

Response of sunflower (*Helianthus annuus* L.) to organic manure and biofertilizer under different levels of mycorrhiza and sulphur in comparison with inorganic fertilizer

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ABSTRACT

Research experiment was conducted in a typical sandy loam soil during rabi seasons of 2013-14 and 2014-15. The experiment was laid out in Randomized Block Design (RBD) with five types of biofertilizers, viz. Phosphate Solubilizing Bacteria (PSB), *Azotobacter* sp., *Trichoderma viride*, *Pseudomonas fluorescens* and *Azospirillum lamos* with three label of VAM (VAM0 kg ha⁻¹, VAM5 kg ha⁻¹ and VAM12 kg ha⁻¹) and four levels of sulphur (S0 kg ha⁻¹, S12 kg ha⁻¹, S25 kg ha⁻¹ and S37 kg ha⁻¹). The results showed that, combination of VAM12 kg ha⁻¹ with sulphur 12 kg ha⁻¹ significantly ($P > 0.01$) increased seed yield, and seed oil content. The highest seed yield of 3354.15 kg ha⁻¹ (2013-14), 3666.84 kg ha⁻¹ (2014-15) and oil content 981.09 kg ha⁻¹ (2013-14), 1072.54 kg ha⁻¹ (2014-15) was obtained for the treatment T1 (Organic manure with biofertilizers 247 kg ha⁻¹ + VAM 12 kg ha⁻¹ + Sulphur 12 kg ha⁻¹). The results also indicated that, treatment T3 (FYM 19753 kg ha⁻¹ + organic manure with biofertilizers 247 kg ha⁻¹ + sulphur 37 kg ha⁻¹) gave approximate nearest result as T1 {seed yield of 3204.86 kg ha⁻¹ (2013-14), 3490.91 kg ha⁻¹ (2014-15) and oil content 925.565 kg ha⁻¹ (2013-14), 1008 kg ha⁻¹ (2014-15)}.

Keywords: Biological yield, organic manure, sulphur, sunflower, vesicular arbuscular mycorrhizae

Oilseeds play a major role in Indian economy. India share 7 per cent of world vegetable oil production. The demand of oil seed increased rapidly in the country, where as its production did not increase as per demand which lead to the shortage of oil. India now imports nearly 50 per cent of the annual consumption. This situation of wide demand-supply gap is because of low and unstable yields of most oilseed crops and uncertainty in returns to investment, high risk production environments (Shenoi, 1989; Jha *et al.*, 2012; <http://icra.in>). Sunflower (*Helianthus annuus* L.) is an important oil crop and can be easily cultivated in different condition of soil (Kaya and Kolsarici, 2011; Lopez-Valdez *et al.*, 2011) Sunflower is cultivated in summer as it has been found to be drought resistant (Meo *et al.*, 2000). Sulphur is an important nutrient for optimal plant growth: it is one of the key macro elements essential for plant growth (Stanislav, 2007). Sulphur is taken up from the soil solution by the plant in the sulphate form (SO₄²⁻) (Goh and Pamidi, 2003). In the plants, sulphur is a component of methionine, cysteine and cystine, which are the essential building blocks of proteins (Jamal *et al.*, 2010). Sulphur is also a component of key enzymes and vitamins in the plant and is necessary for the formation of chlorophyll. Plants which are deficient in sulphur show a pale green colouration of younger leaves, as sulphur is not very mobile in the plant. In severe cases of sulphur deficiency, the entire plant can be stunted and turns green to yellow

with restricted capitulum size, inflorescence may remain covered within the bracts and delayed maturity of capitulum (www.wengfuaustralia.com; <http://agropedia.iitk.ac.in>). Decreased inputs of S have increased the incidence of S deficiency in crops, resulting in decreased yields and quality (Hawkesford, 2000).

The presence of mycorrhiza in rhizosphere provides an advantageous and interactive symbiotic relationship between a higher plant root and a nonpathogenic fungus. Most of the authors by their experiment concluded that colonization of plant roots by mycorrhizal fungi promotes plant growth (Raja, 2006) and enhances the uptake of sulphur and other nutrients (Chen *et al.*, 2005; Hossein, 2012). This study was undertaken to find out the effect of adding various doses of organic manure, inoculation of biofertilizer in presence or absence of VAM-spores, phosphorus on the morphological traits and biological yield of sunflower.

MATERIAL AND METHODS

This experiment was aimed to formulate different combination of organic inputs with biofertilizers, vesicular arbuscular mycorrhizae (VAM) and sulphur to enhance the biological growth, grain yield and oil content of sunflower in comparison to 100 per cent chemical (di-ammonium phosphate) and untreated control. Field experiment was conducted in a typical sandy loam soil at agricultural field of Patanjali Bio

Research Institute, Food and Herbal Park, Village Padartha, Haridwar, Uttarakhand, India during *rabi* season of 2013-14 and 2014-15. The sun flower cv. Sunset (Plantsman's Seeds, Punjab) was used in this study. Different soil parameters like pH, electric conductivity, soil moisture, water holding capacity, nitrogen, potassium, phosphorus and organic carbon were analyzed before sowing and also at the time of

harvesting (Table 1). Soil was slightly alkaline (pH 7.458 and 7.520 for the 1st and 2nd year, respectively), medium in organic carbon (0.518 per cent and 0.564 per cent for the 1st and 2nd year, respectively), medium in total nitrogen (0.0518 per cent and 0.0652 per cent for the 1st and 2nd year, respectively), low in available potassium (38.58 per cent and 37.99 per cent for the 1st and 2nd year, respectively).

Table 1: Some of chemical and physical properties of experimental field soil

Parameters	Before sowing		At the time of harvesting	
	2013-14	2014-15	2013-14	2014-15
Moisture (%)	12.45	12.93	13.46	13.39
Water holding capacity (%)	28.54	27.96	28.61	29.67
pH	7.458	7.520	7.528	7.420
Electrical conductivity ($\mu\text{s cm}^{-1}$)	135.8	138.2	148.2	150.5
Organic carbon (%)	0.518	0.564	0.430	0.521
Nitrogen (%)	0.052	0.065	0.043	0.040
Potassium (%)	38.58	37.99	28.21	29.51
Phosphorus (%)	9.14	9.28	25.72	26.02

Seven treatments including an untreated control were arranged in Randomized Complete Block Design and replicated thrice. Details of treatment have been cited in the table 2. The untreated control (T_0) did not receive

any input. The treatments were delivered in the selected plots @ 50 per cent as basal dose at one week before sowing and the remaining 50 per cent was top dressed at three weeks after crop sowing.

Table 2: Treatment details

Treatments	FYM (kg ha ⁻¹)	Organic manure + Biofertilizer (kg ha ⁻¹)	VAM (kg ha ⁻¹)	Sulphur (kg ha ⁻¹)	*Chemical fertilizer (kg ha ⁻¹)
T_0	0	0	0	0	0
T_1	0	247	12	12	0
T_2	4938	247	5	25	0
T_3	19753	247	0	37	0
T_4	9877	247	12	0	0
T_5	1481	247	0	0	0
T_6	0	0	0	0	148

Note: VAM formulation contain mixture of *Glomus clarum* and *G. fasciculatum*, *DAP (N:P₂O₅=18:46)

Important characteristics of organic manure, biofertilizers and chemical fertilizer used in the experiment

Organic manure	Carbon-24per cent, Nitrogen-2.25 per cent, Phosphorous-2.05 per cent, Potassium-1.775 per cent, Calcium-3 per cent, Magnesium-0.45 per cent, Sulphur-0.4 per cent, Iron-0.6 per cent, Zinc-0.35 per cent, Manganese – 0.09 per cent
Bio-fertilizer	<i>Azotobacter</i> sp., <i>Trichoderma</i> sp., <i>Pseudomonas</i> sp., <i>Azospirillum</i> sp.
Chemical	Di-Ammonium phosphate
NA	Not applied

Seeds were sown in a spacing of 60×45cm in plot measuring 16 m² each and total experimental area was 336 m². Seeds were soaked for 30 minutes in commercial NPK product of Patanjali Bio Research Institute before sowing. The NPK material was liquefied @ 3 ml litre⁻¹ water and used immediately. Flowering stems were harvested when 75 per cent of the ray florets were near horizontal.

Observations were recorded on plant height, shoot diameter, fresh plant biomass, dry plant biomass, fresh root weight, dry root weight, number of leaves, leaf surface area (once at 50 DAS and other at maturity), number of flowers plant⁻¹, number of seeds per flower, grain and oil yield, seed and harvest index. For taking

observation five plants from each replication were selected randomly leaving border rows. The root fresh and dry weight was measured as described by Wenger, 1984; Bashan and de-Bashan, 2005. Data were averaged subjected to statistical analysis of variance using SPSS software.

RESULTS AND DISCUSSION

Plant height

Data on mean plant height has been presented in table 3. The plant height was significantly higher with the treatment comprised of FYM + organic manure + biofertilizer + VAM as compared to other treatments. The maximum plant height at 50 DAS was recorded with T₆ being, 86.70 cm and 86.54 cm for the 1st and 2nd year, respectively; whereas at maturity it was recorded maximum in T₄ being, 206.7 cm and 208.2 cm for the

1st and 2nd year, respectively. Treatment comprised of sulphur did not reveal any positive effect on shoot length. Hence, application of sulphur had no significant effect on plant height and the result is not *at par* with Patra *et al.*, 2013. No significant differences in plant height between treatments were observed.

Shoot diameter

Shoot diameter was observed at 50 DAS and at harvest. At 50 DAS, maximum diameter 7.75 cm was recorded with chemical fertilization i.e. DAP @ 147 kg ha⁻¹ (T₆) for the year 2013-14 whereas, maximum diameter was 7.42 cm in T₃ for the year 2014-15 (Table 3). At harvest shoot diameter was maximum in T₆ during 1st year and T₄ during 2nd year (Table 3). None of the treatments revealed significant difference in shoot diameter.

Table 3: Effect of treatments on plant height and shoot diameter

Treatment	Plant height (cm)				Shoot diameter (cm)			
	50 DAS		At maturity		50 DAS		At maturity	
	2013-14	2014-15	2013-14	2014-15	2013-14	2014-15	2013-14	2014-15
T ₀	73.50 ^a	74.42 ^a	168.9 ^a	168.0 ^a	7.08 ^a	7.25 ^a	7.25 ^a	7.84 ^a
T ₁	70.80 ^a	72.44 ^a	194.0 ^a	195.0 ^a	6.46 ^a	6.59 ^a	7.56 ^a	7.77 ^a
T ₂	71.80 ^a	72.99 ^a	199.5 ^a	200.0 ^a	6.25 ^a	6.22 ^a	6.77 ^a	7.02 ^a
T ₃	74.70 ^a	74.70 ^a	195.6 ^a	196.5 ^a	7.00 ^a	7.42 ^a	7.76 ^a	7.87 ^a
T ₄	76.70 ^a	77.52 ^a	206.7 ^a	208.2 ^a	7.00 ^a	7.17 ^a	7.56 ^a	7.98 ^a
T ₅	75.30 ^a	76.52 ^a	196.0 ^a	197.0 ^a	6.58 ^a	6.38 ^a	7.84 ^a	7.88 ^a
T ₆	86.70 ^a	86.54 ^a	170.0 ^a	170.2 ^a	7.75 ^a	6.86 ^a	8.18 ^a	7.65 ^a

Note: Data marked by common letters are not significant according to Tukey HSD *p*-value at 5% level of probability

Number of leaves per plant and leaf surface area

Data on number of leaves per plant and leaf surface area has been presented in table 4. Maximum number of leaves at 50 DAS (22.83 plant⁻¹ and 23.51 plant⁻¹ for the 1st and 2nd year, respectively) was observed with T₆. At harvest, the number of leaves was observed

maximum in T₂ being, 63.77 plant⁻¹ and 63.14 plant⁻¹ for the 1st and 2nd year, respectively. Total leaf surface area at 50 DAS was maximum 245.83 cm in T₆. There were no significant differences between treatments with respect to leaves per plant and leaf surface area (Table 4).

Table 4: Effect of treatments on number of leaves plant⁻¹ and leaf surface area during 2013-15

Treatment	Number of leaves plant ⁻¹				Leaf surface area (cm)	
	50 DAS		Maturity		50 DAS	
	2013-14	2014-15	2013-14	2014-15	2013-14	2014-15
T ₀	20.66 ^a	22.45 ^a	49.55 ^a	50.45 ^a	186.00 ^a	188.14 ^a
T ₁	19.33 ^a	18.57 ^a	42.22 ^a	44.12 ^a	197.41 ^a	199.85 ^a
T ₂	18.83 ^a	18.55 ^a	63.77 ^a	63.14 ^a	157.83 ^a	159.54 ^a
T ₃	20.50 ^a	21.48 ^a	46.66 ^a	48.54 ^a	213.62 ^a	215.15 ^a
T ₄	22.50 ^a	23.42 ^a	49.55 ^a	49.87 ^a	236.00 ^a	236.21 ^a
T ₅	21.16 ^a	20.54 ^a	41.44 ^a	43.24 ^a	169.16 ^a	170.14 ^a
T ₆	22.83 ^a	23.51 ^a	35.11 ^a	36.55 ^a	245.83 ^a	246.45 ^a

Fresh and dry root biomass

Fresh and dry roots biomass weight was taken at 50 DAS and at maturity. Maximum fresh root weight 76.00 g and 77.14 g was recorded in the treatment T₄ for the 1st and 2nd year, respectively at 50 DAS whereas, at maturity it was maximum with T₂ being 482.66 g and 482.66 g for the 1st and 2nd year, respectively (Table 5).

Fresh roots weight of all the treatments except T₁ was significantly higher over control at 50 DAS whereas,

treatments T₂ was found higher over others during the time of maturity.

Maximum dry weight was recorded in T₂ for both the years at 50 DAS and also at maturity. Analysis of dry root weight at 50 DAS showed that treatments T₂, T₃ and T₅ were significantly higher than control. During the time of maturity only T₂ recorded significantly maximum dry root weight.

Table 5: Effect of treatments on fresh and dry root weight at 50 DAS and at maturity during 2013-15

Treatment	Roots fresh weight (g)				Roots dry weight (g)			
	50 DAS		At maturity		50 DAS		At maturity	
	2013-14	2014-15	2013-14	2014-15	2013-14	2014-15	2013-14	2014-15
T ₀	39.33 ^a	39.21 ^a	245.66 ^a	245.66 ^a	29.83 ^a	29.55 ^a	100.66 ^a	111.47 ^a
T ₁	43.66 ^{ab}	44.24 ^{ab}	319.33 ^{ab}	319.33 ^{ab}	31.09 ^{ab}	32.11 ^{ab}	175.66 ^a	179.54 ^a
T ₂	69.00 ^{bc}	70.42 ^{bc}	482.66 ^b	482.66 ^b	53.14 ^c	55.10 ^c	297.00 ^b	299.15 ^b
T ₃	74.20 ^c	75.12 ^c	256.33 ^a	256.33 ^a	52.39 ^c	53.58 ^c	119.66 ^a	129.01 ^a
T ₄	76.00 ^c	77.14 ^c	294.33 ^{ab}	294.33 ^{ab}	47.28 ^{abc}	48.32 ^{abc}	147.66 ^a	148.54 ^a
T ₅	70.80 ^{bc}	72.12 ^c	298.00 ^{ab}	298.00 ^{ab}	50.37 ^{bc}	49.54 ^{bc}	105.22 ^a	106.34 ^a
T ₆	68.68 ^{bc}	69.54 ^{bc}	249.44 ^a	249.44 ^a	46.05 ^{abc}	47.45 ^{abc}	96.11 ^a	101.00 ^a

Fresh and dry shoot biomass per plant

Data on fresh and dry shoot biomass was recorded at harvest (Table 6). Maximum fresh shoot weight was observed with T₂ being, 2529.00 g and 2522.0 g for the 1st and 2nd year, respectively whereas minimum was recorded in T₆. Also T₁ was found significantly higher than T₃, T₅ and T₆.

To determine the dry biomass, shoots were dried in oven at 80 °C for 4 days to attain the constant weight and data was recorded in gram as presented in table-6. Maximum dry weight 423.00 g (2013-14), 431.00 g (2014-15) was obtained in T₂. Treatment T₁, T₂ and T₄ acquired significantly higher dry shoot biomass over control.

Table 6: Effect of treatments on growth and yield attribute at maturity during 2013-15

Treatment	Fresh shoot biomass		Dry shoot biomass		No. of flower plant ⁻¹		No. of seeds flower ⁻¹		Grain yield (kg ha ⁻¹)		Oil yield (kg ha ⁻¹)	
	plant ⁻¹ (g)		plant ⁻¹ (g)		plant ⁻¹		flower ⁻¹		kg ha ⁻¹		kg ha ⁻¹	
	2013-14	2014-15	2013-14	2014-15	2013-14	2014-15	2013-14	2014-15	2013-14	2014-15	2013-14	2014-15
T ₀	1661.1 ^a	1670.3 ^a	210.7 ^a	222.7 ^a	11.0 ^a	11.0 ^a	249.3 ^a	254.3 ^a	845.9 ^a	864.3 ^a	223.3 ^a	228.2 ^a
T ₁	2031.7 ^b	2038.7 ^b	345.3 ^b	357.0 ^b	14.3 ^b	15.3 ^b	595.5 ^b	604.7 ^b	3354.2 ^b	3666.8 ^b	981.1 ^b	1072.5 ^b
T ₂	2529.0 ^c	2522.0 ^c	423.0 ^{bc}	431.0 ^{bc}	15.8 ^{bc}	16.4 ^{bc}	417.0 ^{ab}	437.0 ^{ab}	2507.6 ^b	2626.3 ^b	697.1 ^b	730.1 ^b
T ₃	1668.0 ^{ad}	1675.0 ^{ad}	253.7 ^{ab}	267.0 ^{ab}	13.0 ^{ab}	14.0 ^{ab}	574.8 ^{bd}	581.0 ^{bd}	3204.9 ^{bcd}	3490.9 ^{bcd}	925.6 ^{bcd}	1008.2 ^{bcd}
T ₄	1869.0 ^{abde}	1875.7 ^{abde}	357.7 ^{bcd}	371.0 ^{bcd}	13.0 ^{ab}	14.0 ^{ab}	512.8 ^b	520.7 ^b	2758.0 ^{bcd}	3026.0 ^{bcd}	783.3 ^{bcd}	859.4 ^{bcd}
T ₅	1377.0 ^f	1388.3 ^f	266.0 ^{abd}	278.0 ^{abd}	11.0 ^a	11.7 ^a	359.3 ^{ad}	365.7 ^{ad}	1490.7 ^a	1611.1 ^a	406.9 ^a	439.8 ^a
T ₆	1227.2 ^e	1242.0 ^e	180.3 ^a	190.3 ^a	12.0 ^{ab}	12.3 ^{ab}	333.0 ^{ad}	338.0 ^{ad}	1400.3 ^a	1456.1 ^a	379.5 ^a	394.6 ^a

Note: Data marked by common letters are not significant according to Tukey HSD p-value at 5% level of probability

Number of flowers per plant and number of seeds per flower

Randomly three plants from each replication were selected for determination of number of flowers per plant as presented in table 6. Maximum number of flowers 15.77 (2013-14), 16.44 (2014-15) were recorded for treatment T₂ followed by treatment T₁ for

both the years. Data of analysis showed that treatments T₁ and T₂ hold significantly more number of flowers than control.

The number of seeds per flower was obtained by random selection of flowers from each treatment and data was recorded as mean and presented in table 6. Maximum number of seeds 595.50 (2013-14),

604.67 (2014-15) per flower was obtained from treatment T₁. Statistical analysis revealed that all the treatments recorded significantly higher number over control.

Grain and oil yield

At the time of maturity flowers were harvested and observations on the grain and oil yields were recorded (Table 6). Maximum grain and oil yields per hectare were recorded with T₁ for the 1st and 2nd year, respectively. Data analysis showed that treatments T₁, T₂, T₃ and T₄ were significantly better over control with respect to grain and oil yields.

Seed index and harvest index

Application of biofertilizer along with VAM without sulphur produced significantly highest seed index (100 seed weight) in T₄ followed by T₁ and T₃. Minimum seed index i.e. 2.60 g for 2013-14 and 2.65 g for 2014-15 was found for the treatment with 100% chemical and untreated control. Treatment T₄, T₃ and T₁ were significantly higher than untreated control. Harvest index was found maximum for treatment T₃ (90%), followed by T₁ (75%) and the lowest for untreated control (40%). T₃ and T₁ were significantly higher than untreated control (Table 7).

Table 7: Effect of treatments on seed and harvest index at maturity during 2013-15

Treatment	Seed Index (100 grains weight in g)		Harvest index (%)	
	2013-14	2014-15	2013-14	2014-15
T ₀	2.63 ^a	2.65 ^a	40 ^a	39 ^a
T ₁	2.96 ^b	2.89 ^b	75 ^b	79 ^b
T ₂	2.73 ^a	2.77 ^a	49 ^a	51 ^a
T ₃	2.95 ^b	2.85 ^b	90 ^b	94 ^b
T ₄	2.99 ^b	3.10 ^b	58 ^a	61 ^a
T ₅	2.77 ^a	2.79 ^a	46 ^a	47 ^a
T ₆	2.60 ^a	2.65 ^a	69 ^a	69 ^a

Note: Data marked by common letters are not significant according to DMRT at 5% level of probability

The analysis of variance (ANOVA) for the studied traits showed that, treatment with VAM and sulphur had significant effect on overall vegetative plant growth, seed yield, seed oil content, seed index and harvest index. This study demonstrated that sunflower was performing better in terms of yield when grown with the treatment combination of organic manure with biofertilizer @ 247 kg ha⁻¹ + Vesicular Arbuscular Mycorrhiza 12 kg ha⁻¹ + sulphur 12 kg ha⁻¹. Also, the elevated label of Sulphur (37 kg ha⁻¹) without VAM (0 kg ha⁻¹) produced the nearest yield.

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