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Response of black cumin to foliar application of some growth substances under limited irrigation conditions

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Abstract

The aim of this experiment was to examine the response of *Nigella sativa* subjected to water stress conditions and the extent of response to exogenous additives that promote the plant's resistance to water stress. The present investigation was conducted as a factorial experiment in the 2020/2021 and 2021/2022 seasons on newly reclaimed land. The main plot designated for the irrigation regime comprised 60%, 80%, and 100% ETc, while the sub-plots included exogenous additives including a control, potassium silicate at 5 ml/l, salicylic acid at 300 ppm, and humic acid at 0.04 %. The highest values for plant growth, seed yield, and fixed oil yield were achieved with the highest irrigation level (100 % ETc), whereas the lowest values were noticed at the lowest irrigation level (60 % ETc) in both seasons. The use of exogenous additives resulted in a significant enhancement of the growth characteristics, seed yield, and irrigation water productivity relative to the control, with salicylic acid exhibiting the most pronounced effect, followed by potassium silicate and humic acid. The combination of 100 % ETc with salicylic acid at 300 ppm or potassium silicate at 5 ml/l produced optimal results. Water stress significantly affected the level of protein, total carbohydrates, and free proline. The highest protein and carbohydrate contents were achieved with 80% and 100 % ETc treatments, however the highest concentration of free proline was recorded at 60% ETc in both seasons. The application of potassium silicate at 5 ml/l resulted in the highest levels of protein and free proline content over both seasons, whereas the highest total carbohydrates were achieved with salicylic acid at 300 ppm during the two seasons.

Keywords: bio-stimulants, drought stress, medicinal plant, irrigation regime, sandy calcareous soil.

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1. Introduction

Black cumin (*Nigella sativa* L.) seeds have a long history of wide therapeutic applications in traditional medicine for such conditions such as asthma, diabetes, inflammation, hypertension, sore throat, bronchitis, headache, fever, eczema, dizziness, and influenza. Additionally, they have been utilized for their carminative, lactagogue, diuretic, and vermifuge properties (Ali and Blunden, 2003; Bayati *et al.*, 2020). Water scarcity, an abiotic stress factor, results in considerable reductions in plant growth, and development, and global crop productivity **(**Hefzy *et al.*, 2020; Li *et al.*, 2015). Obidiegwu *et al.* (2015) and Mostafa *et al.* (2021) assert that climate change includes severe temperature and extended drought globally due to increased evapotranspiration. The irrigation water in Egypt is comparatively scarce and inadequate for irrigation and reclamation (Gewaily, 2019), with the majority of newly reclaimed land consisting of sand or sandy calcareous soils (Abou-Elela, 2002). Consequently, it is imperative to create solutions that improve water use efficiency and augment plant drought resistance. Water stress induces many morphological, physiological, and biochemical changes, including a reduction in leaf area, leaf senescence, and diminished cell growth, stomatal closure, and photosynthetic limitations. Water scarcity and drought are significant characteristics of arid regions. Water is the primary constraint on global agricultural and food production, significantly surpassing other critical restrictions. A substantial quantity of water is utilized in agricultural production of food crops, resulting in a deficit of freshwater resources in several arid or semi-arid regions globally. In areas where water scarcity is the primary limiting factor for cultivation, farmers seek to cultivate drought-resistant crops. Drought stress can damage plant cell membranes and cell wall structure, as well as inhibit photosynthesis and cell division (Hsiao, 1973; Taiz and Zeiger, 2006). Consequently, developing strategies that improve water use efficiency and increase plant drought resistance is highly required. There are several methods to augment plant tolerance in drought stress conditions. The application of bio-stimulants or plant growth regulators may improve the drought resistance of cultivated crops. Humic substances play crucial roles in plant growth, the management of carbon and nitrogen cycles, and integrity of soil structure (El-Naqma, 2020; Ozfidan-Konakci *et al.*, 2018). Moreover, numerous studies have indicated that the application of potassium humate can enhance plant growth and crop productivity across different irrigation regimes in sandy calcareous soil, including black cumin (Abou El-Leel *et al.*, 2019; Ariafar and Forouzandeh, 2017; Aiyafar *et al*., 2015), oregano (Said-Al Ahl *et al*., 2009) and garlic (Badawy *et al.*, 2019). Humic compounds often stimulate photosynthesis, root development, and the absorption of microelements (Shahid *et al.*, 2012; Zhang *et al.*, 2006), while also increasing microbe populations, supplying biochemical substances, and transporting trace elements and growth regulators (Yang *et al.*, 2004). Furthermore, potassium humate facilitates optimal conditions for chemical reactions and biological activity, enhances soil structure, accelerates nutrients transport to plants, and improves pH buffering (Amjad *et al.*, 2010). Conversely, Ali *et al.* (2018) indicated that the use of silicon (Si) can augment the crop's drought tolerance, with plants utilizing Si as a 'quasi-essential' element to alleviate the effects of drought stress (Ma *et al.*, 2004; Pei *et al.*, 2010). The beneficial effects of Si may be ascribed to enhanced antioxidant production, preserving photosynthetic apparatus, and postponement of leaf senescence (Hosseini *et al.*, 2017; Shen *et al.*, 2010). Zargar *et al.* (2019) elucidated several critical functions of silicon in plants, including enhancement of growth, productivity, and quality, facilitation of photosynthesis, nitrogen fixation, and provision of tolerance to abiotic and biotic stresses such as extreme temperature, UV-radiation, nutrient deficiencies, metal toxicity, drought, salinity, and pathogen and fungus attack. Furthermore, Si enhances plant strength by making the plant tissues stronger and rigid, as reported by Marxen *et al.* (2015). Besides, potassium silicate (KS) serves as a source of highly soluble potassium and silicon. It is utilized in agricultural production systems mainly as a silica amendment and additionally provides minor quantities of potassium (Abou-Baker *et al.*, 2011). According to Horvath *et al*. (2007), salicylic acid (SA) functions as a plant signaling molecule that enhances tolerance to abiotic and biotic stresses. Moreover, the effects of salicylic acid on stress adaptation and plant damage vary according to method employed, its concentration, and species of plant (Metwally *et al.*, 2003). Bezrukova *et al.* (2001) and Arfan *et al.* (2007) shown that the application of exogenous hormone SA can exacerbate the effects of drought stress and increase water deficit in many plant species. The beneficial impact of SA on crop plant growth under abiotic stress may be attributed to its influence on nutrient absorption, water management, stomatal regulation, photosynthesis, and overall growth (Arfan *et al.*, 2007; Khan *et al.,* 2003). Therefore, this study aims to mitigate the detrimental effects of drought stress on the growth and productivity of black cumin (*Nigella sativa* L.) by utilizing various growth substances, including potassium silicate, potassium humate, and salicylic acid.

2. Materials and methods

2.1 Plant materials and experimental site

The field experiments were conducted utilizing a drip irrigation system in sandy calcareous soil at the Experimental Farm of Arab El-Awammer Research Station, Agricultural Research Center (A.R.C.), Assiut, Egypt (latitude 27°, 03′ N, longitude 31°, 01′ E and the Altitude 71 m above sea level) during the 2020/2021 and 2021/2022 seasons. Table (1) presents the detailed monthly meteorological data for Assiut for the two growth seasons. The physical and chemical properties of the soil utilized were analyzed following the methodologies outlined by Jackson (1973) and Black *et al.* (1982), as presented in Table (2).

2.2 Experimental design

The experiment was set up in a spilt-plot in Randomized Complete Block Design (RCBD), comprising three replicates. The main plots were designated for irrigation regimes (100%, 80%, and 60% of crop evapotranspiration "ETc"), while the growth substances, including potassium silicate (KS), potassium humate (KH), and salicylic acid (SA) alongside untreated plants (control) were allocated to sub-plots. Each experimental unit (plot) measured 10 m^2 (2 × 5 m). The local cultivar of black cumin seeds was obtained from the Department of Medicinal and Aromatic plants Research, Agricultural Research Center, Ministry of Agriculture, Egypt. Seeds were sowed on both sides of the dripper line in newly reclaimed sandy soil mid-October for both seasons. Four weeks later, the thinning was completed, leaving one plant each hill. All horticultural practices were executed similarly as required. Compost at a rate of 5 tons/feddan (feddan = 4200 m² = 0.420 hectares = 1.037 acres) was uniformly applied to all the plots.

Table (1): Average monthly meteorological data of Assiut weather station during 2020/2021 and 2021/2022 seasons.

Parameter / Month	2020/2021					2021/2022				
	Temperature (°C)		R.H. (%)	Wind speed (km/h)	Sunshine (hours)	Temperature (°C)		R.H. (%)	Wind speed (km/h)	Sunshine (hours)
	Maximum	Minimum				Maximum	Minimum			
October	34.6	20.5	47.8	17.1	10.0	32.4	17.6	44.2	14.9	10.0
November	25.3	12.7	55.0	14.9	9.4	28.6	13.4	50.7	9.9	9.4
December	23.6	9.4	53.6	14.3	9.0	20.3	7.7	54.7	10.7	9.0
January	21.4	7.1	58.9	13.5	8.9	17.1	4.5	57.1	10.3	8.9
February	21.6	7.3	57.4	15.9	9.7	20.2	6.4	51	13.8	9.7
March	27.1	11.3	43.4	18.6	9.9	22.6	8.4	39.2	18	9.9
April	32.0	15.1	34.4	17.1	10.3	34.6	16.2	26.4	15.4	10.3

Table (2): Some of physical and chemical properties of the field experimental soil during 2020/2021 and 2021/2022 season.

Chemical properties									
pH (1:1)	EC dS/m (1:1)	Soluble cations (meq/L)				Soluble anions (meq/L)		Available phosphorus (ppm)	Total nitrogen (%)
		Ca^{++}	Mg^{++}	Na^{+}	K^{+}	$CO_3^{--} + HCO_3^{-}$	Cl^{-}		
8.07	0.55	1.97	1.53	0.57	0.98	2.87	1.89	8.21	0.09
Physical properties									
Particle size distribution (%)			Texture Class	Moisture content (Volumetric %)			O.M (%)	$CaCO_3$ (%)	Bulk density
Sand	Silt	Clay		S. P.	F.C.	W.P.			
89.9	7.0	3.1	Sandy	23.5	11.1	4.7	0.8	28.1	1.60

2.3 Bio-stimulant treatments

Potassium silicate (KS), comprising 10% K_2O and 25% SiO_2, which produced in Egypt by Abo Ghaneima CO. for Fertilizers and Chemical Industries and was applied at a concentration of 5 gl^{-1}. Potassium humate, comprising 85% humic acid and 10% K_2O, which produced in China, was applied at a concentration of 0.4%. Salicylic acid (SA), manufactured by El-Nasr Pharmaceutical Chemicals Co. in Egypt was applied at a concentration of 300 ppm. Plants were sprayed with growth substances 30 days post-planting. Untreated plants were sprayed with tap water as control. Each foliar application was conducted four times at 15-days intervals.

2.4 Irrigation regimes descriptions

The CROPWAT model was employed to calculate reference evapotranspiration (ETo) with Penman Monteith (Smith, 1992). Reference Crop Evapotranspiration (ETc) calculated according to Allen *et al.* (1998):

$$ETc = ETo \times Kc$$

Where: ETc = Crop evapotranspiration, ETo = Reference evapotranspiration, Kc = Crop coefficient (Ghamarnia *et al.*, 2014).

2.5 Irrigation water applied

The actual amounts of irrigation water applied under each irrigation regime were computed using the equation proposed by James (1988) as follow:

$$I.Ra = \frac{ETc + LF}{Er}$$

Where: I.Ra = total actual irrigation water applied mm/interval, ETc = Crop evapotranspiration using Penman Monteith equation, LF = Leaching factor (10%), Er = irrigation system efficiency (85%).

2.6 Economic productivity of irrigation water

The productivity of irrigation water can be defined as economical productivity (EPIW) according to Molden (1997). The calculation was conducted as follows:

$$EPIW = \frac{Grass\ value\ of\ product\ (L.E\ feddan^{-1})}{Total\ amount\ of\ irrigation\ applied\ water\ (m^{3}\ feddan^{-1})}$$

2.7 Yield and yield components

During the harvesting phase (when the plants exhibited yellowed and the capsules dried), ten plants were randomly selected from each experimental unit in both seasons to assess the following growth parameters and yield: plant height (cm), branch number/plant, plant fresh and dry weight (g), capsules number/ plant, seeds weight/plant (g), and seed yield per feddan (kg). The total seed yield/feddan was calculated by multiplying the seed yield/plot by 1050 (plot number per feddan).

2.8 Biochemical analysis

The air-dried seeds were analyzed for fixed oil content using light petroleum ether (80%) in a Soxhlet apparatus for 8h, according to Horwitz *et al*. (1970). To determine the levels of nitrogen (N), phosphorus (P), and potassium (K), dry leaves were oven-dried at 70°C until a constant weight was attained, after which 0.5 g was weighted for wet digestion utilizing a sulfuric acid and hydrogen peroxide mixture (Parkinson and Allen, 1975). Total nitrogen was quantified utilizing the semi-micro Kjeldahl method as outlined by Black *et al*. (1982). Total phosphorus was quantified utilizing a Spectrophotometer in accordance with Jackson (1973). The potassium concentration in leaves was quantified photometrically using a flame photometer, as outlined by Jackson (1973). The percentage of total carbohydrates in dried leaves was colorimetrically determined using the anthrone-sulphuric acid method as described by Hansen and Moller (1975).

The free proline content in dried leaves was assessed according to the methodology outlined by Bates *et al*. (1973). The protein content in dried leaves was calculated by multiplying the percentage of nitrogen content by a ratio of 6.25.

2.9 Statistical analysis

The data collected during the two seasons were analyzed statistically using Statistix 8.1 software. Means were compared utilizing the least significant differences (L.S.D.) test at $P \leq 0.05$, and results are presented as means ± standard deviations in accordance with Gomez and Gomez (1984).

3. Results

3.1 Vegetative growth of N. sativa

3.1.1 Plant height (cm)

Table (3) illustrates the plant height of *N. sativa* influenced by exogenous treatments of potassium silicate, humic acid, and salicylic acid under condition of restricted irrigation. The exogenous treatments and irrigation regime significantly influenced the plant height of *N. sativa* across both seasons of the study. The maximum plant heights of 74.1 cm and 71.9 cm were observed in the first and second season, respectively, with 100% of ETc, in contrast to the minimum heights of 61.2 cm and 55.4 cm reported in the first and second season, respectively, with 60% ETc. The application of humic acid at 0.4% resulted the highest average plant height across the seasons compared to the other treatments. The interaction between the irrigation regime and exogenous applications had no significant influence on the height of *N. sativa* plants.

3.1.2 Branches number/plant

The impact of irrigation regime and exogenous applications on the number of branches per plant of *N. sativa* in the 2020/2021 and 2021/2022 seasons is illustrated in Table 3. The major of these parameters and their interaction on the number of branches per plant is evident only in the first season. The maximum number of branches/plants is achieved with 100% ETc, whereas 60% ETc yielded the minimum in both seasons. The maximum number of branches per plant was observed with salicylic acid at 300 ppm across all irrigation regimes in both seasons. The interaction between the irrigation regime and the applied exogenous treatments was significant only in the first season regarding the number of branches/plants.

3.1.3 Fresh weight (g)/ plant

The fresh weight/plant exhibited significant variation based on the irrigation regime and exogenous inputs (Table 3). In this context, the fresh weight recorded was 91.6, 75.3, 51.8 g/plant for the 100, 80 and 60% ETc treatments in the first season, and 88.4, 70.1, 46.0 g/plant in the second season, respectively. The maximum fresh weight/ plant, 81.7 g and

76.1 g were attained in plants treated with 300 ppm salicylic acid throughout the first and second season, respectively, in comparison to the other treatments. The interaction between the irrigation regime and exogenous applications significantly affected plant fresh weight, with the heaviest plants observed under 100% ETc combined with 300 ppm salicylic acid in both seasons.

Table (3): Plant height (cm), branches number/ plant and fresh and dry weight (g)/plant of *Nigella sativa* L. as affected by exogenous applications of potassium silicate, humic and salicylic acids under limited irrigation condition during 2020/2021 and 2021/2022 seasons.

Irrigation levels (A)	Plant height (cm)									
	2020/2021					2021/2022				
	Exogenous applications (B)									
	Control	Potassium silicate (5 ml/l)	Salicylic acid (300 ppm)	Humic acid (0.4 %)	Mean	Control	Potassium silicate (5 ml/l)	Salicylic acid (300 ppm)	Humic acid (0.4 %)	Mean
100% ETc	68.5	75.4	76.1	76.4	74.1	65.3	72.6	74.6	75.0	71.9
80% ETc	63.3	69.8	70.5	70.6	68.5	59.4	65.0	64.6	66.9	64.0
60% ETc	55.8	62.9	62.6	63.5	61.2	50.8	56.5	56.3	57.8	55.4
Mean	62.5	69.4	69.7	70.2		58.5	64.7	65.2	66.6	
L.S.D.at 0.05	A= 1.5 B= 1.2 A × B= N.S					A= 1.4 B= 1.2 A × B= N.S				
	Branches number/plant									
100% ETc	8.20	10.35	10.60	9.78	9.73	7.30	9.18	9.85	9.15	8.87
80% ETc	7.18	9.60	9.93	8.73	8.85	5.85	7.45	7.58	7.15	7.01
60% ETc	5.45	7.50	7.35	7.40	6.92	4.23	6.00	6.05	5.83	5.53
Mean	6.94	9.14	9.28	8.63		5.79	7.54	7.83	7.38	
L.S.D.at 0.05	A= 0.25 B= 0.30 A × B = 0.51					A = 0.37 B= 0.28 A × B=N.S				
	Fresh weight (g)/ plant									
100% ETc	74.5	97.5	104.8	89.5	91.6	70.8	93.5	98.5	91.0	88.4
80% ETc	56.8	85.5	87.4	71.5	75.3	50.3	77.0	78.5	74.5	70.1
60% ETc	42.0	58.0	53.0	54.0	51.8	35.8	50.5	51.3	46.5	46.0
Mean	57.8	80.3	81.7	71.7		52.3	73.7	76.1	70.7	
L.S.D.at 0.05	A= 4.1 B= 2.2 A × B= 3.8					A = 1.4 B= 2.0 A × B= 3.5				
	Dry weight (g)/plant									
100% ETc	34.5	45.8	48.5	41.5	42.6	32.5	43.5	46.3	42.5	41.2
80% ETc	26.3	40.8	43.3	33.8	36.0	23.0	36.0	37.0	35.0	32.8
60% ETc	19.1	24.8	23.0	23.6	22.6	16.4	22.9	23.4	21.3	21.0
Mean	26.6	37.1	38.3	32.96		24.0	34.1	35.5	32.9	
L.S.D.at 0.05	A= 2.0 B = 1.0 A × B = 1.8					A = 0.7 B= 1.1 A × B= 1.9				

3.1.4 Dry weight (g)/plant

The dry weight/plant exhibited significant variation based on the irrigation regime and exogenous applications (Table 3). The highest dry weight values of 42.6 and 41.2 g/plant were recorded with the 100 % ETc treatment in the first and second season, respectively. The various exogenous applications considerably enhanced dry weight compared to the control, with the maximum dry weights of 38.3 g/plant and 35.5 g/plant resulting from the application of salicylic acid at 300 ppm in the first and second season, respectively. The interaction impact of all treatments was significant for the dry weight, with the greatest observed effect occurring at 100% ETc and 300 ppm salicylic acid in both seasons. A considerable difference was seen in the vegetative growth of nigella plants subjected to varying watering regimes, as illustrated in Table (3). The 100% ETc irrigation regime produced the highest values of plant height, number of branches, and both fresh and dry weight in the two seasons.

3.2 Seed yield of N. sativa

3.2.1 Capsules no/plant

The findings presented in Table (4) indicate that the number of capsules per plant significantly varied due to irrigation regime and exogenous applications in the two seasons. The number of capsules was increased with increasing level of irrigation in both seasons, accounting of 41.3, 35.5, 24.0 for the first season, and 33.8, 25.9, 19.2 for the second season, corresponding to treatments of 100%, 80% and 60% ETc, respectively. In terms of the number of capsules/plants resulting from exogenous applications, salicylic acid at 300 ppm gave the highest results relative to the other treatments over both seasons. The interaction between irrigation level and exogenous applications, was significant for the number of capsules. Salicylic acid at 300 ppm with 100% ETc irrigation level produced the maximum number of capsules per plant in both seasons.

Table (4): Number of capsules, seed yield (g)/plant and seed yield (kg)/feddan of *Nigella sativa* L. as affected by exogenous applications of potassium silicate, humic and salicylic acids under limited irrigation condition during 2020/2021 and 2021/2022 seasons.

Irrigation levels (A)	Capsules number/plant									
	2020/2021					2021/2022				
	Exogenous applications (B)									
	Control	Potassium silicate (5 ml/l)	Salicylic acid (300 ppm)	Humic acid (0.4 %)	Mean	Control	Potassium silicate (5 ml/l)	Salicylic acid (300 ppm)	Humic acid (0.4 %)	Mean
100% ETc	34.9	43.4	46.0	40.9	41.3	27.9	35.3	37.8	34.1	33.8
80% ETc	27.7	38.4	42.5	33.3	35.5	20.7	27.9	28.6	26.6	25.9
60% ETc	19.2	26.5	24.9	25.6	24.0	16.0	20.4	20.8	19.8	19.2
Mean	27.2	36.1	37.8	33.3		21.5	27.8	29.1	26.9	
L.S.D.at 0.05	A= 1.6 B= 1.6 A × B= 2.8					A= 0.9 B= 1.2 A × B= 2.1				
Seed yield (g)/plant										
100% ETc	9.25	12.00	12.75	11.50	11.38	8.38	10.88	11.25	10.75	10.31
80% ETc	7.88	11.00	11.63	10.13	10.16	7.13	8.63	9.00	8.50	8.31
60% ETc	5.75	7.50	7.13	7.13	6.88	5.63	6.88	7.00	6.38	6.47
Mean	7.63	10.17	10.50	9.58		7.04	8.79	9.08	8.54	
L.S.D.at 0.05	A= 0.66 B= 0.39 A × B = 0.68					A = 0.36 B= 0.40 A × B= 0.68				
Seed yield (kg)/ feddan										
100% ETc	469.9	602.5	630.0	564.4	566.7	439.7	567.0	584.1	565.7	539.1
80% ETc	399.3	517.8	550.6	504.6	493.1	394.1	500.7	509.9	491.5	474.0
60% ETc	314.5	400.7	381.0	384.9	370.3	300.1	379.7	381.0	363.9	356.1
Mean	394.6	507.0	520.5	484.6		378.0	482.4	491.6	473.7	
L.S.D.at 0.05	A= 10.0 B= 9.7 A × B= 16.6					A = 16.2 B= 13.2 A × B= 22.9				

3.2.2 Seed yield (g)/plant

The data presented in Table (4) demonstrated that, seed yield varied significantly depending to irrigation levels, exogenous treatments, their interaction across both seasons. The maximum seed yield/ plant was observed in plants irrigated at 100% ETc, whereas the minimum seed yield was recorded in plants irrigated at 60% ETc. The obtained results indicated that the various exogenous applications considerably enhanced seed yield in comparison to the control. The application of salicylic acid at 300 ppm, followed by potassium silicate at 5ml/ L, resulted in the highest seed yield/ plant in both seasons. Moreover, the highest seed yield/ plant was noticed with 100% ETc irrigation level combined with salicylic acid at 300 ppm, in comparison to the other treatments during both seasons.

3.2.3 Seed yield (kg)/ feddan

The data in Table (5) illustrate the seed yield/feddan of *N. sativa* influenced by exogenous treatments of potassium silicate, humic acid, and salicylic acid under restricted watering condition. The exogenous treatments and irrigation levels significantly influenced seed yield/feddan in both experimental seasons. The highest seed yield/feddan were 566.7 kg and 539.1 kg in the first and second season, respectively, achieved with 100% of ETc, while the lowest yields were 370.3 kg and 356.1 kg in the first and second season, respectively, with 60% ETc. On the other hand, the application of salicylic acid at 300 ppm resulted the highest seed yield/ feddan on average across seasons compared to the other treatments. The interaction between the irrigation regime and exogenous applications significantly influenced the seed production per feddan of *N. sativa*. Furthermore, the peak seed yields of 630.0 and 584.1 kg per feddan were seen with 100% ETc combined with salicylic acid at 300 ppm in the first and second seasons, respectively.

Table (5): Fixed oil (%) and fixed oil (kg)/feddan of *Nigella sativa* L. as affected by exogenous applications of potassium silicate, humic and salicylic acids under limited irrigation condition during 2020/2021 and 2021/2022 seasons.

Irrigation levels (A)	Fixed oil (%)									
	2020/2021					2021/2022				
	Exogenous applications (B)									
	Control	Potassium silicate (5 ml/l)	Salicylic acid (300 ppm)	Humic acid (0.4 %)	Mean	Control	Potassium silicate (5 ml/l)	Salicylic acid (300 ppm)	Humic acid (0.4 %)	Mean
100% ETc	28.9	36.1	35.1	35.3	33.8	28.2	37.2	36.4	36.3	34.5
80% ETc	26.7	34.9	34.7	34.2	32.6	26.4	36.3	35.8	35.2	33.4
60% ETc	23.4	33.5	32.8	32.9	30.7	24.7	34.0	34.2	33.0	31.5
Mean	26.3	34.9	34.2	34.10		26.4	35.8	35.5	34.8	
L.S.D.at 0.05	A= 0.7 B= 1.0 A × B= N.S					A= 0.7 B= 0.9 A × B= N.S				
	Fixed oil (kg)/feddan									
100% ETc	135.8	217.6	221.0	199.1	193.4	123.9	210.7	212.7	205.1	188.1
80% ETc	106.7	180.7	190.9	172.4	162.7	103.8	181.9	182.4	173.0	160.3
60% ETc	73.7	125.2	125.0	126.7	112.7	74.1	129.2	130.4	120.1	113.4
Mean	105.4	174.5	178.9	166.1		100.6	173.9	175.1	166.0	
L.S.D.at 0.05	A= 5.0 B= 7.2 A × B = 12.6					A = 6.8 B= 6.2 A × B= 10.8				

3.3 Fixed oil yield of N. sativa

3.3.1 Fixed oil percentage

Table (5) illustrates the impact of irrigation levels and exogenous treatments on the fixed oil percentage of *Nigella* seeds throughout the 2020/2021 and 2021/2022 seasons. The differences among 100%, 80% and 60% ETc irrigation levels were substantial in both seasons regarding oil percentage. The percentage of fixed oil progressively increased with the elevation of irrigation levels, reaching a peak at 100% ETc in the two seasons. The results presented in the same table pointed out that the variations in exogenous applications were significant in both seasons regarding the fixed oil percentage. The maximum oil percentage were seen with potassium silicate at 5 ml/ l (35.9%), followed by salicylic acid at 300 ppm (35.2%), as an average across seasons. The interaction effects of irrigation levels and exogenous applications on oil percentage were not significant in the two studied seasons. The greatest oil percentage (36.6%) was recorded from the combination of 100% ETc irrigation level and the 5 ml/l potassium silicate, while the lowest oil percentage (24.05%) was observed in the control plants receiving 60% ETc irrigation level during the average of the seasons.

3.3.2 Fixed oil (kg)/feddan

The data in Table (5) show the oil yield per feddan of *N. sativa* as affected by exogenous applications of potassium silicate, humic acid, and salicylic acid under limited irrigation condition. Each exogenous application and irrigation level, along with their interactions, significantly affected seed yield/ feddan in both seasons. The 100% ETc irrigation level demonstrated the highest oil yield values, measuring 193.4 and 188.1 kg/feddan in the first and second season, respectively, when compared to alternative treatments. The use of exogenous stimulants significantly enhanced oil yield relative to the control group. The maximum oil yields were 178.9 and 175.1kg/feddan, achieved with salicylic acid at 300 ppm, followed by potassium silicate at 5 ml/l, which yielded 174.5 and 173.9 kg/feddan in the first and second seasons, respectively. Irrigation at 100% ETc with salicylic acid at 300 ppm produced the highest oil yield values (221.0 and 212.7 kg/feddan), whereas the control plants at 60% ETc irrigation level exhibited the lowest values (73.7 and 74.1 kg/feddan) in the first and second season, respectively. Table (5) illustrates that the fixed oil yield *Nigella* plants when subjected to a water regime of 100% ETc combined with either 300 ppm salicylic acid or 5 ml/l potassium silicate, in comparison to other treatments. Exogenous applications to plants resulted in a reduction under drought conditions (60% ETc).

3.4 N, P and K (%) in seeds of N. sativa

The data shown in Table (6) demonstrate that varying irrigation levels and exogenous applications, with their interactions, significantly influenced the nitrogen, phosphorus, and potassium content in the seeds of *N. sativa* plants. The highest nitrogen content was recorded under 80% ETc water regime, whereas the maximum phosphorus and potassium values were detected at the 100% ETc level in comparison to other levels. The minimum contents of these constituents were recorded while planting *N. sativa* at the 60% ETc level over both seasons. Exogenous treatments revealed that the maximum concentrations of N, P, and K% in seeds were achieved with potassium silicate at 5 ml/ l during both seasons. The combined application of 80% ETc and 5 ml/l potassium silicate provide sufficient nitrogen for *Nigella* plants in comparison to alternative treatments. The combined application of 100% ETc and potassium silicate at 5 ml/l resulted the highest concentrations of phosphorus and potassium in the leaves of *Nigella* plants compared to alternative treatments.

Table (6): Nitrogen, phosphorus and potassium (%) in seeds of *Nigella sativa* L. as affected by exogenous applications of potassium silicate, humic and salicylic acids under limited irrigation condition during 2020/2021 and 2021/2022 seasons.

Irrigation levels (A)	Nitrogen (%)									
	2020/2021					2021/2022				
	Exogenous applications (B)									
	Control	Potassium silicate (5 ml/l)	Salicylic acid (300 ppm)	Humic acid (0.4 %)	Mean	Control	Potassium silicate (5 ml/l)	Salicylic acid (300 ppm)	Humic acid (0.4 %)	Mean
100% ETc	3.21	3.58	3.53	3.54	3.47	3.17	3.57	3.52	3.51	3.44
80% ETc	3.29	4.12	3.61	3.56	3.64	3.24	4.01	3.59	3.53	3.59
60% ETc	3.06	3.37	3.55	3.22	3.30	3.04	3.34	3.52	3.20	3.27
Mean	3.19	3.69	3.56	3.44		3.15	3.64	3.54	3.41	
L.S.D.at 0.05	A= 0.03 B= 0.04 A × B= 0.06					A= 0.01 B= 0.02 A × B= 0.04				
	Phosphorus (%)									
100% ETc	0.65	0.96	0.67	0.69	0.74	0.62	0.93	0.66	0.67	0.72
80% ETc	0.53	0.74	0.64	0.64	0.64	0.50	0.71	0.63	0.62	0.61
60% ETc	0.47	0.64	0.61	0.52	0.56	0.43	0.62	0.60	0.51	0.54
Mean	0.55	0.78	0.64	0.62		0.52	0.75	0.63	0.60	
L.S.D.at 0.05	A= 0.02 B= 0.02 A × B = 0.03					A = 0.03 B= 0.02 A × B= 0.02				
	Potassium (%)									
100% ETc	1.82	2.16	1.93	1.90	1.95	1.80	2.09	1.92	1.87	1.92
80% ETc	1.62	1.89	1.71	1.64	1.72	1.58	1.88	1.68	1.62	1.69
60% ETc	1.50	1.69	1.63	1.62	1.61	1.47	1.67	1.61	1.60	1.59
Mean	1.64	1.91	1.75	1.72		1.62	1.88	1.74	1.70	
L.S.D.at 0.05	A= 0.02 B= 0.03 A × B= 0.05					A= 0.02 B= 0.03 A × B= 0.05				

3.5 Protein, total carbohydrates (%) and free proline (mg/g D.W) seeds of N. sativa

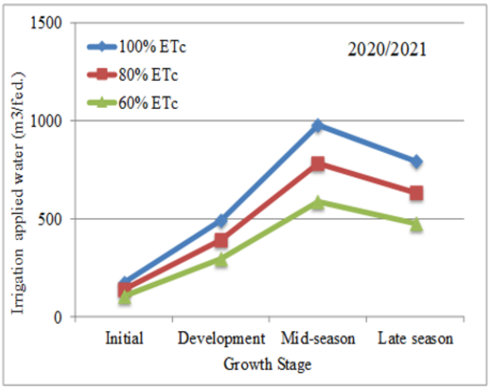
Table (7) displays the effects of exogenous applications of potassium silicate, humic acid, and salicylic acid on the response of protein, total carbohydrates (%), and free proline (mg/g D.W) in the dry seeds of *N. sativa* under condition of inadequate irrigation. The findings indicate that water stress significantly affected these characteristics of *Nigella*. The highest protein and carbohydrate contents in dry seeds were recorded for the 80% and 100% ETc treatments, respectively. The maximum proline concentration was achieved at 60% ETc during both seasons. The application of potassium silicate at a concentration of 5 ml/ l yielded the highest levels of protein and proline content over the two examined seasons. Meanwhile, the highest total carbohydrate levels were achieved by applying salicylic acid at 300 ppm during the two seasons. The interaction effects of water stress and exogenous application treatments were significant for proline and protein contents, but not for total carbohydrate contents in the 2020/2021 and 2021/2022. The maximum protein content was attained at 80 % ETc with potassium silicate at 5 ml/ l, but 100% ETc with salicylic acid at 300 ppm produced the highest total carbohydrate for both seasons. Conversely, water stress at 60% ETc with potassium silicate at 5 ml/ l produced the highest proline level in the dried leaves of *Nigella*. Table (7) indicates that the maximum protein and carbohydrate levels in dry seeds were achieved under 80% or 100% ETc treatments.

Table (7): Protein, total Carbohydrates (%) and free proline (mg/g D.W) in seeds of *Nigella sativa* L. as affected by exogenous applications of potassium silicate, humic and salicylic acids under limited irrigation condition during 2020/2021 and 2021/2022 seasons.

Irrigation levels (A)	Protein (%)									
	2020/2021					2021/2022				
	Exogenous applications (B)									
	Control	Potassium silicate (5 ml/l)	Salicylic acid (300 ppm)	Humic acid (0.4 %)	Mean	Control	Potassium silicate (5 ml/l)	Salicylic acid (300 ppm)	Humic acid (0.4 %)	Mean
100% ETc	20.06	22.39	22.08	22.10	21.66	19.78	22.28	21.99	21.91	21.45
80% ETc	20.55	25.75	22.57	22.24	22.78	20.27	25.08	22.44	22.05	22.46
60% ETc	19.13	21.03	22.19	20.14	20.62	18.99	20.85	22.02	19.99	20.46
Mean	19.91	23.06	22.28	21.49		19.68	22.74	22.15	21.31	
L.S.D.at 0.05	A= 0.16 B= 0.23 A × B= 0.40					A= 0.07 B= 0.15 A × B= 0.26				
	Total carbohydrates (%)									
100% ETc	24.3	31.0	32.0	30.1	29.4	23.1	28.6	29.5	28.3	27.4
80% ETc	19.3	25.8	26.6	24.9	24.1	17.6	24.2	24.8	24.1	22.7
60% ETc	10.9	18.6	17.8	18.3	16.4	10.4	17.5	17.8	17.4	15.8
Mean	18.1	25.2	25.6	24.4		17.0	23.4	24.0	23.3	
L.S.D.at 0.05	A= 0.5 B= 0.7 A × B = N.S					A = 0.9 B= 0.7 A × B= N.S				
	Free proline (mg/g D.W.)									
100% ETc	1.29	1.86	2.01	1.71	1.72	1.22	1.80	1.95	1.67	1.66
80% ETc	1.35	1.93	2.22	1.77	1.82	1.32	1.89	2.15	1.74	1.77
60% ETc	1.42	2.71	2.07	1.91	2.03	1.38	2.68	2.03	1.87	1.99
Mean	1.35	2.17	2.10	1.80		1.31	2.12	2.04	1.76	
L.S.D.at 0.05	A= 0.06 B= 0.05 A × B= 0.08					A= 0.06 B= 0.05 A × B= 0.09				

3.6 Irrigation water applied

The data in Figure (1) indicate that the irrigation water applied changes across different growth stages. The variations were minimal at the starting of the growth season, as the canopy of *Nigella sativa* had not yet developed, resulting in moisture loss primarily through evaporation from the soil surface. A gradual increase in water use was observed in the plant matured. The irrigation water applied reaches its peak during the mid-growth stage. This may be attributable to the plant's growing condition. Following the culmination of vegetative growth, the application rate of irrigation water pronouncedly decreases in the late growing season. The seasonal irrigation water utilized was mostly affected by irrigation levels. The rise in irrigation water utilized at 100% ETc may be attributed to the increase in direct evaporation. Consequently, the seasonal irrigation water utilized is greater at 100% ETc, followed by 80% ETc for *Nigella sativa* across the two growth seasons.



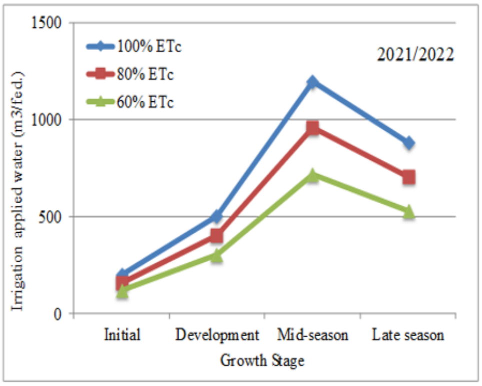


Figure (1): Irrigation water applied (m^3/feddan) at different growth stages of *Nigella sativa* grown under different irrigation regime during the seasons of 2020/2021 and 2021/2022.

*3.7 Economic productivity of irrigation water (EPIW) L E/m^3^*

The economic productivity of irrigation water in agricultural production system is focused on the gross value of product, expressed in LE m^{-3}. The EPIW of *Nigella sativa* is depicted in Figure (2). The minimum EPIW values were 7.46 and 5.94 L E m^{-3} at control under irrigation with 100% ETc, while the maximum values were 10.93 and 8.61 LE m^{-3}, achieved at salicylic acid application under irrigation with 80% ETc, in the first and second seasons, respectively.

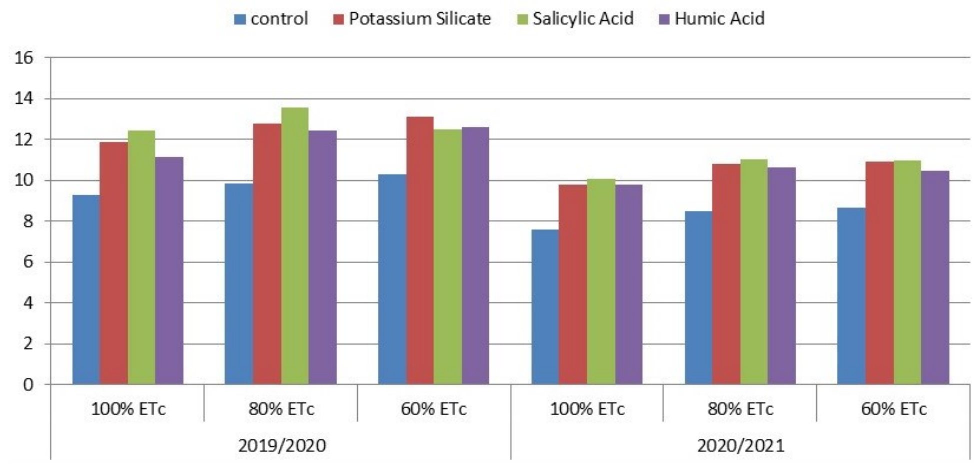


Figure (2): Economic productivity of irrigation water of *Nigella sativa* as influenced by irrigation regime and some growth substances during 2020/2021 and 2021/2022 seasons.

4. Discussion

The impact of reduced irrigation on vegetative development may result from the decreased availability of sufficient moisture in rhizosphere and the reduced absorption of nutrients (Singh *et al*., 1997). These results are consistent with those reported by Ghamarnia and Jalili (2013), Karim *et al*. (2017) and Hendi *et al*. (2021), as well as with findings related to other medicinal plants (Ghamarnia *et al*., 2012; Hefzy *et al*., 2021; Khalid, 2006; Petropoulos *et al*., 2008). On the other hand, our findings indicated that the various exogenous applications improved the vegetative growth parameters of *Nigella* in comparison to the control group. The application of exogenous salicylic acid may mitigate water stress damages to plants (Ahmed *et al*., 2020; El-Esawi *et al*., 2017; Kabiri *et al*., 2012). Dawood *et al.* (2019) indicated that salicylic acid enhances the vegetative growth of *Linum usitatissimum* plants. El-Beltagi *et al*. (2020) spraying salicylic acid as an antioxidant to alleviate the harmful effects of drought stress in chickpea plants. The sequential combination of antioxidants and proline enhanced plant growth under drought stress by upregulating the antioxidant defense system and osmolyte production. Furthermore, the findings on the impact of potassium silicate on *Nigella* aligned with those of El-Leithy *et al.* (2019). The results showed that the maximum values for plant height, branches number/plant, fresh and dry weights/plant with nitrogen application levels up to 100 kg N/fed or with a potassium silicate spray at 8 ml/L alone. Potassium influences various physiological functions in plants, mitigates water stress, increases disease resistance, and improves protein synthesis and quality (Aytac *et al.*, 2017; Wang *et al*., 2013). Furthermore, silicon can modify morphological traits and enhance the growth and quality of ornamental plants, with its effects contingent upon the silicon type, concentration, application method, and plant species (Ashour, 2018; Park *et al.,* 2016). Our results align with those of Safaei *et al.* (2014) and Tarek (2021), who demonstrated varying quantities of humic acid significantly influenced the growth characteristics of *N. sativa*. *N. sativa* is a medicinal plant that exhibits sensitivity to water stress, resulting in a rapid decline in seed yield and its components when water stress intensifies during the growing seasons (Ghamarnia and Jalili, 2013; Ghamarnia *et al.*, 2010). A positive correlation existed between seed yield and aboveground biomass, with yield components, including the number of branches and capsules, directly influencing seed yield (Tuncturk *et al.*, 2012; Sadeghi *et al*., 2009). The result is in alignment with Squire (1990), who stated that enhanced seed production in connected with improved growth characteristics, leading to more solar energy interception and elevated water transpiration during the growing season, contingent upon nutrient availability in the soil. Our results align with those of Roussis *et al*. (2019), who indicated a substantial correlation between growth rate and seed yield in many crop species, including *N. sativa.* Safaei *et al.* (2014) demonstrated that varying concentrations of humic acid on *N. sativa* significantly influenced the number of capsule/plants, the number of seeds/capsules, seed weight, seed yield, biological yield, and harvest index. Conversely, the increased number of umbels/ cumin plant in the non-stress condition results from optimal moisture at field capacity. The quantity of umbels has demonstrated the strongest correlation with seed yield (Jami *et al*., 2015). The reduced yield seen at 60 % ETc, in contrast to 100% or 80% ETc, may be attributed to water stress that increased the depletion of the soil's available water holding capacity. The similar results were also documented by Al-Kayssi *et al.* (2011) and Senyigita and Arslan (2016). Several researchers have indicated that preserving the integrity of cellular membranes during stress conditions is considered a crucial component of drought tolerance mechanisms (Shakirova *et al*., 2003; Shinozaki and Yamaguchi, 1997). Salicylic acid significantly enhances oil production in black cumin plants (Kabiri *et al*., 2012). Our findings align with those of Al-Kayssi *et al.* (2011), Aiyafar *et al.* (2015), and Senyigita and Arslan (2018). Thus, any factor that enhances nutrient absorption might influence the essential oil production pathway and ultimately result in an augmentation of the plant's essential oil content (Moghaddam *et al*., 2013; Moradzadeh *et al*., 2021). The nutrient content results align with those of El-Leithy *et al*. (2019), who indicated that seed concentrations of nitrogen, phosphorus, and potassium grew alongside the growth parameters. Moreover, Hussein and Muhammed (2017) observed that elevated concentrations of potassium silicate enhanced the percentage of nitrogen, phosphorus, and potassium in *Solanum melongena*. This implies that exogenous applications significantly influence the physiological processes involved in leaf pigment synthesis (El-Leithy *et al*., 2019; Rana *et al*., 2012). Conversely, Chen *et al*. (2004) demonstrated that enhancements in yield, when combined with the application of organic fertilizers, resulted from elevated levels of available nitrogen, phosphorus, and potassium in the plant, as well as increased photosynthesis and growth. The enhancement of biological yield influenced by humic acid can be attributed to stimulation of vegetative growth, as well as augmentation of herb and seed yield (Safaei *et al*., 2014). Proteins are essential molecules for all cellular activities. Water stress resulted in diminished protein synthesis, likely attributable to a drop in the number of polysomal complexes in tissues with reduced water content, subsequently leading to decreased plant development and crop yield (Kabiri *et al*., 2014). These findings are in agreement with those of Sara *et al*. (2012), who assert that protein content decreased under extreme drought conditions in chickpea plants, whereas the addition of antioxidants mitigates the detrimental effects of drought. A significant reduction in photosynthesis has led to a decrease in soluble protein during drought stress. The maximum carbohydrate content in dry seeds was recorded for the 80% and 100% ETc treatments, whereas the minimum was observed under the 60% ETc water regime. The findings about the augmentation of total carbohydrate content due to silicon treatments confirmed the reports of Sharifi-Rad *et al*. (2016) concerning *Amaranthus retroflexus*, Ashour and Abdel Wahab (2017) regarding *Jatropha integerrima* and Ashour (2018) pertaining to *Cupressus macrocarpa.* Our results proved that the highest proline content was achieved at 60% ETc in both seasons relative to the other treatments. The results aligned with those of El-Beltagi *et al*. (2020), who observed that exogenous application of ascorbic acid elevated endogenous proline levels during severe drought circumstances. They further stated that proline is an amino acid that significantly benefits plants subjected to diverse stress conditions, functioning as an effective osmolyte, a metal chelator, and an antioxidant defense molecule. Water stress can elevate the concentrations of soluble sugars and proline in Moth Bean and Mulberry plants (Garg *et al*., 2001; Molinari *et al*., 2007). The results demonstrated that the total irrigation water applied in the second season exceeded that of the first season. This difference may be due to the increasing reference evapotranspiration in the second season relative to the first season. These findings align with those reported by, Ghamarnia *et al.* (2010), Refai *et al.* (2019), Mansour *et al.* (2020), Zahran *et al.* (2020), and Dina *et al.* (2021). The application of Salicylic acid enhanced the economic productivity of irrigation water (LE/m^3) at different levels of irrigation water. The resulted are consistent with those obtained by Ghamarnia *et al.* (2010 and 2013), Senyigit and Arslan (2018), Refai *et al.* (2019), Zahran *et al.* (2020), Mansour *et al.* (2020), and Dina *et al.* (2021).

5. Conclusion

The results indicate that the optimal development and productivity of *Nigella sativa* cultivated in the newly reclaimed soil under a modern irrigation system require watering equal to evapotranspiration. The incorporation of salicylic acid at 300 ppm or potassium silicate at 5 ml/l mitigates water stress, promotes robust development, and enhances the production of *Nigella* plants.

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