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Effect of biofertilizers and phytohormone on growth, productivity and quality of sunflower (*Helianthus annuus*. L)

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ABSTRACT

Biofertilizers like Azotobacter can fix atmospheric nitrogen; phosphate solubilizing bacteria (PSB) and vesicular arbuscular mycorrhiza (VAM) are known to increase immobile nutrients particularly phosphorus in the root rhizosphere for increasing crop productivity. A study was carried out to evaluate the effect of biofertilizers and phytohormone on growth, productivity and quality of sunflower. The experiment was laid out in factorial randomized block design with four levels of biofertilizers inoculation (Azotobacter, PSB + Azotobacter, VAM + Azotobacter and PSB + VAM + Azotobacter and two spraying of homo-brassinolide (HBR) @ 0.5ml litre^{-1} of water at budding stage and budding + flowering. The results showed that inoculation of biofertilizers significantly increased aerial biomass production, CGR, test weight, weight of thalamus, number of filled seeds capitulum^{-1} and as well as seed yield, biological yield and oil content. The combined inoculation of PSB + VAM + Azotobacter recorded higher seed yield (3225kg ha^{-1}) over Azotobacter, PSB + Azotobacter and VAM + Azotobacter inoculation. Application of brassinolide at budding + flowering stages significantly recorded higher value of biomass production, CGR, yield parameters and yield as compared to brassinolide spraying at budding stage alone. The maximum seed yield (2838 kg ha^{-1}) and oil yield was obtained from crop receiving the treatment of spraying of brassinolide over only one spraying at budding stage.

***Keywords**: Azotobacter*, homo-brassinolide, PSB, sunflower and VAM

Sunflower (*Helianthus annuus*) is one of the most widely cultivated high quality oil seed crop in world and it has a relatively high concentration of linoleic acid (Seiler, 2007). The application of Biofertilizer like *Azotobacter* can fix atmospheric nitrogen which is very much essential for plants growth and yield. Most Indian soils are deficient in phosphorus. P is generally a limiting factor in sunflower growth and yield because P deficiencies reduce the accumulation of crop biomass (Zubillaga *et al*., 2002). Considering the importance of P nutrition in sunflower and the need for economizing P fertilizer use, microbial P-solubilization as well as mobilization would be the only possible way to increase plant-available P (Peix *et al*., 2001). PSB plays a vital role for making unavailable phosphorus to available phosphorus by mineralization of organic phosphate or by solubilization of inorganic phosphate by production of acids (Rodriguez and Fraga, 1999). Another important biofertiliser, Vesicular-Arbuscular Mycorrhizae (VAM) fungi provides significant amount of nutrients to the plants such as copper, zinc, phosphorus and sulphur by making their widely extended hyphal network on the upper or lower side of the soil layer. Favourable response of phosphate solubilizing bacteria (PSB) and vesicular arbuscular mycorrhizae (VAM) have been noticed by many workers (Tilak and Singh, 1994). The use of these biofertilizers may open the new vistas of phosphorus nutrition. Brassinosteroids (BRs) are common plant-produced compounds and its exogenous application have a broad spectrum effect on physiological responses like cell expansion, vascular differentiation, reproductive development, seed germination, flowering, and fruit set in plants (Cao *et al*., 2005: Yu *et al*., 2004). Pramanik and Bera (2012) also reported that brassinosteroids had significant effect on yield and quality of hybrid groundnut. . Hence, an experiment was conducted to study the effect of biofertilizers and phytohormone on growth, productivity and quality of sunflower (*Helianthus annuus*. L).

MATERIALS AND METHODS

A field experiment was conducted during the *rabi* season of 2010-2011 and 2011-2012 at farmers field adjacent to the farm of the Institute of Agriculture (Palli Siksha Bhavana), Visva-Bharati, Sriniketan, West Bengal. The place is situated at $23^0 39'$ N latitude, $87^0 42'$ E longitude and an elevation of 58.9 m above mean sea level. The soil was slightly acidic (p^H 5.7), low in available nitrogen (130kg ha^{-1}), phosphorus (12.50 kg ha^{-1}) and medium in potassium (163.5 kg ha^{-1}). In the study, “PAC 36” commercial hybrid of oilseed sunflower, which had early maturating, high yield potential, responsive to higher inputs, more tolerant to diseases and pests, higher drought tolerance, more self fertile, superior in their seed filling ability and higher adaptation ability, was used as plant material. The crop was fertilized with respectively dose of 80:100:100 NPK kg ha^{-1}. The experiment was laid out in factorial randomized block design with four levels of biofertilizers

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inoculation (*Azotobacter*, Phosphate solubilizing bacteria (PSB) + *Azotobacter*, Vesicular arbuscular mycorrhizae (VAM) + *Azotobacter* and Phosphate solubilizing bacteria (PSB) + Vesicular arbuscular mycorrhizae (VAM)) + *Azotobacter* and two spraying of homo-brassinolide (HBR) @ 0.5 ml litre^{-1} of water at budding stage and budding + flowering. In all eight treatments replicated three times. The seed was inoculated with *Azotobacter* and PSB by slurry method whereas the soil was inoculated with VAM inoculums. The VAM inoculums were placed at the seeding depth of the soil and then pre-inoculated seeds were sown according to the treatment. The source of homo-brassinolide was "double" and was sprayed according to treatment for achieve higher grain yield. For determining dry matter accumulation, sunflower plant were cut at ground level from 1 meter row length within earmarked area in each plot kept for the purpose of destructive sampling at 30, 60 and 90 DAS. Plant of each plot were separated into green leaves, stem, capsule, and dried in a hot air oven, kept at 65^{0}C for 48 hours till constant weight were obtained. The dry weight of leave, stem, and capsules were recorded and used for determination of aerial dry matter accumulation. Crop growth rate during the period of two growth stage was determined with the following formula given

$$CGR = \frac{W_2 - W_1}{t_2 - t_1} \text{ g m}^{-2}\text{day}^{-1}$$

Where, W_2 and $W_{1,}$ are the final initial total dry weights of all plants per unit land area (m^2) at the time t_2 and t_1 respectively. The yield parameters and yield were recorded at harvesting stage (95 DAS) of plant. The head samples for yield were also dried to constant weight and threshed mechanically. Seed yield was adjusted to a 10% moisture basis. Filled seed and empty hulls were separated by hand. Hereafter, grain number per head refers to filled grains only. Seed oil was determined using the Soxhlet method in seed kernel (dehulled). Data collected were subjected to statistical analysis of variance according to Gomez and Gomez (1984) using MSTAT computer program.

RESULTS AND DISCUSSION

Aerial biomass production

Inoculation of biofertilizers exerted significant effect on crop aerial biomass at all the growth stages in the respective years under study (Table 1). Maximum aerial biomass of crop was recorded in crop with the combined inoculation of PSB + VAM + *Azotobacter* than those recorded at *Azotobacter*, PSB+ *Azotobacter* and VAM+ *Azotobacter* during both the years. Sunflower crop with *Azotobacter* alone produced the lowest aerial biomass as compared to other combined biofertilizers treatments. In respect to aerial biomass production, there was no significant difference between the treatment of PSB + *Azotobacter* and VAM + *Azotobacter*. Increased aerial biomass of crop by PSB + VAM + *Azotobacter* might be due to better development of root systems resulting in tapping larger volume of bound soil water and nutrients especially phosphorus which plays a significant role in several physiological and biochemical plant activities like photosynthesis, transformation of sugar to starch, and transporting of the genetic traits. This result is in conformity with the findings of Mukherjee and Rai (2000) and Pramanik (2003). Twice spraying of homo-brassinolide at budding + flowering stage recorded significantly highest aerial biomass production of crop (377.33g) over one spraying of homo-brassinolide at budding stage at 90 DAS (169.22g) respectively.

Table 1: Aerial biomass (g m^{-2}) of sunflower as influenced by biofertilizers and homo-brassinolide

Treatments	30 DAS			60 DAS			90 DAS		
	2010-11	2011-12	Pooled	2010-11	2011-12	Pooled	2010-11	2011-12	Pooled
Bio-fertilizers									
Azotobacter	10.13	10.58	10.35	85.84	91.76	88.80	161.71	176.74	169.22
PSB + *Azotobacter*	11.45	11.73	11.58	115.61	119.44	117.52	260.78	294.45	277.61
VAM + *Azotobacter*	11.72	12.12	11.92	124.99	129.30	127.14	268.97	299.80	284.38
PSB+VAM+*Azotobacter*	13.06	13.75	13.40	161.32	165.64	163.47	359.15	395.53	377.33
SEm (±)	**0.24**	**0.20**	**0.15**	**3.52**	**3.51**	**2.40**	**5.82**	**6.15**	**4.10**
LSD(P=0.05)	**0.73**	**0.61**	**0.43**	**10.70**	**10.67**	**6.93**	**17.66**	**18.67**	**11.8**
Homo-brassinolide									
Budding stage	11.50	11.97	11.74	120.86	126.53	123.69	241.16	262.67	251.92
Budding + flowering stage	11.66	12.11	11.89	123.02	126.54	124.77	284.13	320.58	302.35
SEm (±)	**0.17**	**0.14**	**0.10**	**2.49**	**7.54**	**1.70**	**4.11**	**4.35**	**2.90**
LSD (0.05)	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**12.49**	**13.20**	**8.37**

Similar types of results were reported by Ramraj *et al*. (1997) and Pramanik *et al*. (2012). Increased dry matter accumulation 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).

Crop growth rate (CGR)

It was found from the data that CGR increased progressively with the advancement of the age of the crop upto 60 to 75 DAS. Application of PSB+VAM+*Azotobacter* recorded significantly higher CGR value over *Azotobacter*, *Azotobacter* +PSB and *Azotobacter* +VAM at all the growth stages during both the years of experiments. The higher crop growth rate among the biofertilizers might be due to higher dry matter accumulation. Since the CGR is a function of total dry matter production. This result is in full agreement with Shinde (1990). Twice spraying of homo-brassinolide at budding + flowering stage was recorded significantly highest CGR value whereas one spraying of homo-brassinolide at budding stage recorded the lowest value at 60 to75 and 75 to 90 DAS.

Test weight (1000 seed weight)

It was revealed from the result (pooled) that PSB + VAM+ *Azotobacter* recorded the maximum test weight which was significantly different from other treatments of biofertilizers (Table 3). The minimum test weight was recorded in the crop receiving *Azotobacter* treatment during both the years of experiment. Hence there is a strong positive synergistic effects that caused to improving photosynthesis by increasing water and nutrients absorption and this leading to producing more assimilate and improving plant growth, as result 1000-seed weight increased as compared with *Azotobacter*, PSB + *Azotobacter* and VAM + *Azotobacter* inoculation. Similar result was reported by Barea *et al*., (1975). The maximum test weight was recorded with two time spraying of homo-brassinolide at budding + flowering stage during both the years of experiments. The minimum test weight was obtained from crop receiving one spraying of homo-brassinolide at budding stage. The results are conformity with those of Mitchell and Gregory (1972).

Weight of thalamus

PSB + VAM + *Azotobacter* recorded the maximum weight of thalamus (45.89 g) whereas the minimum weight of thalamus (29.05 g) was obtained with the treatment of *Azotobacter* inoculation (Table 3). The second best treatment was VAM + *Azotobacter* inoculation (38.67 g) but it was at par with PSB + *Azotobacter* (36.88 g) during both the years. The high response of plant to the PSB + VAM + *Azotobacter* inoculation might be due to mobilization of available P by the native soil microflora, or attributed of increased phosphate solubilizing bacteria activity in the rhizosphere following PSB + VAM + *Azotobacter* application and consequently by enhanced P solubilization. For these reason, it's enhanced P uptake by the crops and to an increase thalamus weight ultimately leading to higher seed yields. Similar result was reported by Barea *et al*., (1975) and Ekin (2010). Two spraying of homo-brassinolide at budding + flowering stage was recorded the highest weight of thalamus (pooled) of 41.25 g over one spray at budding stage (34.00 g) during both the years. The results are conformity with those of Mitchell and Gregory (1972).

Number of filled seed capitulum^{-1}

The combined inoculation of PSB + VAM+ *Azotobacter* treated plant had 35.6 % more number of filled seed capitulum^{-1} (pooled) in compared to *Azotobacter* (Table 3). It means there is a strong synergistic effect among PSB, VAM and *Azotobacter*. It is well known that phosphorous solubilizing bacteria can increase the available phosphorous in the soil which could enhance the seed number in plant (Tohidi-Moghaddam *et al*.,2004). On the other hand, VAM in rhizosphere mobilizes and facilitates the uptake of many inorganic nutrients such as phosphorus, zinc, molybdenum, copper and iron for plant growth and increasing portion of generating organs such as number of filled seed capitulum^{-1}. Growth promoting hormones like indole acetic acid followed by cytokinin generated by *Azotobacter* can cause increasing preserved material through growing side roots and improving root weight and vegetation growth and ultimately number of filled seed capitulum^{-1}. Twice spraying of homo-brassinolide at budding + flowering stage was recorded significant highest (524.5) number of filled seed capitulum^{-1} during both years. The lowest (457.7) number of filled seed capitulum^{-1} was obtained from the crop receiving one spraying of homo-brassinolide at budding stage during the both the years of experiments, respectively.

Seed yield

The highest seed yield (3225 kg ha^{-1}) was produced in crop receiving PSB+VAM+*Azotobacter* inoculation (Table 4).The crop with *Azotobacter* treatment produced the lowest seed yield (1969kg ha^{-1}). The result of pooled analysis showed that combined inoculation of phosphate solubilizing bacteria + Vesicular arbuscular mycorrhizae resulted in 63.78, 37.70 and 21.79 % higher seed yield over *Azotobacter*, PSB + *Azotobacter* and VAM + *Azotobacter* inoculation, respectively. High crop productivity with PSB + VAM + *Azotobacter* inoculation was mainly responsible for better growth of the crop, greater test weight, thalamus weight and seed filling. The result is conformity with those of Jones and Sreenivas (1993).

Table 2: Crop growth rate (g m^{-2} d^{-1}) of sunflower as influenced by biofertilizers and homo-brassinolide

Treatments	**30-45 DAS**		**45-60 DAS**		**60 -75 DAS**		**75 –90 DAS**	
	2010-11	**2011-12**	**2010-11**	**2011-12**	**2010-11**	**2011-12**	**2010-11**	**2011-12**
Bio-fertilizers								
Azotobacter	1.24	1.39	3.81	4.02	3.06	3.06	2.00	2.61
PSB + *Azotobacter*	2.69	2.77	4.26	4.41	4.66	5.49	5.02	6.17
VAM + *Azotobacter*	2.73	2.92	4.82	4.89	4.56	5.06	5.04	6.30
PSB+VAM+*Azotobacter*	3.63	3.72	6.26	6.41	6.01	6.80	7.18	8.53
SEm (±)	**0.10**	**0.08**	**0.26**	**0.24**	**0.31**	**0.33**	**0.39**	**0.48**
LSD (P=0.05)	**0.30**	**0.26**	**0.79**	**0.75**	**0.94**	**1.00**	**1.18**	**1.45**
Homo-brassinolide								
Budding stage	2.56	2.67	4.72	4.96	3.74	3.86	4.27	5.21
Budding + flowering stage	2.57	2.72	4.85	4.90	5.39	6.34	5.34	6.59
SEm (±)	**0.07**	**0.06**	**0.18**	**0.17**	**0.22**	**0.24**	**0.27**	**0.34**
LSD (0.05)	**NS**	**NS**	**NS**	**NS**	**0.66**	**0.71**	**0.84**	**1.03**

Table 3: Yield attributes of sunflower as influenced by biofertilizers and homo-brassinolide

Treatments	**Test weight (g)**			**Weight of thalamus (g)**			**No. of filled seed capitulum^{-1}**		
Bio-fertilizers	**2010-11**	**2011-12**	**Pooled**	**2010-11**	**2011-12**	**Pooled**	**2010-11**	**2011-12**	**Pooled**
Azotobacter	47.0	47.2	47.11	28.71	29.40	29.05	414.8	422.1	418.5
PSB + *Azotobacter*	48.6	49.4	49.05	36.28	37.49	36.88	466.7	475.4	471.1
VAM + *Azotobacter*	49.7	50.5	50.13	38.21	39.13	38.67	503.6	511.0	507.3
PSB+VAM+*Azotobacter*	53.3	54.3	53.84	44.37	47.42	45.89	563.7	571.4	567.6
SEm (±)	**0.54**	**0.60**	**0.40**	**0.97**	**1.05**	**0.69**	**6.8**	**6.9**	**4.6**
LSD (0.05)	**1.63**	**1.82**	**1.15**	**2.96**	**3.19**	**1.99**	**20.6**	**21.0**	**13.2**
Homo-brassinolide									
Budding stage	47.6	48.6	48.17	32.66	35.33	34.00	453.9	461.5	457.7
Budding + flowering stage	51.69	52.10	51.90	41.11	41.38	41.25	520.5	528.4	524.5
SEm (±)	**0.38**	**0.42**	**0.28**	**0.69**	**0.74**	**0.49**	**4.8**	**4.9**	**3.3**
LSD (0.05)	**1.15**	**1.28**	**0.81**	**2.09**	**2.26**	**1.41**	**14.5**	**14.8**	**9.53**

Table 4: Yield, harvest index and oil % of sunflower as influenced by biofertilizers and homo-brassinolide

Treatments	**Seed yields (kg ha^{-1})**			**Biological yield (kg ha^{-1})**			**Oil yield (kg ha^{-1})**		
Bio-fertilizers	**2010-11**	**2011-12**	**Pooled**	**2010-11**	**2011-12**	**Pooled**	**2010-11**	**2011-12**	**Pooled**
Azotobacter	1866	2073	1969	7295	7729	7512	579	667	622
PSB + *Azotobacter*	2264	2421	2342	8337	8838	8587	744	816	780
VAM + *Azotobacter*	2545	2752	2648	8980	9453	9216	871	967	918
PSB+VAM+*Azotobacter*	3189	3263	3225	10271	10300	10289	1175	1245	1210
SEm (±)	**65**	**59**	**44**	**130**	**152**	**103**	**25**	**16**	**14**
LSD (0.05)	**197**	**181**	**127**	**395**	**462**	**297**	**76**	**48**	**40**
Homo-brassinolide									
Budding stage	2171	2337	2254	8106	8471	8288	716	785	750
Budding + flowering stage	2760	2917	2838	9335	9692	9513	968	1061	1015
SEm (±)	**45**	**42**	**31**	**92**	**107**	**72**	**17**	**11**	**10**
LSD (0.05)	**139**	**128**	**89**	**279**	**327**	**207**	**54**	**34**	**28**

Spraying of homo-brassinolide at budding + flowering stage recorded significant higher seed yield (2838 kg ha^{-1}) as compared to one spraying at budding stage (2254 kg ha^{-1}). The result of pooled analysis showed that two spraying of homo-brassinolide increased 25.90 % higher seed yield as compared to one spraying. Similarly higher productivity with two spraying of homo-brassinolide might be due to better growth of the crop, greater test weight, thalamus weight and seed filling. The similar results are in conformity with those of Nayak and Murthy, (1980) and Chowdhary *et al*. (1994).

Biological yield

Biological yield (Table 4) was significantly influenced by biofertilizers inoculation. Inoculation of PSB + VAM + *Azotobacter* recorded significantly higher biological yield as compared to *Azotobacter*, PSB + *Azotobacter* and VAM + *Azotobacter* inoculation. Higher biological yield might be due to better growth and yield. Shinde (1990) and Yadav and Shrivastava (1997) were recorded similar findings. Twice spraying of homo-brassinolide at budding + flowering stage was significantly higher biological yield as compared to one spraying at budding stage. The result is in conformity with those of Meudt *et al*., (1983).

Oil yield

Combined inoculation of PSB + VAM + *Azotobacter* recorded significantly higher oil yield as compared to other treatment combinations (Table 4). The spraying of homo-brassinolide at budding + flowering stage recorded maximum oil yield. The increase in oil yield might be due to higher seed yield. Similar type of result was reported by Mai *et al.* (1989).

Interaction effect

The interaction effect of biofertilizers and homo-brassinolide on biomass accumulation, test weight, weight of thalamus, number of filled seed acpitulam^{-1}, seed yield, biological yield and oil yield were found significant. The maximum biomass accumulation and number of filled seed acpitulam^{-1}; highest test weight, weight of thalamus, seed yield, biological yield and oil yield were recorded in crop receiving PSB+VAM + *Azotobacter* inoculation at twice spraying of homo-brassinolide at budding +flowering stage (Table 5). The minimum biomass accumulation and number of filled seed acpitulam^{-1}; lowest test weight, weight of thalamus, seed yield, biological yield and oil yield were obtained from the crop receiving *Azotobacter* inoculation treatment along with one spraying of budding stage.

Table 5: Interaction effect of biofertilizers and homo-brassinolide on yied and yield parameters of sunflower (Pooled)

Interaction	Biomass 90 DAS	Test weight (g)	Weight of thalamus (g)	No. of filled seed capitulum^{-1}	Seed yield (kg ha^{-1})	Biological yield (kg ha^{-1})	Oil yield (kg ha^{-1})
Azotobacter +HBR at Budding	150.24	45.46	24.72	383.0	1850	7303	579
Azotobacter+ HBR at Budding + flowering stage	188.21	48.76	33.38	454.0	2088	7721	666
PSB + *Azotobacter* + Budding	259.17	48.05	34.96	449.1	2089	7951	744
PSB+Budding + flowering stage	296.05	50.05	38.80	493.0	2595	9223	816
VAM + *Azotobacter*+ Budding	263.29	48.95	36.29	480.1	2328	8554	871
VAM+Budding + flowering stage	305.47	51.31	41.05	534.5	2968	9878	966
PSB+VAM+*Azotobacter*+ Budding	334.97	50.21	40.01	518.7	2749	9346	1175
PSB+VAM+Budding + flowering stage	419.70	57.46	51.77	616.4	3701	11231	1245
SEm (±)	**5.80**	**0.56**	**0.98**	**6.63**	**62.4**	**145**	**20.6**
LSD (0.05)	**16.75**	**1.62**	**2.83**	**19.14**	**180.17**	**418.67**	**59.48**

Note: HBR= Homo-brassinolide

The highest seed yield (3701 kg ha^{-1}) was recorded in crop receiving PSB+VAM+*Azotobacter* inoculation at twice spraying of homo-brassinolide at budding + flowering stage. The lowest grain yield (1850 kg ha^{-1}) was obtained from the crop receiving *Azotobacter* inoculation treatment along with one spraying of homo-brassinolide at budding stage. Based on the above results and discussion, it can be said that inoculation of PSB + VAM+ *Azotobacter* as well as two spraying of homo-brassnolide at budding + flowering stages had a significant influence on aerial biomass production, yield attributes, seed yield, biological yield and oil yield. This can partially encourage farming with the mere use of biological fertilizers (organic systems).

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