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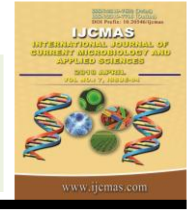

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Effect of Sulphur and Selenium on Yield, Selenium Content and Antioxidant Properties in Sunflower (*Helianthus annuus* L.)

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A B S T R A C T

The present investigation was carried out during 2015-16 at Agricultural Research Institute, PJTSAU, Rajendranagar, Hyderabad, Telangana, India. Four levels of soil applied sulphur (0, 15, 30 and 45 kg S ha^{-1}) in combination with four levels of foliar sprayed selenium concentration (0, 25, 50 and 100 ppm) was tried on sunflower to know their effect on seed yield, selenium content and antioxidant properties. The sunflower crop showed more than 25% in response to sulphur application. Sunflower seed yield increased with selenium application only up to 50 ppm spray and subsequently, the yield decreased. The selenium content in sunflower seed ranged from 9.12 to 20.12 mg kg^{-1}. The antioxidant properties such as phenols and flavonoids significantly increased with the increased levels of soil applied sulphur and foliar sprayed selenium in sunflower crop.

Keywords

DPPH radical scavenging activity, Selenium content, Sulphur, Sunflower, Total flavonoid content, Total Phenol content, Yield attributes

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Introduction

Selenium (Se) is an important trace element in animal and human nutrition but known as a non-essential element for plants, though its beneficial roles have been reported in the plants capable of accumulating large amount of the element (White *et al.*, 2007). It plays an important role in body antioxidation system. It is considered as an individual antioxidant that can cooperate with other antioxidants such as vitamins C and E and in the processes protecting the cells from free radicals. Selenium acts as a cofactor in cellular detoxification of peroxidase. Low Se status in humans may increase the risk of cardiovascular diseases (CVD), cancer and other diseases like Alzheimers, which are caused by free radicals (Fairweather Tait *et al.*, 2011). Selenium participates in thyroid hormone metabolism, immune system, inhibits virulence and slows down the development of AIDS through reducing the speed of HIV development. In India, sunflower is an important oilseed crop. It occupies fourth place among oilseed crops in terms of acreage and production in India (Krishi Jagran, 2015). It is grown in an area of 0.73 m ha and has production of 0.52 million tonnes in India. Sunflower seeds are one of the incredible sources of health benefiting nutrients, minerals, antioxidants and vitamins and are also rich in selenium containing about 79.3 µg in 100 g and come in top 10 foods containing selenium (USDA National Nutrient Database, 2014).

Materials and Methods

A pot experiment was conducted in a net house in Agricultural Research Institute, Rajendranagar on *Alfisol* of Rangareddy district, Telangana state during *rabi* of 2015 to study the performance of sunflower under different sulphur and selenium treatments. Sixteen treatments were taken with combinations of four levels of soil applied sulphur by ammonium sulphate (made at the beginning of the crop @ 0, 15, 30 and 45 kg S ha^{-1}) and four levels of foliar sprayed selenium (0, 25, 50 and 100 ppm Se) by sodium selenite given at 30^{th} day of the crop. The net house is geographically situated at an altitude of 531 m above mean sea level (MSL) on 17^0 19.443' N latitude and 78^0 23.956' E longitude. It is located in the southern agro-climatic zone of Telangana, India.

Pot experiment

The experiment was conducted in completely Randomized design with four replications. The initial properties of the soil are given in Table 1. The required quantity of *DRSH-1* variety seed was taken and sown in pots containing 10 kgs of soil. Each pot was sown with 8 seeds @ 2 seeds for each hill. The RDF for sunflower is 75:90:30 of $N:P_2O_5:K_2O$ kg ha^{-1}. Nitrogen was given in three splits i.e., 50% at the time of sowing, 25% at 30 DAS (vegetative stage) and 25% at 50 DAS. Phosphorus and potassium were given once at the time of sowing in the form of single super phosphate and muriate of potash, respectively. Nitrogen was supplied as ammonium sulphate and urea. Sulphur was given as basal at the time of sowing using ammonium sulphate for 15, 30 and 45 kg sulphur treatments. Selenium was given as foliar spray at 30 DAS (vegetative stage) in the form of sodium selenite (Na_2SeO_3) for 25, 50 and 100 ppm of selenium. Prophylactic plant protection measures were carried out by employing spray application of saaf (carbendazim + mancozeb) @ 2 g/l at vegetative, head forming and flowering stage to control leaf spot disease.

Soil sampling and analysis

Soil was air-dried at room temperature and was sieved using 2 mm sieve. Soil pH was measured using a pH meter (1:2.5 soil water suspension) and Electrical Conductivity was determined by Conductivity meter - Elico CM 180 (Jackson, 1973). Organic carbon was estimated using Chromic acid wet digestion method (Walkley and Black, 1934). Soil available N was determined using Alkaline potassium permanganate method (Subbiah and Asija, 1956), while available P_2O_5 was determined by Olsen's method using 0.5M $NaHCO_3$ (pH 8.5) extraction in ECIL GS 5701 SS colorimeter (Olsen *et al.,*1954). Soil available K_2O was determined by Neutral normal ammonium acetate method (Mervin and Peech, 1951). Available Sulphur by Turbedimetric method (Chesnin and Yien, 1950) and Total Selenium was determined by Azure B colorimetric method (Mathew and Narayana, 2006).

Plant sampling and analysis

The crop was harvested at 90 DAS (head drop stage). The plants were uprooted and harvested separately according to treatments. The heads were separated from the whole plant and kept for shade drying for 3 days. The seeds collected were then oven dried at 65^oC and was powdered.

Total selenium estimation

The method outlined by Levesque and Vendette (1970) was employed for digestion of seed samples for Se determination. The digested samples are then used for determination of total selenium by using Azure B as a chromogenic reagent as outlined by Mathew and Narayana (2006) using spectrophotometer (Model ECIL GS 5701 SS) at 644 nm wavelength. It is expressed in mg kg^{-1}.

DPPH Radical Scavenging Activity

Scavenging of 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was assessed by the method of Koleckar *et al.,* (2007) using ascorbic acid as a standard. Absorbance was measured in spectrophotometer at 517 nm (Model ECIL GS 5701). It is expressed as percentage.

Total flavonoid content was determined using the aluminium chloride colorimetry (Chang *et al.,* 2002) using quercetin as a standard flavonoid compound in spectrophotometer at 415 nm (Model ECIL GS 5701). The results are expressed as mg of quercetin equivalents per gram of the sample.

Total phenolic compounds were determined with Folin-Ciocalteau reagent according to the method of Singleton and Rossi (1965) using chlorogenic acid as a standard phenolic compound The absorbance was measured at 725nm using spectrophotometer (Model ECIL GS 5701).

Statistical analysis

The experimental data was analyzed for statistical significance of different treatments on various characters following standard procedure given by Snedecor and Cochran (1967) using in house built software package at PJTSAU, Rajendranagar, Hyderabad, India.

Results and Discussions

Seed yield

Effect of varying levels of sulphur

It was observed that there was significant difference in seed yield of sunflower due to application of sulphur (Table 2). With the increase in sulphur levels from 0 to 45 kg S ha^{-1}, the seed yield increased from 3.8 to 6.04 g pot^{-1}.The increase in seed yield due to sulphur application was beyond 25 per cent. Similar results were reported by Syed *et al.,* (2006) in sunflower. They have reported that increase in sulphur levels from 0 to 60 kg ha^{-1} increased the seed yield from 8.14 to 10.04 q ha^{-1}. Shamima and Imamul (2002) also reported similar results in sunflower crop. They have reported that the seed yield increased from 1.80 to 3.68 t ha^{-1} with the application of sulphur @ 0 to 60 kg ha^{-1}.

Effect of varying levels of selenium

The sunflower seed yield increased from 4.46 to 5.65 g pot^{-1} with the increase in selenium levels from 0 to 50 ppm and then it decreased due to Se_{100} level of foliar spray. Foliar sprays of selenium at 30 DAS enhanced the yield in the crops but were not found to be beneficial at higher levels of foliar spray. Petr Skarpa (2013) has done similar work on sunflower. He has found that application of Se at 50 g ha^{-1}, increased the achenes yield by 3.1% and application of Se at 150 g ha^{-1} decreased the yield by 6.8%.

Interaction of S x Se levels

There was significant difference in seed yield of sunflower crop due to interaction of sulphur and selenium levels. Seed yield was highest in S_{45} x Se_{50} (7.41 g pot^{-1}) followed by S_{45} x Se_{25} (6.12 g pot^{-1}) and least in S_0 x Se_0 (3.45 g pot^{-1}) treatments, respectively.

Table.1 Initial characteristics of the soil collected for the experiment

pH	EC (dS m^{-1})	OC (%)	Av. N (kg ha^{-1})	Av.P_2O_5 (kg ha^{-1})	Av.K_2O (kg ha^{-1})	Av. S (mg kg^{-1})	Total Se (mg kg^{-1})
7.75	0.29	0.66	188	58	286	9	2.71

Table.2 Effect of sulphur and selenium on yield of sunflower (seed)

Se (ppm)	Bulb yield (g pot^{-1})				
S (kg ha^{-1})	Se_0	Se_{25}	Se_{50}	Se_{100}	Mean
S_0	3.45	3.70	4.23	3.82	3.80
S_{15}	3.98	4.46	5.23	5.32	4.75
S_{30}	5.05	5.42	5.74	5.83	5.51
S_{45}	5.35	6.12	7.41	5.30	6.04
Mean	4.46	4.93	5.65	5.07	
Factors		S	Se	S × Se	
SE(m)±		0.15	0.15	0.29	
C.D at 5%		0.43	0.43	0.86	

Table.3 Effect of sulphur and selenium on selenium content of sunflower seed

Se (ppm)	Selenium Content (µg g^{-1})				
S (kg ha^{-1})	Se_0	Se_0	Se_0	Se_0	Se_0
S_0	10.45	15.42	17.64	20.12	15.91
S_{15}	10.06	14.58	16.34	18.76	14.93
S_{30}	9.58	13.62	15.21	17.11	13.88
S_{45}	9.12	12.27	14.30	15.84	12.88
Mean	9.80	13.97	15.87	17.96	
Factors		S	Se	S × Se	
SE(m)±		0.32	0.32	0.64	
C.D at 5%		0.94	0.94	1.88	

Table.4 Effect of sulphur and selenium on DPPH activity of sunflower seed

Se (ppm)	DPPH (% inhibition) in sunflower seed				
S (kg ha^{-1})	Se_0	Se_0	Se_0	Se_0	Se_0
S_0	53.9	55.8	58.2	61.3	57.3
S_{15}	52.8	53.6	56.1	59.1	55.4
S_{30}	55.5	57.3	58.4	61.5	58.2
S_{45}	57.3	57.5	58.7	61.7	58.8
Mean	54.8	56.1	57.8	60.9	
Factors		S	Se	S × Se	
SE(m)±		1.45	1.45	2.89	
C.D at 5%		NS	4.1	NS	

Table.5 Effect of sulphur and selenium on total flavonoid content of sunflower seed

Se (ppm)	TFC (mg quercetin/100 g) in sunflower seed				
S (kg ha^{-1})	Se_0	Se_0	Se_0	Se_0	Se_0
S_0	160	162	169	176	167
S_{15}	153	159	165	169	162
S_{30}	153	154	156	161	156
S_{45}	147	150	151	154	150
Mean	153	156	160	165	
Factors		S	Se	S × Se	
SE(m)±		4.5	4.5	9.00	
C.D at 5%		13.5	13.5	NS	

Table.6 Effect of sulphur and selenium on total phenol content in sunflower seed

Se (ppm)	TPC (mg gallic acid/100 g FW) in sunflower seed				
S (kg ha^{-1})	Se_0	Se_0	Se_0	Se_0	Se_0
S_0	905	927	946	972	937
S_{15}	936	953	991	1017	974
S_{30}	1005	1027	1049	1083	1041
S_{45}	1020	1046	1061	1103	1058
Mean	967	988	1012	1044	
Factors		S	Se	S × Se	
SE(m)±		28	28	56	
C.D at 5%		84	84	165	

The seed yield in S_{45} x Se_{50} is 17.4% higher when compared to S_{45} x Se_{25} treatment. In the interaction effect, it was found that with the increase in sulphur levels, yield also increased and with the increase in selenium levels, the yield increased till Se_{50} then decreased.

Selenium content in sunflower seed

The selenium content in sunflower seeds (Table 3) significantly increased with increased concentrations of foliar sprays of selenium given to crop at 30th day after sowing. It reached to the highest concentration of 17.96 µg g^{-1} due to 100 ppm foliar spray of selenium. However, the Se content in seeds decreased due to increased levels of soil application of sulphur. Lowest selenium content (12.88 µg g^{-1}) was noticed when sulphur was applied at 45 kg S ha^{-1}. The interaction effect of foliar sprayed selenium and soil applied sulphur on selenium content in sunflower seeds was more pronounced due to combination of Se_{100} and S_{45} treatment.

DPPH radical scavenging activity in sunflower seed

The data presented in the Table 4 indicated that the DPPH radical scavenging activity (% inhibition) increased significantly in sunflower seed due to increased concentrations of foliar spray of selenium given at 30 days after sowing. The highest % inhibition of 60.9% in sunflower seeds was recorded when selenium was sprayed @ 100 ppm. The DPPH content also increased with increasing sulphur levels but the change in DPPH content in sunflower seeds due to sulphur application as basal dosage to the soil was non-significant. The DPPH content at any given level of soil applied sulphur was highest at highest rate of foliar sprayed selenium concentration (Se_{100}). The interaction was more pronounced at both higher levels of selenium and sulphur (61.7% inhibition) in sunflower seeds. Increased DPPH % inhibition with enhanced sulphur application in onion was also reported by Judita *et al.,* (2014).

Total flavonoid contents in sunflower seeds

The data presented in Table 5 indicated that total flavonoid content increased with increasing selenium concentrations (0 to 100 ppm) as foliar spray in sunflower seeds from 153 to 165 mg quercetin/ 100 g. This change in TFC of sunflower seeds due to foliar sprayed selenium was found to be statistically non-significant. The total flavonoid content decreased due to soil application of sulphur and significant decrease was recorded at S_{30} and S_{45} levels compared to control. The interaction was more pronounced on TFC at lowest level of sulphur (S_0) and highest level of selenium (Se_{100}) with 176 mg quercetin/100 g D.W of sunflower seed. Poldma *et al.,* (2013), Reilly *et al.,* (2014) and Judita *et al.,* (2015) reported increase in value of quercetin with the increase of selenium application.

Total phenol content of sunflower seeds

The total phenol content was highest (1044 mg gallic acid/100 g F.W) due to highest selenium spray concentration (100 ppm) and lowest (967 mg gallic acid/100 g) in control. This increase in TPC in sunflower seeds due to enhanced selenium concentration in sprays was statistically non-significant. TPC increased significantly with increase in sulphur application of 30 and 45 kg S ha^{-1} to the crop (Table 6). The combination of highest level of selenium (Se_{100}) and highest level of sulphur (S_{45}) had pronounced effect on total phenol content in sunflower seed (1103 mg gallic acid/100 g D.W). Reilly *et al.,* 2014 reported increased supplementation of selenium increases total phenol content of onion.

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