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RESEARCH ARTICLE

Zinc Fertilizer Applications to *Ocimum basilicum* L. under Water Stress: Changes in the Total Phenolic and Flavonoid Content, Essential Oil Compounds and Morphological Properties

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ABSTRACT

Water stress poses a significant challenge for plant growth and productivity, impacting both yield and quality. With the ongoing changes in global climate, mitigating the adverse effects of water deficiency on plants has become crucial. In this study, the focus is on enhancing the tolerance of *Ocimum basilicum* L., a plant highly susceptible to water stress. To achieve this, in this study examined the effects of zinc fertilizer supplementation at varying rates (2.5 - 5 and 10 mg/kg) on *O. basilicum* grown in silty sandy soil and subjected to water stress conditions. Several parameters, including mineral uptake, morphological characteristics, total phenol and flavonoid contents, and essential oil compounds, were evaluated in sweet basil. The results revealed that water stress had a detrimental impact on the morphological properties and secondary metabolites analysed. Estragole emerged as the main compound in the essential oil analysis, with the highest concentration (69.37%) observed in the group treated with 10 mg/kg of zinc fertilizer. Conversely, the lowest concentration (66.14%) was recorded in the water-stressed group without fertilizer. Notably, the application of zinc fertilizer at concentrations of 5 and 10 mg/kg significantly ameliorated the negative effects induced by water stress. Furthermore, zinc exhibited diverse mechanisms of action concerning the uptake of other nutrients from the soil.

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1. Introduction

Plants are exposed to abiotic stress factors such as drought, abnormal temperature changes, salinity, UV, which can cause harmful effects on plant growth many times throughout their lives. Drought, one of these stress factors, negatively affects the phenological, morphological and physiological characteristics of the plant, causing yield and quality losses (Zulkiffal et al., 2021; Pulvento et al., 2022). In this respect, water deficiency limits the growth and productivity of plants more than other stress factors. Due to the global climate change experienced in recent years, the decrease in the amount of water that can be used in agricultural lands causes a lack of nutrients in the products, resulting in serious yield losses (Weisany et al., 2021). In this context, it will be beneficial for the future of agricultural production to develop alternative methods that can increase the drought tolerance of products that are less sensitive to global climate change or to be grown.

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Ocimum basilicum L. (Sweet basil) is a member of the Lamiaceae family and is renowned for its rich secondary metabolites, including phenolic acids and terpenoids (essential oils), which have been widely studied for their medicinal and aromatic properties (Shahrajabian et al., 2020; Celikcan et al., 2021). This highly valuable plant plays a significant role in various industries such as pharmaceuticals, food, and perfumery, contributing to the economy (Rezaei-Chiyaneh et al., 2021). Sweet basil contains phytochemicals that exhibit diverse therapeutic properties, including antioxidant, anticancer, and antimicrobial effects, making it a crucial component in agricultural practices worldwide (Ahmed et al., 2019; Chu et al., 2022; Perna et al., 2022). However, the sensitivity of basil, especially when exposed to water stress in arid and semi-arid regions, negatively impacts its performance and leads to reduced essential oil production (García-Caparrós et al., 2019). This decline in essential oil output hinders the industrial utilization of the plant. Consequently, several studies have focused on developing strategies to enhance the adaptability and tolerance of basil to abiotic stress factors, resulting in the emergence of various applications and techniques (Farouk et al., 2020; Hozayn et al., 2020; Taha et al., 2020; Kahveci et al., 2021).

While various agricultural practices have successfully enhanced crop yield, improper usage of chemical fertilizers has led to significant issues, causing damage to soil physiology and biochemistry (Ahmadi & Souri, 2020; Zargar Shooshtari et al., 2020). Although plant genetics primarily determine the composition and quantity of phytochemicals, studies have shown that different cultivation techniques employed during plant development can also influence these phytochemicals through distinct mechanisms (Jeffery et al., 2003). Among the commonly employed cultivation techniques, the application of plant nutrients to the soil or directly to the plants remains a popular choice. By utilizing different fertilizers (organic, vermicompost, chemical) at various doses, these techniques have proven to positively impact the phytochemical profiles of medicinal and aromatic plants, offering support for both increased yield and improved quality (Ulusu & Şahin, 2021). It is important to carefully consider and implement appropriate fertilization strategies to maximize the benefits while minimizing any adverse effects on soil health and plant physiology.

Zinc (Zn), a micronutrient, plays a crucial role in vital bio-physicochemical processes within plants, including protein synthesis and gene regulation, among others. Additionally, zinc serves as a cofactor for antioxidant enzymes, helping to mitigate oxidative damage in plant cells during abiotic stress conditions (Marreiro et al., 2017). Moreover, zinc holds a special significance in safeguarding plants against water deficiency stress (Noman et al., 2019). A study demonstrated that the application of zinc fertilizer increased the essential oil production in *Matricaria recutita* L. plants exposed to drought stress (Jeshni et al., 2017). Similarly, in another study, it was reported that the application of zinc fertilizer to *Galanthus elwesii* Hook. significantly enhanced the content of total flavonoids, phenolic compounds, alkaloids, galanthamine, and lycorine (Ay et al., 2023). Furthermore, zinc improves the uptake efficiency of other nutrients such as nitrogen and phosphorus by plants (Shivay et al., 2015), thus, making it vital for minimizing the detrimental effects caused by nutrient deficiencies in plants. In this context, assessing the efficacy of zinc in enhancing the drought tolerance of basil plants holds significant value for agricultural production. Currently, there is limited literature available on the evaluation of various fertilizer applications for medicinal and aromatic plants, particularly in the case of *O. basilicum* (Kalamartzis et al., 2020; Celikcan et al., 2021; Kulak et al., 2021). Thus, this study aims to investigate the potential effects of different dosages of zinc fertilizer on the drought tolerance of basil plants, focusing on alterations in morphological properties and phytochemical components such as total phenolics and essential oils. By examining these aspects, the study seeks to provide valuable insights into optimizing the cultivation practices of basil plants under drought-stress conditions.

2. Materials and Methods

2.1. Plant Material

O. basilicum seeds were obtained from Genta (Simagro Agro & Seed Company) for the experiment. Prior to the planting date, the seeds were stored at 4 °C. To ensure seed sterilization, the basil seeds underwent a surface sterilization process, involving a 1 min incubation in a 1% NaClO solution. The study was conducted in a greenhouse, providing optimal conditions including temperatures ranging from 25 to 30 °C, suitable lighting, and adequate humidity levels. The experiment took place during the 2021-2022 period, following a randomized plot design with three replications to ensure reliable results. For planting, the seeds were spaced 20 cm apart in rows, and the process was carried out in March. The physicochemical properties of the soil samples utilized in the study are detailed in Table 1.

Table 1. The physicochemical properties of the experimental soil.

Physicochemical Properties	Normal Soil	Fertilized Soil
Sand (%)	52.83	51.32
Silt (%)	27.46	26.34
Clay (%)	19.21	19.25
Field capacity (%)	24.58	24.76
pH	7.35	7.13
E.C (mhos/cm)	0.37	0.36
$CaCO_3$ (%)	16.98	17.46
Organic matter (%)	4.12	4.53

Table 1. (continued)

Physicochemical Properties	Normal Soil	Fertilized Soil
N (%)	1.38	1.25
P (%)	8.52	9.67
K (%)	71.37	70.67
Mg (%)	6.26	6.31
Ca (%)	14.14	14.56
Cu (ppm)	3.31	3.31
Fe (ppm)	3.60	3.56
Mn (ppm)	16.25	16.58
Zn (ppm)	1.43	2.25

2.2. Zn Fertilizer and Water Stress Treatments to Plants

After the emergence of plants in all pots, separate applications of zinc fertilizer were administered. The fertilizer was introduced to the soil at three distinct rates (2.5 - 5 and 10 mg/kg) using a stock solution of zinc sulphate (Zn_2SO_4 - 0.22% w/v) (as outlined in Table 2). Pots without zinc fertilizer were designated as the control group for comparative purposes. The plants receiving the fertilization treatment were irrigated every two days with a Zn_2SO_4 solution for a period of 30 days prior to the initiation of water deficiency conditions. Conversely, the control group and without fertilized plants were irrigated solely with distilled water. The drought conditions were maintained for a duration of three weeks. Following the drought period, plant tissues (specifically leaves) were collected for analysis. The freshly harvested tissues were promptly frozen in liquid nitrogen and subsequently stored at -80 °C to maintain their integrity. The drought conditions implemented in this study adhered to the specifications outlined by Bettaieb et al. (2009), as detailed in Table 2. Given that previous research (Kulak et al., 2021) reported 25% of the field capacity to induce stress in basil plants, two distinct water regimes were employed: 100% well-watered (maintaining optimal water levels) and 25% severe water deficit. The control group, on the other hand, was evaluated under 100% field capacity (FC) conditions. The water content at field capacity was expressed as a percentage relative to the maximum pot capacity. To ensure reliability, all measurements were conducted in triplicates.

Table 2. Experimental design of the study.

Treatment	Abbreviation	Water Regime
No-fertilizer + well-watered plants	NF-100 (Control)	100%
No-fertilizer + severe water stressed plants	NF/WS	25%
Zn fertilizer (2.5 mg/kg) + well-watered plants	2.5 ZnF	100%
Zn fertilizer (2.5 mg/kg) + severe water stressed plants	2.5 ZnF/WS	25%
Zn fertilizer (5 mg/kg) + well-watered plants	5.0 ZnF	100%
Zn fertilizer (5 mg/kg) + severe water stressed plants	5.0 ZnF/WS	25%
Zn fertilizer (10 mg/kg) + well-watered plants	10.0 ZnF	100%
Zn fertilizer (10 mg/kg) + severe water stressed plants	10.0 ZnF/WS	25%

NF: No-fertilizer; **ZnF:** Zinc fertilizer; **WS:** Water stress.

2.3. Morphological and Phytochemical Analysis

The effects of all treatments on the morphological characteristics of the plants were evaluated. These morphological properties were plant height (cm), plant weight (g), leaves weight (g), leaves length (cm), leaves width (cm) and root length (cm). In addition, mineral content, total phenolics and flavonoids content, essential oil characterization and ratio were evaluated as phytochemical analysis. All measurements were performed with triplicates.

2.4. Mineral Content Analysis

A comprehensive quantitative elemental analysis was conducted, encompassing nine different elements. The leaves samples, comprising three samples per plant and three plants per replicate, were subjected to rigorous preparation. Prior to analysis, the leaves were carefully washed with deionized water and subsequently dried at a temperature of 70 °C for a duration of 48 hours. The specific analytical techniques employed for each element were as follows: N analysis using the Kjeldahl method, P_2O_5 analysis utilizing spectrophotometric methods, and K_2O, Mg, Fe, Zn, Cu, Mn, and Ca analysis performed through atomic absorption spectroscopy. The methodology employed in this study was based on established protocols outlined in the work of Kulak et al. (2021), ensuring reliable and consistent results.

2.5. Total Phenolics and Flavonoids Content Analysis

To extract the dried and ground leaf tissues, a 5 g sample was mixed with 50 mL of 80% methanol solution in a magnetic stirrer. The mixture was left to stir for 24 hours at room temperature. Subsequently, the solution was filtered using a sterile filter with a pore size of 0.22 µm. The filtrate was then evaporated to dryness using a rotary evaporator set at 40 °C. The resulting green residue, which yielded approximately 9.5% of the initial weight, was stored at +4 °C until further analysis.

The determination of total phenolic content (TPC) was carried out using the Folin-Ciocalteu colorimetric assay, while total flavonoid content (TFC) was assessed using the aluminium chloride assay. The methods utilized in this study were based on the procedures reported by Ulusu et al. (2017) and Ulusu and Şahin (2022). For TPC, the concentration of gallic acid (mg eq. GAE/g DW) was determined using a calibration curve ($y=0.7144x+0.0903$, $R^2=0.9934$) derived from gallic acid standards. On the other hand, TFC was determined by calculating the quercetin equivalent (mg eq. QE/g DW) using a calibration curve ($y=2.0714x-0.0003$, $R^2=0.9925$) generated from quercetin standards.

2.6. Essential Oil Extraction

To extract the essential oil from the dried and ground basil leaves, 1 g of the sample was subjected to hydro-distillation using a Clevenger type apparatus for a duration of 4 h. The solution obtained after hydro-distillation was then subjected to liquid-liquid extraction using n-hexane. The upper phase, containing the essential oil, was carefully collected and transferred to a flask. To remove any remaining water, the solution underwent evaporation, followed by drying with sodium sulphate. The resulting sample was stored at -20 °C until it could be subjected to GC-MS analysis, as described in the methodology outlined by Ulusu and Şahin (2021).

2.7. GC-MS Conditions

The essential oil analysis and identification were conducted using an Agilent 7890A Gas Chromatograph (GC). For the analysis, an HP-5MS capillary column with dimensions of 30 m × 0.25 mm × 0.25 µm was employed, with helium used as the carrier gas at a flow rate of 0.8 mL/min. The injector temperature was set to 240 °C. The oven temperature was initially set at 40 °C and then ramped up to 240 °C at a rate of 4 °C/min. The split ratio used was 1:10. In the electron pulse (EI) mode, the mass spectrometer operated at 70 eV. The scan range for mass detection spanned from 15 to 550 amu. To identify the components, present in the essential oil, reference compounds from the Wiley275 and NIST08 libraries were utilized, allowing for accurate identification based on comparison with known compounds.

2.8. Statistical Analysis

The experimental design involved triplicate replicates for each treatment group, with a total of eight plants analysed. The collected data, which included both fertilizer application and water stress variables, were subjected to statistical analysis using two-way ANOVA. Post-hoc analysis was conducted using Duncan's test. The statistical analysis was carried out using SPSS software, specifically version 24.0 by IBM Corp. located in Armonk, NY, USA. Statistical significance was set at $p<0.05$.

3. Results and Discussion

3.1. Morphological Properties

The response of *O. basilicum* to severe drought conditions in soil treated with different doses of Zn fertilizer was evaluated in terms of morphological characteristics (plant height, plant weight, leaves weight, leaves length, leaves width and root length). Water stress significantly negatively affected all morphological parameters studied in the plants. NF-WS had shorter plant height and leaves length, lighter plant weight (DW) and smaller leaves width compared to the other treatment groups (Table 3). Amending the soil with zinc fertilizer generally affected all morphological parameters positively. In addition, it has been noted that zinc fertilizer has a healing effect in terms of related parameters in plants exposed to water stress. Compared to the control group, shorter plant height and lower plant dry weight were noted at 2.5 ZnF-WS. Water stress caused significant losses in plant and leaf dry weight, while zinc applications supported the increase in plant and leaves dry weight. The dry weight gain of plant and leaves was more pronounced, especially in plants of the applications of 5.0 ZnF and 10.0 ZnF under non-stress conditions. However, in zinc applications were determined higher values in terms of plant dry weight, leaves dry weight, leaves width and root length parameters, relative to plants applied zinc under water stress conditions. In terms of all morphological parameters, 10.0 ZnF treatment showed the best effect in plants. Drought is one of the major constraints on crop yield, quality and productivity of plants. The effects of drought on plants have been reported in different studies (Jeshni et al., 2017; Avila et al., 2020; Ozturk et al., 2021). The first response observed in plants to water stress is generally seen in their phenological, physiological and morphological characteristics (Galmés et al., 2007; He et al., 2020; Ors et al., 2021). In the researches, different fertilizer treatments were carried out to compensate the losses caused by water stress and to increase the tolerance level of the plant (Javan Gholiloo et al., 2019; Ahanger et al., 2021). For instance, in a study investigating the effects of zinc fertilizer treatment on the qualitative properties and oil yield of canola plants grown in different moisture regimes, it was determined that zinc treatments improved all morphological properties of canola exposed to water stress. Especially, it was stated that it contributed to the increase in grain yield (43.82%), biological yield (73.99%) and harvest index (30.04%) (Shahsavari et al., 2014). Gholinezhad (2017), investigated the effects of iron nano fertilizer and different irrigation levels on morphological properties and essential oil percentage in *Anethum graveolens* L. and different properties investigated with the severity of drought stress decreased significantly, but nano Fe contributed towards compensating for these properties. In line with these results determined in the literature, it is extremely important to determine and understand the strengthening of the tolerance to drought stress of medicinal and aromatic plants used in the treatment of diseases with different applications (Sun et al., 2020).

Table 3. The changes in the morphological properties of the plants corresponding to treatments.

Treatments	Plant height (cm)	Plant weight DW (g)	Leaves length (cm)	Leaves weight DW (g)	Leaves width (cm)	Root length (cm)
Control	22.63±1.05^{c}	1.12±0.02^{f}	3.84±0.13^{ab}	0.38±0.03^{e}	1.42±0.15^{bc}	22.54±1.08^{c}
NF/WS	15.42±1.72^{d}	0.86±0.04^{g}	3.28±0.2^{b}	0.24±0.02^{f}	1.33±0.20^{bc}	24.71±1.14^{bc}
2.5 ZnF	21.54±1.35^{c}	1.64±0.05^{e}	3.81±0.14^{ab}	0.61±0.03^{d}	1.84±0.15^{ab}	24.32±1.36^{bc}
2.5 ZnF/WS	19.21±2.12^{c}	1.06±0.05^{f}	3.86±0.61^{ab}	0.52±0.05^{d}	1.61±0.32^{abc}	32.55±2.22^{a}
5.0 ZnF	32.34±1.02^{b}	2.87±0.12^{c}	4.01±0.52^{a}	1.57±0.04^{b}	1.97±0.35^{ab}	27.67±2.13^{ab}
5.0 ZnF/WS	29.12±3.20^{b}	2.14±0.14^{d}	3.94±0.43^{ab}	1.12±0.04^{c}	1.72±0.4^{ab}	31.41±1.11^{a}
10.0 ZnF	35.47±1.13^{a}	3.52±0.06^{a}	4.22±0.26^{a}	1.84±0.05^{a}	2.44±0.10^{a}	27.40±1.53^{b}
10.0 ZnF/WS	28.45±2.64^{b}	3.07±0.1^{b}	3.83±0.42^{ab}	1.61±0.03^{b}	2.14±0.37^{ab}	30.11±1.25^{a}

DW: Dry weight; **NF**: No-fertilizer; **ZnF**: Zn fertilizer; **WS**: Water stress. The same letters in the same column were not differed statistically (Duncan) (p<0.05).

3.2. Mineral Content

When the literature is reviewed, there is little reference to the effect of water stress on *O. basilicum* leaves mineral composition. In this respect, it is worth mentioning the changes in leaf macro and micronutrient concentrations in this study. The changes in the mineral content of plants in response to treatments are listed in the Table 4. In basil leaves, the macroelement (N, P, K, Mg, Ca) and microelement (Cu, Fe, Mn, Zn) contents determined in the control and treatment groups are in a similar range with the previous study (Kulak et al., 2021). In the Table 4, it is seen that the average phosphorus content in basil leaves varies between 0.38 and 0.62 ppm. Phosphorus content is maximum in control while it is minimum in 10.0 ZnF treatment. Zn treatment significantly affected phosphorus in the tissues and the P concentration decreased with the increase of Zn concentration. That is, a negative correlation was determined between Zn and P. This, in turn, is considered as an interference with P uptake by plants as a result of the interaction of these two nutrients (Keram et al., 2012; Samreen et al., 2017). Furthermore, it is clear that Zn treatment causes a negative effect on the Fe uptake of basil plants (Table 4). The highest dose of Zn fertilizer treatment (10.0 ZnF) to the plant reduced the Fe content by 37% compared to the control. The decrease in Fe content may be due to the antagonistic interaction between Zn and Fe in plant roots. In other words, excess Zn can cause a decrease in Fe uptake by plants. Our findings are supported by some studies in the literature (Sresty & Madhava Rao, 1999; Brunetti et al., 2011).

Table 4. Changes in mineral contents in response to treatments.

Treatments	N (%)	P (%)	K (%)	Mg (%)	Ca (%)
Control	2.36±0.02^{d}	0.62±0.01^{a}	0.80±0.01^{g}	0.52±0.01^{d}	1.64±0.04^{f}
NF-WS	1.57±0.01^{g}	0.58±0.02^{b}	0.58±0.01^{h}	0.44±0.02^{e}	1.21±0.02^{g}
2.5 ZnF	2.32±0.02^{d}	0.54±0.03^{b}	1.38±0.15^{d}	0.61±0.01^{c}	2.06±0.03^{e}
2.5 ZnF-WS	1.88±0.01^{f}	0.56±0.03^{b}	1.06±0.02^{f}	0.56±0.01^{c}	2.42±0.01^{c}
5.0 ZnF	2.42±0.01^{c}	0.48±0.01^{c}	1.45±0.02^{c}	0.58±0.02^{c}	2.57±0.02^{b}
5.0 ZnF-WS	2.04±0.15^{e}	0.46±0.01^{c}	1.32±0.01^{e}	0.50±0.03^{d}	2.22±0.02^{d}
10.0 ZnF	2.81±0.02^{a}	0.38±0.03^{d}	1.66±0.01^{a}	0.72±0.02^{a}	2.74±0.04^{a}
10.0 ZnF-WS	2.76±0.01^{b}	0.40±0.02^{d}	1.57±0.01^{b}	0.66±0.01^{b}	2.72±0.01^{a}
	Cu (ppm)	**Fe (ppm)**	**Mn (ppm)**	**Zn (ppm)**	
Control	8.44±0.11^{f}	514.50±12.11^{b}	53.45±0.41^{e}	47.31±1.17^{d}	
NF-WS	6.12±0.12^{g}	489.54±13.10^{b}	42.21±0.54^{g}	39.04±1.40^{e}	
2.5 ZnF	12.11±0.17^{d}	548.72±12.20^{a}	70.88±0.47^{c}	57.24±1.12^{c}	
2.5 ZnF-WS	10.18±0.14^{e}	515.17±11.02^{b}	63.48±0.70^{d}	57.07±1.41^{c}	
5.0 ZnF	14.24±0.26^{c}	423.59±11.11^{d}	102.57±0.30^{a}	69.14±1.33^{b}	
5.0 ZnF-WS	14.52±0.21^{c}	459.83±15.13^{c}	87.45±0.82^{b}	67.06±1.11^{b}	
10.0 ZnF	17.13±0.11^{a}	324.21±16.02^{f}	62.82±0.49^{d}	81.28±2.61^{a}	
10.0 ZnF-WS	16.54±0.20^{b}	382.16±9.15^{e}	51.93±0.52^{f}	79.05±1.60^{a}	

NF: No-fertilizer; **ZnF**: Zn fertilizer; **WS**: Water stress; **DW**: Dry weight. The same letters in the same column were not differed statistically (Duncan) (p<0.05).

It was observed that Zn fertilizer application positively supported the uptake of other nutrients (N, K, Ca, Cu, Mn) by plants. However, the effect of plants on Mg uptake was non-uniform and an increase was observed at 2.5 ZnF and 5.0 ZnF doses compared to the control, while a sharp decrease was observed at 10.0 ZnF doses. This interaction between nutrients is similarly supported by other studies (Fan et al., 2008; Rietra et al., 2017).

According to the data, basil plants have average Zn contents ranging from 39.04 to 81.28 ppm. The Zn content in plants increased significantly with the application of Zn to the soil, resulting in a maximum increase of 67.08% with 10.0 ZnF, followed by a 46.14% increase with 5.0 ZnF. Zinc uptake by plants has a parallel relationship with zinc treatments to the soil. Data supporting our results were also reported by Samreen et al. (2017). They examined the effect of zinc applied in different doses (1 and 2 µM) to the *Vigna radiata* L. plant using hydroponic culture and investigated that on growth, protein and mineral content and determined that the Zn content in the plants increased by 496.6% at the highest dose (2 µM). Nutritional supplementation to the soil facilitates the uptake of mineral elements by plants, and it is a known fact that these nutrients affect important biochemical reactions (photosynthesis, enzyme activity, protein synthesis, etc.) in plants (Prakash et al., 2020; Maitra et al., 2022). According to the findings, water stress generally prevented nutrient uptake in all applications. However, it was observed in the study that zinc can generally modulate other nutrients in plants exposed to water stress and in other treatment groups. With nutritional supplements, plants tolerate environmental stresses better, thus enabling the desired yield and quality increase in addition to growth and development in agriculture.

3.3. Total Phenolics and Flavonoids Content

TPC and TFC obtained from *O. basilicum* leaves samples under water stress and treated with Zn fertilizer are shown in Table 5. While water stress caused an increase in the total phenol content of basil plants, it caused serious decreases in the flavonoid content. However, zinc fertilizer application turned this loss caused by water stress into gain and had an effect on the increase of flavonoid content. In addition, all fertilizer applications significantly increased the total phenolic and flavonoid contents compared to the control (12.30 mg GAE/g DW, 0.53 mg QE/g DW, respectively) and the effect of fertilizer applications on these contents ranged from 14.27-29.42 mg GAE/g DW, 0.81-1.95 mg QE/g DW, respectively. By analyzing the data presented in Table 5, especially, 10.0 ZnF application increased TPC by 107% and TFC by 267% compared to control. In agreement with our results, it was stated in the literature that Zn fertilizer application contributed to the increase in total phenolic and flavonoid content (Ay et al., 2023). Again, it was stated that the application of Zn to *Chrysanthemum balsamita* L. had an effect on increasing the total phenol content (Derakhshani et al., 2011). In addition, Maity et al. (2023) reported that there is a positive correlation between these phytochemicals of nutrients in their study. In previous studies, similar to our study, different fertilizers containing nutrients increased the rate of valuable phytochemicals such as essential oil, saturated and unsaturated oil, phenolic and flavonoid contents (Siddiqui et al., 2020; Ulusu & Şahin, 2021). The main factors that differentiate the synthesis of phenolic compounds in plants are considered to be the amount of water supplied and the exposure time to drought stress (Albergaria et al., 2020). This phenomenon is supported by many studies. McKiernan et al. (2014) determined that the ratio of phenolic compounds in the leaves of 2 different Eucalyptus species (*E. globulus* Labill. and *E. viminalis* Labill.) exposed to drought decreased. In another study, phenolic compound levels of *Salvia officinalis* L. exposed to moderate water deficiency were found to be significantly higher than the control group (Bettaieb et al., 2011). In a study investigating the phenolic and flavonoid contents of 3 different Achillea plants (*A. nobilis* L., *A. millefolium* L. and *A. filipendulina* Lam.) under moderate and severe drought, a significant increase in phenolic and flavonoid contents was determined under moderate water stress (%50 of field capacity) in plants. However, under severe drought (%25 of field capacity), the levels of these compound contents differed within species (Gharibi et al., 2016). Furthermore, *Melissa officinalis* L. exposed to water stress decreased in polyphenols content, while flavonoid content did not change compared to well-watered ones (Szabó et al., 2017). Similar to our study, it was determined that the phenolic content of two basil varieties exposed to drought stress was higher than the control group (regularly irrigated) (Pirbalouti et al., 2017). When we look at the studies, water stress shows intra and inter-species significant differences in plants' secondary metabolite synthesis mechanisms.

Table 5. Total phenolic and total flavonoids content corresponding to the treatments in *O. basilicum* leaves.

Treatments	TPC (mg GAE/g DW)	TFC (mg QE/g DW)
Control	12.30±0.30^{g}	0.53±0.02^{g}
NF-WS	13.21±0.03^{f}	0.35±0.04^{h}
2.5 ZnF	14.27±0.22^{e}	0.81±0.03^{e}
2.5 ZnF-WS	16.33±0.10^{d}	0.72±0.01^{f}
5.0 ZnF	16.47±0.15^{d}	1.57±0.01^{c}
5.0 ZnF-WS	19.86±0.10^{c}	1.14±0.02^{d}
10.0 ZnF	25.50±0.41^{b}	1.95±0.01^{a}
10.0 ZnF-WS	29.42±0.18^{a}	1.83±0.01^{b}

NF: No-fertilizer; **ZnF**: Zn fertilizer; **WS**: Water stress; **DW**: Dry weight; **GAE**: Gallic acid equivalents; **QE**: Quercetin equivalents. The same letters in the same column were not differed statistically (Duncan) (p<0.05).

3.4. Essential Oil Components

The essential oil components identified in *O. basilicum* leaves are shown in the Table 6. The main compound was estragole (phenylpropene) in the range of 66.14-69.37% which was followed by limonene (monoterpen) in the range of 11.38-14.32%. In *O. basilicum*, the interaction of water stress, zinc fertilizer and treatments significantly affected the content of essential oil components. According to the data, all ZnF treatments significantly increased the estragole content. Similarly, the ZnF-WS interaction contributed to an increase in the content of essential oil compound compared to NF-WS. NF-WS caused a drastic decrease in the percentage of all components. Regarding estragole and limonene, 10.0 ZnF treatment significantly affected the increase in the percentage of these two main compounds. Also, compared to control, all zinc fertilizer treatments and zinc fertilizer - water stress interaction treatments contributed to increases of p-cymene, fenchone, methyle eugenol, germacrene D compounds. In *O. basilicum* leaves, the reduction in essential oil content under drought conditions can be associated with a decrease in dry weight.

Water stress is a critical factor that significantly impacts the content of essential oils, particularly in aromatic plants. When plants experience water stress, there is a noticeable decline in the synthesis of phytochemicals (García-Caparrós et al., 2019). The extent and duration of drought conditions, along with the morphological and physiological state of the plant, as well as the plant species and varieties, can influence the essential oil content. Similar to our study, reductions in essential oil content were observed in *Mentha arvensis* L. (Misra & Srivastava, 2000) and *Salvia officinalis* L. (Govahi et al., 2015), both belonging to the Lamiaceae family, when exposed to water stress. However, it is worth noting that literature reports indicate that drought conditions can also lead to an increase in essential oil content in certain Lamiaceae species. For example, in *Thymus caramanicus* Jalas. (Lamiaceae), water stress (at 20% of field capacity) resulted in an 11.9% rise in carvacrol, the primary component of its essential oil (Bahreininejad et al., 2014). Similar findings have been observed in *Salvia officinalis* L. (Bettaieb et al., 2009) and *Satureja hortensis* L. (Baher et al., 2002), both of which belong to the Lamiaceae family. Furthermore, in other studies investigating the effect of Zn fertilizer application on essential oil yield, a significant increase in essential oil content was reported in parallel with fertilizer treatment in *Mentha piperita* L. (Akhtar et al., 2009), *Origanum majorana* L. (Farsi et al., 2017) and *Moringa peregrina* Forssk. (Soliman et al., 2015) plants. In a study evaluating the effect of Zn fertilizer on the *O. basilicum* essential oil content, it was stated that fertilizer application contributed to the production of essential oil, and the results are similar to our findings (Hanif et al., 2017).

In line with our findings, the effects of Zn fertilizer treatment on vegetative growth parameters and essential oil yield of *O. basilicum* plants are similar.

Table 6. Alterations in essential oil components (%) of *O. basilicum* leaves corresponding to the treatments.

Treatments	**p-Cymene**	**Limonene**	**Fenchone**	**Estragole**	**Exo-fenchyle acetate**
RT (min)	13.76	14.57	17.81	20.19	20.81
Control	2.32±0.22^{f}	12.63±0.55^{b}	3.02±0.13^{c}	67.12±1.13^{ab}	8.13±0.40^{ab}
NF-WS	1.87±0.11^{g}	11.38±0.42^{c}	2.78±0.14^{d}	66.14±1.12^{b}	7.24±0.20^{b}
2.5 ZnF	2.34±0.02^{f}	13.54±0.53^{ab}	3.18±0.15^{bc}	68.81±0.80^{ab}	8.66±0.30^{a}
2.5 ZnF-WS	2.98±0.03^{d}	13.11±0.30^{b}	3.06±0.21^{c}	67.86±0.11^{b}	8.12±0.40^{ab}
5.0 ZnF	3.12±0.02^{c}	12.18±0.10^{b}	3.45±0.20^{bc}	69.23±0.57^{a}	8.47±0.32^{a}
5.0 ZnF-WS	2.67±0.17^{e}	12.86±0.44^{b}	3.14±0.15^{bc}	68.70±0.52^{a}	8.82±0.53^{a}
10.0 ZnF	3.31±0.12^{b}	14.32±0.35^{a}	3.66±0.10^{b}	69.37±0.18^{a}	7.54±0.47^{b}
10.0 ZnF-WS	3.56±0.01^{a}	14.18±0.60^{ab}	3.98±0.10^{a}	68.56±0.34^{a}	8.37±0.21^{a}
	Carvacrole	**Methyle eugenol**	**Germacrene D**	**Total (%)**	
RT (min)	22.54	25.36	27. 41		
Control	0.54±0.01^{b}	0.14±0.01^{d}	0.43±0.01^{d}	94.33	
NF-WS	0.42±0.01^{c}	-	0.21±0.02^{e}	90.04	
2.5 ZnF	0.64±0.02^{a}	0.18±0.03^{c}	0.56±0.01^{b}	97.91	
2.5 ZnF-WS	0.31±0.01^{d}	0.15±0.02^{cd}	0.48±0.02^{c}	96.07	
5.0 ZnF	0.67±0.03^{a}	0.23±0.03^{a}	0.59±0.03^{ab}	97.94	
5.0 ZnF-WS	0.52±0.02^{b}	0.20±0.01^{b}	0.55±0.02^{b}	97.46	
10.0 ZnF	0.63±0.01^{a}	0.24±0.02^{a}	0.62±0.01^{a}	99.69	
10.0 ZnF-WS	0.54±0.01^{b}	0.21±0.01^{b}	0.35±0.04^{f}	99.75	

NF: No-fertilizer; **ZnF**: Zn fertilizer; **WS**: Water stress; **DW**: Dry weight; **RT**: Retention time. The same letters in the same column were not differed statistically (Duncan) (p<0.05).

4. Conclusion

In summary, the results of this study indicate that water stress caused serious effects on morphological properties, total phenolics and flavonoids content and essential oil components in *O. basilicum*. In addition, it is seen that Zn application under water stress conditions can reduce the damage caused by water stress. Furthermore, that optimum amount the application of Zn that optimum amount to *O. basilicum* under normal conditions positively affects all the investigated criteria, suggesting that this may be due to the critical roles of Zn in plant nutrition and influencing other nutrients. In general, the results showed that the application of 10 mg/kg Zn fertilizer to *O. basilicum* plant under normal conditions (irrigation based on 100% of field capacity) can both improve the morphological characteristics of the plant and synthesize phytochemicals at an optimum level. Studies involving different experimental groups in *O. basilicum* and evaluating them in terms of their biological activities should be considered.

Conflict of Interest

The author has no conflict of interest to declare.

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