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Effect of Fertilizer Types and Plant Densities on Oil Components of Milk Thistle (*Silybum marianum* L.) Under Rainfed and Irrigated Conditions

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Abstract

The present study was conducted at Bazian location during two winter seasons of 2010-2011 and 2011- 2012 A factorial experiment was conducted using Completely Randomized Block Design (CRBD) with three replications. The study included two different irrigation; Rainfed and Irrigated, combined with three fertilization applications; No fertilization Chemical fertilization and Organic fertilization and two different plant densities; 8and 16 plant m^{-2}.
The most important results of the average of both seasons can be summarized as follows:-
Seed samples were analyzed quantitatively by HPLC for their content of unsaturated fatty acids; α-Linolenic, Linoleic, Oleic and Gadoleic acid. Data showed that effect of irrigation treatment was significant in the amount of Linoleic and Oleic acids, while Gadoleic showed significant differences due to no fertilizer treatment. α-Linolenic respond significantly to the interaction between chemical fertilizer and 8 plant m^{-2}.

Introduction:

Green plants synthesize and preserve a variety of biochemical products, many of which are extractable and used as chemical feed stocks or as raw material for various scientific investigations **[1]**. Milk thistle (*Silybum marianum* L.), belonging to the Asteraceae family, is a recognized medicinal plant which originated in the Mediterranean Basin. As a crop and weed on agricultural plantations **[2** and **3]**. Unsaturated fatty acids have one or more double bonds between carbon atoms. The two carbon atoms in the chain that are bound next to either side of the double bond can occur in a cis or trans configuration. Unsaturated fat, are important for brain development and function, they can even help us maintain mental acuity as we grow old **[4]**.
Possibly due to their antioxidant and membrane stabilizing properties, the compounds have been shown to protect different organs and cells against a number of diseases. Currently the most important medicinal application of milk thistle is its use as a hepatoprotectant and as supportive treatment of chronic inflammatory liver disorders such as cir- rhosis, hepatitis, and fatty infiltration due to alcohol and toxic chemicals **[5]**. The plant also yields 25 – 30 % of edible oil containing essential phospholipids and high content of vitamin E **[6]**. The oil is rich in Linoleic acid and Oleic acid **[7]**. The aims of the study to show the range of variation in the seed oil content of Milk thistle plant depending upon cultivation conditions irrigation, fertilization and plant density and their interaction.

Materials and Methods:

Plant collection and identification

Samples of plants evaluated in this investigation were collected during the 2009-2010 season, at full blooming. Plant samples were identified at faculty of agriculture /university of sulaimani.

Seed Collection:

The seeds were collected from Sulaimani- Bakrajo at late may 2009- 2010. The time of collection has been determined by the taxonomists, when the color of the seeds became dark and just before the bloom explodes **[8]**.

The seeds were separated from the flower heads and kept until sowing season.

Field Site Description:

Field experiment was conducted at Bazian, for two seasons in autumn 2010-2011 and 2011-2012 on the same site, (Latitude 35° 59′ 146″ ; N, Longitude 45° 14′ 094″ ; E, 807 MASL) situated in 22.8 Km far from Sulaimani governorate. The experiment contained 3 factors, first: two irrigation treatment (Rainfed and irrigation), second: fertilization (no fertilization, chemical fertilization and organic fertilization), the third factor was two plant density 8 plant/m^2 and 16 plant/m^2 **[9],** this factorial experiment conducted in Completely Randomized Block Design with 3 replication, each block contained 12 uniform experimental plots of 16 m^2 (4×4) m and 1m apart from each other, the seeds of *Silybum marianum* L. were directly sown in the plots in autumn 2010 and 2011**[10]**. Soil of the experiment prepared for cultivation by ploughing the field two times using mold broad plow and harrow. Weeds were controlled manually whenever necessary, and all other culture practices were conducted uniformly normal for all treatment.

Seed Oil Determination:

2 g of the harvested seed of each treatment was powdered by electric blender. Digital soxhlet instrument used for oil distillation, with n-hexane solvent (BDH, UK), **[11]**, the oil samples put in refrigerator until use, the oil content calculated as follows:

$Oil\% = [(W_2 - W_1) \times 100] / S$ ………. (1)

Where:

W_1 = Weight of Empty Flask (g).

W_2 = Weight of Flask and Extracted Oil (g).

S = Weight of Sample.

Oil samples kept in refrigerator until use for separation of fatty acids. **[12]** ……….(2)

Separation of fatty acids:

Oil samples as prepared in above section were qualitatively and quantitatively analyzed by High Performance Liquid Chromatography (HPLC) {Research Laboratory of the Green Field Company in Baghdad} using a model Shimadzu corporation, Kyoto Japan, LC-AV double delivery pump model LC-10Ashimadzu.

Separation of fatty acids was done using HPLC, on reversed phase C-8 (50×2.6mm ID) column.3µm particle size, mobile phase was acetonitril: tetrahydrofuran: 0.1% phosphoric acid (51:37:12v/v), the flow rate 1ml/minute. The eluted peaks were mentioned by UV detector set at 215 nm, and quantitative analyzed by comparing the area of well known standard with the area of the sample under the same separation condition **[13]**.

Conc. of Sample (µg/ml) = $\frac{\text{Area of sample}}{\text{Area of stanard}}$ × Conc. of Standard × Dilution factor ……….(3)

Statistical Analysis:

Analysis of variance as a general test was don according to analysis of 3 factors in CRBD, and all possible comparisons among the means were carried out by using Least Significant Difference (L.S.D) test at significant levels of 5% **[14]**.

Results and Discussions:

Analysis of fatty acids from Milk thistle seed:

Preparative HPLC analysis for the fatty acids fraction samples showed retention times on HPLC chromatograms as listed, in figure (1). These correspond to α-Linoenic (Omega 3), Linoleic (Omega 6), Oleic (Omega 9) and Gadoleic, as each compound listed to its correspondent retention time 1.172, 2.417, 3.265 and 4.223, respectively. Figure (1) revealed the resolution of these five unsaturated fatty acids with retention time.

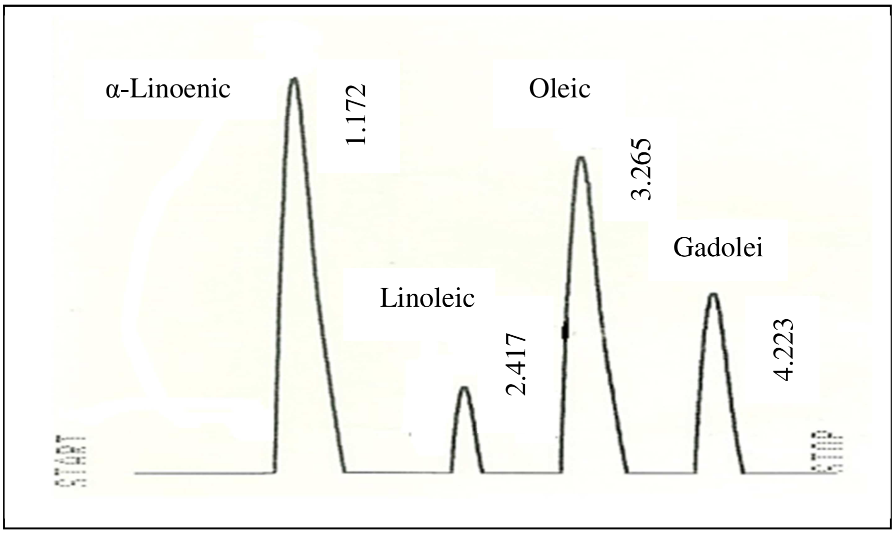

Figure [1]: Retention time (min.) Stander detection of unsaturated fatty acids.

Effects of cultivation conditions and their interactions on the quantitative aspects of unsaturated fatty acids of Milk thistle seed:

- **Effect of irrigation treatments on the quantitative aspects of unsaturated fatty acids of Milk thistle seed**

Data in Table (1) showed the presence of significant and non significant effects between the treatments of irrigated and rainfed on different unsaturated fatty acids contents in both seasons and their average. Although, there were no significant effects obtained on α-Linolenic acid in both seasons and their average, due to the effect of irrigation, but data shows that irrigated treatment increased the amount of α-Linolenic acid by 7.752% and 17.252% in the first and second season, which gave an increasing in the average of both seasons with 14.407%. Linoleic acid showed no significant response due to irrigation treatments in the first season, while it gave significant effects in the second and average of both seasons. Concerning the second and average of both seasons' maximum values were recorded by irrigated treatment with 2.903 and 1.977 mg g^{-1}, respectively.

Mono unsaturated fatty acid, oleic acid showed no significant effects in the first season due to irrigation treatments, while in the second season it produced significant effects and in the average of both seasons significant effects were recorded. Due to irrigated treatment maximum values with 1.134 mg g^{-1} for the second season and 0.857mg g^{-1} for the average of both seasons were obtained. The Gadoleic acid showed no significant effect in both seasons and their average. These results were in agreements with the results obtained by **[10]** who reported that both mono unsaturated fatty acids were increased significantly due to irrigated treatments. Our results differed in the case of polyunsaturated fatty acids; α-linolenic and linoleic which were found to be increased under irrigated condition.

Table 1: Means of unsaturated fatty acids contents of Milk thistle seeds as affected by irrigation treatments (mg g^{-1}).

First season				
Irrigation	***α-linolenic acid (Omega- 3)***	***Linoleic acid (Omega- 6)***	***Oleic acid (Omega- 9)***	***Gadoleic acid***
Rainfed	0.129	1.049	0.586	0.300
Irrigated	0.139	1.050	0.580	1.772
LSD$_{(P\leq0.05)}$	**n.s**	**n.s**	**n.s**	**n.s**
Second season				
Rainfed	0.342	1.702	0.747	0.538
Irrigated	0.401	2.903	1.134	0.877
LSD$_{(P\leq0.05)}$	**n.s**	**0.664**	**0.230**	**n.s**
Average of both seasons				
Rainfed	0.236	1.376	0.667	0.419
Irrigated	0.270	1.977	0.857	1.325
LSD$_{(P\leq0.05)}$	**n.s**	**0.372**	**0.176**	**n.s**

- ***Effect of fertilization treatments on the quantitative aspects of unsaturated fatty acids***

Table (2) showed the effect of fertilization treatment on unsaturated fatty acids in both seasons and their average. Unsaturated fatty acids; α- linolenic, linoleic and oleic recorded no significant effect, due to fertilization treatments in both seasons and their average.

In the first and average of both seasons fertilization treatments recorded significant effect on gadoleic acid, while in the second season no significant effects were obtained. Concerning the first and average of both seasons no fertilization treatments gave maximum values with 0.415 and 0.634 mg g^{-1}, and the lowest value were 0.126 and 0.285 mg g^{-1} due to organic fertilization, respectively. However there were no significant effects in most of fertilization treatments on unsaturated fatty acids, but in realest no fertilization treatment produced an increase in the unsaturated fatty acids contents, these results were in agreement with **[15]** who observed that the highest amount of oleic acid was recorded from no fertilizer treatments, but differ in the case of linoleic acid.

Table 2: Means of unsaturated fatty acid contents of Milk thistle seeds as affected by fertilization treatments (mg g^{-1})

Fertilization	*α-linolenic acid (Omega- 3)*	*Linoleic acid (Omega- 6)*	*Oleic acid (Omega- 9)*	*Gadoleic Acid*
		First season		
Non fer.	0.124	1.357	0.651	0.415
Chemical fer.	0.151	0.799	0.504	0.176
Organic fer.	0.128	0.993	0.594	0.126
LSD $_{(P\leq0.05)}$	**n.s**	**n.s**	**n.s**	**0.161**
		Second season		
Non fer.	0.420	2.420	1.018	0.852
Chemical fer.	0.352	1.956	0.931	0.829
Organic fer.	0.342	2.531	0.872	0.443
LSD $_{(P\leq0.05)}$	**n.s**	**n.s**	**n.s**	**n.s**
		Average of both seasons		
Non fer.	0.272	1.889	0.835	0.634
Chemical fer.	0.252	1.378	0.717	0.503
Organic fer.	0.235	1.762	0.733	0.285
LSD $_{(P\leq0.05)}$	**n.s**	**n.s**	**n.s**	**0.247**

- **Effect of plant density treatments on the quantitative aspects of unsaturated fatty acids of Milk thistle seed**

Table (3) showed no significant effects between the treatments of plant density on unsaturated fatty acids; α- linolenic acid, oleic acid and gadoleic acid) in both seasons and their average.
Linoleic acid showed no significant response in the first and average of both seasons due to plant density treatments, while in the second season significant effects were recorded. The treatment of 16 plant m^{-2} produced maximum value of 2.644 g^{-1}.

Table 3: Means of unsaturated fatty acid contents of Milk thistle seeds as affected by plant density treatments (mg g^{-1})

Plant density (m^{-2})	α-linolenic acid (Omega- 3)	Linoleic acid (Omega- 6)	Oleic acid (Omega- 9)	Gadoleic acid Acid
		First season		
8plant	0.138	1.044	0.578	0.215
16plant	0.131	1.056	0.588	0.262
LSD$_{(P\leq0.05)}$	**n.s**	**n.s**	**n.s**	**n.s**
		Second season		
8plant	0.381	1.961	0.832	0.541
16plant	0.362	2.644	1.049	0.874
LSD$_{(P\leq0.05)}$	**n.s**	**0.664**	**n.s**	**n.s**
		Average of both seasons		
8plant	0.260	1.503	0.705	0.378
16plant	0.247	1.850	0.819	0.568
LSD$_{(P\leq0.05)}$	**n.s**	**n.s**	**n.s**	**n.s**

- **Effect of interaction between irrigation and fertilization treatments on the quantitative aspects of unsaturated fatty acids of Milk thistle seed**

Data in table (4) showed the presence of non significant effects between the treatment interactions of irrigated and fertilization on each of α- linoenic and oleic fatty acids in both seasons and their average. Linoleic acids also showed no significant effects in the first and average of both seasons due to interaction treatments, while in the second season significant effects were recorded. Due to interaction between irrigated and no fertilization treatment maximum value of 3.594 mg g^{-1} was recorded, and the minimum value was recorded due to rainfed and no fertilization treatment which was found to be 1.246 mg g^{-1}in the second season. Gadoleic acid respond significantly to interaction of irrigation and fertilization treatments in the first season, while no significant effects recorded in the second and average of both seasons. The interaction between rainfed and no fertilization treatments in the first season gave maximum value of 0.613mg g^{-1}, and the lowest value was obtained by interaction of rainfed and organic fertilization treatments with the value of 0.108mg g^{-1}.

Table 4: Means of unsaturated fatty acid contents of Milk thistle seeds as affected by interaction between irrigation and fertilization treatments (mg g^{-1})

Irrigation	Fertilization	α-Linolenic acid (Omega- 3)	Linoleic acid (Omega- 6)	Oleic acid (Omega- 9)	Gadoleic Acid
			First season		
Rainfed	***Non fer.***	0.107	1.342	0.591	0.613

	Chemical fer.	0.154	0.780	0.472	0.181
	Organic fer. fer.	0.127	1.027	0.695	0.108
Irrigated	***Non fer.***	0.140	1.373	0.711	0.217
	Chemical fer.	0.148	0.818	0.536	0.171
	Organic fer.	0.130	0.960	0.494	0.144
LSD $_{(P\leq0.05)}$		**n.s**	**n.s**	**n.s**	**0.228**
Second season					
Rainfed	***Non fer.***	0.323	1.246	0.801	0.457
	Chemical	0.428	1.924	0.673	0.662
	Organic fer.	0.276	1.936	0.766	0.496
Irrigated	***Non fer.***	0.518	3.594	1.235	1.248
	Chemical fer.	0.276	1.989	1.188	0.995
	Organic fer.	0.408	3.125	0.978	0.389
LSD $_{(P\leq0.05)}$		**n.s**	**1.150**	**n.s**	**n.s**
Average of both seasons					
Rainfed	***Non fer.***	0.215	1.294	0.696	0.535
	Chemical fer.	0.291	1.352	0.573	0.422
	Organic fer. fer.	0.202	1.482	0.731	0.302
Irrigated	***Non fer.***	0.329	2.484	0.973	0.733
	Chemical fer.	0.212	1.404	0.862	0.583
	Organic fer.	0.269	2.043	0.736	0.267
LSD $_{(P\leq0.05)}$		**n.s**	**n.s**	**n.s**	**n.s**

- **Effect of interaction between irrigation and plant density treatments on the quantitative aspects of unsaturated fatty acids of Milk thistle seed**

The results in Table (5) showed no significant effects between unsaturated fatty acids due to the effect of interaction between irrigation and plant density treatments in both season and their average.

Table 5: Means of unsaturated fatty acid contents of Milk thistle seeds as affected by interaction between irrigation and plant density treatments (mg g^{-1})

Irrigation	*Plant density (m^{-2})*	α-Linolenic acid (Omega- 3)	Linoleic acid (Omega- 6)	Oleic acid (Omega- 9)	Gadoleic Acid
		First season			
Rainfed	*8plant*	0.143	1.021	0.559	0.237
	16plant	0.116	1.077	0.613	0.364
Irrigated	*8plant*	0.133	1.066	0.598	0.193
	16plant	0.145	1.034	0.563	0.161
$LSD_{(P\leq0.05)}$		**n.s**	**n.s**	**n.s**	**n.s**
		Second season			
Rainfed	*8plant*	0.380	1.123	0.499	0.442
	16plant	0.304	2.281	0.995	0.635
Irrigated	*8plant*	0.382	2.799	1.164	0.641
	16plant	0.419	3.006	1.103	1.113
$LSD_{(P\leq0.05)}$		**n.s**	**n.s**	**n.s**	**n.s**
		Average of both seasons			
Rainfed	*8plant*	0.262	1.072	0.529	0.340
	16plant	0.210	1.679	0.804	0.500
Irrigated	*8plant*	0.258	1.933	0.881	0.417
	16plant	0.282	2.020	0.833	0.637
$LSD_{(P\leq0.05)}$		**n.s**	**n.s**	**n.s**	**n.s**

- **Effect of interaction between fertilization and plant density treatments on the quantitative aspects of unsaturated fatty acids of Milk thistle seed**

Table (6) revealed no significant effects between treatment interactions of fertilization and plant density on unsaturated fatty acids: linoleic, oleic and gadoleic acid in both seasons and their average with the exception of α- linolenic fatty acid in second and average of both season.

α- linolenic respond significantly to interaction treatments in the second season, while average of both season recorded significant effects. Regarding the second season the interaction between no fertilizer and 16 plant m^{-2} produced maximum value of 0.634, while in the average of both seasons the interaction between chemical fertilizer and 8 plant m^{-2} gave maximum value with 0.374 mg g^{-1}. The lowest values were recorded by chemical fertilization and 16 plant m^{-2} interactions with the value of 0.130 and 0.130 mg g^{-1}.

Table 6: Means of unsaturated fatty acid contents of Milk thistle seeds as affected by interaction between fertilization and plant density treatments (mg g^{-1})

Fertilization	*Plant density (m^{-2})*	α-Linolenic acid (Omega- 3)	Linoleic acid (Omega- 6)	Oleic acid (Omega- 9)	Gadoleic acid
		First season			
No fer.	*8plant*	0.162	1.186	0.615	0.332
	16plant	0.086	1.529	0.687	0.497
Chemical fer.	*8plant*	0.174	1.098	0.609	0.197
	16plant	0.129	0.500	0.399	0.154
Organic fer.	*8plant*	0.079	0.848	0.512	0.116
	16plant	0.177	1.138	0.677	0.136
$LSD_{(P\leq0.05)}$		**n.s**	**n.s**	**n.s**	**n.s**
		Second season			
No fer.	*8plant*	0.207	2.206	1.070	0.547
	16plant	0.634	2.634	0.965	1.158
Chemical fer.	*8plant*	0.574	1.738	0.788	0.717
	16plant	0.130	2.175	1.074	0.940
Organic fer.	*8plant*	0.362	1.939	0.637	0.360
	16plant	0.321	3.122	1.107	0.525
$LSD_{(P\leq0.05)}$		**0.398**	**n.s**	**n.s**	**n.s**
		Average of both seasons			
No fer.	*8plant*	0.185	1.696	0.843	0.440
	16plant	0.360	2.082	0.826	0.828
Chemical fer.	*8plant*	0.374	1.418	0.699	0.457
	16plant	0.130	1.338	0.737	0.547
Organic fer.	*8plant*	0.221	1.394	0.575	0.238
	16plant	0.249	2.130	0.892	0.331
$LSD_{(P\leq0.05)}$		**0.202**	**n.s**	**n.s**	**n.s**

- **The effect of interaction between irrigation, fertilization and plant density treatments on unsaturated fatty acids of Milk thistle seed**

The results of Table (7) showed no significant effect of interactions between irrigation, fertilization and plant density in both seasons and their average on unsaturated fatty acids content with the exception α-linolenic acid in the first season.

The effects of interactions of rainfed, chemical fertilization and 8 plant m^{-2} treatments on the fatty acid α-linolenic showed maximum value of 0.251mg g^{-1}. The minimum value was obtained by treatment of irrigated, no fertilization and 16 plant m^{-2} which was found to be 0.049 mg g^{-1}.

Table 7: Means of unsaturated fatty acid contents of Milk thistle seeds as affected by interaction between irrigation, fertilization and plant density treatments (mg g-1).

Irrigation	Fertilization	Plant density (m^{-2})	α-Linolenic acid (Omega- 3)	Linoleic acid (Omega- 6)	Oleic acid (Omega- 9)	Gadoleic acid
			First season			
Rainfed	Non fer.	8plant	0.093	1.184	0.606	0.392
		16plant	0.122	1.499	0.575	0.833
	Chemical fer.	8plant	0.251	1.152	0.548	0.252
		16plant	0.058	0.408	0.395	0.109
	Organic fer.	8plant	0.086	0.729	0.522	0.066
		16plant	0.167	1.325	0.868	0.149
Irrigated	Non fer.	8plant	0.231	1.187	0.623	0.271
		16plant	0.049	1.559	0.799	0.162
	Chemical fer.	8plant	0.097	1.044	0.664	0.142
		16plant	0.199	0.591	0.403	0.199
	Organic fer.	8plant	0.072	0.968	0.501	0.166
		16plant	0.187	0.952	0.486	0.123
	$LSD_{(P\leq0.05)}$		**0.174**	**n.s**	**n.s**	**n.s**
			Second season			
Rainfed	Non fer.	8plant	0.188	0.575	0.594	0.367
		16plant	0.456	1.916	1.008	0.547
	Chemical fer.	8plant	0.710	1.780	0.632	0.637
		16plant	0.146	2.063	0.715	0.687
	Organic fer.	8plant	0.241	1.014	0.272	0.321
		16plant	0.310	2.857	1.261	0.671
Irrigated	Non fer.	8plant	0.225	3.036	1.546	0.728
		16plant	0.811	3.352	0.923	1.768
	Chemical fer.	8plant	0.438	1.697	0.944	0.797
		16plant	0.114	2.281	1.433	1.193
	Organic fer.	8plant	0.483	2.864	1.002	0.399
		16plant	0.332	3.386	0.953	0.380
	$LSD_{(P\leq0.05)}$		**n.s**	**n.s**	**n.s**	**n.s**
			Average of both seasons			
Rainfed	Non fer.	8plant	0.141	0.880	0.600	0.380
		16plant	0.289	1.708	0.792	0.690
	Chemical fer.	8plant	0.481	1.466	0.590	0.445
		16plant	0.102	1.236	0.555	0.398
	Organic fer.	8plant	0.164	0.872	0.397	0.149
		16plant	0.239	2.091	1.065	0.410
Irrigated	Non fer.	8plant	0.228	2.112	1.085	0.500
		16plant	0.430	2.456	0.861	0.965
	Chemical fer.	8plant	0.268	1.371	0.804	0.470
		16plant	0.157	1.436	0.918	0.696
	Organic fer.	8plant	0.278	1.916	0.752	0.283
		16plant	0.260	2.169	0.720	0.252
	$LSD_{(P\leq0.05)}$		**n.s**	**n.s**	**n.s**	**n.s**

Conclusions:

According to the results of this study, milk thistle seed oil could be a rich source of polyunsaturated fatty acids, Linolenic (Omega- 3), Linoleic (Omega- 6) and Oleic (Omega-9), which makes it an interesting candidate from a nutritional point of view. From HPLC analysis of seed extract of Milk thistle plant, it can be concluded that unsaturated fatty acids differ in quantitative composition, due to the effect of treatments and their combination in both seasons.

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