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Combined Effect of Sulfur and Sulfofertilizer1 on Physiological Traits and Grain Yield of Wheat Cultivars (*Triticum aestivum* L.) in Mahshahr County (South west of Iran)

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RESEARCH ARTICLE

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ABSTRACT

BACKGROUND: The addition of organic materials along with sulfur fertilizer to alkaline soils provides an effective solution for reducing soil acidity and enhancing nutrient solubility for plants.
OBJECTIVES: To investigate the combined effect of sulfur and sulfofertilizer1 (biofertilizer) on some physiological traits and yield of wheat cultivars, an experiment was conducted during the 2022-2023 cropping season in Mahshahr County.
METHODS: Current research was done by using a split-plot experiment within randomized complete blocks (RCBD) design with three replications. The experimental treatments consisted of three levels of sulfur fertilizer (control, no consumption; 270g of sulfur fertilizer; and 270g of sulfur fertilizer + 6g of Sulfofertilizer1, biofertilizer) and three wheat cultivars (Mehregan, Chamran 2 and Khalil), which were assigned to the main and sub plots, respectively.
RESULT: Significant effects of sulfur fertilizer and wheat cultivars on leaf area index, crop growth rate and grain yield were observed. Moreover, the interaction effects between sulfur fertilizer and wheat cultivars on grain yield were found to be significant. The highest grain yield (averaging $490 g.m^{-2}$) was achieved in the Khalil cultivar with the combined application of sulfur and Sulfofertilizer1, while the lowest grain yield (averaging $346.8 g.m^{-2}$) was observed in the Mehregan cultivar without any fertilizer application. Notably, the combined application of sulfur and Sulfofertilizer1 exhibited the most substantial increase in leaf area index, total dry matter and crop growth rate compared to the control treatment. Moreover, distinct variations in physiological growth traits were observed among the wheat cultivars, with the Khalil cultivar displaying superiority over the others.
CONCLUSION: Based on the findings, cultivating the Khalil cultivar with the application of the biofertilizer Sulfofertilizer1 along with sulfur fertilizer significantly increased grain yield, making it a promising recommendation for the region's conditions.

KEYWORDS: *Biofertilizer, Chlorophyll, Dry matter, Growth indices, Seed yield.*

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1. BACKGROUND

Wheat is the first and most important cereal worldwide, serving as a primary food source for humans in various countries (Ravi *et al*., 2008). Among the critical factors in achieving high wheat yields, having knowledge about genetic diversity and identifying high-yielding and stable cultivars is essential. To identify and introduce high-yielding cultivars, it is necessary to understand the genetic potential of these cultivars under different environmental conditions (Faraji *et al*., 2013). Due to the alkaline nature of most agricultural lands in Iran, wheat yield per unit area is lower than the global average (Moosavi *et al*., 2015). Nutrient deficiency is one of the limiting factors for crop production, especially in wheat, in these calcareous soils. Adding sulfur and inoculating the soil with Thiobacillus bacteria (biofertilizer Sulfofertilizer1) can enhance nutrient availability in calcareous soils and promote the growth of agricultural crops (Khavazi *et al*., 2018). One of the challenges of alkaline and calcareous soils is that despite their high levels of essential nutrients such as iron, zinc and phosphorus, the available and soluble forms of these nutrients are insufficient for plant growth. This nutrient deficiency is one of the major limiting factors for production in such soils (Chaghazardi *et al*., 2013). To address this issue, the use of soil amendments to reduce soil pH for improved nutrient uptake and crop performance is essential (Karimi *et al*., 2014). Conventional methods to compensate for nutrient deficiencies in calcareous soils involve the application of chemical fertilizers, but these methods are not very effective (Wiedenfeld, 2011). Over the years, excessive use of chemical fertilizers has resulted in increased agricultural productivity, but prolonged use has led to soil degradation, environmental issues, rising production costs and reduced agricultural capacity (Yang *et al*., 2011). Sulfur is a vital macronutrient for plant nutrition, involved in the biosynthesis of sulfur lipids, antioxidants, co-factors, secondary metabolites and amino acids essential for human nutrition (Abadie and Tcherkez, 2019). Sulfur oxidation in soils is a useful process, as it converts sulfur to sulfate, which is an absorbable form for plants. Soil also contains sulfur-oxidizing microorganisms and their increased abundance leads to faster oxidation (Chaudhary *et al*., 2019). In comparison to chemical fertilizers, organic fertilizers have a positive effect on soil quality. Organic fertilizers improve soil organic content, increase micronutrients and alter soil pH, thus amending soil properties (Muchuwisin *et al*., 2022). The application of sulfur-oxidizing bacteria in soil is a new and effective method to improve sulfur oxidation and enhance phosphate availability in soils (Tourine *et al*., 2012). The use of Thiobacillus bacteria (biofertilizer Sulfofertilizer1) and sulfur not only directly benefits plant nutrition but also reduces soil pH, improving nutrient accessibility and positively affecting plant growth and yield (Orman and Kaplan, 2007).

2. OBJECTIVES

The aim of this research is to assess effect of sulfur and Thiobacillus bacteria (biofertilizer Sulfofertilizer 1) on some physiological traits and yield of wheat cultivars in the Mahshahr region.

3. MATERIALS AND METHODS

3.1. *Geographical Specifications and Climatic Conditions of studied Site*

This experiment was conducted during the 2022-2023 cropping season on a farm located in Mahshahr County, with geographical coordinates of 49 degrees and 13 minutes east longitude and 30 degrees and 33 minutes north latitude. The site's elevation was 3 meters above sea level. Mahshahr is a coastal city characterized by a warm and humid climate. The temperature in this region varies from 50°C in summer to freezing temperatures in winter. The average annual precipitation in Mahshahr is approximately 233 mm.

3.2. *Experimental design*

The experiment was conducted during the 2022-2023 cropping season in Mahshahr County, utilizing a split-plot design within randomized complete blocks (RCBD) with three replications. The experimental treatments consisted of three levels of sulfur fertilizer [control, no consumption (S_1); 270g of sulfur fertilizer) S2; and 270g of sulfur fertilizer + 6g of Sulfofertilizer1 (S_3)] and three wheat cultivars [Mehregan (C_1), Chamran2 (C_2) and Khalil (C_3)], which were assigned to the main and sub plots, respectively. The soil properties of studied farm was mentioned in table 1.

Table 1. Physiochemical characteristics of field soil

Soil depth (cm)	Soil texture	Clay (%)	Silt (%)	Sand (%)	EC ($dS.m^{-1}$)	pH
0-30	Clay loam	36	39	25	4.54	7.5
Soil depth (cm)	**OM (%)**	**N (%)**	**K (ppm)**	**P (ppm)**	**S (ppm)**	**ρb ($gr.cm^{-3}$)**
0-30	0.71	0.04	190	9.61	45	1.5

3.3. *Implementation Stages of Experiment*

The land preparation operations before planting began in the first half of November and included primary irrigation, plowing with a moldboard plow, two cross-discs harrowing and land leveling. The experiment consisted of 27 plots, each with five planting rows, each row measuring 5m in length and spaced 20 centimeters apart. The plots were positioned 1.5 meters apart from each other, with a 0.5-meter gap between two subplots, and a one-meter gap between two main plots. Before planting, the total required phosphorus was supplied from triple superphosphate, based on a net phosphorus consumption of $80kg.ha^{-1}$. Nitrogen fertilizer was sourced from urea (46%) at a rate of 130 $kg.ha^{-1}$, with half of it broadcasted in the field using a disk harrow, and the other half distributed at the beginning of the tillering stage (early stem elongation). Sulfur fertilizer, in the form of granulated organic fertilizer with a composition of 45% organic matter, 45% sulfur, and 10% bentonite, was applied at a rate of 90 $kg.ha^{-1}$ of pure sulfur. Based on the recommendation of Mehr Asia Biological Technology Company, the biofertilizer Thiobacillus bacteria was applied at a rate of one kilogram per 50 kilograms of sulfur fertilizer, and both were mixed and applied simultaneously before planting.

3.4. *Planting and Crop Management*

After preparing the planting rows, manual planting was carried out on November 28, 2022, at a depth of 3cm, with a density of 400 seeds $.m^{-2}$. The first irrigation was performed one day after planting.

3.5. *Sampling Method and Experimental Traits Estimation*

The final harvest took place on May 18, 2023. For each plot, a two-square-meter area was harvested, and the borders were manually removed before conducting the harvest.

3.6. *Measurement of Physiological Growth Parameters*

Sampling was done at three stages, including booting (49 Zadox stage), beginning of flowering (69 Zadox stage) and beginning of grain filling stages (71 Zadox stage), to determine the changes in Total Dry Weight (TDW) and Leaf Area Index (LAI). During each sampling stage, five random plants were selected from each plot for data collection and analysis. To determine the dry matter of plants per unit area, the samples were placed in an oven at 75°C for 48 hours and then weighed using a digital scale with an accuracy of 0.1 grams (Tarighaleslami *et al.*, 2013). The Leaf area index was calculated using the following formula based of land (SA) and leaf surface area (LA) (Tarighaleslami *et al.*, 2013): LAI = LA/SA.

3.6.1. *Crop Growth Rate (CGR)*

The CGR was determined by calculating difference in dry weight of samples between two consecutive sampling times (from booting to beginning of flowering stages) divided between these two samplings (Tariq-al-Islami *et al.*, 2013): CGR ($g.m^{-2}.day$) = $(W_2-W_1)/(GA(T_2-T_1))$. Where W_1 and W_2 are the dry weights of samples at two consecutive sampling times and T_1 and T_2 are intervals between two samplings.

3.6.2. *Net Assimilation Rate (NAR)*

The net assimilation rate was calculated in grams per square meter of leaf surface per day between two consecutive sampling times (from the booting to the beginning of the flowering stages) using the following formula:

NAR ($g.m^{-2}.d^{-1}$) = $(\mathrm{Ln}\ (LAI_2) - \mathrm{Ln}\ (LAI_1)/(LAI_2 - LAI_1) \times CGR_2$

CGR = crop growth rate ($g.\ m^{-2}.\ d^{-1}$), LAI = leaf area index.

3.6.3. *Chlorophyll Index*

The chlorophyll index (SPAD) was measured by SPAD meter (model SPAD-502) at flowering stage (Jiriaie *et al.*, 2014).

3.6.4. *Grain Yield*

To determine the grain yield, at the ripening stage and after removing the half-meter borders from three central rows, a two-square-meter area was harvested. After threshing grain from the straw, the grain yield was calculated in grams per square meter (Naseri, 2015).

3.7. *Statistical Analysis*

The data analysis and result calculations were conducted using the statistical software package SAS. Mean comparisons were performed using the LSD method at a 5% significance level, and relevant graphs were created using Microsoft Excel software.

4. RESULT AND DISCUSSION

4.1. *Total Dry Weight (TDW)*

Based on the results of the analysis of variance (Table 2), the combined effect of sulfur and Sulfofertilizer1 application and different cultivars had a significant impact on the Total Dry Weight (TDW) during the booting, beginning of flowering and beginning of grain filling stages at 1% of probability level. However, their interactions did not show any significant effects on this trait. The results indicated that the highest TDW during the booting, beginning of flowering and beginning of grain filling stages were obtained with the combined application of sulfur and Sulfofertilizer1, with mean values of 1152.25, 1326.1 and 1300.26g.m^{-2}, respectively. On the other hand, the lowest TDW during these stages was observed in the control treatment (no fertilizer application), with mean values of 868.06, 951.2 and 903.3 g.m^{-2}, respectively (Table 3). Additionally, the highest TDW during the booting, beginning of flowering and beginning of grain filling stages belonged to the Khalil cultivar, with mean values of 1199.61, 1335.11 and 1310.5 g.m^{-2}, respectively. The lowest TDW during these stages was associated with the Mehregan cultivar, with mean values of 876.1, 1012.4 and 965.21 g.m^{-2}, respectively (Table 3). Accumulation of dry matter during the initial stages of plant growth is slow due to the limited leaf area as receiver of solar radiation. So, with an expansion in leaf area, increase in leaf photosynthesis and dry matter synthesis occurs, leading to reaching its maximum value (Gardner *et al*., 1985). These results indicate the high potential of the Khalil cultivar in use available resources and other factors that promote photosynthetic activities and assimilates, ultimately resulting in increased growth of plant organs, especially an increase in biomass and grain yield through increase in number and weight of grains (Zahedian *et al*., 2015). Also, it appears Mehregan cultivar, due to its smaller LAI and lower leaf durability, produced less dry matter compared to other cultivars, which aligns with findings of Ranjbar and Alavi Fazel (2017). In this study, it can be stated the treatments with the combined use of sulfur and Sulfofertilizer1 provided significantly better conditions for improving biological activities within soil, meeting plant's needs and increasing plant photosynthesis, which resulted in an overall increase in TDW in the plant.

Table 2. Results of analysis of variance of studied traits

S.O.V	df	Total dry weight (TDW)			Leaf area index (LAI)		
		Booting stage	Beginning of flowering stage	Beginning of grain filling stage	Booting stage	Beginning of flowering stage	Beginning of grain filling stage
Replication (R)	2	11.01	43.5	20.4 ^{ns}	0.12 ^{ns}	0.05 ^{ns}	0.01 ^{ns}
Sulfur (S)	2	85709.3 **	93461.7 **	89225.1 **	50.74 **	39.1**	43.26**
E_a	4	5744.5	6628	6137.6	0.31	0.43	0.211
Cultivar (C)	2	62725.1 **	77029.03 **	71644.5 **	41.05**	28.7**	36.5**
C × S	4	177.4 ^{ns}	86.1 ^{ns}	905.3 ^{ns}	0.067 ^{ns}	0.013 ^{ns}	0.04 ^{ns}
E_b	12	5531.8	6315.2	5911.4	0.19	0.34	0.174
CV (%)		7.04	6.66	6.75	10.7	11.81	12.52

^{ns}, ** and * not significant and significant at the 5 and 1% probability levels, respectively.

Continue table 2.

S.O.V	df	Crop growth rate (CGR)	Net assimilation rate (NAR)	Chlorophyll index	Grain yield
Replication (R)	2	0.54 ^{ns}	0.21 ^{ns}	0.77	59.2
Sulfur (S)	2	117.3 **	36.72**	673.1**	87731**
E_a	4	4.01	0.84	45.1	3028.5
Cultivar (C)	2	156.19**	66.3**	3.06^{ns}	64751**
S × C	4	0.244^{ns}	0.007^{ns}	0.58^{ns}	102371**
E_b	12	3.71	0.58	33.24	2116.4
CV(%)		11.70	10.46	12.21	11

ns, ** and * represent not significant and significant at the 5 and 1% probability levels, respectively.

Moreover, the application of sulfur fertilizer, by increasing the leaf area, could absorb more radiation, leading to higher dry matter production (Erdem *et al.*, 2016). Similarly, Ravi *et al.* (2008) mentioned that increased dry matter production with sulfur consumption in plants was due to an increase in root growth and chlorophyll formation, resulting in increased photosynthesis. In the study by Ghobadi *et al.* (2013), the use of Thiobacillus bacteria, present in the Sulfofertilizer1 (bio-fertilizer), increased plant dry matter production. The findings of other researchers, such as Mousavi *et al.* (2019) and Shirinzadeh *et al.* (2017), also align with the results of this study.

4.2. *Leaf Area Index* (*LAI*)

The results obtained from the analysis of variance demonstrated that the combined effect of sulfur and sulfofertilizer1 application and different cultivars had a significant and meaningful impact on the Leaf Area Index (LAI) during the booting, beginning of flowering and beginning of grain filling stages at 1% of probability level. However, their interactions did not show any significant effects on this particular trait (Table 2).

Table 3. Mean comparison effect of different level of fertilizer and cultivar on studied traits

Treatment	Total dry weight (TDW)			Leaf area index (LAI)		
	Booting stage	Beginning of flowering stage	Beginning of grain filling stage	Booting stage	Beginning of flowering stage	Beginning of grain filling stage
Fertilizer						
Non-applying (Control)	868.06*	951.2	903.3	3.83	4.47	3.11
Sulfur	1144.82	1297.5	1265.49	4.16	5.04	3.30
Sulfur+ Sulfurfertilizer1	1152.25	1326.1	1300.26	4.24	5.31	3.58
LSD (5%)	3.68	7.34	13.51	0.02	0.11	0.04
Cultivar						
Mehregan	876.1	1012.4	965.21	3.91	4.64	3
Chamran2	1089.42	1227.28	1193.34	4.02	4/78	3.29
Khalil	1199.61	1335.11	1310.5	4.3	5/4	3.71
LSD (5%)	3.25	12.1	15.84	0.03	0.09	0.16

*Mean of treatments that differ from LSD is significantly different at the 5% level.

Continue table 3.

Treatment	Crop growth rate ($gr.m^{-2}.d^{-1}$)	Net assimilation rate ($gr.m^{-2}.d^{-1}$)	Chlorophyll index	Grain yield
Fertilizer				
Non-applying (Control)	14.08	5.71	44.31	352.04
Sulfur	17.2	8	47.78	445.1
Sulfur + Sulfurfertilizer1	18.1	8.15	49.51	464.73
LSD (5%)	0.83	0.01	1.14	10.2
Cultivar				
Mehregan	14.94	6.06	45.71	370.82
Chamran2	15.68	7.3	47.29	413.64
Khalil	18.76	8.5	48.6	477.43
LSD (5%)	0.55	0.08	5.36	15.33

*Mean of treatments that differ from LSD is significantly different at the 5% level.

The results indicated that the highest LAI during the booting, beginning of flowering and beginning of grain filling stages was achieved with the combined application of sulfur and sulfofertilizer1, with average values of 4.24, 5.31 and 3.58, respectively. Conversely, the lowest LAI during these stages was observed in the control treatment (no fertilizer application), with average values of 3.83, 4.47 and 3.11, respectively (Table 3). Furthermore, the highest LAI during the booting, beginning of flowering and beginning of grain filling stages was associated with the Khalil cultivar, with average values of 4.34, 5.45 and 3.71, respectively, while the lowest LAI during these stages was related to the Mehregan cultivar, with average values of 3.91, 4.64 and 3.03, respectively (Table 3). In this study, the use of sulfur and Thubasillus created favorable conditions for increased plant growth. It seems that the presence of Thiobacillus bacteria was necessary for better sulfur absorption. It has been suggested that the localized decrease in soil pH due to the use of sulfur and sulfofertilizer1 provides conditions for enhanced shoot growth and increased Leaf Area Index (LAI) (Malakoti, 2018). It is believed that the main reason behind this is the increased solubility of lime in the soil, which has a positive effect on the soil's physical and chemical properties and enhances the absorption of other nutrients (Singh Shivay *et al*., 2014). Other researchers have also reported a significant increase in the Leaf Area Index (LAI) of canola under sulfur application (Ahmad *et al*., 2006). The results of this study are consistent with the findings of other researchers, such as Mousavi *et al*. (2019), regarding the increase in the Leaf Area Index (LAI) of wheat under the application of biological and chemical sulfur fertilizers. Moreover, the higher Leaf Area Index (LAI) in the Khalil cultivar indicates the production of more tillers per unit area, a larger leaf area and increased light absorption in this cultivar compared to other cultivars, ultimately leading to a better growth trend and higher yield. These findings are in line with the results of Saeedi *et al*. (2021) in wheat plants. Based on the statements of Baygi *et al*. (2017), cultivars with higher Leaf Area Index (LAI), growth rate, relative growth rate and net assimilation rate will exhibit better growth and higher yields. Therefore, selecting the appropriate cultivar for the region can significantly influence the growth and yield of wheat, which is consistent with the results of this study.

4.3. *Crop Growth Rate (CGR)*

The results from the analysis of variance revealed that the combined application of sulfur and sulfovarfertilizer1, along with different cultivars, had a statistically significant effect on the Crop Growth Rate (CGR). However, their interactions did not show a significant effect on this trait (Table 2). Moreover, the highest Crop Growth Rate (CGR) was recorded with the combined application of sulfur and sulfofertilizer1, with an average value of 1.18 $g.m^{-2}.d^{-1}$, while the lowest Crop Growth Rate (CGR) was observed in the control treatment (no sulfur application), with an average value of 0.814 $g.m^{-2}.d^{-1}$ (Table 3). Furthermore, the comparison of mean values indicated that the Khalil cultivar had the highest Crop Growth Rate (CGR) with an average value of 0.7618$g.m^{-2}.d^{-1}$, whereas the Mehregan cultivar showed the lowest Crop Growth Rate (CGR) with an average value of 0.914$g.m^{-2}.d^{-1}$ (Table 3). The findings of the Crop Growth Rate (CGR) analysis demonstrated a similar trend to the changes observed in the Leaf Area Index (LAI). An increase in Crop Growth Rate (CGR) during the growth season could be attributed to an enlargement in leaf area, while a decrease in Crop Growth Rate (CGR) was linked to reduced net photosynthesis and leaf shedding. It has been suggested that sulfur deficiency leads to a reduction in certain sulfur-containing proteins, such as Rubisco and light-harvesting protein complexes in photosystems, which, in turn, negatively affects photosynthesis, ultimately resulting in a decrease in Crop Growth Rate (CGR) (Ferreira and Teixeira, 2005). On the other hand, other researchers have reported that sulfur application positively influences photosynthetic productivity and Crop Growth Rate (CGR) through its impact on the Rubisco enzyme (Khan *et al*., 2002). Additionally, the release of calcium ions in the soil solution under the influence of sulfur application facilitates their exchange with H^+ ions on soil colloids, creating more favorable conditions for wheat plants (Malakoti, 1999). It has also been reported that sulfur application, based on soil properties, especially the absorbable sulfate content, leads to increased plant growth and yield (Erdem *et al*., 2016). The variations in Crop Growth Rate (CGR) among different wheat cultivars indicate that the maximum Crop Growth Rate (CGR) for all cultivars occurred when the leaf area reached its maximum. The Khalil and Chamran 2 cultivars showed higher Crop Growth Rate (CGR) due to their larger leaf area and less shading compared to the Mehregan cultivar. However, the more pronounced reduction in leaf area in the Mehregan cultivar indicated increased shading of the leaves under the canopy, resulting in reduced photosynthesis and, consequently, a lower Crop Growth Rate (CGR) (Gardner *et al*., 1985). According to Asadalahzadeh *et al*. (2019), the Khalil cultivar exhibited the highest Crop Growth Rate (CGR) due to its larger leaf area and longer leaf persistence.

4.4. *Net Assimilation Rate (NAR)*

Based on the results of the analysis of variance (Table 2), the combined application of sulfur and sulfofertilizer1, along with different cultivars, significantly influenced the Net Assimilation Rate (NAR) at 1% of probability level. However, the interactive effects of these treatments on this trait were not statistically significant. The highest NAR was observed in the combined application of sulfur and sulfofertilizer1 with an average of 8.15g.m^{-2}.d^{-1}, while the lowest NAR was recorded in the absence of sulfur application (control) with an average of 5.71g.m^{-2}.d^{-1} (Table 3). Furthermore, the results indicated that the highest NAR belonged to the Khalil cultivar with an average of 5.58 g.m^{-2}.d^{-1} and the lowest NAR was associated with the Mehregan cultivar, averaging 6.06 g.m^{-2}.d^{-1} (Table 3). Net Assimilation Rate is a measure of the photosynthetic efficiency of leaves in a plant community. It has been reported that adequate plant nutrition leads to an increase in photosynthetic efficiency, resulting in an elevation of Net Assimilation Rate (Gardner *et al*., 1985). In this study, the combined application of sulfur and the biofertilizer significantly increased the Net Assimilation Rate compared to the non-application of sulfur. It can be suggested that the use of sulfur fertilizer and the bacterium Thiobacillus enhances the uptake of nutrients, such as nitrogen, which in turn increases photosynthesis per unit leaf area and ultimately boosts the Net Assimilation Rate. These findings are in line with the research conducted by Mosavi *et al*., (2019) and Khadem *et al*. (2012). Moreover, in this study, the Khalil cultivar, with larger and more numerous leaf surfaces, was able to maximize its utilization of environmental resources, leading to higher dry matter production per unit leaf area, resulting in a higher NAR compared to other cultivars. On the other hand, the Mehregan cultivar, with its higher canopy shading and reduced light interception by lower leaves, exhibited lower photosynthesis efficiency and, consequently, a lower overall plant community efficiency, resulting in a lower NAR at the lower leaf level (Gardner *et al*., 1985). This finding is consistent with the results reported by Dadrasi *et al*. (2012), indicating that cultivars with a higher leaf surface area produce more photosynthetic materials, leading to an increased Net Assimilation Rate.

4.5. *Chlorophyll Index*

The results of the analysis of variance (Table 2) indicated that the combined application of sulfur and biofertilizer had a statistically significant effect on the Chlorophyll Index. However, the effects of cultivars and the interaction between sulfur application and cultivars did not show any significant impact on the Chlorophyll Index. Based on the mean comparison results (Table 3), the highest Chlorophyll Index was observed in the treatment where sulfur and Sulfofertilizer1 were applied together, with an average value of 49.51. On the other hand, the lowest Chlorophyll Index was recorded in the control treatment where no sulfur fertilizer was used, with an average value of 44.31. It appears that the application of sulfur fertilizer may enhance chlorophyll biosynthesis and, consequently, improve the plant's photosynthetic efficiency. This effect could be attributed to the role of sulfur in nitrogen metabolism and chlorophyll synthesis. Additionally, the use of chemical and biofertilizers like sulfur might increase nitrogen utilization efficiency and enhance the absorption capacity of other essential nutrients in plants, resulting in an overall increase in chlorophyll content (Marschner, 1995). Soaud *et al*. (2011) also reported that sulfur plays a significant role in chlorophyll formation in plants. Moreover, Heidari *et al*. (2015) stated that sulfur application has a significant impact on the chlorophyll content of leaves, resulting in a 39 percent increase compared to the non-application of sulfur fertilizer. According to the findings of Reif *et al*. (2012), the reduction of sulfur content in plants, due to sulfur deficiency, could be attributed to the influence of free oxygen radicals, which, in the absence of sufficient sulfur, exert damaging effects on photosynthetic membranes. Sulfur deficiency leads to a decrease in photosynthetic pigments, which play a crucial role in photosynthesis, resulting in a reduction of the photosynthetic rate and ultimately impeding plant growth. Furthermore, Orman and Kaplan (2007) found that sulfur fertilizer application leads to an increase in the total chlorophyll content in plants. Similarly, Amin *et al*. (2017) reported a 4.37 percent higher chlorophyll level in the groups treated with sulfur compared to the non-sulfur-treated groups.

4.6. *Grain Yield*

The results of the analysis of variance indicate that the combined effect of sulfur and Sulfofertilizer1 application, as well as the different wheat cultivars and their interaction, significantly influenced grain yield (Table 2). As observed from the mean comparison table (Table 3), the highest grain yield was obtained from the combined application of sulfur and sulfofertilizer1 (with an average of 464.73 g.m^{-2}), while the lowest grain yield was recorded in the non-fertilized control treatment (with an average of 352.04 g.m^{-2}). Among the wheat cultivars, the highest grain yield was associated with the Khalil cultivar (with an average of 477.43 g.m^{-2}) and the lowest was attributed to the Mehregan cultivar (with an average of 370.82 g.m^{-2}) (Table 3). Additionally, the interaction effect of sulfur fertilizer and wheat cultivars showed that the highest grain yield was achieved with the combined application of sulfur and biofertilizer in the Khalil cultivar (with an average of 490.1 g.m^{-2}) (which did not differ significantly from the sulfur fertilizer treatment), while the lowest grain yield was obtained in the non-fertilized treatment (control) with the Mehregan cultivar (with an average of 346.88 g.m^{-2}) (Table 4).

Table 4. Mean comparison interaction effect of treatment on grain yield

Fertilizer	Cultivar	Grain yield (gr.m^{-2})
Non-application	Mehregan	346.8*
	Chamran2	365.2
	Khalil	391.4
Sulfur application	Mehregan	408.3
	Chamran2	429.7
	Khalil	482.3
Sulfur + Sulfofertilizer1	Mehregan	417.5
	Chamran2	455.1
	Khalil	490.1
LSD (5%)		2.37

*Mean of treatments that differ from LSD is significantly different at the 5% Level.

The higher grain yield observed in the high-yielding Khalil cultivar can be attributed to its genotype characteristics, environmental factors and the accumulation of higher dry matter in this cultivar compared to others, which is consistent with the findings of Asadalahzadeh *et al*. (2019) in wheat plants. It can also be noted that improved wheat cultivars usually have higher grain yield due to a larger number of spikes per unit area, as well as a greater number of grains per spike and higher 1000-grain weight, as reported by Mokhtari *et al*. (2015). In this regard, Zahedian *et al*. (2015) stated that selecting suitable cultivars for the region can significantly affect growth, yield and yield components in wheat, leading to increased production and economic yield, which is in line with the results of this study.

Furthermore, it appears that the combined application of sulfur and sulfofertilizer1 resulted in a decrease in soil pH, which in turn enhanced the uptake of essential nutrients, especially nitrogen, phosphorus and trace elements such as iron. The increased phosphorus uptake improved energy use and storage in the plant, ultimately leading to higher grain yield (Yadav and Yuosepur, 2015). Consistent with this, Orman and Kaplan (2007) in their reports mentioned that the consumption of Thiobacillus bacteria (sulfur-containing fertilizer) and sulfur-oxidizing bacteria results in both direct sulfur nutrition effects on plants and a decrease in soil pH, leading to improved access to necessary plant nutrients and having a positive effect on plant growth and yield. According to the findings of Nadeem *et al*. (2021), the use of sulfur and sulfur-oxidizing bacteria had the most significant effect on increasing grain yield-related traits and the number of wheat spikes. Moreover, Hussain *et al*. (2022) stated that sulfur fertilizer application led to an increase in grain yield and 1000-grain weight in the Hiwanto cultivar compared to the Kurdoo cultivar. The results of Hasanpour *et al*. (2018) also indicated that the bio-gypsum (sulfur-containing fertilizer) had a significant effect on grain yield and quantitative growth components of wheat. The consumption of sulfur in soil, in addition to improving soil properties, resulted in an increase in wheat yield, which is consistent with the findings of this study (Erdem *et al*., 2016).

5. CONCLUSION

The simultaneous use of sulfur and biofertilizer (sulfofertilizer1) significantly increased grain yield and physiological indices in wheat plants. This improvement can be attributed to the sufficient supply of sulfur required by the plant, which in turn facilitated the absorption of other essential nutrients such as nitrogen, phosphorus and micronutrients. Thiobacillus bacteria, being free-living, play a vital role in sulfur oxidation, making it readily available to the plant. This underscores the critical importance of sulfur application in agricultural ecosystems. Considering the high lime content in Iran's soils and the cost-effectiveness of sulfur produced in the country's oil and gas industries, it is highly recommended to use sulfur to reduce soil acidity and enhance plant access to this essential element. Additionally, the simultaneous employment of Thiobacillus bacteria is also suggested, as it can further increase sulfur oxidation and increase the activity of beneficial soil microorganisms.

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FOOTNOTES

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