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Original Article

Exploring the Application of Stress Modulators and Gibberellic Acid on Morphological Traits, Yield and Yield Components of Safflower (*Carthamus tinctorius* L.) in Water-Deficiet Conditions

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ABSTRACT

In order to investigate the effect of stress modulator application on safflower tolerance to water deficit stress, an experiment was conducted at the Research Center for Agriculture and Natural Resources of Balochistan in delete 2020 and 2021. The experiment followed a split-plot design based on a randomized complete block design (RCBD) with three replications. The main plot of the experiment consisted of three levels of irrigation, specifically irrigation after 70, 140 and 210 mm of cumulative evaporation. The sub- plot included six levels of stress modulators and gibberellic acid, namely no-foliar application, glycine betaine, proline, ascorbic acid, and tocopherol. Means comparison interaction effects between years under water deficit stress with foliar application showed that in the first year, the irrigation after 210 mm of cumulative evaporation with the application of gibberellic acid led to a significant increase in the number of seed per boll and seed yield by 11.7 and 13.44%, respectively. A significant increase was observed in the number of seeds per boll with ascorbic acid application, the number of boll per m^2 with tocopherol application, and the seed yield with gibberellic acid application, by 13, 77.4 and 109%, respectively, in the second year as compared with no foliar application.Overall, the findings of this study demonstrate that the foliar application of stress modulators and gibberellic acid effectively mitigated the negative effects associated with irrigation after 210 mm of cumulative evaporation in safflower plants.

Keyword: Water-Deficient Conditions, Glycine betaine, Tocopherol, Pan Evaporation

INTRODUCTION

Safflower (*Carthamus tinctorius* L.), belonges to the Asteraceae, holds the distinction of being one of the oldest cultivated plants globally. Its cultivation serves various purposes, including dye production, flavoring in food, medicinal applications, animal and bird nutrition, and oil extraction from its seeds [1]. In recent times, safflower cultivation has gained significant attention due to the escalating demand for oil production, coupled with its favorable attributes such as adaptability, relative drought resistance (owing to its deep-rooted nature), high-quality seed oil, and versatile applications [1].

Drought, being the foremost constraint in plant growth and agricultural productivity on a global scale, poses a particularly significant challenge in dry and semi-arid regions [2]. The impact of drought on each element contributing to yield formation can result in alterations in seed yield. Insufficient water availability not only affects plant growth directly by causing water scarcity but also hampers the accessibility of essential nutrients [3]. Research findings underscore the variability in seed yield, harvest index, and plant height across different safflower cultivars, with a notable decrease observed under drought stress conditions [4].

The utilization of biostimulants presents itself as a viable strategy to mitigate the impacts of both biotic and abiotic stresses, thereby improving crop yield and quality [5]. Noteworthy among these substances are glycine betaine, proline, ascorbic acid, tocopherol, and gibberellic acid. Glycine betaine, in particular, stands out as the most prevalent compatible amino acid in plants. It aids in enhancing plant tolerance to stress by regulating cell osmotic balance, neutralizing the toxicity of various reactive oxygen species, stabilizing membranes, minimizing cellular damage, and safeguarding numerous enzymes [6]. Glycine betaine assumes the role of an osmotic regulator during stress conditions. Extensive research has demonstrated the beneficial effects of glycine betaine application on canola plants under drought stress conditions [7]. Proline plays a pivotal role in facilitating plant adaptation and tolerance to drought conditions. It acts as a compatible solute, regulating cellular osmotic balance, stabilizing protein and cell membrane structures, scavenging reactive oxygen species (ROS), maintaining cellular pH, and participating in oxidation and reduction reactions [8].Researchers have observed a significant increase in plant height, lateral stem number, and fresh and dry weight of chamomile plants with the foliar application of proline[9]. Ascorbic acid serves as a non-enzymatic antioxidant, capable of neutralizing free radicals and safeguarding cells against oxidative damage [10]. Tocopherols, on the other hand, are crucial antioxidants that efficiently scavenge oxygen radicals and lipid peroxyl radicals in lipid-rich environments [11]. In plants, tocopherols primarily function to protect the photosynthetic apparatus from photodamage induced by oxidative stress [12]. The accumulation of tocopherols in growing plant organs is closely related to the physiological state of the plant and its sensitivity to stressors [13]. In tobacco plants, tocopherols play a role in enhancing cellular adaptability under drought stress conditions [11]. Gibberellic acid, apart from stimulating plant growth, enhances photosynthetic capacity, promotes leaf elongation, and improves tolerance to drought stress [14]. The foliar application of ascorbic acid under drought stress conditions has been shown to increase seed yield in safflower [15]. In recent years, the utilization of external sources of stress modulators and plant hormones, including gibberellic acid, has gained recognition for its highly effective role in mitigating the damages caused by abiotic stresses in plants [16].

Based on the information presented, the current research was conducted to examine the effects of specific stress modulators, namely osmoprotectants (proline and glycine betaine), antioxidants (ascorbic acid and tocopherol), and gibberellic acid, on the morphological traits, yield components, and economic yield of safflower under water deficit stress conditions in the Iranshahr region.

MATERIAL AND METHODS

Experimental Sites

The experiment was conducted at the Research Center for Agriculture and Natural Resources of Balochistan during the farming years of 2020 and 2021. The research center is situated at latitude of 27° and 12 mins North, a longitude of 60° and 41 mins East and an elevation of 591 meters above sea level. Soil samples were collected prior to planting from depths of 0-30 cm and 30-60 cm at the experimental site to assess the physicochemical properties and nutrient requirements of the soil (Table 1).

Table 1 Results of soil analysis

Year	Depth (cm)	Soil texture	Clay (%)	Silt (%)	Sand (%)	potassium (ppm)	phosphorous (ppm)	Total nitrogen (%)	Organic carbon (%)	EC (dSm^{-1})	pH
First	0-30	Sandy loam	6	34	60	240	8.04	0.04	0.41	3.47	7.52
	30-60	Sandy loam	8	29	55	253	7.01	0.06	0.58	2.85	7.63
Second	0-30	Sandy loam	7	34	60	260	10.04	0.06	0.41	3.36	7.54
	30-60	Sandy loam	8	26	55	258	9.01	0.07	0.58	2.87	7.60

EC electrical conductivity

The experiment carried out as a split-plot based on randomized complete block design (RCBD) with three replications. The main plot considered was water deficit stress, which included three irrigation levels based on cumulative evaporation from class A pan evaporation (70, 140, and 210 mm). The sub-factor was foliar application, which comprised non-plot application, foliar application with stress modulators (glycine betaine, proline, ascorbic acid, tocopherol), and gibberellic acid. There were six levels of foliar application, utilizing recommended concentrations provided by the manufacturer. Glycine betaine, proline, gibberellic acid, and ascorbic acid were obtained from the Merck company, while tocopherol was obtained from the Sigma company.

Each experimental plot had dimensions of 6 meters in length and 2 meters in width, resulting in a total area of 12 m^2. The planting arrangement consisted of four rows with a spacing of 50 cm between rows and 15 cm between plants within each row. A 2-meter distance was maintained between replications and main plots to prevent moisture leakage. The safflower variety used in this experiment was "Soffeh". Prior to planting, soil test results were considered, and chemical fertilizers including urea, triple superphosphate, and potassium sulfate were applied at a rate of 100 kg per hectare, with an additional 200 kg per hectare of urea applied as a top dressing in two split doses. Planting operations were manually conducted on ridges on December 6, 2020, and December 9, 2021. Throughout the experiment, necessary activities such as thinking, manual weeding, and pest and disease control were carried out based on the specific requirements of the safflower plants. The first irrigation was applied immediately after planting for all treatments, and water deficit stress treatments were initiated during the stem initiation stage once the plants were fully established. The amount of water consumed for each irrigation level was monitored using hoses and a water meter. The foliar application of stress modulators and gibberellic acid was performed in two stages: at the beginning of stem initiation and at the beginning of flowering.

Assessments

Morphological Traits

To determine the morphological traits, including plant height, number of main stem branches, stem diameter, and head diameter, ten plants were randomly selected from the two middle rows within each plot, taking into account the marginal effect. Traits of stem diameter, and boll diameter were measured using a caliper.

Yield and Yield Components

At the stage of physiological maturity, when the safflower heads turned brown, the middle square meters of plants within each experimental plot were harvested, considering the marginal effect. The number of heads was counted, and the calculation of heads per square meter was performed. Subsequently, the heads were threshed, and seed winnowing was carried out to determine the seed yield. To measure the number of seeds per head, a random selection of 20 heads was made from the harvested plants. After separating the seeds from the heads, the count for this trait was obtained. For the determination of thousand seed weight, three thousand-seed samples were randomly taken from the bulk of winnowed seeds within each experimental plot, using a seed counter device. The samples were weighed precisely using a scale, and the average weight in grams was calculated as the thousand seed weight per plot. To determine the biological yield of each experimental plot, the shoot dry weight was weighed and quantified in kg/ha. Additionally, the harvest index, which represents the percentage of seed yield to the total shoot dry weight (biological yield), was calculated [17].

Statistical Analysis

At the last stage, the collected data underwent analysis (ANOVA) using SAS software version 9.2. To assess the homogeneity of variances, the Bartlett's test was performed. Subsequently, the means of the data were compared using the Duncan's multiple range test, considering a significance level of 5%. Microsoft Excel was utilized for generating figures and plots based on the analyzed data.

RESULTS AND DISCUSSION

Morphological Traits

Plant Height

Based on the information provided, plant height was significantly influenced by both water deficit stress and foliar application, as well as their interaction, in both the first and second years (Table 2). The results demonstrated that water deficit stress had a notable impact on reducing plant height in both years. Specifically, the highest plant height was achieved when irrigation was conducted after 70 mm cumulative evaporation, exhibiting a significant superiority of 19.7% and 43.3% compared to irrigation after 210 mm cumulative evaporation in the first and second years, respectively. Moreover, in both years, the foliar application of stress modulators and gibberellic acid led to an increase in average plant height compared to non-foliar application. However, no significant difference was observed between the effects of stress modulators substances and gibberellic acid (Table 3). When comparing the means of the interaction effect between water deficit stress and foliar application in the second year, it was found that the highest plant height of 153.2 cm was obtained under the condition of irrigation after 70 mm cumulative evaporation with the application of ascorbic acid. This height was statistically similar to the application of glycine betaine, proline, and gibberellic acid within the same group. Conversely, the lowest plant height was observed under the condition of irrigation after 210 mm cumulative evaporation with the application of glycine betaine and non-foliar application, with average heights of 99.9 cm and 100.2 cm, respectively. These values were statistically similar to some treatments within the same group (Fig. 1). Water deficit stress has been found to have a significant impact on soil moisture content, resulting in heightened competition among plants for water uptake. This increased competition prompts plants to allocate a greater proportion of their photosynthetic resources towards their root systems, leading to a reduced transport of photosynthetic substances to the shoots, such as the stem. Consequently, plant height and branching tend to decrease as a result [18]. Conversely, it can be argued that as water deficit stress intensifies, vegetative growth diminishes, and plants transition into the reproductive phase with minimal growth, thus accelerating their growth cycle [18]. Hence, the decrease in vegetative growth observed in the safflower plant, including plant height, number of main stem branches, stem diameter, and head diameter, can be rationalized. A study conducted on Thymus daenensis (*Thymus daenensis*), investigating various levels of water deficit stress, reported a decline in growth characteristics as water deficit stress increased, while the highest values for these traits were observed under normal irrigation conditions [19].

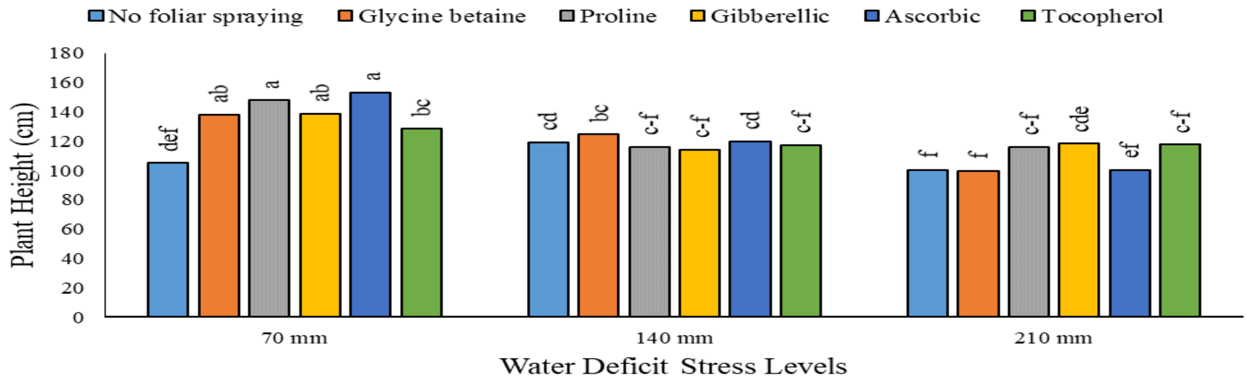

Fig. 1 Comparison of the interaction effects of water deficit stress, foliar application of stress modulators, and gibberellic acid on the height of safflower plants in the second year

Table 2 Analysis of variance for the effects of foliar application of stress modulators and gibberellic acid on morphological traits, yield components, and safflower yield under water deficit stress in two years

Source of variations	DF	Plant Height		Branches Number of Main Stem		Stem Diameter		1000-Seed Weight		Biological Yield	
		First year	Second year	First year	Second year	First year	Second year	First year	Second year	First year	Second year
Replication (R)	2	498.7 ns	66.7 ns	11.41 ns	1.43 ns	1.12 ns	5.42 *	21.48 **	1.89 ns	28.39 ns	569805 ns
Water deficit stress (W*)*	2	30355.3 **	3254.1 **	211.6 *	48.3 *	138.5 *	23.6 *	187.3 *	617.7 **	2097.8 **	91226795.6 **
W × R	4	1414.6	60.4	24.3	5.10	14.94	2.27	21.87	5.47	763.9	325651.7
Spraying(S*)*	5	897.33 *	391.5 **	19.8 *	30.3 **	9.38 *	4.62 *	9.17 **	34.01 **	1100.4 **	25533255.9 **
W × S	10	273.1 ns	388.4 **	1.53 ns	9.63 **	1.22 ns	3.44 *	2.58 ns	18.13 **	251.6 ns	34132651.5 **
Error	30	261.7	86.3	6.19	2.52	2.62	0.52	2.02	3.31	213.2	609792.8
(%)CV	-	11.01	7.68	22.03	12.26	13.25	7.54	6.92	8.73	18.29	9.94

c.v. coefficient of variation, *DF* degrees of freedom, *ns* not significant

*significant at 5%probability level

**significant at 1% probability level

Table 3 Comparison of means for the effects of foliar application of stress modulators and gibberellic acid on morphological traits, yield components, and safflower yield under water deficit stress in two years

	Plant Height (cm)		Branches Number of Main Stem		Stem Diameter (mm)		1000-Seed Weight (g)		Biological Yield (kg/ha)	
	First year	Second year	First year	Second year	First year	Second year	First year	Second year	First year	Second year
Water deficit stress										
70	189.3 a	135.3 a	14.3 a	14.71 a	15.1 a	10.78 a	22.5 a	27.11 a	8136.8 a	9961.0 a
140	143.9 b	118.5 b	11.8 b	12.64 b	11.8 b	9.56 b	22.2 a	19.95 b	7529.8 a	8119.3 b
210	107.3 c	108.7 c	7.61 c	11.47 c	9.68 c	8.49 c	16.8 b	15.50 c	4659.9 b	5482.0 c
Spraying										
No foliar spraying	126.8 b	108.1b	8.36 b	10.9 a	10.17 b	8.52 d	18.57 b	17.3 c	4292 b	5115.5 d
Glycine betaine	148.4 a	120.9 a	11.84 a	11.4 b	12.55 a	9.38 c	20.63 a	20.6 b	5519 b	8941.1 b
Proline	152.8 a	126.6 a	12.10 a	14.1 a	12.97 a	9.30 c	20.62 a	22.5 a	5416 b	7979.8 c
Gibberellic	152.9 a	123.7 a	12.44 a	14.7 a	12.44 a	10.35 ab	21.39 a	22.6 a	8530 a	10131.6 a
Ascorbic	149.0 a	124.5 a	11.28 a	14.9 a	12.67 a	9.67 bc	20.96 a	21.0 ab	8496 a	7573.0 c
Tocopherol	151.1 a	121.1 a	11.72 a	11.4 b	12.53 a	10.44 a	21.08 a	20.7 ab	8400 a	7383.0 c

Mean values of the same category followed by different letters are significant at p≤0.05 level

The Number Branches of Main Stem

The number of primary stem branches was significantly influenced by water deficit stress and foliar application in both the first and second years. Additionally, their interaction also exhibited a significant effect on this trait in the second year (Table 2). The findings indicated that water deficit stress had a significant negative impact on the number of primary stem branches during both years. Specifically, irrigation after 70 mm cumulative evaporation resulted in the highest number of primary stem branches, demonstrating a significant advantage of 22.0% and 46.8% compared to irrigation after 210 mm cumulative evaporation in the first and second years, respectively (Table 3). In the first year, foliar application of stress modulators and gibberellic acid led to an increase in the number of primary stem branches when compared to non-foliar application. However, no significant difference was observed between the effects of stress modulators and gibberellic acid. In the second year, the highest number of branches was achieved through the foliar application of proline, gibberellic acid, and ascorbic acid (Table 3). Comparing the means of the interaction effect between water deficit stress and foliar application in the second year, it was found that the highest number of primary stem branches was obtained when using gibberellic acid with irrigation after 70 mm cumulative evaporation, averaging 17.23 branches per plant. This result was statistically similar to certain treatments at irrigation levels after 70- and 140-mm cumulative evaporation. Conversely, the lowest number of branches was observed in the combination of irrigation after 210 mm cumulative evaporation with the application of tocopherol, averaging 9.93 branches per plant. This result was statistically similar to other foliar application treatments at the same irrigation level, except for the application of proline and the certain treatments at irrigation levels after 70- and 140-mm cumulative evaporation (Fig. 2). Gibberellins are known to stimulate cell division, elongation, or both, thereby promoting the longitudinal growth of shoots [1]. In the context of a study on flax (*Linum usitatissimum* L.), the application of gibberellic acid was found to be beneficial for enhancing plant growth, suggesting its usefulness as a growth stimulant [21]. Glycine betaine plays a role in increasing the osmotic potential within plants, leading to improved water uptake by plant cells and stimulating cell division through the establishment of an osmotic state [7]. As a result, foliar application of glycine betaine has been shown to promote vegetative growth in plants. In a study on *Hyssopus officinalis*, external application of glycine amino acid effectively mitigated the detrimental effects of water deficit stress on vegetative growth and improved the overall growth and yield potential of the plant [22]. Ascorbic acid has been found to mitigate the negative impacts of drought stress and can enhance photosynthesis, ultimately resulting in improved morphological traits, such as increased leaf area, in plants. Researchers have reported significant effects of different levels of ascorbic acid on morphological traits in quinoa plants subjected to drought stress [23].

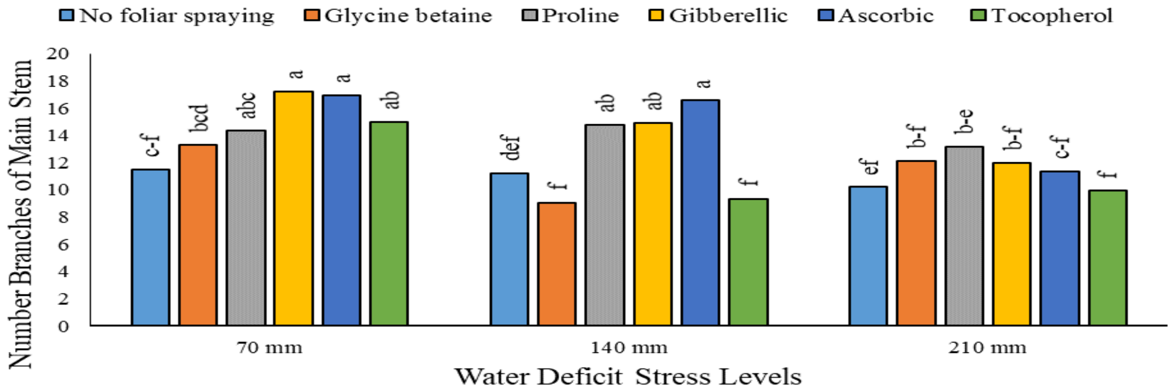

Fig. 2 Comparison of the interaction effects of water deficit stress, foliar application of stress modulators, and gibberellic acid on the number branches main stem of safflower plants in the second year

Stem Diameter

According to Table 2, stem diameter was significantly influenced by water deficit stress and foliar application in both the first and second years. Additionally, their interaction also had a significant effect on this trait in the second year. The results demonstrated that water deficit stress caused a significant decrease in stem diameter during both years. Notably, the largest stem diameter was achieved when using irrigation after 70 mm cumulative evaporation, exhibiting a significant superiority of 21.2% and 35.9% compared to irrigation after 210 mm cumulative evaporation in the first and second years, respectively. In the first year, foliar application of stress modulators and gibberellic acid resulted in an increase in the average stem diameter compared to non-foliar application. However, there was no significant difference observed between the effects of stress-alleviating substances and gibberellic acid. In the second year, the highest stem diameter of 10.44 mm was obtained through the foliar application of tocopherol, which, although not statistically different from the application of gibberellic acid, displayed a statistically significant superiority compared to other levels of foliar application (Table 3). When comparing the means of the interaction effect between water deficit stress and foliar application in the second year, it was observed that the highest stem diameter, with an average of 11.99 mm, was achieved when using gibberellic acid with irrigation after 70 mm cumulative evaporation. This result was in the same statistical group as the application of ascorbic acid. Furthermore, under the condition of irrigation after 210 mm cumulative evaporation, the application of each compound, except for glycine betaine, significantly increased this trait compared to non-foliar application. Conversely, the smallest stem diameter, averaging 5.97 mm, was obtained under the condition of irrigation after 210 mm cumulative evaporation without foliar application (Fig. 3). The decrease in seed count in the head under water deficit stress conditions can be attributed to the sensitivity of most plants to stress during the reproductive stage. Water deficiency during this critical stage leads to a reduction in the production of photosynthetic materials and fertility, resulting in a decrease in the number of seeds produced in the head [24]. The increased competition between physiological processes, specifically vegetative and reproductive processes, and embryo abortion under water deficit conditions also contribute to the decline in seed count in the head [25]. The reduction in photosynthesis, assimilate production, and the impaired transport of photosynthetic materials further contribute to the decrease in seed count in the head under these conditions [24]. In the case of safflower, it is likely that drought stress results in a reduction in the number of lateral branches, which subsequently leads to a decrease in the formation of heads on the plant per unit surface area. Additionally, a significant number of lateral branches may be lost before flowering due to the effects of drought stress. These combined factors contribute to the observed decrease in seed count in the head under water deficit conditions [26].

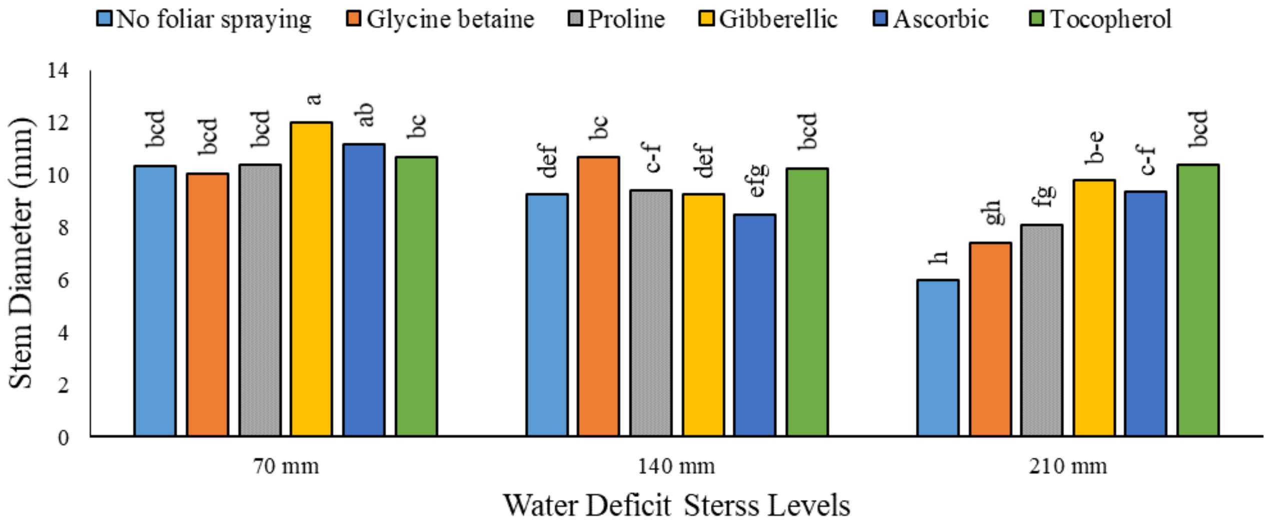

Fig.3 Comparison of the interaction effects of water deficit stress, foliar application of stress modulators, and gibberellic acid on the stem diameter of safflower plants in the second year

Boll Diameter

The diameter of the boll was found to be significantly influenced by water deficit stress and foliar application, as indicated in Table 4. The results revealed that the highest boll diameter was observed in the irrigation treatment after 70 mm cumulative evaporation, with an average measurement of 2.78 cm. This measurement displayed a significant superiority of 31.1% when compared to the irrigation treatment after 210 mm cumulative evaporation. Furthermore, the application of all compounds resulted in a significant increase in the mean boll diameter in comparison to the non-foliar application condition. Specifically, the foliar application of glycine betaine, proline, ascorbic acid, tocopherol, and gibberellic acid led to an increase in boll diameter by 9.7%, 8.9%, 3.8%, 9.7%, and 10.5%, respectively, when compared to the non-foliar application condition (Table 5). The seed filling stage is a critical period in seed-bearing plants that strongly influences the thousand seed weight. Any form of stress, such as heat or drought, during this stage can diminish the potential seed yield by reducing the thousand seed weight. Water deficit stress occurring during the seed filling stage specifically hampers plant photosynthesis, leading to source limitation. Since a large number of seeds are being formed as the storage organ within the plant, there may not be an ample source available to supply the required photosynthetic materials, thereby resulting in a decrease in the thousand seed weight. Furthermore, water deficit conditions can impede the plant's ability to extract the necessary moisture from the soil due to decreased soil moisture levels. Consequently, the plant may experience nutrient deficiency since the required nutrients are primarily available in the soil. This nutrient deficiency further depletes the essential photosynthetic reserves necessary for seed filling, thereby contributing to a decrease in the thousand seed weight [18]. Studies conducted on safflower plants have demonstrated a reduction in both the thousand seed weight and seed yield under water deficit stress conditions [27].

Source of variations	DF	Boll Diameter	Seed Number per Boll	Boll Number Per m^2	Seed Yield	Harvest Index
Year	1	0.0005 ns	116.45 ns	33836.8 ns	8861756.7 ns	2978.9 ns
Y(R)	4	0.080	260.38	1212.6	64813.7	79.88
Water deficit stress(W)	2	3.87 *	1522.2 *	333854.4 ns	8624799.1 ns	164.4 ns
Y × W	2	0.015 ns	52.55 ns	32879.1 ns	820358.5 ns	252.8 ns
YW (R)	8	0.131	228.1	18035.1	520932.8	216.3
Spraying(S)	5	0.147 *	91.34 ns	39843.0 ns	5060311.1 ns	275.1 ns
Y × S	5	0.030 ns	26.11 ns	17687.1 ns	2530679.7 ns	94.8 ns
W × S	10	0.079 ns	70.23 ns	11011.7 ns	1829467.3 ns	634.7 ns
Y × W × S	10	0.042 ns	15104 **	19519.3 **	2815611.4 **	807.3 **
Error	60	0.026	24.60	4160.4	360323.8	81.97
CV (%)	-	6.63	11.78	13.27	25.24	25.10

Table 4 Combined analysis of variance for the effects of foliar application of stress modulators and gibberellic acid on morphological traits, yield components, and safflower yield under water deficit stress

c.v. coefficient of variation, *DF* degrees of freedom, *ns* not significant
*significant at 5%probability level
**significant at 1% probability level

Yield Components

Seed Number per Boll

The number of seeds per boll was significantly influenced by water deficit stress and the interaction effect of the year in water deficit stress in foliar application, as indicated in Table 4. Upon comparing the means for the three-factor interaction, it was found that the treatment involving irrigation after 70 mm cumulative evaporation and the application of tocopherol resulted in the highest number of seeds per boll in the second year, with an average of 58.19. This treatment exhibited a statistical difference when compared to the irrigation treatments after 70 mm cumulative evaporation and the application of gibberellic acid, proline, and glycine betaine in both the first and second years, as well as the treatment involving irrigation after 70 mm cumulative evaporation and the application of ascorbic acid in the second year. All of these treatments fell within the same statistical group. Conversely, the lowest number of seeds per boll in the second year was observed in the treatment involving irrigation after 210 mm cumulative evaporation and the foliar application of tocopherol, with an average of

27.66. However, there was no statistical difference between this treatment and some other treatments in both the first and second years (Fig.4). In a study conducted on cumin plants under dryland conditions, the application of stress modulators, including glycine betaine, exhibited positive effects by increasing the number of seeds per plant [28]. The increase in thousand seed weight and seed yield observed with the application of gibberellic acid can be attributed to its beneficial impacts on nutrient uptake improvement and enhanced transport of assimilates to the seeds during the seed filling stage [14]. Gibberellic acid not only stimulates plant growth but also enhances photosynthetic capacity, leaf elongation, and drought stress tolerance [14].

Table 5 Means comparison of the combined analysis for the effects of foliar application of stress modulators and gibberellic acid on the boll diameter of safflower plants under water deficit stress

	Boll Diameter (cm)
Water deficit stress	
70	2.78 a
140	2.47 b
210	2.12 c
Spraying	
No foliar spraying	2.28 b
Glycine betaine	2.5 a
Proline	2.46 a
Gibberellic	2.52 a
Ascorbic	2.47 a
Tocopherol	2.5 a

Mean values of the same category followed by different letters are significant at p≤0.05 level

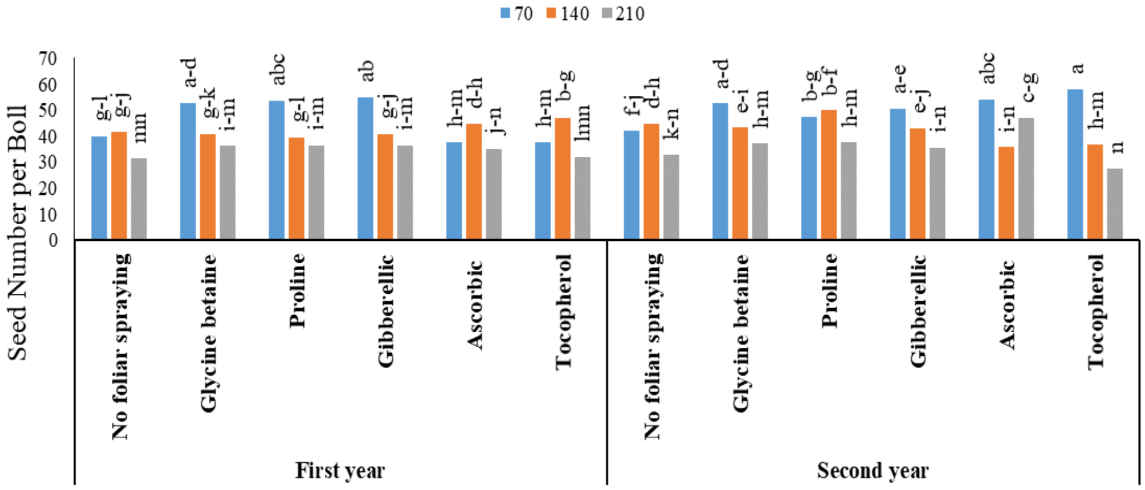

Fig. 4 Comparison of the interaction effects of water deficit stress, foliar application of stress modulators, and gibberellic acid on the seed number per boll of safflower plants

Boll Number per m^2

The number of bolls per square meter was significantly influenced by the interaction effect of the year in water deficit stress in foliar application, as presented in Table 4. When comparing the means for the three-factor interaction, it was observed that the treatment involving irrigation after 70 mm cumulative evaporation and the application of ascorbic acid in the first year yielded the highest number of bolls per square meter, with an average of 818.67. This treatment exhibited a statistical difference when compared to the irrigation treatments after 70 mm cumulative evaporation and the application of gibberellic acid and tocopherol in the second year, as well as the treatment involving irrigation after 70 mm cumulative evaporation and the application of tocopherol in the first year. All of these treatments fell within the same statistical group. On the other hand, the lowest number of bolls per square meter, with an average of 105.33, was observed in the treatment involving irrigation after 140 mm cumulative evaporation and the application of proline in the second year (Fig. 5). Consequently, these effects contribute to an increase in the thousand seed weight. Furthermore, the response of thousand seed weight to foliar application of stress modulators, such as the amino acid proline, can be attributed to the increased accessibility of seeds to photosynthetic materials. Another study reported that the foliar application of the amino acid glycine betaine resulted in increased thousand seed weight and seed yield in cumin plant [29].

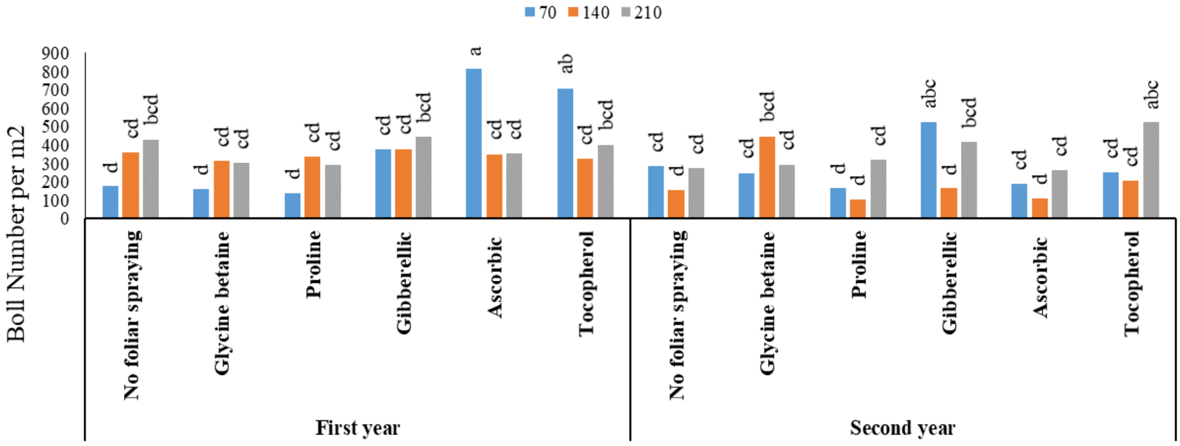

Fig. 5 Comparison of the interaction effects of water deficit stress, foliar application of stress modulators, and gibberellic acid on the boll number per m^2 of safflower plants

1000-seed Weight

The thousand seed weight was significantly influenced by water deficit stress, foliar application, and their interaction in the first and second years (Table 2). The results revealed that water deficit stress caused a substantial reduction in the thousand seed weight during both years. The irrigation method after 70 mm cumulative evaporation resulted in the highest thousand seed weight, exhibiting a significant superiority of 25.3% and 42.8% over the irrigation method after 210 mm cumulative evaporation in the first and second years, respectively (Table 3). In the first year, the foliar application of stress modulators and gibberellic acid led to an increase in the thousand seed weight compared to non-foliar application, although no significant difference was observed among the various stress modulators and gibberellic acid. In the second year, the highest value for this trait was associated with the foliar application of proline, which exhibited no statistical difference when compared to the application of gibberellic acid, ascorbic acid, and tocopherol. However, it demonstrated a statistical superiority of 8.9% and 23.5% over the application of glycine betaine and non-foliar application, respectively (Table 3). Examining the interaction effect of water deficit stress and foliar application in the second year, the highest thousand seed weight was observed under irrigation after 70 mm cumulative evaporation in the treatments involving proline and gibberellic acid, with mean values of 32 g and 29.8 g, respectively. Conversely, the treatment of irrigation after 210 mm cumulative evaporation and non-foliar application yielded the lowest value for this trait, with a mean of 11 g. When irrigated after 210 mm cumulative evaporation, all compounds significantly increased the thousand seed weight compared to non-foliar application (Fig. 6). In a scholarly study, an argument was put forth to explain the decline in biological yield observed in the presence of water deficit stress. This reduction may be attributed to two factors: the preferential allocation of resources to the roots due to limited soil moisture, and a decrease in chlorophyll content, thereby leading to diminished photosynthetic efficiency [30]. Under favorable irrigation conditions, an increase in dry matter production was observed, which can be attributed to the expansion of leaf surface area and an enhanced longevity of leaves. These factors contribute to the creation of an efficient biological source for the optimal utilization of received light, thereby leading to a significant accumulation of dry matter [31]. Another investigation focusing on the medicinal plant Moldavian dragonhead also documented a decline in biological yield when subjected to water deficit stress [32].

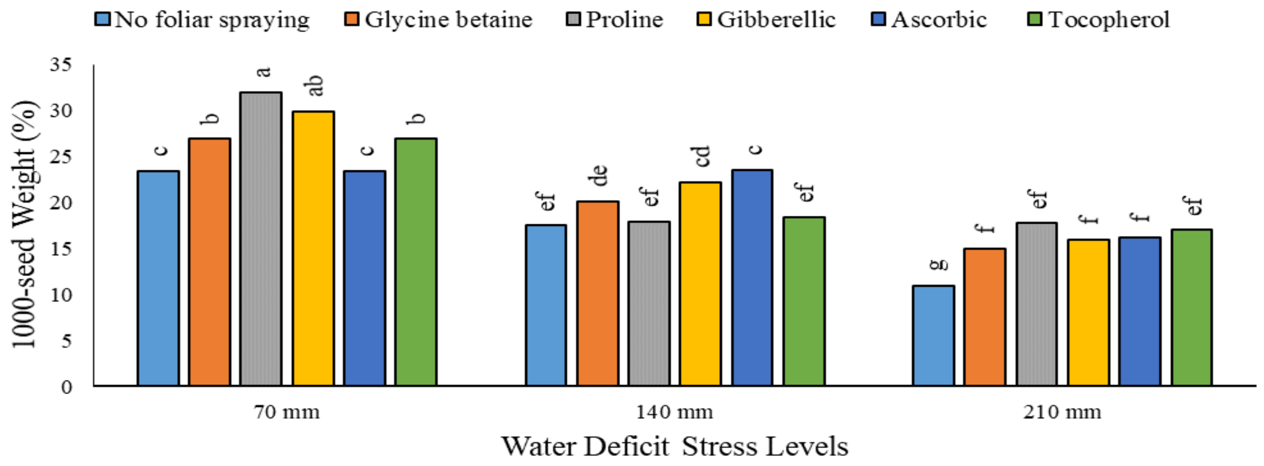

Fig. 6 Comparison of the interaction effects of water deficit stress, foliar application of stress modulators, and gibberellic acid on the 1000-seed weight of safflower plants in the second year

Seed Yield

The seed yield demonstrated a significant influence from the interaction effect of the year, water deficit stress, and foliar application (Table 4). Comparing the means for the three-factor interaction, the highest seed yield in the second year was achieved through the combination of irrigation after 140 mm cumulative evaporation and the application of gibberellic acid, with an average yield of 4593.3 kg per hectare. This yield showed a statistically significant difference from the seed yield obtained in the treatments involving irrigation after 70 mm cumulative evaporation and the application of ascorbic acid, gibberellic acid, and tocopherol in the first year, with average yields of 4526.1 kg/ha, 4389.6 kg/ha, and 4384.1 kg/ha, respectively, falling within the same statistical group. Conversely, the lowest seed yield in the second year was observed when using irrigation after 140 mm cumulative evaporation with the application of ascorbic acid, resulting in an average yield of 871.4 kg per hectare. This yield exhibited a statistical difference when compared to certain treatments, including irrigation after 140 mm cumulative evaporation with the application of proline, as well as irrigation after 210 mm cumulative evaporation and non-foliar application in the second year, all belonging to the same statistical group (Fig. 7). The utilization of gibberellic acid has been found to yield significant benefits in terms of plant growth parameters. Its application has been observed to stimulate shoot growth, ultimately resulting in enhanced biological yield in plants [33]. Notably, a study focusing on rose Golpar plants reported an increase in biological yield following the application of gibberellic acid [34]. The experimental findings strongly suggest that the foliar application of both gibberellic acid and glycine betaine, particularly under water deficit stress conditions, effectively redistributes assimilate allocation between vegetative and reproductive organs. This redistribution is achieved through the promotion of increased leaf area, prolonged leaf longevity, and heightened plant photosynthesis. Consequently, this mechanism significantly contributes to an overall improvement in biological yield, even under water deficit stress conditions. Moreover, amino acids play a pivotal role in facilitating nutrient absorption, thereby providing a solid foundation for cell enlargement. Consequently, under stress conditions, amino acids have been observed to induce an increase in plant weight by promoting cell enlargement [35]. Supporting this, another study reported an increase in shoot weight in Rose geranium plants exposed to moisture stress conditions with the application of glycine betaine [36].

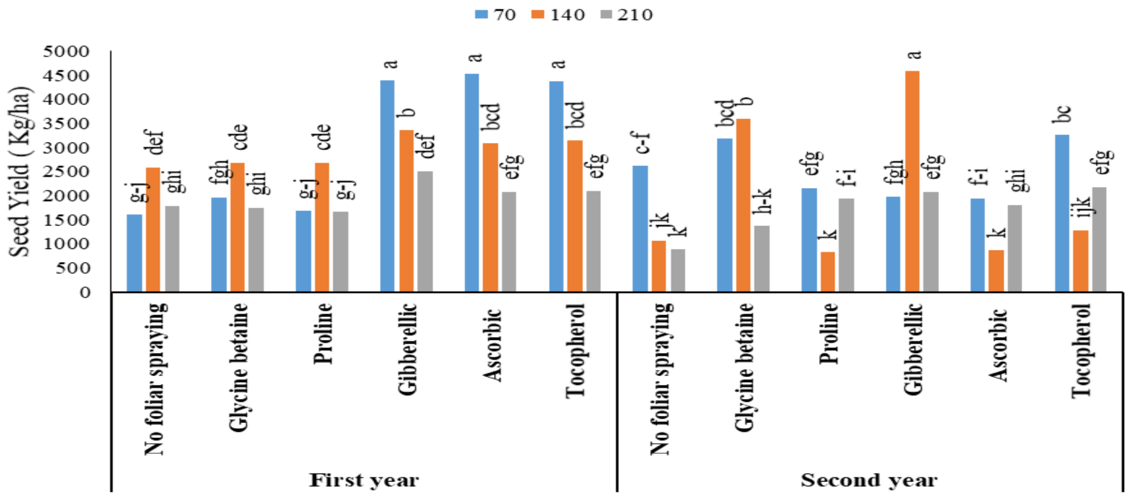

Fig. 7 Comparison of the interaction effects of water deficit stress, foliar application of stress modulators, and gibberellic acid on the seed yield of safflower plants

Biological Yield

The biological yield exhibited a significant influence from the interaction effect of the year, water deficit stress, and foliar application in both the first and second years (Table 2). It was evident that water deficit stress caused a substantial reduction in biological yield during both years. The highest biological yield was achieved through the irrigation after 70 mm cumulative evaporation, displaying a significant superiority of 42.7% and 45% over irrigation after 210 mm cumulative evaporation in the first and second years, respectively. In the first year, foliar application of gibberellic acid, ascorbic acid, and tocopherol resulted in a significant increase of 98.7%, 98%, and 95.7%, respectively, in biological yield compared to non-foliar application. In the second year, the treatment involving foliar application of gibberellic acid showcased the highest level of this trait, demonstrating a significant superiority over other foliar application levels (Table 3). Examining the means for the interaction effect of water deficit stress and foliar application in the second year, the highest biological yield was observed under irrigation after 70 mm cumulative evaporation with the application of gibberellic acid and glycine betaine, with mean values of 11663.4 kg/ha and 11015.6 kg/ha, respectively. Furthermore, under irrigation after 210 mm cumulative evaporation, only the application of tocopherol significantly increased the biological yield. Conversely, the lowest biological yield was obtained when using irrigation after 70 mm cumulative evaporation and non-foliar application, with an average of 4374.2 kg/ha. It exhibited a statistical difference when compared to specific treatments, including irrigation after 140 mm cumulative evaporation and non-foliar application, as well as irrigation after 210 mm cumulative evaporation and non-foliar application, and the use of all compounds except tocopherol, all falling within the same statistical group (Fig. 8). Indeed, drought stress imposed at any stage of plant growth inevitably leads to a decline in the seed harvest index. Under conditions of moisture stress, there exists a direct correlation between seed yield and the seed harvest index. This correlation suggests that enhanced plant growth can be achieved in the presence of moisture stress through three distinct pathways: increased water uptake, improved water use efficiency, and ultimately, a higher seed harvest index. Researchers support this relationship by highlighting the role of deep and extensive root systems in absorbing water from the soil profile, thereby facilitating water uptake. The significance of the other two factors becomes apparent when all available soil moisture is naturally utilized and depleted by the conclusion of the plant growth period. Furthermore, it is worth noting that drought stress has a detrimental impact on the seed harvest index [37].

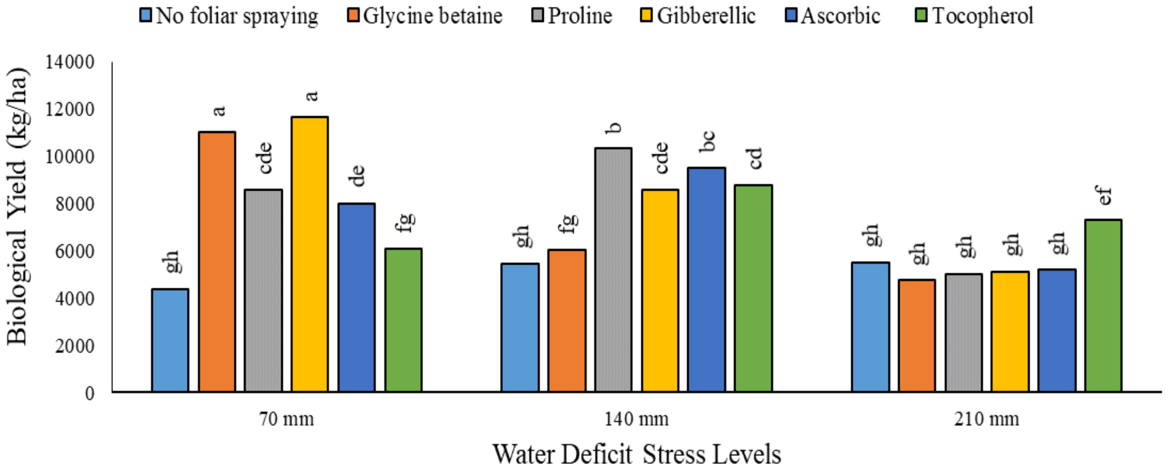

Fig. 8 Comparison of the interaction effects of water deficit stress, foliar application of stress modulators, and gibberellic acid on the biological yield of safflower plants in the second year

Harvest Index

The harvest index demonstrated a significant influence from the interaction effect of the year and water deficit stress in foliar application (Table 4). Comparing the means for the three-factor interaction, it was observed that the highest harvest index, with an average of 37.44%, was observed in the second year under the irrigation after 70 mm cumulative evaporation and non-foliar application. However, this value did not show a statistically significant difference from the treatments involving irrigation after 140 mm cumulative evaporation with the application of gibberellic acid and glycine betaine, as well as irrigation after 70 mm cumulative evaporation with the application of tocopherol, all falling within the same statistical group. Conversely, the lowest harvest index in the second year was obtained when using irrigation after 140 mm cumulative evaporation with the application of ascorbic acid and proline, with average values of 8.4% and 7.5%, respectively. This result exhibited a statistical difference from certain treatments in the second year, all falling within the same statistical group (Fig. 9). Under water deficit stress conditions, the foliar application of glycine betaine can potentially increase the allocation of photosynthetic materials to the seeds. This is achieved by delaying plant senescence, maintaining leaf surface, and extending the seed filling period, thus resulting in an increased harvest index [38]. Some plants can also utilize accumulated reserves in the stem prior to pollination, in addition to current photosynthesis, to contribute to seed filling and consequently enhance the harvest index. Among the treatments examined, the highest harvest index was observed when gibberellic acid was applied under moderate stress conditions. Gibberellic acid is a plant growth regulator hormone that has diverse and varying effects on the growth and development of many plants [39]. Consistent with the findings of this study, the highest harvest index was associated with moderate moisture stress and the foliar application of hormones, while the lowest harvest index was observed under severe moisture stress conditions without foliar application of hormones [40].

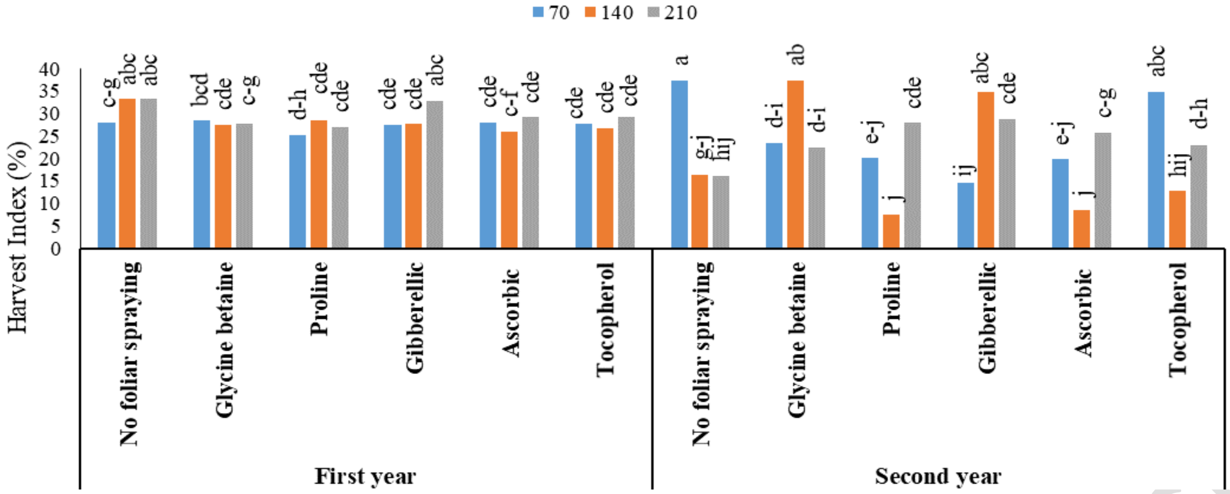

Fig. 9 Comparison of the interaction effects of water deficit stress, foliar application of stress modulators, and gibberellic acid on the harvest index of safflower plants

CONCLUSION

The findings of the present study indicate that water deficit stress had a significant negative impact on the average values of various morphological traits, yield components, and plant yield. However, the foliar application of stress modulators and gibberellic acid resulted in an increase in the average values of many of these mentioned traits compared to the no-foliar application of these compounds. Moreover, under conditions of severe water deficit stress (irrigation after 210 mm cumulative evaporation), the foliar application of stress modulators led to an increase in the average values of specific traits. Furthermore, the results demonstrated that the highest average values for most traits were observed under irrigation conditions after 70- and 140-mm cumulative evaporation, particularly when gibberellic acid was used.

Conflict of Interest

M. Barahouei, S. GH. R. Moosavi, M. J. Seghatoleslami, R. Bradaran and S.M. Javadzadeh declare that they have no competing interests.

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