International Journal of Horticultural Science and Technology
Vol. 3, No. 2; December 2016, pp 113-128
Print ISSN: 2322-1461 Online ISSN: 2588-3143
DOI: 10.22059/ijhst.2016.62912
Web Page: https:// ijhst.ut.ac.ir, Email: ijhst@ut.ac.ir

Influence of arbuscular mycorrhizal inoculation and humic acid application on growth and yield of Roselle (*Hibiscus sabdariffa* L.) and its mycorrhizal colonization index under deficit irrigation

Hamid- Reza Fallahi[1*], Morteza Ghorbany[2], Alireza Samadzadeh[1], Mahsa Aghhavani-Shajari[3] and Amir Hassan Asadian[4]

1. Department of Agronomy and Plant Breeding, Faculty of Agriculture, University of Birjand, Birjand, Iran

2. Department of Biology, Faculty of Science, University of Birjand, Birjand, Iran

3. Department of Agronomy, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran

4. Agronomy and Plant Breeding Department, Sarayan Faculty of Agriculture, University of Birjand, Birjand, Iran

(Received: 26 April 2016, Accepted: 25 January 2017)

Abstract

In this study effect of irrigation managements including irrigation after 100 and 200 mm pan evaporation as normal and deficit irrigation respectively was investigated in Roselle plants. Effects of humic acid (including 0 and 4 kg ha^{-1}) and mycorrhizal inoculants (including *Glomus versiforme* (GV) and *Rhizophagus irregularis* (RI)) were also studied on growth, yield and mycorrhizal symbiosis index of Roselle plants. Drought stress reduced the amounts of morphological indices and yield components, while mycorrhizal treatment particularly RI inoculation and to a lower extent humic acid application reduced the negative impacts of water deficit on growth and yield of Roselle plants. Both inoculants of mycorrhizal fungi increased the economical yield of Roselle under drought stress condition, where the amount of calyx yield for RI, GV and control in 200 mm pan-evaporation treatment was 130, 127 and 66 kg ha^{-1}, respectively. In addition, the highest root mycorrhizal frequency was obtained at normal irrigation × humic application × RI (95%) and the lowest value was observed at deficit irrigation × no-humic × no-mycorrhizal inoculation (31.6%) treatment. In conclusion, combined effects of experimental factors showed that seed inoculation of plants by mycorrhiza and to some extent application of humic acid are two reliable strategies for Roselle production under deficit irrigation.

Keywords: calyx, drought stress, *Glomus versiforme*, medicinal plants, *Rhizophagus irregularis*.

Introduction

Roselle is an annual tropical and sub-tropical medicinal plant belongs to Malvaceae family. Possible origin of this plant is attributed to the West Africa however its cultivation has been reported from other tropical regions such as parts of Asia, Central America, and Australia (Babatunde and Mofoke, 2006; Rahbarian *et al*., 2011; Sonar *et al*., 2013). In Iran,

* Corresponding Author, Email: Hamidreza.fallahi@birjand.ac.ir

Roselle is mainly cultivated in Sistan & Balouchestan province (approximately 300 ha) with a mean dry calyx yield of 700-900 kg ha^{-1}. Roselle is usually cultivated for its stem fibers, leaves, seeds and especially for its red or green inflated edible calyces (Fasoyiro *et al*., 2005a; Satyanarayana *et al*., 2015). The sepals are mainly used in food and cosmetic industries as a source of natural coloring agent to prepare refreshing beverage and jellies (Abo-Bake and Mostafa, 2011; Khalil and Abdel-Kader, 2011; Sonar *et al*., 2013). Roselle fruits contain many essential nutrients such as vitamin A, vitamin C, minerals, carotene and dietary fiber (Fasoyiro *et al*., 2005b). In addition, various medicinal applications such as anti-oxidation effects as well as treatment of diseases like hypertension, pyrexia, cancer, kidney stones and blood pressure have been reported for this plant (Mohd-Esa *et al*., 2010).

Since drought as an abiotic stress has the most dominant role in yield reduction in dry areas (Fallahi *et al*., 2015), adaptability to water deficit conditions for medicinal plants including Roselle in dry regions needs to be investigated. It has been reported that water deficit irrigation in Roselle caused a decrease in relative humidity, chlorophyll and carotenoid contents, while led to an increase in proline content (Sanjari *et al*., 2015). In another study in Roselle, the highest calyx yield was obtained from water deficit treatment by providing of 75% field capacity (Rahbarian *et al*., 2011). These finding suggest that Roselle is relatively a suitable plant for deficit irrigation. However, application of appropriate nutritional methods such as humic acid application and mycorrhizal inoculation is necessary for improvement of its growth under water deficit condition. These practices would provide feasible approaches to conserve limited water resources in arid and semiarid areas (Keshavarz Afshar *et al*., 2014; Koocheki *et al*., 2016).

As eco-friendly fertilizers, humic substances can improve soil physical, chemical and biological properties. This nutritional sources increase the growth and drought tolerance of plants by increasing: water and nutrient absorption, availability of elements, development of root system, plant chlorophyll content and alterations in plant enzymes activity (Rose *et al*., 2014; Bakry *et al*., 2014a; Koocheki *et al*., 2016). Humic acid is a part of the humus compounds which contains most of known trace minerals and plays an important role in plant nutritional balances (Khalil and Yousef, 2014). Former studies have proven that humic substances can affect plant physiology via hormone-like effects, influence on photosynthesis and activating certain enzymes (Bettoni *et al*., 2014). In addition, enhancement of minerals availability, increase in cation exchange capacity, pH adjustment and stimulation of beneficial soil microorganisms might occur when such composts are applied in the soil (Bettoni *et al*., 2014; Fallahi *et al.*, 2017). Likewise, humic substances have an alleviative effect on drought stress mainly by stimulation of antioxidants production, which protect plants from damages caused by reactive oxygen species (Fallahi *et al.*, 2017). These compounds enhance the expression of an enzyme that catalyzes the first main step in the biosynthesis of phenolic compounds in the cells (Canellas *et al*., 2015).

Recently the effects of humic substances on growth and drought tolerance indices of Roselle have been investigated. Results of Sanjari-Mijani (2014) suggested that humic acid is a suitable nutritional source for improving Roselle growth, yield and nutrients uptake under drought stress condition. In another study on Roselle, enhancement of chlorophyll a and b, carotenoids and leaf carbohydrates and a decrease in proline content of Roselle under drought stress condition was reduced (Sanjari-Mijani *et al*., 2015). Furthermore, the positive effect of humic substances on growth and reducing the inhibitory effects of drought stress has been reported on different crops such as onion (*Allium cepa* L.) (Bettoni *et al*., 2014), borage (*Borago officinalis* L.) (Heidari and Minaei, 2014) and safflower (*Carthamus tinctorius* L.) (Yadollahi *et al*., 2015).

Mycorrhizal inoculation protects host plants from negative effects of deficit irrigation and improves their tolerance to drought stress (Sonar *et al*., 2013; Keshavarz Afshar *et al*., 2014). Under drought stress condition mycorrhizal symbiosis helps plants by mechanisms such as maintenance of leaf water potential and turgor pressure, increase in expression levels of drought resistance genes, regulation of abscisic acid production and enhancement of plant recovery to normal condition after stress release (Ahanger *et al*., 2014). In addition, mycorrhizal hyphae facilitate absorption of available forms of nutrients by increase in the effective soil volume and adjustment of soil chemical properties (Sembok *et al*., 2015). Therefore, uptake of nutrients such as phosphorus, nitrogen, zinc, iron, copper, potassium, magnesium, sulfur and other ions, is usually improved by mycorrhiza inoculation in plants (Aulia *et al*., 2009).

Mycorrhizal hyphaes are an alternative mechanism for water and nutrient uptake in inoculated plants, particularly for those elements with low soil mobility, such as phosphorus, zinc and copper (Sonar *et al*., 2013). Based on studies on Roselle plant, 6 and 19% increase in leaf nitrogen and phosphorus contents with mycorrhizal inoculation have been reported (Mohammadpour-Vashvaei *et al*., 2015). Same results have been reported by Subramanian *et al*. (2006) on tomato plants exposed to different levels of drought stress. So far, the positive effects of mycorrhizal inoculation have been reported on Roselle growth, yield and quality. In a study, inoculation of soilless grown Roselle plant induced proliferation of fungal spores and doubled the root volume and dry weight, while root infection was 59-64% (Sembok *et al*., 2015). In another study, mycorrhizal colonization in Roselle showed considerable colonization and salt tolerance potential (Sonar *et al*., 2013). In another research, mycorrhizal inoculation had positive effects on fruit and calyx production in Roselle (Aulia *et al*., 2009). The positive effects of arbuscular mycorrhizal fungi on drought stress tolerance have been previously reported on other plants species such as tomato (Subramanian *et al*., 2006), citrus (Wu *et al*., 2006), marigold (*Tagetes erecta*) (Asrar and Elhindi, 2011) and thymus (Navarro-Fernandez *et al*., 2011). It should be noted that a synergic effect between mycorrhiza fungi and humic substance is exist. This effect is due to improved growth of mycorrhizal mycelium under availability of soil organic matter, including humic substance (Gryndler *et al*., 2005).

The aim of this study was to investigate the effect of mycorrhizal inoculation and humic acid application, on Roselle growth and yield performance under normal and deficit irrigation conditions. In addition, evaluation of sustainable nutritional methods and irrigation managements on mycorrhizal frequency percentage in Roselle roots was studied.

Materials and Methods

Experimental site

This study was conducted at Research Field of Sarayan Faculty of Agriculture (33 ºN, 58 ºE and 1450 masl) in South Khorasan province, east part of Iran. The experimental site is characterized with semi-arid climate with an average annual precipitation and mean annual temperature of 150 mm and 17 ºC, respectively. The values of some climatic parameters for the research station during the experiment period are shown in Table 1.

Experimental design and treatments

For investigating the effect of seed mycorrhizal inoculation and humic acid application on mitigation of drought stress impacts on Roselle and its mycorrhizal symbiosis index a split-split-plot experiment based on a randomized complete block design with three replications was carried out during 2014 and 2015. The main plot was consisted of two water availability levels including irrigation after 100 (normal irrigation) and 200 mm (deficit irrigation) pan evaporation. The sub-plot was assigned to humic acid (0 and 4 kg ha^{-1}) and the sub-sub-plot was including of two species of mycorrhizal fungi (*Glomus versiforme*, *Rhizophagus irregularis* and control). The main chemical properties of humic acid are presented in Table 2. Mycorrhizal species were achieved from TuranBiotech Company (Turanbiotch.ir), which were prepared by trap culture method on berseem clover (*Trifolium alexandrinum* L.).

Table 1. The main climatic indices of Sarayan city during experiment

Growth	Precipitation (mm)	Potential evaporation (mm)	Monthly average humidity (%)	Monthly sunshine hours	Average of minimum temperatures (°C)	Average maximum temperatures (°C)
April	12	135.8	38	248.5	11.7	25.1
May	5.3	297.2	26	287.6	17.1	30.8
June	0	417.6	16	344.1	21.1	35.8
July	0	479.0	16	355.6	24.2	37.5
August	0	418.9	16	368.1	21.5	35.5
September	0	304.3	22	343.9	16.8	32.2
October	1.9	216.1	27	288.4	14.4	29.0
November	9	97.7	45	206.8	8.3	20.2

Table 2. Properties of used humic acid and experimental site based on soil and irrigation water properties

Soil properties

EC ($mS.cm^{-1}$)	pH	O.C (%)	N_{total} (%)	P_{ava} (ppm)	K_{ava} (ppm)	Sand (%)	Silt (%)	Clay (%)	Soil texture
2.27	8.49	0.13	0.016	2.07	194.9	48.5	22.5	29	Loam

Water properties

EC ($mS.cm^{-1}$)	pH	TDS (ppm)	Ca^{2+} (ppm as $CaCo_3$)	Mg^{2+} (ppm as $Caco_3$)	Na^+(ppm)	K^+ (ppm)	Cl^- (ppm)
1.3	7.81	8510	48	51.5	156.4	0.45	170.4

Humic acid (% W/W Total; Brand of Humixtract produced in Spain)

Total humic extract	Humic acids	Polycarboxilic acid	Total organic matter	Calcium oxide	Potassium oxide
70%	38%	32%	70%	1%	10%

Agronomic practices

The main soil physico-chemical characteristics are shown in Table 2. Soil preparation was done in April. Before planting, the plots were supplemented with 30 ton ha^{-1} cow manure. The seeds used in this experiment were from local ecotype of Sistan & Balochestan (The main Roselle production province in Iran). Manual seed planting was carried out on 20 April with three seeds per each planting point. The space between the planting points was 10 cm and the row spacing was 50 cm which resulted in 20 plants m^{-2}. All plots were irrigated two times, one immediately after seed sowing and the second one week after the first irrigation. Irrigation treatments were applied separately in each plot. About 600 m^3 ha^{-1} water was used in each irrigation time. The characteristics of irrigation water are presented in Table 2.

Grown plants were thinned approximately one month after seedlings emergence, so that one plant remained in each planting point. In addition, one time foliar application of micronutrients was done using 2 l/ha multi-purplex (produced in USA and consisted of: 2% N, 5% S, 1.2% C, 0.05% Cu, 0.3% Mg, 0.1% Mn, 0.15% B, 0.2% Fe, 0.5% Zn, 100 mg l^{-1} cytokinins, 0.52 mg l^{-1}gibberlin, 0.33 mg l^{-1} auxin and 0.27%amino acids) about 40 days after seedlings emergence stage. Finally, on November 15, irrigation was stopped until the end of the sepals picking period.

Measured morphological parameters and yield

For measuring morphological parameters and yield components of Roselle plants, five plants were randomly selected from each plot on November 20. The selected plants were cut from above ground and plant height, number of lateral branches per plant, number of fruits per plant, fruit weight, leaf area, leaf weight, plant dry weight and sepal dry weight per plant were determined. After one week, the fruits of remained plants were separately harvested from each plot for measuring biological and economical yields as well as calculation of harvest index.

Measurement of mycorrhizal colonization

For determination of root colonization percent by mycorrhizal fungi, root staining was carried out based on method of Philips and Hayman (1970) and mycorrhizal frequency was determined using equation described by Shirzad and Ghorbany (2015). To do so, five g of proper root of Roselle were prepared and immediately transferred to GEE solution (glycerol + ethanol + distilled water). Stained roots were cleared in 10% KOH for revelation of fungal structures and stained with 0.05% trypan blue in lactophenol. Stained roots were cut into one cm fragments and crushed on slides in a one drop of polyvinyl alcohol-lacto-glycerol (Abbas *et al.,* 2006). In this study, 10 fragments were placed on each slide with 10 replications. Finally, each fragment was evaluated under a microscope to determine mycorrhizal infection. Consequently, mycorrhizal frequency, which indicates the extent of fungal colonization was calculated using Equation (1) (Shirzad & Ghorbany, 2015).

$$\text{Mycorrhizal frequency percentage} = 100(N-n_0)/N \quad (1)$$

N= total number of observed fragments, n_0= number of fragments without mycorrhizae.

Data analysis

Experimental data were statistically analyzed using SAS 9.1 and means were compared by Duncan's multiple range test at 5% level of probability.

Discussion and Results

Interaction effects of deficit irrigation and humic acid application

Combined effects of irrigation management and humic acid application was significant on plant height, number of fruit per plant, leaf area, plant dry weight, biological and economical yields of Roselle (Table 3). Water deficit (irrigation after 200 mm pan evaporation) caused a reduction in all morphological parameters, yield and yield component indices. In addition, humic acid application had positive effects on growth and yield indices of Roselle under water deficit condition (Table 4). Plant height, lateral branches, fruit number, fruit weight, leaf area, leaf weight, plant weight and sepals yield per plant in control plants were 19, 35, 48, 41, 22, 23, 50 and 64% more than their values in water deficit treatment, respectively. Our results are in agreement with those obtained by Sanjari-Mijani (2014). However, Seghatoleslami *et al*. (2013) reported that the highest growth and yield indices of Roselle in semi-arid climatic condition were recorded for the plants with irrigation level of 20% evapo-transpiration of the source plant (ET_O). However, in current experiment, the positive effect of deficit irrigation on water use efficiency of Roselle was similar to results of Seghatoleslami *et al*. (2013). In another study in Egypt, Mandour *et al*. (1979)

showed an increase in quality indices of Roselle calyx which obtained by consumption of 360 m^3 ha^{-1} water with 15 days intervals during the plant life cycle.

Table 3. Results of analysis of variance for effects of irrigation deficit, humic acid application and mycorrhizal inoculation on morphological parameters, yield and yield components and mycorrhizal symbiosis index in Roselle plants

Source of variation	Plant height	Number of lateral branches	Number of fruit per plant	Mean fresh weight of fruit	Leaf area per plant	Leaf dry weight per plant
Replication (R)	**	**	ns	ns	ns	ns
Irrigation (I)	**	**	**	**	**	**
R*I: Error a	*	Ns	ns	ns	ns	ns
Humic acid (H)	ns	Ns	ns	ns	**	ns
I*H	*	Ns	**	ns	*	ns
R*H(I): Error b	ns	Ns	ns	ns	ns	ns
Mycorrhiza (M)	ns	*	**	ns	**	**
I*M	ns	Ns	**	ns	**	*
H*M	ns	Ns	**	ns	**	**
I*H*M	ns	Ns	**	*	ns	ns
C.V.	15.1	30.2	13.3	32.1	13.2	22.6
Source of variation	**Plant dry weight**	**Sepals dry weight per plant**	**Biological yield per hectare**	**Economical yield per hectare**	**Harvest index**	**Mycorrhizal frequency percentage**
Replication (R)	ns	*	ns	ns	ns	ns
Irrigation (I)	**	**	**	**	ns	ns
R*I: Error a	ns	Ns	ns	ns	ns	ns
Humic acid (H)	ns	Ns	ns	ns	ns	**
I*H	**	Ns	**	**	ns	ns
R*H(I): Error b	ns	Ns	ns	*	ns	ns
Mycorrhiza (M)	**	**	**	**	ns	**
I*M	**	**	**	**	*	ns
H*M	**	Ns	**	*	*	*
I*H*M	**	Ns	**	**	*	ns
C.V.	16.4	20.3	16.3	13.8	17.0	09.0

*, ** and ns represent significant at 5% level, significant at 1% level and non-significant, respectively.

Table 4. Interaction effects of irrigation deficit and humic acid application on morphological parameters, yield and yield components and mycorrhizal symbiosis index in Roselle plants

Irrigation management (mm pan evaporation)	Humic acid application ($kg.ha^{-1}$)	Plant height (cm)	Lateral branches ($No.plant^{-1}$)	Number of fruits per plant	Mean fresh weight of fruit (g)	Leaf area per plant (cm^2)	Leaf dry weight per plant (g)
100	0	139.6[a]	4.92[ab]	20.2[a]	1.30[a]	767[a]	9.99[a]
	4	126.4[ab]	5.58[a]	18.5[a]	1.05[a]	615[b]	8.25[ab]
200	0	101.3[c]	3.40[b]	08.9[b]	0.71[b]	541[b]	7.08[b]
	4	113.1[bc]	3.47[b]	11.1[b]	0.69[b]	531[b]	6.95[b]
Irrigation management (mm pan evaporation)	**Humic acid application ($kg.ha^{-1}$)**	**Plant dry weight (g)**	**Sepals yield per plant (g)**	**Biological yield ($kg.ha^{-1}$)**	**Economical yield ($kg.ha^{-1}$)**	**Harvest index (%)**	**Mycorrhizal frequency (%)**
100	0	39.0[a]	1.76[a]	8235[a]	211.9[a]	2.69[a]	54.4a
	4	33.2[a]	1.74[a]	6491[a]	170.7[a]	2.64[a]	72.2a
200	0	16.4[b]	0.62[b]	3381[b]	90.2[b]	2.76[a]	52.2a
	4	19.7[b]	0.73[b]	4069[b]	125.6[b]	3.04[a]	72.7a

In each column, means with at least one similar letter are not significantly different ($P \leq 0.05$) based on Duncan test.

Application of humic acid under water deficit condition improved plant height, lateral branches, fruit number, plant weight and sepals yield per plant by 12, 2, 20, 17 and 15%, respectively, while positive effect of humic acid application was not observed under sufficient water condition (Table 4). These results are in agreement with those reported by Sanjari-Mijani (2014) and Sanjari-Mijani *et al*. (2015). They showed that humic acid can improve the Roselle growth, yield, chlorophyll content, carotenoids content and nutrients uptake under drought stress condition. The highest values of biological and economical yields of Roselle were obtained when humic acid and water stress treatments were not applied. However, humic acid application had a positive effect on these criteria when the crop was exposed to drought stress. So that, in comparisin with control plants, application of humic acid under deficit irrigation led to an increase in the amounts of biological and economical yields by 17 and 28%, respectively. Moreover, water stress combined with humic acid application had an enhancing effect on harvest index of Roselle plant (Table 4).

Drought stress reduces yield of medicinal and aromatic plants due to reduction of leaf area index, leaf area duration and radiation use efficiency (Khalil and Yousef, 2014). Results of Khalil and Yousef (2014) showed that drought stress caused reduction in growth, yield and fruit quality of Roselle. In their study the maximum values for the measured parameters were obtained under combined effect of no-stress × humic acid application. Humic substances improve plant growth condition via providing better soil physical and chemical properties as well as stimulation of beneficial soil microorganisms like mycorrhizal fungi (Bakry *et al*., 2014b). Consistent with these findings our observations revealed that humic acid application improved the root mycorrhizal frequency by 18% in both levels of irrigation regimes (Table 4). The improvement of soil biological activity by humic acid is due to providing of organic carbon for microorganisms feeding and proliferation (Fallahi *et al.*, 2017). However, humic acid has the potential to improve soil physical properties mainly by increasing aggregate stability (Gumus and Seker 2015). These organic complexes also positively affect the soil properties due to their carboxyl ($COOH^-$) and phenolic (OH^-) groups (Khaled and Fawy, 2011). Overall, our findings are in accordance with previous studies (Bettoni *et al*., 2014; Heidari and Minaei, 2014; Yadollahi *et al*., 2015) which showed that humic substance can alleviate the inhibitory effects of drought stress on Roselle growth and development.

Combined effect of deficit irrigation and mycorrhizal inoculation

Combined effect of irrigation treatment and mycorrhizal inoculation significantly affected the morphological parameters, yield and yield components of Roselle (Table 3). Results of current study revealed that mycorrhizal inoculation especially with *Rhizophagus irregularis* mitigated the negative effects of water stress and had a positive effect on growth and yield of Roselle (Table 5). These findings are consistent with the results of Wu *et al*. (2006) which reported that regardless of water status, vegetative parameters in citrus were considerably higher in seedlings inoculated with mycorrhiza fungi. They showed that mycorrhizal inoculation alleviated the negative impacts of water stress. In our experiment, tallest plants were obtained in no-stress × mycorrhizal inoculation treatment, while shortest plants were observed under drought stress condition and no-mycorrhizal inoculation. Similarly, plants were inoculated with mycorrhiza fungi showed more lateral branches and fruit number. On average the number of lateral branches in RI (*Rhizophagus irregularis*) and GV (*Glomus versiforme*) treatments was respectively 35% and 15% more than non-inoculated plants. Number of fruits per plant for RI and GV treatments was 36 and 16% higher than fruit number in control plants. These findings are consistent with the results of Subramanian *et al*. (2006) on tomato and Navarro-Fernandez *et al*. (2011) on thymus which concluded that mycorrhizal inoculation significantly increased shoot dry matter, number of flowers and fruits under well-watered and drought stress treatments.

Table 5. Interaction effects of irrigation deficit and mycorrhizal inoculation on morphological parameters, yield and yield components and mycorrhizal symbiosis index in Roselle plants

Irrigation management (mm pan evaporation)	Mycorrhizal inoculation	Plant height (cm)	Lateral branches ($No.plant^{-1}$)	Number of fruit per plant	Mean fresh weight of fruit (g)	Leaf area per plant (cm^2)	Leaf dry weight per plant (g)
100	*Rhizophagus irregularis*	134.3^{ab}	6.49^{a}	25.7^{a}	1.27^{a}	846^{a}	11.3^{a}
	Glomus versiforme	137.7^{a}	4.43^{b}	16.1^{b}	1.10^{ab}	581^{b}	7.6^{bc}
	Control	127.0^{ab}	4.83^{ab}	16.3^{b}	1.16^{a}	645^{b}	8.4^{b}
200	*Rhizophagus irregularis*	108.8^{bc}	4.26^{b}	11.0^{c}	0.52^{c}	626^{b}	8.2^{b}
	Glomus versiforme	117.8^{abc}	3.83^{bc}	11.8^{bc}	0.89^{abc}	591^{b}	7.7^{bc}
	Control	94.9^{c}	2.21^{c}	07.1^{c}	0.68^{bc}	389^{c}	5.1^{c}

Irrigation management (mm pan evaporation)	Mycorrhizal inoculation	Plant dry weight (g)	Sepals yield per plant (g)	Biological yield ($kg.ha^{-1}$)	Economical yield ($kg.ha^{-1}$)	Harvest index (%)	Mycorrhizal frequency (%)
100	*Rhizophagus irregularis*	47.1^{a}	2.44^{a}	9663^{a}	233.8^{a}	2.49^{a}	84.1^{a}
	Glomus versiforme	30.0^{b}	1.27^{c}	6146^{b}	166.7^{b}	2.41^{a}	69.1^{a}
	Control	31.3^{b}	1.54^{b}	6280^{b}	193.5^{a}	3.09^{a}	36.6^{b}
200	*Rhizophagus irregularis*	20.5^{bc}	0.55^{e}	4236^{bc}	130.4^{b}	3.07^{a}	78.3^{a}
	Glomus versiforme	20.9^{bc}	0.88^{d}	4313^{bc}	127.0^{b}	2.97^{a}	68.3^{a}
	Control	12.8^{c}	0.45^{e}	2626^{c}	66.4^{c}	2.67^{a}	40.8^{b}

In each column, means with at least one similar letter are not significantly different ($P \leq 0.05$) based on Duncan test.

Mean fruit weight in control condition was higher than water deficit treatment in all mycorrhizal and no-mycorrhizal treatments. Mean fruit weight was increased in plants inoculated with GV by 7% when compared with control (Table 5). Highest and lowest leaf area, leaf weight and plant dry weight were obtained in normal irrigation × RI and deficit irrigation × no-inoculated plants, respectively. Sepals yield per plant in inoculated plants was 31% and 37% higher than no-inoculated plants in normal and deficit irrigation treatments, respectively. Irrigation after 100 mm pan-evaporation combined with mycorrhizal inoculation using RI produced the highest biological yield, which was 3.7 times more than non-inoculated plants exposed to deficit irrigation. In addition, RI had an increasing effect on economical yield of Roselle. In inoculated plants, economical yield was 17 and 49% more than control, in normal and deficit irrigation treatments, respectively. Therefore, the positive effect of mycorrhizal inoculation on calyx dry yield was higher when the plants were under low water availability. This finding is in lines with the results of Hazzoumi *et al*. (2015) and Auge *et al*. (2007) which reported that the effectiveness of mycorrhizal fungi can increase with the intensity of drought stress. This is due to mycorrhizal infection capacity to change cellular mechanism of the host plants, resulting in the induction of chemical defense (Hazzoumi *et al*., 2015).

In similar study on tomato the fruit yield of mycorrhizal inoculated plants under severe, moderate and mild drought stresses were respectively 25%, 23% and 16% higher than non-inoculated plants. Moreover, harvest index in water deficit treatment was about 8% higher than harvest index in control irrigation. Furthermore, inoculation of Roselle plants by mycorrhizal fungi under deficit irrigation led to an increase in the value of harvest index by 12% when compared to the harvest index in plants with normal irrigation (Table 5).

The presence of the arbuscular mycorrhizal fungi considered as a common feature in Malvaceae species such as roselle (Sonar *et al*., 2013). In current study the mycorrhizal frequency in plants inoculated with mycorrhizal fungi RI and GV was respectively 81.2% and 68.7% higher than the control. Interestingly this index for non-inoculated plants was 38.7%, for mean of two irrigation levels (Table 5). The value for mycorrhizal frequency in non-inoculated plants showed the presence of native mycorrhizal fungi in studied agro-ecosystem. It has been reported that mycorrhizal symbiosis can increase plant tolerance to various biotic and abiotic stresses (Sonar *et al*., 2013). In a study on marigold,mycorrhizal colonization improved host plant growth, pigments concentration, phosphorous content, flower quality and therefore alleviated the negative impacts of water stress (Asrar and Elhindi, 2011). It has been suggested that mycorrhizal symbiosis has an increasing effect on enzymatic and non-enzymatic antioxidant productions which can helps inoculated plants to enhance their drought tolerance (Wu *et al*., 2006).

The positive effects of biological fertilizers on Roselle plants have been also reported in other studies. Mohammadpour-Vashvaeiet *et al*. (2015) revealed that colonization of Roselle by mycorrhiza (*G. intraradices* and *G. etanicatum*) increased the amount of calyx yield by 15%. Nemati and Dahmadreh (2015) in a study on Roselle have also shown that the highest economical yield was obtained in 10 t ha^{-1} manure + nitroxin biofertilizer 300% higher than control treatment. Results of Gendy *et al*. (2012) on Roselle plants showed that cattle manure and sole biofertilizers or combined form resulted in highest quality and quantity of sepals as well as increased the content of macro-nutrients, protein and total leaf carbohydrates. Overall, our results revealed that mycorrhizal inoculation is a sustainable strategy for Roselle production especially in areas that are affected by drought stress. However, the selection of suitable spices of mycorrhizal fungi for seed inoculation can positively affect the root infection and thereby provide proper condition for plant growth. In this regard, in our study the mycorrhizal symbiosis index in *R. irregularis* was 13% higher than *G. versiforme* in both levels of water availability (Table 5). This indicates the better growth and yield performance when the Roselle plant was inoculated with *R. irregularis*.

Combined effects of humic acid application and mycorrhizal inoculation

Combined effects of humic acid consumption and mycorrhizal inoculation significantly increased the fruit number, leaf area and weight, plant weight, biological yield, economical yield, harvest index and mycorrhizal symbiosis index of Roselle plants (Table 3). Highest and lowest values for plant height were obtained at GV (*Glomus versiforme*) × application of 4 kg ha^{-1} humic acid and no-inoculation × no-humic acid application, respectively. In addition, number of lateral branches per plant was increased by 26% in mycorrhizal inoculation treatment and by 8% in humic acid application. Mycorrhizal endosymbiosis with Roselle plant produced higher fruit number per plant. This induction, with lower extent, was obtained by humic acid application. Moreover, leaf area, leaf dry weight and plant weight were improved by mycorrhizal inoculation particularly with RI (*Rhizophagus irregularis*) inoculation in both levels of humic acid applications. Biomass production and calyx yield of Roselle were positively affected by inoculation with GV and humic acid application (Table 6). These findings are similar with those reported by Bettoni *et al*. (2014) on onion which showed increase in shoot and root biomass after applying humic acid combined with mycorrhizal inoculation. In this regard, it has been reported that application of humic acid can improve root colonization by mycorrhizal fungi (Gryndler *et al*., 2005). In agreement with this finding, in our experiment in comparison with control treatment, application of humic acid led to 20% increase in the amount of mycorrhizal frequency index (Table 6). This result suggests the possibility of stimulating effects of soil organic matter including humic substance for mycorrhizal fungi to promote extensive growth of its mycelium (Gryndler *et al*., 2005).

Table 6. Interaction effects of humic acid application and mycorrhizal inoculation on morphological parameters, yield and yield components and mycorrhizal symbiosis index in Roselle plants

Humic acid application ($kg.ha^{-1}$)	Mycorrhizal inoculation	Plant height (cm)	Lateral branches ($No.plant^{-1}$)	Number of fruit per plant	Mean fresh weight of fruit (g)	Leaf area per plant (cm^2)	Leaf dry weight per plant (g)
	Rhizophagus irregularis	125.7[a]	5.82[a]	22.2[a]	0.98[a]	847[a]	11.68[a]
0	*Glomus versiforme*	126.2[a]	3.88[ab]	11.7[b]	1.18[a]	607[b]	7.95[b]
	Control	109.4[a]	2.77[b]	09.9[b]	0.85[a]	506[b]	5.96[b]
	Rhizophagus irregularis	117.4[a]	4.93[ab]	14.5[ab]	0.81[a]	625[b]	7.82[b]
4	*Glomus versiforme*	129.3[a]	4.38[ab]	16.3[ab]	0.80[a]	565[b]	7.40[b]
	Control	112.4[a]	4.27[ab]	13.6[b]	1.00[a]	528[b]	7.58[b]

Humic acid application ($kg.ha^{-1}$)	Mycorrhizal inoculation	Plant dry weight (g)	Sepals yield per plant (g)	Biological yield ($kg.ha^{-1}$)	Economical yield ($kg.ha^{-1}$)	Harvest index (%)	Mycorrhizal frequency (%)
	Rhizophagus irregularis	40.7[a]	1.58[a]	8371[a]	198.3[a]	2.48[a]	70.8[c]
0	*Glomus versiforme*	24.5[ab]	1.06[a]	5043[b]	129.7[a]	2.56[a]	56.6[d]
	Control	17.9[b]	0.93[a]	4010[b]	125.5[a]	3.14[a]	32.5[f]
	Rhizophagus irregularis	26.8[ab]	1.41[a]	5529[ab]	165.9[a]	3.08[a]	91.6[a]
4	*Glomus versiforme*	26.3[ab]	1.09[a]	5417[ab]	144.0[a]	2.82[a]	80.3[b]
	Control	26.2[ab]	1.05[a]	4896[b]	134.7[a]	2.62[a]	45.0[e]

In each column, means with at least one similar letter are not significantly different ($P \leq 0.05$) based on Duncan test.

Application of humic substances improves soil fertility and amends soil properties such as soil aggregation and structure, pH buffering, cation exchange capacity, water holding capacity, bioavailability of immobile nutrients and reduction of heavy metal toxicity (Rose *et al*., 2014). Moreover, humic acid application can increase the amount of chlorophyll content; as a result enhance photosynthetic activities which consequently result in higher concentrations of non-structural sugars like starch and soluble sugars (Bettoni *et al*., 2014). In the same way, arbuscular mycorrhizal fungi through its hyphae structure can improve minerals absorption particularly phosphorus. In this way, the root can have contact with soil micro-pores, where the root hairs are not able to penetrate (Mohammadpour-Vashvaei *et al*., 2015).

Recently Nur-Amirah *et al*. (2015) showed that vegetative growth of Roselle plants (mainly its root) significantly increased by mycorrhizal colonization (a mixture of *Glomus sp*., *Gigaspora sp.* and *Scutellospora sp*) and consequently improved the quality of produced calyx. In another study, Aulia *et al*. (2009) revealed that mycorrhizal fungi has positive effects on fruit production and calyx yield of Roselle plants. Mycorrhizal fungi increase the absorbing surface of host plants root and thereby the ability of water uptake increases. Consistent with this, results of Subramanian *et al*. (2006) revealed that mycorrhizal tomato plants has more leaf relative water content in different levels of drought stress. In onion, mycorrhizal inoculation led to an increase in the amount of water accumulated in shoot tissues and enhanced the levels of soluble sugars which is possibly due to the enhanced photosynthesis in inoculated plants (Bettoni *et al*., 2014). Therefore, application of humic acid and mycorrhizal inoculation can increase the crop adaptability to drought stress and therefore can be recommended for Roselle production in arid areas. Moreover, our observations revealed that mycorrhizal profitability increases with humic acid application, where the amount of mycorrhizal frequency in roots of Roselle plants under humic acid application increased by 20.8%, 23.7% and 12.5% in RI, GV and control treatments, respectively (Table 6). Accordingly, Gryndler *et al*. (2005) reported that humic acid had a moderate stimulatory effect on root colonization by *G. claroideum* whilst development of the extra-radical mycelium was substantially increased.

Combined effects of deficit irrigation, humic acid application and mycorrhizal inoculation

Triple interaction effects of experimental factors significantly influenced fruit number, fruit weight, plant weight, biomass production, calyx yield and harvest index of Roselle plants (Table 3). Mycorrhizal inoculation using RI (*Rhizophagus irregularis*) and GV (*Glomus versiforme*) improved the plant height in all levels of water availability and humic acid application. Similarly, the number of lateral branches was increased by mycorrhizal symbiosis in both normal and deficit irrigation regimes. In overall, mycorrhizal infection using GV and humic acid consumption had partial positive influence on fruit production under deficit irrigation. However, the negative impacts of deficit irrigation on leaf and plant dry weights were reduced by mycorrhizal inoculation and to some extent by humic acid application. Inoculation with RI resulted in 33 and 38% increase in plant weight under normal and deficit irrigation, respectively. It has been well documented that arbuscular mycorrhizal fungi can stimulate the growth of many associated plants and also can contribute in enhancing plant tolerance to abiotic stresses such as drought (Abbaspour *et al*., 2012). Accordingly, the positive effects of mycorrhizal inoculation on reducing negative effects of drought stress in several plant species such as marigold (Asrar and Elhindi, 2011) thymus (Navarro-Fernandez *et al*., 2011) and pistachio (Abbaspour *et al*., 2012) have been reported.

Sepals yield per plant was increased when plants inoculated with mycorrhiza and supplemented with humic acid and irrigated after 100 mm pan-evaporation. The lowest quantity of Sepals yield was obtained in drought stress × no-inoculation × no-humic acid treatment (Table 7). On average, biomass production of Roselle plants was improved by mycorrhizal inoculation and humic acid application in both levels of water availability. For example, the amount of biomass was enhanced by 29% in plants inoculated with GV and supplemented with humic acid under deficit irrigation regime. Roselle plants produced higher economical yield under drought stress condition when the plant was inoculated by mycorrhizal fungi combined with humic acid application. In water deficit treatment, calyx yield was improved by 32%, 49% and 52% under humic acid, RI and GV applications, respectively. Finally, the highest harvest index of Roselle plants was observed under deficit irrigation combined with mycorrhizal inoculation and humic acid application (Table 7).

Table 7. Interaction effects of irrigation management, humic acid application and mycorrhizal inoculation on morphological parameters, yield and yield components and mycorrhizal symbiosis index in Roselle plants

Irrigation management (mm pan evaporation)	Humic acid application (kg.ha^{-1})	Mycorrhizal inoculation	Plant height (cm)	Lateral branches (No.plant^{-1})	Number of fruit per plant	Mean fresh weight of fruit (g)	Leaf area per plant (cm^2)	Leaf dry weight per plant (g)
100	0	*Rhizophagus irregularis*	149^{a}	7.10^{a}	31.8^{a}	1.58^{a}	1011^{a}	14.41^{a}
		Glomus versiforme	139^{ab}	3.88^{bcd}	14.8^{de}	1.14^{abc}	635^{b}	8.31^{bc}
		Control	130^{ab}	3.77^{bcd}	14.1^{de}	1.18^{ab}	655^{b}	7.24^{bcd}
	4	*Rhizophagus irregularis*	120^{abc}	5.88^{ab}	19.5^{b}	0.97^{bcd}	681^{b}	8.20^{bc}
		Glomus versiforme	136^{ab}	4.98^{abc}	17.4^{bcd}	1.03^{bcd}	527^{bc}	6.89^{bcd}
		Control	123^{abc}	5.88^{ab}	18.6^{bc}	1.14^{abc}	636^{b}	9.67^{b}
200	0	*Rhizophagus irregularis*	102^{bc}	4.55^{a-d}	12.5^{ef}	0.39^{e}	684^{b}	8.96^{b}
		Glomus versiforme	113^{abc}	3.88^{bcd}	08.5^{h}	1.22^{ab}	579^{b}	7.58^{bcd}
		Control	88^{c}	1.77^{d}	05.6^{h}	0.52^{de}	358^{d}	4.69^{d}
	4	*Rhizophagus irregularis*	115^{abc}	3.98^{bcd}	09.5^{fg}	0.64^{cde}	569^{b}	7.45^{bcd}
		Glomus versiforme	122^{abc}	3.77^{bcd}	15.1^{cde}	0.55^{de}	604^{b}	7.90^{bcd}
		Control	101^{bc}	2.66^{cd}	08.6^{gh}	0.85^{b-e}	419^{cd}	5.49^{cd}

Irrigation management (mm pan evaporation)	Humic acid application (kg.ha^{-1})	Mycorrhizal inoculation	Plant dry weight (g)	Sepals yield per plant (g)	Biological yield (kg.ha^{-1})	Economical yield (kg.ha^{-1})	Harvest index (%)	Mycorrhizal frequency (%)
100	0	*Rhizophagus irregularis*	41.8^{a}	2.52^{a}	12702^{a}	289.4^{a}	2.28^{bc}	73.3^{cd}
		Glomus versiforme	29.2^{bcd}	1.28^{bc}	5997^{bc}	157.5^{bc}	2.63^{abc}	56.6^{e}
		Control	26.0^{cde}	1.47^{b}	6005^{bc}	188.8^{b}	3.15^{ab}	33.3^{f}
	4	*Rhizophagus irregularis*	32.2^{bc}	2.37^{a}	6624^{b}	278.2^{bc}	2.69^{abc}	95.0^{a}
		Glomus versiforme	30.8^{bc}	1.26^{bc}	6295^{b}	136.0^{cd}	2.19^{c}	81.6^{bc}
		Control	36.6^{b}	1.60^{b}	6555^{b}	198.1^{b}	3.04^{abc}	40.0^{f}
200	0	*Rhizophagus irregularis*	19.7^{ef}	0.64^{def}	4040^{d}	107.2^{de}	2.67^{abc}	68.3^{d}
		Glomus versiforme	19.9^{ef}	0.84^{de}	4088^{d}	101.9^{def}	2.49^{bc}	56.6^{e}
		Control	09.7^{g}	0.39^{f}	2014^{e}	61.5^{f}	3.13^{abc}	31.6^{f}
	4	*Rhizophagus irregularis*	21.4^{def}	0.45^{ef}	4433^{cd}	153.6^{bc}	3.46^{a}	88.3^{ab}
		Glomus versiforme	21.9^{def}	0.93^{cd}	4538^{cd}	172.1^{bc}	3.45^{a}	80.0^{bc}
		Control	15.8^{fg}	0.51^{ef}	3237^{de}	71.2^{ef}	2.20^{bc}	50.0^{e}

In each column, means with at least one similar letter are not significantly different ($P \leq 0.05$) based on Duncan test.

Our findings in current experiment regarding positive effects of humic acid on drought stress are similar to those reported by Heidari and Minaei (2014) on borage and Yadollahi *et al*. (2015) on safflower. Results of a meta-analysis study indicated the positive response of the plant growth to humic substances, however it is influenced by many environmental and management factors such as source of humic substances, environmental conditions, type of plant, stress conditions, sort of application and the rate of humic substance (Rose *et al*., 2014). Humic substances contains large amount of nutrients which can interfere in osmotic adjustment by maintaining water absorption and cell turgor in drought-imposed plants. In addition, the enzymatic defense mechanism and alkaloids, phenols and tocopherols productions are stimulated by humic substances (Canellas *et al*., 2015). Moreover, humic substances have hormonal effects and chelating capacity that both are important factors positively affect plant growth particularly under nutrients starvation and water deficit conditions (Heidari and Minaei, 2014).

The positive effect of biofertilizers including *Azospirillum* sp., *Bacillus polymyxa* and a mixture of *Glomus* sp., *Gigaspora* sp. and *Scutellospora* sp. on growth, yield and quality of Roselle plants has been previously reported (Abo-Bake and Mostafa, 2011; Sembok *et al*., 2015). improvement of phosphorus nutrition by mycorrhizal fungi during the periods of water deficit and increased relative water content have been suggested as two important mechanisms by which drought tolerance of host plant is enhanced (Subramanian *et al*., 2006). This leaves one possible scenario that the enhanced acid phosphates activity in mycorrhizal plants provides more phosphorus to the leaves by which induce photosynthetic functionality (Bettoni *et al*., 2014). Arbuscular mycorrhiza fungi dominates drought stress by stimulating mechanisms including enhancement of water uptake via extraradical hyphae, improvement of nutrient uptake, development of root system, regulation of polyamine, improvement of osmotic adjustment, enhancement of antioxidant defense systems, induction of glomalin changes in soil structure, alteration in aquaporin expression, biocontrol of pathogens, adjustment of hormonal interactions and adjustment of molecular basis (Auge *et al*., 2007; Wu *et al*., 2013). Alternatively, regulation of plant genes encoding secondary responses and hormone metabolism is used by mycorrhizal host plants for enhancement of plant defenses and drought resistance. Asensio *et al*. (2012) proposed that in compare with non-essential isoprenoids (monoterpenes and sesquiterpenes), the amount of abscisic acid, chlorophylls and carotenoidsin (essential isoprenoids) are increased in leaf when plants are inoculated with, mycorrhizal fungi, particularly when inoculated plants are exposed to drought stress conditions. Moreover, Wu *et al*. (2006) revealed that mycorrhizal roots have lower levels of malondialdehyde, hydrogen peroxide and superoxide with higher values of superoxide dismutase, guaiacol peroxidase and glutathione reductase. Interestingly, these compounds reduce reactive oxygen damages and thereby increase the drought resistance in plants.

Conclusion

Results of this study revealed that Roselle plants grown under semiarid climatic conditions were strongly affected by drought stress. Water deficit significantly restricted the growth and yield of both non-mycorrhizal and mycorrhizal plants. However mycorrhiza colonization exerted a positive effect on Roselle plants growth and yield, under deficit irrigation. Morphological parameters, yield and yield components were reduced in non-mycorrhizal plants when exposed to drought-stress condition.. This suggests that mycorrhizal inoculation could be a suitable strategy for Roselle production in areas which affected by drought stress. In addition, humic acid application positively affects the growth and yield performance of Roselle plants under water deficit condition. Furthermore, seed inoculation of Roselle by mycorrhizal fungi and humic acid application during vegetative growth considerably increased the root mycorrhizal symbiosis index. Taken together, despite of lower yield of Roselle plants under deficit irrigation; here we suggest the arbuscular mycorrhizal inoculation and humic acid application as two feasible approaches which considerably increase the water use efficiency and subsequently drought stress resistance in Roselle plants in arid and semi-arid areas.

Acknowledgments

We wish to thank Vice President for Research and Technology, University of Birjand, Iran for the financial support of this project (Grant number: 1394/D/26676).

References

1. Abbas, Y., M. Ducousso, M. Abourouh, R. Azcon, and R. Duponnois. 2006. Diversity of Arbuscular Mycorrhizal Fungi in *Tetraclinis articulate* (Vahl) Masters Woodlands in Morocco. Ann. For. Sci. 63: 285-291.

2. Abbaspour, H., S. Saeidi-Sar, H. Afshari, and M.A. Abdel-Wahhab. 2012. Tolerance of Mycorrhiza Infected Pistachio (*Pistacia vera* L.) Seedling to Drought Stress under Glasshouse Conditions. J. Plant Physiol. 169: 704-709.

3. Abo-Bake, A.A., and G.G. Mostafa. 2011. Effect of Bio-and Chemical Fertilizers on Growth, Sepals Yield and Chemical Composition of *Hibiscus sabdariffa* at New Reclaimed Soil of South Valley Area. Asian J. Crop Sci. 3(1): 16-25.

4. Ahanger, M.A., A. Hashem, E.F. Abd-Allah, and P. Ahmad. 2014. Arbuscular Mycorrhiza in Crop Improvement under Environmental Stress, pp 69-95. In: Ahmad P. (eds) Emerging Technologies and Management of Crop Stress Tolerance. Elsevier Publication.

5. Asensio, D., F. Rapparini, and J. Penuelas. 2012. AM Fungi Root Colonization Increases the Production of Essential Isoprenoids vs. Nonessential Isoprenoids Especially under Drought Stress Conditions or After Jasmonic Acid Application. Phytochem. 77: 149-161.

6. Asrar, A.A., and K.M. Elhindi. 2011. Alleviation of Drought Stress of Marigold (*Tagetes erecta*) Plants by Using Arbuscular Mycorrhizal Fungi. J. Biologic. Sci. 18: 93-98.

7. Auge, R.M., H.D. Toler, J.L. Moore, K. Cho, and A.M. Saxton. 2007. Comparing Contributions of Soil Versus Root Colonization to Variations in Stomatal Behavior and Soil Drying in Mycorrhizal *Sorghum bicolor* and *Cucurbita pepo*. J. Plant Physiol. 164: 1289-1299.

8. Aulia, R.A., O. Mohamad, M. Marlina, H. Rasli, S.N. Fuad, and A.I. Zainal. 2009. Effects of Mycorrhiza on Productivity of Roselle. Congr. of the 8th Malaysia Congress on Genetics, August 4-6, Malaysia.

9. Babatunde, F.E., and A.L.E. Mofoke. 2006. Performance of Roselle (*Hibiscus sabdariffa* L.) as Influenced by Irrigation Schedules. Pak. J. Nutr. 5(4): 363-367.

10. Bakry, B.A., M.H. Taha, Z.A. Abdelgawad, and M.M.S. Abdallah. 2014a. The Role of Humic Acid and Proline on Growth, Chemical Constituents and Yield Quantity and Quality of Three Flax Cultivars Grown Under Saline Soil Conditions. Agric. Sci. 5: 1566-1575.

11. Bakry, B.A., O.M. Ibrahim, A.R. Eid, and E.A. Badr. 2014b. Effect of Humic Acid, Mycorrhiza Inoculation and Biochar on Yield and Water Use Efficiency of Flax Under Newly Reclaimed Sandy Soil. Agric. Sci. 5: 1427-1432.

12. Bettoni, M.M., A.F. Mogor, V. Pauletti, and N. Goicoechea. 2014. Growth and Metabolism of Onion Seedlings as Affected by The Application of Humic Substances, Mycorrhizal Inoculation and Elevated CO_2. Sci. Horti. 180: 227-235.

13. Canellas, L.P., F.L. Olivares, N.O. Aguiar, D.L. Jones, A. Nebbioso, P. Mazzei, and A. Piccolo. 2015. Review Humic and Fulvic Acids as Biostimulants in Horticulture. Sci. Horti. 196: 15-27.

14. Fallahi, H.R., M. Ghorbany, M. Aghhavani-Shajari, A. Samadzadeh, and A.H. Asadian. 2017. Qualitative Response of Roselle to Planting Methods, Humic Acid Application, Mycorrhizal Inoculation and Irrigation Management. J. Crop Improve. In Press.

15. Fallahi, H.R., R. Taherpour, M. Aghhavani-Shajari, and M.G. Soltanzadeh. 2015. Effect of

Super Absorbent Polymer and Deficit Irrigation on Water Use Efficiency, Growth and Yield of Cotton. Not. Sci. Biol. 7(3): 338-344.

16. Fasoyiro, S.B., S.O. Babalola, and T. Owosibo. 2005a. Chemical Composition and Sensory Quality of Fruit-flavoured Roselle (*Hibiscus sabdariffa*) Drinks. World J. Agric. Sci. 1(2): 161-164.

17. Fasoyiro, S.B., S.O. Babalola, and T. Owosibo. 2005b. Chemical and Storability of Fruit-Flavoured (*Hibiscus sabdariffa*) Drinks. World J. Agric. Sci. 1(2): 165-168.

18. Gendy, A.S.H., H.A.H. Said-Al Ahl, and A.A. Mahmoud. 2012. Growth, Productivity and Chemical Constituents of Roselle (*Hibiscus sabdariffa* L.) Plants as Influenced by Cattle Manure and Biofertilizers Treatments. Australian J. Basic. Appl. Sci. 6(5): 1-12.

19. Gryndler, M., H. Hrselová, R. Sudová, H. Gryndlerová, V. Rezácová, and V. Merhautová. 2005. Hyphal Growth and Mycorrhiza Formation by the Arbuscular Mycorrhizal Fungus *Glomus claroideum* BEG 23 is Stimulated by Humic Substances. Mycorrhiza. 15(7): 483-8.

20. Gumus, L. and C. Seker. 2015. Influence of Humic Acid Applications on Modulus of Rupture, Aggregate Stability, Electrical Conductivity, Carbon and Nitrogen Content of a Crusting Problem Soil. Solid Earth. 6: 1231-1236.

21. Hazzoumi, Z., Y. Moustakime, E.H. Elharchli, and K.A. Joutei. 2015. Effect of Arbuscular Mycorrhizal fungi (AMF) and Water Stress on Growth, Phenolic Compounds, Glandular Hairs, and Yield of Essential Oil in Basil (*Ocimum gratissimum* L). Chem. Biol. Tech. Agric. 2: 1-11.

22. Heidari, M., and A. Minaei. 2014. Effects of Drought Stress and Humic Acid Application on Flower Yield and Content of Macro-elements in Medical Plant Borage (*Borago officinalis* L.). J. Plant Prod. Res. 21(1): 167-182.

23. Keshavarz Afshar, R. M.R. Chaichi, M.H. Assareh, M. Hashemi, and A. Liaghat. 2014. Interactive effect of deficit irrigation and soil organic amendments on seed yield and flavonolignan production of milk thistle (*Silybum marianum* L. Gaertn.). Ind. Crops Product. 58: 166-172.

24. Khaled, H. and H. Fawy. 2011. Effect of Different Levels of Humic Acids on the Nutrient Content, Plant Growth, and Soil Properties under Conditions of Salinity. Soil. Water Res. 6(1): 21-29.

25. Khalil, S.E., and A.S. Abdel-Kader. 2011. The Influence of Soil Moisture Stress on Growth, Water Relation and Fruit Quality of *Hibiscus sabdariffa* L. Grown within Different Soil Types. Natu. Sci. 9(4): 62-74.

26. Khalil, S.E., and R.M.M. Yousef. 2014. Study the Effect of Irrigation Water Regime and Fertilizers on Growth, Yield and Some Fruit Quality of *Hibiscus sabdariffa* L. Inter. J. Adv. Res. 2(5): 738-750.

27. Koocheki, A., H.R. Fallahi, M.B. Amiri, and H.R. Ehyaei. 2016. Effects of Humic acid Application and Mother Corm Weight on Yield and Growth of Saffron (*Crocus sativus* L.). J. Agroecol. 7(4): 1-18.

28. Mohammadpour-Vashvaei, R., A. Ghanbari, and B.A. Fakheri. 2015. Effect of Combined Feeding System on N, P and K Concentration, Biochemical Characteristics and Calyxes Yield of Roselle (*Hibiscus sabdariffa* L.). Iranian J. Filed Crop Sci. 46(3): 497-518.

29. Mohd-Esa, N., F.S. Hern, A. Ismail, and C.L. Yee. 2010. Antioxidant Activity in Different Parts of Roselle (*Hibiscus sabdariffa* L.) Extracts and Potential Exploitation of the Seeds. Food Chem. 122: 1055-1060.

30. Navarro-Fernandez, C.M., R. Aroca, and J.M. Barea. 2011. Influence of Arbuscular Mycorrhizal Fungi and Water Regime on the Development of Endemic *Thymus* Species in Dolomitics Soils. Appl. Soil Ecol. 48: 31-37.

31. Nemati, M., and M. Dahmardeh. 2015. Effect of Application of Bio-Fertilizers and Organic Manure on Yield and Morphological Index of Roselle (*Hibiscus sabdariffa* L.). J. Agroecol. 7(1): 135-136.

32. Nur-Amirah, Y., A.A. Alias, and W.S. Wan Zaliha. 2015. Effects of Mycorrhizal Inoculation on Growth and Quality of Roselle (*Hibiscus sabdariffa* L.) Grown in Soilless Culture System. Malaysian Appl. Biol. 44(1): 57-62.

33. Mandour, M.S., E.N. Abou-Zied, and M. Hassib. 1979. Effect of Irrigation Treatment upon the Chemical Constituents of *Hibiscus sabdariffa* L. Plant. Soil. 52: 485-490.

34. Philips, J.M., and D.S. Hayman. 1970. Improved Procedures Clearing Root and Staining Parasitic and Vesicular Arbuscular Mycorrhizal Fungi for Rapid Assessment of Infections. J. Trans. British Mycol. Soci. 55(1): 158-161.

35. Rahbarian, P., G. Afsharmanseh, and N. Modafea Behzadi. 2011. Effect of Drought Stress as Water Deficit and Planting Density on Yield of Roselle (*Hibiscus sabdariffa*) in Jiroft region. New Find. Agric. 5(3): 249-257.

36. Rose, M.T., A.F. Patti, K.R. Little, A.L. Brown, W.R. Jackson, and T.R. Cavagnaro. 2014. A Meta-Analysis and Review of Plant-Growth Response to Humic Substances: Practical Implications for Agric. Advan. Agron. 24: 37-89.

37. Sanjari-Mijani, M., A. Sirousmehr, and B.A. Fakheri. 2015. The Effects of Drought Stress and Humic Acid on Some Physiological Characteristics of Roselle (*Hibiscus sabdariffa*). Agric. Crop. Manag. 17(2): 403-414.

38. Sanjari-Mijani, M. 2014. Effect of Humic Acid and Drought Stress on Qualitative and Quantitative Indices of oselle (*Hibiscus sabdariffa*) Medicinal Plant. Univ. of Zabol, M.Sc. Thesis.

39. Satyanarayana, N.H., S. Mukherjee, S. Roy, B. Priya, K.K. Sardar, and P. Bandhopashyay. 2015. Genetic Divergence Studies for Fibre Yield Traits in Roselle (*Hibiscus sabdariffa* l.) in Terai Zone of West Bengal. J. Crop. Weed. 11: 90-94.

40. Seghatoleslami, M.J., S.G. Mosavi, and T. Barzegaran. 2013. Effect of Irrigation Levels and Planting Date on Yield and Water Use Efficiency of *Hibiscus sabdariffa* L. Iranian J. Med. Arom. Plants. 29(1): 144-156.

41. Sembok, W.W., N. Abu-Kassim, Y. Hamzah, and Z.A. Rahman. 2015. Effect of Mycorrhizal Inoculation on Growth and Quality of Roselle (*Hibiscus sabdariffa* L.) Grown in Soiless Culture System. Malaysian Appl. Biol. 44(1): 57-62.

42. Shirzad, H., and M. Ghorbany. 2015. Survey of Arbuscular Mycorrhizal Fungi Symbiosis with Cotton Root in North Khorasan Province. Iranian J. Cotton Res. 2(2): 1-12.

43. Sonar, B.A., V.R. Kamble, and P.D. Chavan. 2013. Native AM Fungal Colonization in Three *Hibiscus* Species Under NaCl Induced Salinity. J. Pharm. Biol. Sci. 5(6): 7-13.

44. Subramanian, K.S., P. Santhanakrishnan, and P. Balasubramanian. 2006. Responses of Field Grown Tomato Plants to Arbuscular Mycorrhizal Fungal Colonization under Varying Intensities of Drought Stress. Sci. Horti. 107: 245-253.

45. Wu, Q.S., A.K. Srivastava, and Y.N. Zou. 2013. AMF-induced Tolerance to Drought Stress in Citrus: A Review. Sci. Horti. 164: 77-87.

46. Wu, Q.S., Y.N. Zou, and R.X. Xia. 2006. Effects of Water Stress and Arbuscular Mycorrhizal Fungi on Reactive Oxygen Metabolism and Antioxidant Production by Citrus (*Citrus tangerine*) Roots. Europ. J. Soil Biol. 42: 166-172.

47. Yadollahi, P., M.R. Asgharipour, N. Kheiri, and A. Ghaderi. 2015. Effects of Drought Stress and Different Types of Organic Fertilizers on the Yield and Yield Components of Safflower (*Carthamus tinctorius* L.). J. Oil Plants Prod. 1(2): 27-40.