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Biological and chemical nitrogen fertilizer impact on cumin (*Cuminum cyminum* L) under different irrigation regimens

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ABSTRACT

Introduction: Water and nitrogen deficits are the most important limiting factors for plant growth and crop production in the world. Drought stress would be amplified by the global warming. Moreover, nitrogen scarcity is occurred in most arid and semi-arid areas. Cumin (*Cuminum cyminum* L.) is an important plant due to export benefits and low water demand. This study was aimed to evaluate nitrogen fertilizer effect on yield and some physiological characteristics of cumin under different irrigation regimens.

Methods: The experiment was performed based on a split plot as randomized complete block design. Experiment treatments were irrigation regimens (field capacity, irrigation by draining 40% of soil water as middle stress, and irrigation by draining 80% of soil water as severe stress) and nitrogen fertilizers (60 kg ha^{-1} urea, 30 kg ha^{-1} urea, Nitroxin, and Nitroxin + 30 kg ha^{-1} urea).

Results: Drought stress reduced cumin dry weight, seed yield, and chlorophyll content. In contrary, proline content, malondialdehyde (MDA) rate, phenol content, anthocyanin amount, and activity of catalase (CAT) and peroxidase (POX) increased by water stress. Increment urea use resulted in amending cumin growth and seed yield in the field capacity. Also, nitrogen use and raising its rate under the middle water stress caused to improve cumin drought tolerance. However, under the severe water stress, nitrogen application had not a significant impress on drought acclimation and seed yield.

Conclusion: Nitroxin inoculation with use of 30 kg ha^{-1} urea was the most effective treatment to ameliorate seed yield and drought tolerance.

Implication for health policy/practice/research/medical education:
Drought resistant in cumin increased by nitrogen. Nitrogen-fixing rhizobacteria inoculation with using 30 kg ha^{-1} urea had the most positive effect on cumin seed yield under water stress. Hence, it is recommended to replace half of the nitrogen fertilizer with the nitrogen-fixing rhizobacteria in cumin farming.

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Introduction

Global mean surface temperature increased approximately 1.0°C during 1980 to 2018 higher than the average over the 1850–1900 period. Estimated anthropogenic global warming is currently increasing at 0.2°C per decade and likely would be reached 1.5°C between 2030 and 2052 (1). Hence, evapotranspiration rate is enhanced by the global warming which caused to boost drought stress (2). Therefore, it has been predicted that water stress frequency and intensity would be increased from 1% to 30% in acute drought land area up 2100 (3). Globally, about one-third of the land area is confronted to drought challenge, and Iran also is located in semi dry regions with 240 mm average precipitation (4).

Drought is the most important limiting factor for plant growth and crop production over the world (5). Water stress occurs when water amount loss via transpiration is exceeding of its volume absorbed from the soil (6). Among various abiotic stress, drought is the major reason for reducing plant growth (7) and is gradually rising due

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to global warming subjects (8). Survive of plants under drought stress is dependent on plant species, stage of development, severity and duration of water shortage (9). Drought stress can induce morphological and physiological changes in plants which has been widely studied in recent years (10). Water shortage limited the leaf area expansion by leaf dehydration, rolling, senescence, and reduction meristem cells growth and division (7). Leaf relative water content reduction under drought stress causes stomata closure and gas exchanges finite. In addition, drought reduces the radiation use efficiency and photosynthetic rate which is diminished metabolism of carbohydrates, protein, amino acids and other organic compounds (12). Plants could cope drought condition by osmolyte biosynthesis for adjust water potential, up-regulation enzymatic and non-enzymatic antioxidants, hormone biosynthesis, etc (10).

Nitrogen is the most necessary mineral nutrition for plants. Its content in plants is between 2 to 4 percent on average, which may reach 6 percent (13,14). Moreover, nitrogen is an important constituent in plant biomolecules, such as amino acids, amides, proteins, ribonucleic acid, chlorophyll, enzymes, vitamins, and the like. Nitrogen enables the plant to be faster establishment and produce more photosynthesis compounds. Leaves are greener and succulent by uptake of adequate amounts of nitrogen, which is resulted in more dry matter production. Thus, the plant will be able to provide more carbohydrates for the root, and thus more yield will be obtained (7,15).

Environmental factors such as drought may cause nutrient deficiency because they can impose a negative effect on mineral mobility and absorbance (16). Nitrogen scarcity is occurred in most arid and semi-arid areas, because in these areas the amount of organic matter as a source of nitrogen supply is very low or is rapidly decomposed due to heat (17). Moreover, water stress can reduce the plant nitrogen demand. It has been reported that water deficit can impose a more significant impact on N assimilation than it absorbs from the soil (7). In other hand, nitrogen supplying increased plant tolerance to water scarcity. Zhou et al (17) showed that increment nitrogen use in *Malcolmia africana* and *Bassia hyssopifolia* under water lack conditions resulted in increase leaf number, protein content, and biomass accumulation. In contrary, the content of proline and soluble carbohydrate was reduced.

In recent decades, the chemical fertilizers consumption in agricultural lands has caused many environmental problems, including water resources contamination, decrement quality of agricultural products, and reducing soil fertility (18). Some soil bacteria including *Azospirillum* spp. and *Azotobacter* spp. are capable of fixating atmospheric nitrogen and reducing nitrogen chemical fertilizer consumption. Moreover, these rhizosphere bacteria are able to biosynthesize and secret some biomolecules such as B vitamins, auxin, gibberellin, etc. which have important role in root development (19).

Cumin (*Cuminum cyminum* L.) is an important plant due to export benefits, the arid and semi-arid lands utilization and revitalization, and low water demand (20). Cumin is widely used in the herbal drug formulations and traditional medicine. Cumin seeds active ingredients are applied for improving stomach pain, flatulence, appetite stimulation, and pain relief. Its seed essential oil also has antibacterial properties (21). In regard to the important effects of drought and nitrogen on crop yield, this research was conducted to evaluate nitrogen fertilizer effect on yield and some physiological characteristics of cumin under different irrigation regimens.

Materials and Methods

Farm location, experiment design, and treatments

The experiment was performed based on a split plot as randomized complete block design with 3 replications in Medicinal Plants Research Center of Shahed University, Tehran, Iran at 2016. The experiment treatments were different irrigation regimens as main plot and various nitrogen fertilizers as subplots. Irrigation regimens were as follow: control (field capacity), irrigation by draining 40% of soil water (middle stress), and irrigation by draining 80% of soil water (severe stress). The soil moisture curve was used to determine the irrigation periods, which was obtained by weighing method (gravimetric) sampling from 0-30 cm soil depth. Water stress was applied after the five leaves development. Various nitrogen fertilizers were included 100% nitrogen (60 kg ha^{-1} urea), 50% nitrogen (30 kg ha^{-1} urea), nitroxin (a mixture of nitrogen fixation bacteria), and 50% nitrogen + nitroxin. Urea was divided into two equal amounts and was applied during planting and beginning flowering. Nitroxin was comprised of a mixture of *Azospirillum* and *Azotobacter* strains with 10^8 CFU which purchased from Mehr Asia Biotechnology Company (MABCo) in Iran. Nitroxin was inoculated to seeds before sowing (1 L ha^{-1}). Soil physicochemical properties were determined at 0-30 cm depth before carrying out the experiment (Table 1).

Farm practices, sampling, and growth characteristics

Farm preparation was contained twice plowing, lump crushing, and ground leveling. The plot size was 2 × 3

Table 1. Soil physicochemical properties of experimental farm

Clay (%)	Silt (%)	Sand (%)	Texture	N (%)	P (ppm)	K (ppm)	OC (%)	EC ($ds.m^{-1}$)	pH
10	15	75	Sandy loam	0.19	10.6	241.3	0.1	4.2	7.8

m, and the main plot spacing from the other was 5 m. Each plot was contained 10 sowing rows with 3 m length and a 20 cm interval. Cumin seeds were sown from the Sabzevar population with 10 cm distance on sowing row in February. Other practices were performed according to the plant requirements. Random sampling was conducted at seed physiological ripening for yield and growth parameters. Another sampling was performed before the leaves yellowing for biochemical traits. The plant sample after harvesting was transferred to the lab and accurately weighed with a precision of 0.001 g. Then the samples were shade dried at room temperature (20–25°C) and the yield was calculated for each experimental plot.

Total chlorophyll

The chlorophyll contents were determined by Arnon (22) method. 0.5 g frozen leaf with 80% acetone was homogenized by a mortar and pestle. The homogeneous was filtered over 25 mL volumetric flask. The absorbance was measured at 663 and 645 nm. The chlorophyll content was calculated by the following equation:

$$\text{Total chlorophyll} = 20.2\ (A_{645}) + 8.02\ (A_{663})$$

Anthocyanin content

Frozen leaf tissue (1 g) was homogenized with liquid nitrogen by a mortar and pestle. Then, 30 mL methanolic HCl (1%) was added and rubbing was continued to gain the homogeneous solution. Then, extraction was continued in a dark refrigerator overnight. The extracted solution was filtered and its absorbance was measured at 550 nm. Anthocyanin amount was expressed as μM g^{-1} fresh weight (Ɛ = 33 mM^{-1} cm^{-1}) (23).

Total phenol

The cumin fine powder (0.2 g) was mixed with 20 mL boiling water in a water bath for 1 hour. The extract was filtered over Whatman No.1 paper and was diluted up to 50 mL. Then, 1 mL extract was mixed with 5 mL Folin-Denis reagent. Then, 10 mL saturated sodium carbonate was added and brought to 100 mL with distilled water. The mixture was incubated in the dark and the absorbance was read at 725 nm. The phenol content was calculated based on a gallic acid standard curve (24).

Proline

Proline content in leaf tissue was determined according to Bates et al method (25). The frozen leaf tissue (0.5 g) was crushed in 10 mL aqueous sulfosalicylic acid (3%) and then filtered by Whatman No. 2 paper. The extract (2 mL) was mixed with 2 mL acid-ninhydrin and 2 mL glacial acetic acid in a test tube. The mixture was placed in a boiling water bath for 1 hour. The red solution was extracted with 4 mL toluene and then cooled at room temperature. The absorbance was measured at 520 nm. Proline content was calculated based on a standard curve and expressed as μM g^{-1} fresh weight.

Malondialdehyde (MDA) content

The frozen leaf (0.2 g) was homogenized with 2 mL trichloroacetic acid (TCA) 0.1% and centrifuged at 14 000 rpm for 15 minutes. Then, the supernatant (1 mL) was mixed with 2.5 mL TBA 0.5% in TCA 20% and incubated in a water bath (95°C) for 30 minutes. It was immediately cooled on ice and then centrifuged at 10 000 rpm for 30 min. Absorbance was recorded in 532 and 600 nm. MDA content was expressed as μM g^{-1} fresh weight (155 mM^{-1} cm^{-1}) (26).

Catalase activity (CAT)

Cumin frozen tissue (0.5 g) was rubbed with liquid nitrogen in a mortar by a pestle. Then, 5 mL extraction buffer including 100 mM phosphate buffer, 0.1 mM EDTA, and 2% PVP (pH 7) was added to the mixture and homogenization was continued. The homogenate was centrifuged at 13 000 rpm and 4°C for 20 minutes. The supernatant was separated to determine enzymatic activities (27). The CAT activity was assayed by Cakmak and Horst (28) method. The reaction mixture was included 25 mM phosphate buffer (pH 6.8), 10 mM H_2O_2, and 200 μL extracted enzyme. The absorbance was recorded at 240 nm. The activity of CAT was expressed as mM H_2O_2 per minute per g fresh weight (Ɛ = 43.6 mM^{-1} cm^{-1}).

Peroxidase activity (POX)

The activity of POX was measured by Malik and Singh method (29). The reaction mixture was included 0.25 mL extracted enzyme, 0.25 mL guaiacol, and 5.2 mL phosphate buffer 100 mM (pH 6.8). The reaction was started by adding 25 mL H_2O_2 (5 mM). The absorbance was read at 470 nm. The POX activity was expressed as μM tetraguaiacol per g fresh weight (Ɛ = 26.6 mM^{-1} cm^{-1}).

Statistical analysis

All data were subjected to variance analysis by SAS 9.4 software (SAS Institute, Cary, NC). Means of main factors were compared with protected Fisher's LSD test at $P < 0.05$ and their interactions were compared with Ls means procedure.

Results

Seed yield and dry weight

Irrigation regimens and various nitrogen treatments had a significant effect ($P < 0.01$) on plant dry weight and seed yield. Their interaction had also a significant impact on seed yield ($P < 0.05$) and plant dry weight ($P < 0.01$) (Table 2). Water deficiency reduced the plant dry weight so that water depletion to 40% and 80% from the field capacity reduced the dry weight to 19.3% and 42.7%, respectively, compared to the control. Besides, seed yield reduced to

Table 2. Variance analysis of some measured traits

S.O.V	Df	PDW	SY	CHl	Anthocyanin	Proline	MDA	Phenol	CAT	POX
R	2	24070	2615	0.001	0.00001	1.71	0.030	2.51 [a]	0.03	0.28
Irrigation regimens (I)	2	2584530 [b]	839335 [b]	0.395 [b]	0.007 [b]	660.1 [b]	0.313 [b]	405.9 [b]	3.08 [b]	120.7 [b]
Error a	4	51211	16684	0.003	0.0003	1.60	0.006	1.32	0.02	0.35
Nitrogen (N)	3	455839 [b]	135731 [b]	0.019 [b]	0.011 [b]	22.1 [a]	0.305 [b]	0.89	1.52 [b]	66.2 [b]
I × N	6	162160 [b]	40059 [a]	0.003	0.00098 [b]	22.0 [b]	0.063 [a]	0.20	0.27 [b]	18.03 [b]
Error b	18	27410	14986	0.002	0.00016	4.16	0.014	0.59	0.035	0.88
CV		9.6	12.3	6	6.84	9.87	8.5	6.67	18.03	10.5

PDW: plant dry weight; SY: seed yield; CHl: total chlorophyll; CAT: catalase activity; POX: peroxidase activity; MDA, malondialdehyde.
[a] $P < 0.05$; [b] $P < 0.01$.

20.5% in the middle water stress and 42.1% in the severe water stress as compared to the control group. Nitrogen deficiency (30 kg ha^{-1}) in the control reduced cumin dry weight and seed yield as compared to 60 kg ha^{-1} urea fertilizer. Plant dry weight had not a significant difference with nitroxin inoculation (1541.5 kg ha^{-1}) and the 50% nitrogen (1411.3 kg ha^{-1}) in the middle water stress. Under severe water stress use of 30 kg ha^{-1} urea reduced plant dry weight as compared the other nitrogen treatments (Figure 1). The highest seed yield in the middle water stress was observed at 30 kg ha^{-1} urea + nitroxin (1271.7 kg ha^{-1}). Like to plant dry weight, seed yield was reduced in 60 kg ha^{-1} urea under severe water stress, while other nitrogen treatments had not a significant effect (Figure 2).

Total chlorophyll and anthocyanin content

Irrigation regimens and nitrogen fertilizer had a significant ($P < 0.01$) impact on total chlorophyll and anthocyanin content. Interaction of these factors had also a significant ($P < 0.01$) effect on anthocyanin content (Table 2). Water shortage reduced total chlorophyll as 12% and 38.8% in the middle and severe water stress compared to the control, respectively (Figure 3). In contrary, anthocyanin content increased as 15.3% and 30% by draining 40% and 80%

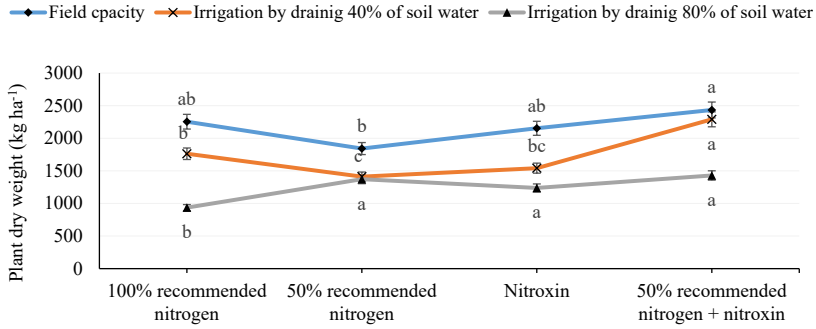

Figure 1. Effect of various nitrogen fertilizers on cumin dry weight under irrigation regimens.

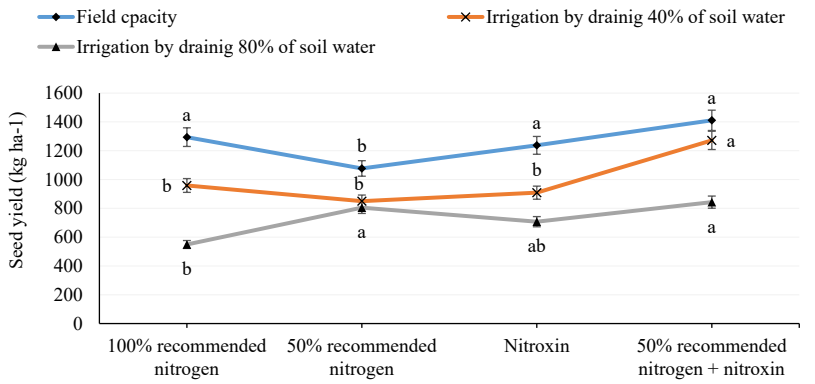

Figure 2. Effect of various nitrogen fertilizers on cumin seed yield under irrigation regimens.

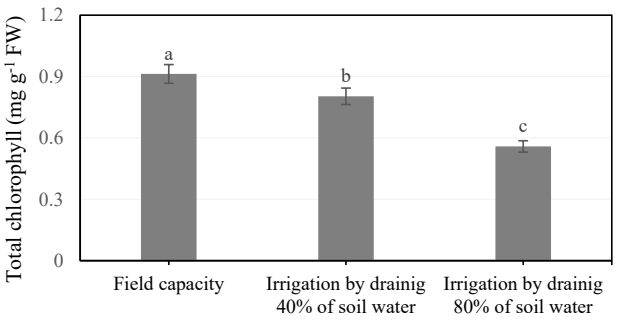

Figure 3. Effect of various irrigation regimens on cumin total chlorophyll.

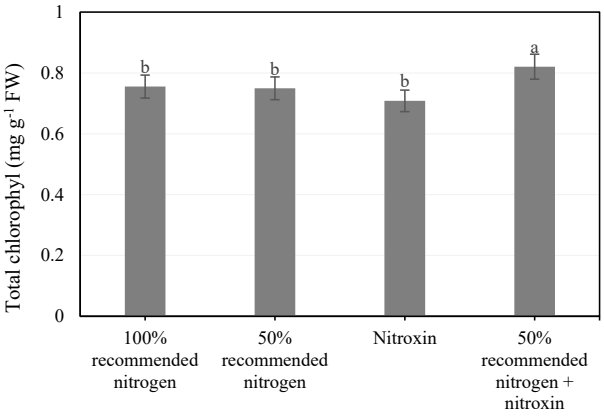

Figure 4. Effect of various nitrogen fertilizers on cumin total chlorophyll.

of soil water compared to the control (0.164 mg g^{-1} FW). Total chlorophyll content had not a significant difference in plant treated by nitroxin, 30 and 60 kg ha^{-1} urea. The highest amount of total chlorophyll was obtained in nitroxin inoculation plus using 50% nitrogen (0.82 mg g^{-1} FW) (Figure 4).

Under good irrigation and the middle water stress conditions, increasing nitrogen application caused a significant increase in anthocyanin content. Severe water stress was responsible for reducing the cumin anthocyanin content in 60 kg ha^{-1} urea application (0.17 µM g^{-1} FW) compared to 30 kg ha^{-1} urea (0.21 µM g^{-1} FW). Nitroxin inoculation alone had not a significant difference with 50% nitrogen in all the irrigation regimens, while nitroxin application plus 50% nitrogen increased significantly the anthocyanin content. Thus, the highest amount of anthocyanin was observed in nitroxin inoculation with using 30 kg ha^{-1} urea in the middle and severe water stress (0.247 and 0.269 µM g^{-1} FW, respectively) (Table 3).

Proline content

The irrigation regimens ($P < 0.01$), nitrogen fertilizers ($P < 0.01$), and their interaction had a significant effect on proline content (Table 2). Drought increased proline content among 47.4% and twice compared to the field capacity (13.6 µM g^{-1} FW). Nitrogen fertilizers had not a significant effect on proline content under severe drought. Under field capacity and the middle water stress, the plant nourished by 60 kg ha^{-1} urea (14.1 and 18.77 µM g^{-1} FW, respectively) encompassed more, but not significant) than the plant fertilized by 30 kg ha^{-1} urea (10.74 and 15.88 µM g^{-1} FW, respectively). Greatest amount of proline in the field capacity and middle drought stress was obtained in nitroxin inoculation (17.32 and 23.98 µM g^{-1} FW, respectively) (Table 3).

Malondialdehyde (MDA) content

The irrigation regimens, nitrogen fertilizers ($P < 0.01$), and their interaction ($P < 0.05$) had a significant influence on MDA content (Table 2). The MDA amount was identical in the control (1.27 µM g^{-1} FW) and middle water stress (1.33

Table 3. The comparison between the mean of various water stress and nitrogen fertilizers on some biochemical traits of cumin

Irrigation regimens	Nitrogen	Anthocyanin	Proline	MDA	POX
Control	N100	0.163 ^{de}	14.1 ^{fgh}	1.083 ^{f}	5.54 ^{fg}
	N50	0.137 ^{f}	10.74 ^{h}	1.339 ^{cde}	3.76 ^{h}
	BN	0.147 ^{ef}	17.32 ^{ef}	1.477 ^{bcd}	4.79 ^{gh}
	BNN50	0.208 ^{b}	12.24 ^{gh}	1.185 ^{ef}	9.32 ^{cd}
Draining 40% of soil water	N100	0.184 ^{cd}	18.77 ^{de}	1.311 ^{cde}	8.5 ^{de}
	N50	0.157 ^{ef}	15.88 ^{efg}	1.207 ^{ef}	6.24 ^{fg}
	BN	0.164 ^{de}	23.98 ^{bc}	1.514 ^{bc}	6.72 ^{f}
	BNN50	0.247 ^{a}	21.53 ^{cd}	1.271 ^{def}	13.16 ^{b}
Draining 80% of soil water	N100	0.167 ^{de}	28.23 ^{a}	1.572 ^{b}	7.14 ^{ef}
	N50	0.212 ^{b}	30.12 ^{a}	1.428 ^{bcd}	15.34 ^{a}
	BN	0.199 ^{bc}	26.84 ^{a}	2.006 ^{a}	10.15 ^{c}
	BNN50	0.269 ^{a}	28.39 ^{a}	1.29d ^{ef}	16.09 ^{a}

The means with the same letters in each column indicate no significant difference between treatments at 5% level of probability. N100: 100% using urea; N50: 50% using urea; BN: Nitroxin; BNN50: Nitroxin + 50% using urea; PDW: plant dry weight; SY: seed yield; CHl: total chlorophyll, CAT: catalase activity; POX: peroxidase activity.

μM g^{-1} FW). Severe water stress increased significantly the MDA content (1.57 μM g^{-1} FW) compared to the control group. The lowest MDA content under full irrigation condition was observed in 60 kg ha^{-1} urea use (1.083 μM g^{-1} FW), which had not a significant difference with nitroxin inoculation along using 30 kg ha^{-1} urea (1.185 μM g^{-1} FW). However, just using nitroxin (1.447 μM g^{-1} FW) or 30 kg ha^{-1} urea (1.339 μM g^{-1} FW) was possessed the highest MDA amount in this condition. The highest MDA content in the middle water stress was acquired by nitroxin inoculation (1.514 μM g^{-1} FW). Under severe water stress, the MDA content had not a significant variation between using 60 and 30 kg ha^{-1} urea. The highest MDA amount in the severe water was observed in the plant inoculated by nitroxin (2.006 μM g^{-1} FW), and its lowest was obtained in nitroxin inoculation with using 30 kg ha^{-1} urea (1.29 μM g^{-1} FW) (Table 3).

Phenol content

Water stress had a significant ($P < 0.01$) influence on the phenol rate. In contrary, the nitrogen fertilizers and interaction with water stress had not a significant impact on phenol amount. Drought conditions increased linearly phenol content. The highest phenol content was observed in the severe water stress (17.9 μg g^{-1} DW) and its lowest was gained in the control group (6.41 μg g^{-1} DW) (Figure 5).

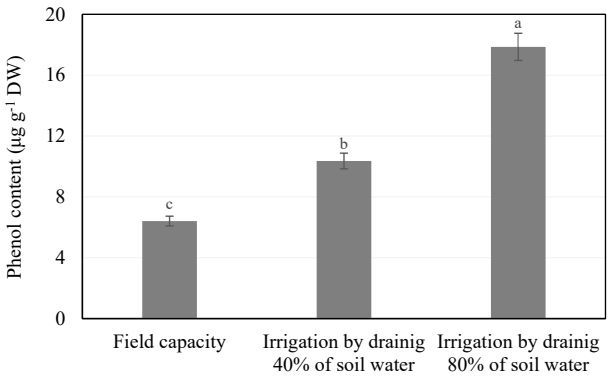

Figure 5. Effect of various irrigation regimens on cumin phenol content.

Catalase activity (CAT)

CAT activity increased significantly ($P < 0.01$) by enhancement draining of water soil (Table 2). The severe water stress caused the highest CAT activity (1.59 mM H_2O_2 g^{-1} FW). In contrast, its minimum activity was observed in the fully irrigated plants (0.6 mM H_2O_2 g^{-1} FW). The CAT activity in nitroxin inoculation, 60, and 30 kg ha^{-1} urea was not significantly different in each of irrigation regimens. The highest CAT activity was obtained in nitroxin inoculation plus use of 30 kg ha^{-1} urea under each irrigation regimen (Figure 6).

Peroxidase activity

POX activity was significantly ($P < 0.01$) affected by the irrigation regimens, nitrogen fertilizers, and their interaction (Table 2). POX activity under the control was the maximum when the plant inoculated by nitroxin plus 30 kg ha^{-1} urea (9.32 μM H_2O_2 g^{-1} FW). Reduction of urea amount under the control and middle water stress caused to decrement POX activity. However, decreasing urea amount in severe water stress increased the POX activity. The highest POX activity was observed in nitroxin inoculation plus using 30 kg ha^{-1} urea (16.09 μM H_2O_2 g^{-1} FW), which was not a significant difference with just using 30 kg ha^{-1} urea (15.34 μM H_2O_2 g^{-1} FW) (Table 3).

Discussion

The results showed that water stress reduced plant dry weight and seed yield. Many studies have shown that drought can reduce plant growth and yield (15,30). Plant productivity is strongly associated with accumulation, partitioning, and transmission of assimilates in drought condition (39). Reducing relative water content and turgor pressure caused to diminish leaves growth because division and elongation of the cells decreased. Moreover, the stomatal opening and conductance, CO_2 fixation, enzyme activity, etc reduced with water shortage (13,31). These agents reduced the plant dry matter production

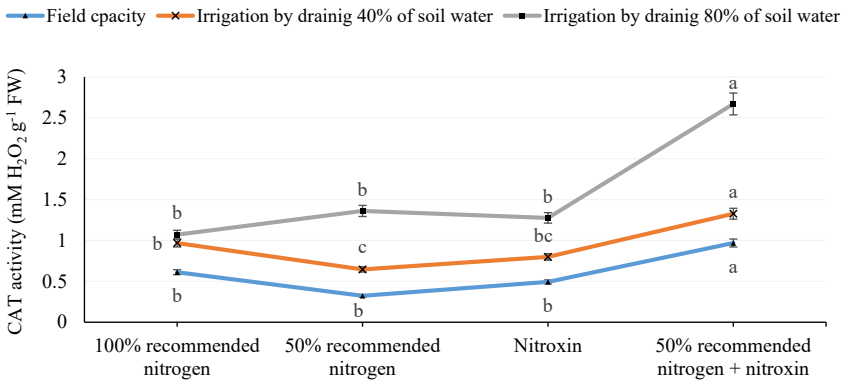

Figure 6. Effect of various nitrogen fertilizers on catalase (CAT) activity under irrigation regimens.

and accumulation. Besides, water losing resulted in boost phloem sap viscosity and the resistance of C flow down. Thus, the assimilated transmission was decreased from the source to the sink. Albeit, the sink strength was also decreased by restricting cell growth, division, and differentiation. (32). Thus, dry matter and seed yield are expected to be decreased with water shortage, which is consistent with the obtained results. Farahza et al (33) showed that field capacity moisture encompassed the highest cumin seed yield, 1000 seed weight, and biomass. Motamedi-Mirhosseini et al (34) reported that the yield of cumin ecotypes was reduced by increasing irrigation interval duration. Vazin (35) also showed that water deficit decreased cumin growth and biomass.

Cumin chlorophyll content was reduced by the water deficit, and in contrary, anthocyanin content increased. The results showed a reduction of plant dry weight and seed yield was connected to chlorophyll content. There are many reports about chlorophyll decreasing in the drought stress conditions (36). Total chlorophyll reduction in drought status pointed to a lesser capacity for light absorbance. Thus, the plant growth and yield would be decreased by a decline in the photosynthetic assimilates (37). It was frequently reported that anthocyanins are accumulated under stress (38). Anthocyanins are capable of absorbing light between 400 and 600 nm. Thus, it is deemed that anthocyanins protected chloroplasts from excess irradiance (39). Moreover, anthocyanins interfered in osmotic regulation because they might regulate water potential. Anthocyanins also can quench ROS, photoprotection, and stress signaling (40).

Proline content, phenol amount and MDA value in leaves were significantly greater under water stress than under the field capacity. It has concurred with other studies (41). The antioxidant enzymes activity of cumin including CAT and POX increased with soil water depletion. Like these results, Zhou et al (17) stated that SOD, CAT, and POX activity in both corn genotypes increased in water stress condition. Salekjalali et al (42) reported that the activities of CAT and POX in barley leaves were increased sharply in flowering and milking stages under water stress. Plants in drought condition expressed more proline biosynthesis genes for moderating the water potential and helped absorb water from the soil (10). Drought stress is provoked ROS generation via disturbance in the electron transport chain, NADPH oxidase activity, etc. Therefore, antioxidant enzymes activities were increased for scavenging ROS. However, one of the major hazards of drought stress is hydroxyl radical production through H_2O_2 reduction by SOD and ascorbate. The hydroxyl radical has a very strong oxidizing potential that reacts with almost any biological molecule. Besides, an enzyme reaction to remove radical hydroxyl is not known. MDA content is a marker for lipid peroxidation by hydroxyl radical (43). Thus, increasing the MDA rate in cumin leaves under drought stress indicated the onset of oxidative stress and destruction of membranes and biological molecules. However, phenolic compounds can quench hydroxyl radical (44), which may be a reason for increasing phenol content with drought stress boosting in this study.

Reduction urea amount in the field capacity and middle water stress decreased plant dry weight and seed yield, while increase urea up to 60 kg ha^{-1} under severe water stress caused to diminish plant weight and seed yield. The soil water content affects the plant's requirements for nitrogen. Thus, plant demand for nitrogen is high in without drought conditions, and in contrary, its demand reduced under drought condition (7). On the other hand, nitrogen overuse in water shortage condition due to increased soil water osmotic pressure resulted in reduced relative water content, chlorophyll amount, photosynthesis rate, leaf area, etc (6). Therefore, deficiency and excessive nitrogen in water shortages could reduce plant growth and yield. Thus, various nitrogen levels are needed to achieve maximum yield in different irrigation regimens. The results of this study showed that in the field capacity, nitrogen enhancing to 60 kg ha^{-1} increased the yield, but under severe drought stress, 30 kg ha^{-1} urea produced the highest yield.

The change of total chlorophyll content was dependent on only water available. So, there was not a significant difference between 60 and 30 kg ha^{-1} urea application under various irrigation regimens. However, anthocyanins rate in the field capacity and middle water stress was reduced with decrement urea to the half. Albeit, it was reverse in the severe water stress and was enhanced. Many authors reported that nitrogen deficiency stimulated anthocyanins accumulation and up-regulation of its synthesis genes in plants (45). It is conformed to the results obtained in the severe water stress.

Nitrogen use increment to 60 kg ha^{-1} in the field capacity raised proline content and reduced MDA amount. Under well-watered condition, nitrogen amount was not impacting on the CAT activity, while POX activity increased by enhancement nitrogen amount. Like to the results, Zhou et al (17) reported that nitrogen supply on adequate irrigation had not a significant effect on proline content in *Malcolmia africana* and *Bassia hyssopifolia*. POX activity in two corn cultivars increased by nitrogen enhancing on adequate water supply. However, MDA content was reduced (46). Under the middle drought stress although proline content was augmented by urea rate enhancing but MDA content had not a significant variation. Also, the activity of enzymes increased in the middle water stress by raising urea rate. Zhou et al (17) reported that under mild water stress (80% field capacity), increment nitrogen caused to raise CAT and POX activity and decline MDA content. Chang et al (47) showed that under middle water stress (70% field capacity), nitrogen rate increment led to reduced antioxidant enzymes activity

and MDA content. The results obtained in this study indicated that MDA and proline contents in severe water stress were not affected by nitrogen amount. POX activity in severe drought stress strongly reduced by decreasing urea, however, CAT activity had not a significant change.

Nitroxin in the full irrigation and middle water stress was capable of substituting 60 kg ha^{-1} urea. However, nitroxin inoculation along with 30 kg ha^{-1} urea had a synergic effect on plant dry weight, seed yield, proline content, and the activity of CAT and POX. Albeit, this synergic effect was more pronounced under the middle water deficit. Some plant growth-promoting rhizobacteria included *Azospirillum* sp. and *Azotobacter* sp. could help drought stress compatibility by biosynthesis and secretion of phytohormones, vitamins, siderophore, etc. Cura et al (48) showed that *Azospirillum* sp. inoculation increased the tolerance of maize to drought stress by reduction ABA, ethylene, and MDA content and in contrary, increment proline content, chlorophyll amount, and RWC value.

Conclusion

Drought stress reduced cumin dry weight, seed yield, and chlorophyll content. In contrast, water stress increased proline content, MDA rate, phenol content, anthocyanins amount, and activities of CAT and POX. Increment use of urea resulted in amending cumin growth and yield in the field capacity. Also, nitrogen use and raising its rate under the middle water stress caused to improve cumin drought tolerance. Proline, and anthocyanins content, CAT and POX activities increased by using urea, hence, the cumin plant could be accumulated more dry matter. However, it was not successful in improving dry matter partitioning to boost seed yield. Under the severe water stress, nitrogen application had not a significant impress on drought acclimation. Nitroxin inoculation with using 30 kg ha^{-1} urea was the most effective treatment to ameliorate seed yield and drought tolerance.

Authors' contributions

All authors contributed to data collection and preparation of the manuscript. ZKP wrote the paper, MAD revised the manuscript, AB designed the experiments, and AMN analyzed the data. All authors read the final version and confirmed it for publication.

Conflict of interests

Authors declare there is not any conflict of interest.

Ethical considerations

Ethical issues (including plagiarism, misconduct, data fabrication, falsification, double publication or submission, redundancy) have been carefully observed by the authors.

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