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Nitrogen Fertilizer Affecting Growth, Seed Yield and Active Substances of Milk Thistle (*Silybum marianum*)

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Abstract: The ripe fruit of milk thistle (*Silybum marianum* L. Gaerth) contain flavonoids that are used to prepare anti - hepatotoxic drugs. This plant is important to pharmaceutical industries. The main aim of this investigation was to study the influence of nitrogen fertilizer on growth, seed yield and active substances (silymarin and silybin) content of milk thistle. The results showed that nitrogen fertilizer had significant effect on growth (plant height, number of capitula per plant, capitula diameter), seed yield, silymarin and silybin content. The highest seed yield (2.35 kg/plot) was obtained from the plots, receiving 200kg / ha N as top dressing after seed germination. But the highest silymarin (9.25%) and silybin (33. 58%) content was accumulated in the seeds of control treatment plants.

Key words: *Silybum marianum,* nitrogen fertilizer, silymarin, silybin

Introduction

Milk thistle (*Silybum marianum* (L.) Gaerth) is an annual over wintering plant belonging to Asteraceae family that reaches a height of 200 - 250 cm. The capitula are 5 - 8 cm in diameter, and ovate. The flowers are purple in colour (Bernáth, 1993; Hornok, 1992). The sunny, stony slopes of the Mediterranean region are the growing locations of warmth-loving milk thistle. It is common in countries of the Mediterranean region. The ripe fruit *(Cardui mariani fructus)* of milk thistle contains flavonoids (Omidbaigi, 1997; 1998).

The main ingredients being silybin, silydianin and silychristin, are collectively termed as silymarin (Fig. 1). The silymarin in the modern pharmaceutical industries is used for curing spleen, liver and gallbladder diseases (Bruneton, 1995; Dewick, 1998; Lipfert and Honerlang, 1971).

According to the results of some investigations the milk thistle productivity is strongly affected by nitrogen fertilizer (Hammouda, 1991; Kushma, 1995; Omer *et al.*, 1990; Omer and Ibrahim, 1995).

Therefore the purpose of this investigation was to clarify the effect of nitrogen fertilizer and to find the most suitable time for N-top dressing which could be used for milk thistle cultivation in order to increase quantity and improve quality of milk thistle productivity.

Materials and Methods

Soil type and weather conditions of field experiment: Physical and chemical analyses of the soil were done before sowing the milk thistle (Table 1). The weather conditions during the experimental seasons were recorded and are presented in Table 2.

Table 1: Physico - chemical characteristics of the experimental soil

Characteristics	Results
Sand (%)	33
Silt (%)	37
Clay (%)	30
Soil texture	clay loam
Available K (ppm)	227.6
Available P (ppm)	15.4
Total N (%)	0.39
Organic C (%)	0.65
Acidity (PH)	8.48
Electrical conductivity (mmhos)	1.01

Field experiment: Seeds of *Silybum marianum* of Hungarian origin were used in this experiment. Experiment with milk thistle was started in the spring of 1995 and carried out at Tehran University, College of Agriculture, Experiment Station of Alborz located in Karaj city situated in west of Tehran. Basic fertilization (250 kg ha^{-1} P_2O_5 (super phosphate triple = Ca $(H_2PO_4)_2$ was applied to the soil of all the treatments during autumn deep ploughing, before sowing the seeds.

In the small plot experiment, the treatment consisted of 3 levels of N and one control group without nitrogen fertilizer. The chemical fertilizer was urea with 46 % essential source. We have studied the effect of increasing (50, 120 and 200kg/ha) N- dosage and top dressing given at three different developmental stages; after seed germination (F_1); at stem initiation stage (F_2) and at flowering stage (F_3) by mixing it into the soil in the rows.

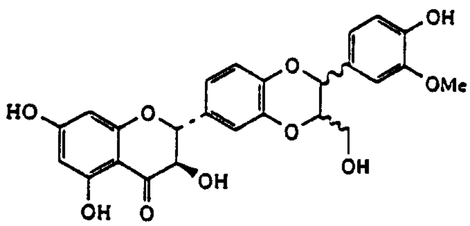
Table 2: Monthly rainfall and temperature of field experiment

Month	Rainfall (mm)	Temperature (°C)
Jan.	308	2.8
Feb.	42.2	6.9
Mar.	148.4	6.5
Apr.	13.3	14.9
May.	89.3	15.1
Jun.	22.4	1.8
Jul.	0.3	22.5
Aug.	0.0	21.8
Sep.	4.1	18.8
Oct.	0.4	16.9
Nov.	5.2	11.8
Dec.	15.6	1.9

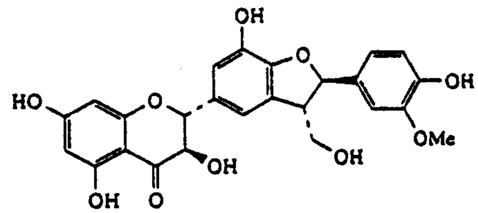
The experimental plots were laid out on sandy soil in randomized complete block design with four replicates in every treatment. The date of sowing was 5th of March. Each plot was 13.5 m^2 consisting of 9 rows with a distances of 50 cm between the rows and 30 cm between each successive plant. Hoeing and mechanical weeding were made regularly. Irrigation was regular during the vegetative period. All agronomic management practices were performed as needed. The effect of nitrogen fertilizers was measured by plant height, capitula number per plant, capitula diameter in full flowering stage, and weight of 1000 seeds, seed yield, silymarin and silybin content were measured after collecting and air drying the seeds.

Analysis of variance was carried out using MSTAT-C Software Package. The statistical mean comparisons were calculated according to Duncan's multiple range test (Nissen, 1989).

Silymarin extraction: Thirty grams of seeds were dried at 50°C



isosilybin



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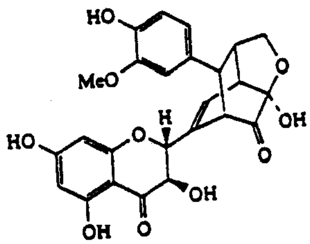

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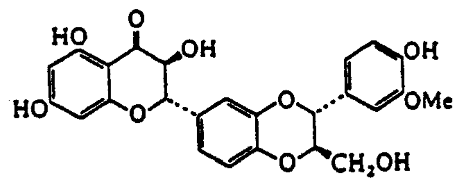

silybin

Fig. 1: Principal flavonoids of milk thistle

Table 3: The growth characteristics of milk thistle from different N - fertilizer

N-fertilizer Kg ha^{-1}			Plant height (cm)	No. of capitula plant^{-1}	Main capit. diameter (cm)
F_1	F_2	F_3			
0	0	0	70.7 a	9.2 b	3.1 d
50	0	0	81.7 ab	16.8 a	3.6 c
120	0	0	80.6 ab	15.1 a	3.8 bc
200	0	0	93.6 a	17.8 a	4.7 a
25	12.5	12.5	72.3 b	17.7 a	3.8 bc
40	40	40	80.2 ab	16.2 a	3.9 b
40	80	80	79.6 ab	16.6 a	3.6 bc

Means followed by the same letters in each column are not significantly different at 5%

Table 4: The seed characteristics of milk thistle from different N - fertilizer on seed characteristics of milk thistle

N - fertilizer Kg ha^{-1}			No. of seed capitula^{-1}	1000 Seeds Weight (g)	Seed yield g plot^{-1}
F_1	F_2	F_3			
0	0	0	86.0b	20.1a	810f
50	0	0	93.2ab	20.2a	1691d
120	0	0	103.1ab	19.9a	2006b
200	0	0	123.4a	19.7a	2343a
25	12.5	12.5	80.0b	20.1a	1346e
40	40	40	97.3ab	19.5a	1283e
40	80	80	111.7ab	20.0a	1937e

Mean followed by the same letters in each column are not significantly different at 5% level according to Duncan's Multiple Range Test .

in a vacuum oven for 14 hours and ground to a fine powder. Oil was extracted from 20 g of powder in 300 ml petroleum ether in a Soxhelt apparatus at 60°C. The samples were dried at 50°C in vacuum oven for 2 hours and then silymarin were extracted by 300 ml methanol at 80°C for 16 hours. The extract, solutions were placed on a rotary evaporator, and dried at 50°C under vacuum oven for 5-6 hours to obtain silymarin (Varma *et al.*, 1980).

From every treatment 0.08 g of silymarin was dissolved completely in 50 ml methanol. The solution was shaken and left at room temperature for 2 hours. Five μl of it was used directly for HPLC.

Silybin analysis: The silybin content of silymarin was determined by a Millipor HPLC system (Fig. 2) with 2 pumps M510, Injector U6K, U.V.Detector 486 and a computer NEC

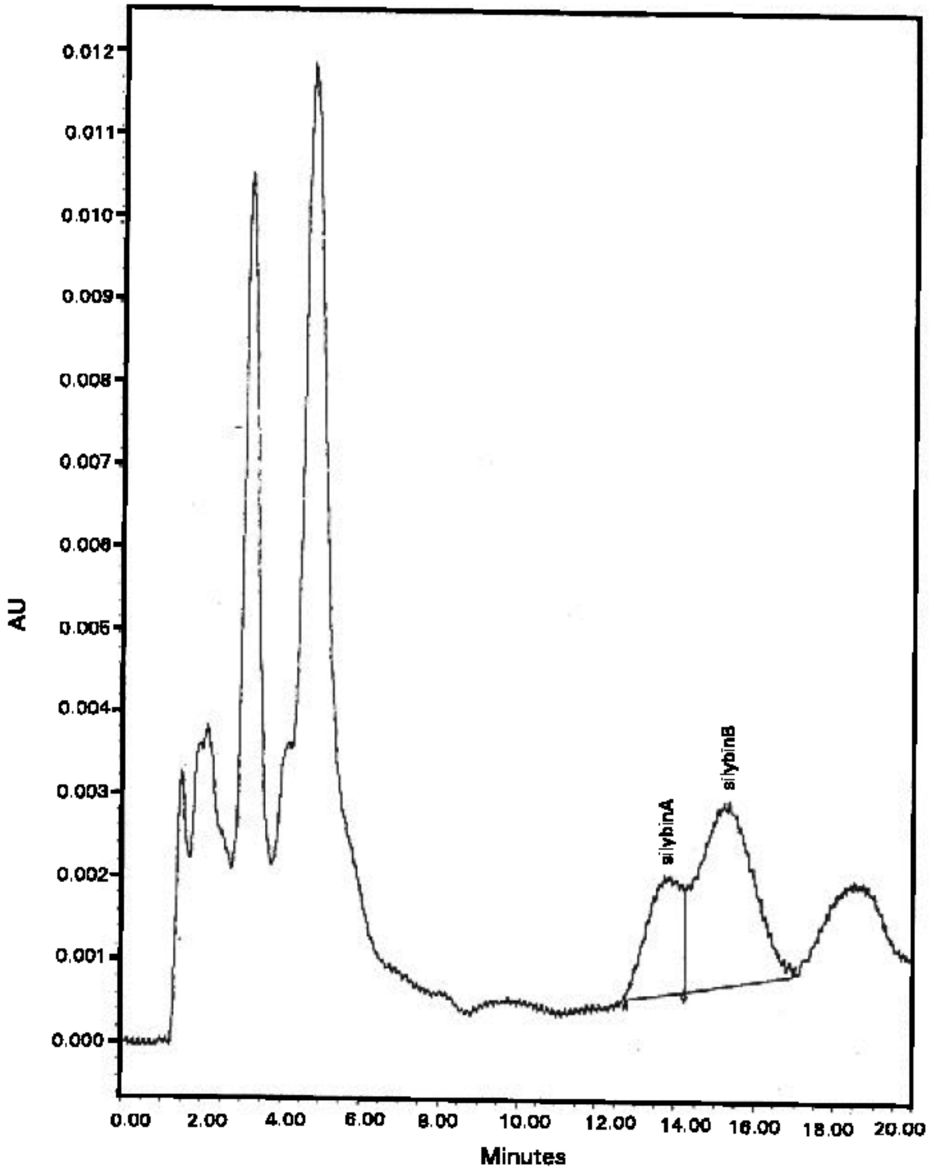

Fig. 2: HPLC chromatogram showing separation of the flavonoids extracted from milk thistle

386/33 with the version 2 of the millennium program. Conditions of analysis: μ Bondapak C18, column 300 × 4.6 mm with guard column, U.V. wavelength 280 nm. Follow: 2ml/ min, injection volume 4μl and the mobile phase consisted of water - acetic acid 61.9% (Tittle and Wagner, 1977).

Dura silymarin capsule containing 29.165 mg silybin (produced by MADAUS Co.) was used as a standard.

Results and Discussion

On growth and seed yield: On the basis of our examination, it could be concluded that nitrogen fertilization at level of 200kg ha^{-1} applied after seed germination caused a significant ($P < 0.05$) increase in plant height. The increase was 38% as compared to control treatment (Table 3).

Number of capitula and main capitula diameter per plant was influenced ($P < 0.05$) by nitrogen fertilization. All nitrogen fertilization treatments increased number of capitula per plant as compared to control. The largest capitula diameter (4.76 cm) was obtained from the plot, which received 200kg $ha,^{-1}$ nitrogen after seed germination (Table 3).

On the basis of our data (Table 4) number of seeds per capitula was significantly affected by nitrogen fertilization. The largest number of seeds per capitula (123.4) produced in the plant having received 200 kg N ha^{-1}. It was 43.5% more than control treatment.

Application of nitrogen fertilizer had no significant ($P < 0.05$) effect on the weight of 1000 seeds. But in agreement with the report of Omer *et al.*, (1990) supply of nitrogen fertilizer influenced the milk thistle yield significantly. Plants receiving 200 kg N ha^{-1} after seed germination displayed 85.1 %

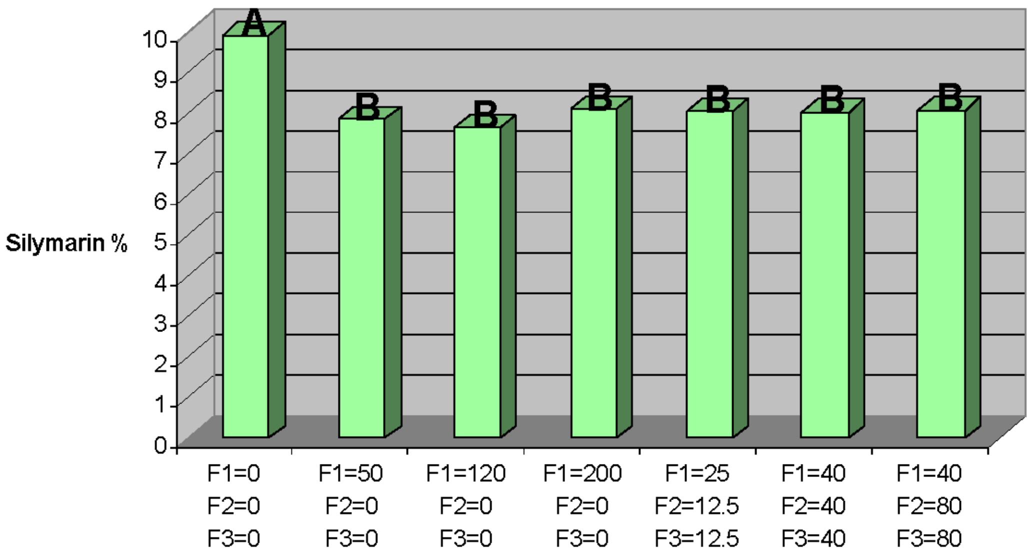

Fig. 3: Effect of N- fertilization on silymarin content of milk thistle

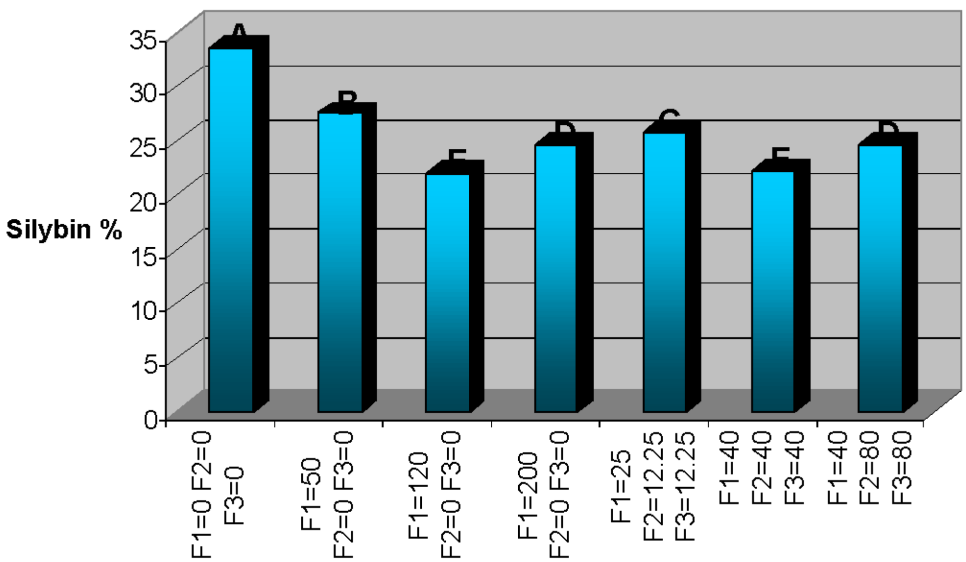

Fig. 4: Effect of N - fertilization on silybin content in silymarin of milk thistle

increase in the seed yield per plot as compared with the control treatment.

On active substances: We have established that accumulation level of silymarin and silybin in silymarin of milk thistle can be affected by supplying nitrogen. As nitrogen fertilization had negative significant ($P<0.05$) effect on percentage of silymarin (Fig. 3) and silybin (Fig. 4). The highest amount of silymarin (9.92%) and silybin (33.58%) was accumulated in the seeds of control treatment plants.

To conclude, present investigation showed that nitrogen fertilization had significant effect on growth parameters of milk thistle plant. Nitrogen fertilization stimulates the growth (plant height, number of capitula per plant and main capitula diameter) of milk thistle. These results are in agreement with those of Omer *et al.* (1990); Omer and Ibrahim (1995) and Kushma (1995).

According to chemical analysis, we have established that nitrogen fertilization had negative significant impact on the silymarin and silybin content in the seeds of milk thistle. As receiving nitrogen fertilization to plots, the percentage of silymarin and silybin significantly decreased. According to Timmerman *et al.* (1993) by increasing nitrogen fertilizer the total phenolic compounds of plants decreased and nitrogen was an inhibitor of phenolic compounds.

Melnikova (1984); Omer *et al.* (1990); Schunke (1992);

Hornok (1992) and Bernáth (2000) reported similar findings.

Acknowledgments

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