Asian Journal of Chemistry

# Investigation of Varying Nitrogen Doses and Row Spacing Applications on the Chemical Composition of Evening Primrose (*Oenothera lamarckiana* L.)

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Evening primrose oil extracted from the seeds of evening primrose (*Oenothera lamarckiana* L.) is one of the most important sources of herbal medicine and food additives, recently. The effects of different nitrogen doses (0, 60, 120 and 180 kg ha<sup>-1</sup>) and row spacing applications (20, 40 and 60 cm) on the seed oil content and its fatty acid composition of evening primrose were studied in the Cukurova ecological conditions of Turkey during 2000 and 2001. In conclusion, varying nitrogen doses and row spacing applications had considerable effect the seed crude oil content and its chemical composition, the highest  $\gamma$ -linolenic acid content was obtained from 180 kg ha<sup>-1</sup> nitrogen dose and 40 cm row spacing application.

Key Words: γ-Linolenic acid, Linoleic acid, Medicinal plants, Crude oil, Nitrogen.

#### **INTRODUCTION**

Oenothera lamarckiana L. (Onagraceae) is a wildflower indigenous probably to northwestern United States and has been grown as a garden plant in other parts of the world<sup>1</sup>. Evening primrose has become a roadside weed in many parts of the world. Evening primrose is an important cash crop and cultivated in many countries for the production of its oil. The total world production of the seed is estimated at 4000 tons<sup>2</sup>. Seeds contain up to 25 % oil, of which about 8-14 %  $\gamma$ -linolenic acid and the rest of the composition is 60-80 % linoleic acid.  $\gamma$ -Linolenic acid is also found in the seeds of *Borago officinalis* and *Ribes nigrum*<sup>2,3</sup>. There are some studies indicating that seed oil content and composition ( $\gamma$ -linolenic acid and linoleic acid contents) are affected by varying development stages, climatic factors and agricultural practices<sup>4-9</sup>. The aim of the study was to determine the optimum nitrogen dose and plant density in order to obtain the highest seed oil content rich in the  $\gamma$ -linolenic acid. 2336 Sekeroglu

Asian J. Chem.

# EXPERIMENTAL

Field and laboratory studies were conducted at Cukurova University, Agricultural Faculty, Field Crops Department of Turkey during 2000 and 2001. In the region during the vegetation period, the average climate values were 25.4°C and 195.3 mm rainfall for 2000 and 25.2°C and 204.4 mm rainfall for 2001<sup>10</sup>. The soil of the research area was a clay-loam with 0.13 % nitrogen, 85.1 kg ha<sup>-1</sup> phosphorus, 24 % lime at a horizon A (0-25 cm) and pH 7.50 of the saturated soil extract. The seeds of evening primrose were sown in seedbeds in the glasshouse in January for both research years. At the beginning of April, the seedlings were transplanted to the fields which prepared before. Field trials were arranged in split plot design with three replications, as the main plots were nitrogen doses (0, 60, 120)and 180 kg ha<sup>-1</sup>); row spacing applications (20, 40 and 60 cm) were the subplots. Nitrogen fertilizers were applied the plots in two parts at the planting time and stem elongation period. After transplanting the plants were irrigated monthly until 75 % of the capsules ripened. The plants were harvested by hand in September and dried under the sun on the fields. After threshing the seeds were cleaned and prepared for laboratory analysis. In the present study, some chemical characteristics of the seeds such as seed oil content and composition in terms of  $\gamma$ -linolenic acid and linoleic acid contents of the oil were studied.

The crude oil analyses were performed with a Raney oilseed crusher. Gas chromatography analyses of essential fatty acids were carried out using a Hewlett Packard 6890 series gas chromatograph [split/splitless injection system equipped with a capillary column ( $30 \text{ m} \times 0.32 \text{ mm i.d.}$ ), initial temp.:  $170^{\circ}$ C for 3 min, final temp.:  $210^{\circ}$ C for 10 min, carrier gas: nitrogen] after methyl esterification by BF<sub>3</sub>·CH<sub>3</sub>OH. Linoleic (18:2, n-6) and  $\gamma$ -linolenic (18:3, n-6) acids were determined in the seed oil<sup>11</sup>. MSTATC, computer package program, was used for variance analysis of data according to split plot design. The least significant differences among the means were compared at the 5 % probability level.

# **RESULTS AND DISCUSSION**

The overall analysis of variance indicated that there was a significant effect of the year factor on the seed crude oil content, whereas all the other factors did not affect this trait (Table-1). There were statistical differences among the seed crude oil contents; the highest value was obtained in the year of 2000. Seed crude oil content of evening primrose determined in the present study varied from 15.33 to 21.00 %. These values are in harmony with reported previous results<sup>4,5,12</sup> for evening primrose; 19.3-29.4 %, 25.5-26.8 % and 17.0-25.0 %. Differences among the values of the seed crude

oil content might be resulted from a number of factors such as the different environmental conditions, growing techniques, crop varieties and soil properties<sup>13</sup>.

TABLE-1
MEAN VALUES FOR THE SEED CRUDE OIL CONTENT (%) OF
EVENING PRIMROSE AFFECTED BY VARIOUS NITROGEN DOSES
AND ROW SPACING APPLICATIONS

Nitrogen (kg ha <sup>-1</sup> )	Year and row spacing (cm)						NT'.
	2000			2001			Mitrogen
	20	40	60	20	40	60	- means
0	18.33	19.67	17.33	16.33	16.33	16.67	17.44
60	18.00	21.00	21.00	17.00	16.33	15.33	18.11
120	19.33	17.00	19.00	16.67	17.00	16.67	17.78
180	18.67	20.00	20.33	17.67	16.33	17.67	18.44
Space means	17.75		17.96		18.13		
Years means	19.14 a			16.75 b			
LOD (5 N) V	1 000						

LSD (5 %): Year, 1.223

There were no statistical differences between means with the same letters.

Table-2 reveals that  $\gamma$ -linolenic acid content of the seed oil of  $\gamma$ -linolenic acidevening primrose was affected by year, year  $\times$  nitrogen dose, year  $\times$  row spacing and year  $\times$  nitrogen dose  $\times$  row spacing. Considering year  $\times$  nitrogen dose  $\times$  row spacing interaction,  $\gamma$ -linolenic acid content of the seed oil varied from 2.63 to 3.97 %. While the highest  $\gamma$ -linolenic acid content of the seed oil was obtained from 180 kg ha<sup>-1</sup> nitrogen dose and 40 cm row spacing application in 2000, the lowest value resulted in 60 kg ha<sup>-1</sup> nitrogen dose and 40 cm row spacing application in 2001. It can be seen easily from the Table-2,  $\gamma$ -linolenic acid content of the seed oil was positively affected by increasing nitrogen doses. A number of factors such as soil, climate, plant variety and agricultural practices affect the fatty acid composition of the seed oils. Levy et al.9 stated that high temperatures in the first vegetation period decrease the formation of the unsaturated fatty acids in the plats oil, and they found that a high temperature regime (32/ 27°C) decreases the  $\gamma$ -linolenic acid content when compared to a low temperature regime in *Oenothera lamarkiana* L. Reiner et al.<sup>5</sup> declared that  $\gamma$ -linolenic acid content of evening primrose oil was much lower in Turkey (5.80-6.90 %) than that of in Germany (10 %). γ-Linolenic acid content determined in the present study were mch lower than the results of different studies on evening primrose<sup>7,12,14</sup>. In the experimental area the temperatures are higher than 30°C during the vegetation period and differences between the years for  $\gamma$ -linolenic acid contents might be resulted from the temperature.

2338 Sekeroglu

Asian J. Chem.

#### TABLE-2 MEAN VALUES FOR THE SEED OIL GAMMA-LINOLEIC ACID CONTENT (%) OF EVENING PRIMROSE AFFECTED BY VARIOUS NITROGEN DOSES AND ROW SPACING APPLICATIONS

Nitrogen $(\log \log^{-1})$		NU					
	2000			2001			means
(kg lia )	20	40	60	20	40	60	means
0	3.18 с-е	3.25 b-е	3.17 с-е	3.71 ab	2.95 ef	3.29 b-e	3.26
60	3.33 b-e	3.55 а-с	3.41 b-e	3.16 с-е	2.63 f	3.32 b-e	3.23
120	3.52 а-с	3.46 b-d	3.94 a	3.21 с-е	3.31 b-e	3.00 d-f	3.41
180	3.61 a-c	3.97 a	3.49 а-с	3.32 b-e	3.31 b-e	2.99 d-f	3.45
Space means	3.38		3.30		3.	33	
Years means		3.49 a			3.18 b		

LSD (5 %): year, 0.1393; year  $\times$  nitrogen  $\times$  spacing: 0.4826

There were no statistical differences between means with the same letters.

Linoleic acid content of the seed oil was statistically affected by row spacing and nitrogen dose  $\times$  row spacing interaction; whereas the remaining factors had no statistical effects on the linoleic acid content (Table-3). The linoleic acid contents of the seed oil varied from 61.73 to 71.22 % by different applications. According to varying row spacing applications, the highest linoleic acid content obtained from 60 cm and the lowest value was determined in 20 cm. There were no statistical differences between the 20 and 40 cm row spacing applications. There are similar results<sup>5-7</sup> for linoleic acid contents of evening primrose oil: 65.2-68.3, 61.9-74.6 and 70.1-72.5%.

#### TABLE-3 MEAN VALUES FOR THE SEED OIL LINOLEIC ACID CONTENT (%) OF EVENING PRIMROSE AFFECTED BY VARIOUS NITROGEN DOSES AND ROW SPACING APPLICATIONS

Nitrogen (kg ha <sup>-1</sup> )	Year and row spacing (cm)						<b>N</b> 71
	2000				Nitrogen		
	20	40	60	20	40	60	- means
0	61.73	64.87	71.22	63.32	65.76	67.13	65.67
60	67.47	64.22	69.41	62.92	65.07	65.89	65.83
120	68.13	63.03	64.45	65.80	61.98	67.94	65.22
180	62.84	65.67	68.04	63.67	63.58	68.20	65.33
Space means	64.48 b		64.27 b		67.78 a		
Years means		65.92			65.10		

LSD (5 %): spacing, 1.622

There were no statistical differences between means with the same letters.

The interaction between the nitrogen doses and row spacing applications for linoleic acid content of the evening primrose oil for the experimental years was significant. Linoleic acid contents determined in the present study changed in the intervals of 69.18-62.50 % for varying nitrogen doses and row spacing applications. The highest value was obtained

#### Vol. 19, No. 3 (2007) Investigation of Nitrogen Doses on O. lamarckiana L. 2339

from 60 cm row spacing application in control plots for nitrogen. However, there were no statistical differences among the other nitrogen doses for this row spacing application by means of linoleic acid content. It can be easily seen from the Fig. 1, in lower plant densities higher linoleic acid contents of the seed oil was obtained from higher nitrogen doses, in contrast, in wider row spacing applications higher nitrogen doses gave the lower linoleic acid contents. Differences in the seed oil formation may be explained by the different nutrition and growing conditions in the experimental area.



Fig. 1. Linoleic acid content for varying nitrogen doses and row spacing

## Conclusion

Varying nitrogen doses and row spacing applications had considerable effect on the  $\gamma$ -linolenic acid and linoleic acid contents of the evening primrose seed oil and the highest  $\gamma$ -linolenic acid content was obtained from 180 kg ha<sup>-1</sup> nitrogen dose and 40 cm row spacing application. Seed crude oil,  $\gamma$ -linolenic acid and linoleic acid contents of evening primrose increased with higher nitrogen doses and row spacing applications.

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2340 Sekeroglu

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(Received: 29 June 2006; Accepted: 4 November 2006) AJC-5251