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ORIGINAL ARTICLE

The Effect of Mineral Sulfur as a Safe Compound on Verticillium Wilt of Pistachio Seedlings under Greenhouse Condition

Masoumeh Haghdel[*1], Amir Hossein Mohammadi[1], Bahman Panahi[1], Mehdi Mohammadi-Moghadam[2]

[1]*Pistachio Research Center, Horticultural Sciences Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Rafsanjan, Iran*

[2]*Crop and Horticultural Sciences Research Department, Semnan Agricultural and Natural Resources Research and Education Center (Shahrood), AREEO, Shahrood, Iran*

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KEYWORDS

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Soil-borne disease;
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ABSTRACT: Verticillium wilt is one of the soil-borne diseases in pistachio orchards that can cause great damage to pistachio trees. The use of safe and low risk fungicide such as sulfur along with other control methods can be effective in reducing of pests and diseases damage in pistachio orchards. In this research, the effect of three concentrations of mineral sulfur (2%,4% and 8%) was evaluated on the disease severity of Verticillium wilt of pistachio seedlings (cv. Ahmad Aghaei) before and after inoculation with *Verticillium dahliae* under greenhouse conditions in Rafsanjan. The experiment was conducted as a factorial in a completely randomized design with five replications. The results showed that concentrations of 4 and 8% of sulfur in the absence of pathogens increased the growth characteristics, chlorophyll concentration and nutrients levels in the aerial parts of pistachio seedlings. In treatment of sulfur 8% before the pathogen inoculation, all the measured traits showed significant increase compared to the pathogen treatment (Vd). The colonization percentage of the pathogen in the stem of seedlings decreased significantly from 97% in treatment of pathogen to 82.4% in treatment of sulfur 8% before pathogen. Also, treatment of mineral sulfur 8% before pathogen with a decrease of 19.3% showed the lowest severity index of Verticillium wilt of pistachio seedlings compared to pathogen treatment. overall, the results of this research demonstrate that applying mineral sulfur 8% before pathogen can improve the growth, biochemical and nutritional characteristics of pistachio seedlings and reduce the severity of Verticillium wilt of pistachio seedlings in greenhouse conditions.

INTRODUCTION

Pistachios are one of the most important horticultural and dried fruit crops in Iran, and they are significant among non-oil exports. The cultivation area of pistachios is expanding due to their high nutritional value and adaptability to adverse environmental conditions, such as water salinity, poor soil quality, and drought Fungal pathogens, especially soil-borne fungi, significantly limit pistachio production in Iran and worldwide, causing to severe damage to pistachio trees [1]. Verticillium wilt is one of the important diseases of pistachio trees and has been

*Corresponding author: m-haghdel@pri.ir (M. Haghdel)
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reported in Greece, the United States, and Iran [2- 5]. This disease, blocks the xylem vessels, causing drying of branches, wilting, and death of seedlings and mature trees [5].

Managing verticillium wilt is challenging and costly due to several factors including the lack of access to the pathogen at the time of host infection, the long-term survival of the fungus in the soil, the wide host range, the lack of resistance sources in the germplasm of different hosts, and the few fungicides effective against the pathogen [6-8]. Sulfur compounds in various formulations are the most effective fungicides approved by the National Organic Program standards (NOPS) [9]. Sulfur is an essential elements for normal plant growth, and its concentration in plants is lower than nitrogen but similar to phosphorus. Sulfur has a critical role in many plants metabolic processes as natural metabolism in plants is dependent on sulfur, and deficiency of this nutrient leads to important metabolic abnormalities in plants and drops the quantity and quality of the product [10].

Numerous studies have shown that in pepper plants, defense mechanisms, including increased phenolic compounds and lignins, along with sulfur production, have a role in controlling the disease caused by *V. dahliae*. Sulfur seems to be involved in a multifaceted reaction that inhibits both the lateral and the vertical spread of the pathogen through physical and chemical barriers [11-12].Among the known phytoalexins, elemental sulfur is one of the simplest, playing a role in defense reactions against Verticillium wilt and is produced in sufficient quantities to prevent the spread of the pathogen in resistant varieties of cocoa and tomato [13- 14]. The sulfur biosynthesis origin seen in the vascular tissues of cocoa trees infected with *V. dahliae* is unknown, but mineral sulfate is converted into organic sulfur compounds such as amino acids after absorption by plants [14]. It has been reported that sulfur accumulation is faster and greater in the vascular tissues of tomato cultivars resistant to *V. dahliae* than in susceptible cultivars [15]. In resistant tomato genotype, a 25 mM concentration of sulfur reduced the infection rate of *V. dahliae* and significantly increased both the rate of photosynthesis and glutathione concentration, regardless of the pathogen's presence or absence [16].

Also, in the pepper cultivar with greater resistance to *V. dahliae*, the accumulation of elemental sulfur was seen 10 days after pathogen inoculation and peaked after 21 days, while in the cultivar with lower resistance, sulfur accumulation was observed 15 days after pathogen inoculation and elemental sulfur was undetectable in the control plants [17]. The main goal in the management of monocyclic pathogens such as Verticillium is to reduce the initial inoculum of the pathogen in the soil, and the soil application of safe and environmentally friendly compounds, such as sulfur compounds, is greatly important. The application of safe and environmentally friendly compounds, such as sulfur, is crucial for this purpose. Therefore, this study investigated the effect of three concentrations of mineral sulfur (2%, 4%, and 8%) on mitigating the severity of Verticillium wilt in pistachio seedlings of the Ahmad Aghaei cultivar.

MATERIALS AND METHODS

Pathogen isolate and inoculum production

In this study, an isolate of *V. dahliae* collected from pistachio trees was used. This isolate has been registered with the code IRAN 2432C in the Iranian Fungal Culture Collection and with the accession number OL361776 in the GeneBank at NCBI. Inoculum production of the pathogen was done based on the method of Huang [18] with slight modifications. In this method, first, a mixture of washed sand-oat flour-distilled water (190 g, 10 g, and 30 ml, respectively) was poured into 500 ml Erlenmeyer flasks, sterilized for 40 minutes at a temperature of 121ºC and 15 psi. Eight discs (6 mm in diameter) from the edge of nine-day-old *V. dahliae* colonies on malt extract agar (MEA) were added to each Erlenmeyer flask and kept at 25°C for 45 days to complete colonization of the medium.

Planting pistachio seeds, sulfur treatments, and inoculation of verticillium dahliae

Pistachio seeds of the Ahmad Aghaei cultivar, susceptible to *V. dahlia* [19],were disinfected in a 10% sodium with tap hypochlorite solution for 10 minutes, washed three times with tap water and soaked in distilled water overnight. A planting substrate containing virgin soil(loamy)-sand (1:2 w/w) was used for planting pistachio seeds. Mineral sulfur (Table 1) treatments at three concentrations of 2%, 4% and 8% (w/w) were applied before and after inoculation with *V. dahliae*.

Table 1. Properties of mineral sulfur.

Description	OM	Ca	S.%	SO_4%	S(Total)%	Solubility%
Mineral Sulfur	15.5	3.1	14.5	8.2	22.7	3.5

1-Mineral sulfur treatment before inoculation with verticillium dahliae (sulfur+vd)

Application sulfur before *Verticillium dahliae* inoculations ,the planting substrate was mixed with concentrations of 2%, 4%, and 8% (w/w) sulfur, and then 6 to 8 pistachio seeds were sown in 2 kg pots containing 1.5 kg of planting substrate. After two weeks, in each pot, four uniform seedlings were kept, and the other seedlings were eliminated. The pots were kept in a greenhouse for 3 months and irrigated using tap water at the field capacity. After three months, a 3 to 4 cm layer of planting substrate mixed with 2%, 4%, and 8% (w/w) mineral sulfur was placed at the bottom of the 4 kg pots. On top of this layer, 100 g of the pathogen inoculum (8% w/w) was added [18]. Then, three-month-old seedlings with the soil around their roots were transferred to 4 kg pots.

2-Mineral sulfur treatment after inoculation of verticillium dahliae (vd+sulfur)

In the post-inoculation mineral sulfur treatment, 6 to 8 pistachio seeds were planted in 4 kg pots containing 3 kg of planting substrate. After two weeks, the number of seedlings in each pot was reduced to four seedlings. After three months, the seedlings and the soil around the roots were removed from the pots. A 3 to 4 cm layer of planting substrate containing 8% (w/w) *V. dahliae* inoculum was poured into the bottom of the pots, then the seedlings were returned to the pots with the soil around their roots and kept in this condition for 3 weeks. Then, concentrations of 2%, 4%, and 8 % (w/w) of mineral sulfur were added to the pots with irrigation water. The greenhouse temperature was set at 25 ± 3 ºC and the light level was 6000 lux during the experiment. The experiment was carried out as a factorial in a completely randomized design with five replications. Data was analyzed by SAS software and means were compared using Duncan's test at 5 % significance level.

Assessing the efficacy of mineral sulfur on verticillium dahliae

1-Harvesting, growth and biochemical characteristics

After observing disease symptoms (wilting and yellowing) in seedlings inoculated with the pathogen, the plants were harvested. The stem height of the seedlings was measured using a ruler, and the leaf area of the pistachio seedlings in each pot was measured using an ADC-VD100 (England) model leaf area scanner. Furthermore, diameter of the stem was measured at three parts (the lower, middle, and upper areas) of the stems using a caliper, then their average was used. The concentrations of chlorophyll *a*, chlorophyll *b*, and total chlorophyll were calculated using the method described by Lichtenthaler [20] by measuring the light absorption of fresh pistachio leaf extract at wavelengths of 647 and 663 nm and employing the following formulas:

$$\text{Chlorophyll a} = (12.25\times A_{66}) - (2.79\times A_{647})$$

$$\text{Chlorophyll } b = (21.5\times A_{647}) - (5.1\times A_{663})$$

$$\text{Chlorophyll total} = (7.15\times A_{663}) - (18.71 \times A_{647})$$

Also, the chlorophyll index was measured using a chlorophyll meter (SPAD-502+) by selecting 20 leaflets from the lower, middle, and upper leaves of the seedlings.

The extract of aerial part was prepared using dry ashing method and digesting with hydrochloric acid (3N). The concentration of nitrogen was measured by the Kjeldahl method [21], phosphorus using the Spectroscopy method and spectrophotometry at a wavelength of 470 nm, and potassium, calcium, magnesium, iron, copper, zinc, and manganese by ICP (PerkinElmer, Optima 7000 DV, USA).

2-Disease severity index and stem colonization

The disease severity index was evaluated using the method described by Morgan [22] with some modifications as follows:

0: No disease symptoms
1:Minor wilting (less than 20% of leaves), mild yellowing of upper leaves
2: Moderate wilting (up to 50% of leaves), necrosis of lower leaves, and severe yellowing of upper leaves
3: Severe wilting (more than 50% of leaves), necrosis and shedding of upper and lower leaves
4: plant death

To determine the percentage of *V. dahliae* colonization, 50 pieces (each piece 5 mm) were randomly collected from the lower, middle, and upper parts of the stems and after disinfection in a 10% sodium hypochlorite solution for a minute and washing three times with sterile distilled water, they were cultured on PDA culture medium containing 50 mg L^{-1} rifampin. The percentage of pathogen colonization in the stem was calculated by counting the infected pieces.

Statistical analysis

The experiment was conducted as a factorial arrangement within a completely randomized design with five replications. The factors included the timing of pathogen inoculation (before and after the addition of sulfur concentrations), sulfur concentrations (0, 2, 4, and 8%), and pathogen presence (with and without pathogen). Data analysis was performed using SAS software, and mean comparisons were carried out using Duncan's test at the 5% significance level.

RESULTS

The effect of mineral sulfur and pathogen treatments on growth characteristics of pistachio seedlings

The results showed that the pathogen treatment (Vd) led to the dry weight of aerial parts of pistachio seedlings decreasing by 39.6% compared to the control. However, aerial dry weight in the 4% and 8% sulfur treatments increased by 12.6% and 21% compared to the control, respectively. The dry weight of aerial parts of seedlings in the sulfur treatments applied before the pathogen inoculation (sulfur+Vd) was higher than that in the pathogen treatment (Vd) (Table 2).

The root dry weight in the sulfur 8% treatment showed an increase of 42.8%, in the pathogen treatment (Vd), and a decrease of 28.9% compared to the control (Table 2). Among the sulfur treatments applied before and after the pathogen inoculation (Vd), only the treatment of sulfur 8%+Vd significantly increased root dry weight of the seedlings compared to the pathogen treatment (Vd) (Table 2). The application of sulfur 8% increase in the height of the seedlings by 16.1% and the leaf area by 13.2% compared to the control. Whereas in the pathogen treatment (Vd), a decrease of 19.2% in the height and 20% in the leaf area of the seedlings, compared to the control (Table 2). In the treatments of sulfur before and after the pathogen (Vd), only the treatment of sulfur 8% +Vd raised the height and leaf area of the pistachio seedlings compared to the pathogen.

Table 2. The effect of different concentrations of mineral sulfur before and after inoculation with *verticillium dahliae* (vd) on the growth characteristics of pistachio seedlings

	Treatment	Shoot dry weight (g pot^{-1})	Root dry weight (g pot^{-1})	Leaf area (cm^2 pot^{-1})	Stem height cm
	Control	6.09 c[ĸ]	1.91 bc	120.6 cd	26.7 bc
Sulfur without Pathogen	**sulfur 2%**	6.43 bc	2.04 b	123.4 bc	27.4 bc
	sulfur 4%	6.86 b	2.19 b	129 b	28.2 b
	sulfur 8%	7.37 a	2.73 a	136.6 a	31 a
Pathogen	***Verticillium dahliae* (Vd)**	3.68 f	1.36 d	95.8 ef	21.6 def
Sulfur before pathogen	**sulfur 2%+ Vd**	3.72 ef	1.4 d	99 ef	22.4 de
	sulfur 4%+ Vd	4.21 e	1.58 cd	102.3 e	23 d
	Sulfur 8%+Vd	5.13 d	2 b	115.7 d	25.6 c
sulfur after pathogen	**Vd + sulfur 2%**	3.58 f	1.39 d	92 ef	20.3 f
	Vd + sulfur 4%	3.88 ef	1.42 d	94.2 f	20.9 ef
	Vd + Sulfur 8%	3.92 ef	1.54 d	98.4 f	22 def

[ĸ]In each column means with the same letter are not significantly different according to Duncan's multiple range test ($P < 0.05$)

The effect of mineral sulfur and pathogen treatments on the concentration of chlorophylls a, b, a+b, and chlorophyll index of pistachio seedlings

The treatment of 8% sulfur enhanced the concentration of chlorophylls *a*, *b*, *a+b*, and chlorophyll index of pistachio seedlings compared to all treatments. The increase in the concentration of chlorophylls *a*, *b*, and *a+b* and the chlorophyll index in this treatment was 45.14%, 46.09%, 45.57% and 14.1% compared to the control, respectively. However, the pathogen treatment (Vd) significantly decreased above criteria compared to the control (Table 3). Additionally, in the treatment of sulfur 8%+Vd, reduction of chlorophyll concentration was not considerably different from the control (Table 3).

Table 3. The effect of different concentrations of mineral sulfur before and after inoculation with *verticillium dahliae* (vd) on the concentration of chlorophylls *a*, *b*, *a+b* and the chlorophyll index of pistachio seedlings

	Treatment	Chlorophyll *a* (mg g^{-1} FW)	Chlorophyll *b* (mg g^{-1} FW)	Chlorophyll *a+b* (mg g^{-1} FW)	Chlorophyll index
	Control	1.9 c[ĸ]	1.6 c	3.54 c	60.2 cd
Sulfur without Pathogen	**sulfur 2%**	2.1 bc	1.66 bc	3.75 bc	61.4 bc
	sulfur 4%	2.4 b	1.88 b	4.33 b	63.8 b
	sulfur 8%	2.8 a	2.36 a	5.15 a	68.6 a
Pathogen	***Verticillium dahlia* (Vd)**	0.95 de	0.66 d	1.61 de	46.3 f
Sulfur before pathogen	**sulfur 2%+ Vd**	1.1 de	0.72 d	1.75 de	47.6 ef
	sulfur 4%+ Vd	1.34 d	0.8 d	2.14 d	48.9 ef
	Sulfur 8%+Vd	1.9 c	1.4 c	3.18 c	58.6 d
Sulfur after pathogen	**Vd + sulfur 2%**	0.89 e	0.5 d	1.34 de	47.2 ef
	Vd + sulfur 4%	1.1 de	0.57 d	1.49 de	48.0 ef
	Vd + Sulfur 8%	1.2 de	0.69 d	1.83 de	49.1 e

[ĸ]In each column means with the same letter are not significantly different according to Duncan's multiple range test (P< 0 .05)

The Effect of Mineral Sulfur and Pathogen Treatments on the Concentration of Macro-Elements (%) in the Aerial Parts of Pistachio Seedlings

In treatments of mineral sulfur before and after inoculation with the pathogen (Vd), treatment of sulfur 8%+Vd increased the concentration of macro-elements in the aerial parts of pistachio seedlings compared to pathogen treatment (Vd). While the concentration of macro-elemnts in the treatment of pathogen (Vd) was severely dropped compared to the control (Table 4).

Nitrogen and calcium in the treatment of sulfur 8%+Vd decreased by 19.4 and 21.8%, respectively, compared to the control. Whereas the other elements in this treatment did not significantly decrease compared to the control. In this study, the treatment of sulfur 8% caused an enhancement in the essential elements of nitrogen, phosphorus, potassium, calcium, and magnesium compared to the control (Table 4).

Table 4. The effect of different concentrations of mineral sulfur before and after inoculation with *verticillium dahliae* on the concentration of macro-elements (%) in the aerial parts of pistachio seedlings

	Treatment	N (%)	P (%)	K (%)	Ca (%)	Mg (%)
	Control	1.28 b[ĸ]	0.24 bc	1.7 c	2.2 b	0.68 c
Sulfur without Pathogen	**sulfur 2%**	1.31 b	0.25 bc	1.8 bc	2.3 b	0.73 bc
	sulfur 4%	1.34 ab	0.29 b	2 b	2.4 b	0.81 b
	sulfur 8%	1.52 a	0.35 a	2.37 a	2.7 a	1.0 a
Pathogen	***Verticillium dahlia* (Vd)**	0.66 de	0.11 ef	0.98 de	1.4 d	0.44 d
Sulfur before pathogen	**sulfur 2%+ Vd**	0.68 de	0.12 ef	1.1 de	1.4 d	0.48 d
	sulfur 4%+ Vd	0.8 d	0.15 de	1.2 d	1.5 d	0.52 d
	Sulfur 8%+Vd	1.03 c	0.2 cd	1.7 c	1.7 c	0.77 bc
sulfur after pathogen	**Vd + sulfur 2%**	0.56 e	0.07 f	0.89 e	1.3 d	0.42 d
	Vd + sulfur 4%	0.65 de	0.08 f	1.0 de	1.4 d	0.45 d
	Vd + Sulfur 8%	0.72 de	0.08 f	1.1 de	1.5 d	0.45 d

[ĸ]In each column means with the same letter are not significantly different according to Duncan's multiple range test (P< 0.05)

The effect of mineral sulfur and pathogen treatments on the concentration of micro-elements in the aerial parts of pistachio seedlings

The results of this study revealed that the concentration of zinc and manganese in two treatments of sulfur 4 and 8%, and the concentration of iron only in the treatment of sulfur 8% increase compared to the control (Table 5). A significant decrease in the concentration of four micro-elements was observed in the treatment of pathogen (Vd) compared to the control. However, the treatment of sulfur 8%+ Vd led to a considerable increase in the concentration of four micro-elements, While the treatment of Sulfur 4%+Vd only increased in the concentration of zinc in the aerial parts of pistachio seedlings compared to the treatment of pathogen (Vd). In the treatments of sulfur after the pathogen, the concentrations of micro-elements did not differ significantly from the treatment of pathogen (Table 5). None of the treatments of sulfur were able to increase the copper concentration in the aerial parts of pistachio seedlings compared to the control.

Table 5. the effect of different concentrations of mineral sulfur before and after inoculation with *verticillium dahliae* (vd) on the concentration of micro-elements in the aerial parts of pistachio seedlings.

	Treatment	Zn (µg g^{-1})	Fe (µg g^{-1})	Mn (µg g^{-1})	Cu (µg g^{-1})
	Control	13.54 c[ĸ]	73.9 b	25.2 c	5.6 a
Sulfur without Pathogen	**sulfur 2%**	14.34 bc	74.4 b	26.2 bc	5.8 a
	sulfur 4%	16.02 ab	76.5 b	28.7 ab	5.8 a
	sulfur 8%	17.3 a	81.1 a	31.2 a	6.2 a
Pathogen	***Verticillium dahliae* (Vd)**	6.9 f	29.3 d	9.2 ef	2.8 c
Sulfur before pathogen	**sulfur 2%+ Vd**	7.19 f	29.9 d	10 ef	2.9 bc
	sulfur 4%+ Vd	9.08 e	33.1 d	12.4 e	3.2 bc
	Sulfur 8%+Vd	10.8 d	43.9 c	16.8 d	3.5 b
sulfur after pathogen	**Vd + sulfur 2%**	7.02 f	28.3 d	8.9 f	2.9 bc
	Vd + sulfur 4%	8.04 ef	30.4 d	9.4 ef	3.0 bc
	Vd + Sulfur 8%	8.28 ef	30.5 d	9.8 ef	3.3 bc

[ĸ]In each column means with the same letter are not significantly different according to Duncan's multiple range test ($P < 0.05$)

The interaction between mineral sulfur and pathogen on the colonization percentage and severity index of verticillium wilt of pistachio seedlings

The lowest percentage of stem colonization by the pathogen (Vd) was observed in the treatment of sulfur 8% +Vd. In this treatment, a 15.1% reduction in colonization was seen compared to the treatment of pathogen (Vd). However, None of the treatments of mineral sulfur after the pathogen

succeeded in reducing the percentage of colonization of the pathogen in the roots of pistachio seedlings (Figure 1).

The severity index of Verticillium wilt in pistachio seedlings in sulfur 8% +Vd had a significant difference compared to the pathogen (Vd). Also, it led to a 19.3% decrease in this index compared to the pathogen treatment (Figure 2). There was no considerable difference in the severity index of Verticillium wilt in pistachio seedlings between the treatments of different sulfur after the pathogen (Figure 2).

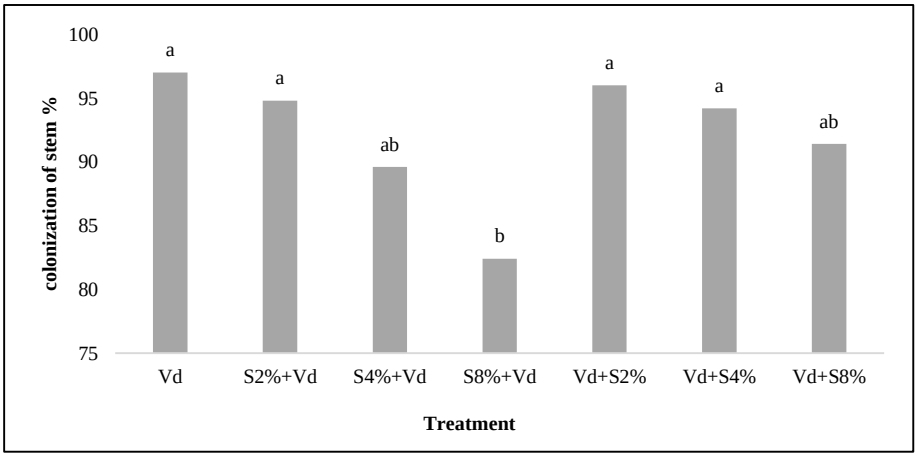
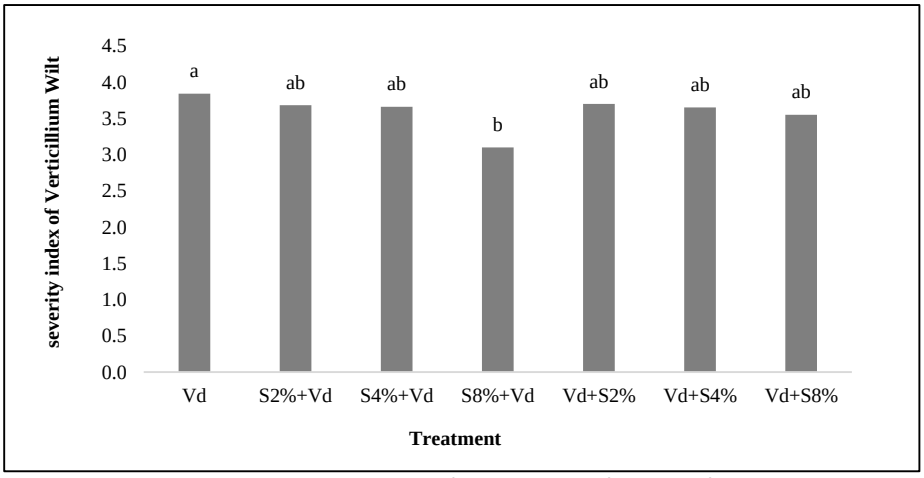


Figure 1. The colonization of stem (%) of pistachio seedlings by *Verticillium dahliae* (Vd) in treatments of mineral sulfur before and after inoculation with the pathogen. Means with the same letter are not significantly different according to Duncan's multiple range test ($P < 0.05$).Vd: *Verticillium dahliae*. S2%, S4% and S8%,



respectively, the concentrations of 2%, 4% and 8% of mineral sulfur.

Figure 2. The severity index of Verticillium wilt of pistachio seedlings in treatments of mineral sulfur before and after inoculation with the pathogen. Means with the same letter are not significantly different according to Duncan's multiple range test ($P < 0.05$).Vd: *Verticillium dahliae*. S2%, S4% and S8%, respectively, the concentrations of 2%, 4% and 8% of mineral sulfur.

DISCUSSION

Verticillium wilt attacks xylem tissues, leading to vascular wilt in plants and causing severe damage to crops [6-7]. Elemental sulfur is known as both a fungicide and a fertilizer [23]. In several farming studies, the application of elemental sulfur resulted better than that of sulfate fertilizers [24], which could be due to the fungicidal role of elemental sulfur with its nutritional role [25]. The results of this research showed that in the absence of pathogens (Vd), concentrations of sulfur 4 and 8% could enhance growth characteristics including the shoot and root dry weight, leaf area, seedling height, chlorophyll concentration and chlorophyll index in comparison with control plants, but sulfur 8%, significantly increased all of the above characteristics. These findingsare consistent with those of [26] highlighting the improvement of date growth indices, [27] and [28] with the positive impact of sulfur on dry weight, grain yield, and vegetative characteristics of wheat. Also, [29] by increasing the fresh weight of flower and the fresh and dry weight of saffron aerial parts, and [30] with a 36 and 114% increase in the shoot dry weight and cucumber yield, resulting from the use of sulfur. Furthermore, using sulfur can increase root growth and chlorophyll concentration and increase the rate of photosynthesis in safflower, leading to increased dry matter production [31]. In addition, nutrition of plants with sulfur significantly raised photosynthesis rates in the both presence and absence of *V. dahliae* [16]. There are several reports show that the use of sulfur, helps increase the concentration of nutrients in various plants. In this study, in treatments of 4 and 8% of sulfur the concentration of macro and micronutrients in the aerial parts of pistachio seedlings, with the exception of copper, increased in the absence of the pathogens. A great increase was reported in the concentration of phosphorus, potassium, manganese, and zinc in the leaves of date palm seedlings treated with sulfur and *Thiobacillus* bacteria and animal manure [26]. In corn, there was a significant increase in the absorption of nitrogen, phosphorus, sulfur, iron, and zinc due to the use of sulfur and *Thiobacillus* bacteria [32]. In contrast, some reports claim that using sulfur has a minor effect on the concentration of elements such as nitrogen, iron [26-33] and zinc [26] in palm leaves. In treatments of sulfur, except for treatment of sulfur 8%+Vd, copper concentration did not show any difference with treatment of pathogen, which is consistent with the findings of [26] regarding the ineffectuality of sulfur on copper concentration in palm tree leaves. In the treatment of sulfur 8%+Vd, all characteristics showed a considerable enhancement compared to the treatment of pathogen (Vd). In other words, the sulfur 8% can mitigate the destructive effects of *V. dahliae* on pistachio seedlings and improve their growth status. These results are consistent with the results of the present study include a great rise in the dry weight of wheat aerial parts in the presence of the pathogen *Gaeumannomyces tritici*, the cause of wheat leaf spot disease, and different concentrations of elemental sulfur with *Thiobacillus* bacteria [27] and a 36 and 114% increase in the dry weight of aerial parts and cucumber yield in the presence of the nematode *Meloidogyne incognita* and different concentrations of elemental sulfur in the soil [30-34].

The treatment sulfur 8%+Vd was able to reduce the stem colonization by *V. dahliae* in the stem of pistachio seedlings by 15.1%. Meanwhile, the severity index of Verticillium wilt of pistachio seedlings also showed a significant reduction in this treatment compared to the pathogen. The lower mortality percentage and also the wilt severity index in the treatment sulfur 8%+Vd compared to the other treatments indicate that using mineral sulfur 8% at the appropriate time positively helps the reduction of mortality percentage and the severity index of Verticillium wilt of pistachio seedlings. Sulfur seems to be a part of a multifaceted reaction avoiding the lateral and vertical spread of the pathogen through

physical and chemical barriers [11-12] . Sulfur accumulates in vascular tissues, and the highest amount of sulfur accumulation is in the parenchymal cells of xylem vessels, vessel walls, vascular gel and tylose, and other structures that are in contact with *V. dahliae*. Also, they associate the defense responses against the pathogen [15].

CONCLUSIONS

The findings of study demonstrated that the use of sulfur, especially at a concentration of 8% before *V. dahliae*, can decrease the percentage of pathogen colonization in the root and the severity index of Verticillium wilt as well as improve the vegetative the characteristics of pistachio seedlings. Since mineral sulfur compounds are extracted from the mine, the amount of elemental sulfur and total sulfur may differ in various parts of the mine, possibly changing the impacts of mineral sulfur on disease severity and growth characteristics.

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Conflict of interests

No conflict

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