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Response of biofertilizers and phytohormone on growth and yield of chickpea (C*icer arietinium* L.)

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ABSTRACT

A field experiment was conducted during the rabi seasons of 2008-2009 and 2009-2010 to find out the response of biofertilizers and phytohormone on growth and yield of chickpea (Cicer arietinium L.). The experiment was laid out in factorial RBD with five levels of biofertilizers (No inoculation, Rhizobium, Phosphate solubilizing bacteria, Vesicular arbuscular mycorrhizae, Rhizobium+ Phosphate solubilizing bacteria + Vesicular arbuscular mycorrhizae) and three levels of homo brassinolide spraying (No spray, Pre-flowering and Pre-flowering + Pod development). Results revealed that inoculation of biofertilizers significantly improved growth parameters like plant height yield parameters like number of pods plant^{-1}, weight of pods plant^{-1}, number of gains plant^{-1}, test weight, grain yield, stalk yield and harvest index. Among the biofertilizers, combined inoculation of Rhizobium + PSB + VAM produced higher grain yield amounting 60.17, 35.35, 17.60 and 13.32 % in first year and 58.64, 35.44, 18.24 and 15.15 % in second year higher kernel yield over no inoculation, rhizobium, phosphate solubilizing bacteria, vesicular arbuscular mycorrhizae respectively. With increase in levels of spraying of homo-brassinolide increased the growth and productivity of chickpea. Spraying of homo-brassinolide at pre-flowering + pod development stages increased higher grain yield by 30.50 and 12.71 % in first year and 29.59 and 12.21 % in second year in comparison with the higher grain yield than spraying of homo-brassinolide at no spray and pre-flowering stage.

Key words**:** Chickpea, rhizobium, PSB, VAM, homo-brassinolide, grain yield.

Pulses form an integral part of the vegetarian diet and the cheapest source of protein for the poor farmers of the Indian sub-continent. Every pulse plant named as an itself mini-fertilizers factory which enriches soil nitrogen It fixes atmospheric nitrogen in symbiotic association with *Rhizobium* bacteria. Pulse crops have deep penetrating root systems which enable them to utilize the limited available moisture more efficiently than many other crop including cereals. Pulses can minimize the magnitude of protein malnutrition and provide a superior quality of food and fodder and fed to the vast human and cattle population, respectively. Amelioration of phosphorus deficiency by application of costly phosphorus fertilizer is also not a viable option to many resource poor farmers (Rao *et al*., 1997). In modern days, intensive crop cultivation requires the use of higher quantity of chemical fertilizer which helps in increasing environment pollution. There is a need to develop a suitable agricultural system which requires lower fertilizer input with higher fertilizer use efficiency. Therefore, the current trend needs to explore the possibility of supplementing chemical fertilizers with organic ones, more particularly bio fertilizers of microbial origin. Research efforts are therefore, needed to develop low input technology for farmers. Several attempts were made to enable technology for substituting or supplementing costly phosphatic fertilizer using micro-organisms capable of solubilizing the native and applied phosphorus. The use of rhizobium, phosphate solubilizing bacteria (PSB) and vesicular arbuscular mycorrhizae (VAM) have opened new vistas of phosphorus nutrition. Brassinolide (BL), considered to be the most important homobrassinolide (HBR) playing a pivotal roles in the hormonal regulation of plant growth and development, so as to increase crop yield. Hence, an experiment was conducted to study the response of biofertilizers and phytohormone on growth and yield of chickpea (*Cicer arietinium* L.).

MATERIALS AND METHODS

A field experiment was conducted during the *rabi* season of 2008-2009 and 2009-2010 at Agricultural Research Farm, (Institute of Agriculture), Visva-Bharati, Sriniketan, Birbhum. The soil was slightly acidic (P^H-5.9), low in available nitrogen (136 kg ha^{-1}), phosphorus (11.50 kg ha^{-1}) and medium in potassium (160.5 kg ha^{-1}).The experiment was laid out in factorial randomized block design with five levels of biofertilizers inoculation (no inoculation, *Rhizobium*, Phosphate solubilizing bacteria (PSB), Vesicular arbuscular mycorrhizae (VAM) and *Rhizobium*+ Phosphate solubilizing bacteria (PSB) + Vesicular arbuscular mycorrhizae (VAM)) and 3 sprayings of homo-brassinolide at No spraying, Pre-flowering stage and Pre-flowering + Pod development stage. The spraying of homo-brassinolide was 0.2 ppm (Double @0.5 ml litre^{-1}, Godrej Agrovet). In all fifteen treatments replicated thrice. The chickpea, 'Mahamaya-2 (B-115)' was sown on November 15 and November 12 during 2008-2009 and 2009-2010 respectively. The seed was inoculated with *Rhizobium* and PSB by slurry method whereas the soil was

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inoculated with VAM inoculum (Mfg. by Symbiotic Sciences, New Delhi). The The pure VAM culture was mixed thoroughly with slightly moisten soil and applied below the seed @ 2g/seed and then pre-inoculated seeds were sown according to the treatment. The yield parameters and yield were recorded at harvesting stage (120 days) of plant. The rainfall received during the cropping period 12.2 and 29.2mm in 2008-2009 and 2009-2010, respectively.

RESULTS AND DISCUSSION

Plant height

The plant height of chickpea was significantly influenced by biofertilizers in both the experimental years (Table 1). The tallest plant height (64.23 and 70.88 cm) was recorded with inoculation of *Rhizobium*+ PSB + VAM in 2008-09 and 2009-10, respectively. The smallest plant height (47.80 and 53.18 cm) was obtained from no inoculation treatment in 2008-09 and 2009-10, respectively. The increase in growth might be due to the enhanced photosynthetic efficiency of *Rhizobium* + PSB + VAM inoculated plant. This showed a strong synergistic effect between *Rhizobium* + PSB + VAM. Inoculations of PSB which are known to produce growth hormones (Sattar and Gaur, 1987) are likely to favour increased plant height. The results are conformity with those of Mukherjee and Rai (2000) and Jain *et al*. (1999). The plant height was influenced by spraying of homo-brassinolide at pre-flowering and pod development stage in both years. The tallest plant height (56.17 and 61.94 cm) was obtained with twice spraying of homo-brassinolide at pre-flowering + pod development stage in 2008-09 and 2009-10, respectively. The smallest plant height (51.17 and 57.90 cm) was obtained without spraying of homo-brassinolide in 2008-09 and 2009-10, respectively. Similar result was reported by Ramraj *et al.* (1997). Increased plant height might be due to positive effect of homo-brassinolide on meristamatic tissues of plant as well as in increasing number and size of cell (Prakash *et al.*, 2008).

Table 1: Plant height, number of pods and grains plant^{-1} as influenced by treatments

Treatments	Plant height (cm) at harvest		No. of pods plant^{-1}			No. of grains plant^{-1}		
	2008-09	**2009-10**	**2008-09**	**2009-10**	**Pooled**	**2008-09**	**2009-10**	**Pooled**
Bio-fertilizers								
No inoculation	47.80	53.18	24.22	26.77	25.50	28.66	32.88	30.77
Rhizobium	49.42	55.74	28.66	31.33	30.00	33.00	37.88	35.44
PSB	52.73	59.45	30.77	34.00	32.39	36.00	41.44	38.72
VAM	53.57	60.76	32.00	35.66	33.83	37.88	42.33	40.11
Rhizobium+PSB+VAM	64.23	70.88	36.89	40.00	38.47	43.89	48.11	46.00
SEm (±)	**0.37**	**0.40**	**0.31**	**0.36**	**0.23**	**0.30**	**0.31**	**0.21**
LSD(0.05)	**0.89**	**0.96**	**0.90**	**1.05**	**0.65**	**0.87**	**0.89**	**0.59**
Homo-brassinolide								
No spray	51.17	57.90	27.26	30.33	28.80	33.00	37.40	35.21
Pre-flowering	53.31	60.18	30.80	33.80	32.32	36.26	40.93	38.60
Pre-flowering + pod development	56.17	61.94	33.46	36.53	35.00	38.40	43.26	40.82
SEm (±)	**0.29**	**0.31**	**0.24**	**0.28**	**0.18**	**0.23**	**0.24**	**0.16**
LSD(0.05)	**0.70**	**0.75**	**0.70**	**0.81**	**0.51**	**0.67**	**0.69**	**0.45**

Number of pods and grains plant^{-1}

Number of pods plant^{-1} and number of grains plant^{-1} (Table 1) were significantly influenced by biofertilizers inoculation. Inoculation of *Rhizobium* + PSB + VAM, VAM, PSB and *Rhizobium* recorded significantly higher number of pods plant^{-1} and number of grains plant^{-1} than control plot (without inoculation). Analysis of table 1 reveals that combine inoculation of *Rhizobium* + PSB + VAM recorded significantly higher pods plant^{-1} of 36.89, 40.00 and 38.47 over the other biofertilizer treatments during 2008-09, 2009-10 and pooled of two years respectively. Combine inoculation of *Rhizobium* + PSB + VAM proved to be better regarding grains plant^{-1} (43.89, 48.11 and 46.00) during 2008-09, 2009-10 and pooled of two years respectively. The percentage increase in number of grains plant^{-1} with *Rhizobium* + PSB + VAM, VAM, PSB and *Rhizobium* was 49.50%, 30.35%, 25.84% and 15.18% over no inoculation in respect of pooled of two years. This increase in yield parameters by *Rhizobium* + PSB + VAM inoculation might be due to more supply of nutrients particularly of phosphorus which helps in increase nodule number and root growth could be ascribed to a better translocation of photosynthate towards the number of pods and grains plant^{-1}. Shinde (1990) and Yadav and Shrivastava (1997) were recorded similar findings. Number of pods plant^{-1} and number of grains plant^{-1} (Table 1) were significantly influenced by spraying of homo-brassinolide. The highest number of pods plant^{-1} (33.46, 36.53 and 35.00) and number of grains plant^{-1} (38.40, 43.26 and 40.82) was obtained with twice sprayings of homo-brassinolide at pre-flowering + pod development stages during 2008-09, 2009-10 and pooled of two years respectively. The lowest number of pods plant^{-1} (27.26, 30.33 and 28.80) and number of grains plant^{-1} (33.00, 37.40 and 35.21) was obtained from control plot (without spraying of homo-brassinolide) during 2008-09, 2009-10 and pooled of two years respectively. The increase in yield attributes might be due to application of homo-brassinolide which was in consonance with the findings of Mai *et al.* (1989).

Weight of pods and grains plant^{-1} and test weight

The biofertilizer exerted significant effect on weight of pods and grains plant^{-1} and test weight during both years and pooled of two years (Table 2). The highest pods weight of 11.64 g, 11.80g and 11.73 g was produced in crop receiving *Rhizobium* + PSB + VAM whereas the lowest pods weight of 8.43 g, 8.48g and 8.45 g was obtained from the crop with no inoculation of biofertilizers during 2008-09, 2009-10 and pooled of two years respectively. Similarly, the highest grains weight plant^{-1} was produced in crop receiving *Rhizobium* + PSB + VAM whereas the lowest grains weight was obtained from the crop with no inoculation of biofertilizers during 2008-09, 2009-10 and pooled of two years respectively. The percentage increase in grains weight plant^{-1} with *Rhizobium* + PSB + VAM, VAM, PSB and *Rhizobium* was 57.93%, 29.48%, 23.85% and 13.04 % over no inoculation in respect of pooled of two years. Test weight was also influenced by inoculation of biofertilizer (Table 2). Combine inoculation of *Rhizobium* + PSB + VAM showed to be better regarding test weight (222.1 g, 222.2 g and 222.2 g) over no inoculation of biofertilizer (217.7 g, 218.2 g and 217.9 g) during 2008-09, 2009-10 and pooled of two years respectively. Similar type of result was reported by Pramanik and Singh (2003). Weight of pods plant^{-1} and grains plant^{-1} (Table 1) were significantly influenced by spraying of homo-brassinolide. The maximum weight of pods plant^{-1} (10.33 g, 10.41 g and 10.37 g) and weight of grains plant^{-1} (8.46 g, 9.33 and 8.89 g) was obtained with twice sprayings of homo-brassinolide at pre-flowering + pod development stages during 2008-09, 2009-10 and pooled of two years respectively. The minimum weight of pods plant^{-1} (9.57 g, 9.72 g and 9.64 g) and weight of grains plant^{-1} (7.67 g, 8.34 g and 8.01 g) was obtained from control plot (without spraying of homo-brassinolide) during 2008-09, 2009-10 and pooled of two years respectively. The pooled of two years also showed that significant variation among different homo-brassinolide spraying and twice sprayings at pre-flowering + pod development stages recorded the highest test weight (220.8 g) as compared to no spraying (219.3 g). Similar types of results were reported by Mai *et al.* (1989) and Pramanik *et al.* (2012).

Table 2: Pods weight plant^{-1}, grains weight plant^{-1} and test weight as influenced by treatments

Treatments	Pods weight plant^{-1} (g)			Grains weight plant^{-1} (g)			Test weight (g)		
	2008-09	2009-10	Pooled	2008-09	2009-10	Pooled	2008-09	2009-10	Pooled
Bio-fertilizers									
No inoculation	8.43	8.48	8.45	6.42	7.07	6.75	217.7	218.2	217.9
Rhizobium	9.44	9.49	9.47	7.23	8.03	7.63	219.0	219.4	219.2
PSB	9.96	10.10	10.02	7.92	8.80	8.36	220.0	220.2	220.0
VAM	10.19	10.47	10.33	8.37	9.10	8.74	220.1	220.5	220.3
Rhizobium+PSB+VAM	11.64	11.80	11.73	10.37	10.95	10.66	222.1	222.2	222.2
SEm (±)	**0.12**	**0.15**	**0.09**	**0.12**	**0.18**	**0.18**	**0.12**	**0.10**	**0.08**
LSD(0.05)	**0.36**	**0.44**	**0.25**	**0.36**	**0.52**	**0.51**	**0.34**	**0.30**	**0.23**
Homo-brassinolide									
No spray	9.57	9.72	9.64	7.67	8.34	8.01	219.1	219.4	219.3
Pre-flowering	9.91	10.07	9.99	8.05	8.69	8.37	219.6	219.9	219.7
Pre-flowering + pod development	10.33	10.41	10.37	8.46	9.33	8.89	220.6	221.1	220.8
SEm (±)	**0.09**	**0.12**	**0.07**	**0.09**	**0.14**	**0.07**	**0.09**	**0.08**	**0.06**
LSD(0.05)	**0.28**	**0.34**	**0.20**	**0.27**	**0.40**	**0.20**	**0.26**	**0.23**	**0.17**

Yield and harvest index

The inoculation of bio-fertilizer exerted significant effect on grain yield, husk yield, stalk yield and harvest index of chickpea during both the years (Table 3). The highest grain yield (19.91 q ha^{-1}, 20.37 q^{-1} and 20.14 q ha^{-1}) was produced in crop receiving the treatment of *Rhizobium* + PSB + VAM, whereas lowest grain yield (12.34 q ha^{-1}, 12.84 q ha^{-1} and 12.63 q ha^{-1}) was obtained from the no inoculated plot during 2008-09, 2009-10 and pooled of two years respectively.

Table 3: Grain yield, husk and stalk yield and harvest index as influenced by treatments

Treatments	Grain yield (qha^{-1})			Husk yield (qha^{-1})			Stalk yield (qha^{-1})			Harvest index (%)		
	2008-09	2009-10	Pooled	2008-09	2009-10	Pooled	2008-09	2009-10	Pooled	2008-09	2009-10	Pooled
Bio-fertilizers												
No inoculation	12.43	12.84	12.63	3.97	4.11	4.04	22.93	23.37	23.14	31.52	31.38	31.70
Rhizobium	14.71	15.05	14.87	4.56	4.65	4.61	25.73	26.16	25.94	32.65	32.77	32.68
PSB	16.93	17.24	17.08	5.11	5.23	5.17	28.68	28.92	28.80	33.37	33.52	33.42
VAM	17.57	17.69	17.63	5.22	5.26	5.24	29.80	29.57	29.68	33.39	33.65	33.51
Rhizobium+PSB+VAM	19.91	20.37	20.14	5.76	5.92	5.84	33.23	33.42	33.32	33.80	34.09	34.13
SEm (±)	**0.22**	**0.17**	**0.14**	**0.05**	**0.07**	**0.04**	**0.51**	**0.45**	**0.34**	**0.09**	**0.11**	**0.25**
LSD(0.05)	**0.63**	**0.51**	**0.40**	**0.14**	**0.20**	**0.11**	**1.48**	**1.30**	**0.96**	**0.26**	**0.32**	**0.71**
Homo-brassinolide												
No spray	14.13	14.46	14.30	4.31	4.42	4.36	24.73	25.13	24.93	32.59	32.75	32.67
Pre-flowering	16.36	16.70	16.53	4.99	5.09	5.04	28.35	28.64	28.49	32.82	33.01	33.86
Pre-flowering + pod development	18.44	18.74	18.59	5.46	5.59	5.53	31.14	31.09	31.11	33.43	33.73	33.73
SEm (±)	**0.17**	**0.13**	**0.11**	**0.04**	**0.05**	**0.03**	**0.39**	**0.35**	**0.26**	**0.07**	**0.09**	**0.19**
LSD(0.05)	**0.49**	**0.39**	**0.31**	**0.12**	**0.14**	**0.08**	**1.13**	**1.01**	**0.74**	**0.20**	**0.26**	**0.54**

The percentage increase in grain yield with *Rhizobium* + PSB + VAM, VAM, PSB and *Rhizobium* was 59.46%, 39.59%, 35.23% and 17.14 % over no inoculation in respect of pooled of two years (Table 3). Inoculations of *Rhizobium* + PSB + VAM recorded higher grain yield of 20.37 q ha^{-1} during 2nd year followed by VAM (17.69 q ha^{-1}), PSB (17.24 q ha^{-1}), *Rhizobium* (15.05 q ha^{-1}) and no inoculation (12.84 q ha^{-1}). Similar trend was found during 1st year also. Inoculations of *Rhizobium* + PSB + VAM recorded significantly higher husk yield, stalk yield and harvest index (%) as compared to no inoculation, *Rhizobium*, PSB and VAM inoculations during both the years. The highest husk yield (5.76 q ha^{-1}, 5.92 q ha^{-1} and 5.84 q ha^{-1}) and harvest index (33.80%, 34.09% and 34.13%)was produced in crop receiving the treatment of *Rhizobium* + PSB + VAM, whereas lowest husk yield (3.97 q ha^{-1}, 4.11 q ha^{-1} and 4.04 q ha^{-1}) and harvest index (31.52%, 31.38% and 31.70%) was obtained from the no inoculated plot during 2008-09, 2009-10 and pooled of two years respectively. The percentage increase in stalk yield with *Rhizobium* + PSB + VAM, VAM, PSB and *Rhizobium* was 43.99%, 28.26%, 24.46% and 12.10 % over no inoculation in respect of pooled of two years. This increase in grain yield, stalk yield and harvest index might be due to higher number of grains plant^{-1} and effect of biofertilizer inoculations. It is well known that PSB produce vitamins (Baya *et al.*, 1981) and IAA, GA like growth substances (Satter and Gaur, 1987). These growth factors in combination with better nutritional condition due to increased availability of phosphorus in soil might have played a role increasing the grain yield, husk yield, stalk yield and harvest index (%). On the other hand, VAM not only supplies essential nutrients but also water to plants resulting in better growth that led to increasing grain yield, husk yield, stalk yield and harvest index (%). Shinde (1990), Yadav and Shrivastava (1997) and Pramanik and Singh (2003) were recorded similar findings. The spraying of homo-brassinolide also exerted significant effect on grain yield, husk yield, stalk yield and harvest index of chickpea during both the years (Table 3).

The highest grain yield (18.44 q ha^{-1}, 18.74 q ha^{-1} and 18.59 q ha^{-1}) was produced in crop receiving the treatment of twice sprayings of homo-brassinolide at pre-flowering and pod development, whereas lowest grain yield (14.13 q ha^{-1}, 14.46 q ha^{-1} and 14.30 q ha^{-1}) was obtained from the no spraying plot during 2008-09, 2009-10 and pooled of two years respectively. The percentage increase in grain yield with pre-flowering + pod development and pre-flowering was 30.00% and 15.59 % over no spraying in respect of pooled of two years. Spraying of homo-brassinolide at pre-flowering + pod development stages recorded higher grain yield of 18.74 q ha^{-1} during 2nd year followed by pre-flowering (16.70 q ha^{-1}) and no spraying (14.46 q ha^{-1}). Similar trend was found during 1st year also. Sprayings of homo-brassinolide at pre-flowering and pod development recorded significantly higher husk yield, stalk yield and harvest index (%) as compared to no spraying and pre-flowering during both the years. The highest husk yield (5.46 q ha^{-1}, 5.59 q ha^{-1} and 5.53 q ha^{-1}) and harvest index (33.43%, 33.73% and 33.73%)was recorded in crop receiving the treatment of pre-flowering + pod development, whereas lowest husk yield (4.31 q ha^{-1}, 4.42 q ha^{-1} and 4.36 q ha^{-1}) and harvest index (32.59%, 32.75% and 32.67%) was obtained from the no spraying plot during 2008-09, 2009-10 and pooled of two years respectively. The percentage increase in stalk yield with pre-flowering + pod development and pre-flowering stage was 24.79% and 14.28 % over no inoculation in respect of pooled of two years (Table 3). The increase in yield due to application of homo-brassinolide was in consonance with the findings of Mai *et al.* (1989), Prakash *et al.*, (2008) and Pramanik *et al*.(2010).

Based on the above results and discussion, following conclusion can be drawn that inoculation of *Rhizobium* + PSB + VAM as well as two spraying of homo-brassinolide at pre-flowering + pod development stages had a significant influence on plant height, yield parameters, yield and harvest index.

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