#### **ORIGINAL ARTICLE / ORIGINALBEITRAG**



# **Effects of** *Thiobacillus* **and Different Levels of Sulfur Fertilizer on Growth and Physiological Indices in Intercropping of Sesame (***Sesamum Indicum* **L.) and Mung Bean (***Vigna Radiata* **L.)**

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Received: 18 December 2020 / Accepted: 29 March 2021 / Published online: 26 April 2021 © Springer-Verlag GmbH Deutschland, ein Teil von Springer Nature 2021

## **Abstract**

The two-location field experiment was conducted to investigate the effect of *Thiobacillus* and different levels of sulfur fertilizer on growth and physiological indices in the replacement intercropping of sesame and mung bean. A factorial experiment was performed based on a randomized complete block design in 3 replications in 2018. The experimental factors consisted of cropping ratio at five levels: 1. sesame sole cropping, 2. mung bean sole cropping, 3. 75% sesame +25% mung bean (3:1), 4. 50% sesame +50% mung bean (1:1), 5. 25% sesame +75% mung bean (1:3). Sulfur fertilizer was used at three levels: control level  $(S_0)$ , 50% of recommended amount  $(S_1)$ , 100% of recommended amount  $(S_2)$ . *Thiobacillus* bacteria was used at two levels:  $T_0$  and  $T_1$ . The results showed that growth indices chlorophyll a, chlorophyll b, total chlorophyll, biological yield, and grain yield of sesame and mung bean were significantly affected by the studied treatments. The highest growth indices, chlorophyll a, chlorophyll b, total chlorophyll, biological yield and grain yield of sesame were obtained by the 3:1 ratio. For the mung bean, the highest growth indices, chlorophyll a, biological yield, grain yield were obtained by mung bean sole cropping and the highest chlorophyll b and total chlorophyll were obtained by the 1:1 ratio. Also, sulfur fertilizer, 100% of the recommended amount, increased all studied indices in both plants except for chlorophyll a in sesame. Interaction effects of cropping ratio and location on growth indices, grain biological yield, chlorophyll a of sesame and mung bean were significant.

**Keywords** Growth indices · Chlorophyll · Intercropping · Sesame · Mung bean

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## **Auswirkungen von** *Thiobacillus* **und verschiedenen Mengen an Schwefeldünger auf Wachstums- und physiologische Indizes im Zwischenfruchtanbau von Sesam (***Sesamum indicum* **L.) und Mungbohne (***Vigna radiata* **L.)**

#### **Zusammenfassung**

Das Feldexperiment wurde durchgeführt, um die Wirkung von *Thiobacillus* und verschiedenen Mengen an Schwefeldünger auf Wachstums- und physiologische Indizes im Zwischenfruchtanbau von Sesam und Mungbohne zu untersuchen. Es wurde ein faktorieller Versuch basierend auf einem randomisierten vollständigen Blockdesign in 3 Wiederholungen im Jahr 2018 durchgeführt. Die Versuchsfaktoren bestanden aus dem Anbauverhältnis in fünf Stufen: 1. Sesam-Alleinanbau, 2. Mungbohnen-Alleinanbau, 3. 75% Sesam +25% Mungbohne (3:1), 4. 50% Sesam +50% Mungbohne (1:1), 5. 25% Sesam +75% Mungbohne (1:3). Schwefeldünger wurde in drei Stufen verwendet: Kontrollstufe (S<sub>0</sub>), 50% der empfohlenen Menge (S<sub>1</sub>), 100% der empfohlenen Menge (S<sub>2</sub>). *Thiobacillus*-Bakterien wurden in zwei Stufen eingesetzt: T<sub>0</sub> und T<sub>1</sub>. Die Ergebnisse zeigten, dass die Wachstumsindizes Chlorophyll a, Chlorophyll b, Gesamtchlorophyll, der biologische Ertrag und der Kornertrag von Sesam und Mungbohnen durch die untersuchten Behandlungen signifikant beeinflusst wurden. Die besten Wachstumsindizes und die höchsten Werte für Chlorophyll a, Chlorophyll b, Gesamtchlorophyll, biologischen Ertrag und Kornertrag von Sesam wurden durch das Verhältnis 3:1 erreicht. Bei der Mungbohne wurden die besten Wachstumsindizes und höchsten Werte für Chlorophyll a, biologischen Ertrag und Kornertrag durch den Mungbohnen-Alleinanbau erreicht, der höchste Chlorophyll-b- und Gesamtchlorophyllwert durch das 1:1-Verhältnis. Auch Schwefeldünger (100% der empfohlenen Menge) erhöhte alle untersuchten Indizes in beiden Pflanzen, außer Chlorophyll a in Sesam. Die Interaktionseffekte des Anbauverhältnisses und des Standorts auf die Wachstumsindizes, den biologischen Kornertrag, das Chlorophyll a von Sesam und Mungbohnen waren signifikant.

**Schlüsselwörter** Wachstumsindizes · Chlorophyll · Zwischenfruchtanbau · Sesam · Mungbohne

## **Introduction**

Intercropping is the cultivation of two or more crops together in one land parcel at the same time to enhance the ratio of land equivalent ratio (LER). It shows that nature always prefers combined species over sole species (Baumann et al. [2002\)](#page-15-0). Intercropping is one of the oldest and most widely used operations in low input agriculture, and one of the cheapest and most affordable ways to increase crop production, which increases production, reduces damage by pest, disease, weed, preserves soil fertility, creates ecological balance, controls erosion, and finally, optimizes utilization of environmental resources by using the plant diversity principle in the field (Lithourgidis et al. [2006;](#page-15-1) Fenández-Aparicio et al. [2007\)](#page-15-2).

Intercropping optimizes the use of space, time and physical resources in both the upper and lower parts of the soil by maximizing the positive effects and minimizing the adverse effects (competition) between the components (Weil Ray, [1991;](#page-15-3) Hauggaard-Nielsen et al., [2001;](#page-15-4) Li et al., [2011\)](#page-15-5).

In designing an intercropping system, the primary success condition is the selection of species in a complementary manner, which requires knowing the plant entirely in terms of its ecological needs and its environmental responses (Mushagalusa et al., [2008\)](#page-15-6). For example, legumes provide an opportunity for sustainable production increase due to high adaptability to different planting patterns and the ability to stabilize nitrogen (Banik et al., [2006\)](#page-15-7).

Generally, increasing yield occurs in the intercropping system when the constituent plants are quite different in terms of their use of resources (Baumann et al., [2002\)](#page-15-0). Accordingly, the use of plant species with different phenological and morphological characteristics, which make the least competition in a fixed ecological niche, both in terms of environmental and temporal factors, is an essential step in the success of intercropping (Mushagalusa et al., [2008\)](#page-15-6). For example, in an experiment by Wenxue et al. [\(2004\)](#page-15-8), it was observed that intercropping of maize and broadbean increased maize grain yield.

Considering the per capita oil consumption in Iran and the low cultivation area for oilseeds, it seems that intercropping of these plants and legumes can be a solution to increase oil production without the need for increased cultivation area. Sesame is an annual plant with a history of about 5000 years of cultivation, apparently making it the oldest oilseed in the world. Meanwhile, sesame is a product of the tropical and subtropical regions, but the modification of its suitable varieties has extended its cultivation to more temperate regions. The role of legumes has also been recognized as an essential source in the human diet, animal feed, and increased soil fertility (Langham, [2007\)](#page-15-9).

Considering the climatic conditions and soil properties in Iran, it is very imperative to pay attention to the use of sulfur in agricultural lands. Failure to use sulfur-containing fertilizers (such as plain superphosphate) in past years, continuous and high-density cultivation of arable lands, presence of

Station	Number of sunny hours	Annual evapo- ration (mm)	Total annual rainfall (mm)	Averarge maximum annual rela- tive humid- ity $(\%)$	Averarge min- imum annual relative hu- midity $(\% )$	Averarge annual relative humidity $(\%)$	Averarge maximum annual tem- perture $(^{\circ}C)$	Averarge minimum annual tem- perture $(^{\circ}C)$	Averarge annual temper- ture $(^{\circ}C)$
Karaj	2899	2184	372.2	68.9	31	49.95	20.8		14.4
Shahriyar 2985		2232	447.7	64.2	24.5	45.4	16.7	6.6	11.65

<span id="page-2-0"></span>**Table 1** Meteorological data of the nearest meteorological station to the test area

<span id="page-2-1"></span>



sodic and saline-sodic soils and abundance and cheapness of sulfur are the most important reasons for justification of using sulfur in soils in Iran. Atmospheric sulfur makes a negative balance in agricultural soil. Since the crop yields increase, so does the dependence on soil for the supply of sulfur, which is required for the synthesis of protein and many essential vitamins and cofactors (Kertesz and Mirleau [2004\)](#page-15-10).

One of the essential solutions to reduce the use of fertilizers is the use of *Thiobacillus* bacteria.

Due to the more efficient use of resources, many reports indicate the effectiveness of plant growth indices in intercropping conditions over the sole cropping. For example, in the study of sesame and cannabis intercropping, the highest and the lowest amount of cannabis dry matter was obtained in the replacement intercropping pattern of 50% sesame and 50% cannabis, and the incremental pattern of 100% cannabis and 50% sesame in 1921 and 929 g/m<sup>2</sup>, respectively. Moving from the sole cropping pattern to intercropping, dry matter accumulation increased. It appears that in the replacement intercropping pattern, reduced intraspecies competition between cannabis plants (as the dominant plant) leads to increased light absorption and nutrient uptake and improved photosynthesis and consequently increased dry matter accumulation (Kouchaki et al. [2010\)](#page-15-11).

In studying the effect of intercropping of soybean and peppermint on the yield and quality of peppermint essential oil, Maffei and Mucciarelli [\(2003\)](#page-15-12) reported that the quantitative and qualitative yield of peppermint in the intercropping is higher than that in sole cropping.

Tsubo et al. [\(2001\)](#page-15-13) compared the efficiency of light in corn and bean intercropping, in which, the efficiency of light absorption in intercropping was higher than that of sole cropping. They attributed the more significant accumulation of dry matter in the intercropping to higher light intake. In corn and bean intercropping, leaf area index, light absorption, dry matter accumulation, and corn light use efficiency were increased in all intercropping treatments compared to

sole cropping ones. Meanwhile, these characteristics were reduced in all intercropping treatments compared to the sole cropping ones. In general, the intercropping in two species of different foliar arrangement and height absorbs more light qualitatively and quantitatively. In fact, in sole cropping treatments, some photosynthetic radiation is always lost due to vacancies in the canopy. However, the amount of these losses in intercropping reduces due to the higher soil cover, and as a result, the radiation absorption increases over sole cropping, which can increase yield (Awal et al. [2006\)](#page-15-14).

The first objective of the current experiment is to collect the logical proof for oil and protein mixture cropping (sesame and mung) as a replacement for sole cropping, the second is the determination of appropriate methods of fertilization (chemical, biological, and the combination of both fertilizers) and finally, if the results showed combined fertilizers were significantly satisfied, it would lead to reduce the environmental pollution and also help to improve rural financial.

## **Materials and Methods**

This study, as a factorial experiment in a randomized complete block design with three replications, was conducted simultaneously in two locations, one in Karaj city (Alborz province) with latitude 35°76'N and longitude 50°94'E and an altitude of 1300m above the sea level and the other in Shahriyar city (Tehran province) with latitude 35°33'N and longitude 51°53'E and an altitude of 1140m above the sea level in the agronomic year 2017–2018 (Tables [1](#page-2-0) and [2\)](#page-2-1).

In this experiment, the cropping pattern was performed in 5 levels, including sesame and mung bean cultivation only, replacement intercropping ratio of 1:3 for sesame and mung bean (75% sesame and 25% mung bean), replacement intercropping ratio of 1:1 for sesame and mung bean (50% sesame and 50% mung bean), replacement intercrop-

Source	DF	<b>LAI</b> of Sesame Days after planting					LAI of Mung bean Days after planting					
		40	55	70	85	100	40	55	70	85	100	
Location $(L)$		$0.022***$	0.004	0.014	0.026	0.015	0.000003	$0.0015*$	$0.002*$	0.007	0.009	
Error <sub>1</sub>	4	0.0003	0.005	0.057	0.21	0.11	0.0003	0.0001	0.0002	0.005	0.009	
Intercropping (I)	3	0.0001	$0.72***$	$0.96***$	$6.68***$	$1.91**$	0.00002	$0.23***$	$0.66***$	$1.38***$	$0.89**$	
Sulfur $(S)$	$\overline{2}$	$0.118***$	$0.28***$	$0.68***$	$1.61**$	$1.1***$	$0.062***$	$0.04***$	$0.36***$	$0.46***$	$0.33***$	
Thiobacillus (T)	1	$0.01***$	$0.035***$	$0.044***$	$0.16***$	$0.11***$	$0.003***$	$0.005***$	$0.025***$	$0.09***$	$0.05***$	
$S \times I$	6	0.0001	0.0008	0.005	$0.02^*$	$0.016***$	0.00003	0.00006	0.0001	0.004	0.002	
$I \times T$	3	0.00001	0.0002	0.002	0.003	0.003	$0.0002*$	0.00002	0.00009	0.005	0.002	
$T \times S$	$\overline{2}$	0.0001	0.002	0.003	0.014	0.009	$0.0008***$	$0.0005***$	$0.005***$	$0.02***$	0.005	
$T \times S \times I$	6	0.00008	0.0002	0.0006	0.006	0.004	0.0001	0.00005	0.0002	0.002	0.001	
$I \times L$	3	$0.002***$	0.0009	$0.03***$	$0.05***$	$0.072**$	0.00007	0.00007	$0.003***$	0.004	0.003	
$S \times L$	$\overline{2}$	$0.001***$	0.0003	0.003	0.014	$0.075***$	0.00009	0.00009	0.00004	0.004	0.0002	
$T \times L$		$0.0008***$	0.0001	0.004	0.008	$0.023***$	0.00004	0.000006	0.0003	0.000001	0.00002	
$S \times I \times L$	6	0.00009	$0.001***$	0.007	0.002	0.0002	0.00005	0.00009	0.0004	0.003	0.003	
$T \times I \times L$	3	0.00007	0.0006	0.004	0.002	0.0005	0.00001	0.00003	0.0006	0.0003	0.0005	
$T \times S \times L$	$\overline{2}$	0.00001	0.0007	0.007	0.004	0.001	0.000001	0.000008	0.00007	0.0002	0.00001	
$TxS\times I\times L$	6	0.0001	0.0005	0.001	0.001	0.0006	0.00007	0.000008	0.0003	0.001	0.002	
Error <sub>2</sub>	92	0.0001	0.0004	0.005	0.007	0.0033	0.00005	0.00006	0.0003	0.002	0.002	

<span id="page-3-0"></span>**Table 3** Results of analysis of variance (mean squared) of leaf area index of sesame and mung bean during the growing season

ping ratio of 3:1 for sesame and mung bean (25% sesame and 75% mung bean). Sulfur fertilizer was used at three levels: control level/no use  $(S_0)$ , 50% of recommended amount  $(S_1)$ , 100% of recommended amount  $(S_2)$ ; and inoculation by *Thiobacillus* bacteria at two levels (no inoculation  $(T_0)$ ) and inoculation with bacteria  $(T_1)$ ). As for the intercropping treatments, a sole crop of a species was cultivated in each of the four rows in every plot. Three rows of sesame and one row of mung bean were cultivated for the ratio of 75% sesame and 25% mung bean, three rows of mung bean and one row of sesame were cultivated for the ratio of 75% mung bean and 25% sesame, and one row of sesame and one row of mung bean were cultivated alternately for the ratio of 50% mung bean +50% sesame. Elemental sulfur fertilizer powder was added as recommended to the plots in lines at a depth of 10 cm 30 days before planting for each treatment and was completely mixed with the soil. *Thiobacillus* was also inserted into the soil as recommended at a depth of 10 cm one week before planting. Two kilograms of *Thiobacillus* (*Halothiobacillus neapolitanus*) was used per 100 kg of sulfur (Besharati et al. [2017\)](#page-15-15). The size of each plot was  $6m \times 2m$ , the distance between plots was 0.5m, and the distance between replications was 2m. Planting was performed manually on June 22, 2017. The distance between plants on each row was 25 cm, and the distance between rows was 50 cm. Irrigation was carried out regularly and every six days. Weeding out was done manually. After eight leaf stage, plants were randomly sampled every 15 days in each plot. Samples were taken from the middle of each plot to reduce the marginal effect.

To calculate growth indices during the growth season, the leaf area index and plant dry weight were measured. Samples were placed in an oven at 75 °C for 48 h to measure dry weight. Leaf area index (LAI) was also measured by a specific leaf area meter at each step. To determine the variations in the crop growth rate (CGR) indices and the net assimilation rate (NAR), the following equations were used:

$$
CGR = (W2 - W1)/(T2 - T1)
$$
 (1)

$$
NAR = CGR/LAI
$$
 (2)

Sampling was performed to determine the chlorophyll content at the beginning of grain filling from the late developed leaves of the plant. 0.2 g of fresh leaves of the plant was thoroughly ground with 5ml of 80% acetone. The solution in the mortar was mixed with another 10ml of acetone through a special filter and reached a volume of 15ml. Three milliliters of the solution were poured into the cuvette and absorbed at 663 and 646 nm by the spectrophotometer (Alpha 1900S Double Beam; South Korea) vs. blank acetone (Lichtenthaler [1987\)](#page-15-16). The amount of photosynthetic pigments was then calculated using the following equations.

Chlorophyll a =  $(19.3 \times A663 - 0.86 \times A646) \text{ V}/100 \text{W}$  (3) Chlorophyllb =  $(19.3 \times A646 - 3.6 \times A663) \text{ V}/100 \text{W}$  (4)



<span id="page-4-0"></span>**Fig. 1** Effect of different sesame (**a**) and mung bean (**b**) treatments on changes in leaf area index (mean data of two locations)



<span id="page-4-1"></span>**Fig. 2** Effect of different treatments of sulfur fertilizer on changes in leaf area index of sesame (**a**) and mung bean (**b**) (mean data of two locations)

Total chlorophyll =  $Chla + Chlb$  (5)

A in these relations is the absorption wavelength of the device.

## **Statistical Analysis**

This experiment was analyzed by combined analysis, Only the basic design of this experiment is factorial. Analysis of variance and mean comparisons of data (at a 5% probability level based on LSD test) were performed using SAS software (Ver. 9.4). MS Excel was also used to plot the diagrams.

## **Results and Discussion**

## **Leaf Area Index (LAI)**

Analysis of variance showed that intercropping, sulfur fertilizer, and *Thiobacillus* bacteria had a significant effect on sesame and mung bean leaf area index during the growing season (Table [3\)](#page-3-0). In different treatments, the increasing trend of sesame and mung bean leaf area index was slow up to 40 days after germinating, but it increased linearly up to 85 days after planting, and then, decreased (Fig. [1\)](#page-4-0). The highest sesame leaf area index was observed at 85 days after germinating for the 75% sesame +25% mung bean intercropping with 3.56, and lowest was observed for 25% sesame +75% mung bean with 2.61. In the case of mung bean, the highest and lowest leaf area index were observed in the sole cropping with 3.13 and in the 50% sesame +50% mung bean with 2.69 (Fig. [1\)](#page-4-0).

Source	DF	TDW of Sesame						TDW of Mung bean					
		Days after planting	Days after planting										
		40	55	70	85	100	40	55	70	85	100		
Location $(L)$	1	$558.18***$	78.03	26533	1202	845	2.1	4.15	4.33	2022	2830		
Error <sub>1</sub>	4	7.89	26.15	4470	3719	1016	0.75	4.16	9.88	1431	51.84		
Intercropping (I)	3	$21.62*$	$13821***$	107519**	299867**	212442**	$5.46***$	$2706***$	60794**	28070**	22252**		
Sulfur $(S)$	$\overline{2}$	4388**	4336.7**	64664**	44071**	$12133***$	692**	$903**$	6622**	12436**	7434**		
Thiobacillus (T)	$\mathbf{1}$	$601.52***$	$780.64***$	6384**	4585**	$858***$	$75.73***$	$93.6***$	$791***$	883**	$651***$		
$S \times I$	6	1.41	$22.73*$	264.43	81.86	39.91	1.28	1.87	$24.61***$	18.44	60.88		
$I \times T$	3	2.83	27.7	90.23	22.28	14.99	0.89	0.59	7.61	41.82	5.58		
$T \times S$	$\overline{2}$	$39.36***$	$60.59***$	623.06	145.8	23.3	$11.06***$	$12.6***$	12.24	246	93.46		
$T \times S \times I$	6	2.6	14.06	133.42	196	17.14	0.54	1.28	6.51	58.08	5.73		
$I \times L$	3	94.79**	23.37	$906.2*$	552.44**	919.16**	2.22	2.73	6.38	0.031	48.65		
$S \times L$	2	8.78	$37.09*$	518.54	1214.15	204.86*	0.08	0.05	2.04	$769**$	33.92		
$T \times L$		1.07	23.8	64.28	340.31	$7.89**$	0.17	0.04	5.55	62.67	0.75		
$S \times I \times L$	6	5.78	7.47	372.28	119.23	73.1	0.89	0.51	0.76	81.5	83.73		
$T \times I \times L$	3	3.57	12.9	103.4	35.96	8.56	0.29	1.23	4.82	20.57	1.85		
$T \times S \times L$	2	1.65	18.3	14.75	55.29	0.91	0.007	0.95	1.78	28.8	2.04		
$T \times S \times I \times L$	6	1.98	11.98	40	122.04	11.3	1.1	1.3	1.47	30.84	17.86		
Error <sub>2</sub>	92	7.47	10.25	249.99	118.2	44.55	0.92	1.08	4.89	98.2	34.52		

<span id="page-5-0"></span>**Table 4** Results of analysis of variance (mean squares) of dry weight accumulation of sesame and mung bean during the growth period

The reason for the increase in the leaf area index of sesame in the intercropping compared to the sole cropping is the positive effects of nitrogen fixation by mung bean. Also, the denser canopy and increased height of sesame compared to the mung bean, in addition to the positive effects of mung bean as a nitrogen-fixing plant in soil, which can increase the growth rate and leaf area index of sesame, thus increasing its competitiveness with mung bean and finally reduced production of mung bean in the intercrop. It is generally believed that to obtain the highest amount of light absorption in intercropping, it is better to have similar species in the intercrop in terms of competitiveness (Gao et al. [2010\)](#page-15-17). In the intercropping of peppermint and soybean, Maffei and Mucciarelli [\(2003\)](#page-15-12) reported that the leaf area index of peppermint was higher than the sole cropping.

The trend of changes in leaf area index of mung beansesame intercrop during the growing season compared to the mung bean sole crop showed that the leaf area index decreased in the intercropping compared to the sole cropping. Since light absorption and nutrient uptake affect vegetative growth and thus photosynthesis, it seems that the growth and photosynthesis of mung bean, and thus its leaf area index increased under sole cropping conditions compared to the mung bean-sesame intercropping. This may be due to the less competitiveness of mung bean than sesame. The higher shading in the intercropping treatments is probably due to the higher leaf area of these plants than their sole cropping. In the study of mung bean-sesame intercrop, Pandita et al. [\(2000\)](#page-15-18) stated that the highest mung bean leaf area was obtained in its sole cropping treatment. In okra and cassava intercropping, the leaf area index of okra was higher in intercropping than the intercropping treatment. The reason for this decrease in the intercropping seemed to be increased competition between species of okra and cassava (Muoneke and Mbah [2007\)](#page-15-19). Ghosh [\(2004\)](#page-15-20) also reported a decrease in the leaf area index of peanut in its intercropping with millet.

Mean comparisons showed that the leaf area index of sesame and mung bean was affected by sulfur fertilizer consumption. The highest leaf area index was obtained in fertilizer treatments  $S_2$  and the lowest in  $S_0$ . The difference in leaf area index observed in sesame was 10.4%, and in mung bean, it was 6.71% (Fig. [2\)](#page-4-1). With increased access to the nutrients, the leaf area growth was also increased, thus leading to an increase in the grain yield. Leaf area index is a major physiological component in crop production, yield, and growth rate, which has its complex characteristics, and its main components are leaf number and leaf size.

The interaction of cropping ratio and location appeared to be significant on the LAI of sesame except 55 days after planting.

#### **Total Dry Weight (TDW)**

As can be seen in Table [4,](#page-5-0) all main characteristics had a significant effect on the amount of sesame and mung bean dry matter during the growing season. Meanwhile, most of the interactive effects were not statistically significant during



<span id="page-6-0"></span>**Fig. 3** Effect of different sesame (a) and mung bean (b) treatments on the trend of changes in dry matter accumulation  $(g/m^2)$  (mean data of two locations)



<span id="page-6-1"></span>**Fig. 4** Effect of different treatments of sulfur fertilizer on the trend of changes in dry matter accumulation (g/m2) in sesame (**a**) and mung bean (**b**) (mean data of two locations)

the growing season. Mean comparison of traits showed that the highest dry matter content was obtained in the 75% sesame and 25% mung bean treatment, and the lowest dry matter content was in the 25% sesame and 75% mung bean treatment. However, the highest dry matter content during the whole growth period of mung bean was obtained in 100% mung bean treatment and the lowest in 75% sesame and 25% mung bean (Fig. [3\)](#page-6-0).

With this ratio, it seems that reduced intra-species competition between sesame bushes appears to increase light and nutrient absorption and improve photosynthesis, resulting in increased dry matter accumulation (Kouchaki et al. [2010\)](#page-15-11). The higher dry matter content in some intercrops compared to the sole crops can indicate a positive effect of intercropping, thus enhancing the sesame efficiency in using environmental capacities (Zaefarian et al. [2010\)](#page-15-21). Increased dry matter accumulation in intercrops compared to the sole crops has been reported in maize and beans intercropping (Kouchaki et al. [2010\)](#page-15-11), sesame and chickpea (Pouramir et al. [2010\)](#page-15-22), anise and fenugreek (Mirhashemi et al. [2009\)](#page-15-23) and soybean and peppermint (Maffei and Mucciarelli [2003\)](#page-15-12).

In general, due to the low growth rate and low shading at the beginning of the growing season, legumes do not have high competitiveness in the early growth stage. Lack of shading of sesame on mung bean and consequently lack of inter-species competition for resources in sole cropping increases the dry matter accumulation in sole cropping of



<span id="page-7-0"></span>**Fig. 5** Effect of *Thiobacillus* inoculation treatments on the trend of changes in dry matter accumulation  $(g/m^2)$  in sesame (**a**) and mung bean (**b**) (mean data of two locations)



<span id="page-7-1"></span>Fig. 6 Effect of different sesame (a) and mung bean (b) intercropping treatments on the trend of changes in crop growth rate  $(g/m<sup>2</sup>d)$  (mean data of two locations)

mung bean treatment compared to the intercropping. In the study of the intercropping of maize and beans, Lindiquist and Mortensen [\(1999\)](#page-15-24) stated that the radiation penetration into the canopy decreased in intercropping compared to sole cropping, which resulted in a decrease in dry matter production and accumulation in the beans.

At the beginning of the growth period, due to small shrubs, there was only a little difference between different treatments of sulfur fertilizer and *Thiobacillus* in terms of an increase in total dry weight of sesame and mung bean. However, within 40 days from germination, total dry matter accumulation started to grow linearly, and after that, it had a decreasing trend in sesame and a consistent trend in mung bean. In both sesame and mung bean, the highest dry matter accumulation was observed in sulfur fertilizer treatment  $S<sub>2</sub>$  and the lowest in non-sulfur treatment. The difference between the two treatments at 85 days after planting (maximum dry matter accumulation) was 13.14% in sesame and 11.74% in mung bean (Fig. [4\)](#page-6-1).

Also, the highest total dry matter accumulation was obtained in bacterial inoculation treatment, and the lowest in non-inoculated treatment in both sesame and mung bean. The difference between the inoculation and non-inoculation treatments was 3.95% in sesame and 4.11% in mung bean, which was obtained after 85 days (maximum dry matter accumulation) (Fig. [5\)](#page-7-0).

Sulfur fertilizer and *Thiobacillus* bacteria increase sesame and mung bean leaf area, photosynthesis, and

<span id="page-8-0"></span>**Table 5** Results of analysis of variance (mean squares) of sesame and mung bean crop growth rate during the growth period

Source	DF	CGR of Sesame				CGR of Mung bean						
		Days after planting					Days after planting					
		40	55	70	85	100	40	55	70	85	100	
Location $(L)$	1	$0.36^{**}$	0.97	105.5	$173*$	18.09	0.003	0.0016	0.00004	8.18	0.297	
Error <sub>1</sub>	$\overline{4}$	0.005	0.2	20.45	17.35	21.23	0.001	0.0075	0.077	5.99	5.87	
Intercropping (I)	3	$0.013*$	$56.81***$	$196***$	$229***$	63.06**	$0.009***$	$12.05***$	$170^{**}$	$37.61***$	$1.68***$	
Sulfur $(S)$	$\overline{2}$	$2.75***$	0.47	$157***$	$10.4***$	$44.57***$	$1.11***$	$0.079**$	$11.53***$	$4.51***$	$2.9***$	
Thiobacillus (T)	1	$0.38***$	0.053	$11.97***$	0.66	$6.56***$	$0.117***$	0.004	$1.51***$	0.012	0.078	
$S \times I$	6	0.0009	0.107	1.2	1.83	0.75	0.002	$0.018*$	0.11	0.026	0.212	
$I \times T$	3	0.0017	0.071	0.11	0.34	0.21	0.001	0.01	0.37	0.025	0.201	
$T \times S$	2	$0.024***$	0.019	1.45	0.75	0.25	$0.018***$	0.0002	0.017	0.67	0.248	
$T \times S \times I$	6	0.0016	0.103	0.66	0.91	0.67	0.001	0.012	0.033	0.16	0.354	
$I \times L$	3	$0.06***$	0.162	$3.72*$	$6.27*$	$5.45***$	0.003	$0.042**$	0.013	0.021	0.192	
$S \times L$	$\overline{c}$	0.005	0.062	1.38	3.37	$9.89**$	0.0002	0.001	0.013	$3.28***$	$2.162*$	
T×L		0.0006	0.064	0.044	0.48	1.08	0.0002	0.002	0.03	0.473	0.344	
$S \times I \times L$	6	0.0038	0.045	1.59	2.82	0.29	0.001	0.006	0.01	0.337	0.283	
$T \times I \times L$	3	0.002	0.059	0.409	0.22	0.17	0.0005	0.007	0.04	0.041	0.146	
$T \times S \times L$	$\overline{c}$	0.00089	0.069	0.279	0.5	0.2	0.00001	0.003	0.02	0.123	0.167	
$T \times S \times I \times L$	6	0.0013	0.063	0.134	0.68	0.58	0.001	0.011	0.017	0.12	0.358	
Error <sub>2</sub>	92	0.0047	0.074	1.072	1.83	0.67	0.0013	0.0076	0.31	0.46	0.47	

ultimately, vegetative growth, dry weight, and biological yield. In the study of growth indices of canola cultivars at different levels of sulfur fertilizer, Fims et al. [\(2000\)](#page-15-18) attributed the increase in total biomass and growth rate of canola crop to expansion in leaf size and area and increased photosynthesis due to the use of sulfur.

The interaction effects of cropping ratio and location were significant on dry matter accumulation of sesame.

#### **Crop Growth Rate (CGR)**

The crop growth rate was low at the beginning of the growing season. The slow crop growth at this stage is due to the imperfect canopy and low light absorption (Kobata and Moriwaki [1990\)](#page-15-25). The highest growth rate of sesame was observed in 75% sesame +25% mung bean intercropping on day 70 after germination (maximum growth rate), and the lowest rate was observed in 25% sesame +75% mung bean intercropping. The rate was negative at the end of the growth season (Fig. [6\)](#page-7-1). It seems that by reducing the intraspecies competition and creating a wave canopy cover in the intercrops, more light would reach the lower leaves of sesame and also nitrogen fixation for sesame by the root of mung bean has increased the crop growth rate in this treatment compared to the sole crop. Of course, the plant may produce more aerial parts to attract more light in the competition. In the investigation of soybean and cotyledon intercropping, Ghosh et al. [\(2006\)](#page-15-26) showed that the growth rate was higher in both crops in the intercropping.

The growth rate of mung bean was relatively uniform in all treatments except in 50% sesame +50% mung bean. The highest crop growth rate in the mung bean was observed in the sole cropping on day 70 after germination (9.42), and the lowest was observed on the same day  $(4.58)$  (Fig. [6\)](#page-7-1). This seems to be due to the reduced inter-species competition between mung bean and sesame, as the mung bean is shaded by sesame in the intercropping, but does not face this problem in sole cropping (Table [5\)](#page-8-0).

Growth rates of sesame and mung bean were also statistically affected by sulfur fertilizer throughout the growing period. The highest growth rate of sesame was obtained from fertilizer  $S_2$  treatment on day 70 after germination (maximum growth rate) and the lowest in the control treatment (no sulfur) on the same day. The difference between these two treatments was 25.8%. Also, the highest growth rate of mung bean was obtained from 100% sulfur fertilizer treatment  $S_2$  on the day 70 after germination and the lowest due to no use of sulfur fertilizer on the same day. The difference between the two treatments in this index was 13.04% (Fig. [7\)](#page-9-0). Since leaves are the main factor in photosynthesis and increase of plant dry matter per unit area, it can be concluded that treatment with a higher leaf area index will have a higher growth rate. On the other hand, sulfur fertilizer reduces soil acidity and dissolves insoluble nutrients and releases essential nutrients to increase crop growth rate (Adhikary and Pandey [2007\)](#page-15-27).

Also, the growth rate of sesame and mung bean was statistically affected by inoculation with *Thiobacillus* only



<span id="page-9-0"></span>Fig. 7 Effect of different sesame (a) and mung bean (b) sulfur fertilizer treatments on the trend of changes in crop growth rate  $(g/m<sup>2</sup>d)$  (mean data of two locations)



<span id="page-9-1"></span>**Fig. 8** Effect of different sesame and mung bean *Thiobacillus* treatments on the trend of changes in crop growth rate (g/m2d) (mean data of two locations)

on day 70 after germination. The highest growth rate of sesame on day 70 after germination was related to bacterial inoculation, which was 8.52% higher than non-inoculated treatment. In the case of mung bean, the growth rate in inoculation with *Thiobacillus* was higher than non-inoculation, with a difference of 8.42% (Fig. [8\)](#page-9-1).

The interaction of cropping ratio and location appeared to be significant on the growth rate of sesame.

## **Net Assimilation Rate (NAR)**

The net assimilation rate represents the net amount of dry matter produced per unit area of leaf per unit time. The net assimilation rate is initially decreasing in most crops, reaching its maximum when the leaves are exposed to full sunlight. With increasing growth, the leaves are expanded, and upper leaves shade on the lower ones. The higher the shading, the more reduced the net assimilation rate. With increasing age in the leaf, photosynthesis also decreases, which in turn steepens the gradient of the net assimilation rate. However, in sugar beet, lettuce and similar plants, the trend is still increasing for some time and then decreases. This is because, at the beginning of the growing season, these plants have small leaves and are closed, so they are unable to utilize the full sun light. Also, after a while, the insider leaves are not capable of photosynthesis, which reduces the net assimilation rate in these plants (Eddowes [1969\)](#page-15-28).

As shown in Fig. [9,](#page-10-0) the trend of changes in the net assimilation rate in the intercropping is the same. In the sesame,



<span id="page-10-0"></span>**Fig. 9** Effect of different sesame (**a**) and mung bean (**b**) treatments on the trend of changes in net assimilation rate  $(g/m^2d)$  (mean data of two locations)

<span id="page-10-1"></span>**Table 6** Results of analysis of variance (mean squares) of net assimilation rates of sesame and mung bean during the growth period

Source	DF	NAR of Sesame				NAR of Mung bean						
		Days after planting					Days after planting					
		40	55	70	85	100	40	55	70	85	100	
Location $(L)$	1	0.001	0.33	16.33	$19.93*$	8.594	0.051	0.092	0.006	0.917	0.082	
Error <sub>1</sub>	4	0.107	0.136	2.75	1.813	5.58	0.035	0.048	0.018	0.666	1.877	
Intercropping (I)	3	$0.249**$	$14.38***$	$13.13***$	$11.43***$	$11.47**$	$0.14***$	$1.12***$	34.93**	$9.39**$	0.312	
Sulfur $(S)$	2	$1.11***$	$1.28***$	$11.31***$	$3.55***$	$8.41***$	$1.223***$	$2.74***$	$0.38***$	0.053	$0.673**$	
Thiobacillus (T)	1	$0.44***$	0.048	$0.843***$	0.229	$1.373*$	0.016	$0.415***$	$0.12***$	0.049	0.022	
$S \times I$	6	0.029	0.062	$0.37***$	0.199	0.364	0.04	$0.08***$	$0.068***$	0.003	0.048	
$I \times T$	3	0.039	0.043	0.017	0.051	0.152	0.039	0.018	0.014	0.027	0.053	
$T \times S$	$\overline{2}$	0.106	0.0007	0.169	0.113	0.036	0.087	0.036	0.031	0.026	0.089	
$T \times S \times I$	6	0.035	0.052	0.09	0.102	0.197	0.031	0.031	0.011	0.02	0.108	
$I \times L$	3	0.089	0.063	$0.671***$	$1.037**$	$1.273***$	0.024	$0.118***$	$0.03*$	0.007	0.056	
$S \times L$	$\overline{2}$	0.062	0.028	0.095	0.339	$2.472**$	0.033	0.001	0.003	$0.477***$	$0.671**$	
$T \times L$		0.133	0.052	0.021	0.044	0.213	0.027	0.004	0.006	0.058	0.085	
$S \times I \times L$	6	0.111	0.029	0.163	0.323	0.113	0.016	0.022	0.005	0.042	0.094	
$T \times I \times L$	3	0.063	0.046	0.029	0.032	0.034	0.013	0.008	0.024	0.003	0.037	
$T \times S \times L$	$\overline{2}$	0.008	0.02	0.05	0.052	0.049	0.0001	0.018	0.013	0.014	0.055	
$T \times S \times I \times L$	6	0.0035	0.043	0.01	0.094	0.022	0.024	0.026	0.007	0.01	0.112	
Error <sub>2</sub>	92	0.056	0.038	0.105	0.188	0.209	0.035	0.026	0.011	0.059	0.141	

the net assimilation rate first declined. Then, it increased from day 55 after germination to day 70, and then the slope of the absorption rate became negative. Meanwhile, the best treatment was 75% mung bean +25% sesame, which had the highest net assimilation rate during the whole growing season, probably because in the intercropping, the sunlight permeated the entire canopy cover, and the maximum rate of photosynthesis was observed in all canopy layers. However, on the day 100 after germination, this index was more affected than other treatments due to leaf aging and higher shading of the upper leaves on the lower ones. The highest net assimilation rate was related to the 75% sesame +25% mung bean treatment with 4.86  $(g/m<sup>2</sup>d)$  on day 70, and the lowest rate was obtained on the same day with  $3.26 \, (g/m^2d)$ (Fig.  $9$ ; Table  $6$ ).

The net assimilation rate of mung bean in the intercropping increased at the beginning of the growing season until day 55 after germination. It declined in the rest of the growing season. The decreasing trend intensified with the growth of sesame bushes (Fig. [10\)](#page-11-0). The highest value of this index was obtained in mung bean sole cropping with 6.82  $(g/m<sup>2</sup>d)$  on day 55 after germination, and the lowest value



<span id="page-11-0"></span>**Fig. 10** Effect of different treatments of sulfur fertilizer on the trend of changes in net assimilation rate (g/m2d) of sesame (**a**) and mung bean (**b**) (mean data of two locations)

was obtained in 75% mung bean  $+25%$  sesame intercropping with  $6.49 \text{ (g/m}^2d)$ . Of course, there was no statistical difference between the intercropping treatments, and only the sole cropping was statistically superior (Fig. [9\)](#page-10-0).

Lower levels of sulfur had higher net assimilation rates than the higher levels in both plants. The highest net assimilation rate throughout the growing season (except day 70) were obtained in treatment  $S_0$  and then in treatment  $S_1$ (Fig. [10\)](#page-11-0). High levels of sulfur appear to increase secondary branch growth and leaf area and accelerate canopy closure. This increases the shading of leaves and reduces carbon assimilation. Reduced photosynthetic capacity, as well as increased respiration, compared to photosynthesis may be due to approaching the plant's physiological maturity. The highest net assimilation rate in sesame was obtained on day 70 after germination in the treatment  $S_2$  with 4.56 (g/m<sup>2</sup>d), and the lowest was obtained on the same day in the treatment  $S_0$ with  $3.67 \, (\text{g/m}^2d)$ . In the case of mung bean, the highest net assimilation rate was obtained on day 55 after germination in the treatment  $S_0$  with 6.9 (g/m<sup>2</sup>d), and the lowest was obtained on the same day in the treatment  $S_2$  (Fig. [10\)](#page-11-0).

Among interactions, the only interactive effect of cropping ratio and location was significant of sesame except 40 and 55 days after planting.

#### **Grain Yield and Biological Yield**

Analysis of variance (Table [7\)](#page-12-0) for grain yield showed that all main effects for both sesame and mung bean, as well as the interactive effects of intercropping with sulfur, intercropping with *Thiobacillus* and sulfur with *Thiobacillus*, were significant for sesame at 1% level.

The mean comparison test results of interactive effects of cropping ratio with sulfur level showed that the highest grain yield in sesame was from 75% sesame +25% mung bean with  $S_2$  treatment (1147 kg/ha). The lowest in the 50% sesame  $+50\%$  mung bean with S<sub>0</sub> treatment (396.5 kg/ha) was obtained, with a difference of 63.43% (Fig. [11\)](#page-13-0).

Also, the mean comparison of interactive effects of cropping ratio with *Thiobacillus* illustrated that the highest seed yield in sesame was related to 75% sesame +25% mung bean.

Moreover, the result of interactive effects of sulfur level with *Thiobacillus* revealed that the highest grain yield in sesame was from fertilizer  $S_2$  treatment with bacterial inoculation (805 kg/ha). The lowest in the control treatment (no sulfur and non-inoculated) was obtained (593 kg/ha) (Fig. [12\)](#page-13-1).

Mean comparison results (Table [8\)](#page-14-0) showed that sesame had the highest grain yield (988.59 kg/ha) in the 75% sesame +25% mung bean intercropping and the lowest  $(445.65 \text{ kg/ha})$  in the 25% sesame  $+75\%$  mung bean, with the difference of 54.92% between the two cultivations. In the case of mung bean, the highest yield was obtained in its sole cropping (976.25 kg/ha) and the lowest in the 50% sesame +50% mung bean treatment (790.88 kg/ha), with a difference of 18.99%.

Among the sulfur fertilizer treatments, the highest yield of both sesame and mung bean was related to the  $S_2$  treatment (sesame 790.67 and mung bean 951.33 kg/ha), and the lowest was related to the control treatment  $(S_0)$  (sesame 602.82 and mung bean 824.07 kg/ha).

The use of *Thiobacillus* bacteria also led to a 4.54% increase in sesame grain yield and a 3.38% increase in mung bean yield.

Source	DF	Sesame		Mung bean Days after planting							
		Days after planting									
		G Y	B Y	Chl a	Chl b	t chl	G Y	B Y	Chl a	Chl b	t chl
Location (L)		40,334	84,535	0.426	0.0007	$0.46*$	552	283,024**	0.00006	0.0019	0.001
Error <sub>1</sub>	4	6136	101,685	0.005	0.009	0.22	3846	5158	0.001	0.0003	0.001
Intercropping (I)	3	2,176,778**	21,244,284**	$1.129***$	$0.262***$	$2.15***$	292,743**	$2,225,250**$	$0.013***$	$0.018***$	$0.009***$
Sulfur $(S)$	2	423,480**	1,213,329**	$0.494***$	0.0007	$0.52***$	194,407**	743,428**	$0.913***$	$0.01***$	$1.12***$
Thiobacillus (T)	1	37,712**	85,800**	$0.053***$	0.00001	$0.052*$	$14,299**$	$65,110**$	$0.071***$	0.001	$0.093**$
$S \times I$	6	$26,350**$	3991	0.002	0.0006	0.003	545	6087	0.0005	0.0004	0.001
$I \times T$	3	4620**	1499	0.00003	0.0008	0.0007	325	558	0.0018	0.0001	0.003
$T \times S$	$\overline{c}$	2438*	2330	0.003	0.0004	0.003	839	9345	$0.004***$	0.00004	$0.004*$
$T \times S \times I$	6	630	1714	0.001	0.001	0.002	39	572	0.0003	0.0001	0.0005
I×L	3	$22,311***$	91,916**	$0.005*$	0.001	0.003	1000	4864	$0.003***$	0.00009	0.003
$S \times L$	2	$2235*$	$20,486^*$	0.001	0.001	0.0002	1395	3391	0.0004	0.00003	0.0007
T×L		175	788	0.00006	0.003	0.003	256	75	0.0014	0.0002	0.003
$S \times I \times L$	6	1162	7309	0.001	0.001	0.003	682	8373*	0.001	0.00008	0.0015
$T \times I \times L$	3	195	856	0.001	0.0009	0.004	69	185	0.0005	0.0001	0.0008
$T \times S \times L$	2	372	91	0.0002	0.002	0.004	400	204	0.0001	0.0001	0.00004
$T \times S \times I \times L$	6	684	1129	0.001	0.001	0.005	132	1786	0.0003	0.0001	0.00009
Error <sub>2</sub>	92	621	4454	0.001	0.001	0.002	521	3451	0.0008	0.0004	0.0011

<span id="page-12-0"></span>**Table 7** Results of analysis of variance (mean squared) of yield and chlorophyll in sesame and mung bean

*G Y* Grain Yield; *B Y* Biological Yield; *chl a* chlorophyll a; *chl b* chlorophyll b; *t chl* total chlorophyll

The 75% sesame +25% mung bean intercropping treatment showed the highest biological yield in sesame (4173.08 kg/ha), but the highest biomass for the mung bean (3406.14 kg/ha) was obtained in the sole cropping treatment.

The highest biological yield was obtained in sulfur treatment (3478.85 kg/ha) for sesame and the  $S_2$  treatment (3248.92 kg/ha) for mung bean, and the lowest biological yield for both was obtained in the control treatment (Table [8\)](#page-14-0).

The use of *Thiobacillus* bacteria for both plants significantly increased yield, with a 2% difference between *Thiobacillus* use and non-use treatments (Table [8\)](#page-14-0).

Higher sesame yield in intercropping than sole cropping indicates a positive interaction between sesame and mung bean in the intercropping. It also shows that the severity of the negative inter-species relationships is less than the intra-species relationships in the sole cropping. So, in the intercropping, competition in resource allocation seems to be avoided (Tavassoli et al. [2010\)](#page-15-29). This may be due to the positive effect of mung bean through nitrogen fixation and the availability of other elements such as phosphorus on sesame growth (Bhatti et al. [2006\)](#page-15-30). There have been many reports of increased plant performance in the intercropping compared to sole cropping (Zhang et al. [2010\)](#page-16-0).

However, the decrease of the yield of mung bean in the intercropping may be due to a decrease in light absorption, a significant factor in photosynthesis, because of sesame shading. Intercropping the mung bean and sun-flower, Kandhro et al. [\(2007\)](#page-15-31) stated that the mung bean yield decreased due to increased competition for resources, especially light.

Sulfur fertilizer increases photosynthesis and thereby carbohydrates in the plant, which in turn, increases both the grain yield and the biological yield. Sulfur also increases the availability of nutrients and the assimilation of elements, influencing the vegetative and reproductive growth, due to its positive effects. On the other hand, *Thiobacillus* activates beneficial soil microbes and facilitates biological oxidation in the soil, thus increasing the yield (Sangale et al. [1981\)](#page-15-32).

#### **Chlorophyll Pigment**

The results of the analysis of variance in Table [7](#page-12-0) show that the intercropping had a significant effect on chlorophyll a, chlorophyll b and total chlorophyll in both plants, and sulfur fertilizer had a significant effect on chlorophyll a, total chlorophyll on sesame and chlorophyll a, chlorophyll b and total chlorophyll on mung bean. The *Thiobacillus* had the same effect on chlorophyll a and total chlorophyll in both plants.

The mean comparison test results of interactive effects of sulfur level with *Thiobacillus* revealed that the highest chlorophyll a and total chlorophyll in mung bean was from fertilizer  $S_2$  treatment with bacterial inoculation



<span id="page-13-0"></span>**Fig. 11** The interactive effects of cropping ratio and sulfur fertilizer (**a**) and cropping ratio and *Thiobacillus* on grain yield of sesame (kg/ha) (**b**) (mean data of two locations)



<span id="page-13-1"></span>**Fig. 12** The interactive effects of sulfur fertilizer and *Thiobacillus* on grain yield of sesame (kg/ha) (mean data of two locations)

 $(chla = 1.245, tch = 1.58 mg/g)$ , and the lowest in the control treatment (no sulfur and non-inoculated) was obtained  $(chla = 0.93, tch = 1.22 \text{ mg/g})$  (Fig. [13\)](#page-14-1).

Mean comparisons of data showed that the highest chlorophyll-a for the sesame (1.34mg/g) was obtained in the 75% sesame +25% mung bean and for the mung bean  $(1.1 \,\text{mg/g})$  in its sole cropping. Also, the highest chlorophyll b and total chlorophyll for the sesame were obtained in the 75% sesame +25% mung bean and for the mung bean in 50% sesame +50% mung bean (Table [8\)](#page-14-0).

According to the analysis of variance, the effect of sulfur fertilizer on chlorophyll a, chlorophyll b and total chlorophyll of mung bean was statistically significant at 1% level (Table [7\)](#page-12-0). The highest chlorophyll a, chlorophyll b and total chlorophyll for the mung bean were obtained in sulfur treatment  $S_2$ , and the lowest chlorophyll indices were obtained in the control treatment; and the difference between the two treatments was 22.13%, 9.38% and 20% for the chlorophyll a, chlorophyll b and total chlorophyll in mung bean. However, in sesame, only chlorophyll a and total chlorophyll were affected statistically. Mean comparisons showed that the highest amount of chlorophyll a  $(1.25 \text{ mg/g})$  and total chlorophyll (1.96mg/g) were obtained from treatment  $S_2$  (Table [8\)](#page-14-0).

The effects of *Thiobacillus* on chlorophyll a and total chlorophyll of both plants was also statistically significant. In both plants, *Thiobacillus* led to a superior statistical group of chlorophyll a and total chlorophyll (Table [8\)](#page-14-0).

In investigating the soybean and sorghum intercropping, Ghosh et al. [\(2006\)](#page-15-26) stated that the chlorophyll content of sorghum leaf was higher in all cropping compounds compared to its sole cropping. They attributed this to the shading of two plants on each other relative to the sole cropping treatment. That is because, under shading, the crops increase their chlorophyll content in order to trap more light to produce a photoassimilate. Another reason mentioned has been fixation and accessibility of nitrogen (due to its role in the production of chlorophyll) by the soybean as a species in the legume family. This is consistent with the result of Tsubo et al. [\(2005\)](#page-15-33).

Wang et al. [\(2003\)](#page-15-34) showed that using sulfur leads to an increase in photosynthetic pigments in alfalfa. According to Marschner [\(1995\)](#page-15-35), most of these compounds have a nitrogen structure, and since sulfur increases nitrogen efficiency as well as increased absorption capacity of other elements in plants, its use partially increases the chlorophyll content in plants. Ravi et al. [\(2010\)](#page-15-36) indicated that the increase in dry matter production with the use of sulfur in safflower is a result of increased root growth and chlorophyll formation that led to increased photosynthesis.

The study of Shinde et al. [\(2004\)](#page-15-37) showed that that the addition of sulfur-oxidizing bacteria leads to a rapid increase in root growth and development as well as an increase in

<span id="page-14-0"></span>

Treatment	Sesame					Mung bean					
	G Y	B Y	Chl a	Chl b	t chl	G Y	B Y	Chl a	Chl b	t chl	
Cropping mix											
Sole sesame	803.2b	3763.33b	1.25 <sub>b</sub>	0.66bc	1.91b	$\overline{\phantom{0}}$					
$S75\% + M25\%$	988.59a	4173.08a	1.34a	0.84a	2.18a	833.98c	2979.78c	1.07b	0.3 <sub>b</sub>	1.37b	
$S50\% + M50\%$	548.95c	2720.50c	1.03c	0.66bc	1.69c	790.88d	2912.64d	1.06b	0.34a	1.41a	
$S25\% + M75\%$	445.65d	2622.39d	0.97d	0.68 <sub>b</sub>	1.65d	953.25b	3336.61b	1.09a	0.3 <sub>b</sub>	1.39a	
Sole mung bean	$\overline{\phantom{0}}$				-	976.25a	3406.14a	1.1a	0.3 <sub>b</sub>	1.40a	
Sulfur fertilizer											
S <sub>0</sub>	602.82c	3160.88c	1.05c	0.7a	1.75c	824.07c	3038.63c	0.95c	0.29c	1.24c	
S <sub>1</sub>	696.3b	3319.19b	1.15b	0.7a	1.86b	890.39b	3150.63b	1.08b	0.31 <sub>b</sub>	1.39b	
S <sub>2</sub>	790.67a	3478.85a	1.25a	0.7a	1.96a	951.31a	3287.13a	1.22a	0.32a	1.55a	
Thiobacillus											
T <sub>0</sub>	680.41b	3295.42b	1.13 <sub>b</sub>	0.7a	1.84b	878.63b	3137.53b	1.06b	0.3a	1.37 <sub>b</sub>	
$T_1$	712.78a	3344.24a	1.17a	0.7a	1.88a	898.56a	3180.06a	1.1a	0.3a	1.41a	

**Table 8** Comparison of the mean of main effects of intercropping, sulfur fertilizer, and *Thiobacillus* on yield and chlorophyll pigment (mean data of two locations)

The means with the same letter in the columns are not significantly different

*G Y* Grain Yield; *B Y* Biological Yield; *chl a* chlorophyll a; *chl b* chlorophyll b; *t chl* total chlorophyll



<span id="page-14-1"></span>**Fig. 13** The interactive effects of sulfur fertilizer and *Thiobacillus* on chlorophyll a and total chlorophyll of mung bean (mg/g) (mean data of two locations)

the protein content of the grain. Biofertilizers can also increase the protein content; because they not only provide the proper conditions for growth and development of the plant but also perform nitrogen fixation, which increases the plant potency for nitrogen assimilation.

# **Conclusion**

In this experiment, the 75% sesame/25% mung bean intercropping treatment showed statistically superior effects on physiological parameters such as leaf area index, total dry matter accumulation, crop growth rate and net assimilation rate compared to other intercropping and sole cropping treatments. However, in the case of mung bean, its sole cropping was superior to all other treatments in all indices. Therefore, the intercropping of mung bean and sesame had a significant positive effect on growth indices in sesame. Because light absorption is a significant factor in growth and photosynthesis, to achieve maximum efficiency in the intercropping despite the many benefits of this cropping pattern it is essential to select the appropriate plants.

The results also showed that sulfur fertilizer increased growth and growth indices in both mung bean and sesame. Also, *Thiobacillus* increased the most growth indices. Due to the high level of lime in the soils of the country, and low cost of sulfur in Iran, it is recommended to use sulfur fertilizer to reduce soil pH and *Thiobacillus* bacteria to facilitate access to sulfur resources.

**Conflict of interest** A. Gilani, H. Abbasdokht and A. Gholami declare that they have no competing interests.

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