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Assess the Effect of Humic Acid Foliar Application on the Seed yield Its Components of Cowpea (*Vigna unguiculata*) under Low Irrigation Conditions in Iran (Southern Khuzestan)

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ABSTRACT

BACKGROUND: Water is the limiting factor for the production of agricultural products in arid and semi-arid climatic conditions, including Iran, and its optimal use is very important. The use of natural fertilizers, including humic acid without harmful environmental effects, can be effective to increase yield.

OBJECTIVES: Current study was done to investigate the effect of different levels of low irrigation and humic acid foliar application on cowpea crop production.

METHODS: This research was conducted according split plot experiment based on randomized complete block design (RCBD) with three replications. The main factor included different levels of low irrigation (I_1:70 or control, I_2: 90, I_3: 110 and I_4: 130 mm of evaporation from the surface of the Class A evaporation pan) and secondary factor included different amounts of humic acid fertilizer (F_1: none use or control, F_2:4 lit.ha-1, F_3: 6 lit.ha^{-1}).

RESULT: The difference between different levels of low irrigation and humic acid in terms of plant height, pod length, number of sub-branches, number of pods per plant, number of seeds per pod, weight of 1000 seeds, seed yield, biological yield and harvest index are statistically significant at the probability level of 1% was significant. The interaction effect of treatments on plant height and number of pods per plant was significant at 1% probability level and pod length, 1000 seed weight and seed yield was significant at 5% probability level. The highest seed yield belonged to the irrigation treatment of 70 mm evaporation and foliar spraying with 6 lit.ha^{-1} with an average yield of 3755 kg.ha^{-1}. The lowest seed yield was obtained from the irrigation treatment of 130 mm of evaporation and without foliar spraying with an average of 987 kg.ha^{-1}. The highest and lowest harvest index belonged to the irrigation treatment of 70 and 130 mm of evaporation pan, respectively (with an average of 41.25 and 25.67%).

CONCLUSION: Based on the results of the research, foliar application of 6 lit.ha^{-1} of humic acid at different levels of low irrigation increased seed yield compared to the control (no foliar application).

KEYWORDS: *Crop production, Nutrition, Organic matter, Pulse, Water stress.*

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1. BACKGROUND

Legumes are the second source of human food after cereals and the main source of vegetable protein. Among legumes in terms of cultivated area, the first place belongs to beans (Davoodi *et al*., 2018). Cowpea is one of the legumes that is cultivated in tropical and subtropical countries, especially Asian, African and South American countries, and is considered as an important source of nutrition. Among legumes, the first place belongs to beans in terms of cultivated area and economic value (Kocheki and Banayan Aval, 2007). Water is the limiting factor for the production of agricultural products in arid and semi-arid climatic conditions, including Iran, and its optimal use is very important. The economics and management of water resources require that the unit volume of water be used to the maximum. In the current situation where there is a shortage of irrigation water, it is very important to know the reaction of plants and determine the sensitivity of different growth stages to water shortage (Hashemi Dezfuli, 2008). Iran is classified as a dry and semi-arid region with an average rainfall of 250 mm, so the occurrence of drought stress during the growth period of agricultural products is inevitable (Tadayyon, 2009). The amount of drought damage to plants depends on factors such as intensity and duration of stress, plant species and plant growth stage (Jaleel *et al*., 2009). Drought, as the most important abiotic limiting factor of growth, has an adverse effect on the growth of agricultural plants (Zhang *et al*., 2003; Cheong *et al*., 2003). Drought stress occurs when the humidity around the root decreases to such an extent that the plant is unable to absorb enough water, or in other words, when transpiration occurs more than water absorption (Benjamin, 2007). In the conditions of mild drought stress, plants are able to prevent or tolerate stunting and prevent a severe reduction in growth with the help of different mechanisms, but in severe stress conditions, due to a severe reduction in cell mass, growth and cell division, it leads to a decrease in vegetative growth of the plant (Ghafari and Pashapour, 2006; Goksoy *et al*., 2004; Rahimzadeh *et al*., 2010). Soil moisture stress is effective on many plant processes such as photosynthesis, cell development and division, and the accumulation and transfer of nutrients in the plant (Boyer and Mcpherson, 1998). The amount of soil moisture causes fluctuations in the rate of photosynthesis of plants and affects the performance of plants through the reduction of stomatal conductance (Ziaiee *et al*., 2017). One of the first reactions to water stress is growth reduction. In general, plants have evolved through morphological and physiological solutions to be able to withstand drought stress (Jazizadeh and Mortezaiee Nejad, 2017). The evaporation pan is one of the most common evaporation meters, and the standard class A pan is a galvanized iron cylindrical pan with a diameter of 121 cm and a height of 12.4 cm (Kocheki *et al*., 1993). Evaporation from this pan is influenced by all atmospheric factors such as air temperature, wind, relative humidity and solar radiation, so it shows short-term fluctuations better than experimental methods that are used to estimate the evaporation and transpiration (Fadaiean, 1996). Nevertheless, and especially in dry climates, one should be careful in relating the amount of evaporation from the pan to the actual evaporation and transpiration. In humid areas, pans can provide a more realistic estimate of potential evaporation and transpiration. Usually, water consumption by crops in such climates is about 60 to 90% of evaporation from the pan (Kocheki *et al*., 1993). A potential tool for improving environmental performance in farms is the use of humic acids as plant stimulators (Popescu and Popescu, 2018). The use of natural fertilizers, including humic acid without harmful environmental effects, can be effective to increase yield. Therefore, humic acid is called as nature-friendly organic fertilizer. Humic acid is one of the most important fertilizers used in plants. Humic acid is a natural polymer that has H^+ positions related to carboxylic benzoic and phenolic acid agents (Cation Exchange Citation) (Rahi *et al*., 2012). One of the ways to reduce the harmful effects of dehydration is to use compounds that increase the tolerance of plants to environmental stresses. Humic acid can be mentioned among these compounds. Humic acid has a high molecular weight, with a natural source and yellow, black and white colors and is resistant to decomposition (Larcher, 2003). Humic acid is one of the best natural chemical products that not only increases cation exchange capacity (CEC) or nutrient holding capacity, but also absorbs calcium and other nutrients in forms that are easy for plants to absorb. Humic acid creates more space for water infiltration by physical modification and soil granularity. In addition, humic acid molecules form bonds with molecules that prevent water evaporation to a large extent (Mirhajian, 2012). Also, folic acid molecules (small part of humic acid molecule) penetrates into plant tissues and by binding to water molecules, it reduces plant transpiration and helps to preserve water inside the plant (Bronick and Lai, 2005).

OBJECTIVES

Considering the positive effect of humic acid in improving plants' response to water deficit stress, this experiment was planned and implemented in order to investigate the effect of humic acid foliar application on the yield and yield components of cowpea under irrigation conditions in southern Khuzestan.

3. MATERIALS AND METHODS

3.1. *Field and Treatments Information*

This research was conducted according split plot experiment based on randomized complete block design (RCBD) with three replications. The main factor included different levels of low irrigation (I_1:70 (control), I_2: 90, I_3: 110 and I_4: 130 mm of evaporation from the surface of the Class A evaporation pan) and the secondary factor included different amounts of humic acid fertilizer (F_1: none use or control, F_2:4 and F_3: 6 liters per hectare). The humic acid used has a liquid phase with the brand name Hume Fert Ultra, including 12% humic acid, 3% folic acid and 3% potassium oxide (K_2O) from Arman Sabz Adina. The levels of stress treatments were applied according to cumulative evaporation from the Class A evaporation pan after the three-leaf stage, and this process continued until harvest. The desired concentrations of humic acid were applied before the beginning of flowering (from the emergence of the third leaflet to the bud formation) in two occasions with an interval of two weeks. Foliar spraying was done after sunset to prevent rapid evaporation of the solution and one to two days before irrigation to maximize the absorption of the solution by the plant. Foliar spraying amounts were based on the consumption of 300 liters per hectare of water after adding different amounts of humic acid using an atomizer sprayer.

3.2. *Farm Management*

Tillage operations including primary irrigation and semi-deep plowing, disc, troweling and fertilizing (urea fertilizer at the rate of 80 kg per hectare, 50 kg of which is preplanting (base) and 30 kg per hectare at the four-leaf stage as root, 80 kg of pure phosphorus from the source of ammonium phosphate and 80 kg of pure potassium from potassium sulfate as a base) were given to the ground. This experiment was carried out in atmosphere and stack in 3 repetitions and 12 treatments and 36 plots. Each experimental unit consisted of 7 planting rows, each 5 meters long, and the distance between the planting rows (stacks) was 0.65 meters, and the width of each plot was 5 meters. Also, the distance between the plants on the row was 15 cm (according to the custom of the region) and the distance between two repetitions was 2 meters. The distance between the two main plots was three uncultivated lines and the distance between the two subplots was two uncultivated lines. In the first half of July 2018, cowpea seeds were planted manually in rows and stacks with a density of 10 plants per square meter. Irrigation method was done in the form of leakage (river and stack). Also, after the greening of the field, according to the cultivation of three seeds in each planting point, in order to reduce intraspecies competition, thinning operation was performed. Thinning of the seedlings was done manually when the seedlings had four leaves after the establishment of the field, and during the growth stages of cowpeas from the time of four leaves simultaneously with the thinning operation and then at different time stages, it was tried by weeding the weeds from the beginning of the establishment. All the weeds in the field should be controlled until the final harvest of beans. Finally, the final harvest was done on November 5, 2018, when the leaves had completely turned yellow, in an area of two square meters from each plot, and after removing the edges, it was done manually.

3.3. *Measured Traits*

3.3.1. *Plant height*

To determine the plant height, the height of 5 plants was randomly measured from the base to the end of the stem and their average was calculated as the plant height for each plot.

3.3.2. *Number of sub-branches*

The number of sub-branches of 10 plants from each plot was counted and its average was known as the number of branches for each plant.

3.3.3. *Pod length*

To calculate length of pods, after collecting all cowpea pods from cultivation lines 2, 3 and 4, 20 complete pods were randomly selected from each plot and the average pod length was determined in centimeters.

3.3.4. *Number of pods per plant*

To determine the number of pods per plant, 20 plants were randomly separated from all harvested plants and their pods were counted, and their average was considered as the number of pods per plant.

3.3.5. *Number of seeds per pod*

To determine the number of seeds per pod, 20 pods were randomly separated from all harvested pods and their seeds were counted, and their average was considered as the number of seeds per pod.

3.3.6. *1000 seed weight*

To determine the 1000 seed weight in each treatment, first 500 seeds were counted and their weight was calculated. Then another 500 seeds were counted and weighed. If the difference in the weight of two samples was less than 5%, their total weight was considered as the 1000 seed weight.

3.3.7. *Seed yield*

To calculate the seed yield, after removing the border lines, all the pods in planting lines 2, 3 and 4 with an area of 2 square meters were collected and after separating the seeds from the pods, the seeds were placed in an oven at a temperature of 75 degrees for 48 hours. and the seed yield was calculated based on kg per hectare.

3.3.8. *Biological yield*

In order to calculate the biological yield, after the final maturity, the plants in the middle three rows of each experimental plot were removed from the area of 2 square meters and placed in plastic bags. After transferring these samples to the laboratory and drying them in the oven (for 48 hours at 72 degrees Celsius), they were weighed and thus the biological yield or total dry matter per unit area of each experimental plot was determined and generalized to hectares.

3.3.9. *Harvest index*

The harvest index in each treatment was calculated by dividing the seed yield by the biological yield of that treatment in terms of percentage.

3.4. *Statistical Analysis*

Analysis of variance and mean comparisons were done via SAS (Ver.8) software and Duncan multiple range test at 5% probability level.

4. RESULT AND DISCUSSION

4.1. *Plant height*

The effect of drought stress, humic acid and their mutual effect on plant height was statistically significant at the probability level of one (Table 1). The highest plant height belonged to the irrigation treatment of 70 mm evaporation with an average of 123.01 cm and the lowest with an average of 90.44 cm to the irrigation treatment of 130 mm evaporation (Table 2). The highest height of the plant in the treatment of foliar spraying with 6 liters per hectare was achieved with a height of 113.14 cm and the lowest height of the plant in the treatment of no application of foliar spraying with an average of 100.22 cm (Table 2). The maximum height of the plant was obtained from the irrigation treatment of 70 mm evaporation and foliar spraying with 6 liters per hectare of humic acid with an average of 125.83 cm. Statistically, with the treatments of 70 mm evaporation and foliar spraying with 4 liters per hectare and 90 mm evaporation and foliar spraying with 6 liters per hectare There was no significant difference in liters per hectare (Table 3). The lowest height of the plant belonged to the irrigation treatment of 130 mm evaporation and no foliar spraying with 82.47 cm. With the increase in the intensity of drought stress, the height of the bean plant also decreased, but it is possible to increase the height of the plant to some extent by increasing the amount of humic acid foliar application in each of the stress conditions; So that the irrigation treatment of 130 mm of evaporation with foliar spraying of 6 liters per hectare was not significantly different from the irrigation treatment of 110 mm of evaporation and no foliar spraying. With the increase of drought stress, the competition for water between the plants increases, so the plant allocates a greater share of photosynthetic materials to the roots, as a result, less photosynthetic materials reach the aerial part, including the stem, which reduces the height of the plant, which is consistent with the results of the research.

Table 1. Result of analysis of variance effect of treatment on studied traits

S.O.V	df	Plant height	Pod length	Number of sub-branch	Number of pod per plant	Number of seed per plant
Replication	2	1.21^{ns}	0.8274^{ns}	1.26^{ns}	2.4042^{ns}	0.6953^{ns}
Water stress	3	1775.43**	32.2451**	62.806**	44.8463**	29.40**
E_a	6	31.89	0.4182	4.164	1.2384	0.4522
Humic acid	2	529.12**	15.2822**	23.903**	13.5974**	12.94**
Water stress × Humic acid	6	26.28**	0.7112*	0.274^{ns}	0.0364^{ns}	0.8305**
E_b	16	3.82	0.2191	0.244	0.1742	0.0824
CV (%)	-	12.95	14.79	16.32	17.07	17.11

^{ns}, * and **: non-significant, significant at 5% and 1% of probability level, respectively.

Continue **table 1**.

S.O.V	df	1000 seed weight	Seed yield	Biological yield	Harvest index
Replication	2	169.78^{ns}	3700^{ns}	475^{ns}	48.934^{ns}
Water stress	3	1281.7**	76814**	173507**	412.79**
E_a	6	37.27	816	912	17547
Humic acid	2	215.63**	24057**	39690**	178.92**
Water stress × Humic acid	6	9.03*	516*	334^{ns}	6.696^{ns}
E_b	16	3.22	154	1503	3.664
CV (%)	-	6.52	36.94	19.36	21.19

^{ns}, * and **: non-significant, significant at 5% and 1% of probability level, respectively.

Zabet and Hoseinzadeh (2011) who stated that the decrease in plant height is the reason that drought stress reduced cell divisions and reduced the vegetative growth of the plant was consistent. Sadeghipour and Aghaei (2012) stated that in the condition of drought stress, the water flow around the growing cells decreases, which stops the elongation of these cells. Also, in the condition of water shortage, the secretion of cytokinin hormone from the root is reduced and through the reduction of cell division, the height of the plant is reduced (Lalinia *et al*., 2012). The height of the plant is considered an indicator of vegetative growth, which due to the significant increase of this attribute due to the use of biological fertilizers and water, the vegetative growth and biomass yield are also improved (Pazoki, 2016). One of the mechanisms of humic substances that leads to an increase in longitudinal growth is related to its gibberellin-like compounds; As a result, during the studies, the application of humic acid in the form of foliar spraying and soil application increased the hormones of auxin, cytokinin and gibberellin in the plant, and with its effect on the metabolism of plant cells, as well as with its chelating power and increasing the absorption of nutrients, it increased growth and height. becomes a plant (Naderi *et al*., 2002). The use of humic acid causes the growth of aerial organs, which is due to the increase in the absorption of elements such as nitrogen, calcium, phosphorus, potassium, manganese, iron, zinc and copper (Erkossa *et al*., 2002). Humic acid increases plant height through hormonal effects and by influencing plant cell metabolism, as well as by chelating power and increasing the absorption of nutrients (Dadnia, 2017).

4.2. *Pod length*

The effect of drought stress and humic acid on pod length was statistically significant at the one percent probability level and their interaction was significant at the five percent probability level (Table 1).

The maximum pod length was obtained in the irrigation treatment of 70 mm of evaporation with an average of 15.5 cm, and the lowest was observed with 11.2 cm in the irrigation treatment of 130 mm of evaporation (Table 2). The maximum and minimum length of pods in the treatment of foliar spraying with 6 liters per hectare and no foliar spraying were obtained with an average of 14.32 and 12.19 cm, respectively (Table 2). The highest length of the pod belonged to the irrigation treatment of 70 mm evaporation and foliar spraying with 6 liters per hectare of humic acid (with an average of 16.07 cm) and the lowest length belonged to the irrigation treatment of 130 mm evaporation and no foliar spraying (with an average of 9.51 cm) (Table 3). Although the pod length decreased with the increase of the stress intensity, but the use of humic acid was able to partially compensate the negative effects of the stress and increase the pod length. The results of this experiment are with the findings of Beheshti *et al*. (2017) who stated pod length decreased with increasing drought stress and the longest pod in the control treatment without stress (50 mm evaporation) and the shortest pod in the severe drought stress treatment (110 mm evaporation) was observed, it was consistent.

Table 2. Mean comparison effect of different level of treatment on studied traits

Treatment	Plant height (cm)	Pod length (cm)	Number of sub-branch	Number of pod per plant	Number of seed per plant
Irrigation					
I_1	123.01_a	15.5_a	19.45_a	15.55_a	12.66_a
I_2	113.82_b	14.45_b	17.68_b	14.43_b	12.2_a
I_3	103.02_c	12.76_c	14.89_c	12.04_c	10.53_b
I_4	90.44_d	11.2_d	13.63_d	10.64_d	8.68_c
Fertilizer					
F_1	100.22_c	12.19_b	14.84_c	11.99_b	9.83_b
F_2	109.36_b	13.92_a	16.82_b	13.43_a	11.46_a
F_3	113.14_a	14.32_a	17.57_a	14.07_a	11.76_a

*Mean which have at least once common letter are not significant different at the 5% level using (DMRT).
I_1:70 or control, I_2: 90, I_3: 110 and I_4: 130 mm of evaporation from the surface of the Class A evaporation pan.
F_1: none use or control, F_2:4 lit.ha^{-1} F_3: 6 lit.ha^{-1}.

Continue **table 2**.

Treatment	1000 seed weight(g)	Seed yield (kg.ha^{-1})	Biological yield (kg.ha^{-1})	Harvest index (%)
Irrigation				
I_1	194.37_a	3460.9_a	8360.5_a	41.25_a
I_2	188.93_b	3022.2_b	7854.3_a	38.25_b
I_3	179.02_c	2076.3_c	6106.4_b	33.84_c
I_4	167.24_d	1411.4_d	5445.0_c	25.67_d
Fertilizer				
F_1	177.61_b	1989.3_b	6301.1_b	30.35_b
F_2	183.85_a	2642.4_a	7109.8_a	36.33_a
F_3	185.7_a	2846.4_a	7413.8_a	37.57_a

*Mean which have at least once common letter are not significant different at the 5% level using (DMRT).
I_1:70 or control, I_2: 90, I_3: 110 and I_4: 130 mm of evaporation from the surface of the Class A evaporation pan.
F_1: none use or control, F_2:4 lit.ha^{-1} F_3: 6 lit.ha^{-1}.

Due to the shedding of flowers during the occurrence of drought stress and the decrease in the number of pods with increasing intensity of drought stress, the physiological indicators are reduced and as a result, the distribution of photosynthetic materials among the pods is less. This has followed the downward trend of pod length reduction and caused no significant difference between high stress levels. Also, by increasing the concentration of humic acid, the length of the pod in the nightingale bean was increased, so that the maximum length of the pod was increased by 2.7% compared to the application of 3 liters per hectare and 14.8% compared to the control. And it was consistent with Beheshti *et al*. (2017) on beans.

4.3. *The number of sub-branches*

The trait of the number of sub-branches plays an important role in terms of forming the number of pods in the plant, the number of seeds and seed yield. The results of analysis of variance showed that the effect of drought stress and humic acid on the number of sub-branches was statistically significant at the probability level of 1%, but their mutual effect was not significantly different (Table 1). The highest number of sub-branches was observed in the irrigation treatment of 70 mm of evaporation with the number of 19.45 sub-branches and the lowest with the number of 13.63 sub-branches in the irrigation treatment of 130 mm of evaporation (Table 2). The highest number of sub-branches was obtained in foliar spraying with 6 liters per hectare (with an average of 17.57 sub-branches) and the lowest in the no-foliar treatment with an average of 14.84 branches (Table 2). The results of this experiment were consistent with the findings of Saeidi Aboueshaghi *et al*. (2014) who stated that in the irrigation treatment of 50 and 75 mm of evaporation, the number of secondary branches of bean increased and in the irrigation of 100 mm of evaporation, it decreased. Lack of water caused a decrease in photosynthesis, a decrease in the expansion of the surface of the leaves, and the lack of formation of new branches. It seems that the increase in inter-species competition, the decrease in absorption of food and water were among the effective factors in reducing the number of sub-branches under stress conditions. The decrease in the number of branches due to the increase in density and lack of water has been confirmed by other researchers (Cox, 1996; Torabi Jefrodi *et al*., 2008; Bashteni, 1997). The results of this experiment were consistent with the findings of Veisi *et al*. (2019) that the highest number of main branches was assigned to the treatment of application of five $kg.ha^{-1}$ of humic acid. The increase in branch growth and plant height under the effect of humic acid has been attributed to the increase in plant nitrogen content (Ayas and Gulser, 2005).

Table 3. Mean comparison of interaction effects of different level of treatment on studied traits

Irrigation	Fertilizer	Plant height (cm)	Pod length (cm)	Number of seed per plant	1000 seed weight (g)	Seed yield ($kg.ha^{-1}$)
I1	F1	120.44_{ab}	14.95_{b}	12.31_{b}	192.42_{a}	3072.5_{c}
	F2	122.77_{a}	15.49_{ab}	12.74_{ab}	194.7_{a}	3555.1_{ab}
	F3	125.83_{a}	16.07_{a}	12.93_{ab}	195.98_{a}	3755.1_{a}
I2	F1	105.17_{d}	12.72_{cde}	10.46_{c}	183.74_{bc}	2297.7_{d}
	F2	115.36_{bc}	15.18_{ab}	12.84_{ab}	190.37_{ab}	3234.2_{bc}
	F3	120.94_{a}	15.45_{ab}	13.3_{a}	192.69_{a}	3534.8_{ab}
I3	F1	92.81_{e}	11.6_{b}	9.36_{d}	173.66_{de}	1599.8_{e}
	F2	106.07_{d}	13.1_{cd}	11.00_{c}	180.69_{cd}	2256.6_{d}
	F3	110.18_{cd}	13.59_{c}	11.24_{c}	182.7_{bc}	2372.5_{d}
I4	F1	82.47_{f}	9.51_{g}	7.19_{e}	160.63_{f}	987.1_{f}
	F2	93.24_{e}	11.93_{ef}	9.26_{d}	169.65_{e}	1523.7_{e}
	F3	95.6_{e}	12.16_{def}	9.58_{d}	171.43_{e}	1723.3_{e}

*Mean which have at least once common letter are not significant different at the 5% level using (DMRT).

I_1:70 or control, I_2: 90, I_3: 110 and I_4: 130 mm of evaporation from the surface of the Class A evaporation pan.

F_1: none use or control, F_2:4 $lit.ha^{-1}$ F_3: 6 $lit.ha^{-1}$.

4.4. *Number of pods per plant*

The results of table (1) showed that the effect of drought stress and humic acid on the number of pods per plant was statistically significant at the probability level of 1%, but the interaction effect of the treatments on this trait was not significant. The highest number of pods per plant was observed in the 70 mm evaporation irrigation treatment with an average of 15.55 pods per plant and the lowest with 10.64 pods per plant in the 130 mm evaporation irrigation treatment

(Table 2). The highest number of pods per plant belonged to the foliar spraying treatment of 6 liters per hectare (with an average of 14.07 pods per plant) and the lowest number to the no foliar spraying treatment (with an average of 11.99 pods per plant) (Table 2). The number of pods is one of the most important components of yield, which can play a decisive role in the number of seeds and finally the yield of seeds. Coincidence of pod formation period with favorable environmental conditions can improve yield by increasing the number of pods per plant, which is one of the main components of yield. The supply of available moisture causes the development of the plant canopy, and as a result, more radiation energy is absorbed by the plant, which leads to an increase in yield components, including the number of pods in the plant and the number of fertile pods (Jalota *et al*., 2007). Foliar spraying of humic acid in beans could significantly increase the number of pods per plant, the number of seeds per plant and the weight of seeds per plant (Beheshti *et al*., 2017). The results of this experiment were consistent with the findings of Gohari Abdzad and Sadeghipour (2019), who announced that increasing the concentration of humic acid to 6 liters per hectare resulted in the highest number of pods per plant. It seems that the increase in the number of pods under the effect of humic acid application is due to preventing the pods from falling by increasing the access to water and nutrients. It has been reported that humic acid has a positive and significant effect on the absorption of copper, zinc, manganese, phosphorus and sodium elements; Therefore, by sprinkling humic acid and increasing the absorption of elements, the growth of the plant increases and the plant has a wider canopy, which is able to feed larger spawning tanks and allocate a sufficient amount of dry matter to them, as a result, the number of pods in the plant increases (Jalota *et al*., 2007).

4.5. *Number of seeds per pod*

The effect of drought stress, humic acid and their mutual effect on the number of seeds in the pod was statistically significant at the probability level of one (Table 1). The highest number of seeds per pod was obtained with 70 and 90 mm evaporation irrigation treatment with an average of 12.66 and 12.2 seeds per pod, respectively, and the lowest with an average of 8.68 seeds per pod was obtained from 130 mm evaporation irrigation treatment (Table 2). The highest number of seeds in the pod was obtained in the foliar spraying treatment with a concentration of 6 and 4 liters per hectare, respectively, with an average of 11.76 and 11.46 seeds in the pod, and the lowest number belonged to the treatment without foliar spraying with an average of 9.83 seeds in the pod (Table 2). The highest number of seeds per pod was obtained from the irrigation treatment of 90 mm evaporation and foliar spraying with 6 liters per hectare of humic acid (with an average of 13.3 seeds per pod). The lowest number of seeds per pod with an average of 7.19 seeds was obtained from the irrigation treatment of 130 mm of evaporation and no foliar spraying (Table 2). It seems that with the increase of evaporation and the decrease of available moisture, the number of seeds in the pod decreased due to the increase of egg abortion. Hosseinzadeh *et al*. (2011) also showed that the number of seeds in the plant will decrease due to water shortage conditions. Kalamian *et al*. (2005) attributed the decrease in the number of seeds in the pod to the sterility of the florets ovary due to drought stress. Mendham and Salisbury (1995) reported that environmental stresses affect the number of seeds in the pod by limiting the supply of photosynthetic materials necessary for seed filling. The results of this experiment were also consistent with the findings of Gergis Davood *et al*. (2019) on green beans, who reported that the application of humic acid increased the number of seeds in pods compared to control. In general, humic acid increases the number of seeds in the pod by increasing the photosynthetic capacity of the plant and providing nutrients including phosphorus, because phosphorus plays an important role in plant seeding. But this element is very less mobile in the soil, especially at high pH. Therefore, humic acid increases plant access to nutrients by reducing soil pH in the rhizosphere and increasing root growth (Yuan *et al*., 2017; Gayathri and Srinivasamurthy, 2016).

4.6. *1000 seed weight*

Based on the results of analysis of variance, the effect of drought stress and humic acid on the weight of 1000 seeds was statistically significant at the one percent probability level and their interaction was significant at the five percent probability level (Table 1). The highest weight of one thousand seeds was obtained in the irrigation treatment of 70 mm of evaporation with an average of 194.37 grams and the lowest with an average of 167.24 grams in the irrigation treatment of 130 mm of evaporation (Table 2). The highest weight of 1,000 seeds in foliar spraying with 6 liters per hectare was obtained with a weight of 185.7 grams and the lowest in the no foliar spraying treatment with an average of 177.61 grams (Table 2). The highest weight per thousand seeds belonged to the irrigation treatment of 70 mm evaporation and foliar spraying with 6.4 liters per hectare and no foliar spraying, respectively with an average of 195.98, 194.7 and 192.42 grams, while the lowest weight was in the 130 mm irrigation treatment. Evaporation and lack of foliar spraying were observed with a weight of 160.63 grams (Table 3). The results showed that humic acid foliar application in different water conditions and between different concentrations did not differ much. Also, the weight of seeds is highly sensitive to the conditions of stress and lack of water, and it causes a significant decrease in the weight of a thousand seeds; However, with humic acid foliar application, further reduction of seed weight can be prevented under stress conditions (Table 3). Drought stress by affecting the degree of opening of the stomata, reducing the activity of Kelvin cycle enzymes, can greatly reduce the production of cultured material and in this way directly cause a decrease in the weight of one thousand seeds (physiological destination capacity) (Pessarakli, 2001). Emam and Ranjbar (2000) stated that the decrease in 1000 seed weight in drought stress treatment can be attributed to the emergence of shriveled seeds with less weight. The reason for this could be the reduction in the length of the vegetative and reproductive growth period due to moisture stress, which shortens the effective period of seed filling and also reduces the production and transfer of photosynthetic materials to the seeds and has caused a reduction in the weight of a thousand seeds. The weight loss of 1000 beans under drought stress was also confirmed by Mahlouji *et al*. (2001). The results of this experiment were consistent with the findings of Shabani and Armin (2017) regarding the increase in the weight of 1000 seeds with humic acid foliar spraying on peas, Gergis Davood *et al*. (2019) on beans. The experimental results on bean plant with humic acid foliar spraying showed its significant effects on 1000 seed weight, so that humic acid foliar spraying at the rate of 3 kg per hectare increased this trait by 15% (Jahan *et al*., 2008). According to reports, humic acid increases the weight of 1,000 seeds in crops by affecting the transfer of photosynthetic materials from leaves to seeds (Chamani *et al*., 2012).

4.7. *Seed yield*

The results of analysis of variance showed that the effect of drought stress and humic acid on seed yield was statistically significant at the 1% probability level and their interaction was significant at the 5% probability level (Table 1). The highest and lowest seed yield was related to the irrigation treatment of 70 and 130 mm of evaporation, respectively, with an average of 3460 and 1411 $kg.ha^{-1}$ (Table 2). The highest seed yield was obtained from the foliar spraying treatment of 6 $lit.ha^{-1}$ (with an average yield of 2846 $kg.ha^{-1}$) and the lowest from the non-foliar spraying treatment (with an average of 1989 $kg.ha^{-1}$) (Table 2). The highest seed yield was observed in the irrigation treatment of 70 mm of evaporation and foliar spraying with 6 l $lit.ha^{-1}$ (with an average yield of 3755 $kg.ha^{-1}$) and the lowest in the irrigation treatment of 130 mm of evaporation and without foliar spraying (with an average of 987 $kg.ha^{-1}$) (Table 3). The increase in seed yield in the irrigation treatment of 70 mm evaporation and foliar spraying with 6 liters per hectare was due to the positive effect of these treatments on the physiological parameters of growth and seed yield components, including pod length, number of pods per plant, number of seeds per pod and weight of 1000 seeds. So that with the increase of soil moisture and humic acid amounts, the seed yield also increased. Water shortage stress reduces the yield of legumes by reducing one or more yield components, such as the weight of 1000 seeds, the number of pods per plant, and the number of seeds per pod, and the highest yield is obtained when the environmental conditions, including moisture, are available at all stages. The growth of the plant is optimal. The decrease in yield under water stress conditions, which is affected by the decrease in yield components, has also been reported by other researchers (Aminifar *et al*., 2012). According to the researchers, under full irrigation conditions, the rate of photosynthesis and the production of plant material increases, and as a result, by increasing the speed of seed filling, the weight of the seed and finally its yield increases (Palmer *et al*., 1995). The economic yield of a plant is the result of many growth processes that take place during the period of growth and development. Drought stress can affect yield by influencing these processes. In optimal irrigation conditions, the plants had the highest height, number of branches, number of pods per unit area, number of seeds per pod and weight of 100 seeds, so they produced more yield. With the occurrence of stress, the seed yield was reduced, so that the plants at different levels of stress yielded less than optimal irrigation. In a study conducted by Jahan *et al*. (2008) under conditions of drought stress and humic acid spraying on beans, they reported that the rate of net assimilation was significantly affected by the interaction of humic acid and drought stress. In this test, humic acid increased the yield at different irrigation levels, so that in the irrigation conditions of 70 mm of evaporation, humic acid 4 and 6 liters per hectare increased the yield by 13 and 18%, respectively, compared to no foliar spraying. The stress conditions of 130 mm of evaporation, humic acid 4 and 6 $lit.ha^{-1}$ increased the seed yield by 35 and 42%, respectively, compared to the conditions of no application of humic acid (Table 3). Giasuddin *et al*. (2007) stated that humic acid membrane permeability It increases the cell and thus facilitates the entry of potassium, the result of which is the increase of intracellular pressure and cell division. On the other hand, increasing the energy inside the cell will lead to an increase in the production of chlorophyll and the rate of photosynthesis. After that, an important factor in growth, i.e., absorption of nitrogen into the cell, is intensified and nitrate production decreases, which ultimately leads to an increase in production (Giasuddin *et al*., 2007).

4.8. *Biological yield*

The effect of drought stress and humic acid on biological yield was statistically significant at the 1% probability level, but the interaction effect of treatments was not significant in terms of biological yield (Table 1). The highest biological yield was observed in the irrigation treatment of 70 and 90 mm of evaporation, respectively, with a yield of 8360 and 7854 $kg.ha^{-1}$. The lowest one belonged to the irrigation treatment of 130 mm of evaporation with a value of 5445 $kg.ha^{-1}$ (Table 2). The highest biological yield was obtained from the foliar spraying treatment with 6 and 4 liters per hectare, respectively, with a yield of 7413 and 7109 $kg.ha^{-1}$, and the lowest from the non-foliar spraying treatment with an average of 6301 $kg.ha^{-1}$ (Table 2). The reason for the decrease in biological yield in the irrigation treatment of 110 and 130 mm of evaporation can be related to the decrease in the height of the plant, the decrease in the number of sub-branches, as well as the decrease in yield and yield components, which is consistent with the results of Emadi *et al*. (2012) on beans. Also, the decrease in biological yield due to water shortage stress can be seen as a result of a decrease in the plant's ability to absorb nutrients and synthesize and transport nutrients due to the lack of water, which has caused a decrease in the accumulation of plant dry matter. Reduction of dry matter of aerial organs and reduction of production of photosynthetic materials due to water shortage stress have been reported by other researchers. On the other hand, the increase in dry matter production under reduced evaporation can be due to the development and expansion of the leaf surface as well as its durability, which by creating an efficient physiological source in order to use the received light energy as much as possible, increases the production of dry matter.

(Moradi *et al*., 2008; Bayat *et al*., 2010). The results of this experiment were consistent with the findings of Gohari Abdzad and Sadeghipour (2019) regarding the increase of bean biomass with the use of 6 liters per hectare of humic acid. Mozaffari and Gorgin Shabankareh (2016) showed in research that foliar application with humic acid increases protein and nutrient absorption rate in bean plant and ultimately increases biomass yield. El-Bassiony *et al.* (2010) reported that humic acid increased plant nitrogen content and biomass production in beans by 15%. Through positive physiological effects, such as increasing the metabolism inside the cells and also adding the amount of chlorophyll in the leaves, humic acid makes the leaves last longer, and as a result, the yield and production biomass of the cowpea plant increased, and these results are in line with the findings of Naderi *et al.* (2002) was consistent. Ayas and Gulser (2005) reported that humic acid increases plant growth, height and biological yield by increasing nitrogen content.

4.9. *Harvest index*

The harvest index expresses how the cultivated material is distributed between the vegetative parts of the plant and the seed. The effect of drought stress and humic acid on harvest index was statistically significant at the probability level of 1%, but their interaction effect on this trait was not significant (Table 1). The highest harvest index was obtained in the 70 mm evaporation irrigation treatment with an average of 41.25% and the lowest in the 130 mm evaporation irrigation treatment with 25.67% (Table 2). The highest harvest index belonged to the foliar spraying treatment with 6 and 4 lit.ha^{-1}, respectively, with an average of 37.57 and 36.33%, and the lowest in the no foliar spraying treatment with 30.35% (Table 2). Humic acid increases plant performance through positive physiological effects, including the effect on the metabolism of plant cells and increasing the concentration of leaf chlorophyll (Naderi *et al*., 2002). It seems that humic acid prolongs the life of photosynthetic tissues and increases seed yield. In addition to the accumulation of dry matter, it is also important to distribute the grown material between different organs of the plant. With the increase of drought stress, most of the produced photosynthetic materials are used for the growth of roots to provide more water for the plant, therefore, in such conditions, the harvest index decreases (Taleei *et al*., 2008). The reduction of the harvest index in the treatment of drought stress in the vegetative and reproductive stages can be due to the reduction of the photosynthesizing level and the reduction of re-transmission of the photosynthesized materials in the seed filling stage (Khoshvaghti, 2006). Moradi *et al*. (2008) also considered the lack of available water as the possible reason for the reduction of the harvest index under drought stress conditions, because as a result, the intensity of photosynthesis and the transfer of nutrients to the seed are reduced and it leads to a decrease in seed yield. Also, reducing the number of pods per plant, which has an important contribution to yield, is one of the important reasons for reducing the harvest index in stress treatment.

5. CONCLUSION

The general results of this research showed that in the irrigation treatment of 70 mm of evaporation, the highest yield and components of seed yield were obtained and the positive effect of humic acid on all the measured traits was observed. Also, considering that the increase of drought stress up to 110 and 130 mm of evaporation caused the reduction of all investigated traits, but humic acid foliar application was able to reduce the negative effects of drought stress. In the meantime, in order to save irrigation water consumption and prevent its wastage, it is possible to achieve a favorable yield compared to the control by irrigating 90 mm of evaporation along with foliar spraying of 6 lit.ha^{-1} of humic acid as an organic fertilizer that does not pollute the environment.

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