

The Studies of Effect of Bio Fertilizers Rhizobium, Phosphobacteria, and Root Nodule Extract (R.PB, R+PB & RNE) on the Growth and Certain Biochemical Changes in the Seedlings of Black Gram & Maize

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ABSTRACT

A pot culture experiment was carried out and to the find out the effect of Rhizobium, Phosphobacteria and Root Nodule extract on the vegetative growth and biochemical changes in Black gram and Maize. The Black gram and Maize is vegetative parameters such as seed germination, shoot and root length, leaf fresh weight, leaf dry weight, shoot and root dry weight had analyzed and biochemical studies of both Black gram and Maize was carried such as total chlorophyll content, leave soluble protein and in vivo nitrate reductase activity. Among microbial inoculants, the Rhizobium + PB mixer was found most effective in terms of seed germination, shoot and root length, leaf fresh weight, leaf dry weight, shoot and root dry weight and also showed increase total chlorophyll content, soluble protein and nitrate activities in both Black gram and Maize.

Keywords: Black gram, Maize, Rhizobium, Phosphobacteria, Root nodule, Bio-fertilizer.

Introduction

Bio-fertilizers are very cheap and renewable source of plant nutrients as they are living organisms. Though the use of Rhizobium Culture for leguminous crop is known for long the field application of Azotobacter, Azospirillum, Rhizobium and Phosphobacteria etc. bio fertilizers form excellent supplementary manure for various cultivated crops as well as substitute. Pulses are cultivated in India in as area of 24 million hectares annually. The grains form the major source of protein for those who are no accustomed to non-vegetarian diet. The per capita consumption of pulses at all Indian level is 35 at present which was 75g in 1960-61. Hence efforts are being made to increase the pulses production. Phosphobacteria (Phosphate Solubilizing Bacteria) one of the bio-fertilizer and it is mainly used in the cereals crops. Phosphobacteria also enhances of the plant growth and biochemical level. Phosphobacteria now day widely used for the agricultural purposes.

Black gram is one of the important pulse crops grown throughout of India. It is grown almost entirely as a dry crop in tracts with a rainfall not exceeding about 35” in the tracts of heavier rainfall it is raised only in the season following the cessation of the rain. Black gram is grown in two seasons, as an early or midseason crop it is sown in the early part of May, as a midseason crop is shown in June or July as a late crop in the month of October.

Maize, also called Indian corn (or merely “CORN” in the U.S.A.) is a food grain of considerable importance in many parts of the world, notably in North, Central and South America. To an even greater extent the grain is used as food for all kinds of farm animals, while the plant themselves furnish large quantities of succulent green fodder and are grown almost as much for their value as green fodder and silage as for the grain. The crop said to be a native of Central America and Mexico from where it has spread to other parts of the world.

In the present studies as attempt has been made to know the effect of biofertilizers (R, PB, R+PB & RNE) on the growth and certain biochemical changes in the seedlings of Black grown & maize. The growth parameters such as root length, shoot length, root fresh weight, dry weight, shoot fresh weight and dry weight were studied. The biochemical parameters such as total chlorophyll content soluble protein content and NRA activity as studied.

Materials and Methods

The effect of bio fertilizers (R, PB, R+PB and RNE) on the growth and certain bio chemical changes were studied on the Black gram and Maize. The Black gram variety Co5 and maize Co2 were procured from Agricultural Research Centre, Kovilpatti, Tamil Nadu, India. In this study the bio fertilizers were inoculated through the Nutrient medium.

Preparation of Nutrient Medium

The nutrient solution was prepared by mixing major & minor Nutrient solution by standard methods. This nutrient solution was treated with bio fertilizer of R, PB, R+PB mixture and RNE in separate containers.

Inoculation of Bio-fertilizers

The bio-fertilizer were treated in the Nutrient Solution by dissolving 5gm of R, PB, mixture of R+PB (2.5 gm each) root nodules extract in separate containers.

Root Nodule Extract

Root nodules were selected & harvested freshly from leguminous plant (*Teprosia purpuriea*) and it was thoroughly cleaned/washed in distilled water. 5gm of fresh nodules were ground in 5 ml of sterile water. This was used as root nodules extract. This extract was treated with nutrient solution.

The above prepared bio fertilizes inoculated Nutrient solutions were used for our study. Control was made by using nutrient solution without mixing bio fertilizers.

Cultivation of plants

Soil was thoroughly washed in the water for two to three times for the removal of unwanted seeds, spores and other materials. The washed soil was dried in the Sun light for two days. The dried Soil was again sterilized in the hot air oven at 160°C for 2 hours. The sterilized soil was used for raising of plants. The sterilized soil was taken in the earthen pot. The seeds of Black gram and maize were soaked in the water for 2 hrs. Later the seeds were planted in earthen pot separately (maize and black gram).

Percentage of Germination

Healthy and uniform sized deeds of black gram and maize were soaked in the nutrient solution of R, PB, R+PB mixture and RNE for 24 hours in a Petridis (20 seeds per plate) lined with sterilized cotton.

Enough amount of the above nutrient solutions was added to the Petridis and the control was made by using nutrient solution (without bio fertilizer) only on 3rd day; the percentage of seed germination was calculated by using of following formula.

Percentage of seed germination

$$\text{Percentage of seed germination} = \frac{\% \text{ No of seed germinated}}{\text{No of seed sown}} \times 100$$

Shoot and Root Length

The intact seedling of both control and treated planted were harvested (without damage) on 7th day, 14th, 21st and 28th day the shoot length root length, was measured in cm.

Fresh Weight in Shoot and Root

The intact seedling of both control and treated planted were harvested (without damage) on 7th day, 14th, 21st and 28th day the shoot length root length, was measured in mgs.

Dry Weight in Shoot and Root

The intact seedling of both control and treated planted were harvested (without damage) on 7th day, 14th, 21st and 28th day and the dry weight of root and shoot was measured after drying in the shoot and root in the oven at 120^oc for 24 hours.

Biochemical Analysis

(1) Total Chlorophyll (ARNON'S 1949)

Total chlorophyll analysis was used Aron's methods.

Chlorophyll content is calculated by using the following Aron's (1949) formula.

$$\text{Chlorophyll a mg lit} = 12.7 \times A663 - 2.69 \times A645 \times$$

$$\text{Chlorophyll b mg lit} = 22.9 \times A645 \times V/W \times 100$$

$$4.68 \times A663 \times V/W \times 100$$

$$\text{Total Chlorophyll mg/lit} = 20.8 \times A645 + 80.2 \times A663$$

$$\times V/W \times 100$$

V – Volume of the extract & W – Weight of the leaf materials

(2) Leaf Soluble Protein (LOWRY'S METHOD 1951)

The soluble protein was analyzed by Lowry's methods.

The amount of protein was estimated by using a standard graph.

(3) In Vivo Nitrate Reductase Enzyme (n.r.e.c.1.6.6.1) activity (JAWORSKI 1971)

100 ml of leaf bits were prepared from the control and treated plant and it was place in a vival containing 5 ml of incubation medium (assay medium PH 7.5). Incubation was carried out in dark for ½ hour at room temperature in ice cold condition with by giving frequent shaking. After ½ hour, 0.5ml of assay medium was taken and it was

added with 1.5ml distilled water and 1 ml of sulphanimide, 1 ml of N -1-N (1 – Naphyl ethylene diamine hydrochloride). It was allowed to keep in an undisturbed condition for 10 minutes for the development of blue color. After 10 minutes the solution was subjected for taking OD at 540 nm by using proper blank solution. 2 ml of distilled water, 1 ml sulphanimide, and 1 ml N – 1 – N.

The NR activity was calculated by finding the nitrate content of the incubated assay medium. The Nitrate content was directly proposanal to the activation of enzyme (NRA) related by which nitrate is converted to nitrate.

Results

The effect of bio fertilizer on the growth and certain biochemical changes were studied in black gram and maize. The results were as follows:

Germination Percentage

The germination percentage was increase in all the treated condition in both black gram and maize when compare with control. It was noted that only 5% was increased in R+P mixture were as in R, PB, RNE treated condition 12% was increased than the control there was no significant difference observed among maize and black gram (Table 1).

Table 1. Germination in Percentage

S. No.	Treatment	Black gram in %	Maize in %
1.	C	100	100
2.	R	112	112
3.	PB	112	112
4.	R+PB mix	105	105
5.	RNE	112	112

Shoot Fresh Weight

The fresh weights of the shoot were noted on 7th day 14th, 21st, and 28th day. There was no significant levels of fresh weight were noted on the above date of harvest but it was gradually increased. However the fresh weight on 28th day analysis were 300,324,306.60,304 & 305 mgs from the control and treated plant of R,PB, R+PB mixture and RNE (respectively) in black gram and 830,910.90,868.40,847 &856.00 mgs from the control and treated plant of P,PB,R+PB and RNE (respectively) in maize (Table 2).

Table 2. Shoot Fresh Weight in mg

S. No.	Treatment	7 th Day		14 th Day		21 st Day		28 th Day	
		B	M	B	M	B	M	B	M
1.	C	270.80	800	280.80	806.00	290.30	826.10	300.00	830.00
		100%	100%	100%	100%	100%	100%	100%	100%
2.	R	310.00	847	313.50	861.40	323.80	871.20	324.20	910.09

		114%	106%	112%	107%	112%	105%	108%	110%
3.	PB	290.60	830	296.60	840.00	299.80	850.20	306.60	868.40
		107%	104%	106%	104%	103%	103%	102%	105%
4.	R+PB mix	279.40	810	284.00	815.00	294.00	835.20	304.00	847.00
		103%	101%	101%	101%	101%	101%	101%	102%
5.	RNE	280.90	812	288.80	819.00	298.80	849.50	305.00	856.00
		104%	102%	103%	102%	103%	103%	102%	103%

Note; B= Black gram, M= Maize

This result showed that the fresh weight of the shoot from all the treated plant was increased than that of control in both black gram and maize. The fresh weights were moderately increased in all the treated condition ranges from in 1% to 8% in black gram and 2% to 10% in maize. It was also noted that the fresh weight of the maize was higher than that of black gram.

Root Fresh Weight

The fresh weights of the root were noted on 7th day 14th, 21st and 28th day. There was no significant levels of fresh weight were noted on the above date of harvest but it was gradually increased. However the fresh weight of root on 28th day analysis were 109.00, 157.00, 136.70, 128.60 mg from the control and treated plants of P,PB,R+PB mixture and RNE (respectively) in black gram and 172.00, 280.00, 222.30, 201.80, 208.20 mgs from the control and the treated plants of P,PB,R+PB mixture and RNE (respectively) in maize (Table 3).

Table 3. Root Fresh Weight in mg

S. No.	Treatment	7 th Day		14 th Day		21 st Day		28 th Day	
		B	M	B	M	B	M	B	M
1.	C	99.90	149	101.00	152.00	108.70	162.00	109.00	172.00
		100%	100%	100%	100%	100%	100%	100%	100%
2.	R	113.30	250	123.60	267.30	133.70	272.30	157.60	280.00
		113%	168%	122%	176%	124%	168%	145%	163%
3.	PB	102.94	180.5	116.34	195.30	128.38	205.30	136.70	222.30
		103%	121%	115%	128%	119%	127%	125%	129%
4.	R+PB mix	101.70	158	110.00	162.00	115.60	172.00	120.60	201.80
		102%	106%	110%	107%	107%	106%	110%	117%
5.	RNE	104.12	174	112.14	184.00	118.50	194.50	128.60	208.20
		109%	117%	111%	121%	110%	120%	118%	121%

Note; B= Black gram, M= Maize

This result showed that, the fresh weight of root from all the treated plant was increase than that of control in both black gram and maize. The fresh weights were moderately increased in all the treated condition ranges from 10% to 45% in black gram and 10% to 63% in maize. It was also noted that the fresh weight of maize was higher than that of black gram.

Shoot Dry Weight

The dry weights of the shoot were noted on 7th day 14th, 21st and 28th day. The dry weight was gradually increased in the above date of harvest. However the dry weight on 28th day analysis were 28.10, 39.00, 29.26,32.14 mgs from the control and treated plants P,PB,R+PB mixture and RNE (respectively) in black gram & 72.19, 99.56, 99.99, 78.34 & 86.64 mgs from the control and treated plants of P,PB,R+PB mixture and RNE (respectively) in maize (Table 4).

Table 4. Shoot Dry Weight in mg

S. No.	Treatment	7 th Day		14 th Day		21 st Day		28 th Day	
		B	M	B	M	B	M	B	M
1.	C	20.24	65.68	22.24	69.04	25.30	70.08	28.10	72.19
		100%	100%	100%	100%	100%	100%	100%	100%
2.	R	32.05	79.00	34.04	84.00	37.64	86.08	39.00	99.56
		158%	120%	153%	122%	149%	123%	135%	138%
3.	PB	23.45	74.00	25.34	79.90	39.64	36.90	39.99	99.99
		116%	113%	114%	116%	157%	124%	138%	139%
4.	R+PB mix	21.30	68.60	23.00	70.50	26.24	76.50	29.26	78.34
		106%	104%	103%	102%	104%	109%	101%	109%
5.	RNE	22.59	70.96	23.29	72.74	29.14	78.74	32.14	86.64
		112%	108%	105%	105%	115%	112%	111%	120%

Note; B= Black gram, M= Maize

This result showed that the dry weight of the shoot from all the treated plants was increased than that of control in both black gram and maize. The dry weights were moderately increased in all the treated condition ranges from 1% to 38% in black gram and 9% to 30% in maize. It was also noted that the dry weight maize was higher than that of black gram.

Root Dry Weight

The dry weights of root were noted on 7th day, 14th, 21st and 28th day. The dry weight was gradually increased in the above date of harvest. However the dry weight on 28th day analysis were 17.93,19.93,18.96,18.30 and 19.29 mgs

from the control and treated plants of P,PB,R+PB mixture and RNE (respectively) in black gram & 31.64, 46.96, 48.74, 32.96 & 38.98 mg from the control and treated plants of P,PB,R+PB and RNE (respectively) in maize (Table 5).

Table 5. Root Dry Weight in mg

S. No.	Treatment	7 th Day		14 th Day		21 st Day		28 th Day	
		B	M	B	M	B	M	B	M
1.	C	15.00	20.65	15.80	23.45	16.96	27.45	17.93	31.64
		100%	100%	100%	100%	100%	100%	100%	100%
2.	R	16.88	35.50	17.88	38.86	18.96	42.86	19.98	46.96
		113%	172%	113%	166%	112%	156%	111%	148%
3.	PB	16.00	34.69	16.80	39.54	17.93	42.64	18.96	48.74
		107%	168%	106%	164%	106%	155%	106%	154%
4.	R+PB mix	15.96	22.99	16.00	24.00	17.63	28.30	18.30	32.96
		106%	111%	101%	102%	104%	103%	102%	104%
5.	RNE	16.40	29.89	17.80	30.62	18.99	34.69	19.29	38.98
		109%	145%	113%	131%	112%	126%	108%	123%

Note; B= Black gram, M= Maize

This result showed that the dry weight of the shoot from all the treated plants was increased than that of control in both black gram and maize. The dry weights were moderately increased in all the treated condition ranges from 2% to 11% in black gram and 4% to 54% in maize. It was also noted that the dry weight maize was higher than that of black gram.

Shoot Length

The shoot lengths were noted on 7th day, 14th, 21st and 28th day. The shoot length was gradually increased in the above date of harvest. However, the shoot length on 28th day analysis was 29.86,32.06,32.46,30.50 & 31.04 cm from the control and treated plants of P, PB, R+PB mixture and RNE (respectively) in black gram (plate -1) & 34.20,38.30,37.60,35.40 & 36.40 cm from the control and treated plants of P, PB, R+PB and RNE (respectively) in maize (Table 6).

Table 6. Shoot Length in cm

S. No.	Treatment	7 th Day		14 th Day		21 st Day		28 th Day	
		B	M	B	M	B	M	B	M
1.	C	20.35	30.84	23.84	31.64	29.63	32.63	29.86	34.20
		100%	100%	100%	100%	100%	100%	100%	100%

2.	R	23.45	33.74	26.06	35.94	28.63	36.73	32.06	38.30
		115%	109%	109%	114%	116%	113%	107%	112%
3.	PB	22.63	32.64	25.02	34.76	27.53	34.83	32.46	37.60
		111%	106%	105%	110%	112%	107%	107%	110%
4.	R+PB mix	21.63	31.54	24.40	32.64	26.63	33.72	30.56	35.40
		106%	102%	102%	107%	108%	103%	102%	104%
5.	RNE	21.73	32.64	25.94	34.54	27.43	35.42	31.04	36.40
		107%	106%	109%	109%	111%	110%	104%	106%

Note; B= Black gram, M= Maize

This result showed that the shoot length from all the treated plant was increases than that of control in both black gram and maize. In all the treated plants the shoot length were moderately increased from 2% to 7% in black gram and 4% to 12% in maize. It was also noted that the shoot length of the maize was higher than that of black gram.

Root Length

The root lengths were noted on 7th day, 14th, 21st and 28th day. The root length was gradually increased in the above date of harvest. The root length on 28th day analysis was 8.50,11.64,11.84,9.00 &10.40 cm from the control and treated plants of P, PB, R+PB mixture and RNE (respectively) in black gram & 9.98,14.20,14.48, 11.30,12.99 cm from the control and treated plants of P, PB, R+PB and RNE (respectively) in maize (Table 7).

Table 7. Root Length in cm

S. No.	Treatment	7 th Day		14 th Day		21 st Day		28 th Day	
		B	M	B	M	B	M	B	M
1.	C	6.40	8.64	8.40	9.54	8.30	9.63	8.50	9.98
		100%	100%	100%	100%	100%	100%	100%	100%
2.	R	9.40	10.60	10.12	11.70	11.35	13.20	11.64	14.20
		147%	123%	120%	123%	137%	137%	136%	142%
3.	PB	9.66	10.50	10.22	11.72	11.45	13.60	11.84	14.48
		150%	122%	122%	123%	138%	141%	139%	145%
4.	R+PB mix	7.00	9.50	8.49	10.50	8.70	10.80	9.00	11.30
		109%	110%	101%	110%	105%	112%	106%	113%
5.	RNE	8.60	10.00	9.60	11.00	9.80	11.99	10.40	12.99
		134%	116%	114%	115%	118%	125%	122%	130%

Note; B= Black gram, M= Maize

This result showed that the root lengths of all the treated plant were increased than that of control in both black gram and maize. The root lengths were moderately increased in all treated plants ranges from 6% to 39% in black gram and 13% to 45% in maize. It was also noted that the root length of the maize was higher than that of black gram.

Total Chlorophyll

The total chlorophyll content was noted on 7th day, 14th, 21st and 28th day. The chlorophyll content was gradually increased in the above period of harvest. However, the total chlorophyll content on 28th day analysis was 0.3195,0.3565,0.3969,0.3295,0.3334 mg from the control and the treated plants of P, PB, R+PB and RNE (respectively) in black gram and 0.3089,0.3065,0.2869 &0.2887 mg/-fwt. from the control and the treated plants of P, PB, R+PB and RNE (respectively) in maize (Table 8).

Table 8. Total Chlorophyll Content in mg/g FWT

		7 th Day		14 th Day		21 st Day		28 th Day	
S. No.	Treatment	B	M	B	M	B	M	B	M
1.	C	0.0378	0.0358	0.0622	0.0622	0.2856	0.2392	0.3195	0.3731
		100%	100%	100%	100%	100%	100%	100%	100%
2.	R	0.0531	0.0555	0.0777	0.1591	0.3179	0.2751	0.3565	0.3089
		141%	155%	123%	156%	111%	115%	111%	113%
3.	PB	0.0446	0.0447	0.0666	0.1586	0.3131	0.2726	0.3469	0.3065
		118%	124%	107%	155%	110%	113%	106%	110%
4.	R+PB mix	0.0388	0.0372	0.0640	0.0659	0.2926	0.2492	0.3295	0.2869
		102%	103%	103%	106%	104%	104%	103%	105%
5.	RNE	0.0400	0.0397	0.0656	0.0675	0.0322	0.2562	0.0334	0.2887
		106%	111%	105%	109%	113%	107%	104%	106%

Note; B= Black gram, M= Maize

This result showed that the chlorophyll content of all the treated plants was increased than that of the control in both black gram and maize. The total chlorophyll content was moderately increased in all treated plants ranges from 3% to 11% black gram and 5% to 13% in maize. It was also noted that the total chlorophyll content of the maize was higher than that of black gram.

Leaf Soluble Protein

The soluble protein content was noted on 7th day, 14th, 21st and 28th day. The protein content was also gradually increased in the above date of harvest. However, the soluble protein content on 28th day analysis was 6.66, 6.69,

6.78, 6.74, & 6.76 mg/g fwt. the control and treated plants of P, PB, R+PB mixture and RNE (respectively) in maize (Table 9).

Table 9. Leaf Soluble Protein in mg/Fr.wt

S. No.	Treatment	7 th Day		14 th Day		21 st Day		28 th Day	
		B	M	B	M	B	M	B	M
1.	C	2.40	2.10	2.88	2.30	6.12	6.43	6.66	6.54
		100%	100%	100%	100%	100%	100%	100%	100%
2.	R	2.70	3.90	3.30	4.14	6.54	6.72	6.96	7.82
		112%	185%	115%	180%	107%	104%	105%	112%
3.	PB	3.00	3.00	3.42	4.04	6.43	6.90	6.78	7.14
		125%	143%	148%	176%	106%	106%	102%	104%
4.	R+PB mix	2.50	2.60	2.94	3.74	6.23	6.56	6.74	6.68
		104%	124%	102%	162%	101%	101%	101%	102%
5.	RNE	2.70	3.30	3.00	4.20	6.30	6.72	6.76	6.72
		112%	159%	104%	183%	103%	104%	102%	103%

Note; B= Black gram, M= Maize

This result showed that the soluble protein of all the treated plant were increases than that of control in both black gram and maize. The soluble proteins were moderately increased in all the treated plants ranges from 1% to 5% in black gram. 2% to 12% in maize. It was also noted that the protein of the maize was higher than that of black gram.

Nitrate Reductase Activity

The nitrate reductive activity was noted on 7th day, 14th, 21st and 28th day. The NR activity was gradually increased in the above date of harvest. However, the nitrate reductive activities on 28th day analysis were 3.50, 4.00, 4.86, 3.60 & 3.67 mol/no₂ form/gfwt/hour from the control and treated plants of P, PB, R+PB and RNE (respectively) in maize (Table 10).

Table 10. NRA activity of moles of NO₂ form/g FWT

S. No.	Treatment	7 th Day		14 th Day		21 st Day		28 th Day	
		B	M	B	M	B	M	B	M
1.	C	2.18	2.50	2.33	2.67	2.66	3.17	3.50	2.83
		100%	100%	100%	100%	100%	100%	100%	100%
2.	R	2.83	3.00	2.67	3.17	3.33	3.66	4.00	4.01
		131%	120%	116%	119%	125%	115%	114%	141%

3.	PB	2.50	2.89	4.16	3.00	4.30	3.50	4.86	3.96
		116%	116%	178%	112%	180%	110%	139%	140%
4.	R+PB mix	2.27	2.65	2.47	2.80	2.76	3.20	3.60	2.93
		105%	106%	106%	105%	103%	101%	103%	104%
5.	RNE	2.33	2.69	2.56	2.83	3.17	3.33	3.67	3.50
		108%	108%	107%	109%	119%	105%	105%	124%

Note; B= Black gram, M= Maize

This result showed that the nitrate reductase activities of all the treated plants were increased than that of control in both black gram and maize. The nitrate reductive activity was moderately increase in all the treated plants ranges from 3% to 14% in black gram and 4% to 41% in maize. It was also noted that the nitrate reductase activity of the maize was higher than that of black gram. The growth parameters (root, shoot length, fresh weight of root and shoot, dry weight of shoot and root) and the biochemical parameters were decreased in the dual infected (R+PB) plants (higher than the control) in both maize and black gram (Table 11, and 12).

Table 11. Interaction of Rhizobium + Phosphobacterium Mixture in Black Gram

Parameter	Predicted	Observed	Interaction
Seed germination	112	105	Antagonistic
Shoot Fresh Weight	315.30	304.00	Antagonistic
Root fresh weight	147.15	120.60	Antagonistic
Shoot dry weight	39.49	29.26	Antagonistic
Root dry weight	19.47	18.30	Antagonistic
Shoot length	32.26	30.56	Antagonistic
Root length	11.74	9.00	Antagonistic
Total chlorophyll	0.3517	0.3295	Antagonistic
Soluble Protein	6.87	6.74	Antagonistic
NRA	4.43	3.60	Antagonistic

Table 12. Interaction of Rhizobium + Phosphobacterium Mixture in Maize

Parameter	Predicted	Observed	Interaction
Seed germination	112	105	Antagonistic
Shoot Fresh Weight	889.24	847.00	Antagonistic

Root fresh weight	251.15	201.80	Antagonistic
Shoot dry weight	99.78	78.34	Antagonistic
Root dry weight	47.35	32.96	Antagonistic
Shoot length	37.95	35.40	Antagonistic
Root length	14.34	11.30	Antagonistic
Total chlorophyll	0.3077	0.2864	Antagonistic
Soluble Protein	7.48	6.68	Antagonistic
NRA	3.98	2.93	Antagonistic

Discussions

Seed Germination

The percentage of seed germination was higher than that of control in all the treated condition of P, PB, R+PB and RNE (fig-1) it may be due to various growth regulators presents at any time may affect germination and growth rate of plant organ. In addition, synthesis of growth substance depends on the medium composition of raise are fall in concentration of medium. (Thiman 1972). The inoculated bacteria may produce substance such as auxin, gibberellinee and cytokinin as well as plant growth inhibitors. The high percentage of germination may be due to the formation of auxin, gibberellinee and cytokinins and other growth regulators. This may have enhanced the percentage of seed germination than the control.

Plant growth

In both black gram and maize, the fresh weight dry with of shoot and root, shoot length and root length of the treated plants (R, PB, R+P, RNE) were increased than the control. It is positively correlated with findings of Alagawadi and A.C. Gaur (1987) In Soybean, Hernandez and Hill (1983) In Cowpea G. Ghosh (1989) In Summer Moong, Vashimalsi and Subramaniyan (1980) In Legumes, R. Chandra (1991). In Green gram S.K. Kothari and C.S. Sarf (1990) In Mung.

Bhatnagar *et al.*, (1979) In Soybean. Misra and Singh (1967) in Soybean. This may be due to the following reasons. The efficient strains of inoculated rhizobium well established inside the root by forming nodules which fix Nitrogen symbiotically, rhizohium & phorphobactrium however must become established in the rhizosphere competition with many other microorganisms. They are disturbing the normal biological balance in the root zone. It has been stated that inoculation can be expected to improve plant growth mainly if the bacteria are established and growned in the rhizosphere.

The biofertilizer can improve the bacterial growth and the establishment of a large population of inoculated microorganism. Cooper (1959). The inoculated bio fertilizers may produce substances belonging to auxin, gibberellins cytokinin as well as plant growth inhibitors. This was supported by findings of Lu *et al.*, (1958).

Microorganism able to form symbiotic association with plant such as vesicular arbuscular mycorrhizae (VAM) and rhizobium legumes have been described to improve plant growth nutrition much more efficiently than free living microorganism. The proportion of various hormones present at any time determines the growth rate of plant.

The improved plant growth may be due to interaction of hormones from the both host plants and bacteria in the rhizosphere. Different kinds of unknown substance were also synthesized.

Combined inoculation of rhizobium and phosphobacterium promote growth. It may be due to cumulative effect such as supply of N and P to the crop in addition to growth promoting substances produced by the organisms Pareek and Gaur (1973).

The microorganism in the rhizosphere can increase or decrease the absorption of inorganic nutrients by plant roots. These effects depend on the availability of elements and the physical conditions around the roots.

Regarding the biomass accumulation (fresh weight, dry weight, root length, shoot length) was comparatively higher in the maize than the black gram. This may be due to the individual characters of the plant body by which the maize can synthesize more biomass normally than the black gram.

Biochemical Parameters

The biochemical parameters such as total chlorophyll content soluble protein content and NR activity was studied. It was comparatively higher in all the treated plants (R, P, R+P, & RNE) than the control in both black gram and maize.

The total chlorophyll content was also high in the treated plants. This is due to the formation of more amounts of nitrate and phosphate formed in the soil by the application of Rhizobium and phosphobacterium.

The total chlorophyll content and protein content was also higher in the treated plants than the control in both black gram and maize. It is directly correlated with the growth of the plants. The growth of the plants depends up on the available nutrients phosphorous and nitrate in the soil.

The inoculation of rhizobium and phosphobacterium synthesis more amount of NO_4 and phosphate from the atmosphere by symbiotically. So that the plants were able to grow well than that of control by absorbing the minerals NO_4 and PO_4 by root system. The increased chlorophyll content is also correlated with the height level of photosynthetic process. High rate of photosynthetic process increases the synthesis of starch. The increased starch content may increase the level up protein content.

The NR activity was also higher in the treated plants (R, PB, R +P, RNE) than the control in both black gram and maize. This is positively correlated with the finding of Maiti C.C. Das B.N. Chatterjee and K. Sengupta 1988 in green gram and lentil Alagawdi and A.C. Gaur (1988) in chickpea, Sullia (1968) in chick pea.

The NR activity is depending upon the reacting substance NO_4 available in the soil. The inoculated biofertilizers enhances the synthesis of nitrate from the atmospheric free nitrogen by symbiotic N₂ fixation in the soil. This NO_4 is absorbed by the root system of the plant and it will enhance the NR activity in the leaf. Phosphorous availability generally enhances symbiotic nitrogen fixation

In the Rhizobium + Phosphobacterium treated plants the total chlorophyll contents protein content and NR activity was decreased than the other treated plants. (Higher than the control). This may be due to the interaction of growth regulating substance from the bacteria and from the host and also by the formation of growth inhibitor. The growth and multiplication of rhizobium and phosphobacterium will be inhibited by the synthesis of inhibitors. This will directly affect the growth.

The growth parameters (root and shoot length, fresh dry weight of root shoot) and the observed bio chemical parameters were decreased in the dual infected plants R+PB mixture (higher than the control) than the other treated plants in both maize and black grams. It shows antagonistic effect in all the observed parameters. It may be due to the combined effects of Rhizobium and phosphobacteria activity in the rhizosphere. Some growth inhibitors may be synthesis by the interaction of microorganism which ma suppresses the growth of the plants.

Summary

The growth parameters such as the fresh wt, dry wt. of root and shoot, root length was increased in all the treated plants than the control in both black gram and maize. The biochemical parameters such as total chlorophyll content, soluble protein content and NRA activity were also increased than the control in both black gram and maize. This may be due to the formation of hormones such as auxin, gibberellins and cytokines in the inoculated plants. The hormones were enhancing by the inoculation of bio fertilizers in the treated plants. The growth and biochemical parameters were also increased due to the rapid growth of plants by taking more amounts of nitrate and phosphate from the soil. This phosphate and nitrate content was increased in the soil by the action of bio fertilizers by symbiotic fixation process. The overall biomass of maize as a C₄ plant and it can fix more amount of CO₂ than the black gram.

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Competing Interests Statement

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Consent for publication

Authors declare that they consented for the publication of this research work.

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