T_2=rhizobium; T_3=phosphorus; T_4= phosphorus+ rhizobium; T_5=sulphur; T_6= sulphur+ rhizobium; T_7= phosphorus+ sulphur and T_8= phosphorus+ sulphur+ phizobium, Figures having common letter in a column are not significantly different at 5% level, SE=Standard error of means CV=Co-efficient of variation

The highest grain yield (1203kg ha^{-1}) and straw yield (1503kg ha^{-1}) were found for the treatment of T_8 (Table 5). Khan *et al. (1985*), and Ghosh and Poi (1998) found similar findings who reported that biofertilizer increased the nodule numbers. At 30 DAS, interaction effect of chemical fertilizers and biofertilizer was found insignificant for most of the growth parameter. But at 55 DAS, most of the growth and yield parameters showed significant variations. Both Grain yield and straw yield were maximum for T_8. The Lowest weight of dry root (40.10 mg $plant^{-1}$), grain yield (802.0 kg ha^{-1}), straw yield (1102 kg ha^{-1}) were found for the control T_1.Besides this ,most of the other growth and yield parameters were statistically different. These results revealed that the application of lone biofertilizer was significant compared the to control (without any fertilizer).

The data on the growth and yield of blackgram were significantly influenced by the combine effect of biofertilizer and chemical fertilizers.The interaction of biofertilizer and chemical fertilizers were more efficient to produce higher number of nodules and higher yields of

blackgram compared to the control as well as application of *Rhizobium* inoculant alone.

Overall results of experiment revealed that chemical fertilizer (P, S) in addition to biofertilizer inoculant produced higher shoot weight,grain yield, leaf number, straw yield, nodule number, dry weight of nodule, plant height of blackgram under the present study which is similar to Rahman *et al.* (2008) reported that phosphorus (P), molybdenum (Mo) along with *Rhizobium* produced higher dry weight of plant tops,grain yield, straw yield,nodule number, dry weight of nodule and leaf number of blackgram.

Table 4. Effects of phosphorus, Sulphur and Rhizobium on Root length, Root dry weight, numberof leaves and leaves dry weight at 30 and 55 days after sowing (DAS)

Treatments	Root length(cm)		Root dry weight (mg)		Number of leaves		Leaves dry weight (mg)	
	30	55	30	55	30	55	30	55
T_1	9.700 c	11.92 c	40.10 d	602.7	5.333 e	11.33 e	310.5 d	2681.
T_2	11.20 ab	13.37 bc	48.03 bc	616.3	7.333 cd	13.33 bcd	340.5 abc	2738.
T_3	10.60 bc	12.70 bc	43.57 cd	608.0	6.333 de	12.33 cde	328.5 bcd	2702.
T_4	11.53 ab	14.23 b	53.50 ab	622.4	8.66 ab	14.00 b	352.5 ab	2761.
T_5	10.23 bc	12.07 c	40.73 d	603.2	6.333 de	12.00 de	320.7 cd	2692.
T_6	11.23 ab	14.07 b	50.43 bc	618.2	7.667 bc	13.67 bc	348.5 ab	2746.
T_7	10.77 bc	13.10 bc	45.53 cd	612.6	6.667 cd	12.67 bcde	330.5 bcd	2722.
T_8	12.47 a	16.43 a	57.33 a	630.5	9.667 a	15.67 a	360.4 a	2767.
$LSD_{0.05}$	1.21	1.72	6.45	32.59	1.06	1.35	24.80	186.10
Level of sign.	**	**	**	NS	**	**	**	NS
SE (±)	0.401	0.568	2.13	10.74	0.347	0.445	8.18	61.36
CV (%)	6.35	7.30	7.77	3.03	8.31	5.87	4.21	3.90

T_1=control; T_2=rhizobium; T_3=phosphorus; T_4= phosphorus+ rhizobium; T_5=sulphur; T_6= sulphur+ rhizobium; T_7= phosphorus+ sulphur and T_8= phosphorus+ sulphur+ phizobium, Figures having common letter in a column are not significantly different at 5% level, SE=Standard error of means CV=Co-efficient of variation

Table 5. Effects of P, S and Rhizobium on Seeds plant^{-1},1000 seeds weight, Grain yield and Strover yield

Treatments	Seeds plant^{-1}	1000 seed weight(g)	Grain yield (kg/ha)	Stover yield (kg/ha)
T_1	48.33 d	30.23 c	802.0 e	1102.d
T_2	60.00 c	33.33 ab	1002.cd	1320 abc
T_3	52.00 d	32.70 bc	930.0 d	1252 bcd
T_4	75.33 a	34.17 ab	1151.ab	1431 ab
T_5	49.67 d	30.70 c	895.0 de	1122 cd
*T_6	67.67 b	34.03 ab	1083.bc	1403 ab
T_7	59.67 c	32.73 bc	951.0 d	1307 abc
T_8	76.33 a	35.70 a	1203.a	1503 a
$LSD_{0.05}$	5.50	2.42	111.70	185.40
Level of significance	**	**	**	**
SE (±)	1.81	0.796	36.81	61.13
CV (%)	5.14	4.19	6.36	8.11

T_1=control; T_2=rhizobium; T_3=phosphorus; T_4= phosphorus+ rhizobium; T_5=sulphur; T_6= sulphur+ rhizobium; T_7= phosphorus+ sulphur and T_8= phosphorus+ sulphur+ phizobium, Figures having common letter in a column are not significantly different at 5% level, SE=Standard error of means CV=Co-efficient of variation

The foregoing results showed that biofertilizer *(Rhizobium* inoculant) in combination with chemical fertilizers (P + S) were beneficial for growth, yield and yield contributing characters of blackgram variety (Bina mash-1). Considering the obtained result it may be inffered that- (i). Such study is needed in different agro-ecological zones (AEZs) of Bangladesh for regional adaptability and other performance, (ii). Combine effect of phosphorus and Sulphur with biofertilizer appeared promising for obtaining the higher yields of blackgram.

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