Soybean photosynthesis responses, yield, and grain quality affected by vermicompost and sulfur

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Abstract

Soybean (*Glycine max* L.) is considered as one of the major sources of oil and protein. In this study, the effects of vermicompost (VC) and sulfur (S) on soybean photosynthesis responses, yield, and grain quality were studied. To this end, a split-plot design field experiment based on randomized complete blocks with nine treatments and three replications were conducted in Aligudarz city, Lorestan province, Iran during 2017. The First factor included vermicompost treatments (0, 4, and 8 t h^{-1}) as the main plot and the second factor included sulfur treatments (0, 250, and 500 kg h^{-1}) as the sub plot. Results revealed that VC application significantly increased the stomatal conductance (g_s), transpiration rate (E), net photosynthetic rate (P_N), chlorophyll *a* (*Chl a*), chlorophyll *b* (*Chl b*), chlorophyll *a*+*b* (*Chl a*+*b*), carotenoids (Car), grain yield (GY), grain oil content (GOC), and grain protein content (GPC) of soybean. Furthermore, S application (250 kg h^{-1}) improved the important traits in comparison to the control treatments. A significant interaction was found between VC and S treatments, suggesting the combined application of VC and S may improve soybean physiological responses, yield, and grain quality in comparison with VC and S treatments alone.

Keywords: *Glycine max* L; photosynthetic pigments; sulfur; vermicompost; yield

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Introduction

Soybean seed contains protein and oil and depending on the end use, other important constituents such as specific amino acids (nutritionally essential and non-essential), non-protein-based amino acids, sugars, and fatty acids whose relative concentration contributes to oil stability (Bellaloui et al., 2015). In recent decades, the use of chemical fertilizers in agricultural lands has caused environmental problems such as water pollution, quality loss of agricultural products, and negative effects on biological properties of soil. Long-term use of chemical fertilizers can affect microbial communities and reduce microbial activity (Sturz and Christie, 2003). One of the fertilizers used in sustainable agriculture is vermicompost (VC). This fertilizer is produced as a result of the process of digestion and conversion of organic waste such as livestock manure and plant leftovers and passing through the digestive

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system of earthworms (Azarpoor et al., 2012).

Table 1
Soil physicochemical properties of the experimental location

E.C [ds m^{-1}]	pH	O.C [%]	P [mg kg^{-1}]	K [mg kg^{-1}]	Sand [%]	Silt [%]	Clay [%]	Soil texture	S [mg kg^{-1}]	Fe [mg kg^{-1}]	Zn [mg kg^{-1}]	B [mg kg^{-1}]
0.79	7.19	0.41	25.00	273.70	43.20	40.80	16	loam	400.00	9.00	0.04	0.58

Table 2
Temperature, precipitation, and relative humidity during the experimental period (2017)

	Mean Air Temperature [°C]				
Month	Maximum	Minimum	Average	Precipitation [mm]	RH [%]
May	24.5	9.3	16.9	43.7	52.0
June	29.8	13.5	21.6	0.0	42.0
July	34.3	17.7	26.0	32.8	28.0
August	32.5	17.7	25.1	0.0	23.0
September	29.2	12.2	20.7	0.0	22.0

Table 3
Properties of vermicompost fertilizer

E.C [ds m^{-1}]	pH	O.C [%]	N [%]	P [%]	K [%]	S [%]	Pb [%]	Moisture [%]
0.81	7.28	15.00	1.60	1.37	0.68	0.09	6.15×10^{-6}	38.00

Vermicompost improves the soil condition, increases sulfur oxidation, and as a carbon source, aggravates the activity of phosphorus solubilizing and oxidizing sulfur microorganisms (Arancon et al., 2004). The use of bio fertilizers such as vermicompost improved leaf chlorophyll content, grain oil, and grain yield in soybean (Pirdashti et al., 2010); and chlorophyll and carotenoids in milk thistle (Nikkah Naeeni et al. 2017). Application of vermicompost in beans was reported to improve net photosynthetic rate (P_N) (Beyk Khurmizi et al., 2015).

The range of biological compounds that contain sulfur is vast. Sulfur is found in vitamins such as biotin and thiamine and many secondary compounds. It also serves important structural, regulatory, and catalytic functions in the context of proteins, and as a major cellular redox buffer in the form of the tripeptide glutathione and certain proteins such as thioredoxin, glutaredoxin, and protein disulfide isomerase (Leustek, 2002). As a nutrient, sulfur has a role in the formation of amino acids and increases the amount of protein and oil in soybeans (Pathak and Singh, 2014). In soybean, S increases leaf chlorophyll content and grain yield (Sharma 2014).

The present study was conducted to evaluate the effects of vermicompost and sulfur on physiological responses, yield, and quality of soybean (*Glycine max* L.).

Materials and Methods

Field experiments were performed in Aligudarz, Lorestan province, Iran in 2017. Site altitude was 2058 m above sea level (33°28' N, 49°41' E). The farm’s soil was loam and alkaline with low organic carbon and high available P and K (Table 1). Fertilizers were applied to the field according to the fertilizer recommendation. The amounts of K and other available nutrients measured before adding any fertilizer to the soil. The precipitation and temperature information of the site are shown in Table 2.

The field experiment was arranged in a split-plot design based on randomized complete blocks with nine treatments and three replications. Experimental treatments included vermicompost (VC) at three levels (0, 4, and 8 t h^{-1}) as the main factor and sulfur (S) at three levels (0, 250, and 500 kg h^{-1}) as the sub plot. The VC fertilizer was prepared from Gilan Golbaranesabz Co. Characteristics of the fertilizer are shown in

Table 3. The applied S fertilizer contained 98% S and was prepared from Isfahan Taban Powder Co. Treatments were applied to Hobbit soybean cultivar, which was recommended for planting in the site of the experiment.

Each plot consisted of five planting rows with a spacing of 50 cm and a length of 5 m. The distance between the main and sub plots was considered as 2.5 and 1.5 m, respectively. The sowing was carried out manually and at a depth of three cm with a planting density of 50 plants m^{-2}. The fertilizer recommendations were applied based on soil analysis results (Table 1). Accordingly, 30 kg h^{-1} of manganese sulfate, 40 kg h^{-1} of zinc sulfate, 20 kg h^{-1} of copper sulfate, 50 kg h^{-1} of potassium sulfate, and 100 kg h^{-1} of urea were added to the field. The whole fertilizer was applied before sowing time. Due to the high soil phosphorus content, the use of phosphate fertilizer was avoided in the study. Irrigation, weed, pest, and disease control were performed regularly during the experiment period. Weed control was done manually. The water requirement for irrigation was estimated about 500 mm using equation (1) (Gholinezhad et al. 2009); about 45 mm of it was supplied by rainfall.

$$V = ((FC - \theta m) \times \rho m \times D_{root} \times A) / IE \qquad (1)$$

where V is irrigation water volume (mm); FC is field capacity; θm is soil weight moisture percentage; ρm is soil external specific density (g cm^{-3}); D_{root} is root depth (m^{-1}); *A* is irrigated area (m^{-2}); and IE is irrigation efficiency.

Irrigation was carried out by surface (leakage) method and when the average soil moisture reached less than 50% available moisture content. Soil samples were taken from two depths of soil 0-30 and 30-60 cm for determining soil moisture content. Then weight moisture percentage was determined by a pressure plate (Armfield CAT.REF: FEL13B-1 Serial Number: 6353 A 24S98). The sowing and harvesting soybeans were May 20^{th} and September 06^{th}, respectively.

Measurements

The stomatal conductance (g_s), transpiration rate (E), and net photosynthetic rate (P_N) were measured in the measuring chamber (6.25 cm^2) at the full flowering stage (R_2) using a photosynthesis meter (Li-6400, Li-COR Inc., NE, USA) between 9:30 and 11 h (Chen et al., 2016) (to prevent high temperature and low air vapor pressure) on the middle leaflet of each leaf (the leaf before last). The target leaflet was placed in a chamber with the upper surface of the leaflet facing upwards to receive sufficient light. In each experimental unit, measurements were performed on five random plants and their average was reported. Chl *a*, Chl *b*, and carotenoids (Car) were extracted by 80% cold acetone and determined at 663 (Chl *a*), 646 (Chl *b*), and 470 nm (Car) by UV/VIS spectrophotometer (DU800, Beckman, USA) according to the method of Dere et al. (1998).

The grain yield was determined at maturity stage and through the harvest of all plants from the level of 1 m^{-2} per plot and after removing 0.5 m from the beginning and end of the respective planting rows. To determine the grain oil content (GOC) and grain protein content (GPC), the near-infrared (DA 7250 NIR Analyzer) spectroscopy was used (Mourtzinis et al., 2017).

Analysis of variance was performed using a general linear model (GLM) procedure of the statistical analysis system (SAS version: 9.3). The means were compared using the least significant difference (LSD) method at $P \leq 0.05$ (LSD 0.05).

Results

Analysis of the obtained data showed that VC and S treatments significantly affected the stomatal conductance (g_s) (Table 4). VC and S increased the g_s by 37.40 and 25.45%, respectively, compared to control (Table 5). The maximum and minimum g_s were obtained in the combined treatment with VC (8 t h^{-1}) + S (250 kg h^{-1}) and non-using of both VC and S, respectively (Table 5).

In addition, VC and S treatments significantly affected the transpiration rate (E) (Table 4). In fact, VC and S increased the E by 39.13 and 29.78% respectively, compared to the control (Fig. I). The maximum and minimum E were obtained in the combined treatment with VC (4 t h^{-1}) + S (250 kg h^{-1}) and non-use of VC + S (control) (Fig. I).

Table 4
Analysis of variance for g_s (stomatal conductance), P_N (net photosynthetic rate), Chl *a* (chlorophyll *a*), Chl *b* (chlorophyll *b*), Chl *a+b* (chlorophyll *a+b*), Car (carotenoids), GY (grain yield), GOC (grain oil content), and GPC (grain protein content) of soybean (*Glycine max* L.) affected by VC (vermicompost) and S (Sulfur). Ns, *, and ** denote non-significant, significance at P≤0.05, and significance at P≤ 0.01, respectively.

Sources of Variance	DF	g_s	E	P_N	Chl *a*	Chl *b*	Chl *a+b*	Car	GY	GOC	GPC
Block	2	0.0006^{ns}	0.05^{ns}	4.65^{ns}	1.09^{ns}	0.33^{ns}	4.21^{ns}	0.02^{ns}	35,833.33	2.03	4.71
VC	2	0.0037**	4.16*	26.32**	17.46**	12.14**	20.94**	0.24**	386,811.11**	12.47**	18.08**
Error(a)	4	0.0013	0.04	9.06	1.70	0.79	3.97	0.02	40,879.63	1.93	3.38
S	2	0.0022*	2.22*	17.31*	12.24**	9.15**	14.13**	0.15**	296,944.46**	8.50**	14.67**
VC×S	4	0.0029**	1.65*	21.69**	15.86**	10.09**	17.53**	0.17**	316,972.28**	10.75**	15.56**
Error(b)	12	0.0010	0.03	4.23	1.45	0.60	3.11	0.01	38,611.18	1.09	3.07
CV (%)		13.37	12.42	10.31	7.62	6.63	8.32	10.35	11.56	8.10	7.64

Furthermore, VC and S treatments had significant effects on the net photosynthetic rate (P_N) (Table 4). VC and S increased the P_N by 62.63 and 44.88%, respectively, compared to control (Fig. II). Maximum and minimum P_N were obtained in the combined treatment with VC (8 t h^{-1}) + S (250 kg h^{-1}) and non-use of VC + S (control) (Fig. II).

Also, VC and S treatments had significant effects on the *Chl a* content of the plants under study (Table 4). VC and S increased *Chl a* by 42.33% and 32.98%, respectively, compared to control (Table 5). Maximum *Chl a* was obtained in the combined treatment with VC (8 t h^{-1}) + S (250 kg h^{-1}) while minimum *Chl a* was recorded in non-use of VC + S (control) (Table 5).

Moreover, VC and S treatments also significantly affected *Chl b* contents (Table 4). In fact, VC and S increased Chl *b* by 40.65% and 22.67%, respectively compared to control (Table 5). Maximum and minimum *Chl b* were obtained in the combined treatment with VC (8 t h^{-1}) + S (250 kg h^{-1}) and non-use of VC+ S (control), respectively (Table 5).

Findings also suggested that VC and S treatments had significant effects on the Chl *a+b* (Table 4). VC and S increased the *Chl a+b* by 41.52% and 28.27%, respectively, compared to control (Table 5). Maximum and minimum Chl *a+b* were recorded in the combined treatments with VC (8 t h^{-1}) + S (250 kg h^{-1}) and non-use of VC + S (control), respectively (Table 5).

Furthermore, the findings showed that VC and S treatments significantly affected the carotenoid contents of the plants (Table 4). VC and S increased the Car by 46.21% and 22.89%, respectively, compared to control (Table 5). The

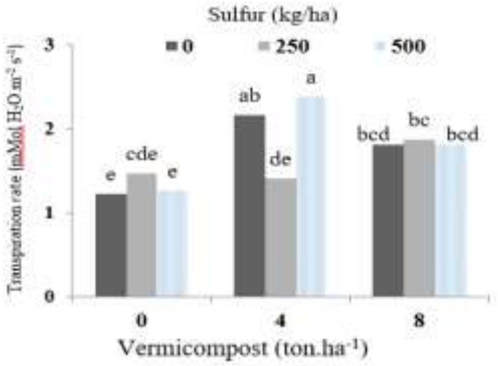

Fig. I. Effect of vermicompost and sulfur on soybean leaf transpiration rate; columns followed by the same letter are not significantly different, according to LSD, p<0.05.

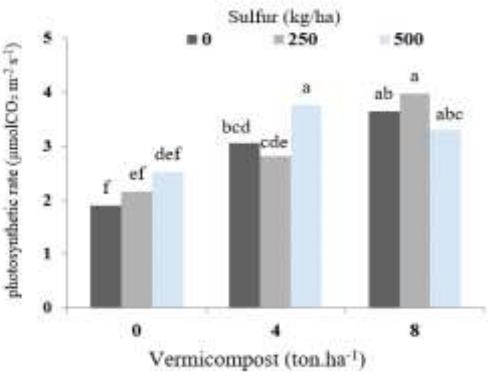

Fig. II. Effect of vermicompost and sulfur on soybean net photosynthetic rate; columns followed by the same letter are not significantly different, according to LSD, p<0.05.

maximum and minimum Car were found in the combined treatment with VC (8 t h^{-1}) + S (250 kg h^{-1}), and non-use of VC + S (control), respectively (Table 5).

Table 5
Mean values for g_s (stomatal conductance), P_N (net photosynthetic rate), *Chl a* (chlorophyll *a*), *Chl b* (chlorophyll *b*), *Chl a+b* (chlorophyll *a+b*), Car (carotenoids), GY (grain yield), GOC (grain oil content), and GPC (grain protein content) of soybean (*Glycine max* L.) affected by VC (vermicompost) and S (sulfur) in a split-plot design with three replications. Column means followed by *the same letter* are not significantly different at 0.05 probability level using LS means of *SAS*.

Treatments		g_s [mol(H_2O) m^{-2} s^{-1}]	Chl *a* [mg g^{-1} (FM)]	Chl *b* [mg g^{-1} (FM)]	Chl *a+b* [mg g^{-1} (FM)]	Car [mg g^{-1} (FM)]	GY [kg h^{-1}]	GOC [%]	GPC [%]
VC [t h^{-1}]	S [kg h^{-1}]								
	0 (control)	0.041^{g}	3.82^{f}	3.70^{h}	7.52^{h}	0.32^{e}	1.440.11^{g}	16.92^{h}	30.78^{i}
0 (control)	250	0.060^{e}	5.12^{e}	5.02^{e}	10.14^{fg}	0.33^{e}	1.575.21^{f}	17.50^{g}	32.42^{g}
	500	0.050^{f}	6.28^{cd}	4.55^{f}	10.83^{ef}	0.50^{d}	1.704.29^{e}	21.10^{a}	31.60^{h}
	0 (control)	0.071^{d}	6.60^{c}	6.03^{d}	12.63^{cd}	0.68^{c}	1.990.21^{c}	18.76^{e}	36.65^{b}
4	250	0.092^{b}	7.85^{b}	7.12^{ab}	14.97^{ab}	0.93^{ab}	2.120.25^{b}	20.50^{b}	35.80^{c}
	500	0.102^{a}	7.83^{b}	6.80^{b}	14.63^{b}	0.68^{c}	1.983.78^{c}	18.12^{f}	37.46^{a}
	0 (control)	0.060^{e}	6.65^{c}	6.44^{c}	13.09^{c}	0.51^{d}	1.835.29^{d}	19.93^{c}	33.29^{f}
8	250	0.103^{a}	9.01^{a}	7.27^{a}	16.28^{a}	1.01^{a}	2.250.25^{a}	21.12^{a}	35.00^{d}
	500	0.083^{c}	6.62^{c}	6.41^{c}	13.03^{c}	0.85^{b}	1.850.63^{d}	19.34^{d}	34.16^{e}
LSD 5%	–	1.33	1.15	0.45	1.62	0.085	126.43	0.57	0.80

Means in each column, following same letter(s) are not significantly different at 5% probability level by LSD test.

Results also revealed that VC and S treatments had significant effects on the grain yield (Table 4). In fact, VC and S increased the GY by 24.70 and 12.17%, respectively, compared to control (Table 5). Maximum and minimum GY were recorded in the combined treatment with VC (8 t h^{-1}) + S (250 kg h^{-1}) and non-use of VC + S (control), respectively (Table 5).

VC and S treatments significantly affected the grain oil content (GOC) (Table 4). VC and S increased the GOC by 2.42% and 2.38%, respectively, compared to control (Table 5). Results also showed that maximum and minimum GOC were obtained in the combined treatment with VC (8 t h^{-1}) + S (250 kg h^{-1}) and non-use of VC +S (control), respectively (Table 5).

Finally, grain protein content (GPC) showed significant increase under VC and S treatments (Table 4). VC and S increased the GPC by 4.19% and 1.23%, respectively, compared to control (Table 5). Maximum and minimum GPC were obtained in the combined treatments with VC (4 t h^{-1}) + S (500 kg h^{-1}) and control, respectively (Table 5).

Discussion

In the present experiment, it was observed that the use of vermicompost significantly increased all measured traits, i.e. g_s, E, P_N, Chl *a*, Chl *b*, Chl *a+b*, Car, GY, GOC, and GPC compared to the non-use of vermicompost. A study on beans showed that transpiration rate (E) in all vermicompost treatments except in 10% were significantly increased. Increased E in the presence of vermicompost, may be due to the physical, chemical, and biological structure of vermicompost. Vermicompost increases the amount of water entering roots due to its capacity of holding water and the microorganisms including mycorrhizal fungi (Beyk Khurmizi et al., 2015). Therefore, the transpiration rate rise along with the increases in plant water. Hossein Zadeh et al. (2016) reported that the use of vermicompost resulted in an increase in P_N and *Chl a + b*, a finding which is in line with the results of the present study. Vermicompost has a high capacity for ventilation, proper drainage, and water storage in the soil and by limiting the closure of the stomps, increases the CO_2 required for photosynthesis (Arancon et al., 2004). In addition, vermicompost has been reported to increase CO_2 production in the rhizosphere (Marinari et al., 2000). The increase in P_N as a result of the use of vermicompost in the present experiment is not unexpected. Reduction of g_s decreases leaf internal carbon dioxide concentration and consequently decreases the activity of Rubisco and P_N (Rahbarian et al., 2011). Therefore, any factor that increases g_s can also increase P_N. Applying vermicompost in the present study increased g_s, which could justify an increase in P_N as a result of the use of vermicompost. In their study on in soybeans, Yadavi et al. (2014) found that using vermicompost had a significant effect on Car. These findings were inconsistent with the results of the present experiment. Heidari and Salehi (2017) reported that the use of vermicompost increased the Car via increasing the absorption of nitrogen, phosphorus, potassium, and the role of these elements in the production of photosynthetic organs. These findings can justify the results of the present experiment. Nitrogen is one of the main components of the structure of amino acids, nucleic acids, chlorophylls, alkaloids, and purines (Lopez Cantarero and Romero, 1994). Organic fertilizers, especially vermicomposts, have been reported to increase nitrogen uptake by increasing the maintenance of nutrients and growth regulating hormones; in fact, with increasing nitrogen, chlorophylls (*Chl b and Chl a*) also increase (Azizi et al., 2008). Therefore, increasing Chl *a*, Chl *b*, and *Chl a + b* in the present study is related to the role of vermicompost in increasing nitrogen availability and uptake. Azarpoor et al. (2012) reported an increase in GY of soybean due to vermicompost application compared to control. They attributed this increase to the role of vermicompost in the release of macro and micro nutrients such as nitrogen, phosphorus, potassium, calcium, and magnesium in the soil and increased absorption of these elements by soybean plant.

The use of vermicompost in soybeans increased the GOC relative to the control (Kazemi Moghadam et al., 2014). In addition, Zahedi Fard et al. (2014) reported a GOC increase as a result of the use of vermicompost in rapeseed. These findings were consistent with the results of the present experiment. Kazemi Moghadam et al. (2014) stated that the application of different levels of vermicompost was not significant on soybean GPC. These findings were inconsistent with the results of the present experiment. Regarding the role of nitrogen in increasing GPC, it can be attributed to the role of vermicompost in releasing nitrogen (Orozco et al., 1996) and its absorption by the plant.

In the present experiment, traits such as g_s, P_N, *Chl a, Chl b, Chl a + b*, Car, GY, GOC, and GPC were improved significantly by S application. D'Hooghe et al. (2013) reported that P_N is affected using S and S shortage in oily products reduces P_N. Raza et al. (2018) also found similar results, reporting an increase in P_N because of the use of S as the main component of ferredoxin in the process of photosynthesis. They attributed the increase in P_N as a result of application of S to the role of this element in the formation of chlorophylls (*Chl b and Chl a*) and the ratio between Rubisco and plant-soluble protein. These findings are in line with the results of the present study. Kumar et al. (2017) stated that S application increased the synthesis of various chlorophylls including *Chl a*, *Chl b*, and *Chl a + b*. This may be due to the role of sulfur in enzyme structure, nitrogen uptake and metabolism, and increased molecular nitrogen uptake by root nodules.

In addition, S resulted in an increase in *Chl a, Chl b, Chl a + b*, and Car (Bakry et al., 2015). This may be related to the role of S in the nitrogen and chlorophyll contents in leaves and photosynthetic enzymes. These findings were consistent with the results of the present experiment. It has been reported that increasing soybean GY because of S application (Prakasha et al., 2010). It has been reported that S, as the acid producing agent in the soil, could improve the soil reaction by increasing the activity of the rhizobium, stabilizing nitrogen, and improving the growth and yield of soybeans. The results of the present experiment were consistent with the findings of the above researchers. In a study, GOC and GPC were reported to increase in rapeseed due to the use of sulfur (Malhi et al., 2002). This may be related to the role of sulfur in reducing the oxidation of fatty acids, especially unsaturated ones, and their subsequent increase in the synthesis of oil. The increase in GPC because of application of S is related to the role of this element in the structure, composition, and function of proteins, as well as the biosynthesis of amino acids as a protein constituent (Kesare et al., 2015). Sulfur is used in the formation of amino acids and proteins and is a structural component of two amino acids (cysteine, Methionine) that participate in the structure of proteins. Sulfur plays an essential role in the making of proteins and the presence of this element in plants can increase the protein content of seeds (Marschner, 2007). This is consistent with the results of the present study.

A significant interaction was found between VC and S treatments, suggesting the combined application of VC and S may improve soybean physiological responses, yield, and grain quality in comparison to the VC and S treatments alone.

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