Medicinal pumpkin responses to Thiobacillus and sulfur under water stress

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Abstract

The present study aims to assess the effect of Thiobacillus and sulfur on morphological and biochemical properties of medicinal pumpkin properties under deficit irrigation based on a split-plot design. For this purpose, water stress was applied as a main factor in three levels (control, no irrigation in flowering stage, no irrigation in fruiting), and Thiobacillus and sulfur fertilizers as sub-plots (250 kg/ha). The results revealed water stress significantly reduced stem diameter, fruit weight, fruit yield, and 1000-seed weight. In contrast, Thiobacillus + sulfur significantly increased fruit weight, fruit yield, 1000-seed weight, seed yield, and oil content. Furthermore, deficit irrigation and fertilizers influenced fatty acid of medicinal pumpkin. The interaction of fertilizer and deficit irrigation was significant on all seed oil compositions, fruit weight and yield as well as seed weight and yield. The fatty acid profile of the oil showed that it is composed primarily of oleic, linoleic, palmitic, stearic, and linolenic acids.

Keywords: *Cucurbita Pepo;* Thiobacillus; sulfur; deficient irrigation; oil composition

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Introduction

Medicinal pumpkin (*Cucurbita pepo* convar. *pepo* var. *styriaca*) known as an eminent medicinal plant in developing countries belongs to the Cucurbitaceae Family. The family is one of the largest families in plant kingdom consisting of the largest number of edible plant species (Saboo et al., 2013). Pumpkin has been traditionally used for its medicinal value in many countries, including Iran, China, Korea, India, Yugoslavia, Argentina, Mexico, and Brazil. It has many uses including antibiotic, antidiabetic, and anti-inflammatory effects, lowering of blood pressure and cholesterol, and as treatment for intestinal parasites (Saboo et al., 2013). The leaves and rind of the pumpkin contain several proteins, named PR-1, PR-2, and PR-5, that have been found to be antibiotic and antifungal (Saboo et al., 2013). It has been reported that proteins extracted from the rind inhibit the growth of several fungi, including *Candida albicans* that cause oral and genital yeast infections in adults and diaper rash in infants. *C. albicans* can become resistant to the antimycotics used to treat the infection, such as fluconazole. The seeds of the pumpkin are best known as a healthy fall snack. Nutritionally, the seeds contain essential fatty acids, potassium, phosphorus, magnesium, iron, and beta-carotene (Zho, 2017). They are also a good source of fiber. The protein in pumpkin seeds, called cucurbitin, is effective in resolving tapeworm infestations (Rezig et al.,

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2012). Moreover, it has been reported that pumpkin seeds can help prevent the most common type of kidney stone by reducing levels of substances that promote stone formation (Rezig et al., 2012).

Drought is considered to be one of the most important environmental stresses limiting plant growth and crop productivity, and it emerges from changes in the world's climate. Drought stress physiologically and morphologically influences plants. Under such conditions, stomatal closing is a common response of plants, which decreases CO2 uptake and increases the accumulation of NADPH (Terzi and Kadioglu, 2006). In that case, oxygen is final electron acceptor instead of limited NADP, which generates superoxide (Zlatev et al., 2006). Through a variety of reactions, superoxide leads to the formation of hydrogen peroxide, hydroxyl radicals, and other reactive oxygen species (ROS), all of which can cause damage in different ways (Sairam et al., 1998). Producing ROS results in lipid peroxidation, protein degradation, and nucleic acid damages. Plants develop defense mechanisms against free oxygen radicals under stress conditions (Asada, 1987). Some of these mechanisms depend on enzymatic cascades and include reactions for the neutralization of harmful effects of these radicals; a number of these mechanisms are related to non-enzymatic ways and substances. Many studies have shown the reduction of growth and yield of different plants under drought stress (Gutbrodt et al., 2011; Chen et al., 2017; Sade and Moshelion, 2017). For example, Zhou et al. (2017) revealed that shoot fresh and dry weight, leaf area and relative water content of tomato significantly decreased under drought as compared to control.

Poor nutrient availability despite high total nutrient content in the soil is an eminent parameter resulting in crop nutrient deficiency as observed in alkaline and calcareous soils. Nutrient deficiencies restrict plant growth and yield (McCray and Rice, 2013). Appropriate use of fertilizers and/or amendments can improve nutrient balance in soil, resulting in increased crop yield and enhanced fertilizer use efficiency. For the proper soil nutrient balance, it is very important to use adequate amount of S in the soil along with nutrients in plans (Scherer, 2001). pH adjustment of soils is a strategy that is used to increase availability of pH-sensitive nutrients. Elemental sulfur application has been recommended to decrease soil pH and consequently improve nutrient availability to crops (Schueneman, 2001). The effectiveness of sulfur to reduce soil pH is dependent on the oxidation of elemental sulfur into sulfate. The rate of oxidation depends upon factors like microbiological populations in soil and environmental conditions including temperature, moisture and soil pH (Jaggi et al. 2005).

Sulfur serves many functions in plants. It is used in the formation of amino acids, proteins, and oils. It is necessary for chlorophyll formation, promotes nodulation in legumes, helps develop and activate certain enzymes and vitamins, and is a structural component of two of the 21 amino acids that form protein. A wide variety of soil microorganisms are capable to oxidize elemental sulfur, but Thiobacillus bacteria are the most important sulfur oxidizing microorganisms in agricultural lands (Tabatabai, 1986) so that application of these bacteria along with sulfur, accelerates the oxidation of elemental sulfur and plants will benefit from its advantages. Many researchers have reported the beneficial effects of Thiobacillus inoculation along with sulfur application (Pathiratna, et al., 1989; Ghani, et al., 1991; Salimpour, et al., 2010; Salimpour, et al., 2012). Also, several studies have confirmed the importance of soil inoculation with S-oxidizing bacteria to improve the S oxidation process in calcareous soils (Besharati and Saleh-Rastin, 1999). Abdou et al. (2011) reported that soils inoculated with Paracoccus versutus has a significant effect on the concentration of SO_4, phosphorus and micronutrients, so they suggested that elemental sulfur is an effective agent for the amendment of sandy calcareous soils, and application of elemental sulfur accompanied with sulfur oxidizing bacteria, phosphorus, and micronutrients are essential for nutrient availability in calcareous soils. In a similar research application of S with Thiobacillus spp. in Iranian calcareous soils significantly increased the availability of phosphorus and the uptake of phosphorus in maize plants grown under greenhouse conditions (Besharati and Saleh-

Rastin, 1999). Hence, the aim of our study was to investigate the effect of Thiobacillus and sulfur fertilizers on morphological and biochemical properties of medicinal pumpkin under deficit irrigation.

Materials and Methods

Site description

The present study was conducted in an experimental field in Saveh, central Iran. The climate of Saveh is known as a local steppe climate. According to Köppen and Geiger, this climate is classified as BSk. The temperature of Saveh averages 16.6° C. About 223 mm of precipitation falls annually. The soil properties of our study field have been presented in Table 1.

Table 1
Soil properties of the medicinal pumpkin filed in Saveh

Soil property	Value
pH	7.8
EC (ds/m)	4.5
K (mg/kg)	256
P (mg/kg)	84.5
Clay (%)	35
Silt (%)	40
Sand (%)	25

Treatments and traits

The study was carried out as a split-plot design. Irrigation as a main factor was applied at three levels including control (normal irrigation), no irrigation in flowering stage, and no irrigation in fruiting stage. The fertilizers as sub-plots included sulfur fertilizer as 250 kg/ha in the form of TIGER 90CR® Sulphur fertilizer (0-0-0-90). Thiobacillus biofertilizer used as 250 kg/ha with the number of active bacteria equal to 108 per gram (CFU). For this purpose, stem diameter, plant height, fruit weight, fruit yield, seed yield, 1000-seed weight, oil content, and fatty acid profile were measured.

Lipid Extraction for the Determination of % Oil and Fatty Acid Profile

Samples (2 g) were finely ground (1.0 mm mesh size) using a Moulinex Optiblend 2000. The oil from the finely ground samples was extracted by the procedure described by Savage et al., (1997). Briefly, oil was extracted with 6 ml hexane/isopropanol (3:2, v/v) at room temperature under vigorous stirring for 1 h in glass beakers to facilitate homogenization of the food. The food preparations were filtered through a vacuum and the residues were washed twice with 4 ml hexane/isopropanol solvent. Thereafter, 7 ml of 6.7% sodium sulfate (w/v) were added and the samples were vortexed for 30 s and centrifuged at 2,000 rpm for 10 min. The solvent layer was removed, dried under nitrogen and the pure oil was weighed to calculate the percentage of yield.

Preparation of fatty acid methyl esters

Fatty acid methyl esters (FAME) were prepared from extracted oil by the method of Slover and Lanza (1979). Briefly, approximately 40 mg extracted oil was treated with 1 ml methanolic NaOH at 100° C for 15 min in 25 × 150 mm Pyrex culture tube with Teflon-lined screw cap. The tubes were cooled on ice before 2 ml boron trifluoride were added and the tubes were boiled for a further 15 min. The tubes were cooled on ice, then 1 ml isooctane and 2 ml saturated sodium chloride were added, shaken vigorously, and left to stand to allow the layers to separate. The upper hexane layer containing the FAME was transferred to a small tube and stored at −20° C until further analysis by gas chromatography (GC).

FAME analysis by GC

For FAME analysis, a DB-WAX capillary column (30 m × 0.32 mm i.d.; J and W Scientific, Folsom, California, USA) was used. The column was connected to a Shimadzu GC-14A (Kyoto, Japan) gas chromatograph equipped with a flame-ionization detector. Nitrogen was used as the carrier gas. The temperature program was as follows: initial temperature 50° C; increase to 200° C at 10° C/min, hold for 25 min and increase to 230° C at 10° C/min, hold for 20 min. Injector and detector temperatures were 250° C. chromatograms were recorded using Millennium 32 chromatography manager software (Waters Corporation, Milford, Massachusetts, USA).

Table 2
Analysis of variance for quantitative and qualitative properties of medicinal pumpkin

Variable source	df	MS						
		Stem diameter	Plant Height	Fruit weight	Fruit yield	1000-seed weight	Seed yield	Oil percent
Water stress	2	0.371^{**}	0.127^{ns}	16.36^{**}	1308.8^{**}	7270.86^{**}	3425^{ns}	9.25^{ns}
Main error	4	0.019^{ns}	0.037^{ns}	0.034^{*}	2.66^{ns}	43.70^{**}	1954^{ns}	14.58^{ns}
Fertilizer	3	0.215^{**}	0.038^{ns}	0.037^{*}	19.62^{**}	93.65^{**}	1339^{**}	48.76^{**}
Water stress* Fertilizer	6	0.015^{ns}	0.004^{ns}	0.042^{*}	31.40^{**}	76.26^{**}	2601^{*}	5.76^{ns}
Sub error	18	0.014	0.154	0.011	3.17	12.84	837.8	7.26
CV (%)		8.08	9.9	4.72	3.9	2.43	3.57	6.61

**, *, and ns show significance at 0.01%, 0.05% and no significance, respectively.

Table 3
The effect of fertilizers on quantitative and qualitative properties of medicinal pumpkin

Treatment	Treatment	Stem diameter (cm)	Fruit weight (kg)	Fruit yield (ton/ha)	1000-seed weight (g)	Seed yield (kg/ha)	Oil percent
Fertilizer	Control	$1.2±0.11^{c}$	$2.19±0.15^{b}$	$43.6± 2.4^{b}$	$147. 4±18^{b}$	$773.8±1.75^{c}$	$38.1±0.35^{c}$
	Thiobacillus	$1.35 ±0.15^{bc}$	$2.31±0.35^{a}$	$44.3±2.7^{b}$	$147±1.3^{b}$	$778.7±4.83^{bc}$	$40.5±1.15^{bc}$
	Sulfur	$1.42±0.17^{b}$	$2.3±0.14^{a}$	$46.8±4.1^{b}$	$143. 2±2.8^{c}$	$805.5±2.08^{b}$	$41.2±0.42^{ab}$
	Thiobacillus + Sulfur	$1.61±0.28^{a}$	$2.33±0.15^{a}$	$51.1 ±3.9^{a}$	151. 2± 1.38 a	$862. 2±0.55^{a}$	$43.66±0.36^{a}$

Each value represents the mean ± SE (n=3). Mean values in each column followed by the same lower-case letter are not statistically significant by Duncan multiple range test (P≤0.05).

Statistical analysis

All data were submitted to SAS. Duncan multiple range test was applied to mean comparison at the 0.05 level.

Results

Analysis of variance showed that the simple effect of water stress on stem diameter, fruit weight, fruit yield, and 1000-seed weight was significant (P≤0.05). On the other hand, Thiobacillus and sulfur fertilizers significantly influenced stem diameter, fruit yield, fruit weight, 1000-seed weight, seed yield, and oil content (P≤0.05). The interaction of deficit irrigation and fertilizer was significant on fruit yield, fruit weight, 1000-seed weight, and seed yield (P<0.05) (Table 2).

Plant height and stem diameter

Plant height was not influenced by water stress and fertilizers (P≥0.05). In contrast, the effect of water stress was significant on stem diameter (P≤0.05). The highest and lowest stem diameter was recorded in Thiobacillus + sulfur (1.61 cm) and control (1.2 cm), respectively.

Fruit weight and yield

Deficit irrigation significantly influenced the fruit weight (P≤0.05). The lowest fruit weight was observed in the flowering stage as 1.15 kg, whereas the highest fruit weight was recorded in control to be 3.48 kg. In fertilizer treatments, the minimum fruit weight was obtained in control as 2.19 kg, while the maximum value was recorded as 2.33 kg in sulfur + Thiobacillus. On the other hand, deficit irrigation significantly reduced the fruit yield. It ranged from 34.83 ton/ha for deficit irrigation in flowering stage to 55.66 ton/ha in control. Sulfur and Thiobacillus reached the highest fruit yield to be 51.51 ton/ha. The highest fruit weight and yield was recorded in control (no stress)* Sulfur + Thiobacillus, whereas the lowest values were observed in water stress in fruiting stage* control (Fig. I).

1000-seed weight and seed yield

Sulfur and Thiobacillus significantly increased 1000-seed weight (P≤0.05). The maximum 1000-seed weight was observed in 151.22 g, whereas the minimum value was recorded in sulfur to be 143.22 g. Moreover, the

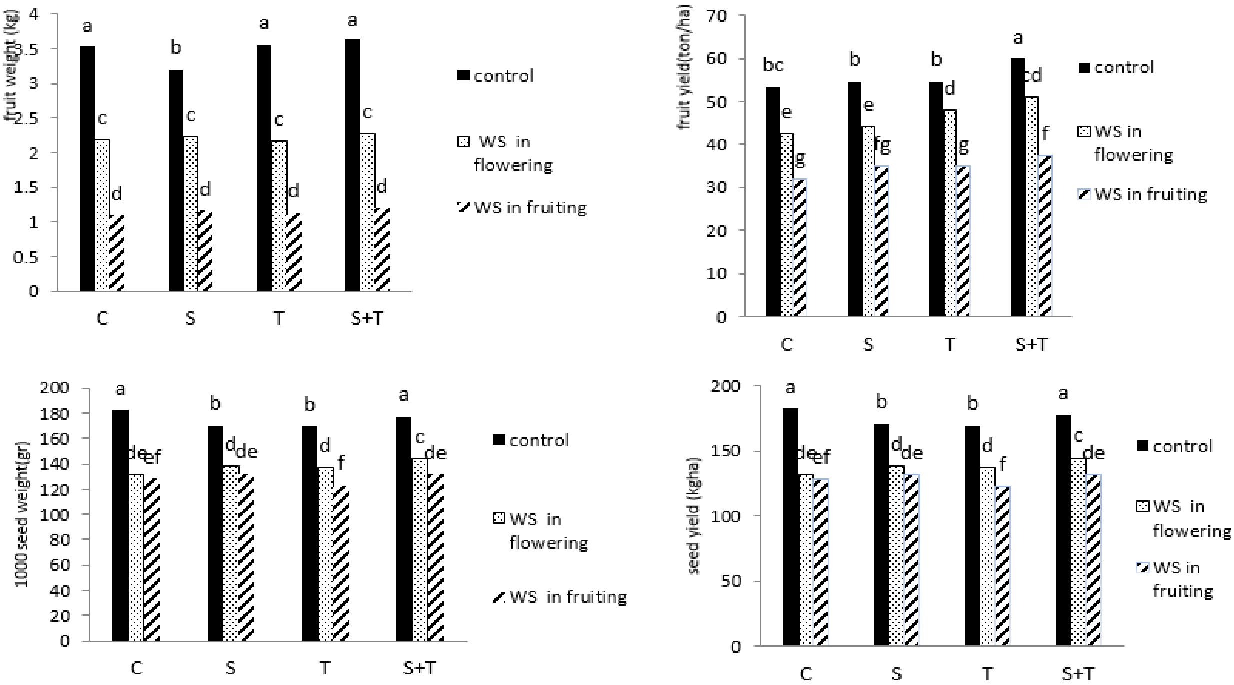

Fig. I. The interaction of water stress (WS) and fertilizers for fruit weight and yield, seed weight, and yield of medicinal pumpkin

Table 4

The effect of water stress on quantitative and qualitative properties of medicinal pumpkin

Treatment	Stem diameter (cm)	Fruit weight (kg)	Fruit yield (ton/ha)	1000-seed weight (g)	Seed yield (kg/ha)	Oil percent
Control	1.61±0.11^{a}	3.48±0.15^{a}	55.6 ±5.1 ^{a}	175.5 ± 0.4 ^{a}	817.1±0.05^{a}	41.7±0.15^{a}
Deficit irrigation in flowering stage	1.41 ±0.15^{b}	2.22±0.15^{b}	45.2 ±3.6^{b}	137.5± 1.54 ^{b}	788.6±0.11^{a}	39.5±0.19^{a}
Deficit irrigation in fruiting stage	1.28±0.17^{b}	1.15±0.38^{c}	34.8±3.3 ^{c}	128.5 ± 0.38 ^{c}	816±0.18^{a}	40.6±0.38^{a}

Table 5

Analysis of variance for seed oil compositions of medicinal pumpkin

	df	MS				
Variable source		Palmitic acid	Stearic acid	Oleic acid	linoleic acid	linolenic acid
rep	2	0.103^{ns}	20**	68.24**	39.91**	0.23^{ns}
Deficient irrigation	2	15.4**	3.38**	172.5**	323.30**	6.27**
Main error	4	2.74^{ns}	0.06^{ns}	0.48^{ns}	2.69^{ns}	0.21^{ns}
Fertilizer	3	6.65**	4.52**	172.9**	159.18**	3.86**
Deficient irrigation * Fertilizer	6	7.60**	2.49**	209.02**	100.96**	5.09**
Sub error	18	6.31**	4.44**	132.73**	107.09**	3.29**
CV (%)		11.67	7.44	0.99	7.74	75/49

Each value represents the mean ± SE (n=3). Mean values in each column followed by the same lower-case letter are not statistically significant by Duncan multiple range test (P≤0.05).

highest and lowest 1000-seed weight was obtained in normal irrigation (175.55 g) and in the irrigation at fruiting stage (128.55 g), respectively. Deficit irrigation had no significant impact on seed yield, but an increase was found in seed yield using sulfur and Thiobacillus, where 773 kg/ha and 862 kg/ha were recorded for control and sulfur + Thiobacillus, respectively (Tables 3 and 4). The interaction showed that the highest 100 seed weight was observed in control (normal irrigation)*no fertilizer, whereas the lowest value was recorded in the deficit irrigation at fruiting stage* Thiobacillus (Fig. I).

Table 6
Seed oil compositions of medicinal pumpkin under deficit irrigation and fertilizers

Deficit irrigation	Fertilizer	Palmitic acid	Stearic acid	Oleic acid	linoleic acid	linolenic acid
Control	Control	12.29bc	5.84cd	55.69c	20.64de	0.47b
	Sulfur	11.96cd	6.96ab	53.16d	23.62cd	0.1cd
	Thiobacillus	13.31a	7.36a	57.29b	17.83fg	0.39b
	Sulfur+ Thiobacillus	10.5de	4.89ef	42.32i	35.43a	0.62a
Deficit irrigation in flowering stage	Control	9.49de	4.59fg	41.26j	36.27a	0.13a
	Sulfur	12.59ab	6.01cd	58.59a	18.23fg	0.16fg
	Thiobacillus	11.88cd	6.92ab	52.19e	24.15c	0.26c
	Sulfur+ Thiobacillus	13.8a	5.95cd	44.68g	28.46b	0.1b
Deficit irrigation in fruiting stage	Control	8.7e	3.99g	32.26k	17.08fg	0.1fg
	Sulfur	9.79de	5.48de	47.48f	16.32fg	0.24fg
	Thiobacillus	9.28e	5ef	43.42h	14.92g	0.22g
	Sulfur+ Thiobacillus	12.39ab	6.38bc	55.18c	19.01ef	0.1ef

Each value represents the mean ± SE (n=3). Mean values in each column followed by the same lower-case letter are not statistically significant by Duncan multiple range test (P≤0.05).

Oil content

There was no significant change of oil content in samples treated by deficit irrigation. However, fertilizers increased oil content, in which the highest value was obtained in sulfur + Thiobacillus as 43.66%.

Fatty acid composition

Analysis of variance showed the interaction of deficit irrigation and fertilizer was significant for all seed oil compositions (P≤0.1) (Table 5). Fertilizer and irrigation treatments significantly affected the fatty acid composition. The fatty acid profile of the oil showed that it is composed primarily of oleic, linoleic, palmitic, stearic, and linolenic acids (Table 6).

Discussion

Water stress significantly reduced stem diameter. The reduction in stem diameter is due to the problem in cell division induced by water stress (Ohashi et al., 2006). The decrease of stem diameter in soybean (Ohashi et al., 2006) and peach trees (Garnier et al., 1986) under water stress have been reported.

Fruit weight and yield decreased by water stress. Many researchers have reported similar effects of water stress on fruit yield and/or fruit weight for a range of other agricultural and horticultural crops including sorghum (Chaudhuri and Kanemasu, 1982), tomato (Rudich et al., 1977; Tan, 1988), and strawberry (Kirnak et al. 2001). Sulfur has a significant role in soil emendation and improvement in other nutrients uptake. Moreover, Thiobacillus helps more appropriate uptake of sulfur (Wild, 2003). Hence, treatments of Thiobacillus + sulfur achieve haugher values of most studied traits. Cui et al. (2004) reported that the elemental sulfur acidified the soil and increased shoot concentration of Zn and Cd. According to Wild (2003), the deficiency of micronutrients in soil, such as Zn, limits the crop growth which results in fruit weight and yield, but can be corrected by the application of fertilizers containing the required elements.

Sulfur and Thiobacillus significantly increased 1000-seed weight. This increase may not be attributed to the association of Thiobacillus with organic matter in this treatment because these bacteria are mainly obligate chemolithotrophs, which use CO as the major carbon source under all growth conditions and organic materials seem not to play an important role in their metabolism (Kuenen and Veldkamp, 1973). Other types of sulfur oxidizing bacteria in the soil may have interacted with the organic materials existing in the fertilizer. Therefore, the fertilizers comprising only sulfur + Thiobacillus may have more fruitful results. Basharati et al. (2000) also indicated that sulfur alone significantly caused an increase in the availability of phosphorus, but adding Thiobacillus doubled the positive effect of sulfur. A reduction in pumpkin and squash fruit variability was found to be associated with irrigation (Fandika, 2012). The Seed yield, harvest index, 1000-seed weight, and seed germination percentage in *C. pepo* were significantly affected by irrigation quantity (Amer, 2011). Similar seed yields were also obtained for confectionary pumpkin (Cakir, 2000). Yegul et al., (2012) reported a 360–570 kg ha^{-1} in the seed yield of oil-seed pumpkin. Yavuz et al. (2015) found a reduction in seed yield of confectionary pumpkin under deficit irrigation. Ghanbari et al. (2007) obtained maximum seed yield (1700 kg ha^{-1}) with a 7-day irrigation interval and 100 × 40 cm plant spacing for pumpkin. Nerson (2005) studied the effects of plant spacing on the seed yield with confectionary pumpkin and obtained a seed yield of 1100 kg ha^{-1} while sowing 4 plants/m^2. A high seed yields of *C. pepo* was reported at high irrigation levels (full irrigation), which decreased significantly at low irrigation levels (no irrigation) (Al-Omran et al., 2005). In furrow irrigation on squash, the fruit yield significantly increased in a linear relationship from 22.4 to 44.7 t ha-1 as the irrigation water use increased from 279 to 475 mm in a deficit irrigation, where no deep percolation occurred (Ertek et al., 2004).

The fatty acid composition of pumpkin oil is important in determining nutritional quality and the possible uses of oil in industrial applications. The fatty acid profile of the oil showed that it is composed primarily of oleic, linoleic, palmitic, stearic, and linolenic acids. Schinas et al. (2009) indicated that the fatty acid profile of the oil is composed primarily of linoleic, oleic, palmitic, and stearic acids. The oil was chemically converted via an alkaline transesterification reaction with methanol to methyl esters, with a yield nearly 97.5 wt%. Fatty acid profile can be changed in different ecological conditions (Lelley et al., 2009). In our study the main fatty acids of pumpkin were altered by irrigation and fertilizers. In total, irrigation resulted in reduction of fatty acids and fertilizers increased this value. The contents of oleic acid in the kernel oil increased due to water stress with a subsequent decrease in linoleic acid causing an increased oleic/linoleic ratio of kernel oil from drought stressed plants. Similar changes in the contents of oil oleic and linoleic acids as a result of drought stress have already been demonstrated in some other reports in different plant species, e.g. sunflower (Flagella et al., 2000, 2002) and Moringa oleifera (Anwar et al., 2006). Such changes in seed oil contents depend on different factors such as intensity of drought stress and temperature (Specht et al., 2001; Triboi and Triboi-Blondel, 2002), type of genotype, and timing of full maturity of cultivars (Triboi and Triboi-Blondel, 2002). In agreement with our present findings a marked decline in seed oil content of maize with water deficit was reported in both maize cultivars (Champolivier and Merrien, 1996). Furthermore, Carvalho et al. (2005) reported that drought is a major factor responsible for any significant decrease in oil content in two lupine cultivars. Similarly, Asbagh et al. (2009) reported a significant decrease in sunflower seed oil contents under water deficit condition.

Conclusion

Different studies have shown that sulfur Thiobacillus in calcareous and alkaline soils reduces pH and improves soil properties. In calcareous soils, the intake of nutritional elements by the plant are impaired which result in the reduction of yield and productivity. Furthermore, the plants do not efficiently benefit from the fertilizers applied to the soil. On the other hand, deficient irrigation can reduce the quality and quantity of seed and fruit. In our study, deficient irrigation reduced fruit weight, fruit yield, and 1000-seed weight, but these properties were significantly improved by both sulfur and Thiobacillus. Fatty acid profile was changed under deficient irrigation and fertilizers. It can be applied by producers to obtain the appropriate treatment of sulfur and Thiobacillus under deficient irrigation.

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