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The Effect of Different Concentrations of Salicylic Acid on the Biochemical-Physiological Characteristics and Essential Oil Yield of Basil under Various Moisture Regimes

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ABSTRACT

Drought stress, as a non-living limiting factor, has a highly adverse effect on the growth and productivity of crops. A study was conducted to evaluate the impact of salicylic acid on the characteristics of basil subjected to drought-stress in Karaj, Iran, during the cropping seasons of 2015-2016 and 2016-2017. Irrigation was performed at four levels: I_1 (100% water requirement), I_2 (80% water requirement), I_3 (60% water requirement), and I_4 (40% water requirement) in the main plots. At the same time, salicylic acid was applied as a secondary factor at five concentrations: S_1 (no application, spraying with pure water), S_2 (50 µM), S_3 (100 µM), S_4 (150 µM), and S_5 (200 µM) in the sub-plots. The results indicate that the application of salicylic acid has a more significant impact on increasing dry weight under drought-stress conditions. A significant increasing trend in essential oil yield was observed with the increase in salicylic acid concentration from zero to 200 µM. Under drought-stress conditions, the total sugar and protein levels increase to enhance the plant's tolerance to stress. Moreover, relative water content and chlorophyll index increased with the rise in salicylic acid concentration from zero to 50, 100, 150, and 200 micromolar. These findings suggest the positive effects of foliar application of salicylic acid, particularly at higher concentrations (150 & 200 micromolar), under different moisture regimes, especially drought stress, in basil plants. Hence, applying salicylic acid may be suggested as an effective management strategy for basil production under drought-stress conditions.

Keywords: Dry weight, Essential oil, *Ocimum basilicum* L., Water stress

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INTRODUCTION

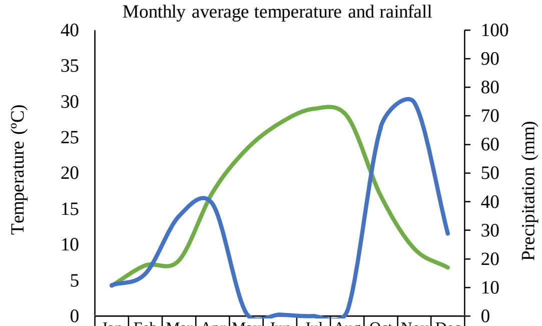
The significance of medicinal plants has led to an increasing number of farmers each year shifting their cultivation from conventional crops to medicinal plants [1]. Sweet basil (*Ocimum basilicum* L.) is known for its numerous medicinal properties [2]. This annual herbaceous plant exhibits significant morphological diversity, secondary metabolites, and particularly essential oil content [3, 4]. Drought stress, as a non-living limiting factor, has a highly adverse effect on the growth and productivity of crops [5, 6]. Today, with the growing population, increased food demand, and limited water resources, improving crop yield per unit of water used has become more critical than simply focusing on yield per unit of area [7, 8]. Various methods, such as plant breeding and the use of growth regulators, are employed to enhance plant resistance to stress. Compared to breeding techniques, often long-term and costly, the use of chemical substances like salicylic acid is simpler and more cost-effective [9]. Salicylic acid plays a role in mitigating the effects of stresses by promoting cell elongation and division and increasing the production of essential oils and aromatic compounds in plants [10]. Reduced water content in plant tissues restricts plant growth under drought-stress conditions [11]. Water-stressed plants typically have shorter heights and smaller leaves [12]. Afkari [13] found that water stress significantly affected traits of basil, such as dry matter yield, carbohydrate content, essential oil content, and essential oil yield. Moghadam *et al.* [14] reported that different levels of water stress had significant effects on the number of secondary branches, essential oil content, and fresh weight in the studied varieties. The impact of drought stress on growth, by reducing turgor pressure and cell expansion, decreasing light absorption, and reducing the overall photosynthetic capacity of the plant, particularly in stems and leaves, results in reduced plant growth and delays in germination. In a study on basil, Ghaemi et al. [15] reported that with decreased soil moisture, plant height, leaf number, and essential oil content decreased, while stem diameter increased, especially under severe stress during the flowering stage. Salicylic acid plays a central role in regulating various physiological processes of plants [16, 17]. It is recognized as a signaling molecule involved in plant defense responses and in resistance to both biotic and abiotic stresses through regulating physiological and biochemical functions. It also influences plant growth, membrane structure, ion absorption and transport, photosynthesis rate, stomatal conductance, flowering, and fruit ripening [9]. Salicylic acid appears to improve nutrient uptake under drought conditions and to promote growth (affecting plant height, length, and number of internodes) in turn [18, 19]. The application of salicylic acid depends on its concentration, method of application, and plant condition [20]. Reports by [19] indicate that salicylic acid acts as an elicitor, increasing the active compounds in the plants studied. [21] revealed that the application of salicylic acid to basil plants grown under drought-stress conditions led to an increase in growth indices and yield. Similarly, [19] suggested that salicylic acid is an effective approach to improving pumpkin growth under water stress. [22] examined the effects of drought stress on the physiological characteristics of basil and reported that the application of salicylic acid increased basil growth under drought-stress conditions. Due to the negative impact of drought stress on basil's growth and productivity, the use of salicylic acid to mitigate drought stress appears essential. Therefore, the current study was conducted to examine the effects of drought stress (various irrigation levels) and foliar application of different concentrations of salicylic acid on the performance of the medicinal basil plant.

Table 1 Climatic conditions of the experimental site during the 2016-2017 and 2017-2018 growing seasons

Climatic conditions	2016-2017	2017-2018
Average annual rainfall	503.3 mm	456.1 mm
Absolute maximum annual temperature	(ºC) 42.5	(ºC) 42.5
Average annual temperature	(ºC) 16.2	(ºC) 15.4
Absolute minimum annual temperature	(ºC)- 8.6	(ºC) 7.9-
Annual evaporation rate	(mm) 2377	(mm) 2016
Average annual humidity	%40	%41
Sunshine hours	2981	2992
Number of rainy days	72	64
Number of frost days	68	84

MATERIALS AND METHODS

This experiment was carried out during the 2016-2017 and 2017-2018 crop years in a private farm located in the Mahdasht region in Karaj (Iran), with coordinates of 51° 6' E longitude, 35° 49' N latitude, and an elevation of 1321 meters above sea level. Table 1 shows the weather conditions at the experimental site, and Figure 1 illustrates the monthly average temperature and rainfall at the site during the 2016-2017 and 2017-2018 cropping seasons.



	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Oct	Nov	Dec
Mean Monthly Temperature	4.25	7.1	7.8	17.3	23.3	27	29	28	16.9	9.45	6.8
Mean Monthly precipitation	10.8	14.8	34.9	39.2	1.1	0.5	0	1.6	66	74.7	28.8

Fig. 1 Monthly average temperature and rainfall at the experimental site during the 2016-2017 and 2017-2018 crop years

Soil Physicochemical Properties

To determine the physical and chemical characteristics of the soil, before the experiment was carried out, and when the soil moisture was at field capacity, several soil samples were collected from a depth of 0 to 30 cm from different parts of the field in a sigmoid pattern. A composite sample was sent to the soil science laboratory for analysis after mixing the samples. The soil texture was determined to be loamy using Texture Autolookup software, v. 4.2.

Table 2 Physicochemical properties of the soil at the experimental field before the start of the experiment

Soil properties	Equivalent word/symbol	Optimal range	2016-2017	Status	2017-2018	Status
Salinity (Ds/m)	Ec (ds/m)	0-2	1.9	Salt free	1.8	Salt free
pH (Acidity)	pH	6-7	7.94	Weakly alkaline	7..92	Weakly alkaline
Organic carbon (%)	(%) OC	>2	0.79	Deficient	0.87	Deficient
Total Nitrogen (%)	Total N (%)	>0.2	0.12	Deficient	0.14	Deficient
Neutralizing Value (%)	(TNV) (%)	0.15	32	-	34	-
Phosphorus (mg/kg)	Available P	15	8.8	Deficient	8.6	Deficient
Potassium (mg/kg)	Available K	300	383	Sufficient	385	Sufficient
Sand (%)	Sand (%)	-	40	-	41	-
Silt (%)	Silt (%)	-	36	-	34	-
Clay (%)	Clay (%)	-	24	-	25	-
Soil texture class	Soil texture class	-	-	Sandy loam	-	Sandy loam
Bulk density (g cm^3)	Bulk density	-	1.20	-	1.20	-

Experimental Methodology

The experimental treatments were as follows:

A) Different Irrigation Levels (Four Levels):

- 100% water requirement, consuming 6000 cubic meters per hectare (I_1),
- 80% water requirement, consuming 4800 cubic meters per hectare (I_2),
- 60% water requirement, consuming 600 cubic meters per hectare (I_3),
- 40% water requirement, consuming 2400 cubic meters per hectare (I_4), applied to the main plots.

B) Salicylic Acid Foliar Application (five levels):

- No salicylic acid application (foliar spraying with pure water) as the control treatment (S_1),
- Foliar application with 50 µM salicylic acid (S_2),
- Foliar application with 100 µM salicylic acid (S_3),
- Foliar application with 150 µM salicylic acid (S_4),
- Foliar application with 200 µM salicylic acid (S_5) was applied to the sub-plots.

Foliar application of salicylic acid was performed in three stages, starting 30 days after planting and repeated every 10 days.

Land preparation operations included deep plowing with a moldboard plow in the fall, surface plowing, and disc harrowing on March 29. Before planting, furrows were made 60 cm apart using a furrower. The seeds were sown on both sides of the ridges, spaced 60 cm apart, with a planting distance of 10 cm between seeds on the planting rows (planting arrangement: 30×10 cm). The distance between main plots was 1.8 meters (three unsown rows), the distance between sub-plots was one unsown row, and the distance between iterations was 4 meters.

Seed sowing was carried out on May 28 in both years. Holes 1 to 2 cm deep and spaced 10 cm apart were created along the planting rows for sowing. To ensure optimal germination and achieve the desired plant density, 3 to 4 seeds were sown in each hole. After full germination and seedling establishment, thinning was performed at the 3-4-leaf stage, leaving one plant in each hole by removing excess seedlings. After thinning, the final plant density was set at 33.33 plants per square meter.

Fertilizer Application and Irrigation Management

The amounts of nitrogen and phosphorus fertilizers used in both experimental years were determined based on soil test results and fertilization recommendations. In total, 50 kg/ha of urea and 110 kg/ha of triple superphosphate were applied. Potassium fertilizer was not used in this study, as the available potassium in the soil was above the critical threshold.

The first irrigation was conducted immediately after planting. Subsequent irrigations were carried out regularly every 5 days until the plants reached the 4-leaf stage, following the typical practices of the region. After the 4-leaf stage, the irrigation treatments were applied. A water pump was used to ensure the required pressure and energy for all irrigations, and a water meter was employed to measure the exact volume of water used in irrigation. Weed removal was manually conducted during the 2-4-leaf stage and continued every 7 days until the flowering stage. Foliar application of salicylic acid was carried out using a backpack sprayer during the coolest hours of the day.

Evaluated Traits and Measurement Methods

The traits were measured in two stages. The first measurement occurred at the 50% flowering stage to determine dry plant weight and essential oil content. The second measurement was conducted at the end of the growth period when the seeds were fully mature. To determine the essential oil percentage, after drying the samples from four middle rows in the shade at ambient temperature for 5 days, 40 grams of the dried plant samples were used for essential oil extraction through water distillation [23]. The percentage of essential oil was then calculated by dividing the weight of the extracted oil (in grams) by the sample weight (40 grams) and multiplying it by 100. Essential oil yield was obtained by multiplying the essential oil percentage by the dry weight of the plants and dividing by 100. The results were reported in kg/ha. The leaf chlorophyll index (LCI) was measured by a chlorophyll meter (SPAD, Minolta Camera Co., Osaka, Japan). The chlorophyll meter provides a rapid and nondestructive approach that enables users to measure chlorophyll content in the field [24]. The soluble sugar content (SSC) was estimated by the phenol sulfuric acid reagent method as mg per g dry weight [25]. To determine soluble protein content (SPC), 10 leaves from each sample were washed with distilled water and homogenized in 0.16 M Tris buffer (pH = 7.5) at 4°C. Then, 0.5 ml of total homogenized solution was used for protein determination by the [26] method. Relative water content (RWC) was calculated by the method of [27] and based on the below-given equation:

RWC % = [(sample weight – dry weight)/(saturated weight – dry weight)] × 100

Statistical Analysis

Mixed ANOVA for all traits was carried out based on data from both experimental years, using a split-plot design within a randomized complete block design in SAS software (v. 9.1). The comparison of treatment means was performed using Duncan's multiple range test at a 5% probability level. The correlation coefficients between the traits were also calculated using SAS software. Graphs were drawn using Excel, and tables were created with Word.

RESULTS AND DISCUSSION

The Dry Weight of the Plant at the Flowering Stage

The comparison of means (Fig. 2) showed that at the irrigation level of 40% water requirement, increasing the salicylic acid concentration from zero to 50, 100, 150, and 200 µM resulted in dry weight increases of 15.32%, 24.02%, 31.36%, and 36.95%, respectively. At the irrigation level of 100% water requirement, the increases in dry weight were 1.92%, 4.86%, 6.69%, and 7.86%, respectively. The results of the study by [28] revealed that drought stress reduced basil dry matter. Drought stress reduces the leaf surface area, decreasing light absorption and consequently lowering the plant's overall photosynthetic capacity. Thus, the plant's dry matter yield decreases with limited photosynthetic products under drought-stress conditions. In the present study, the highest dry weight of basil plants – 3105.45 kg/ha and 3144.88 kg/ha – was obtained with the application of 150 µM and 200 µM salicylic acid, respectively, under 100% water requirement conditions (Fig. 2). The results indicate that elevating the concentration of salicylic acid across all drought stress treatments significantly enhances plant dry weight. These findings suggest that foliar application of salicylic acid has a more pronounced effect in increasing dry weight under water deficit conditions. Drought stress reduces leaf water content, causing cell shrinkage and weakening of the cell wall. Consequently, leaf area and leaf number decrease, reducing photosynthesis. As photosynthesis decreases, plant vegetative growth, fresh weight, and ultimately dry weight are reduced, too [29].

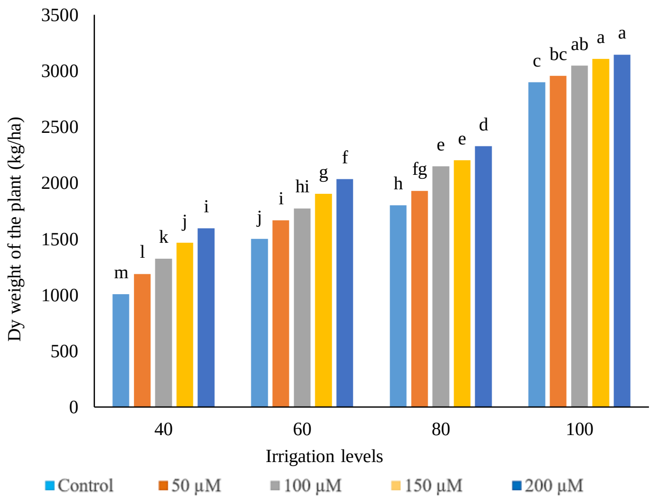


Fig. 2 Mean comparisons of the interaction effect of irrigation × salicylic acid on dry weight of the plant

Essential Oil Percentage

The essential oil percentage was significantly affected ($P < 0.01$) by the independent effects of irrigation and salicylic acid foliar application, as well as by the interaction between these two factors (Table 3). The results showed that drought stress increased the essential oil percentage in basil (Fig. 3). The highest essential oil percentage across all salicylic acid concentrations was observed under the 40% water requirement treatment, followed by the 60% water requirement treatment, consistent with the findings of [30]. Besides, the results suggested that the impact of salicylic acid on increasing the essential oil percentage was lower under full water availability (100% water requirement) compared to drought-stress conditions (Fig. 3). [13] reported that the essential oil percentage in basil increased with higher salicylic acid application under drought-stress conditions. [31] reported that the essential oil percentage in mint was directly related to soil moisture, and to achieve optimal yield and quality, soil moisture should be maintained at 80% during the growing season, with regular irrigation from three to four weeks after emergence until before flowering.

Table 3 Summary of combined F significance from analysis of variance

S.O.V.	Df	MS						
		Dry weight	Essential oil percentage	Essential oil yield	Relative water content	Leaf chlorophyll index	Total protein	Soluble sugar
Y	1	12.3301 n.s	0.038 n.s	2284.8 n.s	35.66 n.s	50.28 ns	2.58 ns	161.32 ns
I	1	43615.37 **	0.038 **	120.414 *	17458.16 **	861.53 **	24.39 **	345.74 **
Y × I	1	22.572 n.s	0.00003 n.s	11.933 n.s	79.14 **	21.32 n.s	1.86 ns	7.31 ns
F	2	944.67 **	0.0037 **	511.16 **	285.14 **	327.28 **	11.38 **	187.56 **
Y × F	2	3.56 n.s	0.0001 n.s	1.001 n.s	5.11 n.s	13.57 n.s	0.87 ns	11.37 ns
I × F	2	88.35 **	0.0001 **	15.71 **	5.40 **	29.82 **	6.35 **	67.83**
Y× I × F	2	10.4 n.s	0.000002 n.s	0.993 n.s	0.72 n.s	3.18 ns	0.74 ns	5.48 ns
CV (%)		2.89	1.80	8.72	4.81	17.67	4.53	4.58

ns: non-significant, * and **: Significant at 5% and 1% probability levels, respectively
Y: year, I: Irrigation, F: Foliar application

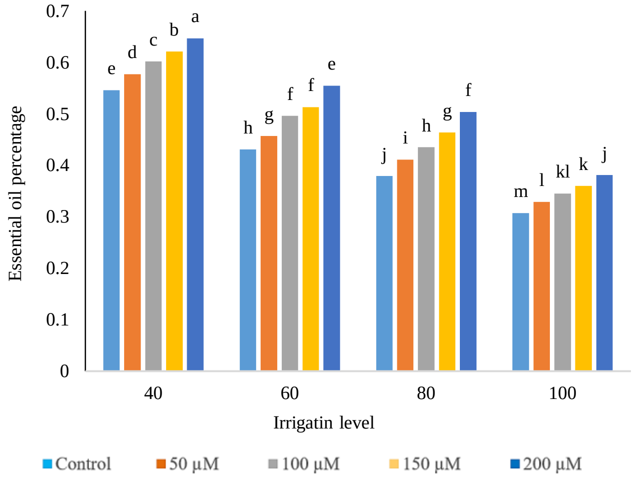


Fig. 3 Mean comparisons of the interaction effect of irrigation × salicylic acid on essential oil percentage

Essential Oil Yield

Essential oil yield was calculated by multiplying the dry weight of the plant (per unit area) by the essential oil percentage. The variance analysis results (Table 3) indicated that essential oil yield was significantly influenced by different irrigation levels, salicylic acid foliar application, and the interaction between these two factors (P<0.01). The comparison of the mean values for different irrigation levels (Fig. 4) showed that increasing salicylic acid concentration under various water regimes led to an increase in the essential oil percentage.

In this study, the highest essential oil yield (11.982 kg/ha) was achieved with 200 µM salicylic acid under the 100% water requirement treatment. The highest essential oil yields under 40% water requirement (severe drought stress), 60% water requirement (moderate drought stress), and 80% water requirement (mild drought stress) with 200 µM salicylic acid were 11.730 kg/ha, 11.287 kg/ha, and 10.329 kg/ha, respectively. Given that essential oil yield is the product of plant dry weight and essential oil percentage, the results of this study showed that the higher essential oil yield under 100% water requirement conditions, compared to other moisture conditions (drought stress), was primarily due to the increase in plant dry weight. The essential oil percentage had a smaller role in enhancing the yield. Most plants under drought stress exhibited limited growth, and due to smaller leaf area and reduced biomass, the aerial part's weight decreased. The reduction in essential oil yield because of lower soil moisture and increased irrigation intervals may be due to the detrimental effects of stress on the plant's vegetative growth and yield [31]. [13] reported that increasing drought stress significantly reduced basil's essential oil yield. However, in this study, increasing salicylic acid concentration led to an increase in basil's essential oil yield.

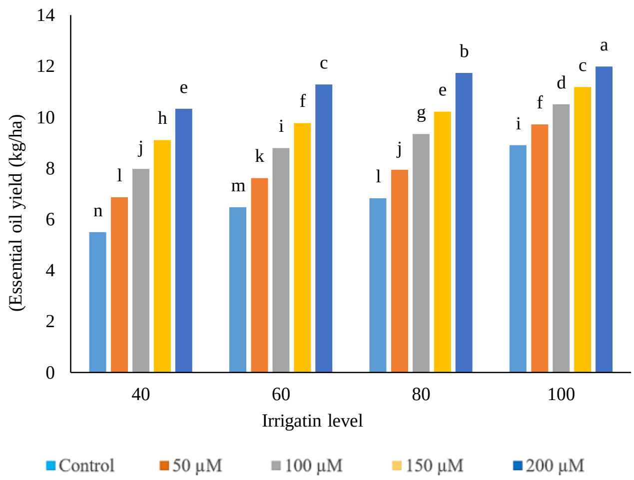


Fig. 4 Mean comparisons of the interaction effect of irrigation × salicylic acid on essential oil yield

Relative Water Content

The mean comparison of the interaction effect between different irrigation levels and salicylic acid (Fig. 5) demonstrated that with an increase in available moisture from 40% of the water requirement to 60%, 80%, and 100%, the relative water content of basil leaves increased. Moreover, relative water content increased with the rise in salicylic acid concentration from zero to 50, 100, 150, and 200 micromolar. Under limited available moisture (increased drought stress), salicylic acid exhibited more positive effects on the relative water content of the plant. Due to an increase in salicylic acid concentration from zero to 50, 100, 150, and 200 micromolar, the highest percentage increase in relative water content belonged to the 40% water requirement treatment, amounting to 6.52%, 8.16%, 10.75%, and 13.36%, respectively (Fig. 5). This finding exhibits the greater positive effects of salicylic acid under 40% water requirement (severe drought stress) compared to full irrigation supply (100% water requirement). The increase in leaf relative water content by salicylic acid can be attributed to its role in enhancing the antioxidant defense system, reducing stress, improving membrane stability and cohesion, and regulating osmotic balance by increasing potassium content, a crucial ion in maintaining cellular turgor [32, 33]. Furthermore, the improvement in plant water status may result from enhanced water absorption due to salicylic acid treatment or greater control over water loss [19].

Leaf relative water content stands for one of the key indicators for determining leaf water levels, being superior to cell water potential in this regard [34]. [35] reported that with increasing stress levels, the relative water content in basil leaves decreases. A reduction in relative water content and stomatal closure is the first impact of drought stress, disrupting the production of photosynthetic compounds and leading to a decline in seed yield [36].

Chlorophyll Index (SPAD Value)

The mean comparison of the interaction effect between different irrigation levels and salicylic acid (Fig. 6) demonstrated that with an increase in available moisture from 40% of the water requirement to 60%, 80%, and 100%, the chlorophyll index of basil leaves increased. Moreover, the chlorophyll index increased with the rise in salicylic acid concentration from zero to 50, 100, 150, and 200 micromolar. Under limited available moisture (increased drought stress), salicylic acid exhibited more positive effects on the chlorophyll index of the plant. Conversely, the lowest percentage increase in the chlorophyll index with the rise in salicylic acid concentration from zero to 50, 100, 150, and 200 micromolar was observed in the 100% water requirement treatment (Full irrigation supply), amounting to 2.38%, 4.06%, 5.14%, and 6.26%, respectively (Fig. 6). Drought stress has been shown to disrupt the lamellar and blade structure of chloroplasts, leading to chlorophyll degradation, resulting in a reduction in chlorophyll concentration and yellowing of the leaves [37]. Studies have revealed that salicylic acid prevents chlorophyll degradation, protects the photosynthetic apparatus, and increases the chlorophyll index in barley crops [38]. Furthermore, the role of salicylic acid in increasing the content of photosynthetic pigments under environmental stress conditions has been reported in some crop plants, such as wheat and barley [39].

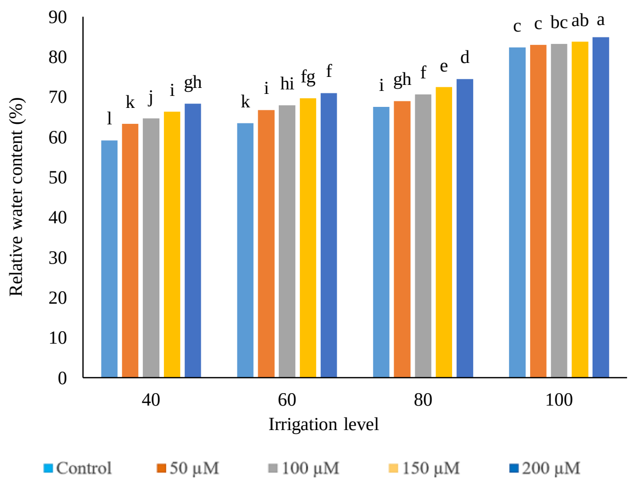


Fig. 5 Mean comparisons of the interaction effect of irrigation × salicylic acid on relative water content

Total Protein

The mean comparison of the interaction between different irrigation levels and salicylic acid (Fig. 7) demonstrated that with an increase in available moisture from 40% of the water requirement to 60%, 80%, and 100%, total leaf protein decreased. In contrast, total protein in leaves increased with the rise in salicylic acid concentration from zero to 50, 100, 150, and 200 micromolar. Under limited available moisture (increased drought stress), salicylic acid enhanced total leaf protein content more significantly than in well-watered conditions.

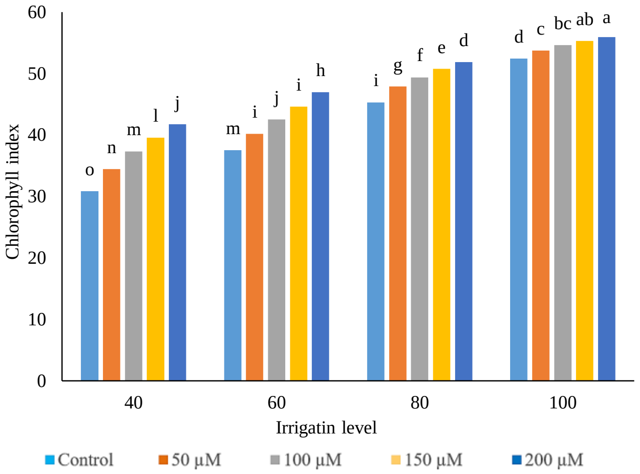


Fig. 6 Mean comparisons of the interaction effect of irrigation × salicylic acid on chlorophyll index

The highest percentage increase in total soluble protein with the rise in salicylic acid concentration from zero to 50, 100, 150, and 200 micromolar was recorded at 5.41%, 23.85%, 28.91%, and 32.10%, respectively, in the 40% water requirement treatment (Fig. 7). Recent findings indicate that total protein content (as osmolytes) increased in response to drought stress in peanut leaves. They also stated that soluble proteins play a key role in peanut plants' response to water stress, enhancing plant resistance to drought stress and improving peanut yield depending on the severity of the stress [40]. Another study found that variations in soluble protein content were highly dependent on the degree of drought resistance [41]. [42] reported a significant relationship between osmotic regulatory compounds and antioxidant enzyme activity under environmental stress conditions in cotton. The findings demonstrate that by regulating soluble protein content and the activity of SOD, CAT, and POD enzymes, osmotic balance was maintained, thereby reducing oxidative damage.

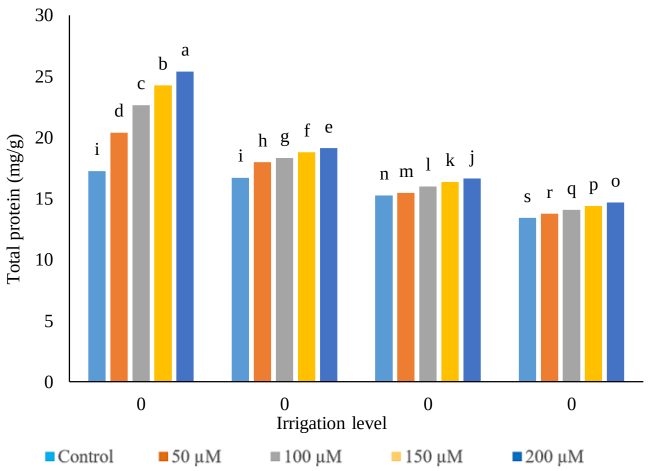


Fig. 7 Mean comparisons of the interaction effect of irrigation × salicylic acid on total protein

Total Soluble Sugar

The mean comparison of the interaction effect between different irrigation levels and salicylic acid (Fig. 8) exhibited that with an increase in available moisture from 40% of the water requirement to 60%, 80%, and 100%, total leaf sugar content decreased. Conversely, total leaf protein content increased with an increase in salicylic acid concentration from zero to 50, 100, 150, and 200 micromolar. Under limited available moisture (increased drought stress), salicylic acid enhanced total leaf sugar content more significantly than in well-watered conditions (Fig. 8). Foliar application of salicylic acid increased total soluble sugar content under all studied moisture regimes; however, this increase was lower in treatments with higher water supply (Fig. 8), suggesting that the application of salicylic acid enhances plant resistance to drought stress through improving biochemical characteristics (increasing total soluble sugar as compatible osmolytes). Plants mitigate drought-stress-induced damage through the accumulation of soluble sugars [43]. The role of soluble sugars in water retention within plant tissues and their osmoprotective function has been previously reported [44]. Moreover, according to previous studies [40, 45, 46], the increase in soluble sugar content due to foliar application of salicylic acid has been documented, aligning with the present research findings.

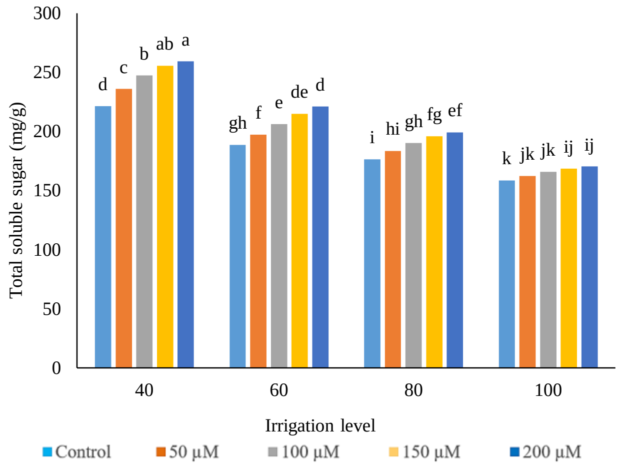


Fig. 8 Mean comparisons of the interaction effect of irrigation × salicylic acid on total soluble sugar

CONCLUSION

Given the water scarcity, the chlorophyll index, relative water content, and consequently, dry weight and essential oil yield decreased, while greater water availability improved these parameters. Under drought-stress conditions, the total sugar and protein levels increase to enhance the plant's tolerance to stress. Besides, salicylic acid improved essential oil yield by enhancing biochemical characteristics (increasing total sugar and protein as compatible osmolytes) and physiological traits (increasing the chlorophyll index and relative water content), ultimately, dry weight under all studied moisture conditions increased. These findings suggest the positive effects of foliar application of salicylic acid, particularly at higher concentrations (150 & 200 micromolar), under different moisture regimes, especially drought stress, in basil plants. Hence, application of salicylic acid may be recommended as an effective management strategy for basil production under drought-stress conditions.

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