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Influence of different irrigation and nitrogen levels on crude oil and fatty acid composition of maize (*Zea mays* L.)

M. Kaplan[a], H. Kale[a], K. Karaman[b,✉] and A. Unlukara[c]

[a]Department of Field Crops, Faculty of Agriculture, University of Erciyes, Kayseri, Turkey
[b]Department of Agricultural Biotechnology, Faculty of Agriculture, University of Erciyes, Kayseri, Turkey
[c]Department of Biosystem Engineering, Faculty of Agriculture, University of Erciyes, Kayseri, Turkey

✉Corresponding author: kevserkaraman@erciyes.edu.tr

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SUMMARY: The effect of irrigation and nitrogen fertilizer levels on the crude oil and fatty acid composition of maize cultivars was studied. Three levels of irrigation (50, 75 and 100% of field capacity) and nitrogen (100, 200 and 300 kg·ha^{-1}) were used for treatment groups. After harvest, the crude oils were extracted and fatty acid profiles were determined by Gas Chromatography system. The study was repeated for two years and the interaction effects of fertilizer and irrigation were determined. Our results show that the crude oil content was affected positively by the fertilizer and the irrigation applications. As expected, the most abundant fatty acid was linoleic and the harvest year did not alter it. The highest linoleic acid content value was obtained with a 50% field capacity and 300 kg·ha^{-1} fertilizer treatment combination. In addition, fatty acid contents varied with the changing of interaction effects except for myristic and palmitic acid. Oleic acid was the second abundant fatty acid in the oil samples and the lowest oleic acid value was obtained with a 50% field capacity and 300 kg·ha^{-1} fertilizer treatment combination. Oleic acid content tended to increase with 75% field capacity but 100% field capacity treatment decreased in it.

KEYWORDS: *Fatty acid profile; Fertilizer; Irrigation; Maize;* Zea mays *L.*

RESUMEN: ***Influencia de niveles de riego y nitrógeno en la composición del maíz (*****Zea mays** ***L.).*** Se estudió el efecto de niveles de riego y fertilizantes nitrogenados sobre la composición de aceites y ácidos grasos de cultivares de maíz. Se utilizaron tres niveles de riego (50, 75 y 100% de capacidad de campo) y nitrógeno (100, 200 y 300 kg·ha^{-1}) para los grupos de tratamiento. Tras la cosecha se extrajeron los aceites y se determinó el perfil de ácidos grasos mediante cromatografía de gases. El estudio se repitió durante dos años y se determinó los efectos de la interacción del fertilizante y el riego. Los resultados mostraron que el contenido de aceite se ve afectado positivamente por el fertilizante y las aplicaciones de riego. Como era de esperar, el ácido graso mayoritario fue linoleico y el año de cosecha no lo altera. El mayor contenido de ácido linoleico se obtuvo con riego del 50% de la capacidad de campo en combinación con fertilización de 300 kg·ha^{-1}. Los contenidos de los demás ácidos grasos varían con los cambios de interacción riego/fertilización, excepto los ácidos mirístico y palmítico. El ácido oleico fue el segundo ácido graso más abundante en los aceites y su valor más bajo se obtuvo con la combinación de una irrigación del 50% de la capacidad de campo y 300 kg·ha^{-1} de fertilizantes. El ácido oleico tiende a aumentar con una irrigación del 75% de la capacidad de campo, pero el 100% del tratamiento de capacidad de campo lo hace disminuir.

PALABRAS CLAVE: *Ácidos grasos; Fertilizantes; Maíz, Riego;* Zea mays *L.*

ORCID ID: Kaplan M http://orcid.org/0000-0002-6717-4115, Kale H http://orcid.org/0000-0003-4736-1498, Karaman K http://orcid.org/0000-0003-0729-6185, Unlukara A http://orcid.org/0000-0003-4931-8100

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1. INTRODUCTION

Cereal grains are predominantly composed of carbohydrates (generally starch forms), proteins, lipids, vitamins, and minerals and the amount and quality are affected by genetic and environmental conditions (Ali *et al*., 2010). Among the cereals, maize (*Zea mays* L.) plays an important role in food, feed and bio fuel processes worldwide. In a growing strategy to obtain high grain and biomass yields from the grains, some applications such as increasing nitrogen (N) fertilizer levels have been used (Zhu *et al*., 2016). N is an important element which controls the growth of the plant. It is also important for photosynthetic activity and it also has an important function for the growth of oil seeds and oil fruits. Chemical fertilizers have been used to provide an increment in the yield of oil seeds for a long time (Yalcin *et al*., 2011). At the same time, doses of N play a vital role in plant nutrition and a deficit or excess of N can cause some adverse effects on plant growth. Ashraf *et al*. (2006) reported that high nitrogen doses cause a decrease in some enzyme activities and thereby the photosynthetic rate decreases. In addition, nitrogen affects the dry matter production of cells by influencing the leaf area development (Shahrokhnia and Sepaskhah 2016).

Maize is well-known as a crop which demands water. High grain yields can be achieved (10–12 $t{\cdot}ha^{-1}$) when the water and nutrients are not limited during growing. Maize is a crop which is sensitive to water shortage and grain yield response to deficit irrigation depends on optimum use of N (Pandey *et al*., 2000). Several researches showed that unsuitable conditions, especially drought, had an effect on the change of the seed composition and related quality parameters such as oil level or structure. It has been reported that the lack of water is the limiting factor for seed growth and affects its composition (Ali *et al*., 2009; Ali *et al*., 2010).

The main structural parts of maize kernels are endosperm (82%) and germ (embryo and scutellum) (12%). 80 to 84% of total kernel oil is present in the germ region followed by 12% in the aleurone and 5% in the endosperm. The typical profile of fatty acids in a maize kernel is composed of 57.9% linoleic acid, <1% linolenic acid, 25.2% oleic acid, 11.6% palmitic acid and 1.8% stearic acid (White *et al*., 2003; Ashgan Abou Gabal and Amera Zaitoun, 2015). In earlier studies it has been reported that water shortage, along with N application at low or high doses can affect the chemical composition of different seeds by different metabolic processes (El-Deen *et al*., 1997, Yang *et al*., 2004, Xing *et al*., 2001 Pavlista *et al*., 2016a, Pavlista *et al*., 2016b.). However, the effects of fertilizer and water shortage on the fatty acid composition of maize oil have been poorly investigated. In particular, little or no information has been obtained about the effects of simultaneous application of irrigation and fertilizer on maize oil. Therefore, the objective of this study was to evaluate the change in the crude oil content and fatty acid composition of maize with three levels of fertilizer (N1:100, N2:200 and N3:300 $kg{\cdot}ha^{-1}$), three levels of irrigation (I 50: 50%; I 75: 75%; and I 100: 100% field capacity). The interaction effects of irrigation and fertilizer treatments were also displayed as well as the effect of harvest year.

2. MATERIALS AND METHOD

2.1. Plant material and treatment

The experiments were carried out in the Kayseri Province of Turkey (39°48’N; 38°73’E) during the growing seasons of 2013 and 2014. For the plant material, Simon hybrid maize cultivar was used. Seeds were sown over 6 x 4.2 m plots with 70 x 15 cm on-row plant spacing. Experimental design was conducted in split-split plots conducted with three replications. Three different irrigation levels based on field capacity (50, 75 and 100%) were placed over main plots and three nitrogen doses (100, 200 and 300 $kg{\cdot}ha^{-1}$ N) were placed over sub-plots. Soil moisture content was measured with a neutron probe. The amount of irrigation water was determined before each treatment and applied through drip irrigation. Based on soil analysis, half of nitrogen was applied at sowing and the other half was applied when the plants had a height of 50 cm. Together with nitrogen fertilization, 80 $kg{\cdot}da^{-1}$ P_2O_5 was applied at sowing. Plants were harvested for chemical analyses at physiological maturity.

2.1.1. Soil and climate characteristics of research site

Seeds were sown on 23rd of April in the first year and 28th of April in the second year treatment. Experimental years generally had similar temperatures to long-term averages. Precipitations were lower than the long-term averages in the first year and higher than the long-term averages in the second year. Relative humidity levels of the experimental years were generally lower than those of the long-term averages. Table 1 shows the precipitation, temperature, and relative humidity data of the experimental site. Soils of the experimental site are classified as sandy-loamy sampled at 0-30 cm and 30-60 cm depths. Calcareous and salt were low while potassium and phosphorus were rich in the soil. The pH of the soil was slightly alkaline but organic matter content was quite low. In Table 2, physical and chemical characteristics of the experimental soils were tabulated.

2.2. Oil extraction and preparation of fatty acid methyl esters (FAME)

Impurities were removed from the seeds and the cleaned seeds were ground using a ball mill to prepare powdered samples. The samples were placed in

TABLE 1. Precipitation, temperature and relative humidity data of the experimental site

	Temperature (°C)			Precipitation (mm)			Relative Humidity (%)		
Months	**2013**	**2014**	**Long Term***	**2013**	**2014**	**Long Term***	**2013**	**2014**	**Long Term***
April	12.1	14.1	10.7	43.6	2.9	54.8	56.2	44.3	62.6
May	18.1	16.7	15.1	31.3	39.7	52.0	44.7	50.4	60.8
June	21.1	19.7	19.1	12.6	52.9	39.1	38.7	46.8	55.3
July	22.5	25.2	22.6	3.4	0.0	10.3	36.9	33.7	49.5
August	22.5	25.1	22.0	0.8	47.4	5.3	36.0	37.4	49.8
September	17.0	18.8	17.1	10.3	85.4	13.3	44.1	54.2	54.4
October	9.2	11.7	11.5	52.5	54.4	30.5	58.9	68.1	64.0
Mean	17.5	18.7	16.8	-	-	-	45.0	47.8	56.6
Total	-	-	-	154.5	282.7	205.3	-	-	-

*from 1970 to 2013

TABLE 2. Physical and chemical characteristics of soils of the experimental site

	2013		2014	
Property	**0-30 cm**	**30-60 cm**	**0-30 cm**	**30-60 cm**
Clay (%)	13.10	8.94	12.58	9.18
Silt (%)	4.16	10.40	5.11	9.55
Sand (%)	82.74	80.66	82.31	81.27
Class	Sandy-Loam	Sandy-Loam	Sandy-Loam	Sandy-Loam
pH	7.94	7.75	7.48	7.60
Organic Matter (%)	1.05	1.27	1.09	1.14
$CaCO_3$ (%)	0.28	0.27	0.24	0.29
K_2O (kg·ha^{-1})	1092.20	755.14	1184.20	842.34
P_2O_5 (kg·ha^{-1})	89.63	11.56	110.41	12.58
EC (mmhos/cm^{-1})	0.96	0.23	0.83	0.27

a hexane/isopropanol (2:1 v/v) solution to extract lipids from the structure overnight in a laboratory type shaker. The obtained extracts were centrifuged at 10000 *g* for 5 min and filtered. After that, the solvent was removed onto a rotary evaporator at 40 ºC to prepare the maize oil samples. After the extraction procedure, the fatty acids of the maize oil samples were converted to methyl esters by means of 2% sulphuric acid (v/v) in methanol.

2.3. Determination of fatty acid composition using a GC system

The fatty acid methyl esters (FAME) of the oil samples were analyzed using a GC system (Schimadzu, GC 2010 plus) equipped with a flame ionization detector (Schimadzu, Kyoto, Japan), a 100-m fused silica capillary column (i.d. 0.25 mm) and H_2 as the carrier and fuel gas to characterize the fatty acids. The injected sample level was 0.6-µL at a split ratio of 1:50. The FAME were separated using a temperature gradient program (Chilliard *et al*., 2013), and the peaks were identified by comparing retention times with the authentic standard (Supelco #37, Supelco Inc., Bellefonte, PA, USA; L8404 and O5632; Sigma). The analysis was repeated twice with two replicates.

2.4. Statistical analysis

Data were subjected to variance analysis using SAS (SAS Inst., 1999) statistical software. The LSD multiple range test was employed to compare the treatment means as a complement of the ANOVA procedure.

3. RESULTS AND DISCUSSION

In this study the effects of different irrigation and nitrogen levels on the crude oil content and fatty acid composition of *Zea mays* L. were investigated. Table 3 shows the effects and their importance between the interactions and means of fertilizer and irrigation, statistically. Figure 1 shows the irrigation

Table 3. Irrigation and nitrogen effects on fatty acid composition

Irrigation Level	Fertilizer Doses N1	N2	N3	Means
Myristic acid (C14:0)				
I 50	0.04[ab]	0.03[b]	0.03[b]	0.03[b]
I 75	0.13[a]	0.07[ab]	0.04[ab]	0.08[a]
I 100	0.03[ab]	0.11[ab]	0.09[ab]	0.05[a]
Means	**0.07**	**0.07**	**0.05**	
*Irri: *; Fert: **; Irri x Fertilizer:*; Year:***				
Palmitic acid (C16:0)				
I 50	11.48	11.36	11.57	11.47[b]
I 75	11.74	11.68	11.66	11.69[a]
I 100	11.37	11.53	11.62	11.51[b]
Means	**11.53**	**11.52**	**11.62**	
*Irri: *; Fert: NS; Irri x Fertilizer:NS; Year:NS*				
Stearic acid (C18:0)				
I 50	2.12[ab]	2.00[b]	1.73[c]	1.95[b]
I 75	2.28[a]	2.09[ab]	1.94[bc]	2.10[a]
I 100	1.98[bc]	2.04[ab]	1.99[bc]	2.00[ab]
Means	**2.13[a]**	**2.04[b]**	**1.88[c]**	
*Irri: **; Fert: **; Irri x Fertilizer:**; Year:***				
Oleic acid (C18:1)				
I 50	28.72[ab]	28.67[ab]	24.94[d]	27.44[b]
I 75	29.34[a]	27.86[bc]	28.69[ab]	28.63[a]
I 100	27.3[c]	28.19[bc]	27.83[bc]	27.78[b]
Means	**28.45[a]**	**28.24[a]**	**27.16[b]**	
*Irri: **; Fert: **; Irri x Fertilizer:**; Year:NS*				
Linoleic acid (C18:2)				
I 50	56.40[c]	56.80[c]	60.64[a]	57.94[a]
I 75	55.26[d]	57.10[c]	56.47[c]	56.28[c]
I 100	58.07[b]	56.88[c]	57.33[bc]	57.42[b]
Means	**56.58[b]**	**56.92[b]**	**58.15[a]**	
*Irri: **; Fert: **; Irri x Fertilizer:**; Year:NS*				
Linolenic acid (C18:3)				
I 50	1.24[ab]	1.15[abc]	1.09[c]	1.16[b]
I 75	1.25[a]	1.20[ab]	1.21[ab]	1.22[a]
I 100	1.24[ab]	1.25[a]	1.14[bc]	1.21[ab]
Means	**1.24[a]**	**1.20[b]**	**1.14 [c]**	
*Irri: **; Fert: **; Irri x Fertilizer:**; Year:***				

__I 50:__ 50%; __I 75:__ 75%; and __I 100:__ 100% field capacity; __N1:__100, __N2:__200 and __N3:__300 kg•ha^{-1} N; __Irri:__ Irrigation level; __Fert:__ Fertilizer doses; __:__ P ≤ 0.05; __**:__ P ≤ 0.01; __NS:__ non-significant; Different superscript small letters show significant differences between the interactions of irrigation and fertilizer and significant differences between the means of irrigation and fertilizer*

and nitrogen effects on the crude oil content of *Zea mays* L. The fertilizer doses positively affected positively the crude oil content and the % oil content increased with increasing nitrogen levels. It can be seen that increasing irrigation levels caused an increase in oil content. On the contrary to our results, Sebei *et al*. (2004) studied four levels of N fertilization (none, low, medium and high) in rapeseed cultivation and they stated that the highest N fertilizer dose decreased the oil content of rapeseed. Ghassemi-Golezani *et al*. (2015) aimed to evaluate the effects of water deficit and nitrogen fertilizer on the grain yield, oil and protein contents of maize and similarly to our results grain oil percentage

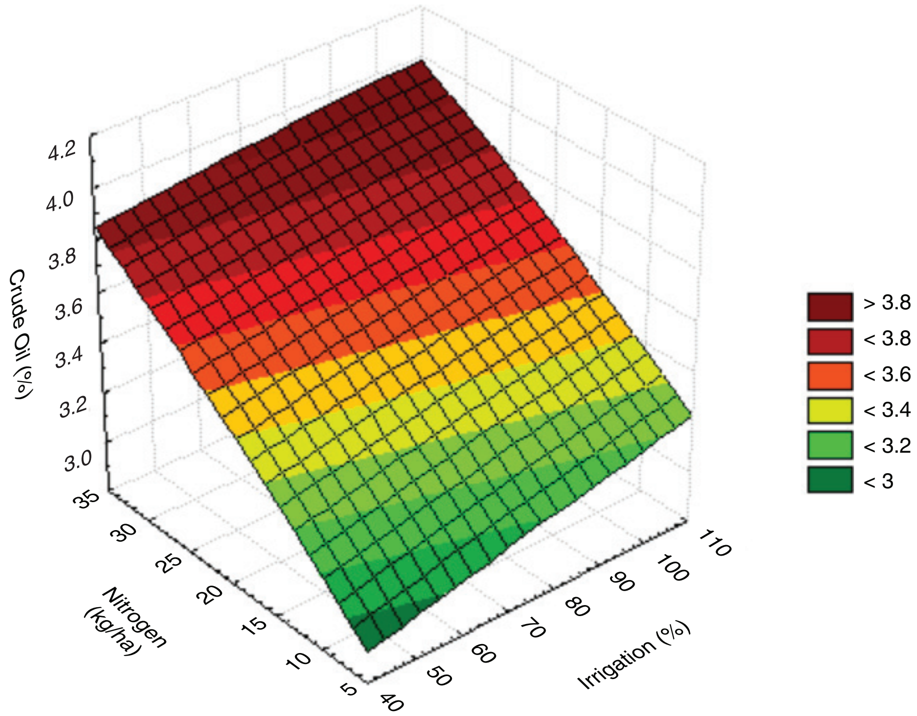

FIGURE 1. Irrigation and nitrogen effects on crude oil content.

significantly decreased as a result of water deficit but unlike in our study, a nitrogen application decreased the oil percentage while oil yield per unit area increased. Ray *et al*. (2006) aimed to study the effect of high rates of N applied at planting on seed protein and oil content of soybean and as in our study, oil yield increased with the application of fertilizer N at planting. The authors explained these increases due to the increase in seed yield with the application of fertilizer. In agreement with our results, Pavlista *et al*. (2016a) informed that the highest seed oil content was determined in fully irrigated camelina samples. On the contrary, the same authors concluded that the fatty acid profile was not substantially altered by irrigation in canola seed oil (Pavlista *et al*. 2016b).

The lowest content among the fatty acids in the maize samples was determined to be myristic acid. The interaction effect between irrigation and fertilizer was not found to be statistically significant on myristic acid content. But the mean value of myristic acid measured in 50% irrigated samples was determined to be lower than the 75 and 100% water ($p < 0.05$). In addition, the harvest year caused a significant change in its content. White *et al*. (2007) informed that environmental factors such as temperature, soil type, planting date, year, and location influence the composition of seeds, resulting in quality changes.

Palmitic acid is the third most abundant fatty acid and neither harvesting year nor fertilizing treatment had a significant effect on the amount of it. There was only significant change in palmitic acid content with irrigation treatment. It could be seen that stearic acid values changed with year, irrigation and fertilizer parameters. The interaction effect between irrigation and fertilizer was found to be statistically significant ($p < 0.01$). Similarly, Kiani *et al* (2016) reported that there is a strong interaction between irrigation and N for sunflower seed oil. When the fertilizer doses increased the mean values of stearic acid showed a decrement. When the irrigation level was increased from 50% field capacity to 75%, the mean values showed an increment but at the 100% water level the stearic acid value decreased slightly.

As expected, the linoleic and oleic acids were the most abundant fatty acids present in the maize samples. The harvesting year did not affect the oleic acid content while the other parameters were determined to be statistically significant ($p < 0.01$). The lowest oleic acid value was obtained from a 50% field capacity and 300kg ha-1 fertilizer treatment combination. The oleic acid content tended to increase with 75% field capacity but 100% field capacity treatments caused a decrease in it. The highest linoleic acid content value was determined in a 50% field capacity and 300 kg·ha^{-1} fertilizer treatment combination while the lowest value was obtained from a 75% field capacity and 100 kg·ha^{-1} fertilizer treatment combination. The mean values of linoleic acid increased with the highest fertilizer dose but no trend was found with the increasing fertilizer doses.

Shao-Wen *et al*. (2004) investigated the effects of the level of N fertilization (125–25 kg·ha^{-1}) on the fatty acid composition of corn and they pointed out that a moderate N application at 175–225 kg·ha^{-1} significantly increased the contents of total fatty acids, unsaturated fatty acids, linoleic acid and oleic acid while higher N treatment levels had a negative effect on these fatty acid contents.

Linoleic acid was not affected by the harvesting year. The mean values of linoleic acid showed a decrement with the increment of irrigation level from 50% field capacity to 75%, but an increment was observed with 100% field capacity treatments once again. In accordance with our results the amount of oleic acid (C18:1) and linoleic acid (C18:2) decreased from 21 to 18% and 20 to 19%, respectively with adding water during the season for camelina (Pavlista *et al*., 2016a). Ashraf *et al*. (2006) reported that linoleic acid showed no change in their amounts at varying levels of N for black cumin seed oil.

The combination of 50% field capacity and 300 kg·ha^{-1} fertilizer negatively affected the linolenic acid content and the lowest value was obtained. The mean values of linolenic acid decreased with the increasing fertilizer doses, while increasing irrigation levels caused an increase in the mean values of linolenic acid. Pavlista *et al*. (2016a) determined the growth, seed yield, and oil characteristics of camelina exposed to four levels of applied water and stated that linolenic acid (C18:3) as a main constituent increased with irrigation from 32 to 35% in concordance with our results.

CONCLUSION

In conclusion, irrigation and nitrogen fertilizer might be considered as visible factors which affected the crude oil and fatty acid composition of maize. Irrigation and nitrogen application altered the oil content and fatty acid composition of maize seed oil but the pattern of increase or decrease in fatty acids at varying N and I levels was not constant. In particular, the interaction effects induced remarkable variations in fatty acids. These results might be used for future breeding studies.

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