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# Increased fatty acids of Purslane by nitrogen and Arbuscular Mycorrhiza under drought stress

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**Abstract**
To investigate combined effect of nitrogen and mycorrhiza under water deficit stress on the purslane, a factorial split experiment based on a randomized complete block design with three replications was conducted in a semi-arid region of Iran in 2015 and 2016. The main plots were factorial combination of two irrigation conditions (non-stressed control and stressed condition) and two treatments, i.e. control and inoculation with arbuscular mycorrhiza fungi (AMF, *Rhizophagus irregularis*). The subplots consisted of unfertilized control, 100% farmyard manure (8.5 Mg FYM $ha^{-1}$), 100% N (120 kg urea $ha^{-1}$), 75% FYM and 25% urea, 50% FYM and 50% urea, 25% FYM and 75% urea. In both of years, the drought stress reduced AMF colonization (by 30.3 and 15.3%), phosphorous content in purslane, while contributing to the production of saturated and unsaturated fatty acids. The AMF increased unsaturated fatty acid content of leaf in purslane. Application of nitrogen fertilizers enhanced phosphorous uptakes and fatty acid contents of the leaf under both irrigation conditions. It can be stipulated that the treatments with mixed application of FYM, urea and mycorrhiza tend to not only produce the highest values for qualitative and quantitative traits, but also reduce the need for water and nitrogen fertilizer.
**Keywords**: Colonization, Fatty acid, Manure, Urea, Water stress

## Introduction

Purslane (*Portulaca oleracea* L.) is an annual C4 plant in the family Portulacaceae. Acknowledged as one of the most important medicinal herbs, purslane has been called "Global Panacea" by the World Health Organization (Jin *et al*., 2015; D'Andrea *et al*., 2015). Purslane is a rich source of omega-3 (α-linolenic acid) and omega-6 (linoleic acid) fatty acid. Thanks to the abundance of unsaturated fatty acids, particularly omega-3 instances, and the presence of antioxidants in the composition of the plant, its consumption by humans can neutralize free radicals and hence strengthen the immune system, thereby preventing cardiovascular diseases, cancers, asthma, type-I

diabetes, and infectious diseases. These advantages have made the plant an excellent vegetable for a healthy diet for humans (Montoya-García et al., 2018).

In terms of distribution, purslane can be found worldwide and have been seen to grow well under various meteorological conditions. The widespread distribution of the lands where the plant is grown will expose the plant to abiotic stresses including the water deficit stress during the growth season (Jin *et al*., 2015). Researchers have reported that purslane can tolerate high temperatures and moisture stress. This characteristic is controlled by multiple mechanisms (i.e., antioxidant production, secondary metabolites, increased expression of the protein and lipid metabolism genes, and a change of carbon stabilization state from C4 to Crassulacean (CAM)), which make the plant suitable for growing in arid and semi-arid regions of the world (Jin *et al*., 2015; D'Andrea *et al*., 2015; Montoya-García *et al*., 2018). Furthermore, upon irrigation and elimination of the unfavorable conditions, the plant is capable of switching back to the C4 photosynthesis system to not only compensate for the delayed growth, but also further proceed to grow (D'Andrea *et al*., 2014). Growing medicinal herbs under stress conditions represents a solution for increasing medicinal contents and secondary metabolites in medicinal herbs. However, it should be noticed that, the increase in the content of such composition may be offset by a decrease in the produced biomass or crop yield, making the approach economically inefficient (Baghbani-Aran *et al*., 2017).

Given adverse environmental effects of the ever-increasing consumption of industrial inputs such as chemical fertilizers, the importance of sustainable agriculture in human health follows an ever increasing trend, particularly when it comes to the production of medicinal herbs (Baghbani-Arani *et al*., 2017). In this respect, application of organic fertilizers has gained attention as a suitable approach toward sustainable agriculture (Baghbani-Arani *et al*., 2017). Long-term studies have shown that, continuous application of chemical fertilizers reduces the yield of agronomic plants due to degradation of desirable physical and chemical soil properties and lack of micro-nutrients in the composition of such fertilizers. Animal manure can not only provide the plant with the required nutrient elements during the growth season, but also improve biological activities and physiochemical properties of soil, and attenuate the impacts of water deficit by enhancing water-retention capacity of the soil (Jalilian *et al*., 2012). Montoya-García *et al*. (2018) suggested that fertilization can maximize medicinal contents, unsaturated fatty acids and antioxidant capacity of purslane.

Application of biotic fertilizers such as mycorrhiza in a sustainable agriculture-based system not only keeps the environment healthy, but also enhances the quality and sustainability of the yield, especially in the production of medicinal herbs (Safari Sinegani and Elyasi Yeganeh, 2017). Accordingly, the plants treated with such fungi exhibit better growth characteristics and crop yield along with higher water efficiency and tolerance to biotic and abiotic stresses due to enhanced uptake of nutrients and water from soil (Grover *et al*., 2010).

It seems necessary to identify and domesticate the medicinal herbs with high tolerance to adverse environmental conditions while providing sustainable and reasonable yield, so as to promote organized growth of such plants and enhance their yield to achieve sustainable agriculture and produce healthy and high-quality products. As such, the present experiment was design to investigate combined effect of different fertilizers and mycorrhizal symbiosis on tolerance to water deficit stress, and their contributions into quantitative and qualitative traits of purslane as a medicinal herb.

## Material and method

Characteristics of the site of experiment

A two-year field experiment was performed at a farm located in Zavarian Village, 8 km away from Salafchegan Township, Qom Province, Iran (50°24´E, 34°26´N, altitude: 1500 m above mean sea level) in 2015 and 2016. According to available meteorological information retrieved from the closest meteorological station in the city of Salafchegan, the region has a semi-arid climate with the long-term mean annual precipitation of 210mm and annual mean, maximum, and minimum temperatures of

14.8℃, 36℃, and 2℃, respectively. Fig. 1 depicts the daily meteorological parameters recorded during the growth season in the two years studied herein. Two months prior to the experiment, soil samples were taken to determine physiochemical characteristics of the soil, with the results presented in Table 1.

**Agronomic practices and experimental design**

A factorial split experiment was arranged in a randomized complete block design with three replications. The main plots were factorial combination of two irrigation conditions (non-stressed control and stressed condition: irrigation at 70% and 50% of field capacity (FC), respectively) and two treatments, i.e. control and inoculation with arbuscular mycorrhiza fungi (AMF, *Rhizophagus irregularis*). The subplots consisted of unfertilized control, 100% farmyard manure (8.5 Mg FYM $ha^{-1}$, sheep manure (50%) and chicken manure (50%)), 100% N (120 kg urea $ha^{-1}$), 75% FYM and 25% urea, 50% FYM and 50% urea, 25% FYM and 75% urea. Plots were 4m in length and consist of five rows. Distances between main and sub plots were 2- and 1.2-m, respectively. Seeds were planted manually in June into a depth of 1-2 cm along 40 cm-spaced rows, with the spacing between successive seeds on the same line being 25 cm.

As an inoculum, *Rhizophagus irregularis* was used to apply the arbuscular mycorrhizal fungi treatment. Procured from Zist Fanavar Turan Company (Shahrood, Iran), the inoculum was commercially designated as Mycopersica and comprised of a mixture of spore (50-150 living fungal spores $g^{-1}$ soil), hyphae, mycorrhizied plant roots, and hyphae of the mycorrhizal fungi (20-50 g $g^{-1}$ soil). A minimum of 100 g of the inoculum per square meter of area was applied to the soil at planting. Fresh root subsamples were cleared in 10% KOH solution and then washed with 1 N HCl, and stained in trypan blue solution for 30 min and then washed (Philips and Hayman, 1970). The gridline intercept method was used to measure the AM colonization (Giovannetti and Mosse, 1980).

All experimental plots were fully irrigated until 10-15-leaf stage (30 days after planting, DAP). The irrigation treatment was applied based on field capacity (FC), permanent wilting point (PWP), and soil moisture, where the soil moisture at a depth of 30 cm was obtained using a time-domain reflectometer (TDR). Equations (1) and (2) were used to obtain the water volume used to irrigate each of the plots (Mokhtassi-Bidgoli *et al*., 2013).

(1) $$\mathrm{MAD} = \left(\frac{\mathrm{FC} - \theta}{\mathrm{FC} - \mathrm{PWP}}\right)$$

where MAD is the maximum allowable drainage, FC is the volumetric soil moisture content at field capacity, $\theta$ is the volumetric soil moisture measured by TDR, and PWP is the volumetric soil moisture at permanent wilting point. The required volume of irrigation water was calculated from the following two equations:

(2) $$ASW = \mathrm{FC} - \mathrm{PWP}$$

$$Vd = \mathrm{MAD} \times \mathrm{ASW} \times \mathrm{Rz} \times 10$$

where ASW is the available soil water, $V_d$ is the irrigation water volume in mm, $R_z$ is the root zone depth (30 cm), and 10 is a constant for unit conversion (cm to mm). Accordingly, the irrigation water volume was measured through a counter.

To prepare and extract samples (1 g), extraction liquid (10ml 80% methanol-water) was added into the samples and extracted during 1 h at 50ºC. The extraction was done in closed vessels to inhibit loss of the extraction liquid. After extraction process, the eluate was filtered.

To obtain the profile of fatty acids content of purslane leaf, oil extraction was performed via cold-solvent method to minimize the damage to temperature-sensitive fatty acids. In order to extract the oil from the sample, chloroform-methanol mixture with a mixing ratio of 1:2 was utilized. For this purpose, the powdered plant sample was mixed with the solution at a mixing ratio of 1:10 and stirred for 24 hr. Subsequently, suspended particles were eliminated by centrifugation at 3,800 rpm for 15 min and the solvent was separated using a rotating evaporator at 40℃, followed by examining the sample for fatty acid composition. The fatty acid profile was determined using gas chromatography (GC). The GC apparatus used in the present research was equipped with a flame ionization detector (FID) and a fused-

silica capillary column (length: 50 m, diameter: 0.25 mm, operating temperature range: 150-240℃). Serving as mobile phase gas, hydrogen was flown at 1.2 ml/min, with nitrogen serving as support gas ad flown at a flowrate of 30 ml/min. The fatty acids were identified and measured based on the extracted peaks compared to those of standard samples of fatty acids (Liu *et al.*, 2000).

**Statistical analysis**

All of the statistical analyses were performed utilizing SAS v. 9.2 software (SAS Institute Inc., 2002). The Bartlett's test indicated that, for most of the studied traits, the variance was not uniform between different years, so that the data pertaining to each year was analyzed separately. When the *F*-test was significant, comparisons between treatment means were analyzed via the least significant difference (LSD) at a significance probability level of 0.05. When two-way or three-way interaction among different treatments went significant for a trait, their main effects were no more referred to or interpreted (Baghbani-Arani *et al.*, 2017).

## Results and discussion

**AMF colonization**

In each of the two years of experiment, the three-way interaction among irrigation, AMF, and fertilizing factors on the AMF colonization was significant (Table 3). In both years, minimum AMF colonization was achieved in the treatments exposed to water deficit stress (Table 4). In addition, the highest AMF colonization was achieved with the mixed fertilizer treatment (75% FYM + 25% urea) (Table 4). In agreement with the results of the present research, a study on 48 species of medicinal herbs (of a total of 9 families) in Hamedan (Iran), the symbiosis rate between AMF and purslane root was reported to be 30% (Safari Sinegani and Elyasi Yeganeh, 2017). Various researchers have referred to the infection of plant roots with the AMF under drought stress (Soleymani and Pirzad, 2016; Habibzadeh *et al.*, 2013; Al-Karaki *et al.*, 1998). An important indicator of the activity of AMF is the colonization rate of the root system of the plant by such fungi, which is affected by a wide spectrum of factors including apparent and structural characteristics of the root system, quantity and quality of the root exudates, application of phosphorus fertilizers, and high concentration of heavy elements (Al-Karaki *et al.*, 1998). It has been also reported that the highest colonization percentage with the application of AMF could be achieved when the plant is not affected by water deficit stress (Wu and Xia, 2006). Upon a decrease in soil moisture, quantity and quality of the root exudates changes, thereby affecting the spore germination directly (Wu and Xia, 2006). Smith and Read (2008) reported that, the reduction observed in the growth of subaerial organs of plants under soil moisture stress conditions can be a result of the reduced root colonization which leads to reduced uptake of nutrients. Moreover, it was reported that a mixture of AMF and a chemical fertilizer (NPK) produced the lowest root colonization percentage (RCP) of sorghum without fungal inoculation (control), while the highest RCP was observed with the mixed FYM and nitroxin (Kamaei *et al.*, 2016). In another research, Bilalis *et al.* (2015) showed that the RCP associated with AMF was higher in all four chickpea cultivars with application of FYM, as compared to chemical fertilizer, thanks to enhanced microbial biomass and improved biological structure of the soil.

**Leaf phosphorous content**

Analysis of variance (ANOVA) showed that, phosphorous content of purslane leaf affected by all main effects of the experimental factors as well as their two-way interactions, with the only exception being the interaction between irrigation and AMF in both of the studied years and also the interaction between AMF and fertilizer in the first year (Table 3). In both years, the highest and lowest phosphorous contents of the leaf were observed in the non-stressed control mixed with 25% FYM + 75% urea and those with water deficit stress without applying any fertilizer, respectively, so that the water deficit stress reduced phosphorous content of the plant in both years (5.8 and 7.7%) (Table 5). Moreover, application of AMF, either separately or in combination with fertilizer can enhance phosphorous uptake of the plant (Table 6 and Fig. 2), so that the application of AMF enhanced

phosphorous uptake of the leaf by more than 7% (Table 6).

Given that nutrients tend to exhibit reduced mobility under water deficit stress, the AMF can impose significant effects on the growth of plant under drought stress, as compared to normal irrigation system (Boomsma and Vyn, 2008). The uptake and mass transfer mechanisms in soil and the plant (e.g., diffusion, mass flow, and osmosis) are functions of the moisture contents of soil and root, so that the mechanisms are disturbed upon a deficit in soil water/moisture content, thereby limiting the photosynthesis process which ends up with reduced growth and crop yield (Izzo *et al.*, 1991).

The main advantage of AMF over non-mycorrhizal plants is the extension of nutrient uptake zone by the mycorrhizal roots. It has been estimated that 80% of phosphorous uptake by the plant is performed via the AMF. This fungus further improves nitrogen, potassium magnesium, copper, and zinc uptake in poor soils (Rillig, 2004).

**Fatty acid profile**

ANOVA indicated that, in both years, the three-way interaction of irrigation, fertilizer and AMF on total contents of saturated and unsaturated fatty acids of the purslane leaf was significant (Table 3). In both years, the highest saturated fatty acid (palmitic and stearic acids) contents of the purslane leaf was obtained with the water deficit stress and application of AMF and only urea, while the lowest value was produced under the non-stressed control with application of AMF and 25% FYM+75% urea (Table 4). However, the lowest palmitic acid content in the second year was obtained under the non-stressed control with application of AMF and 75% FYM+25% urea, although its difference to the treatment non-stressed control with application of AMF and 25% FYM+75% urea was not significant (Table 4). Moreover, in both years of the study, the treatment combinations of water deficit stress with application of AMF and 25% FYM+75% urea, and water deficit stress without application of AMF and fertilizer produced the highest and lowest contents of unsaturated fatty acids (linoleic and linolenic acids) in the purslane leaf, respectively. An increase in the content of saturated and unsaturated fatty acids in the leaf was observed with decreasing the soil water content (Table 7). However, application of only urea and 25% FYM+75% urea enhanced the contents of saturated and unsaturated fatty acid contents in the purslane leaf (Table 4). Regardless of treatments, fatty acid composition of the purslane leaf was in the order of α-linolenic acid > palmitic acid > linoleic acid > stearic acid. In agreement with the results obtained in this research, Montoya-García *et al.* (2018) reported maximum accumulation of fatty acids of the purslane in its leaf, with its profile being composed of two saturated fatty acids, namely palmitic acid (C16:0) and stearic acid (C18:0), and two unsaturated fatty acids, namely linoleic acid (18:2, ω6) and α-linolenic acid (18:3, ω3), where the α-linolenic acid and palmitic acid were the dominant components (Petropoulos *et al.*, 2016; Montoya-García *et al.*, 2018).

In a research on purslane, D'Andrea *et al.* (2015) showed that, expression of the genes involved in encoding a ω6-fatty acid desaturase increased under drought stress condition.

Environmental conditions during the growth season (particularly during late growth stage) can impose extensive impacts on the content and composition of unsaturated fatty acids (Sezen *et al.*, 2011). As can be inferred from Fig. 1 and Tables 4 and 7, higher temperature during the growth season in the first year decreased the amount of unsaturated fatty acids. It has been suggested that high temperature induced decrease in the content of unsaturated fatty acids (e.g., linoleic acid) by a reduction in the activity of oleat desaturase enzyme. This enzyme has a high sensitivity to temperature and its activity decreases at high temperatures (Schneiter and Miller, 1981). Sezen *et al.* (2011) reported larger saturated/unsaturated fatty acid content ratios for sunflower in a relatively drier year, as compared to a relatively wetter year. Moreover, they found that larger saturated fatty acid contents (palmitic and stearic acids) were more sensitive to water deficit stress, so that an increase in the severity of the drought stress further added to the contents of stearic acid.

Application of nitrogen fertilizer resulted in enhanced content of fatty acids (unsaturated fatty acids, in particular) in the purslane

(Montoya-García *et al.*, 2018). In a research on the effect of nitrogen sources on the oil content and fatty acid composition of sunflower, it was shown that, application of animal manure in combination with chemical fertilizer increased the contents of unsaturated fatty acids, in particular linoleic acid (Shoghi-Kalkhoran *et al.*, 2103).

It can be proposed that, organic (animal-sourced and AMF) and mixed (chemical and animal-sourced) nutrient systems have provided the plant with all of the required elements during the growth season. Indeed, at the start of the growth season, nitrogen content of chicken manure is released and accelerates vegetative growth of the plant which ends up with larger green leaf area. Subsequently, nitrogen release from sheep manure further fuels the photosynthesis process to achieve even improved vegetative growth, so that not only biomass of the plant, but also oil content of the leaf increases. Under water deficit stress, the purslane is likely to face problems in regarding uptake of nutrient elements, especially nitrogen and phosphorous. In this case (limited availability of necessary elements), the plant increases the synthesis of carbon-based compounds via, for example, biosynthesis of secondary metabolites (e.g., oils) in its tissues as a response to abiotic stresses, and given that a large portion of the oil is composed of fatty acid chains, then an increase in the content of oil under abiotic stress conditions (drought and nitrogen deficit) is expectable (Montoya-García *et al.*, 2018). Moreover, from the results, it can be inferred that, negative impacts of water deficit condition on TDM of purslane is offset against enhanced medicinal quality (unsaturated fatty acids) regarding the higher content of the α-linolenic fatty acid compared to other fatty acids and larger effect of water deficit stress on unsaturated fatty acids.

It is worth noting that, higher concentration of secondary metabolites and medicinal contents of plants under stress conditions may not necessarily end up with economic advantages, because the higher concentration is often times accompanied with the smallest biomass (Baghbani-Arani *et al.*, 2017).

## Conclusion

Although purslane is classified as a weed in some cultures, it exhibits much resistance to tough environmental conditions, is rich in unsaturated fatty acids (ω3 and ω6), vitamins, minerals, and antioxidants in its vegetative organs and seeds, making it a valuable agronomic-medicinal herb, especially for arid regions of the world. As such, the significance of this research is doubled for identification of the behavior of this plant in response to agronomic treatments to obtain suitable composition of chemical, organic, and biological fertilizers and their interactions with the aim of enhancing the health state of usable vegetative organs of the purslane while maximizing its secondary medicinal contents.

Limited water reduced the fungal colonization, phosphorus content of the leaf as well as total dry matter of purslane, but rather increased secondary medicinal metabolites (saturated and unsaturated fatty acids) in the purslane leaves in both years. Nitrogen application, especially with mixed organic and chemical inputs could decrease lipid peroxidation by boosting the activity of antioxidant enzymes under drought condition. Increasing the fungal colonization with the purslane root, application of AMF increases water, phosphorous and nitrogen uptake and alleviates negative impacts of water deficit stress, there by improving quantitative (total dry weight) and qualitative traits of purslane. Finally, it can be concluded that, negative impacts of water deficit stress on total dry matter of purslane is compensated by the increase in the medicinal quality (unsaturated fatty acids (α-linolenic and linoleic acids).

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**Table 1. Physico-chemical properties of the experimental soil before the beginning of the experiment.**

| Characteristics | Soil texture | EC (dS.m$^{-1}$) | pH | Organic Matter (%) | Bulk density (g.cm$^{-3}$) | Total nitroge (%) | Available K (mg.kg$^{-1}$) | Available P (mg.kg$^{-1}$) | Total P (mg.kg$^{-1}$) | PWP (% by volume) | FC (% by volume) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Loamy | 3.8 | 8.14 | 1.59 | 1.5 | 0.095 | 372.6 | 9 | 25 | 31 | 14 |

**Table 2. Chemical characteristics of animal manure (FYM) used in experiment.**

| Manure | EC (dS.m$^{-1}$) | pH | P (%) | K (%) | N (%) | Organic carbon (%) | Organic matter (%) | C/N |
|---|---|---|---|---|---|---|---|---|
| Sheep | 12.66 | 8.28 | 0.39 | 3.8 | 1.43 | 20.1 | 34.57 | 14 |
| Chicken | 7.88 | 7.02 | 0.69 | 1.3 | 2.08 | 17.6 | 30.2 | 8.46 |

**Table 3. Analysis of variance (mean squares) for the effects of irrigation (I), arbuscular mycorrhiza fungi (AMF) and fertilizer (F) on P uptake and fatty acid in purslane leaves in 2015 and 2016**.

| S.0.V 2015 | df | AMF colonization | Phosphorus | Palmitic acid | Stearic acid | Linoleic acid (ω6) | α-linolenic acid (ω3) |
|---|---|---|---|---|---|---|---|
| Repliaction | 2 | 0.99ns | 4121.26ns | 33ns | 3.64ns | 6.99ns | 1087.54ns |
| I | 1 | 143.27* | 84975.03** | 39985.46** | 2016.93** | 21006.76** | 115159.9** |
| AMF | 1 | 4473.24** | 21476.28** | 9.18ns | 0.002ns | 396.43ns | 2115.46* |
| I×AMF | 1 | 143.27* | 34.03ns | 1607.36** | 119.53** | 1721.1** | 10397.43** |
| Error a | 6 | 10.56 | 1438.37 | 113.43 | 6.71 | 84.99 | 303.76 |
| F | 5 | 24.63** | 382104.8** | 1117.62** | 171.24** | 5002.7** | 26786.21** |
| I×F | 5 | 18.7** | 4778.43** | 406.75** | 35.75** | 691.07** | 3425.23** |
| AMF ×F | 5 | 24.63** | 1826.13ns | 253.98** | 13.92** | 77.25ns | 385.03ns |
| I× AMF ×F | 5 | 18.7** | 963.88ns | 779.11** | 33.26** | 453.05** | 2792.84** |
| Error b | 40 | 4.23 | 1167.49 | 104.86 | 3.15 | 70.08 | 288 |
| C.V (%) | - | 26.1 | 7.32 | 9.82 | 8.98 | 9.85 | 8.41 |
| Repliaction | 2 | 2.21ns | 688.01ns | 107.84ns | 8.75ns | 151.37ns | 53.03ns |
| I | 1 | 28.54ns | 33088.78** | 51704.07** | 2312.48** | 26019.75** | 127619.2** |
| AMF | 1 | 4154.16** | 33735.03** | 1.01ns | 5.48ns | 425.75ns | 1891.44ns |
| I× AMF | 1 | 28.54ns | 1164.03ns | 266.47ns | 40.5ns | 0.01ns | 1209.91ns |
| Error a | 6 | 5.26 | 804.62 | 222.08 | 10.62 | 186.77 | 969.39 |
| F | 5 | 11.09ns | 224433** | 1742.23** | 168.61** | 4511.31** | 26431.66** |
| I×F | 5 | 25.27** | 1320.43* | 862.09** | 55.77** | 1079.87** | 3534.31** |
| AMF ×F | 5 | 11.09ns | 3454.68** | 1030.81** | 33.97* | 229.02$^{ns}$ | 1949.87* |
| I× AMF ×F | 5 | 25.27** | 225.63ns | 1986.04** | 69.62** | 788.96** | 4170.44** |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Error b | 40 | 5.26 | 458.79 | 207.58 | 10.11 | 153.05 | 588.87 |
| C.V (%) | - | 30.19 | 4.96 | 13.17 | 14.95 | 13.87 | 11.84 |

* ns: non significant., *Significant at the 0.05 probability levels.,**Significant at the 0.01 probability level

**Table 4. Interactions of irrigation, arbuscular mycorrhiza fungi (AMF) and fertilizer on mycorrhizal colonization and oil fatty acids in purslane leaves in 2015 and -2016.**

| Treatments | | | AMF colonization | | Palmitic acid | | Stearic acid | | Linoleic acid (ω6) | | α-linolenic acid (ω3) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| I | M | N | % | | ml/g | | ml/g | | ml/g | | ml/g | |
| | | | 2015 | 2016 | 2015 | 2016 | 2015 | 2016 | 2015 | 2016 | 2015 | 2016 |
| I1 | M1 | F1 | 15.84cd | 16.21bcd | 79.13$^{ef}$ | 88.38$^{g}$ | 17.01$^{gh}$ | 19.78$^{cdef}$ | 44.78$^{m}$ | 50.87$^{jk}$ | 108.54$^{m}$ | 112.33$^{jkl}$ |
| | | F2 | 16.88cd | 17.48a-d | 76.55$^{ef}$ | 78.98$^{g}$ | 15.35$^{hij}$ | 17.46$^{efg}$ | 63$^{kl}$ | 69.8$^{hij}$ | 151.59$^{kl}$ | 154.41$^{ghi}$ |
| | | F3 | 24.82a | 20.68a | 75.19$^{ef}$ | 70.86$^{g}$ | 12.9$^{jk}$ | 12.74$^{gh}$ | 71.57$^{jk}$ | 73.25$^{hi}$ | 169.43$^{ijk}$ | 168.71$^{fghi}$ |
| | | F4 | 21.60ab | 13.94de | 79.78$^{ef}$ | 84.38$^{g}$ | 9.81$^{lm}$ | 11.39$^{h}$ | 82.62$^{hij}$ | 93.82$^{efg}$ | 197.65$^{gh}$ | 205.16$^{def}$ |
| | | F5 | 18.48bc | 19.73ab | 69.61$^{f}$ | 74.42$^{g}$ | 8.17$^{m}$ | 10.34$^{h}$ | 75.05$^{jk}$ | 81.42$^{fgh}$ | 176.29$^{h-k}$ | 200.48$^{ef}$ |
| | | F6 | 13.89de | 10.67e | 78.01$^{ef}$ | 86.28$^{g}$ | 15.97$^{hi}$ | 19.10$^{def}$ | 55.33$^{lm}$ | 66.63$^{hij}$ | 127.71$^{lm}$ | 142.60$^{ijk}$ |
| | M2 | F1 | 0.00g | 0.00f | 82.48$^{ef}$ | 80.24$^{g}$ | 18.01$^{fgh}$ | 19.40$^{def}$ | 45.96$^{m}$ | 49.31$^{jk}$ | 108.07$^{m}$ | 103.00$^{kl}$ |
| | | F2 | 0.00g | 0.00f | 79.14$^{ef}$ | 76.33$^{g}$ | 16.24$^{ghi}$ | 14.95$^{efgh}$ | 63.47$^{kl}$ | 57.92$^{ijk}$ | 154.17$^{jkl}$ | 147.92$^{hij}$ |
| | | F3 | 0.00g | 0.00f | 77.90$^{ef}$ | 85.02$^{g}$ | 13.80$^{ij}$ | 14.54$^{fgh}$ | 65.39$^{kl}$ | 67.32$^{hij}$ | 157.09$^{jk}$ | 167.16$^{fghi}$ |
| | | F4 | 0.00g | 0.00f | 74.44$^{ef}$ | 73.07$^{g}$ | 12.57$^{jkl}$ | 12.66$^{gh}$ | 76.27$^{ijk}$ | 73.37$^{ghi}$ | 182.17$^{hij}$ | 182.59$^{efghi}$ |
| | | F5 | 0.00g | 0.00f | 86.01$^{ef}$ | 79.65$^{g}$ | 10.69$^{klm}$ | 10.08$^{h}$ | 90.01$^{ghi}$ | 75.29$^{ghi}$ | 214.29$^{fg}$ | 178.07$^{fghi}$ |
| | | F6 | 0.00g | 0.00f | 110.70$^{d}$ | 113.50$^{f}$ | 23.3$^{de}$ | 24.86$^{bc}$ | 81.77$^{hij}$ | 83.27$^{fgh}$ | 194.57$^{ghi}$ | 192.63$^{efg}$ |
| I2 | M1 | F1 | 14.70d | 11.44e | 127.28$^{bcd}$ | 125.17$^{def}$ | 25.36$^{cd}$ | 27.26$^{b}$ | 68.96$^{jkl}$ | 72.03$^{hi}$ | 159.16$^{jk}$ | 166.66$^{fghi}$ |
| | | F2 | 10.86ef | 16.09bcd | 122.34$^{cd}$ | 115.94$^{ef}$ | 25.51$^{cd}$ | 25.57$^{b}$ | 98.73$^{efg}$ | 98.58$^{def}$ | 240.8$^{ef}$ | 219.76$^{de}$ |
| | | F3 | 19.00bc | 18.58abc | 124.54$^{bcd}$ | 133.07$^{cdef}$ | 25.51$^{cd}$ | 27.73$^{b}$ | 103.97$^{def}$ | 104.57$^{cde}$ | 248.17$^{de}$ | 244.73$^{cd}$ |
| | | F4 | 10.33ef | 11.55e | 117.61$^{cd}$ | 122.56$^{def}$ | 20.53$^{ef}$ | 23.49$^{bcd}$ | 124.47$^{bc}$ | 126.27$^{b}$ | 290.23$^{bc}$ | 305.76$^{ab}$ |
| | | F5 | 13.41de | 15.39cd | 139.54$^{b}$ | 139.55$^{cde}$ | 23.88$^{d}$ | 25.37$^{b}$ | 146.29$^{a}$ | 146.96$^{a}$ | 341.43$^{a}$ | 323.84$^{a}$ |
| | | F6 | 9.37f | 10.55e | 166.44$^{a}$ | 191.68$^{a}$ | 37.40$^{a}$ | 38.38$^{a}$ | 113.57$^{cd}$ | 115.36$^{bcd}$ | 275.54$^{cd}$ | 277.34$^{bc}$ |
| | M2 | F1 | 0.00g | 0.00f | 86.52$^{e}$ | 81.98$^{g}$ | 18.02$^{fgh}$ | 17.7$^{efg}$ | 43.96$^{m}$ | 45.41$^{k}$ | 104.93$^{m}$ | 98.99$^{l}$ |
| | | F2 | 0.00g | 0.00f | 119.81$^{cd}$ | 164.54$^{b}$ | 25.18$^{cd}$ | 33.81$^{a}$ | 92.35$^{fgh}$ | 127.15$^{ab}$ | 216.93$^{fg}$ | 277.47$^{bc}$ |
| | | F3 | 0.00g | 0.00f | 133.78$^{bc}$ | 143.13$^{bcd}$ | 27.62$^{bc}$ | 28.29$^{b}$ | 108.12$^{de}$ | 117.05$^{bcd}$ | 262.43$^{cde}$ | 279.70$^{bc}$ |
| | | F4 | 0.00g | 0.00f | 131.31$^{bc}$ | 156.07$^{bc}$ | 23.87$^{d}$ | 28.33$^{b}$ | 111.59$^{cde}$ | 124.99$^{bc}$ | 264.79$^{cde}$ | 293.35$^{ab}$ |
| | | F5 | 0.00g | 0.00f | 132.70$^{bc}$ | 127.86$^{def}$ | 19.31$^{fg}$ | 19.87$^{cde}$ | 133.81$^{ab}$ | 135.68$^{ab}$ | 304.51$^{b}$ | 293.30$^{ab}$ |
| | | F6 | 0.00g | 0.00f | 132.65$^{bc}$ | 132.72$^{cdef}$ | 28.66$^{b}$ | 27.50$^{b}$ | 79.35$^{hij}$ | 84.44$^{efgh}$ | 192.49$^{gi}$ | 184.58$^{efgh}$ |

$I_1$= non-stressed, irrigation at 70% of FC; $I_2$= water deficit stress, irrigation at 50% of FC; $M_1$ & $M_2$: inoculated with AMF and non-inoculated; $F_1$, $F_2$, $F_3$, $F_4$, $F_5$ and $F_6$: unfertilized control, 100% FYM, 75% FYM + 25% urea , 50% FYM + 50% urea, 25% FYM + 75% urea, 100% urea, respectively.

Means in each column followed by similar letter (s) are not significantly different at 5% probability level using LSD Test.

**Table 5. Interactions of irrigation and fertilizer on phosphorus purslane leaves in 2015 and 2016.**

| Treatments | | Phosphorus | |
|---|---|---|---|
| | | mg/kg | |
| Irrigation | Fertilizer | 2015 | 2016 |
| I1 | F1 | 222.25h | 259.75i |
| | F2 | 468.75d | 418.00e |
| | F3 | 536.50c | 472.00d |
| | F4 | 679.00b | 597.00b |
| | F5 | 745.50a | 633.50a |
| | F6 | 354.50f | 337.75g |
| I2 | F1 | 209.25h | 239.75i |
| | F2 | 418.25e | 388.50f |
| | F3 | 478.00d | 422.00e |
| | F4 | 555.75c | 518.50c |
| | F5 | 639.50b | 585.00b |
| | F6 | 293.50g | 307.00h |

$I_1$= non-stressed, irrigation at 70% of FC; $I_2$= water deficit stress, irrigation at 50% of FC; $F_1$, $F_2$, $F_3$, $F_4$, $F_5$ and $F_6$: unfertilized control, 100% FYM, 75% FYM + 25% urea , 50% FYM + 50% urea, 25% FYM + 75% urea ,100% urea, respectively.

Means in each column followed by similar letter (s) are not significantly different at 5% probability level using LSD Test.

**Table 6. The main effects of irrigation, fertilizer and arbuscular mycorrhiza fungi (AMF) on phosphorus in purslane leaves.**

| Treatments | Phosphorus |
|---|---|
| | mg/kg |
| Irrigation | 2015 |
| I1 | 501.08a |
| I2 | 432.38b |
| Fertilizer | |
| F1 | 215.75f |
| F2 | 443.5d |
| F3 | 507.25c |
| F4 | 617.38b |
| F5 | 692.5a |
| F6 | 324e |
| AMF | |

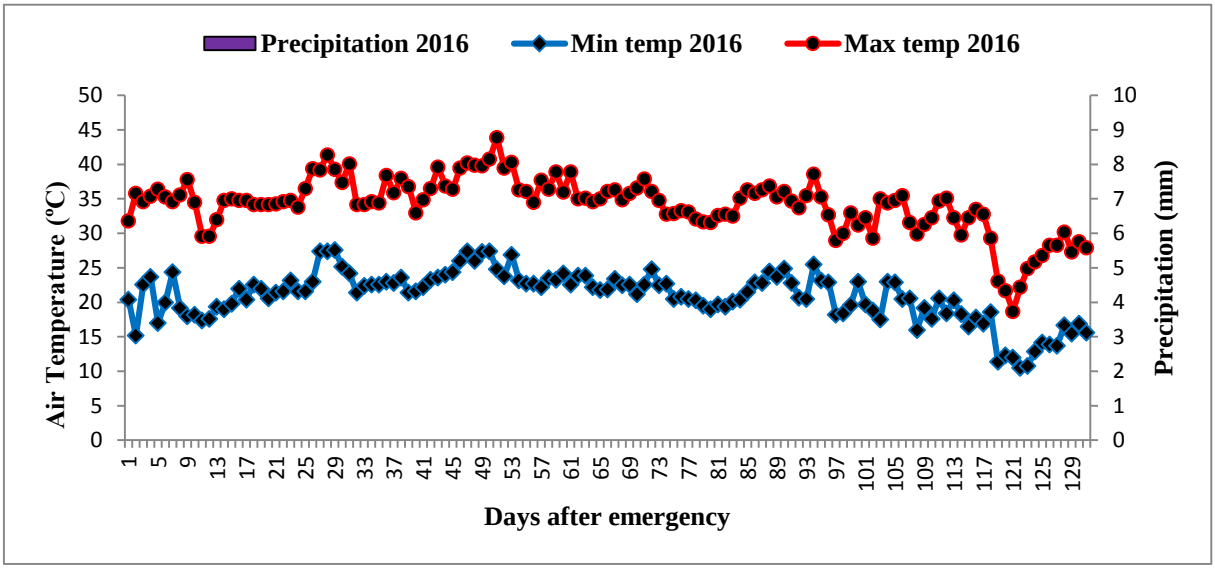
| | |
|---|---|
| M1 | 484.00a |
| M2 | 449.46b |

$I_1$= non-stressed, irrigation at 70% of FC; $I_2$= water deficit stress, irrigation at 50% of FC; $M_1$ & $M_2$: inoculated and non-inoculated with AMF; $F_1$, $F_2$, $F_3$, $F_4$, $F_5$ and $F_6$: unfertilized control, 100% FYM, 75% FYM + 25% urea , 50% FYM + 50% urea, 25% FYM + 75% urea ,100% urea, respectively. Means in each column followed by similar letter (s) are not significantly different at 5% probability level using LSD Test.

**Table 7. The main effects of irrigation on fatty acids (saturated and unsaturated ) and arbuscular mycorrhiza fungi (AMF) in purslane leaves.**

| Treatments | Palmitic acid | | Stearic acid | | Linoleic acid (ω6) | | α-linolenic acid (ω3) | | AMF colonization | |
|---|---|---|---|---|---|---|---|---|---|---|
| | ml/g | | ml/g | | ml/g | | ml/g | | % | |
| Irrigation | 2015 | 2016 | 2015 | 2016 | 2015 | 2016 | 2015 | 2016 | 2015 | 2016 |
| I1 | 80.74 b | 82.59 b | 14.49b | 15.61b | 67.93b | 70.19 b | 161.8 b | 162.92b | 9.29 a | 8.23 a |
| I2 | 127.88a | 136.19a | 25.07a | 26.94a | 102.1a | 108.21a | 241.78a | 247.12a | 6.47 b | 6.97 a |

$I_1$= non-stressed, irrigation at 70% of FC; $I_2$= water deficit stress, irrigation at 50% of FC. Means in each column followed by similar letter (s) are not significantly different at 5% probability level using LSD Test



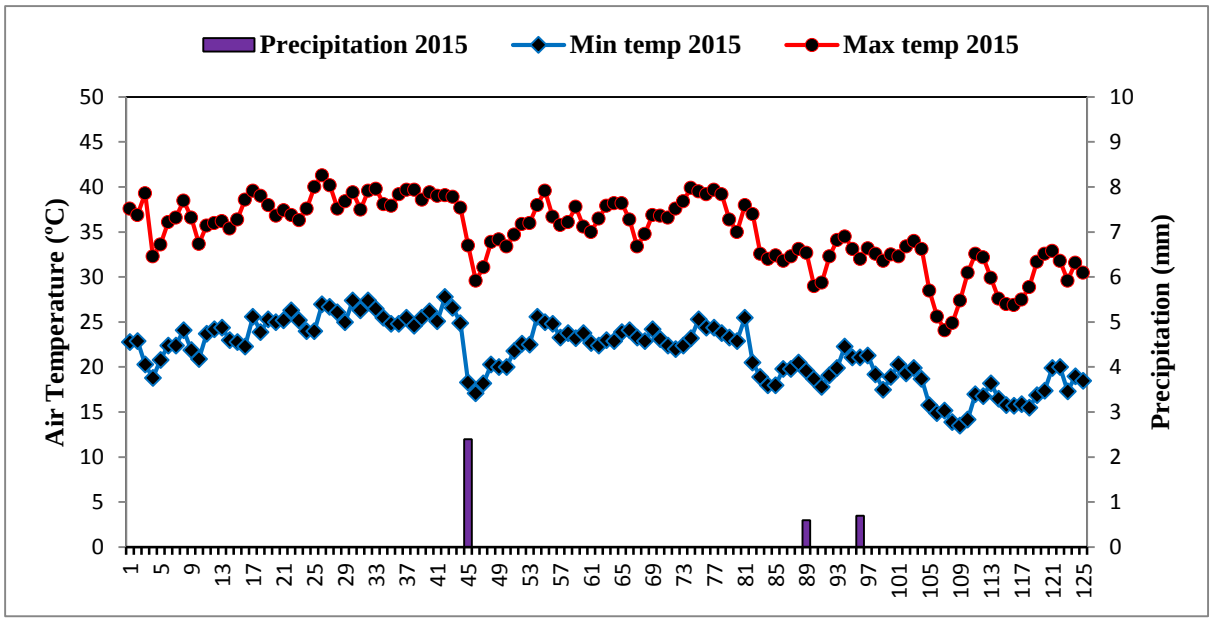



Figure 1. Daily maximum and minimum air temperatures (°C), and precipitation (mm) recorded during the growing season in 2015 and 2016. The arrows show the start of inflorescence emergence (flowering) in both growing season.

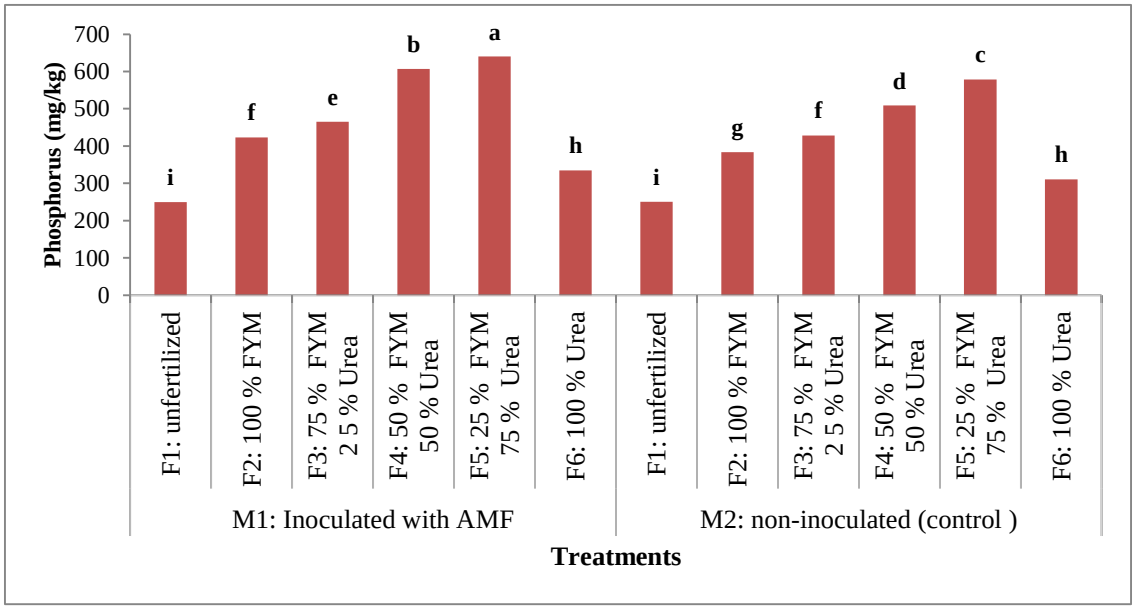



Figure 2. Interactions of arbuscular mycorrhiza fungi (AMF) and fertilizer on phosphorus in 2016.Means in each column followed by similar letter (s) are not significantly different at 5% probability level using LSD Test.

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