Irrigation Regime and Organic Fertilizers Influence on Oil Content and Fatty Acid Composition of Milk Thistle Seeds

R. Keshavarz Afshar,* M. R. Chaichi, K. Rezaei, M. H. Asareh, M. Karimi, and M. Hashemi

ABSTRACT

Milk thistle (Silybum marianum L. Gaertn.) seeds contain relatively high amounts of edible oil. Influences of irrigation regime (full irrigation, moderate deficit, and severe deficit irrigation) and organic fertilizers (vermicompost [VM] and poultry manure [PM]) on the oil content and fatty acid (FA) composition of milk thistle seed were evaluated in a field trial. Averaged across treatments, seed oil content was 27% whereas unsaturated FAs constituted 78% of the oil composition. Linoleic acid (41.4%) and oleic acid (35.1%) were the most abundant unsaturated FAs whereas palmitic, stearic, arachidic, and behenic acids were major saturated FAs. Oil content was significantly influenced by irrigation regimes, but not by organic fertilizers. Severe deficit irrigation reduced oil content by 7.4% compared with full irrigation. Deficit irrigation had a minor effect on the FA composition of milk thistle oil where total unsaturated fatty acids (UFA) was lower in stressed plants. The application of soil organic amendments did not induce significant changes in unsaturated fatty acids but reduced the content of saturated ones which totally imposed positive effects on oil quality of milk thistle. According to the results, it seems that milk thistle has potential to be considered as an oil seed crop in low-input agricultural systems in arid and semiarid areas and moderate deficit irrigation can be implemented without deleterious effects on its oil content and quality.

With an increasing growth of the world population (surpassing 9 billion by 2050 and exceeding 10 billion in 2100 [United Nations, 2011]), the demand for high-quality oils continues to rise. To meet the global demand, increasing the production of traditional oil seed crops seems inevitable. Moreover, diversification of edible oil sources and introducing alternative oil crops, especially those that require less off-farm inputs with higher resiliency to environmental stresses is also necessary (Geleta et al., 2011).

Milk thistle is a recognized medicinal plant belonging to the Asteraceae family, and originated in the Mediterranean Basin. Silymarin, a derivative of milk thistle, has been used as an herbal remedy to treat liver disorders for more than 2000 yr (Afshar et al., 2014b). Milk thistle is commercially grown in Europe where Poland is the biggest producer of milk thistle seeds and its derivatives in the world with cultivation area of more than 2000 ha (Andrzejewska et al., 2011). In addition to silymarin, milk thistle seeds contain relatively high amounts of oil which needs to be removed from the seeds before extraction of silymarin (Hadolin et al., 2001). It has been reported that milk thistle seeds contain 25 to 30% high-quality edible oil, essential phospholipids and a relatively high content of vitamin E (Fathi-Achachlouei and Azadmard-Damiri, 2009). Consumption of oils with high content of unsaturated fatty acids (UFAs) such as linoleic, oleic, and linolenic acids and low content of saturated fatty acids (SFAs) such as palmitic, stearic, arachidic, and behenic acids has positive effect on human health (Zheljazkov et al., 2009). Due to high quality of milk thistle oil, it seems that this crop could be considered as a potential source of edible vegetable oil thus needs to be investigated more.

This crop can easily be cultivated with traditional machinery and equipment. It can be directly seeded in soils in fall or spring (depending on the climatic condition), at the sowing depth of 1 to 2 cm, with row spacing of 40 to 75 cm, and 20 to 30 cm in row spacing. Milk thistle sown in spring fulfills its life cycle within 120 to 140 d and can be harvested by a regular cereal combine equipped with a sunflower (Helianthus annuus L.) header (Andrzejewska et al., 2011).

Although a plant’s genotype is the primary factor determining the oil content of the seeds and their FA composition, environmental factors such as moisture availability and soil fertility may also have a significant effect on oil quantity and quality (Ashrafi

Abbreviations: C, control (no organic fertilizer); FA, fatty acid; I50, providing 50% of the estimated water requirements or severe deficit irrigation; I75, providing 75% of the estimated water requirements or moderate deficit irrigation; MUFA, monounsaturated fatty acids; OLR, Oleic/linoleic acid ratio; PM, poultry manure; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; V, providing 100% of the estimated water requirements or full irrigation; VM, unsaturated fatty acids; VM, vermicompost.
and Razmjoo, 2010). Drought is considered to be the single most important abiotic stress that limits crop production in arid and semiarid regions (Keshvarz Afshar et al., 2012). Deficit irrigation systems have been considered as practical strategies to maximize water use efficiency and increase yields per unit of applied irrigation water in these regions (Jahanzad et al., 2013; Keshvarz Afshar et al., 2014a). Deficit irrigation is watering crops with less water than that used in full irrigation systems. In this strategy, the crop is exposed to a certain level of drought stress, either during a particular growth stage or throughout the whole growing season (Bekele and Tilahun, 2007). The effect of deficit irrigation and drought stress on oil yield and quality of oil crops in Asteraceae family such as sunflower (Anastasi et al., 2010; Baldini et al., 2002; Jalilian et al., 2012; Sezen et al., 2011) and safflower (Ashrafi and Razmjoo, 2010) have been extensively studied but very limited information exists about milk thistle. Depending on the genotypes and environmental conditions, plants may respond to deficit irrigation differently. Reduction in unsaturated FAs content (Anastasi et al., 2010; Jalilian et al., 2012) and increase in the content of saturated FAs (Dwivedi et al., 1993; Hamrouni et al., 2001; Laribi et al., 2009) are among the general responses observed in oil seed crops when exposed to water deficiency.

Optimum plant growth and crop yield, requires adequate supply of essential nutrients. However, continuous application of synthetic fertilizers has often caused environmental problems including water pollution, greenhouse gas emissions, and soil degradation (Yadav, 2003). Alternatively, application of soil organic amendments, such as PM and VM, which are good sources of essential macronutrients and micronutrients, are considered as sustainable means to improve soil physical, chemical and biological properties and therefore, to enhance soil quality and productivity (Bohme et al., 2005). Application of fertilizers due to altering the nutrient status in the soil and subsequently in plant organs may influence the oil content and the FA composition in oilseed crops. Gao et al. (2010) reported that the application of N fertilizer increased protein but decreased oil content of canola. Reducing FAs (Dwivedi et al., 1993; Hamrouni et al., 2001; Laribi et al., 2009) have been extensively studied but very limited information exists about milk thistle. Depending on the genotypes and environmental conditions, plants may respond to deficit irrigation differently. Reduction in unsaturated FAs content (Anastasi et al., 2010; Jalilian et al., 2012) and increase in the content of saturated FAs (Dwivedi et al., 1993; Hamrouni et al., 2001; Laribi et al., 2009) are among the general responses observed in oil seed crops when exposed to water deficiency.

MATERIALS AND METHODS

The study was conducted in 2012 and 2013 at the Research Farm of the College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran (N35°56’ N, E50°58’ E). The climate type of the region is considered as arid to semiarid with long-term (50-yr average) air temperature of 13.5°C and 262 mm of rainfall. The weather condition at the experimental site during the two growing seasons is shown in Fig. 1. Soil at the site is clay loam in texture and classified as a Typic Haplocambid in the USDA classification (Afshar et al., 2014a).

Experimental Design and Factors

The study was arranged in a factorial arrangement based on a randomized complete block design with four replicates. Three levels of irrigation regimes including normal irrigation (I100), moderate deficit (I75), severe deficit irrigation (I50), and two organic fertilizers comprised of VM and PM plus no fertilizer (C) were studied.

Irrigation

Before the study began, soil samples were taken at the depths of 0 to 80 cm at 20-cm increments using a 10-cm diam. soil core sampler. Soil water retention curves were determined using pressure plates. The volumetric soil water content at field capacity (θFC) was 0.24, 0.24, 0.22, and 0.21 m³ m⁻³ for depth increments of 0 to 20, 20 to 40, 40 to 60 and 60 to 80 cm, respectively. Permanent wilting point was measured and was 0.12, 0.11, 0.10 and 0.10 m³ m⁻³ in the above mentioned depths. The difference between field capacity and permanent wilting point at each depth was considered as the soil available water content. The times of irrigation in all regimes were similar and done after 50% depletion of the soil available water in full irrigation plots. Every 4 d, samples were taken from full irrigation plots to determine effective root depth and volumetric soil water content at the rooting depth (θ̅). The amount of irrigation water required to bring the soil water content back to the field capacity point was calculated using the following equation (Afshar et al., 2014a):

\[ Vₚ = (θ_{FC} - θ̅) × D × A \]

where \( Vₚ \) is the volume of water (m³) used at each irrigation interval, \( D \) is the effective vertical rooting depth (m), and \( A \) is the plot surface area (m²).

![Graph showing temperature and precipitation](image-url)
In both years, full irrigation (furrow irrigation system) was applied to all experimental plots until plants were fully established and deficit irrigation treatments were imposed after establishment of plants. In the full irrigation treatment ($I_{100}$) the entire volume of estimated water was used while in moderate ($I_{75}$) and severe deficit irrigation ($I_{50}$) only 75 and 50% of the estimated water was supplied, respectively. Therefore, at each irrigation time, 25 and 50% of the water was saved in moderate and severe deficit irrigation, respectively. To avoid runoff from plots after irrigation, both ends of the plots were blocked by soil. A border of 2 m between adjacent plots in each replication and 5 m between replicates were maintained to avoid entering of drainage water from neighboring plots. A water flow meter was used for accurate and uniform irrigation. The total amount of irrigation water used during the plant life cycle was 2430 m$^3$ ha$^{-1}$ during the first year, and 2780 m$^3$ ha$^{-1}$ during the second year of the experiment in $I_{100}$.

**Organic Fertilizers**

Before planting, soil samples were taken from 0- to 30-cm depth and were analyzed for selected physical and chemical characteristics which are presented in Table 1. The rate of organic fertilizer was determined based on milk thistle P requirement (69 kg P$_2$O$_5$ ha$^{-1}$) (Karkanis et al., 2011) and P$_2$O$_5$ content of the organic amendments. Accordingly, vermicompost was applied at the rate of 3965 and 4082 kg ha$^{-1}$ in 2012 and 2013, respectively, and poultry manure was used at the rate of 3833 and 3689 kg ha$^{-1}$ in the two experimental years (Table 2). Before planting, organic amendments were evenly spread on the soil surface and incorporated manually into the top 20 cm of the soil.

**Crop Management**

Milk thistle seeds (cultivar Budakalaszi) were sown by hand on 10 Mar. 2012 and 3 Mar. 2013 at the depth of 1.5 to 2.0 cm. Each plot consisted of five rows, 6 m long and 0.5 m wide. Initially plants in each row were spaced 0.1 m which were thinned to 0.2 m 2 wk after planting. Thus, the final plant population density in all plots was 10 plants m$^{-2}$.

**Measurements**

Milk thistle was hand harvested after ripening and before seed dispersion (26 June 2012 and 20 June 2013). After harvesting, seeds were thrashed with a mini thrasher, cleaned with a blower, and oven dried at 30$^\circ$C and adjusted to 10% moisture content. Harvested seeds in each plot were thoroughly mixed and a sample of 100 g was separated for further analysis.

Chemical analysis was performed on whole achene. Oil of the seeds (w/w) was extracted using a Soxhlet apparatus and petroleum ether 40 to 60° (Merck Chemical Co., Darmstadt, Germany).

A PerkinElmer Clarus 500 GC system (PerkinElmer, Shelton, CT) equipped with a Supelco SP-2560 column (100 m by 0.25 mm by 0.2 µm film thickness) was used for FAs analysis. Oven temperature started at 110°C and increased to 190°C at a rate of 3°C min$^{-1}$ and was held at 190°C for 15 min. Temperature was then increased to 235°C with the same rate and held for 10 min. A final increase in the temperature at a rate of 2°C min$^{-1}$ was applied until it was reached to 240°C. Both injector and detector (FID) were set at 250°C. Half a microliter sample was injected and a split ratio of 20:1 was selected in the injection system. Integration of peak areas was performed using Total Chrome software.

Fatty acid components were calculated using following formula:

\[
\text{oleic/linoleic acid ratio (OLR)} = \frac{\text{oleic (C}_{18:1})}{\text{linoleic (C}_{18:2})}.
\]

**Table 1. General properties of the soil of the experimental site (depth of 0–30 cm).**

<table>
<thead>
<tr>
<th>Year</th>
<th>Soil texture</th>
<th>pH</th>
<th>EC†</th>
<th>Organic matter</th>
<th>Total N</th>
<th>Available P</th>
<th>Available K</th>
</tr>
</thead>
<tbody>
<tr>
<td>2012</td>
<td>clay loam</td>
<td>8.2</td>
<td>1.6</td>
<td>0.15</td>
<td>0.05</td>
<td>5.3</td>
<td>126</td>
</tr>
<tr>
<td>2013</td>
<td>clay loam</td>
<td>7.9</td>
<td>1.9</td>
<td>0.05</td>
<td>0.07</td>
<td>8.0</td>
<td>143</td>
</tr>
</tbody>
</table>

† EC, electrical conductivity.

**Table 2. The application rate of organic fertilizers and amount of N, P$_2$O$_5$, and K$_2$O provided by each source based on their percentage of nutrients (2012 and 2013).**

<table>
<thead>
<tr>
<th>Source</th>
<th>2012</th>
<th>2013</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rate kg ha$^{-1}$</td>
<td>Percentage in the source</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VM</td>
<td>3965</td>
<td>1.52</td>
</tr>
<tr>
<td>PM</td>
<td>3833</td>
<td>4.58</td>
</tr>
<tr>
<td>VM</td>
<td>3965</td>
<td>1.74</td>
</tr>
<tr>
<td>PM</td>
<td>3833</td>
<td>1.80</td>
</tr>
<tr>
<td>VM</td>
<td>3965</td>
<td>0.32</td>
</tr>
<tr>
<td>PM</td>
<td>3833</td>
<td>0.12</td>
</tr>
</tbody>
</table>

**Nitrogen**

**Phosphorus (P$_2$O$_5$)**

**Potassium (K$_2$O)**
Polyunsaturated fatty acid (PUFA) = linoleic (C\(_{18:2}\)) + linolenic (C\(_{18:3}\)).

Unsaturated fatty acid (UFA) = MUFA + PUFA

### Statistical Analysis

For statistical analyses an ANOVA technique for factorial design was performed using Proc GLM procedure of SAS software. Main effects were year, irrigation regime, and fertilizer treatments which were considered as fixed effects while only block was treated as a random effect. Means were separated using LSD test at \(P < 0.05\) significance level. Since ANOVA indicated no significant differences between the 2 yr of the experiment for the majority of the variables, the values reported herein are averaged over 2 yr.

## RESULTS AND DISCUSSION

### Oil Content and Quality

Averaged across all treatments, the oil content of milk thistle seeds was 27%, which is within the range previously reported for milk thistle (Fathi-Achachlouei and Azadmard-Damirchi, 2009). Nineteen different FAs were detected in the oil of milk thistle seeds where 11 of them comprised more than 97% of the oil composition and are discussed herein. The major FA found in milk thistle oil was linoleic acid constituting 41.4% of the total oil composition followed by oleic acid with a proportion of 35.0% (Table 3). Linolenic acid and eicosenoic acid were other high nutritionally valuable unsaturated FAs, which existed in small amount in milk thistle oil (Table 3). The most abundant saturated FAs in milk thistle oil were palmitic acid, stearic acid, arachidic acid and behenic acid with a proportion of 8.5, 6.1, 2.8, and 1.4% of the oil composition, respectively (Table 4).

Overall, UFA constituted 78.2% (Table 5) and SFA formed 19.1% (Table 4) of the oil composition. Out of 78.2% proportion of unsaturated FAs, 42.4% belonged to PUFA and 35.8% belonged to MUFA, therefore, the MUFA/PUFA ratio was 0.85 (Table 5). The amount of PUFA in the oil is an important factor due to their various functions such as influence on cellular signaling, membrane structure, nervous, endocrine, and immune system mediations (Yehuda, 2001). Averaged across treatments, OLR ratio of milk thistle oil was 0.85. Linoleic and oleic acids are among the essential UFAs and play important roles in human metabolism and prevention of several nutrition-related illnesses (Wijendran and Hayes, 2004). Therefore, the suitability of edible oils and their commercial values is highly

### Table 3. Oil content and the percentages (± SE) of unsaturated fatty acids including: palmitoleic (C\(_{16:1}\)), margaroleic (C\(_{17:1}\)), oleic (C\(_{18:1}\)), linoleic (C\(_{18:2}\)), linolenic (C\(_{18:3}\)), and eicosenoic (C\(_{20:1}\)) of milk thistle oil as influenced by irrigation regimes and fertilizers.

<table>
<thead>
<tr>
<th>Irrigation</th>
<th>Fertilizer</th>
<th>Oil content</th>
<th>Palmitoleic</th>
<th>Margaroleic</th>
<th>Oleic acid</th>
<th>Linoleic</th>
<th>Linolenic</th>
<th>Eicosenoic</th>
</tr>
</thead>
<tbody>
<tr>
<td>(I_{100})</td>
<td>C</td>
<td>27.8 ± 0.6</td>
<td>0.10 ± 0.01</td>
<td>0.04 ± 0.00</td>
<td>34.96 ± 0.38</td>
<td>42.77 ± 0.73</td>
<td>0.58 ± 0.02</td>
<td>0.67 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>VM</td>
<td>27.6 ± 0.5</td>
<td>0.11 ± 0.00</td>
<td>0.04 ± 0.00</td>
<td>33.84 ± 0.65</td>
<td>43.50 ± 0.83</td>
<td>0.71 ± 0.02</td>
<td>0.66 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>PM</td>
<td>27.9 ± 0.4</td>
<td>0.11 ± 0.01</td>
<td>0.07 ± 0.01</td>
<td>35.38 ± 0.84</td>
<td>41.78 ± 1.01</td>
<td>0.64 ± 0.03</td>
<td>0.67 ± 0.04</td>
</tr>
<tr>
<td>(I_{75})</td>
<td>C</td>
<td>27.5 ± 0.5</td>
<td>0.09 ± 0.00</td>
<td>0.07 ± 0.01</td>
<td>34.62 ± 0.78</td>
<td>40.39 ± 0.51</td>
<td>0.88 ± 0.06</td>
<td>0.60 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>VM</td>
<td>27.6 ± 0.5</td>
<td>0.08 ± 0.01</td>
<td>0.06 ± 0.01</td>
<td>37.24 ± 0.49</td>
<td>40.15 ± 0.73</td>
<td>1.33 ± 0.22</td>
<td>0.61 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>PM</td>
<td>27.0 ± 0.5</td>
<td>0.08 ± 0.00</td>
<td>0.09 ± 0.01</td>
<td>32.82 ± 0.25</td>
<td>42.32 ± 0.38</td>
<td>0.86 ± 0.02</td>
<td>0.68 ± 0.02</td>
</tr>
<tr>
<td>(I_{50})</td>
<td>C</td>
<td>26.0 ± 0.5</td>
<td>0.08 ± 0.01</td>
<td>0.04 ± 0.00</td>
<td>35.14 ± 0.95</td>
<td>40.75 ± 0.48</td>
<td>1.46 ± 0.17</td>
<td>0.64 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>VM</td>
<td>25.8 ± 0.2</td>
<td>0.09 ± 0.01</td>
<td>0.04 ± 0.00</td>
<td>35.83 ± 0.61</td>
<td>40.32 ± 0.47</td>
<td>1.39 ± 0.09</td>
<td>0.61 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>PM</td>
<td>25.3 ± 0.4</td>
<td>0.10 ± 0.01</td>
<td>0.07 ± 0.01</td>
<td>35.59 ± 0.54</td>
<td>40.88 ± 0.55</td>
<td>1.03 ± 0.15</td>
<td>0.62 ± 0.02</td>
</tr>
<tr>
<td>LSD†</td>
<td></td>
<td>1.3</td>
<td>0.02</td>
<td>0.03</td>
<td>1.84</td>
<td>1.88</td>
<td>0.32</td>
<td>0.07</td>
</tr>
<tr>
<td>(I_{100})</td>
<td></td>
<td>27.8 ± 0.3</td>
<td>0.11 ± 0.00</td>
<td>0.05 ± 0.01</td>
<td>34.73 ± 0.39</td>
<td>42.68 ± 0.50</td>
<td>0.64 ± 0.02</td>
<td>0.67 ± 0.02</td>
</tr>
<tr>
<td>(I_{75})</td>
<td></td>
<td>27.4 ± 0.3</td>
<td>0.09 ± 0.00</td>
<td>0.07 ± 0.01</td>
<td>34.89 ± 0.53</td>
<td>40.95 ± 0.38</td>
<td>1.02 ± 0.09</td>
<td>0.63 ± 0.01</td>
</tr>
<tr>
<td>(I_{50})</td>
<td></td>
<td>25.7 ± 0.2</td>
<td>0.09 ± 0.00</td>
<td>0.05 ± 0.01</td>
<td>35.52 ± 0.40</td>
<td>40.65 ± 0.28</td>
<td>1.29 ± 0.09</td>
<td>0.62 ± 0.01</td>
</tr>
<tr>
<td>C</td>
<td></td>
<td>27.1 ± 0.3</td>
<td>0.09 ± 0.01</td>
<td>0.05 ± 0.01</td>
<td>34.91 ± 0.41</td>
<td>41.30 ± 0.41</td>
<td>0.97 ± 0.10</td>
<td>0.64 ± 0.01</td>
</tr>
<tr>
<td>VM</td>
<td></td>
<td>27.0 ± 0.3</td>
<td>0.09 ± 0.01</td>
<td>0.04 ± 0.00</td>
<td>35.64 ± 0.47</td>
<td>41.32 ± 0.53</td>
<td>1.14 ± 0.11</td>
<td>0.63 ± 0.01</td>
</tr>
<tr>
<td>PM</td>
<td></td>
<td>26.7 ± 0.4</td>
<td>0.10 ± 0.00</td>
<td>0.07 ± 0.01</td>
<td>34.60 ± 0.44</td>
<td>41.66 ± 0.41</td>
<td>0.85 ± 0.06</td>
<td>0.66 ± 0.02</td>
</tr>
<tr>
<td>Mean§</td>
<td></td>
<td>27.0</td>
<td>0.09</td>
<td>0.06</td>
<td>35.05</td>
<td>41.43</td>
<td>0.99</td>
<td>0.64</td>
</tr>
<tr>
<td>LSD¶</td>
<td></td>
<td>0.8</td>
<td>0.01</td>
<td>0.00</td>
<td>1.12</td>
<td>0.85</td>
<td>0.07</td>
<td>0.03</td>
</tr>
</tbody>
</table>

\* \(P \leq 0.05\).

\** \(P \leq 0.01\).

† Abbreviations: \(I_{100}\), full irrigation; \(I_{75}\), moderate deficit irrigation; \(I_{50}\), severe deficit irrigation (based on soil moisture depletions of available water). C, no fertilizer; VM, vermicompost; PM, poultry manure.

‡ LSD value for comparison of means as affected by interaction of irrigation and fertilizers.

§ Mean: mean value of each variable averaged across treatments.

¶ LSD value for comparison of means as affected by main effect of irrigation and fertilizers.

†† ns, not significant at \(P \leq 0.05\).
depends on the proportions of these FAs. Being rich in essential FAs with an appropriate portion of PUFA, containing valuable FAs such as linolenic, and finally having low content of unhealthy saturated FAs makes milk thistle oil a remarkable source of edible oil with high nutritional value. More details on milk thistle seed and oil yield are presented in our earlier report (Afshar et al., 2014a).

### Effect of Irrigation Regime on Oil Content and Fatty Acids Composition

The irrigation treatment showed a significant influence on oil content of milk thistle seed (Table 3). Although moderate deficit irrigation (I50) did not significantly influence oil content compared with full irrigation, when deficit irrigation was intensified (I75), oil content decreased by 7.4% (Table 3). Similarly, in many oil seed crops deficit irrigation imposed negative impact on oil accumulation (Anastasi et al., 2010; Betaieb et al., 2009; Flagella et al., 2002; Sezen et al., 2011). Drought stress usually causes degradative processes such as the inhibition of lipid biosynthesis and stimulation of lipolytic and peroxidative activities resulting in decreased oil content (Upchurch, 2008). The adverse effect of drought stress on oil accumulation could also be related to translocation of higher level of abscisic acid from leaves to seeds, which contributes to inhibition of oil biosynthesis (Connor and Sadras, 1992).

Deficit irrigation also affected milk thistle FA composition. Linoleic acid content significantly reduced in response to moderate deficit irrigation, however, severe water deficiency did not impose further changes (Table 3). Palmitoleic and eicosenoic acids decreased, linolenic acid increased, and no change was observed in oleic acid content in response to water deficiency. As a consequence of changes in the content of unsaturated FAs, OLR ratio increased whereas PUFA and UFA decreased under deficit irrigation condition (Table 5).

Among saturated FAs, palmitic acid reduced only when plants exposed to severe drought stress; while no changes were observed in the content of margaric, arachidic, and behenic acids content in response to irrigation treatment (Table 4). Overall, SFA increased in moderate deficit irrigation but when severe drought stress was induced, a considerable reduction in SFA was detected (Table 4).

Water stress could affect oil FA composition through accelerating embryo development and stimulating enzymatic activities of FAs biosynthesis, including oleoyl Δ-12 desaturase (Baldini et al., 2002). However, in this study the deficit irrigation had only minor effect on milk thistle FA composition and its oil

<p>| Table 4. The percentages (± SE) of saturated fatty acids including: palmitic (C16:0), margaric (C17:0), stearic (C18:0), arachidic (C20:0), behenic (C22:0), and total saturated fatty acids (SFA) of milk thistle oil as influenced by irrigation regimes and fertilizers. |</p>
<table>
<thead>
<tr>
<th>Irrigation</th>
<th>Fertilizer</th>
<th>Palmitic</th>
<th>Margaric</th>
<th>Stearic</th>
<th>Arachidic</th>
<th>Behenic</th>
<th>SFA</th>
</tr>
</thead>
<tbody>
<tr>
<td>I100†</td>
<td>VM</td>
<td>8.70 ± 0.12</td>
<td>0.22 ± 0.08</td>
<td>6.04 ± 0.06</td>
<td>2.76 ± 0.03</td>
<td>1.37 ± 0.17</td>
<td>19.10 ± 0.18</td>
</tr>
<tr>
<td>I75</td>
<td>PM</td>
<td>8.37 ± 0.21</td>
<td>0.16 ± 0.04</td>
<td>5.98 ± 0.07</td>
<td>2.76 ± 0.09</td>
<td>1.50 ± 0.01</td>
<td>18.79 ± 0.15</td>
</tr>
<tr>
<td>I50</td>
<td>C</td>
<td>8.75 ± 0.30</td>
<td>0.31 ± 0.05</td>
<td>6.41 ± 0.09</td>
<td>2.75 ± 0.08</td>
<td>1.51 ± 0.03</td>
<td>19.73 ± 0.43</td>
</tr>
<tr>
<td>I50</td>
<td>VM</td>
<td>8.28 ± 0.13</td>
<td>0.23 ± 0.09</td>
<td>6.16 ± 0.19</td>
<td>2.61 ± 0.04</td>
<td>1.46 ± 0.02</td>
<td>18.73 ± 0.05</td>
</tr>
<tr>
<td>I50</td>
<td>PM</td>
<td>8.82 ± 0.11</td>
<td>0.23 ± 0.03</td>
<td>6.23 ± 0.29</td>
<td>2.77 ± 0.11</td>
<td>1.28 ± 0.12</td>
<td>19.32 ± 0.22</td>
</tr>
<tr>
<td>I50</td>
<td>C</td>
<td>8.48 ± 0.20</td>
<td>0.18 ± 0.02</td>
<td>6.04 ± 0.10</td>
<td>2.95 ± 0.07</td>
<td>1.60 ± 0.02</td>
<td>19.25 ± 0.16</td>
</tr>
<tr>
<td>I50</td>
<td>VM</td>
<td>8.07 ± 0.18</td>
<td>0.17 ± 0.02</td>
<td>5.99 ± 0.15</td>
<td>2.71 ± 0.03</td>
<td>1.38 ± 0.15</td>
<td>18.32 ± 0.20</td>
</tr>
<tr>
<td>I50</td>
<td>PM</td>
<td>8.44 ± 0.14</td>
<td>0.31 ± 0.02</td>
<td>5.90 ± 0.28</td>
<td>2.74 ± 0.05</td>
<td>1.46 ± 0.04</td>
<td>18.85 ± 0.21</td>
</tr>
</tbody>
</table>

LSD‡ 0.13 0.14 0.51 0.24 0.28 0.84

### Notes

* P ≤ 0.05.
** P ≤ 0.01.
† Abbreviations: I100, full irrigation; I75, moderate deficit irrigation; I50, severe deficit irrigation (based on soil moisture depletion of available water). C, no fertilizer; VM, vermicompost; PM, poultry manure.
‡ LSD value for comparison of means as affected by interaction of irrigation and fertilizers.
§ Mean: mean value of each variable averaged across treatments.
¶ LSD value for comparison of means as affected by main effect of irrigation and fertilizers.
# ns, not significant at P ≤ 0.05.
compared with well-watered plants which are in agreement with irrigation. The OLR ratio was also improved in stressed plants capability of the crop to tolerate even severe levels of deficit drought condition was fairly small (Table 5) which reflects the study, the extent of reduction of PUFA of milk thistle oil under or adjust FA unsaturation (Upchurch, 2008). In the current instance, it has been shown that the mid-seasonal drought had lower than non-stressed plants, which reflects damages caused by the stress (Upchurch, 2008). It can be summarized that the application of severe deficit irrigation caused 7% reduction in oil content with very small influence on unsaturated FAs and no changes in the content of saturated FAs. It is worth noting that, the time of drought stress induction determines the effect of drought stress on plant metabolism including FAs synthesis and composition. For example, it has been shown that the mid-seasonal drought had no effect on FA composition of peanut oil, while late season drought significantly reduced its linoleic acid content and increased stearic and oleic acid contents (Dwivedi et al., 1993). In the current study, deficit irrigation was employed during most the earlier findings in standard and high oleic acid hybrids of sunflower (Baldini et al., 2002; Flagella et al., 2002). Another important finding in this study was improvement of linolenic acid content in response to drought stress. It has been postulated that in non-tolerant plants that experienced drought stress, level of linolenic acid in their cell membranes is usually lower than non-stressed plants, which reflects damages caused by the stress (Upchurch, 2008). This can be an indication of milk thistle resiliency to drought stress.

quality in general was not highly influenced by irrigation treatment. This is an important outcome since in traditional oil seed crops, water deficiency usually caused major variations in FA composition, often resulting in a decrease in unsaturated FAs proportion like oleic acids (Anastasi et al., 2010; Bettia et al., 2009; Baldini et al., 2002; Hamrouri et al., 2001; Jalilian et al., 2012) and linoleic acid (Dwivedi et al., 1993; Jalilian et al., 2001; Jalilian et al., 2012; Laribi et al., 2009) in favor of saturated ones (Dwivedi et al., 1993; Hamrouri et al., 2001; Jalilian et al., 2012; Laribi et al., 2009), which lowers the quality of the oil. In fact, the degree of fatty acids unsaturation may be reduced by drought stress due to inhibiting biosynthesis of PUFA and denaturizing activities leading to a reduction in oil content and a change in FA composition (Ashrafi and Razmjoo, 2010). It has been suggested, therefore, tolerance of plants to drought greatly depends on the inherent level of FA unsaturation and/or the ability to maintain or adjust FA unsaturation (Upchurch, 2008). In the current study, the extent of reduction of PUFA of milk thistle oil under drought condition was fairly small (Table 5) which reflects the capability of the crop to tolerate even severe levels of deficit irrigation. The OLR ratio was also improved in stressed plants compared with well-watered plants which are in agreement with

Table 5. Oleic/linoleic acid ratio (OLR), polyunsaturated fatty acids (PUFA), monounsaturated fatty acids (MUFA), MUFA/PUFA ratio, total unsaturated fatty acids (UFA), and unsaturated/saturated fatty acids ratio (UFA/SFA) of milk thistle oil as influenced by irrigation regimes and fertilizers. (For PUFA, MUFA, and UFA results are given as % of total fatty acids ± SE. For OLR, MUFA/PUFA, and UFA/SFA results are given as ratio ± SE.).

<table>
<thead>
<tr>
<th>Irrigation</th>
<th>Fertilizer</th>
<th>OLR</th>
<th>PUFA</th>
<th>MUFA</th>
<th>MUFA/PUFA</th>
<th>UFA</th>
<th>UFA/SFA</th>
</tr>
</thead>
<tbody>
<tr>
<td>I100†</td>
<td>C</td>
<td>0.82 ± 0.02</td>
<td>43.56 ± 0.64</td>
<td>35.77 ± 0.40</td>
<td>0.82 ± 0.02</td>
<td>79.32 ± 0.72</td>
<td>4.21 ± 0.18</td>
</tr>
<tr>
<td>I100†</td>
<td>VM</td>
<td>0.78 ± 0.03</td>
<td>44.76 ± 0.66</td>
<td>34.65 ± 0.66</td>
<td>0.78 ± 0.03</td>
<td>79.41 ± 0.37</td>
<td>4.16 ± 0.06</td>
</tr>
<tr>
<td>I100†</td>
<td>PM</td>
<td>0.85 ± 0.04</td>
<td>42.53 ± 0.98</td>
<td>36.22 ± 0.86</td>
<td>0.86 ± 0.04</td>
<td>78.75 ± 0.38</td>
<td>4.19 ± 0.04</td>
</tr>
<tr>
<td>I75</td>
<td>C</td>
<td>0.86 ± 0.03</td>
<td>41.05 ± 0.52</td>
<td>35.38 ± 0.77</td>
<td>0.86 ± 0.02</td>
<td>76.43 ± 0.77</td>
<td>3.88 ± 0.09</td>
</tr>
<tr>
<td>I75</td>
<td>VM</td>
<td>0.93 ± 0.03</td>
<td>40.94 ± 0.75</td>
<td>37.98 ± 0.50</td>
<td>0.93 ± 0.03</td>
<td>78.92 ± 0.39</td>
<td>4.21 ± 0.02</td>
</tr>
<tr>
<td>I75</td>
<td>PM</td>
<td>0.78 ± 0.01</td>
<td>43.07 ± 0.41</td>
<td>33.67 ± 0.25</td>
<td>0.78 ± 0.01</td>
<td>76.73 ± 0.35</td>
<td>3.97 ± 0.04</td>
</tr>
<tr>
<td>I50</td>
<td>C</td>
<td>0.86 ± 0.03</td>
<td>42.21 ± 0.45</td>
<td>35.89 ± 0.93</td>
<td>0.85 ± 0.03</td>
<td>78.10 ± 0.76</td>
<td>4.06 ± 0.06</td>
</tr>
<tr>
<td>I50</td>
<td>VM</td>
<td>0.89 ± 0.02</td>
<td>41.70 ± 0.49</td>
<td>36.57 ± 0.61</td>
<td>0.88 ± 0.02</td>
<td>78.27 ± 0.44</td>
<td>4.28 ± 0.06</td>
</tr>
<tr>
<td>I50</td>
<td>PM</td>
<td>0.87 ± 0.02</td>
<td>41.91 ± 0.58</td>
<td>36.39 ± 0.54</td>
<td>0.87 ± 0.02</td>
<td>78.30 ± 0.34</td>
<td>4.16 ± 0.06</td>
</tr>
</tbody>
</table>

** | Mean§ | 0.85 | 42.41 | 35.84 | 0.85 | 78.25 | 4.12 |
| Year (Y)   | 0.04 | 0.85 | 1.80 | 1.84 | 0.07 | 1.52 | 0.23 |
| Irrigation (I) | ns# | # | ** | ns | ** | ** | ns |
| Fertilizer (F) | ns | ns | ns | ns | ns | ns | ns |
| I × F | ns | ns | ns | ns | ns | ns | ns |
| Y × I X F | ns | ns | ns | ns | ns | ns | ns |

* P ≤ 0.05. ** P ≤ 0.01. † Abbreviations: I100, full irrigation; I75, moderate deficit irrigation; I50, severe deficit irrigation (based on soil moisture depletions of available water). C, no fertilizer; VM, vermicompost; PM, poultry manure. ‡ LSD value for comparison of means as affected by interaction of irrigation and fertilizers. § Mean: mean value of each variable averaged across treatments. ¶ LSD value for comparison of means as affected by main effect of irrigation and fertilizers. # ns, not significant at P ≤ 0.05.
deficiency from establishment through physiological maturity. This could explain why deficit irrigation did not greatly change the FA composition of milk thistle oil, although the inherent ability of the crop to tolerate drought would be the primary reason for this observation.

**Effect of Organic Fertilizers on Oil Content and Composition**

Oil content of milk thistle seed was not significantly affected by the application of organic fertilizers (Table 3). Generally, application of N fertilizer lowers oil content in favor of protein content in oil seed crops (Brennan et al., 2000; Rathke et al., 2005). It has been shown that excessive use of fertilizers, especially chemical N fertilizer caused a reduction in oil content (Brennan et al., 2000). However, in this study soil amendments were used based on their P content, not N, thus; plants were not supplied with high amounts of N.

Although application of soil organic amendments did not influence seed oil content but it caused some alterations on FA composition. While the main effect of fertilizer was only significant on margaroleic and linolenic acids content, the interaction of irrigation and fertilizer was significant for palmitoleic, margaroleic, oleic, linoleic, and linolenic acids content (Table 3). In full irrigation regime, the application of organic fertilizers did not make any changes in the content of above mentioned UFAs, but when deficit irrigation was induced differences were observed between control and fertilized plants. In all irrigation regimes, the greatest content of margaroleic acid was observed in plants fertilized with PM. However, the application of VM was more effective than PM in regard to improve linolenic acid content. Averaged across irrigation regimes, no significant differences were observed between control and organic fertilizer treatments in regard to OLR, PUFA, MUFA, MUFA/PUFA, and UFA (Table 5). Among SFAs, the content of palmitic and arachidic acids were influenced by the application of soil organic amendments whereas margaric, stearic, and behenic acids content were not influenced by fertilizing treatments (Table 4). The application of both organic amendments reduced the content of palmitic and arachidic acids; therefore, resulted in significant changes of SFA. In full irrigation regime, the SFA value was stable and did not change by organic fertilizers but when deficit irrigation was imposed, application of both organic amendments lowered SFA (Table 4). The UFA/SFA ratio was also influenced by the application of organic fertilizers. While in $I_{100}$ no difference was observed between control and fertilizer treatment, application of VM in $I_{50}$ and $I_{50}$ improved the ratio (Table 5). Overall, the results indicated that the application of soil organic amendments did not significantly influence UFA but reduced SFA which totally imposed positive effects on oil quality of milk thistle. Vick et al. (2004) reported that growing conditions, including plant nutrition, plays an important role in the relative proportion of SFA in sunflower oil and Zheljazkov et al. (2009) revealed that the application of N fertilizer increased SFA of sunflower. It seems that the degree of FA saturation–unsaturation may change in response to fertilization which supports findings by Žanetti et al. (2009). However, very limited information is available in regard to the effect of fertilizers especially organic amendments on the seed oil content and FA composition of various crops and more specifically in milk thistle. Further studies are needed to investigate the influence of plant nutrition on FA composition in milk thistle to confirm the results of the current study.

**CONCLUSION**

Saving 25% of irrigation water by implementation of moderate deficit irrigation resulted in no changes in oil content of milk thistle seeds compared with full irrigation. However, severe deficit irrigation reduced the oil content of milk thistle seeds by 7%. Deficit irrigation had only a minor effect on the FA composition of milk thistle oil and overall quality of the oil was not altered by water limitation. The oil content of milk thistle seed was not influenced by application of VM and PM, but both organic amendments had a positive effect on oil quality by decreasing the percentage of saturated FAs. Accordingly, even a 50% savings of irrigation water, which is crucial for sustainability of farming in arid and semiarid regions, did not considerably affect milk thistle oil content and quality taking into account the amount of water saved in this system. Based on the results obtained in this study, it seems that milk thistle has potential to be considered as a good source of edible oil in arid and semiarid areas. The findings of this study and earlier reports by the authors (Afshar et al., 2014b) indicate that cultivation of milk thistle as a high quality oil seed crop in regions with limited irrigation water is quite feasible. However, more research trials are needed to evaluate milk thistle suitability in crop rotation and its potential as becoming a noxious weed in agricultural plantations.

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