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Research Article

Significance of selenium in ameliorating the effects of irrigation deficit via improving photosynthesis efficiency, cell integrity, osmo-protectants, and oil profile of anise crop

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Abstract

Anise is one of the plants with therapeutic potential, which is classified among the most important medicinal plants with interesting biological effects. Its components could be perceived so as "natural" and "safe" alternatives to antibiotics as well as they are applied in different industries such as food and cosmetic purposes. Selenium (Se) is an essential micronutrient, however, its importance to improve oil yield and quality of anise has not been adequately investigated, specifically under drought. Therefore, two successive seasons were conducted to investigate the effect of selenium foliar application upon anise plants under drought stress. Selenium was applied at three different concentrations (0.0 1.0 and 2.0 mM denoted Se_0, Se_1 and Se_2, respectively) along with two levels of crop evapotranspiration (ET): Full irrigation, 100% of ET (FI) and 60% of ET (DI). The promotive effect of combinations of DI × Se_1 or Se_2 (for Fv/Fm, RWC%, and MSI% in the first season) and DI × Se_2 (for Fv/Fm, and MSI% in the second season) were as similar as FI × Se_2. Compared to the counterpart control treatment (DI × Se_0), the highest increases in total free amino acids (31.5 and 31.6%), total soluble sugars (84.2 and 86.4%) and free proline content (84.2 and 86.4%) were recorded with application of DI × Se_2 practice in both seasons, respectively. Under DI, Se_2 recorded the maximum values of

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root length, shoot fresh weight and shoot dry weight in the second season. Under drought, the increases in seed yield due to application of Se_1 and Se_2 amounted to 1.72 and 1.62 folds in the 1st season and 1.50 and 1.43 folds in 2nd one, respectively. The most effective practice for improving IWUE was $Se_1 \times$ DI in both seasons, followed by $Se_2 \times$ DI. Based on the chromatographical detection, the maximum values of Anethole were recorded with FI × Se_2 while L-Linalool has greatly increased with DI × Se_2. In conclusion, the growers in arid and semi-arid zones are advised to use selenium (2.0 mM) in anise fertilization to mitigate the adverse impacts of drought, and keeping crop yield and quality.

Keywords: anise oil profile; cell moisture content; drought tolerance; GC mass; photosynthetic efficiency

Introduction

Anise (*Pimpinella anisum,* Linn.) is as an important medicinal and aromatic plant that contains about 1.5-5.0% volatile oil (Ullah and Honermeier, 2020). This oil consists of trans-anethole, as a main component and estragol, cis-anethole, anisaldehyde, and γ-himachalene as a secondary component (Omidbaigi *et al.*, 2003; Tabanca *et al.*, 2006). Hence anise may be used as an anti-vomiting, analgesic, kidney reinforcement, diuretic, anti-phlogistic, odontalgic, antiseptic, anti-spasmodic and antibacterial (Afify *et al.*, 2011; Sergio *et al.*, 2013), tonicardiac and stomachic (Hossain *et al.*, 2014), antifungal (Kosalec *et al.*, 2005) and anticancer activity (El-Sayed *et al.*, 2015). As well, it has antimicrobial, insecticidal, and antioxidative effects (Özcan and Chalchat, 2006; Afify *et al.*, 2012) and carminative and mild expectorant characteristics of its essential oil were highlighted (El-Beltagi *et al.*, 2020; Aly *et al.*, 2023a). Furthermore, the role of anise oil in increasing the weight and the rate of conversion (FCR) of broiler hens was showed (El-Beltagi *et al.*, 2023). Moreover, the aqueous extract of anise has a beneficial effect against neurological turbulence caused by lead intoxication (Ciftci *et al.*, 2005). Therefore, the medicinal crops trade could serve as a promising source of people income.

Hence, the quality of anise oil is determined by its contents and composition. Nevertheless, yield and quality of such composition can be influenced by many factors like temperature, rainfall, agronomic treatments, soil texture and nutrients (Zheljazkov *et al.*, 2008). Nowadays, agriculture aims to produce crops with high levels of vitamins and nutrients for resistance to oxidization process in plants, animals and humans (Dwivedi *et al.,* 2023). Nutrient supply had the beneficial action to address soil nutrients deficiency while improving crop yield and quality (Oyetunji *et al.*, 2022). So, selenium-rich products have been given particular attention for its importance in the alimentary chain (Stefîrță *et al.*, 2017). Selenium (Se) is an important microelement, which exists in small quantities in humans, animals, microorganisms, and plants.

Selenium has an essential effect on the metabolism of animals and humans in trace quantities (Terry *et al.*, 2000; Farag *et al.*, 2023), resistance to specific cancers, protection of atherosclerosis, change in immunological functions as well as arthritis (Germ and Stibilj, 2007), and the reduction of prostate cancer when the human diet supplied with yeast containing SeMet (Duffield-Lillico *et al.*, 2003). Moreover, Se supported the function of vitamin E and electron transport in muscles.

As well, Se is an important nutrient for plant growth (Pilon-Smits and LeDuc, 2009). This may be through its role in increasing plant antioxidant levels (Hartikainen, 2005; Semidaet *et al.*, 2021). Since Egyptian lands suffering Se deficiency (Yin *et al.*, 2012), it must be provided to plants by fertilizer application through i.e. seed soaking, seed coating as well as soil, and foliar treatments (Shedeed *et al.,* 2018). Previous studies reported that Se foliar treatment was significant to improve drought tolerance and to avoid Se translocation from root to shoot (Nawaz *et al.*, 2014; Winkel *et al.*, 2015; Semida *et al.*, 2023). Moreover, the foliar application could give a fast chance of increasing Se level in the edible part of plants, as well as avoiding toxicity to nearby ecosystems by long-term use of soil application (Keskinen *et al.*, 2011).

Doubtless, growing crops in stressed environments led to depression in plant growth and development, hence low economic outputs (Langstroff *et al.*, 2022). Drought has a severe effect on the productivity of different crops (Aly and Latif, 2011; Abdou *et al.*, 2021; Rady *et al.*, 2021a; Abd El-Mageed *et al.*, 2022). The most limiting factor for food production and agriculture in the world is the degree of irrigation water availability particularly in dry countries, such as Egypt (Christoforidou *et al.*, 2023). In these areas, deficit irrigation (DI) threatens crop productivity. Deficit irrigation causes biochemical, physiological, and metabolic modifications in most plants which make a negative effect on growth characteristics, yield components, water relations and status in plants membrane stability and integrity, pigments content and activity of photosynthesis (Saudy *et al.*, 2023). However, DI stress stimulates water use efficiency (iWUE) (Rady *et al.*, 2021b; Abou-Sreea *et al.*, 2022; Abd El Mageed *et al.*, 2023), osmotic regulation adaptation as well as antioxidant accumulation (Mohamed *et al.*, 2009; Praba *et al.*, 2009; Meena *et al.*, 2016).

Crop productivity was decreased under DI stress due to decreasing all leaf pigments and their activities then decreasing net assimilation rate (Ekmekci *et al.*, 2005). Furthermore, nutritional imbalance occurs as a result of water deficiency (Abd–Elrahman *et al.*, 2022). Thus, utilizing more effective irrigation approaches is a very important and a big challenge against DI circumstances to increasing crop productivity (El-Shirbeny *et al.*, 2014; Abd El-Mageed *et al.*, 2021; Ibrahim *et al.*, 2022; Ramadan *et al.*, 2023).

Rare knowledge is available about the response of anise as a medical plant to exogenous supply of selenium, especially under drought. This work hypothesized that a specific rate of Se could cause distinctive changes in anise physiology under drought in favor of crop yield and oil profile. Therefore, this research amid to study the effectiveness of three selenium rates as a cofactor for ameliorating the detrimental impacts of deficit water on morphological, cell integrity, osmoprotectants, aniseeds yield and chromatogram profile of the oil.

Materials and Methods

Site of experiments

The experiments were done in 2017/2018 and 2018/2019 seasons, at the Faculty of Agriculture Experimental Farm, Fayoum University, (29°19′33′N, 30°51′43′E), Egypt. According to Klute and Dirksen (1986) and Page *et al.* (1982), the chemical and physical analysis of the experimental soil was assessed (Table 1).

Table 1. Physico-chemical characteristics and water status of the experimental soil

Depth (cm)	Particle types %			Texture	B_d (g cm^{-3})	Water status %			ECe (dS m^{-1})	pH	OM%	CaCO$_3$%
	Sand	Silt	Clay			FC	WP	AW				
0-25	10.0	20.0	70.0	Clay	1.40	34.33	19.73	14.60	2.41	7.55	1.31	3.28
25-50	7.0	21.0	72.0	Clay	1.36	32.19	19.13	13.06	1.89	7.66	0.96	3.66

B_d: bulk density, FC: field capacity, WP: wilting point, AW: available water, ECe: electrical conductivity, pH: acidity, OM: organic matter, CaCO$_3$: calcium carbonate.

Irrigation requirements

Using the surface irrigation method, irrigation was done every 20 days interval by different treatments of irrigation. IR treatments were calculated as a percentage of the evapotranspiration (ETc) i.e. full irrigation, I_{100} (FI= = 100% of ETc) and deficit irrigation (DI = 60% of ETc). ETc) was calculated using equation 1 according to Allen *et al.* (1998).

$$\mathrm{ETc} = \mathrm{Kpan} \times \mathrm{Epan} \times \mathrm{Kc} \qquad (1)$$

Where: ETc is the evapotranspiration (mm d^{-1}), Kpan is the coefficient of pan evaporation, Epan is the pan class A evaporation (mm d^{-1}) and Kc is crop coefficient. Irrigation requirements were calculated by the equation 2.

$$\mathrm{IR} = \frac{\mathrm{ETc} \times \mathrm{A} \times \mathrm{Kr} \times \mathrm{Ii}}{\mathrm{Ea} \times 1000} \qquad (2)$$

Where IR is irrigation requirements (m^3), ET_c is the evapotranspiration (mm d^{-1}), A is the area (m^2), Kr is the covering factor, Li is the irrigation intervals (day) and Ea is the application efficiency (%), The quantity of irrigation water was controlled and moved to cover the plot by using one plastic pipe (spile) of 50 mm diameter for each plot. The quantity of conveyed water in each pipe was estimated by equation 3 according to Israelsen and Hansen (1962).

$$\mathrm{Q} = \mathrm{CA}\sqrt{2\mathrm{gh}} \times 10^{-3} \qquad (3)$$

Where: Q is the irrigation water discharge (l sec^{-1}), C is discharging coefficient, A is irrigation pipe cross section area (cm^2), g is acceleration of gravity (cm sec^{-2}) and h is the effective head of water average (cm).

Experimental design and treatments

A split-plot design with three replications was the experimental layout. The studied treatments were two irrigation requirements (IR) and three selenium (Se) levels. Irrigation requirements treatments (FI and DI) were presented on the main plots. While Se foliar treatments (Se_0 = 0 mM Se, used as control, Se_1 = 1 mM Se, and Se_2 = 2 mM Se), applied at 40, 60 days after planting, were allocated in the sub-plots. Se (Na_2SeO_4, Sigma-Aldrich, St. Louis, MO, USA) as a wetting factor, Triton B was added to the spray solution in a few drops. Using a hand pressure sprayer, foliar spray was applied at 8-10 am. Distilled water was used to spray control plants. Each experimental plot area was 10.8 m^2 (3 m × 3.6) and about 0.25 m between plants within rows (60 cm apart) including 6 rows each row 3 m in length.

Fields were prepared before sowing with incorporating 75 kg P_2O_5 ha^{-1} (calcium superphosphate, 15% P_2O_5) and compost (25 m^3 ha^{-1}). Sowing was done into 1-2 cm to have 20 kg of seed ha^{-1}. Seeds of anise were brought from the Department of Medicinal and Aromatic Plants, Ministry of Agriculture, Giza, Egypt. Sowing was done in the 1st week of October in the two seasons. After 45 days from sowing, the plants were thinned to two plants per hill. 200 kg N ha^{-1} (urea 46.5% N) and 96 kg K_2O ha^{-1} as potassium sulfate, 48% K_2O were added in three equal doses (45, 75, and 105 days after sowing.

Measured data

Morpho-physiological traits

After 150 days from planting, nine plants from each treatment were chosen to estimate the morphological characters as; plant height, stem diameter, root length, and shoot fresh and dry weight were measured at the beginning of flowering stage, during February of both seasons (Aly *et al.,* 2023b).

Chlorophyll fluorescence

Chlorophyll fluorescence was determined using PEA fluorometer (Hansatech portable Instruments Ltd, Kings Lynn, UK). Measurements of maximum *Fv/Fm* and PI quantities were performed using (Clark *et al*., 2000; Maxwell and Johnson, 2000) methods. Leaf chlorophyll content (SPAD-value) was determined using SPAD502, KONICAMINOLTA. Inc., Tokyo.

Leaf relative water content and membrane stability index

Relative water content (RWC %) of anise leaf was determined according to Hayat *et al.* (2007) by equation 4.

$$\mathrm{RWC}(\%) = \left[\frac{(\text{fresh weight} - \text{dry weight})}{(\text{turgid weight} - \text{dry weight})}\right] \times 100 \dots .(4)$$

Moreover, membrane stability index (MSI %) was assessed by method of Premachandra *et al.* (1990) using the equation 5.

$$\text{MSI (\%)} = \left[1 - \left(\frac{\text{C1}}{\text{C2}}\right)\right] \times 100 \qquad (5)$$

Where: C1 is the solution EC at 40 °C and C2 is the solution EC at 100 °C.

Osmo-protectants

Free proline (mg per g of leaf dry weight) was extracted by sulfosalicylic acid according to Bates *et al.* (1973). Total soluble sugars (TSS) (mg per g of leaf dry weight) were measured as described by Irigoyen *et al.* (1992). Total free amino acids (mg per g of leaf dry weight) were detected according to Dubey and Rani (1989).

Yield characters and irrigation water use efficiency

At harvest (195 days from sowing), the yield characters (umbels number plant^{-1} and fruit yield plant^{-1} were estimated from plants of the central ridge of each experimental unit. Furthermore, irrigation water use efficiency (WUE) was estimated according to Jensen (1980) by equation 6.

$$\text{IWUE} = \frac{\text{Seed yield (kg ha}^{-1}\text{)}}{\text{Applied water (m}^{3}\text{ ha}^{-1}\text{)}} \qquad (6)$$

Essential oils distillation

Essential oil was determined gravimetrically by hydro-distillation according to the European Pharmacopoeia (EP) (C.O.E., 1997). Fruit sample from each plot was hydro-distilled for 2 h using a Clevenger-type apparatus. The concentration of oil was estimated as the quantity of oil (g) per dry fruits weight (g), then, oil yield per area was calculated using equation 7.

$$\text{Oil yield} = \frac{\text{Fruit yield} \times \text{oil content \%}}{100} \qquad (7)$$

The essential oil content (v/w%) per seeds was determined by extract 100 g of crashed anise samples in 0.5 L of water. The oil was dried using anhydrous sodium sulphate Na_2SO_4 and kept at 4 °C in dark glass containers for further lab analyses. 1.0 ml (density = 1.04 g/ml) (Cheronis, 1963)

Gas chromatography-mass spectrometry analysis (GC-MS)

The GC-MS system (Agilent Technologies) was done at Central Laboratories Network, National Research Centre, Cairo, Egypt.\, using gas chromatograph (7890B) and mass spectrometer detector (5977A). Samples were diluted with hexane (1:19, v/v). The GC was equipped with HP-5MS column (30 m × 0.25 mm internal diameter and 0.25 μm film thickness). Analyses were conducted by helium gas as the carrier with a flow rate of 1.0 ml/min at a split 1:10 of injection volume of 1 µl and the temperature program was 40 °C for 1 min then the temperature was increased from 4 °C /min to 150 °C and then it was decreased to 6 min and then it was increased from 4 °C/min to 210 °C and then it decreased to 1 min. The injector and detector were fixed at 280 °C and 220 °C, respectively. Mass spectra was received by electron ionization (EI) at 70 eV, using a spectral range of m/z 50-550 and solvent delay of 3 min. Different components were estimated by matching the spectrum fragmentation appearance with those in Wiley and NIST Mass Spectral Library data.

Statistical analysis

The analysis of variance for data of each of the two growing seasons was carried out according to Steel (1997) using Gen STAT computer software package. The LSD test at the 1.0 and 5.0% level of significance was used in means comparison.

Results

Anise response attributes as affected by irrigation and selenium can be explained under the following headings:

Growth characters

The absence of selenium with either with deficit or normal irrigation was inferior values of anise growth characters i.e., plant height, stem diameter, root length, shoot fresh and dry weight in both seasons were observed (Table 2). While, obvious beneficial influences of Se on anise growth were achieved whether with FI or DI. Under FI, plant height (with Se_1), root length (with Se_1 or Se_2) as well as shoot fresh and dry weight (with Se_2) gave the maximum values in both seasons. On the other site, plant height in the second season and stem diameter in both seasons possessed the highest values under drought stress (DI). Also, under DI, Se_2 recorded the maximum values of root length, shoot fresh weight and shoot dry weight in the second season.

Table 2. Effect of Se foliar spray on plant height, stem diameter, root length, shoot fresh weight and shoot dry weight of anise plants grown under full and deficit irrigation in 2017/18 (SI) and 2018/19 (SII) seasons

Variable		Plant height (cm)		Stem diameter (cm)		Root length (cm)		Shoot fresh weight (g)		Shoot dry weight (g)	
WR	Se (mM)	SI	SII	SI	SII	SI	SII	SI	SII	SI	SII
FI	Se_0	86.0^{c}	87.0^{b}	1.4^{a}	1.3^{a}	11.7^{d}	11.3^{d}	56.7^{d}	53.3^{c}	28.0^{c}	24.0^{c}
	Se_1	100.0^{a}	92.0^{a}	1.5^{a}	1.5^{a}	16.7^{ab}	17.0^{ab}	73.3^{b}	76.7^{b}	34.5^{b}	37.7^{b}
	Se_2	91.0^{c}	92.0^{a}	1.5^{a}	1.4^{a}	18.0^{a}	17.3^{a}	100.0^{a}	96.7^{a}	52.0^{a}	46.0^{a}
DI	Se_0	69.7^{f}	71.7^{c}	1.0^{b}	0.97^{b}	9.5^{e}	9.8^{d}	31.7^{e}	28.3^{d}	16.17^{d}	13.0^{d}
	Se_1	90.0^{d}	90.0^{a}	1.4^{a}	1.4^{a}	14.3^{c}	14.0^{bc}	65.0^{cd}	78.3^{b}	35.7^{b}	39.0^{b}
	Se_2	93.0^{b}	90.0^{a}	1.5^{a}	1.3^{a}	16.0^{b}	16.3^{ab}	80.0^{b}	88.3^{a}	40.0^{b}	44.0^{a}
ANOVA (df)		% sum of the squares									
WR (1)		304.2^{**}	150.0^{**}	1.49^{**}	1.5^{*}	21.1^{**}	15.0^{*}	1422.2^{**}	501.4^{**}	275.3^{**}	68.1^{**}
Se (2)		1016.3^{**}	602.3^{**}	3.3^{**}	2.7^{*}	135.2^{**}	130.0^{**}	6319.4^{**}	8477.7^{**}	1661.9^{**}	2280.1^{**}
WR×Se (2)		250.1^{**}	193.4^{**}	1.16^{*}	0.63^{*}	0.1^{*}	3.3^{*}	219.4^{*}	544.4^{*}	182.1^{**}	122.1^{**}
Residual (10)		5.00	21.07	0.49	2.1	7.9	26.0	213.9	213.9	93.6	20.6

FI: full irrigation (100% of water requirements, WR), DI: deficit irrigation (60% of WR), Se_0, Se_1 and Se_2: spraying of selenium by 0, 1.0 and 2.0 mM, respectively. Mean pairs followed by different letters are significantly different, ** and * indicate differences at $p \leq 0.05$ and $p \leq 0.01$ probability level, respectively. Differences between means were compared by the Duncan's multiple range test (LSD, $P \leq 0.05$).

Fv/Fm, PI, SPAD, MSI% and RWC%

The combination between irrigation and selenium had significant influences on all traits of chlorophyll florescence and cell water status of anise plants in 2017/18 and 2018/19 seasons (Table 3). Spraying the well-watered anise plants (FI) with Se at a rate of 2 mM (Se_2) showed the maximal increases in Fv/Fm, PI, SPAD, RWC% and MSI% in both seasons. However, such effective practice statistically at par with the combinations of DI × Se_1 or Se_2 (for Fv/Fm, RWC% and MSI% in the first season) and DI × Se_2 (for Fv/Fm and MSI% in the second season). Furthermore, as an average of the two seasons, the increases in Fv/Fm, PI, SPAD, RWC%

and MSI% owing to Se$_2$ reached 7.8, 161.6, 29.3, 11.2 and 14.1%, respectively, compared to the counterpart control treatment (DI × Se$_0$).

Table 3. Effect of selenium (Se) foliar spray on chlorophyll florescence (Fv/Fm and PI), leaf greenness (SPAD), leaf relative water content (RWC%) and membrane stability index (MSI%) of anise plants grown under full and deficit irrigation in 2017/18 (SI) and 2018/19 (SII) seasons

Variable		Fv/Fm		PI		SPAD		RWC%		MSI%	
WR	Se (mM)	SI	SII	SI	SII	SI	SII	SI	SII	SI	SII
FI	Se$_0$	0.83^{a}	0.81^{b}	5.9^{bc}	5.2^{c}	38.7^{bc}	37.2^{bc}	77.5^{b}	77.3^{c}	80.3^{a}	81.3^{a}
	Se$_1$	0.83^{a}	0.83^{a}	6.6^{b}	6.0^{bc}	40.8^{b}	46.8^{a}	82.3^{a}	80.8^{b}	80.5^{a}	81.6^{a}
	Se$_2$	0.84^{a}	0.83^{a}	8.7^{a}	8.8^{a}	46.0^{a}	46.3^{a}	82.4^{a}	84.9^{a}	80.9^{a}	81.9^{a}
DI	Se$_0$	0.77^{b}	0.77^{c}	2.6^{d}	2.6^{e}	29.5^{e}	29.6^{d}	70.6^{c}	71.4^{d}	69.2^{b}	69.8^{c}
	Se$_1$	0.83^{a}	0.81^{b}	5.2^{c}	4.1^{d}	34.5^{d}	35.0^{c}	79.3^{ab}	77.4^{c}	77.7^{a}	77.9^{b}
	Se$_2$	0.83^{a}	0.83^{a}	6.7^{b}	6.9^{b}	37.5^{c}	38.9^{b}	79.0^{ab}	78.9^{bc}	78.5^{a}	80.1^{ab}
ANOVA (df)		% sum of the squares									
WR (1)		0.002^{*}	0.001^{*}	20.2^{*}	20.4^{*}	173.6^{*}	285.0^{*}	87.6^{*}	119.7^{*}	134.5^{*}	145.3^{*}
Se (2)		0.005^{*}	0.007^{*}	32.4^{*}	48.9^{*}	290.4^{*}	359.1^{*}	177.8^{*}	175.8^{*}	43.7^{*}	96.4^{*}
WR×Se (2)		0.004^{*}	0.002^{*}	3.18^{*}	0.42^{*}	6.6^{*}	18.1^{*}	14.1^{*}	7.01^{*}	71.1^{*}	78.6^{*}
Residual (10)		0.0004	0.001	3.30	2.5	27.1	32.8	39.3	23.5	67.3	16.5

FI: full irrigation (100% of water requirements, WR), DI: deficit irrigation (60% of WR), Se$_0$, Se$_1$ and Se$_2$: spraying of selenium by 0, 1.0 and 2.0 mM, respectively. Mean pairs followed by different letters are significantly different. ** and * indicate respectively differences at $p \le 0.05$ and $p \le 0.01$ probability level. Differences between means were compared by the Duncan's multiple range test (LSD, $P \le 0.05$).

Osmo-protectants

Remarkable effects of irrigation and selenium combination on the osmo-protectants of anise plants were obtained in 2017/18 and 2018/19 seasons (Table 4). In this respect, total free amino acids, total soluble sugars and free proline content recorded the highest values with application of DI × Se$_2$ practice in both seasons. The effective combinations along DI × Se$_2$ practice for producing high values were DI × Se$_1$, and FI × Se$_1$ or Se$_2$ (for total free amino acids and free proline content in the first season), DI × Se$_1$ (for total free amino acids in the second season, and total soluble sugars and free proline content in the first season) as well as FI × Se$_1$ (for total soluble sugars in both seasons).

Table 4. Effect of selenium (Se) foliar spray on total free amino acids, total soluble sugars and free proline content of anise plants grown under full and deficit irrigation in 2017/18 (SI) and 2018/19 (SII) seasons

Variable		Total free amino acids mg g^{-1} DW		Total soluble sugars mg g^{-1} DW		Free proline content (µg g^{-1}DW)	
WR	Se (mM)	SI	SII	SI	SII	SI	SII
FI	Se$_0$	0.647^{c}	0.657^{d}	1.89^{bc}	1.88^{c}	0.583^{b}	0.613^{cd}
	Se$_1$	0.870^{ab}	0.847^{b}	2.30^{ab}	2.30^{ab}	0.677^{ab}	0.670^{c}
	Se$_2$	0.867^{ab}	0.863^{b}	2.37^{b}	2.41^{b}	0.683^{ab}	0.697^{c}
DI	Se$_0$	0.730^{bc}	0.737^{c}	1.52^{c}	1.55^{d}	0.543^{b}	0.553^{d}
	Se$_1$	0.950^{a}	0.953^{a}	2.41^{ab}	2.56^{b}	0.810^{ab}	0.823^{b}
	Se$_2$	0.960^{a}	0.970^{a}	2.80^{a}	2.89^{a}	0.980^{a}	0.990^{a}
ANOVA (df)		% sum of the squares					
WR (1)		0.033^{*}	0.043^{**}	1.62^{**}	1.97^{**}	0.023^{*}	0.32^{**}
Se (2)		0.054^{*}	0.045^{**}	1.14^{*}	1.26^{**}	0.033^{*}	0.328^{**}
WR×Se (2)		0.145^{**}	0.136^{**}	0.191^{ns}	0.274^{**}	0.022^{ns}	0.016^{ns}
Residual (10)		0.074	0.018	1.44	1.99	0.265	0.030

FI: full irrigation (100% of water requirements, WR), DI: deficit irrigation (60% of WR), Se$_0$, Se$_1$ and Se$_2$: spraying of selenium by 0, 1.0 and 2.0 mM, respectively. Mean pairs followed by different letters are significantly different, ** and * indicate differences at $p \le 0.05$ and $p \le 0.01$ probability level, respectively, ns indicates not significant difference. Differences between means were compared by the Duncan's multiple range test (LSD, $P \le 0.05$).

Yield attributes, oil content and IWUE

Anise yield attributes and IWUE significantly responded to the combinations of irrigation and selenium in 2017/18 and 2018/19 seasons (Table 5). FI × Se_2 was the potent practice for enhancing umbels number and seed yield in both seasons, significantly equaling FI × Se_1 and DI × Se_2 for seed yield in 2018/19 season. Se_2 × FI or DI and Se_1 × DI (for oil yield and oil% in both season) in addition to Se_1 × FI (for oil % in the second season) exhibited the maximum increases. The most effective practice for improving IWUE was Se_1 × DI in both seasons, followed by Se_2 × DI. Under deficit irrigation water (DI), the increases in seed yield due to application of Se_1 and Se_2 amounted to 1.72 and 1.62 folds in 2017/18 season and 1.50 and 1.43 folds in 2018/19 season, respectively, higher than the counterpart control treatment (Se_0). Furthermore, IWUE of drought- stressed anise plants increased by about 72.1 and 50.0% in 2017/18 season and 62.1 and 43.3% in 2018/19 season due to spraying of Se_1 and Se_2, respectively, compared to Se_0.

Table 5. Effect of Se foliar spray on umbels number, seed yield, oil yield, oil % and irrigation water use efficiency (IWUE) of anise plants grown under full and deficit irrigation in 2017/18 (SI) and 2018/19 (SII) seasons

Variable		Umbels number		Seed yield (kg ha^{-1})		Oil yield (L ha^{-1})		Oil %		IWUE (kg m^{-3})	
WR	Se (mM)	SI	SII	SI	SII	SI	SII	SI	SII	SI	SII
FI	Se_0	27.7^{b}	28.33^{b}	1387^{d}	1418^{c}	14.47^{b}	15.56^{c}	0.110^{b}	0.143^{a}	0.324^{d}	0.354^{d}
	Se_1	32.7^{b}	31.00^{b}	1618^{bc}	1627^{abc}	15.20^{b}	16.87^{bc}	0.113^{b}	0.120^{ab}	0.378^{cd}	0.407^{cd}
	Se_2	50.3^{a}	53.00^{a}	1880^{a}	1853^{a}	19.77^{a}	19.92^{a}	0.150^{a}	0.150^{a}	0.439^{c}	0.463^{c}
DI	Se_0	20.0^{c}	19.00^{c}	987^{e}	1058^{d}	11.92^{c}	12.68^{d}	0.097^{b}	0.103^{b}	0.384^{cd}	0.441^{c}
	Se_1	28.7^{b}	31.33^{b}	1698^{b}	1716^{ab}	18.67^{a}	19.11^{ab}	0.140^{a}	0.110^{b}	0.661^{a}	0.715^{a}
	Se_2	33.7^{b}	32.33^{b}	1480^{cd}	1516^{bc}	20.46^{a}	20.24^{a}	0.153^{a}	0.153^{a}	0.576^{b}	0.632^{b}
ANOVA (df)		% sum of the squares									
WR (1)		624.2**	355.6**	259207**	185382**	1.31^{ns}	0.048^{ns}	0.001^{ns}	0.002^{ns}	0.115**	0.158**
Se (2)		1409.3**	1267.0**	931655**	774954**	144.1**	109.82**	0.007**	0.006**	0.102**	0.098**
WR×Se (2)		139.1*	345.4**	230405**	192023**	27.26*	20.05*	0.001*	0.001^{ns}	0.038**	0.038*
Residual (10)		104.33	110.7	84428	113123	19.49	16.68	0.009	0.002	0.013	0.016*

FI: full irrigation (100% of water requirements, WR), DI: deficit irrigation (60% of WR), Se_0, Se_1 and Se_2: spraying of selenium by 0, 1.0 and 2.0 mM, respectively. Mean pairs followed by different letters are significantly different, ** and * indicate differences at $p \leq 0.05$ and $p \leq 0.01$ probability level, respectively, ns indicates not significant difference. Differences between means were compared by the Duncan's multiple range test (LSD, $P \leq 0.05$).

Chromatographical analysis of oil

Concerning the essential oil components (Table 6 and Figure 1), GC mass detected that the maximum Anethole percentage (94.83%) was produced from FI × Se_2. FI × Se_0 recorded the highest percentage of D-Limonene and Isobutyrophenone as compared with other treatments. However, L-Linalool has greatly increased with DI × Se_2. FI × Se_1 had the highest increment for alpha-Himachalene, gamma-Himachalene, alpha-Longipinene, and Calarene (beta-Gurjunene) by 0.31, 3.75, 0.2, and 0.11, respectively. DI × Se_0 was of high influence in rising up the number of different components such as alpha-Curcumene, sesquiterpene, (R)-beta-bisabolene, Pseudoisoeugenol 2-methylbutanoate, and Thellungianin G over other treatments. As well, DI × Se_1gave the highest record of both delta-Elemene and Isospathulenol.

Table 6. Mass chromatographical analysis of the essential oil components for anise plant as influenced by selenium under full and deficit irrigation

Variable	FI × Se_0		FI × Se_1		FI × Se_2		DI × Se_0		DI × Se_1		DI × Se_2	
	RT	Area sum %	RT	Area sum %	RT	Area sum %	RT	Area sum %	RT	Area sum %	RT	Area sum %
Oil component												
D-Limonene	8.9	0.32	---	---	---	---	---	---	8.9	0.17	---	---
L-Linalool	12.0	0.24	---	---	---	---	---	---	12.0	0.14	12.2	0.34
Isobutyrophenone	15.7	1.81	15.9	0.39	15.9	0.21	15.9	0.34	15.8	1.46	15.9	0.38
Anethole	19.4	92.94	19.5	93.38	19.4	94.83	19.4	93.00	19.4	92.45	19.5	94.76
delta-Element	---	---	---	---	20.6	0.11	---	---	20.6	0.13	20.7	0.10
alpha-Himachalene	24.2	0.24	24.2	0.31	24.2	0.23	24.2	0.16	24.18	0.22	24.2	0.22
(R)-beta-bisabolene	---	---	24.9	0.10	---	---	---	---	---	---	---	---
gamma-Himachalene	25.2	2.78	25.2	3.75	25.2	2.68	25.2	1.97	25.2	2.70	25.2	2.49
alpha-Curcumene	25.4	0.15	25.4	0.27	25.4	0.31	25.4	0.51	25.4	0.17	25.4	0.20
sesquithujene	25.7	0.17	25.8	0.23	25.8	0.26	25.8	0.52	25.7	0.23	25.8	0.23
alpha-Longipinene	25.8	0.14	25.8	0.20	25.8	0.16	25.8	0.15	25.8	0.15	25.9	0.15
(R)-beta-bisabolene	26.1	0.14	26.2	0.21	26.1	0.22	26.2	0.27	26.1	0.18	26.2	0.21
Isospathulenol	---	---	---	---	---	---	---	---	29.8	0.11	---	---
Calarene (beta-Gurjunene)	---	---	26.4	0.11	---	---	---	---	---	---	---	---
Pseudoisoeugenol 2-methylbutanoate	38.9	0.85	38.9	0.81	38.9	0.79	39.0	2.48	38.9	1.46	38.9	0.73
Thellungianin G	40.8	0.21	40.8	0.24	40.8	0.19	40.8	0.59	40.8	0.42	40.8	0.20

FI: full irrigation (100% of water requirements, WR), DI: deficit irrigation (60% of WR), Se_0, Se_1 and Se_2: spraying of selenium by 0, 1.0 and 2.0 mM, respectively, RT retention time.

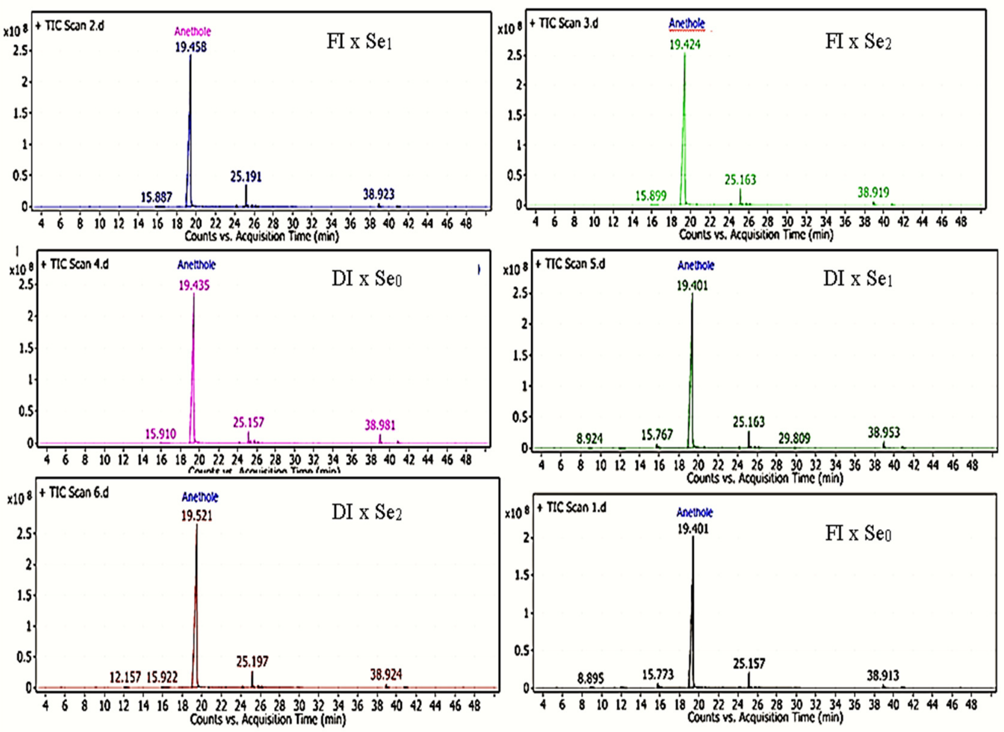

Figure 1. GC mass image illustrates the fractionation of the essential oil components for anise plant as influenced by selenium under full and deficit irrigation. FI: full irrigation (100% of water requirements, WR), DI: deficit irrigation (60% of WR), Se_0, Se_1 and Se_2: spraying of selenium by 0, 1.0 and 2.0 mM, respectively

Discussion

In general, semi-arid and arid areas soils are alkaline, so its nutrients and structure are very weak (Rady *et al.*, 2020). Besides, water deficit could be another problem counteracting such regions (Saudy and El-Metwally, 2023). In this study, the findings showed that deficit irrigation caused reduction at different degrees in anise photosynthetic pigment contents and efficiency, RWC, MSI, growth characters and yield. On the other hand, contents of osmo-protective substances as free amino acids, total soluble sugars, proline content and irrigation water use efficiency (IWUE) were increased under DI. The increases obtained in protective substances and IWUE produced from different mechanisms which plants maintained to improve growth characters and yield under deficit irrigation. Such positive results needed positive modifications in plant pigments, RWC% and MSI% as well as further improvements in protective substances which were already reached by foliar feeding with Se. Although IWUE increased, anise plants were not grown and produced seed better yield under deficit irrigation. Such negative results under deficit irrigation may be due to the reduction in RWC% and MSI% and/or the dehydration of the leaf cells' protoplasm, causing a reduction in plant pigments, hence the reduction in the activity of the photosynthesis process (Awad *et al.*, 2021)

Despite that, in comparison with the control plants, Se foliar treatment enabled DI-stressed anise plants to grow better. Latif and Iqbal (2001) reported that Se foliar spray is effective and applicable approach for fertilizers application and increasing fertilizer use efficiency. Selenium is implicated in antioxidants and the organization of reactive oxygen types, rebuilding cell membrane, structures of chloroplast, and protecting photosynthetic systems. The growth-promoting function of Se in this study was correlated with a significant increase in photosynthetic pigments content and efficiency. The increasing of Se concentrations leads to decrease chloroplast damage and improve chlorophyll content (Chu *et al.*, 2010; Yao *et al.*, 2011; Malik *et al.*, 2012). Senescence can be delayed with Se supply and encourage the growth of old seedlings. The application of Se strengthens the antioxidative level through increasing tocopherol and superoxide dismutase (SOD) levels (Xue *et al.*, 2001). Senescence probably slows down by antioxidation, which correlated with the increasing of glutathione peroxidase activity delay (Hartikainen *et al.*, 2000). Many other studies showed that Se significantly improved the activity of CAT (Yao *et al.*, 2011; Malik *et al.*, 2012). Reductions in the ROS capacity through Se supply to stressed plants have been showed in rape seed seedlings (Wang, 2011; Hasanuzzaman and Fujita, 2011).

Despite its essentiality for plants under debate, many studies have proved Se positive contribution to plants growth under each of stressed and non-stressed environments. Many studies stated Se efficacy for plants resistance against certain abiotic stresses (Yao *et al.*, 2009; Malerba and Cerana, 2018). Previous studies showed that Se could regulate plant and water relationship under drought stress (Hasanuzzaman and Fujita, 2011; Kuznetsov *et al.*, 2003). It has been reported that the increase in plant height under stress illustrates the role of Se in moisture regulation in seedlings under stress (Djanaguiraman *et al.*, 2005) and increased plant hormones which responsible for cell elongation (Hartikainen, 2005). Kahakachchi *et al.* (2004) revealed that the young parts of plant accumulate more quantities of Se which improve osmoregulation process in plants. Yao *et al.* (2009) showed that root system activity affected on plants growth and development. Selenium treatments gave the highest root length with extensive root hairs, indicating the Se role in improving drought tolerance. Increasing Se supply led to increasing in root growth and water uptake under stress. Singh *et al.*, (1980) reported that Se application enhanced growth and yield of Indian mustard (*Brassica juncea* L.). Recently, Hasanuzzaman *et al.* (2011) showed that applied Se at low levels, stimulates growth and antioxidative levels. Selenium foliar application enhanced growth characteristics in green tea (Hu *et al.*, 2011). Furthermore, Se enters an important enzyme composition (Birringer *et al.*, 2002), which enhances the antioxidants (Wang, 2011; Hasanuzzaman and Fujita, 2011), which is produced in plants under stress conditions and disrupt cell membranes, proteins, etc (El-Beltagi and Mohamed, 2010; Gupta and Gupta, 2017). In the same concern, Stefîrţă *et al.* (2016) showed that foliar treatment of garlic plants with potassium selenate increased the ability of plants adaptation via reducing water deficit negative effect and enhanced substances accumulation that increase the ability of water retention in the leaves and improves growth and productivity. Selenium treatment increased the physiological indices which supported the positive function of Se in increasing tolerance to drought in anise plants (Table 2 -5). In different *Brassica* species, exogenous application of Se has increased vegetative growth and reproductive ability, regarding seed yield and seed viability (Lyons *et al.*, 2009; Hajiboland, 2012). Selenium foliar spray was reported to enhance the growth of garlic and onion bulbs (Poldma *et al.*, 2011; Kápolna *et al.*, 2012). Under normal or stressed conditions, Se was effective to achieve the best growth, photosynthetic pigment, quality of oil, endogenous proline content, catalase activity and increased phosphorus and magnesium contents (Hashem *et al.*, 2013). In this study, although free amino acids, total soluble sugars and proline contents were increased under water stress, they were not enough increase anise plants tolerance to drought stress to give high growth and seed yield. Such improvements increased adaptive mechanisms, including keeping proper cellular turgor in the side of physiological functions (Agami *et al.*, 2019; Rady *et al.*, 2020; Awad *et al.*, 2021), which make plants more tolerant to stress and give acceptable growth and seed yield (Tables 2 and 5). For increasing seed yield, the water-stressed anise plants needed stable osmotic adjustment, which was obtained by increasing the osmo-protectants via the treatment of Se.

In aromatic plants, water deficit stresses have proved to stimulate the biosynthesis and accumulation of different secondary metabolites across various organs (Dunja *et al.*, 2021). Terpenoids, the main constituents of essential oils are playing an important role in plant stress tolerance by efficiently scavenging reactive oxygen species (ROS) and protect the cell from drought induced oxidative damages (Yadav, 2021). Selenium positively increased gradually the results of all seed and oil yield parameters as well as the most essential oil components, Anethole maximum percentage (Table 6 and Figure 1) which could go back to Se essentiality for cell membranes and chloroplasts safety (Latif and Iqbal, 2001). The Se foliar treatment in the current study prevented the change in the chemotype of anise essential oil that the anethol content in the WD was not changed compared with the FI, the most recent study demonstrated that the composition of essential oils was altered from t-anethole/estragole in normal irrigation conditions to t-anethole/β-bisabolene under water deficit, changes in EO composition may be attributed to changes in the enzymatic activity in response to water deficit stress (Mehravi *et al*., 2023). The present study could be the first definitive evidence to date of the improvement of anise essential oil quality (Table 6) as a result of Se treatment. However, Se was found to have a positive effect on canola oil quality (Mohtashami *et al*., 2020).

Conclusions

Under the current climatic changes in the whole world and the occurring struggles over the fresh water especially in the African continent, selenium foliar spray could be used as an effective method to improve growth and seed yield as well as physiological and biochemical characters of anise plants under deficit irrigation stress. In this context, selenium at a rate of 2.0 mM had the potential to enhance anise growth and yield via stimulating photosynthetic rate, maintaining cell membrane stability and relative water content, and adjusting the osmo-protectants under deficit irrigation. Therefore, to get an adequate seed yield and quality under deficit irrigation stress, plants must keep higher water content in cells beside to higher antioxidants content and osmo-protectants, the thing that was achieved by foliar feeding with selenium. Hence, a viable recommendation is to use selenium (2.0 mM) for arid and semi-arid areas lacking water. This can be an effective agro-management for anise production, as one of the most important medicinal plants, under drought stress conditions.

Authors' Contributions

Conceptualization: KMAR, HSEB, TAA, KEM, GFM, MTS, FMAE, MHHR, HSS and AIBA; Data curation: KMAR, HSEB, TAA, KEM, GFM, MTS, FMAE, MHHR, HSS and AIBA; Formal analysis: MTS, FMAE, MHHR, HSS and AIBA; Funding acquisition: KMAR and HSE-B; Investigation: HSEB, KMAR and HSS; Methodology: KMAR, HSEB, TAA, KEM, and GFM; Project administration: HSEB, KMAR and HSS; Resources: AAR, IME-M, ESB and HSS; Software AAR, IME-M, ESB and HSS; Supervision: HSEB, KMAR and HSS; Validation:KMAR, HSEB, TAA, KEM, GFM, MTS, FMAE, MHHR, HSS and AIBA; Visualization KMAR, HSEB, TAA, KEM, GFM, MTS, FMAE, MHHR, HSS and AIBA; Writing - original draft: TAA, KEM, GFM, MTS, FMAE, MHHR, HSS and AIBA; Writing - review and editing: KMAR, HSEB, TAA, KEM, GFM, MTS, FMAE, MHHR, HSS and AIBA; All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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