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EFFECT OF DIFFERENT IRRIGATION LEVELS ON BLACK CUMIN SEED YIELD, FIXED AND ESSENTIAL OIL CONTENTS IN SOUTHERN TURKEY

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Abstract

Limited information is available on irrigation practices of black cumin. This study aimed to evaluate the effect of different irrigation levels on seed yield, fixed oil content, and essential oil content and composition. The experiment was carried out for one crop season. The trial was arranged based on a randomized complete block design, with five treatments and four replications. Seed yield, fixed oil and essential oil contents were significantly affected by the different treatments. It can be concluded that supplying quantities of water below black cumin's water requirement does not adversely affect its seed yield and oils content.

***Key words**: medicinal plant, pharmaceutical potential, evapotranspiration, water management.*

INTRODUCTION

Black cumin (*Nigella sativa* L.) is a medicinal plant. It is an annual herb native to Mediterranean and western Asia (D'Antuono et al., 2002). The dried seeds have high contents of fatty acids (Ali et al., 2012) and also contain essential oil, which has shown to have large therapeutic potential to treat various diseases (Nickavar et al., 2003) including COVID-19 (Koshak et al., 2021; Shirvani et al., 2021). Turkey has been increasing both its sown area and production in the last years (Figure 1); going from a sown area of 230 ha and a production of 161 tons in 2012 to a sowing area of 3,377 ha and a production of 3,412 tons in 2020 (Turkish Statistical Institute, 2021).

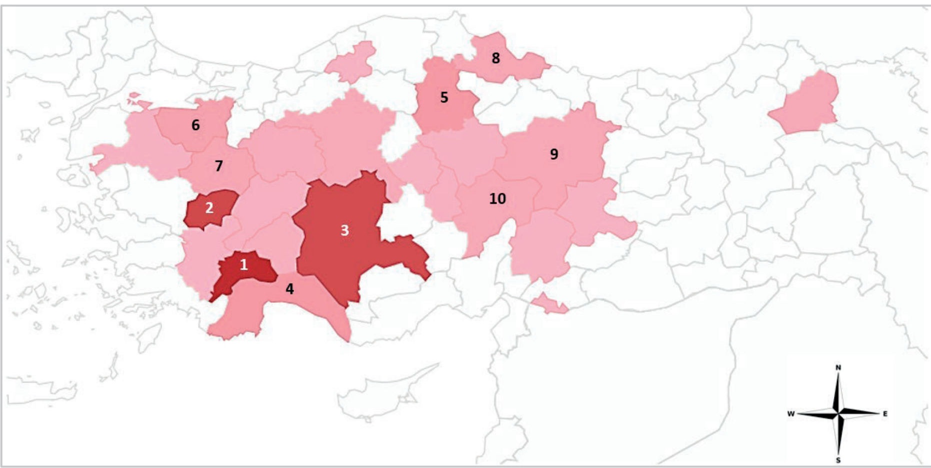

Figure 1. Black Cumin seed production on the basis of provinces in Turkey in 2019.
1) Burdur: 929 tons; 2) Uşak: 779 tons; 3) Konya: 753 tons; 4) Antalya: 203 tons; 5) Çorum: 199 tons; 6) Bursa: 132 tons; 7) Kütahya: 93 tons; 8) Samsun: 90 tons; 9) Sivas: 77 tons; 10) Kayseri: 70 tons.
Turkey's overall production of black cumin is 3,603 tons.
Source: Adapted from DrDataStats (2019)

Despite the enormous potential of this species as a medicinal plant and its increasing demand in the pharmaceutical industry, limited information about its cultivation is available.

Provided that agriculture is the largest user of water and its demand for water will increase to meet the rising need for food, fiber, and biofuels (FAO, 2017) and that climate change will significantly increase temperatures and reduce the annual precipitation in the future (Konapala et al., 2020), the need to find new cultivation strategies for stressful weather conditions to reduce the water consumption and maximize final productivity is evident. One promising strategy might be regulated deficit irrigation (RDI), originally developed for fruit orchards (Santesteban et al., 2011), that purposely stresses a crop at a specific developmental stage such that there is little or no negative impact on the yield of marketable product (Goldhamer et al., 2007). This technique might as well be adopted for other crops such as black cumin as we did in this study. The present experiment aimed to evaluate the effect of different levels of irrigation during seed maturation on black cumin on seed and oil yields. Our hypothesis is that certain degree of water shortage during the seed maturation stage will lead to increases in the parameters measured.

MATERIALS AND METHODS

Experiment site and plant material

The experiment was carried out at the experimental field of the field crops department of Çukurova university, Adana-Turkey (37° 2′ 2.23" N, 35° 20′ 52.12" E). Black cumin seeds belonging to Çameli variety were brought from the institute of agricultural research of Eskişehir (Eskişehir-Turkey) and used as vegetal material.

Climate

Adana city is situated in southern Turkey, on the Mediterranean Sea, where climate is characterized by mild wet winters and hot dry summers (Table 1).

Table 1. Weather data during the growing cycle

Parameter	Nov 2016	Dec 2016	Jan 2017	Feb 2017	March 2017	April 2017	May 2017	June 2017
Maximum Temperature (°C)	23.2	17.1	13.9	17.6	21.1	24.7	27.4	32.1
Minimum Temperature (°C)	11.4	7.2	5.08	5.59	10.3	13.0	17.0	21.3
Precipitation (mm)	58.4	131.0	51.8	0.80	65.4	65.9	45.9	17.3
Relative Humidity (%)	61.3	66.8	62.1	50.7	62.8	60.7	68.8	69.1

Experiment design

The experimental design was randomized complete block. Treatments corresponded to different percentages of the irrigation requirement (IR) of the plant, which is defined as the amount of water needed to compensate water loss through evapotranspiration (ET) (Brouwer and Heibloem, 1986). Five treatments were established, namely 0% IR, 10% IR, 50% IR, 70% IR, and 100% IR, each one replicated for four times. Thus, having 20 experimental plots. The experiment was conducted for one crop season. The trial initiated on November 8th, 2016, and finalized on June 5th, 2017. Treatments were applied during the seed maturation stage (from May 15th, 2017, until June 5th, 2017). The area of each plot was 3.75 m^2 (1.25 m x 3 m), distance between treatments was 1.5 m and distance between blocks 1 m. The total area of the experiment was 168 m^2.

Water management and water requirement calculation

Sprinkler-irrigation was done three times, including the irrigation applied at sowing. During the seed maturation stage, drip irrigation was used for applying the different treatments. Irrigation requirements were calculated by using the formula below (Equation 1) (Sampathkumar et al., 2013) and a Class-A pan, which provided the evaporation datum, which is referred to as CPE in the equation:

$$\textbf{IR (Liters)} = \text{CPE (mm)} * K_p * K_c * W_p\,(\%) * S\,(m^2) \qquad \text{(Equation 1)}$$

Where: IR is the volume of water to apply [liters/plant]; CPE corresponds to cumulative pan evaporation during the days between two consecutive irrigation events [mm], for the case seven days; K_p is the pan coefficient, which depends on the relative humidity, the wind speed and the distance between the crop and the pan (Allen et al., 2006); K_c is the crop coefficient, this value depends on the type of crop, the growth stage of the plant, and the weather. For this experiment the crop coefficient of the Seed Maturation development stage was taken from a study carried out by Ghamarnia et al. (2014), which so far is the only known reporting of it; W_p is the percentage of crop area wetted by the drip irrigation system, it was assumed to be 0.8 [%]; and S corresponding to the crop density [m^2], which was 0.15 m*0.25 m.

Measured parameters and sampling

The measured parameters *were seed yield*, *fixed oil content*, *essential oil content*, and *essential oil composition*. To measure all parameters, forty plants were randomly taken from each treatment.

- *Fixed oil content*. To determine the content of fixed oil of black cumin seeds, three grams of powder seeds were extracted with hexane as the extraction solvent using a Soxhlet apparatus for three hours (Ghourchian et al., 2016).

- *Essential oil content.* The essential oil content (%) produced by a 50-gram seed sample was determined by extraction by hydro-distillation for 3 hours using a Clevenger apparatus.

- *Essential oil composition.* The analysis of the essential oil was carried out on a 7000 Series Triple Quad GC/MS apparatus [Agilent], equipped with split-splitless injector and automatic liquid sampler, attached to HP-5MS capillary column (30 m x 0.25 mm x 0.25 µm film thickness, 5% phenyl methyl poly siloxane). The carrier gas flow rate (He) was 1 ml/min, split ratio 1:30, oven temperature program was started at 50°C (held for 3 min) while column temperature was linearly programmed from 50-240°C (at rate of 3° min^{-1}). For this measurement only three samples were taken from each treatment and two of them were randomly selected to run the essential oil composition analysis.

Statistical analysis

Analysis of Variance (ANOVA) was employed to test differences among treatments. Difference between means was identified by using the Tukey's honestly significant difference (HSD) test at $P<0.05$. Analyses were performed in InfoStat software versions 2014 and 2020 (Di Rienzo et al., 2020).

RESULTS AND DISCUSSIONS

Evapotranspiration was measured by using a Class-A pan. This data along with other parameters (Equation 1) were used to calculate the amount of water to apply to plants from each treatment (Table 2).

Table 2. Cumulative evaporation in a Class-A pan on each of the dates when differentiated irrigation was applied and water supplied by drip irrigation to plants from each treatment during the seed maturation stage

Date	Evaporation (mm)	Water applied to the different treatments (L)				
		0%	10%	50%	70%	100%
15.05.2017	3.9	0	0.12	0.60	0.85	1.22
22.05.2017	1.6	0	0.05	0.25	0.35	0.50
29.05.2017	2.3	0	0.04	0.22	0.30	0.72
05.06.2017	2.9	0	0.05	0.27	0.38	0.90

Seed yield, fixed oil content, and essential oil content

Analysis of variance (ANOVA) showed that the amount of water applied during the seed maturation stage significantly affected seed yield ($F=28.06$, $P<0.05$), fixed oil content $F=25.10$, $P<0.05$), and essential oil content ($F=35.70$, $P<0.05$) of black cumin.

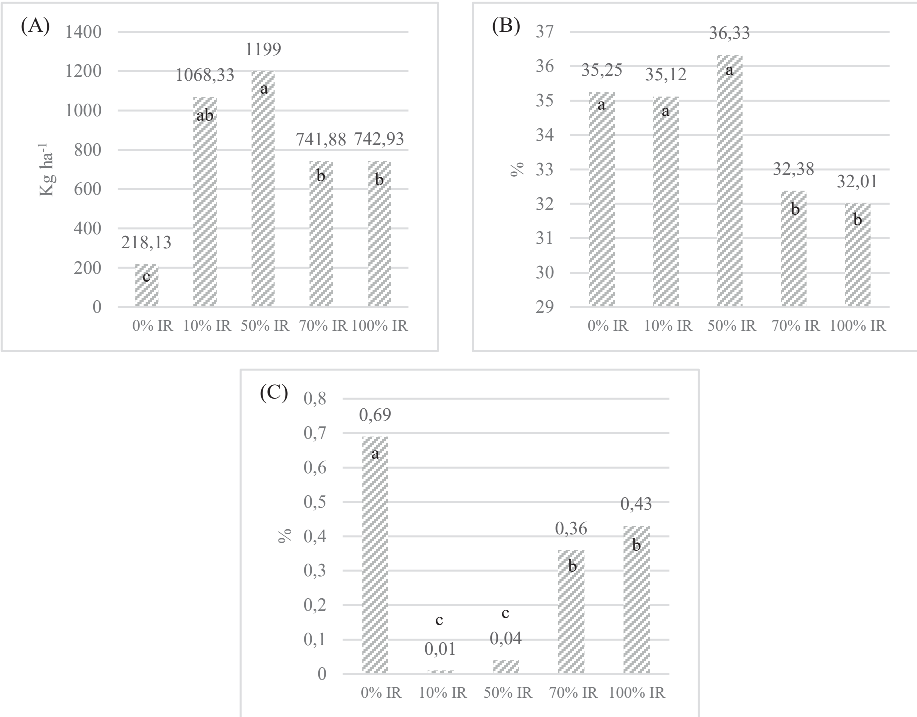

Figure 2. Effect of different irrigation levels on: (A) Seed yield; (B) Fixed oil content; (C) Essential oil content. Means with different letters are significantly different (P<0.05)

Essential oil composition

According to the analysis of variance, contents of α-terpinene (F = 42849.0, P<0.05), farnesol (F = 8.20, P<0.05), γ-terpinene (F = 41.40, P<0.05), lauryl-alcohol (F = 25.66, P<0.05) ρ-cymene (F = 12.23, P<0.05), and β-caryophyllene (F = 24.21, P<0.05) were significantly affected by treatments (Table 3).

Seed yield

The results of the present study (Figure 2) do not agree with studies previously performed by the research carried out by Ghamarnia et al. (2010) and Ghamarnia and Jalili (2013) who concluded that higher amounts of water applied produced higher seed yields. On the other hand, Bannayan et al. (2008) established an experiment with four different irrigation regimes. Researchers concluded that the start of the blooming stage was the most sensitive to a water deficit. This indicates the possibility of obtaining higher seed yields with less water applied if irrigation is ceased over a tolerant developmental stage (Figure 2).

Table 3. Composition of essential oil (%) from black cumin seeds as affected by different irrigation levels

COMPOUND	0% IR	10% IR	50% IR	70% IR	100% IR
α-terpinene	1.04^{b}	0^{a}	0^{a}	0^{a}	0^{a}
Citronellal	1.8^{a}	1.35^{a}	2.53^{a}	1.27^{a}	1.82^{a}
Farnesol	3.55^{a}	14.58^{ab}	20.79^{b}	3.04^{a}	20.05^{ab}
γ-terpinene	42.03^{b}	7.81^{a}	0^{a}	2.76^{a}	5.43^{a}
Guaiyl-acetate	2.98^{a}	0^{a}	0^{a}	0^{a}	2.65^{a}
Lauryl-alcohol	0.76^{ab}	1.56^{bc}	2.12^{c}	1.5^{bc}	0^{a}
ρ-cymene	10.45^{c}	4.58^{abc}	1.74^{ab}	0^{a}	6.78^{bc}
Nerolidol	0^{a}	2.24^{a}	0^{a}	0^{a}	0^{a}
β-caryophyllene	0^{a}	0^{a}	0^{a}	1.23^{b}	0^{a}
Other compounds*	37.39	67.88	72.82	90.2	63.27

Values with different letters are significantly different (P<0.05)
*Compounds corresponding to solvents or present in values less than 1.0%

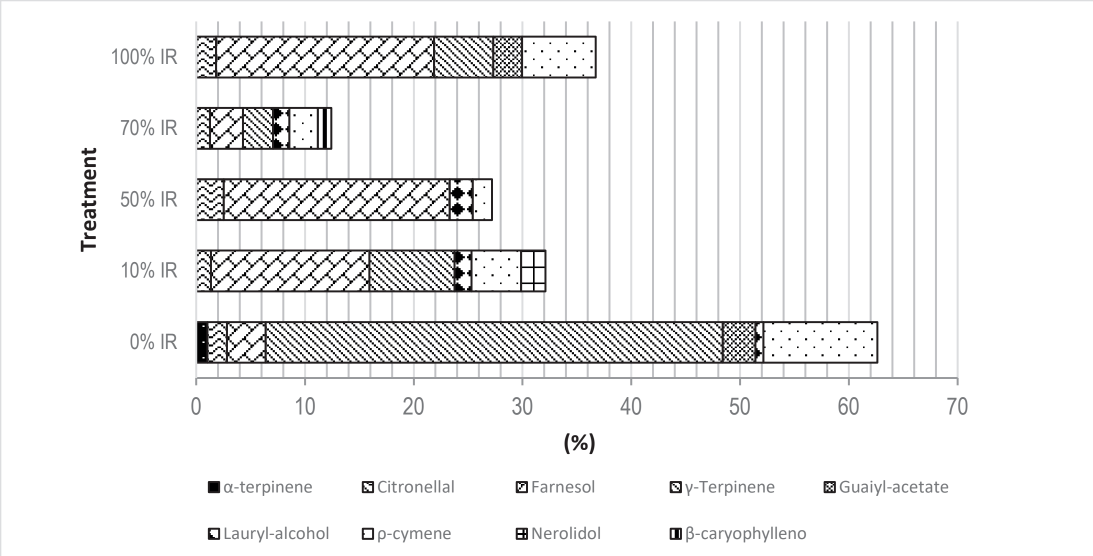

Figure 3. Composition of essential oil from black cumin seeds as affected by different irrigation levels applied during the seed maturation stage

El-Mekawy (2012) performed an experiment to study the effects of sowing date and irrigation regimes on growth and yield of black cumin. This research concluded that decreasing the soil moisture content (increasing the period between irrigation events) during the seed maturation stage caused the decrease of seed yield. The results do not agree with the findings of the present study, according to which, shortening the amount of water provided to black cumin plants during the seed maturation stage by 90% and 50% percent produced the highest seed yields.

Fixed oil content

According to the results, the applied treatments affected black cumin fixed oil content (Figure 2). The highest content belonged to the 50% IR-treatment with 36.33% fixed oil; there was no significant difference between the treatments 50% IR, 10% IR, and 0% IR. Fixed oil contents observed in the present research are higher than those reported by Ghamarnia et al. 2010, which showed that the higher the amount of water applied, the higher the fixed oil content. On the other hand, Ghamarnia and Jalili (2013) found out that contents of fixed oil decreased as a result of increasing water stress.

Essential oil content

Results showed that the amount of water applied to black cumin affected significantly essential oil content (Figure 2). The highest value of essential oil was obtained from the 0% IR-treatment, which had a content of 0.69% essential oil, followed by treatments of 100% IR (0.43%), 70% IR (0.36%), 50% IR (0.04%), and 10% IR (0.01%). The results of the present study suggest that water stress applied to black cumin should be either quite severe or very slight in order to obtain high yields. Although the 0% IR and 10% IR treatments are pretty close to one another in terms of the amount of water applied to the plants, contents of essential oil obtained from treatments are very contrasting, which might suggest that there is a critical and very narrow threshold at which the water stress turns from having a positive effect on the production of secondary metabolites (i.e. essential oils) to having a detrimental effect. The present findings agree with the results of the increasing research carried out in recent years on secondary metabolites, and particularly with studies about the effect of water stress on aromatic and medicinal plants' production of essential oil production. Studies indicate that water stress induces an increase in the production of secondary metabolites such as essential oils, which provide plants with characteristic aroma and biological properties (Chrysargyris et al., 2016; Mandoulakani et al., 2017; Morshedloo et al., 2017).

Ghamarnia and Jalili (2013) established a trial to study the effect of water stress on yield components of black cumin. Results showed that black cumin essential oil yield decreased rapidly as a result of increasing water stress. Researchers concluded that black cumin is sensitive to water stress and has as threshold value 80% of water requirement; a water stress level above this value is not recommended.

Essential oil composition

The essential oil extracted from black cumin seeds in the present study did not contain thymoquinone, which is thought to be largely responsible for its medicinal properties. In investigations carried out by Hamrouni-Sellami et al. (2007) and Wajs et al. (2008), thymoquinone was not detected either. D'Antuono et al. (2002) and Nickavar et al. (2003), reported concentrations of thymoquinone lower than 5%. While thymoquinone was not found in the essential oil, another compound with well-known pharmaceutical properties was detected; farnesol was found in never before reported amounts for black cumin essential oil. As shown in Figure 3 and Table 3, farnesol concentration was significantly affected by treatments ($F = 8.20$, $P<0.05$). Researchers like Kokoska et al. (2008) and Benkaci-Ali et al. (2011) did find farnesol to be present in the essential oil of *Nigella sativa* seeds, however, concentrations ranged from less than 0.05% to as much as 0.9%. In the present study, on the other hand, farnesol concentrations ranged from 3.04% to 20.79%. the highest concentration corresponded to 50% IR-treatment. This result is worth highlighting for farnesol suppresses the formation of histopathological manifestations of colon cancer, which adds weight to the hypothesis that this agent possesses chemopreventive activity against colon carcinogenesis (Rao et al., 2002). Farnesol can also intensify the effect of antimicrobial agents on *Escherichia coli* and *Mycobacterium smegmatis* (Jing et al., 2010; Constantino et al., 2016).

CONCLUSIONS

As hypothesized, the amount of water applied during the seed maturation stage differentially affected seed yield, fixed oil content as well as essential oil content and composition of black cumin. Plants receiving ten and fifty percent of the water requirements were more efficient at seed production. Concerning the fixed oil content, the highest yields were obtained when water quantities equal to fifty percent or less of the irrigation requirements were applied. The treatment consisting of no-irrigation showed the highest essential oil content. Ultimately, results show that providing black cumin plants with hundred percent of the water requirement during the seed maturation stage does not produce higher yields.

The results obtained in the present study constitute an initial yet important contribution to the knowledge of black cumin water management. Further studies should be carried out applying regulated deficit irrigation during the seed maturation stage to validate the findings here obtained. Also, future studies are recommended on other phenological stages of black cumin in order to examine which of the phenological stages is the most sensitive to water shortages.

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