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Effect of Biofertilizers and Sulphur on Growth, Yield, and Oil Content of Hybrid Sunflower (*Helianthus annuus.* L) In a Typical Lateritic Soil

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Abstract

Field experiments were conducted in a typical lateritic soil, during the *rabi* season of 2009-2010 and 2010-2011 at Agricultural Research Farm, Institute of Agriculture, Visva-Bharati University, Sriniketan, India, to find out the effect of biofertilizers and sulphur on growth, yield, and oil content of sunflower (*Helianthus annuus* L.). The experiment was laid out in factorial RBD with three types of biofertilizers, *viz*. Phosphate Solubilizing Bacteria (PSB)+*Azotobacter,* Vesicular Arbuscular Mycorrhizae (VAM)+*Azotobacter* and Phosphate Solubilizing Bacteria+Vesicular Arbuscular Mycorrhizae+*Azotobacter*, and four levels of sulphur (S_0, S_{20}, S_{40} and S_{60} kg ha^{-1}). The crop was fertilized with respective dose of NPK of 80:100:100 kg ha^{-1}. Results revealed that inoculation of biofertilizers significantly affected plant height and total chlorophyll content. Biofertilizers also significantly increased yield attributes, *viz.* thalamus diameter, weight of thalamus, filled seeds $capitulum^{-1}$, and 100 seed weight (g), as well as seed and biological yield and oil content. The combined inoculation of PSB+VAM+*Azotobacter* recorded higher values of these parameters, as compared to PSB+*Azotobacter* and VAM+*Azotobacter* inoculation. Application of sulphur @40 kg ha^{-1} significantly improved plant height and total chlorophyll content, as well as yield attributes, yield, and oil content, as compared to other levels of sulphur application. PSB+VAM+*Azotobacter,* as well as application of sulphur @40 kg ha^{-1}, was found to be the best treatment for hybrid sunflower.

Keywords: *Azotobacter*; Hybrid sunflower; PSB; Sulphur; VAM

Introduction

Sunflower (*Helianthus annuus* L.) is one of the most important oilseed crops containing high quality edible oil. It is easy to cultivate and grown in different conditions and soils [1,2]. Sunflower oil has excellent nutritional properties, and has a relatively high concentration of linoleic acid [3]. Since the environmental and health problems arising from chemical fertilizers usage, attention has been drawn to the application of biological fertilizers in agriculture. Biological fertilizers or biofertilizers contain useful microorganisms, which could colonize the rhizosphere and promote plant growth through increasing the supply or availability of essential nutrients to the plants [4]. Considering the significant role of N, S and P in sustainable production of oil seed crops, an experiment was conducted to study the effect of biofertilizers and sulphur on growth, yield and oil content of sunflower (*Helianthus annuus*. L).

Materials and Methods

Field experiments were conducted in a typical lateritic soil at Agricultural Research Farm, Institute of Agriculture, Visva-Bharati University, Sriniketan (23˚03' N and 87˚04' E), West Bengal, India, with hybrid sunflower (*Helianthus annuus*. L), during the rabi season of 2009-2010 and 2010-2011. The soil was slightly acidic (pH-5.6), low in available nitrogen (127 kg ha^{-1}), phosphorus (14.50 kg ha^{-1}), sulphur (8.00 mg kg^{-1}), and medium in potassium (167.5 kg ha^{-1}). The experiment was laid out in factorial randomized block design, with three levels of biofertilizers inoculation *viz.* Phosphate Solubilizing Bacteria (PSB)+*Azotobacter,* Vesicular Arbuscular Mycorrhizae (VAM)+ *Azotobacter* and Phosphate Solubilizing Bacteria (PSB)+Vesicular Arbuscular Mycorrhizae (VAM)+*Azotobacter,* and four levels of sulphur *viz.* 0 kg ha^{-1} (S_0), 20 kg ha^{-1} (S_{20}), 40 kg ha^{-1} (S_{40}) and 60 kg ha^{-1} (S_{60}), as elemental S. The seed was inoculated with *Azotobacter* and PSB by slurry method, whereas the soil was inoculated with VAM inoculums (Mfg. by Symbiotic Sciences, New Delhi). The VAM inoculums were placed at the seeding depth of the soil, and then pre-inoculated seeds were sown according to the treatment. The yield parameters and yield were recorded at harvesting stage (95 DAS) of plant. The crop was fertilized with respectively dose of 80:100:100 NPK kg ha^{-1}. Oil from the sunflower seeds were extracted with petroleum ether in Soxhlet apparatus. It is then distilled off completely, dried, the oil weighed, and the per cent oil is calculated [5]. Total chlorophyll content was measured adopting the method of Hiscox and Israelstam [6], and calculated by using the formula given by Arnon [7], and expressed as mg g^{-1} of fresh leaf.

Statistical analysis

Data collected were subjected to statistical analysis of variance according to Gomez and Gomez [8], using MSTAT computer program.

Results

Effect of biofertilisers and sulphur on plant height

Data on mean plant height (cm) recorded at the harvest stage of crop are presented in table 1. Inoculation of Phosphate Solubilizing Bacteria+Vesicular Arbuscular Mycorrhizae+*Azotobacter,* recorded significantly higher plant height during the both years, as compared to other treatments. In the second year, PSB+*Azotobacter* were at par with VAM+*Azotobacter* inoculation. This showed a strong synergistic effect between PSB+VAM+*Azotobacter*. The results are in conformity with those of Mukherjee and Rai [9]. Application of S@40 kg ha^{-1} recorded significantly higher plant height, as compared to S@0 and 20 kg ha^{-1},

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Treatments	Plant height at harvest (cm)		Total chlorophyll content at different growth stages (mg g^{-1} of fresh leaf)							
			30 DAS		45 DAS		60 DAS		75 DAS	
Bio-fertilizers	2009-10	2010-11	2009-10	2010-11	2009-10	2010-11	2009-10	2010-11	2009-10	2010-11
PSB+*Azotobacter*	81.47	85.66	1.066	1.077	1.437	1.452	1.615	1.628	1.733	1.851
VAM+*Azotobacter*	82.33	86.53	1.104	1.117	1.477	1.497	1.684	1.701	1.802	1.927
PSB+VAM+*Azotobacter*	87.63	94.03	1.163	1.177	1.539	1.559	1.759	1.775	1.891	2.028
SEm (±)	0.34	0.38	0.002	0.002	0.003	0.004	0.01	0.011	0.009	0.011
CD(P=0.05)	0.8	0.9	0.005	0.005	0.007	0.009	0.024	0.026	0.021	0.026
Sulphur levels (kg ha^{-1})										
S_0	79.32	83.52	1.06	1.071	1.431	1.446	1.51	1.524	1.571	1.663
S_{20}	82.06	86.26	1.09	1.101	1.464	1.481	1.677	1.693	1.785	1.894
S_{40}	87.2	93.1	1.145	1.159	1.521	1.541	1.778	1.795	1.939	2.107
S_{60}	86.66	92.09	1.15	1.163	1.522	1.543	1.779	1.795	1.94	2.107
SEm (±)	0.4	0.44	0.003	0.003	0.004	0.004	0.012	0.012	0.011	0.013
CD(P=0.05)	0.95	1.04	0.007	0.007	0.009	0.009	0.028	0.028	0.026	0.031

Table 1: Plant height and total chlorophyll content as influenced by biofertilizers and sulphur of sunflower.

Treatments	Thalamus diameter (cm)		Weight of thalamus (g)		Filled seeds capitulum^{-1}		100, seed weight (g)	
Bio-fertilizers	2009-10	2010-11	2009-10	2010-11	2009-10	2010-11	2009-10	2010-11
PSB+*Azotobacter*	11.12	11.32	28.45	30.25	288.58	295.58	3.84	3.99
VAM+ *Azotobacter*	12.42	12.7	35.2	37.17	297.25	303.83	4.02	4.21
PSB+VAM+*Azotobacter*	13.35	13.54	37.79	40.02	321.83	332.04	4.28	4.45
SEm (±)	0.05	0.03	0.23	0.26	2.02	2.31	0.01	0.02
CD(P=0.05)	0.12	0.07	0.54	0.62	4.78	5.47	0.02	0.05
Sulphur levels (kg ha^{-1})								
S_0	11.64	11.84	30.9	32.72	279.55	287.05	3.93	4.1
S_{20}	12.1	12.4	33.41	35.32	289.88	298.17	3.98	4.15
S_{40}	12.93	13.13	35.85	38.32	321	329.61	4.14	4.33
S_{60}	12.52	12.71	35.08	36.88	319.77	327.11	4.13	4.27
SEm (±)	0.07	0.04	0.27	0.3	2.33	2.66	0.02	0.03
CD(P=0.05)	0.17	0.09	0.64	0.71	5.51	6.29	0.05	0.07

Table 2: Yield attributes (thalamus diameter, weight of thalamus, filled seeds capitulum^{-1} and 100, seed weight), as influenced by biofertilizers and sulphur of sunflower.

but application of S@40 kg ha^{-1} was at par with S@ 60 kg ha^{-1}, during the both year, respectively. Similar results have been reported by Raj et al. [10].

Effect of biofertilisers and sulphur on total chlorophyll content

Total chlorophyll content was higher in the second year, as compared to first year (Table 1). Data also indicated that total chlorophyll content increase with advancement of crop age, up to 75 DAS, during the both years. Total chlorophyll content in leaf was increased by inoculation of biofertilizers. Inoculation of PSB+VAM+*Azotobacter* was recorded significantly higher chlorophyll content in leaf, as compared to PSB+*Azotobacter* and VAM+ *Azotobacter* inoculation, and the difference between PSB+*Azotobacter* and VAM+*Azotobacter* was also statistically significant in the both years at all the crop growth stage. The result is in conformity with those of Jones and Sreeniras [11]. Total chlorophyll content in leaf increased significantly by the successive dose of added sulphur. Consequently, the total chlorophyll content in leaf was in order of $S_{60}>S_{40}>S_{20}>S_0$, but S_{60} kg ha^{-1} was *at par* with S_{40} kg ha^{-1} during both the years. Similar results have been reported by Yadav et al. [12].

Effect of biofertilisers and sulphur on yield attributes

Data on thalamus diameter (cm), weight of thalamus (g), filled seeds capitulum^{-1} and 100 seed weight (g), as affected by biofertilizers inoculation and levels of sulphur application are presented in table 2. Inoculation of PSB+VAM+*Azotobacter* recorded significantly higher thalamus diameter, weight of thalamus, filled seeds capitulum^{-1} and 100 seed weight, as compared to PSB+*Azotobacter* and VAM+*Azotobacter* inoculation. Application of sulphur @40 kg ha^{-1} recorded significantly higher thalamus diameter, weight of thalamus, filled seeds capitulum^{-1} and 100 seed weight, as compared to S_0, S_{20} and S_{60} kg ha^{-1}. Similar results have been reported by Yadav et al. [12].

Effect of biofertilizers and sulphur on seed yield, stalk yield, biological yield, harvest index, and oil content

Seed yield, stalk yield, biological yield, harvest index, and oil content, as affected by biofertilizers and sulphur application are presented in table 3. The seed yield was more in the second year, as compared to first year. Inoculation of PSB+VAM+*Azotobacter* showed significant effect on grain yield, stalk yield, biological yield, harvest index, and oil content, as compared to PSB+*Azotobacter* and VAM+*Azotobacter* inoculation. The result is in conformity with those of Jones and Sreeniras [11]. The average seed yield had significant effect of sulphur levels at crop harvest. The yield increased progressively and significantly, with each successive doses of sulphur application. In S_0 level of sulphur, the seed yield was 13.77 and 15.97, as against 16.08 and 18.27; 20.98 and 23.18 and 19.81 and 21.90 q ha^{-1} recorded in S_{20}, S_{40} and S_{60} levels of sulphur, during the both years, respectively (Table 3). Thus, the difference in yield resulting from S application was significant. This confirms the findings of Mishra

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Treatments	Seed yield (q ha^{-1})		Stalk yield (q ha^{-1})		Biological yield (q ha^{-1})		Harvest index (%)		Oil content %	
Bio-fertilizers	2009-10	2010-11	2009-10	2010-11	2009-10	2010-11	2009-10	2010-11	2009-10	2010-11
PSB+*Azotobacter*	14.15	16.35	61.05	67.77	75.2	84.15	18.78	19.41	27.44	27.57
VAM+*Azotobacter*	17.12	19.4	69.65	74.83	86.76	94.27	19.65	20.5	27.91	28.11
PSB+VAM+*Azotobacter*	21.72	23.75	75.47	80.81	97.2	104.61	22.24	22.64	28.21	28.43
SEm (±)	0.26	0.28	0.91	0.74	1.09	0.96	0.17	0.16	0.08	0.08
CD(P=0.05)	0.62	0.66	2.15	1.75	2.58	2.27	0.4	0.38	0.19	0.19
Sulphur levels (kg ha^{-1})										
S_0	13.77	15.97	57.1	64.22	70.87	80.43	19.3	19.77	26.25	26.39
S_{20}	16.08	18.27	63.45	70.64	79.53	88.95	20.1	20.47	27.73	27.89
S_{40}	20.98	23.18	78.13	82.98	99.12	106.23	21.02	21.72	28.88	29.12
S_{60}	19.81	21.9	76.21	79.84	96.02	101.77	20.48	21.42	28.57	28.75
SEm (±)	0.3	0.33	1.05	0.85	1.26	1.11	0.2	0.18	0.09	0.1
CD(P=0.05)	0.71	0.78	2.48	2.01	2.98	2.63	0.47	0.43	0.21	0.24

Table 3: Seed yield, stalk yield, biological yield, harvest index and oil%, as influenced by biofertilizers and sulphur of sunflower.

and Agarwal [13] in soybean, Ghosh et al. [14] in mustard, Ravi et al. [15] in safflower, and Gangadhara et al. [16] in sunflower.

Discussion

A wide range of bacteria such as *Rhizobium, Azospirillum, Azotobacter, Pseudomonas, Bacillus*, and *Enterobacter* have been used as biofertilizer because of their positive effects on growth and productivity of plants *via* several mechanisms including plant hormones production, N_2 fixation, antagonism against phytopathogenic microorganisms and solubilization of nutrients [17-20].

The higher grain yield due to biofertilizers inoculation might be due to increase in plant height and total chlorophyll content and yield component (thalamus diameter, weight of thalamus, filled seeds capitulum^{-1} and 100 seed weight, as well as seed and stalk yield, and oil content). The phosphate solubilizing bacteria is known produce vitamins and IAA and GA like growth substances [21].

Phosphorus (P) is an essential plant nutrient required for higher and sustained productivity of oil from sunflower. Its influence on seed yield, oil yield, and oil quality has been well established [22-26], and application of phosphorus has become an essential part of sunflower fertilizer program. In general, phosphorus is added to soil as inorganic phosphates, because the free inorganic P in soil solution plays a central role in P-cycling and plant nutrition [27]. However, a large portion of soluble inorganic phosphate applied to soil as chemical fertilizer is immobilized rapidly after application due to phosphate fixation by aluminum, calcium, iron, magnesium, and soil colloids [28], and becomes unavailable to plants [29]. Therefore, P is often a limiting nutrient in agricultural soils. Micro-organisms are also involved in a range of process that affect the transformation of soil P, and thus, an integral part of the soil P cycle [30]. In particular, P-solubilizing micro-organisms (bacteria or fungi) are able to solubilize unavailable soil P and increase the yield of crops [31]. Plant Growth-promoting Rhizobacteria (PGPR) and rhizosphere bacteria are free-living soil organisms that can benefit plant growth by different mechanisms [19]. P-solubilization ability of micro-organisms is considered to be one of the most important traits associated with plant P nutrition [30]. Several bacterial species, in association with plant rhizosphere, are capable of increasing availability of Phosphorus to plants, either by mineralization of organic phosphate, or by solubilization of inorganic phosphate by production of acids [28]. Phosphorus is commonly a limiting factor in sunflower growth and yield because P deficiencies reduce the accumulation of crop biomass [26]. This is attributable to (i) a reduction in the partitioning of assimilates to the formation of leaf area, or (ii) a decrease of the efficiency with which the intercepted radiation is used for the production of above-ground biomass [32]. Rodriguez et al. [33] reported that under P deficiencies sunflower showed a reduction in the rate of leaf expansion, and in photosynthetic rate per unit of leaf area. However, P application produced greater and more consistent effects on crop performance as P fertilization allowed more efficient use of supplied N (soil+fertilizer). Loubser and Human [23] also noted that the response of seed and oil yield of sunflower was in agreement with the P absorption by the plants.

Inoculations of PSB which are known to produce growth hormones are likely to favour increased plant height. Inoculation with PSB+VAM+*Azotobacter* recorded higher chlorophyll content, which might be due to higher content of nitrogen and magnesium, which is core component of chlorophyll [21]. 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 due to increased PSB activity in the rhizosphere, following PSB+VAM+*Azotobacter* application, and consequently, by enhanced P solubilization. These reasons might have contributed towards its enhanced P uptake by the crops, an increase in thalamus diameter, weight of thalamus, filled seeds capitulum^{-1} and 100 seed weight, ultimately leads to higher seed yields. Stimulated photosynthetic activity and synthesis of protein due to sulphur application might have also contributed towards the improvement of better yield attributes.

Various nutrients and micronutrients are required for oilseed production, but the nutrient which plays a multiple role in providing nutrition to oilseed crops, particularly those belonging to *cruciferae* family is "Sulphur". Sulphur is the fourth most important nutrient after nitrogen, phosphorus and zinc for Indian agriculture [34]. Its role in balanced fertilization and consequently in crop production is being increasingly realized. Considering similar sulphur and phosphorus requirements of crops, sulphur can rightly be called as the fourth major nutrient in Indian agriculture. Sulphur is best known for its role in the synthesis of proteins, oils, vitamins, and flavoured compounds in plants. It is a constituent of three amino acids *viz.* Methionine (21% S), Cysteine (26% S) and Cystine (27% S), which are the building blocks of protein. About 90% of plant sulphur is present in these amino acids [35]. Sulphur is also involved in the formation of chlorophyll, glucosides and glucosinolates (mustard oils), activation of enzymes and sulphydryl (SH-) linkages that are the source of pungency in onion, oils, etc. [36]. This is why adequate sulphur is so crucial for oil seed crops. Sulphur is also a constituent of vitamins biotine and thiamine (B_1), and also of iron sulphur proteins called ferrodoxins. Sulphur is associated with the production of crops of superior nutritional and market quality. Each unit of fertilizer sulphur generates 3-5 units of edible oil, a commodity needed by every family. Sulphur application also has marked effect on soil properties, and is used as soil amendment to improve the availability of other nutrients in soil as gypsum and pyrite. Presently, S is being required as fourth major nutrient. S, which is mostly applied to oilseed and pulses, has been found to benefit more than one crop in a sequence due to its significant residual response [37]. S and P have synergistic and antagonistic effect with each other on their varying levels of application, as well as level of availability in the soil [29,38].

Increase in oil content by sulphur application might be attributed to involvement of sulphur in the biosynthesis of oil. Sulphur is involved in the formation of glucosides and glucosinolates and sulphydril-linkage and activation of enzymes, which aid in biochemical reaction within the plant. The higher oil yield by sulphur application was obviously because of higher seed yield and oil content.

Based on the experiment, it can be concluded that inoculation of biofertilisers and sulphur have significant effect on yield and yield attributes of sunflower. However, PSB+VAM+*Azotobacter*, as well as application of sulphur @40 kg ha^{-1} may be considered as the best treatment for sunflower, with respect to height, total chlorophyll content, thalamus diameter, weight of thalamus, filled seeds $capitulum^{-1}$ and 100 seed weight, grain yield, stalk yield, biological yield, harvest index and oil content [39].

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