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Effect of humic acid on grain yield and yield components in chickpea under different irrigation levels

Abbas Abhari and Esmaeil Gholinezhad*

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Department of Agricultural Sciences, Payame Noor University, Tehran, Iran.
*Corresponding author; Email: gholinezhad1358@yahoo.com

Abstract
In order to investigate the effect of soil application of humic acid on grain yield and yield components in chickpea (*Cicer arietinum* L.), an experiment was conducted as factorial based on randomized complete block design with three replications in 2016 and 2017. Factors were irrigation at two levels of full irrigation and drought stress (20% of full irrigation from flowering to harvesting) and humic acid at four levels of 0, 2.5, 5 and 7.5 mg per pot. The results showed that drought stress reduced dry weight of leaf, number of grains and pods, total dry weight and grain yield. Application of humic acid in two years increased the value of all studied traits under full irrigation. In the first year, application of 2.5 and 5 mg humic acid increased 1000-grain weight, and total and leaf dry weight under drought stress conditions, respectively. In the second year, with higher consumption of humic acid, grain yield, total dry weight and harvest index increased significantly under drought stress conditions. Application of 7.5 mg humic acid in the second year, produced the highest proline under optimum irrigation (393.10 mg kg^{-1} DW) and drought stress (507.90 mg kg^{-1} DW) conditions.

Keywords: Grain number; Grain yield; Pod weight; Proline; Total dry weight.

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Introduction

Chickpea (*Cicer arietinum* L.) is the second most important legume in the world. Considering the importance of legumes as one of the most important sources of plant proteins in dry and semi-arid regions, many efforts have been made to determine tolerance to drought stress based on the yield reduction in drought stress conditions (Daryanto *et al*. 2015). Chickpea is one of the plants cultivated mainly in the west and northwest of Iran, and it is faced with drought stress in most of its growing period. Cultivated area, production and grain yield of chickpea in the world is 12.6 Mha, 12.09 Mt and 956 kg ha^{-1}, respectively (FAO 2016). This plant with a cultivated area of 433356 ha, production of 177493 and grain yield of 409 kg ha^{-1} ranks first among the beans planted in Iran (FAO 2016).

Irrigation generally increases the value of most traits in chickpea, so that there is a linear relationship between biomass, grain yield and supplemental irrigation (Johansen *et al*. 1994). Biotic or abiotic environmental stresses limit chickpea yield (Saeed and Darvishzadeh 2017). Nakhzari Moghaddam *et al*. (2017) in a study on chickpea indicated that with increasing drought stress grain yield, pod per plant, grain per plant 1000-grain weight and protein yield decreased significantly. Beheshti *et al*. (2016) showed the reduction in number of pods per plant, 1000-grain weight, gain yield and harvest index of lima bean (*Phaseolus lunatus* L.) due to drought stress.

Humic acid is a natural polymer found in soils and due to insolubility in water is not mobilized in the environment. Humic acid regulates environmental processes, contributes to fluidity and porosity of the soil and acts as adsorbent and reservoir of water and plant nutrients (Davies *et al.* 1995). According to Mohsen-Nia and Jalilian (2011), safflower yield was significantly affected by the humic acid under drought stress conditions. Humic material reduces the adverse effects of chemical fertilizers in the soil (Rady 2011). In a study on bentgrass, it was found that humic acid significantly increased the rate of photosynthesis, dry matter of the root and the content of plant nutrients, especially at the concentration of 400 mg L^{-1} (Liu *et al*. 1998). Tahir *et al.* (2011) have indicated the positive effect of medium dose of humic acid (60 mg kg^{-1} soil) on wheat growth (plant height, and shoot fresh weight, shoot dry weight), and also nitrogen uptake; however, the highest rate of humic acid (90 mg kg^{-1} soil) had a negative effect on growth and nutrient uptake of wheat as well as nutrient accumulation in soil. Also, humic substances stimulate root and shoot growth; however, root growth is generally more pronounced than shoot growth (Chen and Aviad 1990).

According to Barzegar *et al.* (2016), foliar application of humic acid improved the yield of okra under water deficit stress conditions as compared to the control treatment. Celik *et al.* (2011) indicated beneficial effects of foliar application of humic acid under calcareous soil conditions for the improvement of plant growth and nutrients uptake. In a study on millet, spraying with humic acid had positive effects on plant height, grain yield, 1000-grain weight, grain number and crude protein (Saruhan *et al.* 2011). It was shown that application of humic acid on chickpea, increased number of grains per plant, grain yield and harvest index (HaghParast *et al.* 2012). Beheshti *et al*. (2016) also reported that humic acid increased yield of pods per plant, 100-seeds weight, grain yield and harvest index under drought conditions. Albayrak and Camas (2005) showed the increased root and leaf yield and yield components of forage turnip as affected by foliar application of humic acid. Gad El-Hak *et al.* (2012) reported a significant positive effect of humic acid on yield and yield components of the pea plant.

The objective of this study was to investigate the effect of humic acid on grain yield and yield components of chickpea under different irrigation conditions.

Materials and Methods

This research was conducted in the greenhouse of Payame Noor University of Sabzevar during growing seasons of 2016 and 2017. The greenhouse conditions were as follows; temperature 25 °C, relative humidity 55% and light 4800 lx. The city of Sabzevar is located at northeast of Iran. This research was conducted as a factorial experiment based on randomized complete block design with three replications. Factors included irrigation at two levels of full irrigation and drought stress (20% of full irrigation from flowering to harvesting) and humic acid at four levels of 0, 2.5, 5 and 7.5 mg per pot (0, 1.6, 3.3 and 5 kg ha^{-1}). Field capacity was determined by the weighted method. In this method, the field capacity was measured according to the soil texture. The physical and chemical properties of the soil in the experimental site are presented in Table 1.

Seeds were planted in the pots (25 cm in diameter) filled with a proper soil. After emergence, the number of plants was reduced to five plants per pot. Fertilization included 85 kg of elemental nitrogen (source: urea), 70 kg of pure phosphorus (source: ammonium phosphate) and 75 kg of elemental potassium (source: potassium sulfate) per hectare, which added to the pot every year. Fertilizers if each pot were 0.24 g nitrogen, 0.2 g phosphorus and 0.22 g potassium.

After imposing treatments, the dry weight of the whole plant, leaf weight, stem weight, number of pods per pot, grain number per pot, 1000-grain weight and grain yield were measured at the end of first year. In the second year, in addition to these traits, proline (Bates 1973) was also measured. Means were compared by LSD test at 5% probability level. SAS (9.1), MSTATC and Excel (2007) software were used for data analysis and drawing graphs.

Results

The interactions of irrigation × year, humic acid × year and irrigation × humic acid × year were significant for all traits, except grain number per pod, 1000-grain weight and harvest index; however, for grain number per pod, 1000-grain weight and harvest index irrigation × humic acid was still significant. Therefore, the results for all traits were interpreted based on the interaction of year by irrigation × humic acid combination (Table 2).

Number of pods: In 2016 and 2017, drought stress, compared to the optimum irrigation conditions, reduced number of pods per pot about 52 and 32%, respectively. In the first and second year, under optimum irrigation conditions, application of humic acid, compared to the pots with no humic acid, on the average increased number of pods per pot 38 and 13%, respectively. In 2017, under drought stress conditions, application of humic acid, compared to no humic acid, increased number of pods about 70%. There were no significant differences among humic acid concentrations for this trait in 2016 (Tables 3 and 4).

Grain number per pod: In the first year, the highest grain number per pod was obtained from the application of 7.5 mg humic acid under full irrigation (11.66) and 5 mg of humic acid under drought stress (5.00) (Table 3). In the second year, the highest number of grains was obtained from 5 mg humic acid under full irrigation (11.3) and from 7.5 mg humic acid under drought stress (8.83) (Table 4).

Pod weight: In 2016, under full irrigation conditions, application of 7.5 mg of humic acid, compared with non humic acid condition, increased pod weight about 62%. However, under drought stress conditions the pod weight did not increase significantly by application of humic acid (Table 3). In the second year, under full irrigation conditions, the maximum pod weight (1.85 g) was obtained using 5 mg of humic acid (Table 4). However, under drought stress conditions, pod

Table 1. Chemical and physical properties of the soil in two years of testing.

Property	Unit	2016	2017
EC	ds/m	1.28	1.42
pH	-	7.4	7.4
Clay	%	14	14
Silt	%	24	24
Sand	%	62	62
Organic Carbon	%	0.66	0.64
Nitrogen	%	1.03	0.99
Phosphorus	mg kg^{-1}	16	16.4
Potassium	mg kg^{-1}	315	312
Soil texture	-	Sandy loam	Sandy loam

weight decreased either significantly or insignificantly by the application of humic acid (Table 4).

1000-Grain weight: In the first and second years, drought stress, compared to full irrigation, decreased 1000-grain weight about 3 and 36%, respectively (Tables 3 and 4). In 2016, under full irrigation and drought stress conditions, application of 2.5 mg humic acid increased 1000-grain weight about 31 and 8%, respectively (Table 3). In 2017, under full irrigation and drought stress conditions, application of 7.5 mg humic acid increased 1000-grain weight about 59 and 27%, respectively (Table 4).

Grain yield: In the first and second years, drought stress, compared to full irrigation, decreased grain yield about 53 and 47%, respectively (Tables 3 and 4). In 2016, under full irrigation and drought stress conditions, application of 7.5 mg humic acid increased grain yield about 63 and 36%, respectively (Table 3). In 2017, under full irrigation and drought stress conditions, application of 7.5 mg humic acid increased grain yield about 37 and 39%, respectively (Table 4).

Total dry matter: In the first and second years, drought stress, as compared with full irrigation, decreased total dry matter about 47 and 16%, respectively (Tables 3 and 4). In 2016, under full irrigation and drought stress conditions, application of 7.5 and 5 mg humic acid increased total dry matter about 50 and 31%, respectively (Table 3). In 2017, under full irrigation and drought stress conditions, application of 5 and 7.5 mg humic acid increased total dry matter about 24 and 22%, respectively (Table 4).

Leaf dry weight: In the first and second years, drought stress decreased leaf dry weight about 24 and 10%, respectively, as compared to full irrigation conditions (Tables 3 and 4). In 2016, under full irrigation and drought stress conditions, application of 7.5 and 5 mg humic acid increased leaf dry weight about 13 and 50%, respectively (Table 3). In 2017, under full irrigation and drought stress conditions, application of 5 and 7.5 mg humic acid increased leaf dry weight about 24 and 5%, respectively (Table 4).

Stem dry weight: There was no significant difference among humic acid treatments (first year) for stem dry weight in both full irrigation and drought stress conditions (Table 3); however, in 2017, comparison of means showed that under full irrigation conditions, the highest stem dry weight was obtained by application of 5 mg humic acid (Table 4). On the other hand, there were no significant differences among humic acid treatments under drought stress (Table 4).

Harvest index: In the first year (2016), it was determined that under full irrigation and drought stress conditions, application of 2.5 mg of humic acid produced the highest harvest index (Table 3). In the second year (2017), mean comparisons showed that under full irrigation and drought stress conditions, the highest harvest index was obtained from 7.5 mg humic acid (Table 4, Figure 1).

Proline: A 21% increase in the proline content was observed under drought stress as compared to full irrigation, averaged over humic acid concentrations (Table 4). A 36 and 35% increase in the proline content was realized under full irrigation and drought stress conditions, respectively, by application of 7.5 mg humic acid, compared to the conditions with no humic acid (Table 4).

Table 2. Analysis of variance of studied traits of chickpea during two successive years 2016 and 2017.

SOV	df	Pod No. per pot	Grain No. per pod	Pod weight per pot	1000-grain weight	Grain yield per pot	Total dry weight per pot	Leaf dry weight per pot	Stem dry weight per pot	Harvest index	df proline	Proline
Year (Y)	1	21.3	1.24	25.5**	40.9	5.5**	9.9**	2.25**	10.8**	0.5	-	-
Replication/Y	4	2.9*	1.38	0.001	7.8	0.001	0.009	0.01	0.004	0.79	2	12.9
Irrigation (I)	1	126	97.3**	2.36	1.0	1.45	29.57	0.93	1.46	24.0**	1	4550**
Humic acid (HA)	3	9.1	9.7**	0.37	3076**	0.15	3.45**	0.49	0.06	115.7**	3	26906**
I * HA	3	15.2	13.1**	1.24	1118**	0.288	5.61**	0.41	0.08	52.7**	3	16764**
I * Y	1	16.3**	1.25	9.57**	2.5	97.33**	1.45**	7.3**	1.45**	1.7	-	-
HA * Y	3	12.2**	0.15	12.5**	0.2	9.98**	0.25**	9.8**	0.15**	0.1	-	-
I * HA * Y	3	11.5**	0.88	11.1**	0.1	3.07**	0.288**	3.7**	0.5**	0.8	-	-
Error	28	1.04	0.99	0.006	17.7	0.008	0.013	0.01	0.02	1.43	14	4.41
C.V (%)		15.47	16.48	8.53	3.52	13.25	3.18	7.76	14.7	6.31	7.8	0.58

Table 3. Means of different chickpea traits for humic acid levels under full irrigation and drought stress conditions in 2016.

Irrigation	Humic acid (mg per pot)	Pod No. per pot	Grain No. per pod	Pod weight per pot (gr)	1000-grain weight (g)	Grain yield per pot (g)	Total dry weight per pot (gr)	Leaf dry weight per pot (g)	Stem dry weight per pot (g)	Harvest index
Full irrigation	0	6.11	5.55	0.86	104.66	0.53	3.56	1.65	1.23	15.00
	2.5	7.22	6.66	0.67	150.00	1.00	3.63	1.56	1.23	27.54
	5	10.00	8.33	1.30	101.33	0.79	4.82	1.75	1.22	17.16
	7.5	12.22	11.66	2.23	123.33	1.41	7.02	1.88	1.61	20.08
	LSD	1.84	2.71	0.2	7.73	0.25	0.34	0.32	0.45	3
Drought stress	0	4.44	3.88	0.83	135.3	0.33	2.39	1.07	0.77	21.56
	2.5	4.45	3.88	0.84	146.6	0.53	2.43	1.05	0.80	21.87
	5	5.00	5.00	0.48	87.60	0.38	3.42	2.11	0.90	10.90
	7.5	3.33	3.33	0.40	100.0	0.51	1.91	1.02	0.80	17.44
	LSD	2.21	1.1	0.09	7.9	0.08	0.06	0.1	0.13	1.50

Table 4. Means of different chickpea traits for humic acid levels under full irrigation and drought stress conditions in 2017.

Irrigation	Humic acid (mg per pot)	Pod No. per pot	Grain No. per pod	Pod weight per pot (gr)	1000-grain weight (g)	Grain yield per pot (g)	Total dry weight per pot (g)	Leaf dry weight per pot (g)	Stem dry weight per pot (gr)	Harvest index	Proline (mg kg^{-1} DW)
Full irrigation	0	11.00	6.25	1.42	107.2	0.70	5.47	1.58	1.88	12.79	253.43
	2.5	6.63	4.09	1.14	169.5	0.69	4.39	1.27	1.79	15.79	309.22
	5	20.66	11.3	1.85	110.6	1.11	7.15	2.07	2.39	16.87	319.04
	7.5	10.55	4.30	1.01	258.5	1.25	3.89	1.12	1.59	28.67	393.10
	LSD	0.74	0.3	0.03	5.93	0.21	0.11	0.41	0.4	0.97	5.54
Drought stress	0	3.00	3.03	1.53	95.00	0.39	3.90	1.43	2.01	6.61	331.40
	2.5	8.10	4.00	1.22	100.80	0.40	3.95	1.14	1.92	10.19	433.70
	5	10.60	6.68	1.26	95.30	0.58	4.87	1.41	1.99	13.07	341.30
	7.5	12.00	8.83	1.28	128.50	0.63	4.95	1.49	2.02	17.16	507.90
	LSD	0.77	0.39	0.26	1.94	0.07	0.26	0.37	0.12	0.9	2.99

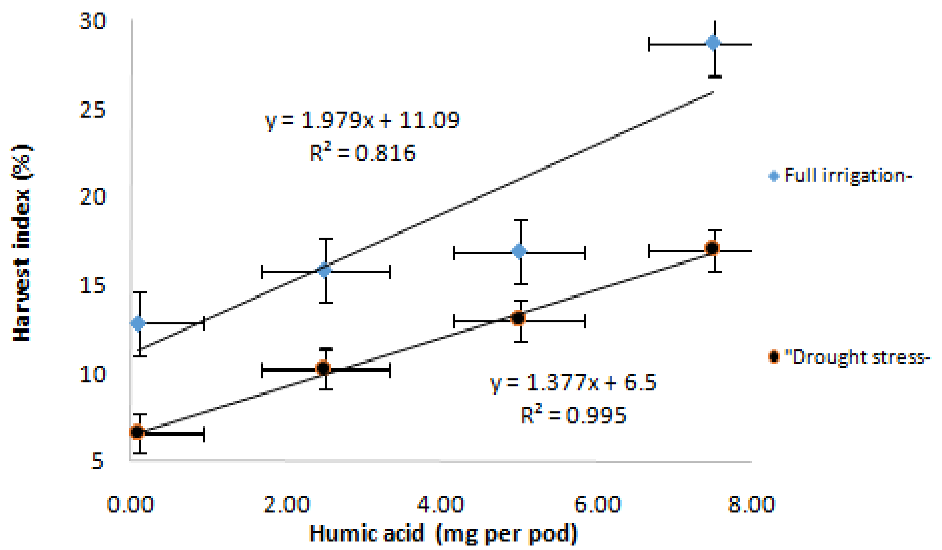

Figure 1. The relationship between humic acid content and harvest index under full irrigation and drought stress conditions.

Discussion

In this research, it was determined that drought stress reduced grain yield and yield components, while increased proline. One of the reasons for reducing the number of pods per pot with increasing drought stress is that irrigation increased the number of pods and prevented falling flowers and pods. Along with our findings, other researchers also reported such results (Raei *et al*. 2008; Nakhzari Moghaddam *et al*. 2017). Factors that affect cell division and development, such as the amount of water in the tissue and the concentration of effective plant hormones, such as abscisic acid, are responsible for regulating the number of pods under drought stress conditions (Saini and Westgate 2000). On the other hand, application of humic acid, increases the auxin, cytokinin and gibberellin hormones in the plant, and it seems that this mechanism is a reason for increasing the number of pods per plant, along with other factors. Abdel-Mawgoud *et al*. (2007) reported the increase in plant hormones (gibberellic acid and indole acetic acid) by application of a humic-based fertilizer in tomato. In our study, the use of humic acid in both full irrigation and drought stress conditions increased grain yield of chickpea due to increasing all yield components. HaghParast *et al*. (2012) reported the positive effect of humic acid on number of pods, grain yield and harvest index of chickpea under drought stress conditions.

Drought stress reduced number of grains of chickpea in both years (Tables 3 and 4). The reduction in amount of available water may have negative effect on pollination and, thus, reduces the number of pods per plant. On the other hand, under drought stress conditions, number of grains increased linearly with increasing of humic acid in 2017; however, the highest value in 2016 was obtained at 5 mg humic acid concentration, which was significantly different than the control. A significant increase in the number of grains per plant in chickpea was also reported in another experiment by application of humic acid, as compared with the control, when drought stress was imposed (HaghParast *et al.* 2012).

Drought stress reduced 1000-grain weight in our study. Reduction of 1000-grain weight under drought stress may have been due to the shortening of grain filling period, and thus has reduced the seed weight. However, the positive effect of humic acid on grain weight at drought stress conditions was inconsistent from one year to another. In 2016, application of humic acid at the rate of 2.5 mg per pot improved 1000-grain weight over the control, but other concentrations were significantly lower than the control. On the other hand, in 2017, the highest 1000-grain weight was obtained at the rate of 2.5 mg humic acid per pot, which was significantly higher than the control treatment. Jahan *et al*. (2012) emphasized the increase in dry matter accumulation and grain yield in beans with the use of humic acid under drought stress conditions, as compared to the no-humic acid treatment. El-Ghamry *et al*. (2009) in a study on beans, reported that soil consumption of humic acid increased the growth by increasing the rate of nutrients absorption. Khaled and Fawy (2011) also showed an increase in the uptake of nutrients through the use of 2 g/kg humic acid in the soil and its foliar application at the rate of 0.1%. It was shown that humic acid substances are involved in the adaptation of plants to phosphorus availability (Jindo *et al.* 2016).

Some levels of humic acid, as compared to the control, improved total, leaf and stem dry weight significantly under full irrigation conditions in both years and under drought stress in the first year; however, humic acid was effective only on total dry weight under drought condition of 2017. According to Osman and Rady (2012), humic acid significantly increased leaf area, shoot dry weight and grain yield. The increase in leaf, stem and total dry matter can be attributed to the improvement of soil structure, increasing of soil water holding capacity and good ventilation and drainage, which expands root growth, enhances the absorption of nutrients and may provide tolerance to drought stress. Cacco *et al*. (2000) provided evidence on the promoting effect of humic acids on the molecular expression of proteins in the nitrate transport system. On the other hand, some researchers were not confident about the positive effects of humic acid on the nutrient uptake by plants. For example, Delfine *et al*. (2005) indicated that foliar application of humic substances don't improve consistently the nutritional status of durum wheat and doesn't compensate for the imbalance of mineral nutrition. They concluded that humic acid had limited effects on photosynthesis, growth and grain yield of durum wheat crops grown in a typical Mediterranean environment.

Soil application of 7.5 mg humic acid led to the highest value in proline content under full irrigation and drought stress conditions (393.10 and 507.90 mg kg^{-1} DW, respectively). This increase was about 36 and 35% higher than the control under full irrigation and drought stress conditions, respectively. The increase in proline content under drought stress was, on the average, 21% higher than full irrigation. Increasing of proline under drought stress has been reported by Beheshti and Tadayyon (2017) in lima bean and Sanchez *et al*. (1998) in pea plants. Proline is probably the most abundant substance that accumulates under drought stress. Proline, as a soluble agent, reduces water loss from the cell and maintains turgor. The increase in proline concentration under drought stress may indicate the potential role of this amino acid in osmotic regulation (Kuznetsov and Shevyakova 1999). Munns (2002) indicated that proline accumulates under water stress and is found at high concentrations in plants adapted to dry soils. Kaur and Asthir (2015) indicated that proline plays a vital role in plants' abiotic stress tolerance. However, the role of proline in drought tolerance has not been confirmed by all scientists (i.e. Souza *et al*. 2004; Tavakoli ***et al.*** 2016; Danyali *et al.* 2019). Souza *et al*. (2004) reported a small increase of proline level in cowpea after stress. They stated that this increase may be a consequence and not a beneficial response induced by stress. Tavakoli ***et al.*** **(**2016) and Danyali *et al.* (2019) have shown that proline content in the sensitive cultivars was larger than in the tolerant cultivars under salt and drought stresses, respectively.

Conclusion

Drought stress treatments in both years reduced leaf and total dry weight, number of grains and pods, and grain yield. Soil application of humic acid at some concentrations, increased grain yield and yield components under full irrigation in both years. Mean comparison in the first year showed that under full irrigation and drought stress conditions, soil consumption of 7.5 mg of humic acid in the pot produced mainly the highest grain yield. Based on the results of this research, in the first year and under drought stress conditions, application of 2.5 and 5 mg of humic acid increased 1000-grain weight, and leaf and total dry weight, respectively. In the second year, with higher consumption of humic acid, grain yield, total dry weight and harvest index increased linearly under drought stress conditions. Harvest index also increased in the second year by increasing the humic acid concentration under full irrigation conditions. Under full irrigation and drought stress conditions, application of 7.5 mg humic acid produced the highest proline; however, the increase was higher under drought stress, as compared to the full irrigation conditions. This indicates the use of humic acid could moderate and reduce the effect of drought stress.

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تأثیر اسید هیومیک بر عملکرد و اجزای عملکرد دانه در نخود تحت شرایط مختلف آبیاری

عباس ابهری و اسماعیل قلی نژاد*

گروه علمی علوم کشاورزی، دانشگاه پیام نور، تهران.

*مسئول مکاتبه؛ Email: gholinezhad1358@yahoo.com

چکیده

به‌منظور بررسی تأثیر کاربرد خاکی اسید هیومیک بر عملکرد و اجزای عملکرد دانه در نخود (*Cicer arietinum* L.)، آزمایشی به‌صورت فاکتوریل در قالب طرح پایه بلوک‌های کامل تصادفی با سه تکرار در سال‌های ۱۳۹۵ و ۱۳۹۶ انجام شد. تیمارهای آزمایشی شامل آبیاری در دو سطح آبیاری کامل و تنش خشکی (۲۰ درصد آبیاری کامل از مرحله گل‌دهی تا برداشت) و اسید هیومیک در چهار سطح صفر، ۲/۵، ۵ و ۷/۵ میلی‌گرم در گلدان بودند. نتایج نشان داد که تیمار خشکی وزن خشک برگ، تعداد دانه و غلاف، وزن خشک کل و عملکرد را کاهش داد. کاربرد اسید هیومیک در دو سال مقادیر تمامی صفات مورد مطالعه را تحت شرایط آبیاری کامل افزایش داد. در سال اول کاربرد ۲/۵ و ۵ میلی‌گرم اسید هیومیک به ترتیب وزن ۱۰۰۰ دانه و وزن خشک کل و برگ را تحت شرایط تنش خشکی افزایش داد. در سال دوم در شرایط تنش خشکی با افزایش مصرف اسید هیومیک عملکرد دانه، وزن خشک کل و شاخص برداشت به‌صورت معنی‌داری افزایش یافت. در سال دوم کاربرد ۷/۵ میلی‌گرم اسید هیومیک، بیشترین مقدار پرولین را تحت شرایط آبیاری مطلوب (۳۹۳/۱۰ میلی‌گرم پرولین بر کیلوگرم وزن خشک) و تنش خشکی (۵۰۷/۹۰ میلی‌گرم پرولین بر کیلوگرم وزن خشک) تولید کرد.

واژه‌های کلیدی: پرولین؛ تعداد دانه؛ عملکرد دانه؛ وزن خشک کل؛ وزن غلاف.