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Effects of Different Sowing Times and Densities on Milk Thistle *(Silybum marianum)* Seeds' Oil Ratios and Fatty Acids

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Abstract - The purpose of this study was to determine the effects of different sowing times (summer and winter), row spacings (25 and 50 cm) and intra-row spacings (10, 20, 30 and 40 cm) on Milk Thistle seeds' oil ratios and fatty acids. The results obtained demonstrated that the oil ratio in Milk Thistle seeds ranges from 22.02% to 25.97% and the effects of different sowing densities on oil ratio were statistically insignificant. Furthermore, oil ratio was approximately 3.9% higher in summer sowing than that in winter sowing. In terms of fatty acids, the ratios of linoleic acid, oleic acid, palmitic acid, and stearic acid in winter sowing and summer sowing were 43.77% and 46.87%, 31.31% and 28.78%, 8.93% and 8.47%, and 6.72% and 6.68%, respectively.

Keywords: *Fatty acids, Milk Thistle, Silybum marianum, sowing density, sowing time.*

1. Introduction

As one of the medicinal plants which has recently come to the fore and been the subject of different studies in Turkey, Milk Thistle is an annual plant within the genus *Silybum* of the family *Asteraceae (Compositae*), both growing naturally and being commercially cultivated [17]. Milk Thistle seeds contain 1% to 6% silymarin. Produced in America and Europe, condensed Milk Thistle extracts have a silymarin content of 70% to 80%. Silymarin is a pharmacologically active substance contained in Milk Thistle seeds and composed of flavonolignans such as silychristin, silydianin, diastereoisomers silybin and isosilybin [9, 4, 17]. Many Milk Thistle seed preparations (either unaccompanied or as a mixture) are commercially available in the market and recommended for the treatment of toxicant-induced liver injury, cirrhosis and chronic liver injuries [14, 17].

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Milk Thistle plant grows in South-West Europe, Australia, North Africa and Western Asia. It is widespread in Mediterranean countries and the southern parts of Russia and naturally exists in Kashmir, India and Pakistan. In Turkey, Milk Thistle grows along the roads and fields and in the wastelands of İzmir, Aydın, Denizli, Mersin, Adana and Antakya in Western and Southern Anatolia and of the coastal areas of Marmara Region [9]. Consumed as a vegetable especially in the countries around the Mediterranean Sea, Milk Thistle has been used in treating liver diseases for long years. Every part of the plant can be used for nutritive or other purposes [13].

Germany is the leading country in the production of *Silybum marianum*. It is also produced in California on a large scale. In California and Germany, seeds are used in herbal medicine industry. The U.S. is reported to have generated an income of $380 millions from Milk Thistle in 2000.

Milk Thistle seeds contain fixed oil (20%-30%), starch, tannin, silymarins (silybin, silydianin and slychristin) as well as tyramin, flavonid, histamine, resin, amine, albumin and agmatine [19]. According to a study, 75.1% of total fatty acids in seeds are unsaturated fatty acids [20]. *S. marianum* seeds are reported to be rich in linoleic acids (50%-60%), followed by oleic acids (20%-30%), palmitic acids (9%) and stearic acids (6.6%) [21, 6].

In a study conducted on *S. marianum* seeds in Iran, the focal point was fatty acid amount and components, tocopherols, and antiradical activity. To determine antiradical activity, tocopherol amount (Vitamin E), and fatty acid components, researchers used DPPH radical, HPLC, and GC, respectively. The results of the study revealed that total oil content was 25%. Besides, they identified 9 fatty acids; namely, palmitic acid (8.25%), palmeotic acid (0.07%), stearic acid (6.67%), oleic acid (31.58%), isomer oleic acid (0.53%), linoleic acid (45.36%), linolenic acid (0.87%), arachidic acid (4.11%), eicozantoic acid (0.088%) and behenic acid (2,6%). α, γ, δ-tocopherol contents were calculated as 563.157 mg, 88.87 mg, and 163.791 mg, respectively. The studies performed suggest that *S. marianum* seed is a rich source of antioxidants [10].

This study was conducted to determine oil ratio and fatty acid composition in the seeds of Milk Thistle plant, which can be cultivated in Tokat region. Milk Thistle not cultivating in Turkey, also, grows along the roads and fields and in the wastelands of İzmir, Aydın, Denizli, Mersin, Adana and Antakya in Turkey and Western-Southern Anatolia and of the coastal areas of Marmara Region [9].

2. Material and Methods

2.1. Material

Seed material was provided from a previous study, conducted by Yılmaz et al. [23]. The seeds were harvested during the physiological maturity period of milk thistle crops. Following blending, the seeds were not processed in any manner. Experimental design was split plot in a randomized complete block with three replications. Seeds were sowed as two row spacings (25 and 50 cm) and four intra-row spacings (10, 20, 30 and 40 cm) on October and March months. Each plot consisted of four rows which were 4 m long. Equal amounts of nitrogen (10 kg/ha N), phosphorus (80 kg/ha P_2O_5) were applied homogenously to the plots. Half of nitrogen and all phosphorus were given to prepared plots and mixed with the soil before the transplanting of seedlings. The other half of nitrogen was given in bolting time. Study was conducted under rainfed conditions. The seeds were harvested during the physiological maturity period of milk thistle crops.

2.2. Methods

2.2.1. Oil Extraction

For each different sowing seasons and density, approximately 50 grams of milk thistle seed were ground by a laboratory blender. Of the said 50 grams of ground milk thistle seed, 10 grams were extracted with 30 mL hexane for 24 hours at room temperature. Following filtering by a filter paper and transferring to a tared flask, the solvent of the filtrate was removed under low pressure at 40°C. The total amount of oil was calculated based on 0% humidity, taking weight loss into consideration. For fatty acid analysis, the oil obtained was put into amber glass containers and kept at +4°C [2].

2.2.2. Formation of Fatty Acid Methyl Esters

For fatty acid analysis, fatty acid methyl esters were formed. To do so, 30 milligrams of extracted oil was solved in 3 mL hexane. After adding 3 M KOH solution prepared in 3 mL methanol, it was vortexed for 3 minutes. Then, it was kept waiting for 5-10 minutes at room temperature for phasing. 0,5 mL hexane containing methyl esters was put into vials and analyzed by GC-FID. The amount of each fatty acid was determined in percentages according to the rate of peak areas. In the determination of fatty acids, standard fatty acid mixture (Supelco 37 Component FAME mix 47885-U) was used [5].

2.2.3. Gas Chromatography Analysis Program

Fatty acids were analyzed by Perkin Elmer Clarus 500 Series gas chromatography (GC) using a FID (Flame Lonization Detector) detector. Apolar capillary column (TR-FAME 30 m X 0,25 mm X 0,25µm I.D) was employed. Split rate was set to 50:1. As the carrier gas, helium was used at a flow rate of 0,5 ml/min. Injection temperature and detector temperature were set to 250ºC and 260ºC, respectively. Being originally 100ºC, column furnace temperature was increased for 2ºC per minute and fixed to 220ºC.

2.2.4. Statistical Analysis

The results obtained were subjected to variance analyses according to Randomized Block Design. Averages were compared in accordance with Duncan Test [1].

3. Results and Discussion

The present results revealed that the oil ratio of Milk Thistle plants sowed in summer (24.10%) was higher than those sowed in winter (23.20%) (Table 1). During the study, different sowing densities were applied in both sowing seasons. According to the findings related to the most suitable row spacing and intra-row spacing for sowing the plant, oil content in seeds varied by density but the differences between densities in terms of oil ratios were found to be statistically insignificant.

Table 1 Effects of different sowing density on oil ratio and oil yield in summer and winter sowing of milk thistle

Sowing density (cm)	Summer		Winter	
	Oil ratio (%)	Oil yield (kg/da)	Oil ratio (%)	Oil yield (kg/da)
25x10	25.97	53.35 a	22.98	130.35 b
25x20	23.10	49.60 abc	24.16	162.52 a
25x30	22.89	38.97 cde	23.09	123.77 b
25x40	24.64	50.29 ab	23.40	135.75 b
50x10	24.65	40.27 bcd	23.89	108.49 bc
50x20	25.00	40.64 bcd	22.02	92.68 cd
50x30	22.37	28.78 e	22.74	66.25 de
50x40	24.21	33.57 de	23.33	54.52 e
Mean	24.10	41.93	23.20	109.29
Duncan [a,b,c]	ns	0.72**	ns	0.88***

*ns= no significant, **=p<0.01, ***=p<0.001 In each column, the same letter was not significantly different*

In such plants, not only oil ratio but also oil yield per decare is important. Accordingly, oil yield per decare ranged from 28.78 kg/da to 53.35 kg/da in summer sowing and from 54.52 kg/da to 162.52 kg/da in winter sowing, depending on sowing density, and was found to be statistically significant. Oil ratio was higher in summer sowing, while winter sowing had a higher seed yield. Accordingly, oil yield per decare was found to be 41.93 kg in summer sowing but 109.29 kg in winter sowing (Table 1). The highest oil yield was produced by the density of 25x10 cm (53.35 kg/da) which was the thickest one in summer sowing and by the density of 25x20 cm (162.52 kg/da) in winter sowing (Table 1).

It is a known fact that, in oilseeds, the effect of temperature on oil synthesis differs by plant species, ecology, and genetic structure. In a study, oil ratio in the seeds of Milk Thistle plants sowed in summer was found to be higher. However, high temperature and insufficient humidity resulted in lower yield, giving rise to lower oil yield [23]. In summer sowing, oil synthesis in seeds is generally higher as a result of higher temperature. In winter sowing, however, unless any cold damage occurs, plants grow much better, below- and above-ground parts are stronger, and factors positively affecting yield improve. As a result, seed yield per decare increases. Katar et al. [11] analyzed the effects of 8 different sowing times on oil ratio, oil yield and fatty acids in camelina plant and obtained the highest oil ratio from the plants sowed in November 15 and the lowest oil ratio from the ones sowed in May 1. On the other hand, the highest oil yield was produced by the first sowing date, October 1. Flagella et al. [7] examined the effects of different sowing times on yield and yield components in hybrid sunflower plant. They reported that the oil ratio of the plants sowed earlier was higher. They also reported that the plants sowed earlier produced higher seed yield and consequently higher oil yield. In another study, the effect of different sowing times on oil ratio in safflower was found to be insignificant [12].

Table 2 Effects of different sowing date on oil ratio and oil yield

Sowing date	Oil ratio (%)	Oil yield (kg/da)
Summer	24.10 a	41.93 b
Winter	23.20 b	109.29 a
Mean	23.65	75.61
Duncan [a,b,c]	0.73*	0.99 ***

**=p<0.05, ***=p<0.001, In each column, the same letter was not significantly different*

In the present study, 16 different fatty acids namely myristic, decanoic, decenoic, palmitic, palmiteoleic, heptadecanoic, heptadecenoic, stearic, oleic, linoleic, linolenic, arachidic, eicosanoic, arachidonic, cis-8,11,14-eicosatrienoic acid and eicosapentanoic acids were detected in fixed oil of Milk Thistle plant, 8 of which (palmitic, stearic, oleic, linoleic, arachidic, eicosanoic, cis-8,11,14-eicosatrienoic and eicosapentanoic acids) had a higher ratio and were found to be statistically significant as shown in Tables 3 and 4.

Findings related to the distribution of fatty acids in the seeds of Milk Thistle plants sowed in winter are given in Table 3. Accordingly, the effect of different sowing densities on the changes in stearic, oleic, linoleic, arachidic, cis-8,11,14-eicosatrienoic and eicosapentanoic fatty acids was not statistically significant. However, changes in palmitic ($p<0.05$) and eicosanoic ($p<0.001$) fatty acids were found to be statistically significant. The highest palmitic fatty acid ratio was obtained from the sowing density of 50x10 cm, while the sowing density of 25x10 cm resulted in the lowest palmitic fatty acid ratio. In eicosanoic fatty acids, the highest ratio and the lowest ratio were produced by the sowing densities of 25x30 cm and 25x10 cm, respectively (Table 3). As shown in the figures, increase in the number of plants per unit area gave rise to a decrease in palmitic and eicosanoic fatty acid ratios.

Table 3 Effects of different row spacing and row on fatty acids ratio of winter sowing in milk thistle

Row spacing (cm)	Intra-row spacing (cm)	Palmitic asid	Stearic asid	Oleic asid	Linoleic asid	Arachidic asid	Eicosanoic asid	Cis-8,11,14-eicosatrienoic asid	Eicosapentanoic asid
25	10	8.86 a	6.71	31.26	43.97	4.24	0.78 a	2.98	0.76
	20	8.89 a	6.58	31.56	43.45	4.25	0.94 bc	2.97	0.76
	30	8.73 a	6.62	31.38	43.97	4.23	0.98 c	2.96	0.76
	40	9.02 ab	6.75	31.37	43.42	4.30	0.96 bc	3.02	0.77
	Mean	8.88	6.67	31.39	43.70	4.26	0.92	2.98	0.76
50	10	9.36 b	6.99	31.25	43.14	4.37	0.88 b	2.91	0.72
	20	8.74 a	6.65	31.52	44.12	4.19	0.91 bc	2.83	0.72
	30	8.83 a	6.69	31.31	43.83	4.27	0.94 bc	3.00	0.78
	40	8.99 ab	6.73	30.85	44.30	4.13	0.95 bc	2.95	0.75
	Mean	8.98	6.77	31.23	43.85	4.24	0.92	2.92	0.74
Duncan [a,b,c]		0.43 *	ns	ns	ns	ns	0.61 ***	ns	ns

*ns= no significant, *=p<0.05, ***=p<0.001, In each column, the same letter was not significantly different*

In the seeds of Milk Thistle plants sowed in summer, different sowing densities had a statistically significant ($p<0.05$) effect only on the change in eicosanoic fatty acid ratio (Table 4). The sowing density of 25x20 cm produced the highest eicosanoic fatty acid ratio while . the lowest content of this fatty acid was detected in the sowing density of 50x10 cm. Upon examining each fatty acid group in itself, it was concluded that, compared to thick sowing, thin sowing positively affected fatty acids other than linoleic acid ratio and that more linoleic acids were synthesized in thick sowing. Besides, linoleic acid synthesis was higher in the seeds of Milk Thistle plants sowed in summer (Table 5).

Table 4 Effects of different row spacing and row on fatty acids ratio of summer sowing in milk thistle

Row spacing (cm)	Intra-row spacing (cm)	Palmitic asid	Stearic asid	Oleic asid	Linoleic asid	Arachidi c asid	Eicosanoic asid	Cis-8,11,14-eicocsatrienoic asid	Eicosapentanoic asid
25	10	7.49	6.44	28.11	48.34	4.01	0.95 bc	2.83	0.75
	20	8.63	6.47	29.03	46.70	4.10	0.98 c	2.93	0.76
	30	8.29	6.69	29.12	47.00	4.16	0.96 bc	2.99	0.79
	40	8.63	6.71	28.84	46.67	4.16	0.91 ab	2.98	0.80
	Mean	8.26	6.58	28.78	47.18	4.11	0.95	2.93	0.78
50	10	8.96	6.75	28.82	46.39	4.13	0.89 a	2.94	0.76
	20	8.67	6.78	29.08	46.20	4.18	0.94 abc	2.98	0.77
	30	8.63	6.70	28.14	47.40	4.11	0.95 bc	2.89	0.81
	40	8.51	6.87	29.10	46.25	4.25	0.95 abc	2.96	0.77
	Mean	8.69	6.78	28.77	46.56	4.17	0.93	2.94	0.78
Duncan [a,b,c]		ns	ns	ns	ns	ns	0.42*	ns	ns

*ns= no significant, *=p<0.05, In each column, the same letter was not significantly different*

Milk Thistle seeds' fatty acid ratio varied by sowing time; namely, summer and winter. Accordingly, palmitic (p<0.01), oleic (p<0.001), linoleic (p<0.001), arachidic (p<0.001) and eicosapentanoic (p<0.05) fatty acids' changes were found to be statistically significant (Table 5). In winter sowing, palmitic, oleic and arachidic fatty acids' ratios were higher. On the other hand, linoleic and eicosapentanoic fatty acids had higher ratios in summer sowing.

Table 5 Effects of different sowing season on fatty acids

Sowing season	Palmitic asid	Stearic asid	Oleic asid	Linoleic asid	Arachidic asid	Eicasonoic asid	Cis-8,11,14-eicocsatrienoic asid	Eicosapentanoic asid
Winter	8.93 a	6.72	31.31 a	43.77 b	4.25 a	0.92	2.95	0.75 b
Summer	8.47 b	6.68	28.78 b	46.87 a	4.14 b	0.94	2.94	0.78 a
Mean	8.70	6.70	30.05	45.32	6.32	0.93	2.95	0.77
Duncan [a,b,c]	0.14**	ns	0.74 ***	0.70 ***	0.15 ***	ns	ns	0.06 *

*ns= no significant, *=p<0.05, **=p<0.01, ***=p<0.001*
In each column, the same letter was not significantly different

In Milk Thistle sown in summer, oleic acid ratio decreased, while linoleic fatty acid ratio increased. As studies demonstrate, with temperature rise, there may be a decrease in the activity of the enzymes that catalyze the synthesis of linoleic and linolenic acids from oleic acid such as oleayl-PC desaturase and linoleayl-PC desaturase, respectively [3]. As a result, high temperature can sometimes affect linoleic and linolenic acid synthesis negatively but oleic acid synthesis positively [22, 18, 15]. Another study they conducted in Victoria, presented that fatty acid composition in rapeseed (*Brassica napus*) varied by regions and years. While oleic acid content ranged from 0.4% to 60.3%, low temperature and precipitation reduced oleic acid content. In spite of higher temperature and lower precipitation, linoleic (0.3%-19.7%) and linolenic acid (0.3%-10.4%) had lower variability between regions. In a study performed by [8] in the leading rapeseed producing regions of Australia for 3 years, saturated fatty acid ratios were found to be lower in the rapeseeds grown in the regions with higher temperature.

Not different sowing densities but sowing time (summer and winter) had a statistically significant effect on the change in the oil ratios of Milk Thistle plant seeds. Compared to winter sowing, summer sowing led to an increased oil ratio of 3.9% on average. On the other hand, oil yield per decare was considerably lower in summer sowing than that in winter sowing. The reason of a higher oil yield per unit area in winter sowing compared to summer sowing is higher seed yield. The significant point here is seed and oil yield per unit area. Milk Thistle seeds' oil ratio is approximately 25% on average; oil yield per decare is nearly 110 kg in winter sowing and 42 kg in summer sowing. Therefore, it is recommended that, under the ecological conditions of Kazova, Tokat, Milk Thistle is sowed in winter and each decare is populated by minimum 10.000 plants.

As revealed by this study, thick sowing gave better results than thin sowing and the sowing density of 25 cm was more advantageous than that of 50 cm. Likewise, although there was no difference between intra-row spacing, thick sowing gave better results. In a study carried out by [23], thick sowing of Milk Thistle plants produced fewer branches. As a result, plants to be harvested were more homogenous than the ones sowed thinly. The same researchers reported that, in thin sowing, depending on branch creation, there were more differences between the fruits on each branch in terms of growth and maturation. Further density studies can be performed on the subject.

In Milk Thistle seeds sowed in winter, the effect of sowing density on fatty acid distribution was found to be statistically significant only in palmitic and eicosanoic acid content. The sowing density having the lowest palmitic acid ratio and the highest eicosanoic acid ratio was 25x30 cm. In summer sowing, the highest difference in eicosanoic acid content was generated by the sowing density of 25x20 cm. In other fatty acids, the difference was statistically insignificant in all sowing densities in both summer and winter sowings; they produced approximate values. The ratios of stearic, palmitic and oleic fatty acids decreased while linoleic fatty acid ratio increased in summer sowing. In a sowing time study conducted on 3 different safflower varieties, late sowing dates resulted in higher oleic acid, palmitic acid and stearic acid ratios but a lower linoleic acid ratio [16]. In Mediterranean Region, early sowing of high oleic type sunflower hybrids led to a decrease in oleic and palmitic acid contents but an increase in linoleic and linolenic acid ratios [7].

Milk Thistle seeds contain a low number of polyunsaturated fatty acids but a high number of unsaturated fatty acids, making its oil ideal for human nutrition. Besides, the plant contains slymarin, a substance used for the treatment of serious liver diseases such as cirrhosis and cancer. So, if included in the agricultural products of Kazova region in Tokat, where there are no ecological or physiological restrictions, Milk Thistle may contribute to both regional and national economy.

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