Plant Archives Vol. 20 Supplement 1, 2020 pp. 2341-2344 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

EFFECT OF BIOFERTILIZERS [*AZOTOBACTER* & PSB] ON DIFFERENT GROWTH PARAMETERS OF SOYBEAN (*GLYCINE MAX* L.) IN THE PRESENCE OF *RHIZOBIUM JAPONICUM*

Saba Naaz and Zia-ul-Hasan

Department of Botany, Saifia Science College, Bhopal, (M.P.), India.

Abstract

Biofertilizers are eco friendly and are pollution free. They provide plant nutrients at very low cost and are required in small quantity thereby increase vegetative yield of plant growth by 20-30%. They fasten the vegetative growth in soybean plant and restore natural soil fertility and improve all the properties of soil. The two varieties of *Glycine max* L. JS-9305 and JS 9560 were taken for the study of growth parameters. In the present study the comparative effect of Biofertilizers *Rhizobium, Azotobacter*, PSB single treatment as well as mixed strain on plant growth characters of *Glycine max* L. The highest plant height (cm) observed in mixed strain of *Rhizobium* + PSB in both varieties of *Glycine max* L. Number of branches per plant were significantly increased in co-inoculation of *Rhizobium* + PSB. The highest fresh weight and dry weight (gm) of Root/ Shoot and Root/Shoot Ratio were observed in the treatment of *Rhizobium* + *Azotobacter*.

Key words: Biofertilizers, *Azotobacter*, PSB, *Rhizobium,* FWR, DWR, FWS, DWS, growth parameters.

Introduction

Soybean (*Glycine max* L.) belong to family fabaceae is a globally important oilseed crop and source of high quality protein for human consumption, used as fodder for animal and is also important in improved crop rotation system (Corskey *et al.*, 1997) *Rhizobium japonium* symbiotically associate with the soybean plant and form root nodules where the bacteria reside. Biofertilizers are increase the production of crop plant quality and it's responsible with good agriculture environment. They are eco-friendly, non hazardous and non toxic. *Azotobacter* and phosphate solubilizing bacteria (PSB) are presently being used as biofertilizers (Kannaiyan *et al.,* 2004) Nitrogenous and phosphate biofertilizers are to be used to produce plant growth promoting substances. *Azotobacter* is a non symbiotic biofertilizers which fixes 20-30 kg. N/hectare and can be used in various plants also it protects roots of plant from pathogenic attack which are present in soil. Phosphorus is an important plant nutrients which help in root formation and plant growth and thereby better vegetative growth and yield. Phosphate solubilizing bacteria solubilize the phosphate and increase uptake efficiency of phosphorus to plants.

***Author for correspondence :** E-mail: naazsaba902@gmail.com

The present study to see the effect of Biofertilizers (*Azotobacter* and PSB) with *Rhizobium japonicum* on growth parameters of *Glycine max* L.

Material and Method

Material

Two common cultivars JS-9305 and JS-9560 of Central India of *Glycine max* L. (soybean) were collected from Sehore Agriculture College Sehore, which is commonly grown black cotton soil around Bhopal. Biofertilizers, *Rhizobium japonicum*, *Azotobacter chroococcum* and PSB *Pseudomonas putida* (Phosphate solubilizing bacteria) single and their co-inoculation were mixed strain, used in soil inoculation, collected from M.P. Agro industries, Bhopal.

Experimental site

Two experiments were conducted, one in Botany department laboratory of Saifia Science College Bhopal *i.e.* pot experiment and second field experiment, simultaneously in middle June to September during 2011 and 2012.

Black soil was used for better healthy growth of this crop. Such soil samples were collected from 0-15 cm. depth with the help of soil auger. Before sowing the

selected seeds of *Glycine max* L. variety JS-9305 and JS-9560 were treated with fungicide *i.e.* 1% sodium hypochloride for 10 minutes then soil inoculated with *Rhizobium, Azotobacter* and PSB culture 20 gm. Individually and mixed strain of *Rhizobium* + *Azotobacter, Rhizobium* + PSB and *Azotobacter* + PSB, 20 + 20 gm. Respectively. First the soil inoculated with biofertilizers and seed of pot and field experiments with their control (Uninoculated). In pot experiments 24" pots were taken, number of treatments were 6+1=7, depth of sowing of treated seeds was 1.5 to 2.0 cm. and total number of replicates were three. In field experiment, the treated seeds of soybean two varieties JS-905 and JS-9560 were sawn in randomized block design. After 15 days germination thinning was done to maintain uniformity and desirable plant population. To protect the crop from weed interaction, first and second hand weeding was done 15 days and 30 days after spraying Theydon 35 EC @ 10 m/10 liters twice after 25 days and 50 days of seed germination. 25 plants were selected randomly and tagged for the observations of growth and yield characters. Root/shoot length of 20 days old seedlings, Root/shoot ratio, fresh weight and dry weight of Root and Shoot, plant height at maturity. no. of branches per plant, dry weight of plant. The observations were recorded during different stages of growth and statistically analyzed.

Result & Discussion

In the present study to see the effect of non-symbiotic biofertilizers on the growth of *Glycine max* L. (Soybean) in the presence of symbiotic biofertilizers. *Rhizobium japonicum* lives in root nodules of soybean, forms the nodules and fix atmospheric N_2 symbiotically. *Azotobacter chrooccocum* non symbiotic biofertilizers, which fix the atmospheric N_2, in soil independently, Phosphate solubilizing bacteria (PSB) which is *Pseudomonas putida* convert the insoluble phosphate into soluble forms. Various treatments of biofertilizers, *Rhizobium, Azotobacter* and PSB in single and mixed inoculation showed a significant effect on root length and shoot length of 20 days old seedlings of soybean.

In both varieties JS-9305 and JS-9560 Root length was significantly increased by the treatment of mixed strain, *Rhizobium* + *Azotobacter, Rhizobium* + PSB and *Azotobacter* + PSB 9.6 cm, 9.3 cm and 9.4 cm. respectively.

Table 1: Effect of different doses of biofertilizers (*Rhizobium, Azotobacter* and PSB) and their combination on 20 days old seedling, Root length, Shoot length and Root/shoot ratio in two varieties of soybean (*Glycine max* L. Merril.).

S. No.	Treatment of Biofertilizers	Doses	20 Days old seeding		
			Root Length (cm)	Shoot Length (cm)	Root/ Shoot ratio
			Variety - JS - 9305		
1.	Control (uninoculated)	-	7.2	2.8	2.57
2.	*Rhizobium*	20 gm	8.1	3.0	2.70
3.	*Azotobacter*	20 gm	8.4	2.9	2.89
4.	PSB	20 gm	7.9	2.4	3.29
5.	*Rhizobium* + *Azotobacter*	20+20 gm	9.6	3.9	2.46
6.	*Rhizobium* + PSB	20+20 gm	9.3	2.7	3.44
7.	*Azotobacter* + PSB	20+20 gm	9.4	3.0	3.13
	CD =		2.1	2.3	
	SE ± =		0.02	0.02	
			Variety – JS- 9560		
1.	Control (uninoculated)	-	7.0	2.7	2.59
2.	*Rhizobium*	20 gm	8.3	3.1	2.67
3.	*Azotobacter*	20 gm	8.6	2.8	3.07
4.	PSB	20 gm	7.5	2.3	3.26
5.	*Rhizobium* + *Azotobacter*	20+20 gm	9.9	3.8	2.60
6.	*Rhizobium* + PSB	20+20 gm	9.4	3.5	2.68
7.	*Azotobacter* + PSB	20+20 gm	9.6	3.3	2.90
	CD =		2.2	2.4	
	SE ± =		0.01	0.02	

Table 2: Effect of different doses of Biofertilizers (*Rhizobium, Azotobacter* and PSB) and their combination on FW & DW root and FW & DW shoot in two varieties of soybean (*Glycine max* L. Merril.).

S. No.	Treatment of Biofertilizers	Doses	FW root per plant	DW root (g) per plant	FW shoot (g) per plant	DW shoot (g) per plant
			Variety - JS - 9305			
1.	Control (uninoculated)	-	14.21	7.26	28.20	11.12
2.	*Rhizobium*	20 gm	18.14	8.82	45.21	13.01
3.	*Azotobacter*	20 gm	19.21	8.87	42.31	12.42
4.	PSB	20 gm	21.31	10.10	38.12	11.01
5.	*Rhizobium* + *Azotobacter*	20+20 gm	38.43	18.34	56.21	18.14
6.	*Rhizobium* + PSB	20+20 gm	32.14	12.01	56.13	16.23
7.	*Azotobacter* + PSB	20+20 gm	34.11	13.12	55.10	15.98
	CD =		1.41	0.96	2.4	1.01
	SE ± =		0.92	0.08	0.97	0.12
			Variety – JS- 9560			
1.	Control (uninoculated)	-	13.87	7.21	28.91	12.01
2.	*Rhizobium*	20 gm	18.34	8.89	46.19	13.41
3.	*Azotobacter*	20 gm	20.01	8.93	43.12	12.62
4.	PSB	20 gm	22.30	10.18	39.72	11.51
5.	*Rhizobium* + *Azotobacter*	20+20 gm	37.72	18.81	58.12	18.20
6.	*Rhizobium* + PSB	20+20 gm	33.14	18.54	57.32	16.37
7.	*Azotobacter* + PSB	20+20 gm	35.20	12.92	57.97	16.92
	CD =		1.32	0.97	2.12	1.01
	SE ± =		0.97	0.09	0.97	0.18

Fw = Fresh Weight, DW = Dry Weight

Shoot length of 20 day old seedling in both varieties of JS-9305 and JS-9560 were significantly increased under the mixed strains of biofertilizers *i.e.* 3.9 cm, 3.8 cm, 3.0 cm, in comparison of control 2.8 cm. The best response was recorded in treatment of *Rhizobium* + *Azotobacter* where Root length and shoot length was highest 9.6 cm and 9.9 cm in comparison of control 7.2 and 7.0, table 1.

The fresh weight (FW) and dry weight (DW) of Root per plant when plant become mature, highest fresh weight and dry weight observe under the treatment of *Rhizobium* + *Azotobacter* 20 gm + 20 gm it was (FW) 38.43, (DW) 18.34 in the variety of JS-9305 and 37.72 and 18.81 in the variety of JS-9560 respectively. The same pattern of FW and DW of Shoot under the treatment *Rhizobium* + *Azotobacter* in both varieties JS-9305 and JS-9560 were observed. (Table 2).

Plant height were recorded when the plants become mature. In this parameter there was significant difference in height of plants, treated with mixed strains of *Rhizobium* + *Azotobacter, Rhizobium* + PSB and *Azotobacter* + PSB. In single treatments of biofertilizers, the height was not significantly increase in both the varieties JS-9305 and JS-9560 of Soybean. The highest plant height was observed under the treatment of *Rhizobium* + PSB mixed strain, it was 96.34 cm in variety JS 9305 against the control 62.43 cm. (Table 3). In variety JS-9560 the plant height was 98.92 cm observed by the same mixed strain.

In this parameter mixed strains of biofertilizers are again more effective than that of individual treatment of Biofertilizers (Table 3) Highest number of branches observed under mixed treatment of *Rhizobium* + PSB 24.24 in variety JS-9305 and 28.97 in variety JS-9560 in comparison of control 12.31 and 12.17 respectively. The combination of *Rhizobium* + *Azotobacter* highest dry weight of plant observed *i.e.* 24.13 gm in variety. JS-9305 and 23.01 gm in variety JS-9560. Single PSB treatment in both varieties was lowest. (Table 3).

Plant height is an important index of plant growth which indicate the effect of different treatments. In two cultivars of soybean, height of plant significantly effected, statistical data show the increase in height in mixed strain *Rhizobium* + PSB treatment is prominent. PSB increase the solubilization of phosphate which with nitrogen increase the proliferation of root thus increase the

Table 3: Effect of different doses of biofertilizers (*Rhizobium, Azatobactor* and PSB) and their combination on growth parameter (Height and Dry weight of plant) in two varieties of soybean (*Glycine max* L. Merril.).

S. No.	Treatment of Biofertilizers	Doses	Height (cm)	Number of Branches per plant	Dry weight of plant at (g) maturity
			Variety - JS - 9305		
1.	Control (uninoculated)	-	62.43	12.31	11.21
2.	*Rhizobium*	20 gm	76.32	16.37	19.15
3.	*Azotobacter*	20 gm	75.97	17.10	19.21
4.	PSB	20 gm	68.76	14.01	15.04
5.	*Rhizobium* + *Azotobacter*	20+20 gm	94.90	23.13	24.13
6.	*Rhizobium* + PSB	20+20 gm	96.34	24.24	27.42
7.	*Azotobacter* + PSB	20+20 gm	91.21	22.10	23.01
	CD =		2.98	1.78	1.10
	SE ± =		1.16	0.20	0.04
			Variety – JS- 9560		
1.	Control (uninoculated)	-	62.14	12.17	11.34
2.	*Rhizobium*	20 gm	75.28	16.42	19.12
3.	*Azotobacter*	20 gm	75.12	17.30	19.34
4.	PSB	20 gm	67.34	15.10	15.92
5.	*Rhizobium* + *Azotobacter*	20+20 gm	95.12	24.13	23.01
6.	*Rhizobium* + PSB	20+20 gm	98.92	28.97	26.19
7.	*Azotobacter* + PSB	20+20 gm	90.41	26.11	24.72
	CD =		3.12	1.87	1.12
	SE ± =		1.04	0.21	0.03

absorption of nutrient and ultimately increase the height of plant. Various treatments of biofertilizers, *Rhizobium, Azotobacter* and PSB in mixed and single inoculation showed a significant effect on root length and shoot length of 20 days old seedlings of soybean varieties JS-9305 and JS-9560 which is due to leaching out some growth promoting substances by biofertilizers. Similar results observed in Root/Shoot ratio in all the inoculation, particularly in mixed strains of biofertilizers, fresh weight and dry weight of Root/Shoot significantly increased which indicate the healthiness of 20 days old seedlings. The present investigation is carried out to evaluate reliable information for the effect of biofertilizers on growth characters of *Glycine max* L. with special reference of *Rhizobium japonicum*.

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