

## The Effect of Times and Type of Stress Modulator on Quantitative and Qualitative Yield of Cumin under Rainfed and Irrigated Conditions

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### ABSTRACT

A split-plot factorial experiment was conducted with 3 replications to evaluate the impacts of different type and time applications of stress modulators on the yield and yield components of cumin (*Cuminum cyminum* L.) under rainfed and irrigated conditions during 2016-2018. The studied factors were the cultivation method at 2 levels (rainfed and irrigated conditions) as the main plot and times of foliar applications with 2 levels (vegetative and vegetative + flowering growth stage) and sources of stress modulators with the 4 levels (control, 1.5 M Glycine Betaine (GB), 10-7 mM Brassinosteroids (BRs), and 1.5 mM Sodium Nitroprusside (SNP)). The results showed that irrigated cultivation led to the productions of higher plant heights, more lateral branches, larger numbers of umbrellas and seeds per plant, greater 1000-seed weights, more grain and essential oil yields, and less essential oil contents as compared to the treatments undergoing the rainfed condition. The lateral branches, umbrellas per plant, seeds per plant, and 1000-seed weights were affected by the application times. The foliar applications of Brassinosteroids (BRs) increased plant heights, lateral branches, umbrellas per plant, seeds per plant, 1000-seed weights, and grain and essential oil yields with less essential oil contents compared to those of the control. Under the irrigated condition, spraying of BRs enhanced plant height (7.64%), lateral branches (3.57%), umbrellas per plant (14.3%), seeds per plant (47.4%), 1000-seed weight (3.69%), and grain yield (74.5%), and lowered essential oil content (11.22%) when compared to those undergoing the rain-fed condition. Overall, the modulators for reducing drought stress impacts by elevating the yields and yield components could be classified as BRs>GB>SNP.

### Keywords

Brassinosteroids

Cumin

Drought stress

Glycine Betaine

Sodium Nitroprusside

### INTRODUCTION

Cumin (*Cuminum cyminum* L.) is an annual plant belonging to the Apiaceae family. It is one of the most important medicinal plants, which is cultivated in arid and semi-arid climates in many countries, such as those located in the Mediterranean environments, Iran, India, and Egypt [1]. Environmental stresses like drought are considered as one of the major factors limiting the growths and productions of medicinal plants in many parts of the world, as well as Iran. Drought stress restricts plant growth more than any other environmental stresses [2]. Such characteristics as a short growing season, leaf shape, canopy structure, and low water

requirement enable the rainfed cultivation of cumin [3]. However, when the rainfall is lower than the crop requirements, its rainfed cultivation does not produce proper economic yields. In a related study, it was reported that cumin phenology, yield, and yield components were influenced by drought stress. An increasing drought level from 70 to 200 mm was seen to decrease the days to flowering and maturity by 4.66 and 3.83 days, respectively. Branches/plant, number of umbels/plant, and number of umbellets/umbel were observed to have no responses to the drought stress levels, whereas the number of seeds and biological yield dramatically decreased by increasing drought stress [2]. Armin and Miri [4]

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reported higher yields under irrigated compared to rainfed conditions although yield components were not affected by the cultivation method. It was also reported that cumin had higher plant height, number of lateral branches, number of umbrellas per plant, number of seeds per umbrella, biological yield, and economic yield under the irrigated condition compared to dry cultivation [5]. Despite the negative impacts of drought stress on the yield and yield components of cumin, many studies have generally reported enhanced qualities of medicinal plants under drought stress conditions [4-6].

Some agronomic and physiological approaches have been adopted to reduce the harmful effects of drought stress and improve plant tolerance to it. The use of plant hormones is one of the promising and realistic strategies for enhancing crop productivities under stressful conditions [7,8]. Applications of hormones (e.g., BRs or compatible osmolytes Proline, Glycine betaine, etc.), which are capable of modulating the effects of drought stress on crops would provide some efficient ways for alleviating drought stress impacts. BRs are the recently introduced steroidal plant hormones that play an essential role in plant growths and developments. Studies have identified that these compounds are involved in a wide range of physiological processes and morphological reactions, such as stem and root elongations, activation of protein pumps, nucleic acid and protein syntheses, regulations of carbon assimilation and allocation, photosynthesis activation, photomorphogenesis, and flowering initiation [9]. BRs have been also witnessed to take part in plant responses to abiotic stress [10]. Some researchers have suggested that exogenous BRs can alleviate water deficit in such crops as Fennel [11], Sage [12], Marigold [13], Chili pepper [14], and maize [15].

Sodium Nitroprusside (SNP) is another material that has been recently tested to lower stress effects on plants. This compound as a plant growth regulator acting as a mediator has been shown in many studies to be involved in signaling and responding to environmental stress [16,17]. Farooq, *et al.* [18] found drought stress to significantly reduce seedling growth in rice, while treatment with SNP led to an improved plant growth under this condition. They maintained that the external application of nitric oxide could significantly augment the plant water uptake under drought stress. Water stress can be

alleviated by the exogenous application of SNP in crops like safflower [16], thymus [17], and cut leaf ground-cherry [19].

Glycine Betaine (GB) is a quaternary ammonium compound, which is abundantly found in chloroplasts and plays a critical role in regulating and protecting the thylakoid membrane [20]. GB accumulation in response to stress in many crops, including sugar beet, spinach, wheat, and barley, has been already proven [21]. The results of different studies have indicated that the external application of GB can enhance the yields of various plants under water deficit stress conditions even if the intended plant acts as an accumulating one. The reduced adverse effects of environmental stresses by the exogenous application of GB have been further reported in cumin [4], marigold [22], wheat [23], and barley [24]. Several agronomic and physiological methods were employed for alleviating the adverse effects of drought and elevating plant resistance to it. The use of plant hormones as one of the promising and achievable approaches can contribute to the productivity enhancement of crops under stressful conditions. Unfortunately, there are few studies examining the use of such substances as stress modulators in medicinal crops. It would be important to cultivate cumin as a medicinal plant under both rainfed and irrigated conditions and augmenting its yield, especially when the rainfall is below its normal level. Accordingly, this research was conducted to investigate the effects of stress modulators on the yields and yield components cumin under rainfed and irrigated conditions.

## MATERIALS AND METHODS

This study was carried out at a private farm in Jouin County located at 57° 34' East latitude and 36° 22' North longitude with a height of 980 m above sea level during 2016-2018. First, the top soil samples of 0-30-cm depths randomly selected from the soil layers of the experimental site. According to the soil analysis results (Table 1), the studied soil was of a loamy type with a pH of 7.2, EC of 0.72 ds/m, and total N, P, and K contents of 0.02%, 10 mg/kg, and 284 mg/kg, respectively. The data on the rainfalls and mean temperatures during the growing stages of cumin are presented in Table 2.

**Table 1** Physical and Chemical analysis of the field soil

Organic matter	EC	pH <sub>(1:5)</sub>	N	K	P	Texture	Sand	Silt	Clay	Depth (cm)
%	dS/m		%	Mg/kg			%			
0.19	0.72	7.2	0.02	284	10	Loam	46	20	34	0-30

**Table 2** Average temperature and total rainfall in growing season in two years

Month	Average temperature (°C)		Total rainfall (mm)	
	2017	2018	2017	2018
January	5.9	5.76	15	1.6
February	6.5	9.99	49.9	20
March	12.5	17.1	21.4	2
April	18.9	17.9	29.4	32.2
May	26.9	23.3	14.2	17.8
June	30.86	29.52	0	0.7

The split-plot factorial experiment was done based on a randomized complete block design in 3 replicate. The studied factors were the cultivation method with 2 levels (rainfed and irrigated conditions) as the main plot and times of foliar applications with 2 levels (vegetative and vegetative + flowering growth stage) and sources of stress modulators with the 4 levels (control, 1.5 M Glycine Betaine (GB),  $10^{-7}$  mM Brassinosteroids (BRs), and 1.5 mM Sodium Nitroprusside (SNP)) as the sub plot. The experimental field underwent wheat and barley cultivations in the previous first and second years. For soil preparation, the land was plowed in autumn. The fertilizers consisted of  $P_2O_5$  (75 kg/ha),  $K_2O$  (100 kg/ha), and N (100 kg/ha) representing ammonium diphosphate, potassium sulfate, and a form of urea, which were added at sowing under the irrigated condition. In the rain-fed condition, the amount of nitrogen fertilizer was reduced to 50 kg/ha. The experimental units were  $4 \times 1.5$  m with 6 planting rows of 20-cm spaces and a 2-m space between each unit. The local landrace (Sabzevar), which is common and compatible with the region, was used. Cumin was sown in December, 15<sup>th</sup>, 2017 and December, 25<sup>th</sup>, 2018. The first irrigation was done after planting. In irrigated condition, 4 times of irrigation, i.e., at planting, branching, flowering, and seeding stages, were considered, while only once irrigation was regarded at the planting stage under the rainfed condition. After the plant emergence and establishment, its density on the field was arranged to be 1,200,000 plants per hectare. The experimental farm was kept free of weeds by hand-weeding. Cumin seeds germinated on the 18<sup>th</sup>-30<sup>th</sup> of March. 80 days after germination, 50 kg/ha of nitrogen was

added as the top dressing under the irrigated condition. Other cultivation practices were managed according to the crop requirements. Due to the use of stress modulators in this experiment, herbicides and pesticides were not utilized so as to avoid any possible adverse effects on the results. No pests or diseases were observed on the farm during the growing season. The first foliar spraying was carried out on the 4<sup>th</sup> and 15<sup>th</sup> of April and the second foliar application was followed on the 5<sup>th</sup> and 15<sup>th</sup> of May in the first and second years, respectively. During spraying, Triton X100 as a surfactant was applied at the concentration of 1% for better leaf area coverage. At the end of the growing season, 10 plants were randomly selected from each plot and their cumin yield components, including final plant height, number of branches, number of umbels per plant, number of seeds per plant, and 1000-seed weight, were measured. To determine grain yield, as well as biological yields, an area of 2.5 m<sup>2</sup> was harvested in each plot after removing the margins. To measure the essential oil, the Clevenger apparatus was employed based on the steam distillation method. For this purpose, 50 grams of the seeds in each treatment were selected and after being crushed with a laboratory mill, they were dissolved in 500 mL of water for 3 h. The essential oil yield was obtained by multiplying the essential oil percentage by seed yield. Data analysis was performed using SAS software (ver. 9.4) and tables and charts were drawn using Word and Excel software. Mean data was compared with LSD method.

## RESULTS AND DISCUSSION

The analysis of variance revealed that the planting method and external applications of the modulators had significant effects on the final plant height, number of lateral branches, number of umbrellas per plant, number of seeds per plant, 1000-seed weight, economic yield, essential oil percentage, and essential oil yield. The lateral branches, umbrellas per plant, seeds per plant, and 1000-seed weights were significantly affected by the application times. The interaction between the planting methods and application times showed significant impacts on the final height, number of umbels per plant, number of seeds per umbrella, 1000-seed weight, essential oil percentage, and essential oil yield. Although the number of lateral branches, number of umbrellas per plant, number of seeds per umbrella, seed yield, essential oil percentage, and essential oil yield were influenced by the interaction effects of the planting methods and modulators, the interaction between the application times and modulator types had significant impacts only on the number of lateral branches and essential oil yield (Table 3).

### Plant Height

Cultivation under the irrigated condition led to the plant height of 8.4% higher than that achieved under the rainfed condition (Table 3). The reduced plant height under the rainfed condition could be attributed to the lack of turgor pressure for cell division. In line with the above results, Nasrabadi, *et al.* [5] reported a 12% reduction in the final height of cumin under rainfed compared to irrigated condition. It was also reported that in addition to reducing cellular turgidity, which decreased cell division and plant final height, the declined allocations of photosynthetic materials to the stem might result in the lowered plant height [8, 25]. Plant height reduction in *Matricaria recutita* caused by drought stress was also reported by Baghalian, *et al.* [26].

Among osmotic modulators in our research, foliar application with BRs produced the highest plant height, which was not significantly different from the case of GB application. Although foliar application with SNP increased the final plant height compared to the control, it produced less height compared to when other modulators were utilized. It seemed that the reason for the decrease in plant height caused by SNP application was related to the increased SNP concentration (Table 4). This was while some other

results revealed that SNP had negative effects on plant growth and yield at high concentrations [16]. Nevertheless, the enhanced plant height was attributed to the positive effects of BRs on the plant meristematic tissues resulting in its augmented cell number and size [27].

In this study, the plant height had a greater response to the modulators under the rainfed compared to the irrigated condition. Although the use of BRs produced the highest plant height in both planting methods, fewer responses to the stress modulators were observed under the irrigated condition (Table 5). Under the rainfed condition, foliar application with BRs increased plant height by 18.7%, while its height was enhanced by 15.1% under the irrigated condition, indicating that the use of these compounds under the former condition had greater effects on the mentioned component. The same responses were observed for GB and SNP applications. Our results were similar to the findings of da Silva Leite, *et al.* [19], who reported the decreased height of *Physalis angulata* during a water deficit period. Although SNP application at low concentrations (25 and 50  $\mu\text{M}$ ) mitigated drought stress effects on the plant height, increasing SNP above 50  $\mu\text{M}$  further reduced this variable. The decrease in the plant height under the stress condition could be ascribed to the disturbed relations of leaf water and gas exchange. Anyhow, the use of BRs improved growth under the stress condition. BRs ameliorate transports of photosynthetic materials, which stimulate growth and result in an enhanced plant height [28]. The findings of Tanveer, *et al.* [8], who demonstrated that 24-Epibrassinolide promoted or elevated plant growth by increasing carbon assimilation rate and maintaining a balance between ROS and antioxidants, as well as playing a major role in the solvent accumulation and water relationships, further support our conclusions.

### Number of Lateral Branches

Although no statistically significant difference was observed between the rainfed (3.88) and irrigated (4.18) conditions for lateral branches, the irrigated condition resulted in more lateral branches (Table 4). A similar result of fewer branches than normal [26] under drought stress has been shown with Baghalian *et al.*, [9].

**Table 3** Analysis of variance for studied traits in cumin plants.

SOV	df	Plant height	Lateral branches	Umbrella plant	per	Seed per plant	1000 seed weight	Grain yield	Essential content	oil	essential oil yield
Year (Y)	1	395.00 **	11.70 **	1197 **		9563631 **	141 **	497880 **	3.89 **		572 **
Year (rep)	2	10.90	1.59	4.93		3472	0.012	13029	0.06		30.3
Cultivation method (A)	1	59.50 **	2.16 **	47.81 **		1569246 **	0.92 *	4641869 **	0.65 **		649 **
A × Y	1	29.50 **	11.70 *	25.80 *		698344 **	4.04 **	1744393 **	0.74 **		34.81 ns
Error a	4	1.17	0.93	0.58		2085	0.15	10140	0.04		18.21
Time (B)	1	0.24 ns	5.41 **	44.51 **		75141 *	0.86 *	23969 ns	0.06 ns		50.10 ns
Type (C)	3	34.80 **	3.43 **	847 **		2002880 **	0.85 **	1080393 **	2.07 **		1378 **
A × B	1	0.19 ns	0.14 ns	2.34 ns		10938 ns	0.20 ns	91014 ns	2.01 **		237 **
A × C	3	22.70 **	1.11 *	7.82 ns		381913 **	0.90 **	568553 **	0.25 *		28.60 ns
B × C	3	0.49 ns	1.88 **	14.30 *		29849 ns	0.17 ns	6775 ns	0.15 ns		24.61 ns
A×B × C	3	0.19 ns	0.59 ns	19.4 **		1491 ns	0.18 ns	9463 ns	0.18 ns		41.90 *
Y × B	1	8.90 ns	0.02 ns	0.01 ns		154866 **	1.17 *	4710 ns	0.37 *		644 **
Y × C	3	9.25 *	1.23 **	108 **		1455383 **	0.21 ns	297238 **	1.84 **		925 **
Y × A × B	1	0.01 ns	2.53 **	7.59 ns		95933 *	0.19 ns	121902 *	0.15 ns		76.31 *
Y × A × C	3	1.15 ns	1.41 **	19.10 **		292819 **	0.72 *	12683 ns	0.34 **		115 **
Y × B × C	3	0.30 ns	0.39 ns	17.51 **		28348 ns	0.16 ns	32212 ns	0.32 **		137 **
Y × A × B × C	3	0.70 ns	0.66 ns	16.11 **		2631 ns	0.25 ns	8697 ns	0.13 ns		3.84 ns
Error b	-	2.93	0.28	3.85		15806	0.18	31472	0.07		13.77
CV %	-	9.56	13.10	13.38		26.91	5.94	12.61	14.25		14.31

ns: not significant; (\*) and (\*\*) represent significant difference over control at  $P < 0.05$  and  $P < 0.01$ , respectively.

**Table 4** Effect of planting method, time of application and type of stress moderator on yield and yield components of cumin

Experimental factor	Plant height	Lateral branches	Umbrella per plant	Seed per plant	per 1000 seed weight	Seed yield (Kg/ha)	Essential oil (%)	Essential oil yield (Kg/ha)
Cultivation method								
Irrigated	18.71 a	4.18 a	15.40 a	595 a	3.18 a	1627 a	1.75 b	28.81 a
Rain-fed	17.10 b	3.88 a	14.01 b	339 b	2.99 a	1187 b	1.91 a	23.20 b
LSD(0.05)	0.61	0.55	0.43	25.81	0.22	57.10	0.12	2.42
Application time								
Veg	17.91 a	4.26 a	14.01 b	439 b	3.18 a	1423 a	1.86 a	26.90 a
Veg + Flow	18.01 a	3.79 b	15.30 a	495 a	2.99 b	1391 a	1.81 a	25.12 a
LSD(0.05)	0.70	0.22	0.80	51.4	0.17	72.5	0.11	1.52
Stress modulator type								
Control	16.31 c	3.58 b	7.22 d	364 d	3.37 a	1153 c	1.44 c	16.81 d
SNP	17.70 b	3.85 b	12.51 c	441 c	3.02 b	1320 b	1.77 b	22.90 c
GB	18.62 ab	4.35 a	18.80 b	524 b	2.98 b	1528 a	2.02 a	30.61 b
BRs	19.12 a	4.33 a	20.12 a	638 a	2.98 b	1626 a	2.09 a	33.80 a
LSD(0.05)	0.99	0.31	1.13	62.7	0.24	102	0.15	2.14

\* Values followed by the same letter within the same columns do not differ significantly at  $p = 5\%$  based on LSD.

SNP: Sodium Nitroprusside, GB: Glycine Betaine and BRs: Brassinosteroids.

**Table 5** Interaction between planting method and stress modulator type on plant height, number of lateral branches, number of umbrellas per plant, number of seeds per plant, 1000 seed weight and essential oil yield

Modulator type	Plant height (Cm)		Lateral branches		Umbrella per plant		Seed per 1000 seed weight (g)		Grain yield		Essential oil (%)			
	Rain-fed	Irrigated	Rain-fed	Irrigated	Rain-fed	Irrigated	Rain-fed	Irrigated	Rain-fed	Irrigated	Rain-fed	Irrigated		
Control	15.51 d	17.21 bc	3.55 d	3.60 d	6.32 e	8.21 d	23 4f	495 ef	3.27 ab	3.46 a	943 d	1361 c	1.38 d	1.51 cd
SNP	16.82 cd	18.60 ab	3.60 d	4.10 bc	12.50 c	12.62 c	378 de	506 d	2.80 c	3.23 ab	1079 d	1560 b	1.94 b	1.61 c
GB	17.90 bc	19.32 a	3.95 cd	4.75 a	18.41 b	19.21 b	429 c	619 b	2.94 bc	3.02 bc	1313 c	1743 a	2.12 ab	1.91 b
BRs	18.41 ab	19.81 a	4.40 ab	4.25 bc	18.72 b	21.40 a	517 c	760 a	2.93 bc	3.02 a	1411 c	1841 a	2.22 a	1.97 b

\* Values followed by the same letter within the same columns do not differ significantly at  $p = 5\%$  based on LSD.

SNP: Sodium Nitroprusside, GB: Glycine Betaine and BRs: Brassinosteroids.

**Table 6** Interaction between planting method and foliar application time on the number of umbel per plant, essential oil percentage and essential oil yield

Application time	Umbrella		Essential oil (%)		Essential oil yield (t/ha)	
	Irrigated	Rainfed	Irrigated	Rainfed	Irrigated	Rainfed
Veg	14.51 b	13.41 b	1.91 ab	1.79 b	30.81 a	22.51 d
Veg + Flow	16.20 a	14.47 b	1.57 c	2.03 a	26.30 b	24.20 c

\* Values followed by the same letter within the same columns do not differ significantly at  $p = 5\%$  based on LSD.

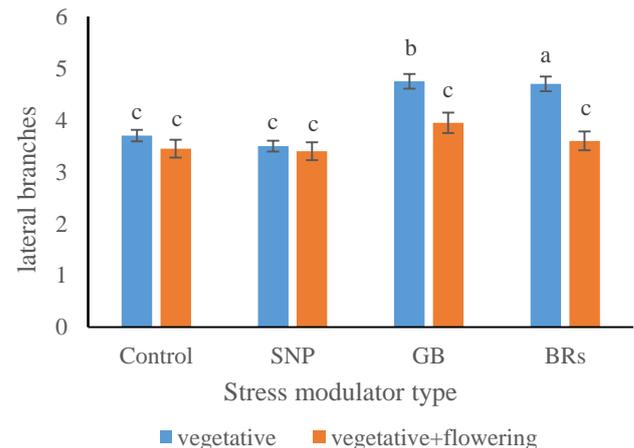
Contrary to our results, Alinian and Razmjoo [2] found that drought stress did not affect the numbers of branches per plant and umbrells per umbel. Armin and Miri [6] reported that more lateral branches were produced under the rainfed condition due to finding more vacant spaces as more plants disappeared during the vegetative period. Under both the rainfed and irrigated conditions, the twice compared to the once foliar application resulted in the growth of more lateral branches. The twice foliar application at the vegetative + flowering periods led to increased numbers of lateral branches by 11.17% and 7.9% under the irrigated and rainfed conditions, respectively (Table 6).

Among the stress modulators, foliar application of BRs produced the largest number of lateral branches, which was not significantly different from the case of GB application. SNP produced statistically similar lateral branches to the control treatment (Table 4). There was a significant and high correlation between plant height and the number of lateral branches. Since the foliar application of BRs produced higher heights, this type of treatment resulted in more lateral branches. Our results were in line with the findings of Jamimoeini [29], who stated that there was no statistical difference between salicylic acid and GB applications in terms of producing lateral branches in cumin, but the use of GB increased the number of lateral branches compared to salicylic acid.

Under both the rainfed and irrigated conditions, the use of stress modulators produced more lateral branches than the control treatment. In both conditions, the highest numbers of lateral branches were produced by using BRs. Compared to the irrigated condition, GB and SNP uses decreased the numbers of lateral branches, whereas the use of BRs increased them under the rain-fed condition. SNP applications were not different in both conditions though increasing the numbers of lateral branches compared to the control treatment (Table 5). These results are in general agreement with those obtained by Mohammadi, *et al.* [12], who reported that drought stress resulted in fewer branches per plant in sage, but foliar application of BRs increased their numbers.

Our twice foliar applications with all the stress modulators in the vegetative + flowering stage reduced the numbers of lateral branches per plant compared to once spraying in the vegetative stage (Fig. 1). In the flowering stage, the production of

lateral branches was completed and thus, the twice foliar applications of the stress modulators did not affect production of any new ones. Since the use of modulators enhanced other yield components, such as the number of umbrellas per plant, most of the photosynthetic materials produced were spent on the developments of the mentioned traits. In this case, due to the remobilization of the materials from bottom to top, the lateral branches were produced at the bottom of the dry canopy and hence, their total numbers were declined.



**Fig. 1** Interaction effects of stress modulator type and application time on lateral branch.

\* Values followed by the same letter do not differ significantly at  $p = 5\%$  based on LSD.

SNP: Sodium Nitroprusside, GB: Glycine Betaine and BRs: Brassinosteroids.

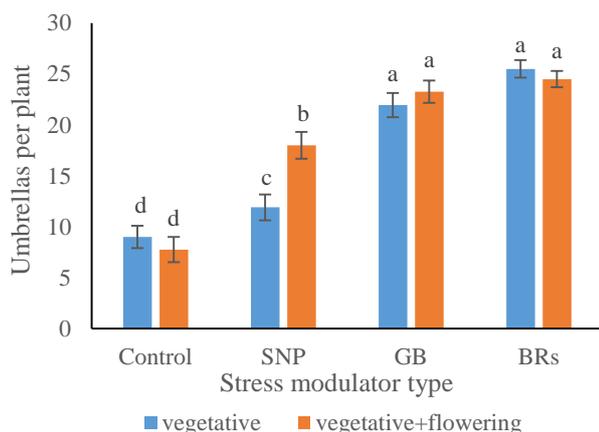
### Number of Umbrellas per Plant

Compared to the irrigated condition, there were fewer umbrellas per plant (14) under the rain-fed condition (15.4) (Table 4). The large number of lateral branches under the irrigated condition was the main reason for increasing the number of umbrellas per plant. Aminpour and Mousavi [30] reported that the number of irrigations played an important role in their different productions under both the rainfed and irrigated conditions in a way that their numbers per plant significantly increased by enhancing the number of irrigations up to 3 or 4 times during the growing season. Nonetheless, they were not different under the rain-fed condition and in the case of twice or 3 times of irrigation during the growth period, which ultimately led to the augmented final yield of the plant. A similar observation was reported for cumin [4,29].

Foliar application of BRs increased the number of umbrellas per plant by 6.48, 60.8, and 179% compared to the applications of GB, SNP, and control

treatment, respectively (Table 4). The greater positive effect of GB increasing the number of umbrellas per plant compared to salicylic acid was reported by Jamimoeini [29], which is consistent with the results of this study.

The number of umbrellas per plant demonstrated the same response as that indicated by the number of lateral branches based on the stress modulator types under the rainfed and irrigated conditions. SNP consumptions were not significantly different in both conditions, whereas the highest incremental response to BRs (14.4%) was well witnessed.



**Fig. 2** Interaction effects of stress modulator type and application time on umbrella per plant.

\*Values followed by the same letter do not differ significantly at  $p = 5\%$  based on LSD.

SNP: Sodium Nitroprusside, GB: Glycine Betaine and BRs: Brassinosteroids.

As shown in Table 5, there was a significant difference between the treatments under the two mentioned conditions and the control treatment, while the irrigated cultivation caused a 30% increase in the number of umbrellas per plant compared to the rainfed cultivation. SNP application produced similar umbrellas per plant in both conditions. Applications of stress moderators could enhance leaf areas because of the delay in leaf deterioration and loss. The enhanced leaf area associated with the plant ability to absorb lighter and thereby improve photosynthetic efficiency was caused by brassinolide application.

Twice foliar application with GB in the vegetative + flowering stage led to the greatest increase in the number of umbrellas per plant (31.9%) compared to once foliar application in the vegetative stage (Fig. 2). However, the twice foliar application with BRs resulted in a 1.25% decrease in the number of umbrellas per plant, while their highest number was observed with once foliar spray over the application

of BRs (Fig. 2). It seemed that the use of hormones, such as BRs, at the vegetative stage produced the same height and number of lateral branches as those achieved by their twice applications in the vegetative + flowering stage. Since there was a self-regulation mechanism in the plant from the standpoint of yield components and the lateral branches showed maximum potential numbers and heights, maximum values were obtained in this treatment, while the increasing numbers of foliar applications had no significant effects on the increasing umbrellas per plant.

### Number of Seeds per Plant

The number of seeds per plant was lower in the rainfed compared to the irrigated condition (Table 4). It seemed that the higher number of seeds per plant in the irrigated condition was due to the larger number of umbrellas per plant. Although the increasing number of umbrellas per plant was associated with the decreasing number of seeds per umbrella, the increase in the number of umbrellas was more than the decrease in the number of seeds per umbrella under the irrigated condition, which led to the augmented number of seeds per plant in that condition compared to the dry season. Our result was supported by the finding of [2,31-33] reporting that drought stress had a negative effect on the number of seeds per plant.

The twice foliar application produced 12.7% more seeds per plant compared to once foliar spray (Table 4). The increasing number of seeds per plant by twice foliar application was associated with leaf area maintenance since the second time of foliar application was performed at the reproductive stage and thus, more seeds could reach their full growth stage. The enhancing leaf area and life in the reproductive stage could also reduce seed loss, which led to an increase in the number of seeds per plant.

The use of BRs resulted in a greater enhancement in the number of grains per plant compared to the use of other stress modulators (Table 4). The increasing numbers of lateral branches and umbrellas per plant were the main reasons for the elevating number of seeds per plant in this treatment. The previous studies have shown that BRs can stimulate plant growth when used at the right dose and stage for plant production [34].

The interaction between the planting methods and stress modulator types showed that the number of

seeds per plant was higher in the irrigated compared to the rainfed condition both in the control and osmotic modulator treatments. Under both the rainfed and irrigated conditions, osmotic adjustments increased the number of grains per plant while foliar application with BRs produced the highest number of grains per plant (Table 5). It seemed that the decreasing number of seeds per plant in the rainfed condition was due to the smaller numbers of lateral branches and umbrellas per plant. Under the irrigated condition, the plant produced more heights and numbers of branches and umbrellas per plant, which resulted in more seeds per plant as well. However, there were slight enhancements in the mentioned traits under the rainfed condition despite the positive effects of stress modulators on the increasing numbers of umbrellas per plant and lateral branches.

### 1000-grain Weight

There was a lower 1000-grain weight under the rainfed (2.99 g) compared to the irrigated (3.18 g) condition (Table 4). The decreasing 1000-grain weight under the rainfed condition could be attributed to the reduced available photosynthetic materials resulted by the leaf falls under the stress conditions. It was reported that water deficit would shorten this time and reduce grain weight during the grain filling period [25].

The use of stress modulators reduced 1000-grain weight (Table 4). It seemed that the main reason for this reduction was the increased number of grains per plant induced by the modulators, which led to the enhanced number of photosynthetic targets and subsequent contribution of each grain to augmenting the amounts of photosynthetic materials.

The highest 1000-grain weight was observed in the irrigated condition without applying the stress modulators (Table 5). The foliar application of BRs lowered the grain yields by 10.39 and 12.71% under the rain-fed and irrigated conditions compared to the control treatment, respectively (Table 5). This implied that 1000-grain weight as one of the components of grain yield was affected by the environment and more strongly by the increases in the other yield components. Although the lowest 1000-grain weight was produced by SNP consumption under the rainfed condition, the consumption of this compound led to the highest changes in 1000-grain weight under the rainfed and irrigated conditions. 1000-grain weight was affected by other yield components, which themselves

underwent fewer changes in this treatment. Therefore, under the irrigated condition, each grain received more photosynthetic materials and hence, the grain weight increased, while it was enhanced by the lower other yield components under the rainfed condition. Contrary to our results, it was reported that the use of GB further increased the weight of 1000 grains per plant compared to salicylic acid application [29].

### Grain Yield

The comparison results of the means showed a higher grain yield under the irrigated compared to the rainfed condition (Table 4). The grain yield decline caused by drought stress could be attributed to the decreased days of flowering and maturity and some yield components [2] besides the inadequate photosynthesis resulted from stomata closure and subsequent reduction in CO<sub>2</sub> absorption [6]. Most studies have shown that drought stress in cumin lowers grain yield, which is congruent with the results of this study [4, 31-33].

Grain yield (1626 kg/ha) was further enhanced by BR treatment as compared to the other modulators although no significant difference was observed between it and GB (1528 kg/ha) (Table 4). BRs improve the absorptions of water and minerals, especially nitrogen, which ameliorates protein synthesis, growth, and ultimately grain yield. BRs inhibit degradation of photosynthetic pigments by activating the enzymes involved in chlorophyll biosynthesis and stimulating their productions [35]. They would enhance photosynthetic carbon fixation efficiency by overcoming stomach constraints and augmenting stomatal conductance [9]. It has been reported that BRs stimulate the synthesis or activation of carbonic anhydrase [36]. The positive impacts of external BRs on the efficiency of photosystem II have been observed in many plants. Recent studies have indicated that the plants treated with BRs express some genes involved in photosynthesis, which can play a critical role in elevating plant tolerance against non-biological stresses. These factors would increase yield [8-10, 37,38]. Similar results have shown that plant treatment with SNP under normal growth conditions (FC=100%) reduce the fresh weight of the shoots by about 56% in safflower, whereas SA-treated plants have been reported to improve the dry weight of roots, shoot length, leaf area, and leaf number as compared to the controls [16].

In this investigation, the interaction between the planting methods and stress modulator types revealed higher grain yields both in the control and osmotic modulator treatments under the irrigated compared to the rainfed condition. In both conditions, the osmotic adjustment application augmented grain yield and foliar application with BRs produced the highest grain yield (Table 5). Generally, achieving more grain yield via obtaining more seeds and umbrellas per plant would be the main objective under irrigated compared to rain-fed conditions.

### Essential Oil Percentage

Compared to the cultivation in the irrigated condition, cultivation in the rainfed condition led to 9.14% more essential oil (Table 4). The enhanced essential oil percentages in rainfed conditions are due to the physiological responses of plants to increase drought resistance. Under these conditions, a plant tries to maintain cellular turgor for its growth and photosynthesis by producing secondary metabolites. In line with our results, Bettaieb, *et al.* [39] showed that under moderate water stress, the essential oil contents of the aerial parts of cumin increased, while being decreased by severe water stress. In contrast, Armin and Miri [4] showed that rain-fed and irrigated conditions produced the same oil contents in cumin. According to the above results, Alinian and Razmjoo, [3] reported enhanced essential oil percent in a susceptible thyme cultivar by increasing drought stress up to 60 and 80% of the field capacity resulting from its elevated drought tolerance, while increasing stress intensity to 40% of the capacity resulted from a decrease in the essential oil percentage.

In this research, the foliar application of BRs and the control treatment produced the highest and lowest essential oil percentages, respectively. There was no statistically significant difference between the amounts of essential oil produced by the applications of BRs and GB (Table 4). The results obtained from earlier studies have indicated the positive effects of 24-eBL application on essential oil contents. In this study, foliar application of up to 1.5 mg/L had significant effects on the essential oil content, whereas enhancing its concentration to more than 1.5 mg/L significantly reduced the essential oil content [34]. 24-eBL stimulates biosynthetic pathway enzymes, such as linalool synthase and linalool acetyltransferase, which elevate productions of essential oils. The same result was reported by

Eskandari and Eskandari [40], who found that 28-homobrassinolid made a significant increase in the essential oil content of savory when applied at a concentration of 10<sup>-6</sup> M and its essential oil components were modified.

The average essential oil percentage obtained in the rainfed condition at all the modulator levels was higher than that produced in the irrigated condition. The highest percentage of essential oil was observed with the foliar application of BRs in the rainfed condition. This was not significantly different from the case of using GB foliar solution. Under the irrigated compared to the rainfed condition, less difference between the essential oil percentages obtained from the foliar treatments was witnessed (Table 5). It has been reported that there is an inverse relationship between the amounts of photosynthetic materials and the production of secondary metabolites. This is indicative of the fact that any factors capable of decreasing the production of photosynthetic materials would increase the percentages of secondary metabolites and essential oils of medicinal plants. Thus, in this research, the rainfed condition provided more essential oil content compared to the irrigated condition [41].

### Essential Oil Yield

The highest (28.8 kg/ha) and the lowest (23.2 kg/ha) essential oil yields were achieved under the irrigated and rain-fed conditions, respectively (Table 4). The low essential oil yield in the rain-fed condition was related to its lower grain yield though there was no statistically significant difference in the essential oil percentages produced under the rain-fed and irrigated conditions. The previous studies have also shown that essential oil yields decrease under drought stress, which is consistent with the results of this research [2, 6,32,39].

In this investigation, foliar applications of stress modulators enhanced the essential oil yields compared to the control treatment, while the highest essential oil yield was produced via the use of BRs (Table 5). The essential oil yield of the plant as reflected by the grain yield and its essential oil content was concomitantly elevated by treating it with BRs.

An increase in the spraying times decreased and increased the essential oil yields in the irrigated and rain-fed conditions, respectively. Despite the decrease in the essential oil percentage in the

vegetative + flowering treatment under the irrigated condition, this type of treatment provided a higher essential oil yield compared to twice spraying under the rain-fed condition (Table 6). It has been reported that there is a high correlation between essential oil yield and seed yield, while essential oil percentage represents little response to agronomic management. Therefore, the treatments leading to higher grain yields would provide higher essential oil yields as well. This finding is in agreement with the results of this study. By increasing the drought severity, cumin essential oil percentage was observed to be enhanced linearly. Hence, the highest and lowest essential oil contents were obtained by the severe drought stress and non-stress treatments, in which irrigations were performed after having 160 and 60 mm of evaporations from the evaporation pans, respectively [42]. Essential oils are terpenoid compounds and their constituents require essential elements, such as nitrogen and phosphorus. Therefore, foliar applications with osmotic modulators can increase their percentages and yields by positively affecting nitrogen and phosphorus uptakes.

## CONCLUSION

Our results demonstrated that cumin treatments with exogenous stress modulators induced its vegetative and reproductive growths under the rain-fed and irrigated conditions. Its reproductive components, such as the numbers of umbrellas and seeds per plant were significantly more responsive to the stress modulators application compared to its vegetative components, including height and number of lateral branches. Grain yield enhancements triggered by the foliar applications of these materials were mainly caused by the increased numbers of grains per plant. The essential oil contents did not respond to the modulator consumptions, but the foliar applications of the modulators augmented the essential oil yields by increasing the grain yields. The times of stress modulators had fewer effects on the vegetative growths, especially in the irrigated condition. The twice foliar application of SNP had negative impacts on the vegetative and reproductive growths, while the use of BRs and GB had more positive effects compared with their once spraying. Overall, twice spraying with BRs provided the best treatments for enhancing cumin yields and yield components in the rainfed and irrigated conditions. Hence, twice application of BRs in rainfed and irrigated conditions can be recommended for augmenting yield in cumin.

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