

Original Article

Assessment of growth characteristics of basil (*Ocimum basilicum* L.) as affected by sulfur treatments and *Thiobacillus* inoculation

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ARTICLE INFO	ABSTRACT
<p>Corresponding Author: Ghasem Gandomkar gh.gandomkar@gmail.com</p> <p>Received: 20 May 2024 Accepted: 27 May 2024</p> <p>Keywords: Basil Essential Oil Sulfur <i>Thiobacillus</i></p>	<p>To investigate the effect of the use of sulfur and <i>Thiobacillus</i> bacteria on the yield and the amount of the effective substance of the basil, a factorial experiment was conducted in the form of completely randomized blocks with three replications in the research lands of Ran Agricultural Company located in Firuzkoh region in 2013. The levels of 0, 100, 200, 300, 400, and 500 kg of sulfur per hectare were considered as the first factor and <i>Thiobacillus</i> bacteria (no inoculation and inoculation) as the second factor. The results showed that the effect of sulfur is significant for the traits of plant height, number of leaves, leaf fresh weight, stem fresh weight, leaf dry weight, stem dry weight, essential oil content, leaf area index, leaf length and width, chlorophyll and essential oil percentage. The effect of <i>Thiobacillus</i> bacteria inoculation was also significant for plant height, number of leaves, fresh weight of leaves, dry weight of leaves, amount of essential oil, leaf surface index, chlorophyll and percentage of essential oil. The beneficial effect of <i>Thiobacillus</i> bacteria and sulfur was also significant for the characteristics of leaf dry weight, essential oil percentage, leaf surface index, chlorophyll, essential oil bruneol percentage, essential oil yield and borneol yield. Among the levels of sulfur treatment, 500 kg/ha had the highest value for all traits except chlorophyll. Leaf fresh weight (59.22 grams per plant), stem fresh weight (56.16 grams per plant), and leaf dry weight (9.644 grams per plant) were the highest. The results showed that <i>Thiobacillus</i> bacteria could be effective in sustainable production systems.</p> <p>Copyright © 2022 Union Medicinal Plants of Iran. All rights reserved.</p>

1. Introduction

Sustainable agriculture based on the use of organic and biological fertilizers with the aim of eliminating or significantly reducing the use of chemical inputs is considered a desirable solution to overcome these problems. Sustainable agriculture is a system that, while enjoying economic dynamism, can improve the state of the environment and optimal use of available resources, and also play a significant role in providing human food needs and improving the quality of life of human societies. In addition, sustainable agriculture by observing ecological principles can increase the efficiency of resource use while creating balance in the environment and provide the basis for long-term human productivity. Therefore, the use of organic and biological fertilizers is a fundamental and reliable step towards achieving the goals of sustainable agriculture (Motiei Langroudi et al. 2010; Menozzi et al. 2015).

The use of sulfur to reduce the pH of alkaline soils and increase the ability to absorb nutrients depending on soil pH has always been considered. However, the main

problem that arises after the use of sulfur in agricultural soils is its oxidation. This action is possible with the help of *Thiobacillus* bacteria that live in aerobic conditions in the soil. These bacteria are able to grow and multiply under favorable conditions, especially high organic matter and suitable humidity, and as a result, increase the biological oxidation of sulfur (Oliveira et al., 2014).

Thiobacillus bacteria are the most important sulfur oxidizers in soil. The presence of these types of bacteria will increase the rate of oxidation in the soil. Normally, in calcareous soils, due to the lack of organic matter, the activity of microorganisms effective in sulfur oxidation is reduced, and the use of sulfur in these types of soils is effective when it is combined with organic fertilizers or is used together with Thiobacillus inoculant (Amal et al. 2014).

Basil (*Ocimum basilicum* L.) is a herbaceous plant belonging to the mint family and rich in essential oils. Basil is used as a medicinal plant, spice and also as a fresh vegetable, which is used in traditional medicine as an expectorant, anti-flatulent, to relieve stomach pain, anti-



parasitic, appetite suppressant and stimulant and effective in improving lung and chest diseases (Sonmezdag et al., 2018). Also, basil essential oil has antifungal and bacterial properties and repels insects, which is widely used in the food industry, perfumery, oral and dental products (Zeynali et al., 2019).

2. Materials and Methods

This experiment was carried out in the spring of the agricultural year 2011 in the research farm of Ran Company located in Firuzkoh region with longitude 52 degrees 44 minutes north and latitude 35 degrees 45 minutes east and altitude 1930 meters above sea level.

In order to determine the physical and chemical properties of the soil, before conducting the experiment, samples were taken from the field soil from a depth of 0-30 cm in several places and the samples were mixed and a composite sample was prepared. Soil analysis was done in the soil science laboratory of Peshahang Azmoun soil company, located in Qom province, and the results can be seen in Table 1.

Table 1. Physical and chemical properties of the soil of experiment site.

Texture	Loamy-Clay
pH	7.6
T.N.V (%)	25
O.C (%)	1.86
Total N (%)	0.12
P (ppm)	48
K (ppm)	720
Fe (mg/Kg)	8
Zn (mg/Kg)	1.1
Cu (mg/Kg)	1.2
Mn (mg/Kg)	6.6

The experiment was carried out as a factorial in the form of a randomized complete block design with three repetitions. Inoculation with *Thiobacillus* bacteria at two levels (no inoculation and inoculation) were considered as the first factor and sulfur fertilizer at six levels (0, 100, 200, 300, 400 and 500 kg/ha) as the second factor.

The basil seeds used in this research were also provided by the Medicinal Plants Department of Isfahan Agricultural Research Center. *Thiobacillus* bacteria was obtained from Iran Water and Soil Institute located in Karaj. For every 50 kg of sulfur, half a kg of *Thiobacillus* was mixed with it and before cultivation, it was placed in strips on the rows and mixed with the soil.

Different amounts of sulfur and Tubacillus bacteria were applied in Kurt Howe based on the design plan. The seeds were planted at a depth of 2-3 cm and with a planting distance of 15 cm, and 5-7 seeds were planted at each planting point. Due to three consecutive days of rain, irrigation was done on the fifth day. Irrigation operations were also carried out once every 3-5 days during the

growth period. Irrigation was also done with the help of drip tapes.

Since more seeds were placed in the ground at the beginning of the cultivation in order to ensure the desired and desirable number of plants, after germination and establishment of the plant in the ground at the stage of 3-4 leaves, additional plants were planted with gardening shears. And the weak and unfit was removed from the earth. Due to the absence of pests and diseases in the field, no spraying was done in the field and all the weeds in the field were manually weeded.

During plant growth, samples were randomly selected by removing marginal effects from each plot, and characteristics such as plant height, number of leaves, leaf fresh weight, stem fresh weight, leaf dry weight, stem dry weight, surface Leaf, leaf length and width, leaf chlorophyll and amount of effective substance were measured and studied.

To measure the height of the plant, 4 plants were randomly selected from each experimental unit and measured by one meter from the tip of the plant to the soil surface. To measure the wet weight of 5 plants from each plot, and in each sample, the wet weight of leaves and stems was measured by a digital scale. The plants measured to determine the dry weight were placed inside the oven in the laboratory of the agriculture department for 48 hours at a temperature of 75 degrees Celsius.

To measure chlorophyll, the average leaf chlorophyll of 10 randomly selected plants from each plot was measured using the SPAD device of the Agriculture Laboratory of the Islamic Azad University of Roudhen.

DELTA-T device was used to determine the surface of the leaves. After calibrating the device, the surfaces of the leaves of each plot were measured. This measurement was done in the laboratory of Agriculture Department of Tarbiat Modares University. To measure the effective substance, first the plants of each plot were spread at room temperature and transferred to the laboratory of Biochemistry and Biophysics Research Institute of Tehran University for essential oil extraction.

In order to extract and calculate the essential oil of the basil plant, the plant was first dried, then the leaves and flowering branches, which are the essential and medicinal parts of the plant, were ground, then 20 grams of each sample was poured into a balloon and filled to 2.3 of the balloon volume. Distilled water was poured into it (because it is solvent-free and does not affect the essential oil under the influence of heat) and the essential oil was distilled with distilled water by a Cloninger machine for 5 hours.

As a result of heat, the water vapor pressure increases and the glands containing the essential oil are broken and the essential oil enters the refrigerant along with the water vapor. Condensation takes place in the refrigerant and the drops of essential oil in the water move towards the

graduated tube in two distinct phases. Due to the fact that the essential oil is lighter than water, the essential oil accumulates on the water and the excess water returns through the balloon connection tube.

To collect the essential oil, before we open the outlet part of the device valve, we pour N pentane into the tube so that all the essential oil inside the tube is removed, so that the water and essential oil come out. Then, the essential oil was collected in small vials that were previously weighed with a scale of 0.0001. After dehydrating the essential oils with sodium sulfate (Na₂SO₄) and rapid evaporation of N-pentane, the essential oils inside these vials were weighed with a laboratory scale with an accuracy of 0.0001, and the weight of the essential oils and its performance were calculated. Then the vials containing the essential oil are kept in the refrigerator to be used for analysis (Padalia et al., 2017).

To measure the amount of Berneol, basil extract was first prepared. In this way, consider water and methanol in a ratio of 1:1 as solvents and pour it into an Erlenmeyer flask and add 10 grams of powdered basil to it and put a magnet in it and cover the opening of the Erlenmeyer so that the butanol does not evaporate. and put it on the cyclor to spin for 1 hour, and then pass it through the filter for 24-72 hours and prepare it for loading on the chromatography paper as a sample.

The investigated tests for the normality of the distribution of errors and the homogeneity of variances showed that all traits had normal distribution of errors and all traits had homogeneity of variance.

SAS statistical software was used for statistical analysis of data. Mstat-c software was also used to compare the average treatments. Excel software was also used to draw graphs. SAS software was used to perform the normality test.

3. Results

3.1 Plant Height

According to the results of variance analysis table, the main effect of *Thiobacillus* bacteria and sulfur content on plant height was significant at 1% level, and the interaction effect of *Thiobacillus* bacteria and sulfur was not significant (Table 2). The comparison of the average treatments showed that there is a significant difference between different levels of sulfur fertilizer, so that the height of the plant with the consumption of 500 kg of sulfur fertilizer was about 43.62% higher than without sulfur application (Figure 1).

3.2 Leaf Number

The leaf number per plant was statistically affected by the inoculation with *Thiobacillus* at the level of five percent and by the effect of sulfur at the probability level of one percent, and the statistical results did not show a significant interaction effect (Table 2).

With 500 kg of sulfur, compared to the control, the number of leaves increased by 25.40%, so that the maximum number of leaves was obtained with 400 and 500 kg of sulfur, which were 104.8 and 108.7, respectively, and were statistically at the same level. and the lowest was obtained in the treatment without sulfur (77.5 numbers) and 100 kg (80.23 numbers) (Figure 2). The number of leaves per plant in the treatment with *Thiobacillus* inoculation (95.87 numbers) was significantly higher than without inoculation (88.57 numbers) (Table 3).

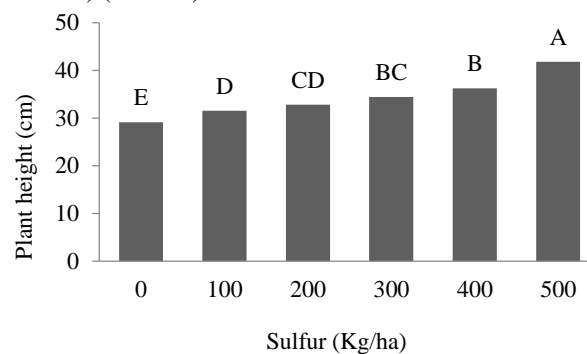


Fig1. Effects of sulfur on plant height

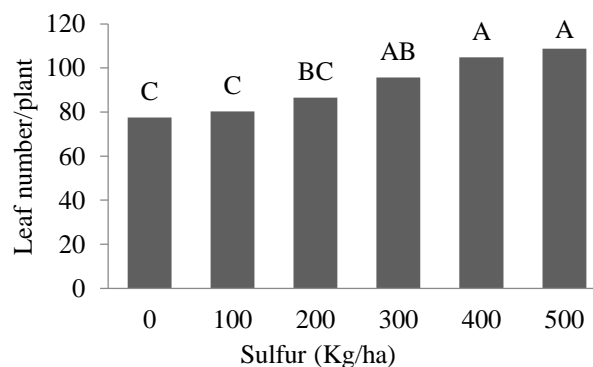


Fig2. Effects of sulfur on leaf number

3.3 Chlorophyll

The results of analysis of variance (Table 2) showed that the main effects of *Thiobacillus* bacteria and sulfur fertilizer were significant at the level of 1% and their mutual effects were significant at the level of 5%.

The results of the average comparison showed that the highest effect is related to the level of 200 kg per hectare with 11.86% and the lowest number is related to the control treatment with 9.847%. Regarding the main effect of *Thiobacillus* bacteria, the average comparisons showed that the lowest effect is related to the absence of Bacterial inoculation and the highest amount was obtained in bacterial inoculation.

Regarding the interaction between *Thiobacillus* and sulfur, the most effective was the treatment of 500 kg/ha of sulfur along with bacterial inoculation (Figure 3), which is due to the increase in the chlorophyll content of the leaves in the treatment of simultaneous use of sulfur and *Thiobacillus*.

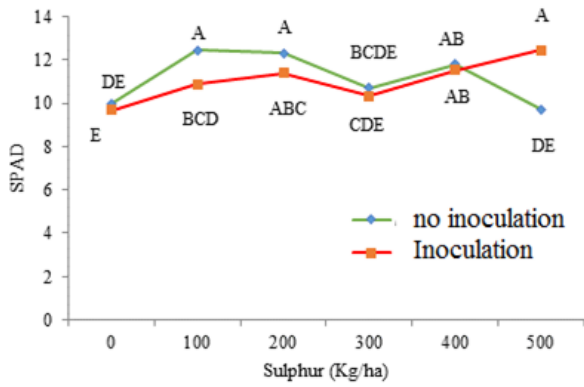


Fig 3. Interaction effects of sulfur and *Thiobacillus* bacteria on leaf chlorophyll

3.4 Leaf Fresh Weight

Fresh weight of leaf and stem was statistically affected by the main effect of *Thiobacillus* bacteria and sulfur at the level of 1%, while their interaction was not significant (Table 2). With the application of sulfur up to 500 kg per hectare, the weight of fresh leaves and stems compared to the control showed an increasing trend, so that the highest fresh weight of stems (73.67 grams per plant) and fresh weight of leaves (76.33 grams per plant) Under the main effect of 500 kg of sulfur and the lowest fresh weight of the stem (31 grams per plant) and leaves (33 grams per plant) was obtained in the control treatment.

In other levels of stem wet weight, statistically, the levels of 200 and 300 kg of sulfur were at the same level and did not show a significant difference (Figure 6-4). And for leaf wet weight, the levels of 300 and 400 kg and 100 and 200 kg were placed in a lower level (Figure 4).

3.5 Leaf Dry Weight

Fresh weight of leaf and stem was statistically affected by the main effect of *Thiobacillus* bacteria and sulfur at the level of 1%, while their interaction was not significant (Table 2). From the analysis of variance table, it can be seen that the dry weight of leaf and stem was significant under the direct effect of sulfur at the level of one percent, and the main effect of *Thiobacillus* bacteria on the dry weight of leaves was at the level of five percent. Also, the

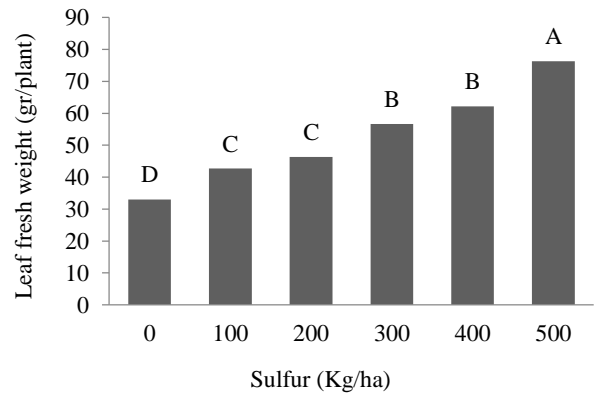


Fig 4. Effects of sulfur on leaf fresh weight

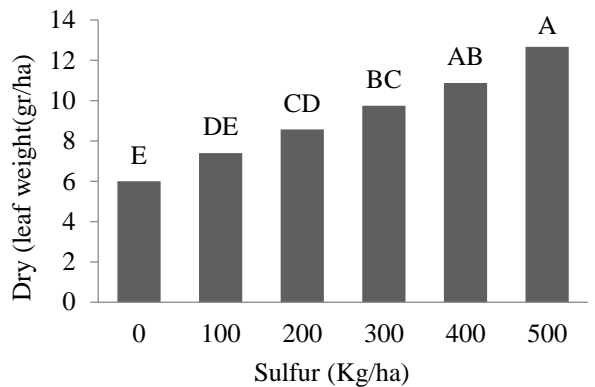


Fig 5. Effects of sulfur on leaf dry weight

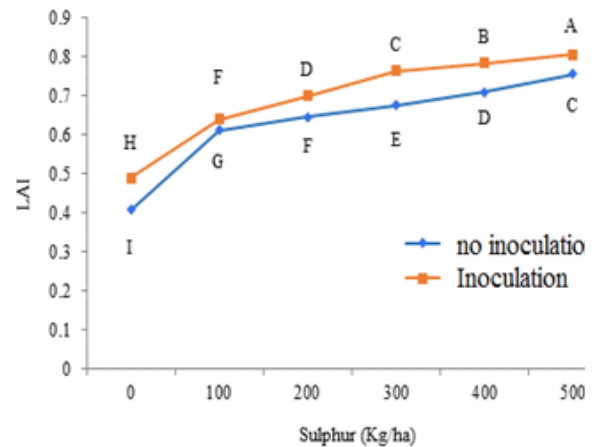


Fig 6. Interaction effects of sulfur and thiobacillus on LAI

Table 2. Analysis of variance (mean of squares) for the effect of sulfur and *Thiobacillus* bacteria on basil

S.O.V	df	Height	Leaf Number	Leaf weight		LAI	Leaf Length	Leaf Width
				Fresh	Dry			
R	2	7.145	1.750	5.025	0.290	0.290	41.541	63.501
T	1	225.484**	13.925**	152.469**	13.216**	13.215**	73.003**	27.729**
S	5	430.243**	4.083**	27.234**	0.168**	0.168*	52.501	6.671
T S	6	1.732	1.009	3.279	1.645	1.644*	26.551	4.736
Error	22	2.516	1.477	5.583	1.176	1.468	17.283	18.99
CV%		4.55	8.48	13.09	2.42	11.34	20.42	14.24

* and **: Significant at the 5% and 1% probability levels, respectively.

Table 3. Means comparison of *Thiobacillus* bacteria effects on measured traits of basil

<i>Thiobacillus</i>	Chlorophyll	LAI	Leaf Dry Weight	Stem Fresh Weight	Leaf Fresh Weight	Leaf Number	Plant Height
Inoculation	13.45 a	21.25 a	9.64 a	56.17 a	59.22 a	95.88 a	35.42 a
Non-Inoculation	10.85 b	22.52 b	8.78 b	43.56 b	46.50 b	88.57 b	33.22 b

Means followed by similar letters in each column do not significantly differ at $\alpha=5\%$ probability level based on Duncan's test.

interaction effect of *Thiobacillus* bacteria and sulfur content was not significant (Tables 2).

Regarding the main effect of sulfur treatment, the lowest stem dry weight (6.00 grams per plant) and the lowest leaf dry weight (6.167 and 7.45 grams per plant) were obtained under the treatment of no sulfur consumption (control) and the highest leaf dry weight (12.67 g/plant) with the application of 500 kg/ha of sulfur and the highest dry weight of the stem (16.65 and 13.43 g/plant) was obtained under the treatment of 500 and 400 kg/ha of sulfur (Figure 5).

3.5 Leaf Area Index

The leaf surface index was statistically affected by the main effects of *Thiobacillus* bacteria and sulfur fertilizer at the probability level of one percent, and also the interaction effect of bacteria and sulfur showed a significant difference at the five percent level (Table 2). Average comparisons showed that with bacterial inoculation, the highest leaf surface index (0.697 square meters) and the lowest in non-inoculation (0.634 square meters) are obtained. The highest leaf surface was obtained with the application of 500 kg of sulfur and the lowest was obtained under the treatment without application of sulfur.

The lowest leaf area was obtained under the interaction effect of non-inoculation \times without sulfur application (0.406 square meters) and the highest leaf area was obtained under the interaction effect of inoculation treatment with *Thiobacillus* \times 500 kg/ha of sulfur (0.805 square meters) (Figure 6).

3.6 Leaf Length and Width

The results of analysis of variance between different treatments showed that the main effect of sulfur fertilizer was significant at the 1% level, and the main effect of *Thiobacillus* bacteria and the interaction effects of *Thiobacillus* bacteria and sulfur were not significant (Table 2).

The comparison of the average treatments showed that there is a significant difference between different levels of sulfur fertilizer, so that the length of the leaf with the increase of sulfur fertilizer was about 14.03% and the width of the leaf was 32.28% more than without sulfur application. The maximum leaf length (48.44 mm) and maximum leaf width (28.11 mm) were obtained under the main effect of 500 kg of sulfur. The lowest leaf

length (42.48 mm) and leaf width (21.25 mm) were obtained in the sulfur control treatment.

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4. Discussion

4.1 Plant height

The comparison of the average treatments showed that there is a significant difference between the different levels of *Thiobacillus* bacteria, so that the growth of the stem was the highest in the inoculation with *Thiobacillus* (Table 3). The reason for this is that sulfur oxidation is a mainly biological process. The realization of this process requires the presence of sulfur oxidizing microorganisms, of which *Thiobacillus* bacteria is one of the most important types of oxidizers (Amal et al., 2014).

Regarding the effect of sulfur, (Clarke and Murray., 1990) stated in an experiment on Eucalyptus that cell division is controlled by a balance between oxidized sulfur and reduction of elemental sulfur, and this can explain how oxidized sulfur affects growth. Leaves and growth of the tip of the plant (height). It is also possible that the change in the allocation of resources between the roots and the above-ground parts leads to bigger leaves and an increase in height. Also, the positive effects of bacteria on increasing the height in various plants such as beans, oranges, and bananas have been proven (Wani et al., 2006; Qiangsheng et al., 2006; Gavito et al., 2000; Yano-melo et al., 1999).

4.2 Leaf Number

In other words, the consumption of appropriate amounts of sulfur and also the inoculation of *Thiobacillus* due to the presence of *Thiobacillus* bacteria, the oxidation of sulfur and acidification of the soil has taken place, and it has increased the ability to use soil nutrients by the

plant and increased the amount of photosynthesis, and this ultimately leads to the increase of aerial organs. Dayalmi et al. (2009) in their study, which was conducted on Barhi date palm seedlings, showed that the increase in vegetative growth indicators due to the application of sulfur along with *Thiobacillus* inoculum could be attributed to the positive effects of this substance in improving the nutritional status of the plant.

The hormonal effect induced in the plant by growth-stimulating bacteria may either directly cause changes in the stem morphology of the inoculated plants (thickening of the stem, increase in foliage and the number of flowering branches) or by increasing the growth of the root and consequently increasing the area. Access to water and salts makes the growth of the aerial part of the plant possible (Gilani et al., 2021). The results of this research are consistent with the results obtained by (Rivaudo et al., 2006) in the tomato plant and (Azzaz et al., 2009) in the fennel.

4.3 Chlorophyll

Regarding the interaction between *Thiobacillus* and sulfur, the most effective was the treatment of 500 kg/ha of sulfur along with bacterial inoculation (Figure 3), which is due to the increase in the chlorophyll content of the leaves in the treatment of simultaneous use of sulfur and *Thiobacillus*. The increase in the availability of elements is caused by the decrease in soil pH. In other words, consuming amounts of sulfur through participation in the essential amino acids cysteine and methionine plays an essential role in protein synthesis, and on the other hand, another important role of sulfur is by participating in the structure of sulfolipids in the structure of chlorophyll and protein to absorb different light spectrums, Whose deficiency disrupts photosynthesis (Gilani et al., 2021).

In this context, the results (Marius et al., 2005) showed that the effect of bacterial inoculation on the sunflower plant caused an increase for pigments, before and after flowering, and ultimately improved the growth of sunflower in the biofertilizer treatment compared to the control has been observed. The presence of these bacteria in the root environment of some medicinal plants, including basil, has also been reported (Karthikeyan et al., 2007).

4.4 Leaf Fresh Weight

Regarding the effect of sulfur fertilizer on the fresh weight of leaves and stems, it should be said that this was probably due to the oxidation of sulfur, especially in the rhizosphere zone, in the dissolution of insoluble food compounds and the release of essential elements and the increase of plant growth (Amal et al., 2014). *Thiobacillus* bacteria has been able to help the solubility of elements in calcareous soil by reducing the pH of the

soil around the roots and increasing plant growth. Such synergistic effects between sulfur oxidizing bacteria have been proven by other researchers (Anandham et al., 2007) on the other hand, *Thiobacillus* bacteria by oxidizing sulfur while providing sulfate needed by the plant; Lowering the pH of the soil around the plant roots increases the solubility of low-use elements and leads to an increase in yield (Amal et al., 2014).

In an experiment, they reported that if the total level of phosphorus in the soil is high, the use of sulfur in the presence of *Thiobacillus* causes the soil phosphorus to dissolve and it is a suitable alternative to phosphate fertilizers, which increases the yield and fresh weight of the plant (Gilani et al., 2021).

4.5 Leaf Dry Weight

The dry weight of leaves under the main effect of non-inoculation of *Thiobacillus* bacteria had the lowest value (8.788 g/plant) and the highest dry weight of leaves was obtained under the main effect of inoculation with *Thiobacillus* bacteria (9.644 g/plant) (Table 3) The reason for this is the use of growth-stimulating bacteria in the plant through the production and secretion of growth-stimulating hormones (auxin and gibberellin) can increase the number of leaves, dry weight of different parts of the plant, and crop production (Govahi et al, 2006). According to the findings of Ravi et al., (2008) in sunflower, with increasing sulfur levels, an increase in the dry weight of the studied crops was observed. It is stated that the main effects of *Thiobacillus* and sulfur on corn plants increased dry matter and improved dry yield (Anandham et al. 2007)

4.6 Leaf Area Index

Comparisons of the average effect of *Thiobacillus* inoculation with sulfur showed a significant increase in the leaf surface. Also, there is a significant difference in different levels of sulfur and *Thiobacillus* inoculation on the leaf surface index. Sulfate and iron of the soil and absorption of phosphorus by the plant and increased the yield and leaf surface index of the plant. In calcareous and alkaline soils due to the presence of high pH and high concentration of calcium ions, some nutrients such as phosphorus, iron and zinc that can Their absorption depends on pH, they are stabilized and they are out of the reach of plants (Amal et al., 2014).

By intensifying sulfur oxidation in such soils, *Thiobacillus* thiooxidans bacteria can be effective in reducing pH and improving the soil, providing sulfate needed by the plant, dissolving some nutrients and increasing their absorption capacity, and they can withstand the harsh conditions of removing substances from accumulated solutions. . The report of some researchers indicates the positive results of the above treatments on the leaf surface index (Singh et al., 1998; Deluca et al., 1989; Tisdal et al., 1993). Studies

(Annandeham et al., 2006) on the peanut plant also expressed the above theory.

4.7 Leaf Length and Width

According to the results obtained, the cause can be attributed to the effect of bacteria through the production of hormone-like substances, reducing ethylene levels, providing food, biological control, and creating plant resistance to environmental stresses, such as lack of food and reducing the toxicity of elements (Mena-Violante and Portugal 2007).

The increase in the growth of aerial organs of some medicinal plants in the presence of biological bacteria effective on growth was previously reported (Kapoor et al., 2004, Yadgari and Barzegar, 2019).

5. Conclusion

There is clear evidence of the improvement of the characteristics of basil due to the application of sulfur and thiobacillus bacteria, which is due to the improvement of the structural and physical characteristics of the soil and the improvement of the physiological processes of the plant. The results of this experiment also showed that treatments had a positive and significant effect on the traits studied on basil and can be a solution to meet plant nutritional needs in sustainable production systems based on ecological principles, and therefore, reduce dependence on artificial chemicals in agricultural systems.

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