INNSPUB

Journal of Biodiversity and Environmental Sciences (JBES)
ISSN: 2220-6663 (Print) 2222-3045 (Online)
Vol. 11, No. 1, p. 287-295, 2017
http://www.innspub.net

RESEARCH PAPER OPEN ACCESS

Effect of humic acid on some morphological traits of Guar (*Cyamopsis tetragonoloba*) in Karaj region

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Article published on July 30, 2017

Key words: Fertilizer, Morphological traits, Leguminous, Seed yield

Abstract

Humic acid is one of the most organic substances that has been used today due to its advantages, such as improving drainage and soil air, causing the development of microorganisms, increasing plant yield and growth, and also reducing the consumption of other fertilizers. In order to evaluate humic acid on some morphological traits of guar plant, a randomized complete block design experiment with three replications was conducted in 2015 and 2016. Fertilization treatments included four levels of humic acid (0,1,3 and 6 liters per Hectare). The evaluated traits was including the emergence index, emergence percentage, leaf area index, number of leaves, lateral shoots, seeds per pod, pods per plant, seed yield, weight of 100 seeds, stem diameter in two steps and plant height in three steps. Analysis of variance showed that humic acid had a significant effect on evaluated traits except number of lateral shoots and weight of 100 seeds. Based on the results, the highest leaf area index, number of leaves, stem diameter, number of pods per plant, number of seeds per pod, emergence index, emergence percentage and seed yield were related to the fertilization treatment of 6 liters per hectare, however, no significant difference was observed with fertilization treatment of 3 liters per hectare. Also, the results showed that fertilization reduced the plant height at different steps.

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Introduction

In recent decades, extensive research has been done on medicinal plants and drugs with natural active ingredients, has opened new horizons for researchers. So that now about a third of the drugs used in human societies and herbal medicines are made of natural origin. Many researchers have turned their attention to the cultivation, production and study of medicinal plants. Guar (*Cyamopsis tetragonoloba*) is an annual plant of beans family (*Leguminous*) and is the source of guar gum (Whistler and Hymowitz, 1979). Many parts of it are used to humans and animals, but its seed which contains gel is the most important part (Mudgil *et al.*, 2011). Guar is one of the unique beans which its large spherical endosperm contains a significant amount of galactomonas that is used in a wide range of food and industrial applications (Marina *et al.*, 2007; Murwan and Abdalla, 2008). This plant can be used in various textile, paper, explosive and pharmaceutical industries as adhesives or as a decomposition agent in pills and the main ingredient in some of the laxatives, In the cosmetics industry as a concentrator, in toothpaste, shampoos, oil industry and gas industry (Alexander *et al.*, 1988; Narayan, 2012). Organic fertilizers have a positive effect on agricultural sustainability due to the fact that authentic and safe products are present (Najafi and Rezvani Moghadam, 2001). According to the report, organic fertilizers reduce bulk density and, by linking with soil molecules, increase water holding in soil (Sharifi Ashoor Abadi, 1998). One of the most important compounds in soil structure is humic acid, which results from organic matter decomposition in soil. In general, humic substances are used as soil modifiers. Humic acid polymers act like an organic adhesive and glued together the particles of the mineral material and, while creating larger granules, a suitable space for microscopic and macroscopic organisms, provides greater penetration of air, water and roots. Similarly, humic acid molecules form with soil minerals and create interconnected networks that, in general, can store large volumes of water in their own stores. The lighter the soil texture, the greater the effect. This operation improves water holding capacity and improves water use efficiency in products (Singer & Bissonnais, 1998).

Humic acid can increase cellular proliferation throughout the plant, especially in the roots, and it does this by producing amino acids and nucleic acids (Dursun *et al.*, 2002). Humic acid, with physical modification and improved soil gradation, creates more space for water penetration. Humic acid molecules interfere with water molecules, eliminate head loss and evaporate water (Bronick & Lai, 2005) (Mirhajian, 2012). The molecules of folic acid (a division of the humic acid molecule), which penetrate the plant tissues and bind to water molecules, reduce the sweating and transpiration of the plant and help maintain water inside the plant and hence, they have a positive effect on the growth and development of the plant (Bronick & Lai, 2005). Therefore, the aim of this experiment was to investigate the effect of humic acid on some vegetative and reproductive traits in Guar's medicinal plant.

Previously, researchers worked on weeds, salt stress, drought stress and etc. on guar plant and they reported that removing weeds at 20 or 30 DAS increased the number of pods per plant, water use efficiency and seed yield of cluster bean. Water consumption was higher in unweeded plots. Water use efficiency decreased with the increase in time of weed removal beyond 20 days after crop sowing (R. S. Yadav, 1998).

Several workers reported that water stress caused more shedding of flowers, immature pods and smaller seeds, e.g. (Boutraa and Sanders, 2001). However, in this ex-periment, stressed plants at 65 DAS did not show this esponse. Increased amounts of irrigation water or sam-pling errors are possible reasons for this clear discrep-ancy. Moreover, (Pandey *et al.*, 1984) reported that pod density was most affected by water stress, among yield components with pod reduction averaging more than 50%, mainly due to the reduced flower production and the greater abortion of flowers.

In the current age, considering the high application of guar and gum obtained from it, as well as the limitation of adequate water and cultivated land, it will be questioned that can increasing the yield of this plant and some other vegetative and reproductive factors by fertilization. So, the aim of this study was to investigate the effect of humic acid on valuable Guar herb.

Materials and methods

Study areas

The study was conducted in March of 2015 and 2016 in Karaj (longitude 50 degrees 54 minutes latitude 35 degrees 55 minutes and 1312.5 m above sea level). Karaj with hot-dry summers and cool-wet winters, with the average of 239.5 mm annual rainfall, is arid and the annual average of minimum and maximum temperatures of Karaj is respectively 8.7 and 21.1 degrees Celsius. The soil type of experimental farm was silty-sandy.

Design type and analysis

This experiment was conducted in a randomized complete block design with three replications. Data analysis was performed using SAS software and Duncan's multi-domain test was used to compare averages.

Evaluated traits

After the stages of growth, some morphological characteristics such as the emergence index, emergence percentage, leaf area index, number of leaves, lateral shoots, seeds per pod, pods per plant, seed yield, weight of 100 seeds, stem diameter in two steps and plant height in three steps were measured.

Farm preparation

In the autumn of the 2014 and 2015 tillage including plowing, make the bed and Kurt were carried and the type of soil cultivation before planting tested and evaluated. Planting operations was done in March of 2015 and 2016 in Karaj region on the personal farm. As well as pre- and post-emergence weed control was carried out. Each plot consists of 5 rows of 2 × 3 m, plant spacing on rows 25 cm and between rows 50 cm was considered. In this experiment, planting was done by clumped seeds and immediately after planting, watering was done.

Treatments

In this study, four levels of fertilization were considered. First level was without fertilizer (control). Second level was fertilizing with 1 liters per hectare of Humic Acid (F1). Third level was fertilizing with 3 liters per hectare of Humic Acid (F2) and Finally, the last level was fertilizing with 6 liters per hectare of Humic Acid (F3).

Methods

The different stages of sampling and data collection were as follows:

To measure the emergence index and emergence percentage, the number of emergence seeds per day in each experimental unit was counted and after confirming the number of seedlings per plot, the emergence percentage and then the emergence index were calculated (Shekari, 2001). The emergence index as a criterion for the rate of emergence was obtained according to the following formula (Shekari *et al.*, 2010):

$$EI= (E1 \times 14) + (E2\times 13) + \ldots + (E14\times 1)$$

E: Respectively, the number of greenery seedlings from the first count to the last count. In this regard, any treatment that can produce more seedlings in the earliest days, will produce a larger number, comparing with the treatment that produces the same number of seedlings in the last days and therefore, it is considered as a criterion for seedling emergence rate.

Also, to calculate plant height at certain times, 5 plants from each plot were randomly selected and measured their height from cotyledon to plant ends by the ruler and their mean was considered as plant height.

Coles were used to measure stem diameter at specified times.

To calculate number of lateral shoots, number of leaves and leaf area index, at an area of 1 square meter, 3 plants per plot were randomly taken out and immediately inside the nylon bags were transferred to the lab and then in the laboratory after separating the leaves from the plants, the number of lateral shoots and number of leaves were counted and the leaf area of the plants was measured with the Leaf Area Meter.

In order to measure seed yield at the experimental units, the plants were harvested and the pods of the plants were thrown and the seeds were weighed accurately and they were recorded as seed yield per unit area (kg/ha).

Finally, in order to measure the yield components, at the end of the growth period, when more than 90% of the pods were turned yellow, from each plot 10 plants were randomly harvested and the number of pods, seeds per pod and weight of 100 seeds were calculated.

Results and discussion

Emergence index and Emergence percentage

Bean treatment with humic acid significantly increased germination rate of seeds due to its metabolic and biochemical changes in the plant (Koo, E.S. 2006), which was in line with the result of this experiment. Humic acid increased germination rate of guar seeds by increasing germination speed and also, by increasing seedling establishment, it improved the germination level in the field. The effect of humic acid in different phonological stages on the index and percentage of emergence was significant (Table 1). The highest emergence index was related to F3 treatment (212.833), which did not have a significant difference with F2 treatment (208.333), but both treatments had a significant difference with control (118) and F1 (121) treatments (Fig. 1). The highest percentage of emergence was related to F3 treatment (51.85), which did not have a significant difference with F2 treatment (50.89), but both treatments had a significant difference with control (42.71) and F1 (43.09) treatments (Fig. 2).

Table 1. Analysis of variance of evaluated traits of Guar in four different levels of humice acid.

(S.O.V)	(DF)	(MS)			
		Number of leaves in flowering time	Number of lateral shoots	Seed yield	Number of pods per plant
(Y)	1	155.041667*	2.66666667ns	114.97504ns	2.666667ns
REP(Y)	4	20.416667	0.91666667	1134.85862	11.041667
(Treat)	3	375.486111**	0.55555556ns	21043.02746**	504.944444**
(Treat*Y)	3	59.486111ns	011111111ns	192.73810ns	0.111111ns
ERROR	12	23.194444	0.750000000	1135.61377	52.819444
(CV)	-	8.238451	22.59197	6.436717	10.52019

ns=nonsignificant, * and ** = respectively significant in level of 5% and 1%.

Continue of Table 1. Analysis of variance of evaluated traits of Guar in four different levels of humice acid.

(S.O.V)	(DF)	(MS)		
		Weight of 100 seeds	Number of seeds per pod	Number of leaves in harvest time
(Y)	1	0.03153750ns	2.04166667ns	117.041667*
REP(Y)	4	0.01263333	3.33333333	2.708333
(Treat)	3	0.02114861ns	4.37500000**	501.486111**
(Treat*Y)	3	0.00587083ns	0.04166667ns	42.152778ns
ERROR	12	0.11277222	0.66666667	17.652778
(CV)	-	9.951325	11.87631	5.907235

ns=nonsignificant, * and ** = respectively significant in level of 5% and 1%.

Continue of Table1. Analysis of variance of evaluated traits of Guar in four different levels of humice acid.

(S.O.V)	(DF)	(MS)			
		Emergence index	Emergence percentage	Stem diameter in podding time	Stem diameter in harvesting time
(Y)	1	35.04167ns	0.00000418ns	0.37500000ns	8.16666667**
REP(Y)	4	997.79167	0.00295604	0.58333333	1.41666667
(Treat)	3	16621.59722**	0.01447084**	2.48611111*	8.11111111**
(Treat*Y)	3	402.37500ns	0.00001072ns	0.04166667ns	0.94444444ns
ERROR	12	274.40278	0.00020355	0.47222222	0.52777778
(CV)	-	10.03692	3.026524	13.19394	7.925271

ns=nonsignificant, * and ** = respectively significant in level of 5% and 1%.

Continue of Table 1. Analysis of variance of evaluated traits of Guar in four different levels of humice acid.

(S.O.V)	(DF)	(MS)			
		Leaf area index	Plant height in flowering time	Plant height in podding time	Plant height in harvesting time
(Y)	1	10922.6667*	6.00000000*	13.50000000*	18.3750000ns
REP(Y)	4	692.3333	1.58333333	3.45833333	4.8333333
(Treat)	3	121767.0000**	12.77777778**	30.33333333**	258.4861111**
(Treat*Y)	3	3670.7778ns	0.55555556ns	0.05555556ns	20.2638889ns
ERROR	12	1668.2222	0.91666667	2.0694444	19.166667
(CV)	-	6.010872	6.679724	6.346572	7.894170

ns=nonsignificant, * and ** = respectively significant in level of 5% and 1%.

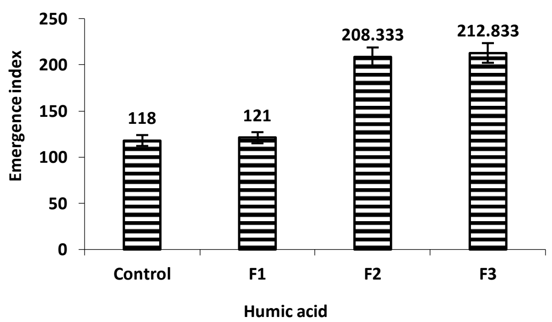

Fig. 1. Effect of humic acid on emergence index in guar plant.

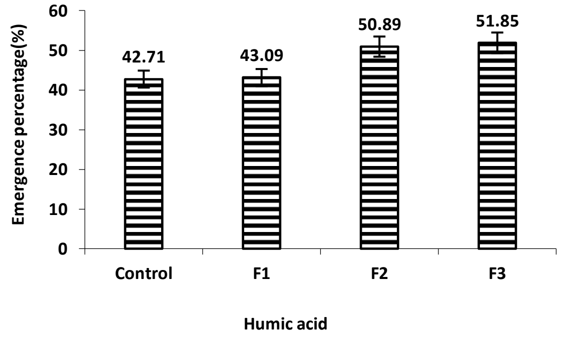

Fig. 2. Effect of humic acid on emergence percentage in guar plant.

Leaf area index

Humic acid has been responsible for the persistence of photosynthetic tissues and also the humic acid has been shown to increase the leaf yield of plants through positive physiological effects such as increasing leaf chlorophyll content and increasing the metabolism and permeability of cells relative to water and food (Nardi *et al.*, 2002), that the positive effect of humic acid on photosynthetic tissues is clearly evident in the results of this study. The effect of humic acid in different phonological stages on the leaf area index was significant (Table 1).

The highest leaf area index was related to F3 treatment (808.50), which did not have a significant difference with F2 treatment (797), but both treatments had a significant difference with control (551) and F1 (561.50) treatments (Fig. 3). Similar to the results of this experiment, it was reported that increasing leaf area and producing more chlorophyll content in bean leaves has a direct relation to the use of humic acid (Astaraei and Ivani, 2008).

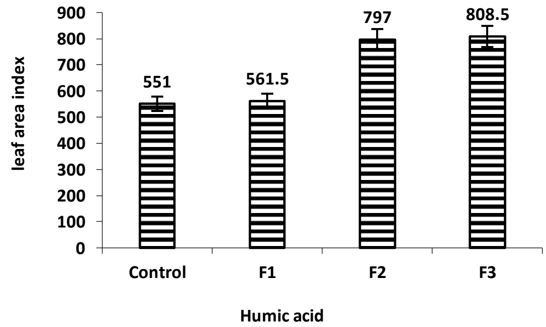

Fig. 3. Effect of humic acid on leaf area index in guar plant.

Number of leaves

The effect of humic acid in different phonological stages (flowering time and harvesting time) on number of leaves was significant (Table 1). In flowering time, the highest number of leaves was related to F3 treatment (66.333), which did not have a significant difference with F2 treatment (64.167), but both treatments had a significant difference with control (51) and F1 (52.333) treatments. In harvesting time, the highest number of leaves was related to F3 treatment (80), which did not have a significant difference with F2 treatment (78), but both treatments had a significant difference with control (62.667) and F1 (63.833) treatments (Fig. 4).

The results of this experiment were found in wheat plants (Sabzevari *et al.*, 2009), Plants of eggplant, cucumber and pepper (Padem *et al.*, 1999) and Peppers, Strawberries and Parsley (Norman *et al.*, 2003).

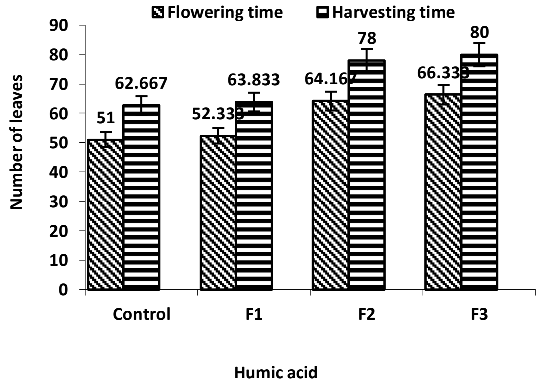

Fig. 4. Effect of humic acid on number of leaves in guar plant.

Stem diameter

The results showed that humic acid had a significant effect on the stem diameter in podding time and harvesting time (Table 1). In podding time, the highest stem diameter was related to F3 treatment (5.8333) and the lowest stem diameter was related to control treatment (4.5) and they had a significant difference. In horvesting time, the highest stem diameter was related to F3 treatment (10.6667) and the lowest stem diameter was related to control treatment (8.1667) and they had a significant difference (Fig. 5). The same conclusion was reported by (Turkmen *et al.*, 2004) in pepper and (Albayrak and Camas, 2005) in Arugula (*Eruca sativa*).

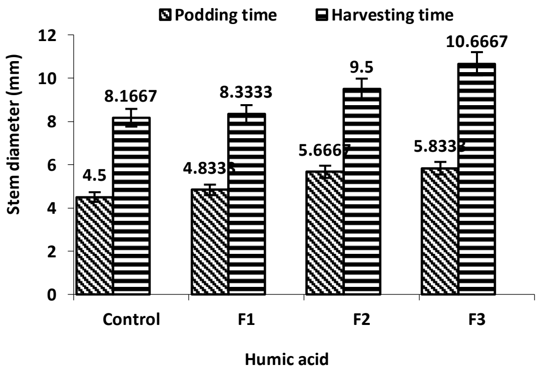

Fig. 5. Effect of humic acid on stem diameter in guar plant.

Plant height

Humic material acts to reduce plant growth by increasing the production of hormones and the antagonistic effects of these hormones on growth and plant height. According to the effect of different concentrations of humic acid on plant height in grass, height was higher in control treatment than other treatments (Daneshvar Hakimi Meibodi *et al.* 2011; Cooper *et al.* 1988), which was in line with the result of this experiment.

The results showed that humic acid had a significant effect on the plant height in flowering time, podding time and harvesting time (Table 1), so that the control treatment showed the highest plant height in the three stages of flowering (15.666), podding (25) and harvesting time (61.667) and F1, F2 and F3 treatments respectively with mean values of 15.5, 13.333 and 12.833 at flowering time, 24.1667, 21 and 20.5 at podding time and 60.5, 50.833 and 48.833 at the harvesting time (Fig. 6), were placed in the next steps and there was no significant difference between control and F1 treatments, but both treatments were significantly different with F2 and F3 treatments. But some results (Turkmen *et al.*, 2004; Nardi *et al.*, 2002; Abdel-Mawgoud *et al.*, 2007) were contrary to this experiment results, which is about the ability of humic substances to increase stem growth in different cultivars of plant species under various conditions.

However, the mechanism responsible for it is not well understood, it may be one of the reasons for the positive effect of humic acid on growth and stem height due to the effect on roots H^+, ATPase activity as well az the distribution of nitrates in root and stem, which in turn lead to changes in The specific distribution of plant hormones, including cytokines, polyamines and ABAs. Thus, they affect the growth of the stem (Rubio *et al.*, 2009). The reasons for not responding positively to humic acid on plant height can be due to the type of humic material used or because of the lack of reactivity of guar gum to humic acid. Therefore, based on the obtained results, the use of humic material used in this study did not have a positive effect on increasing the height.

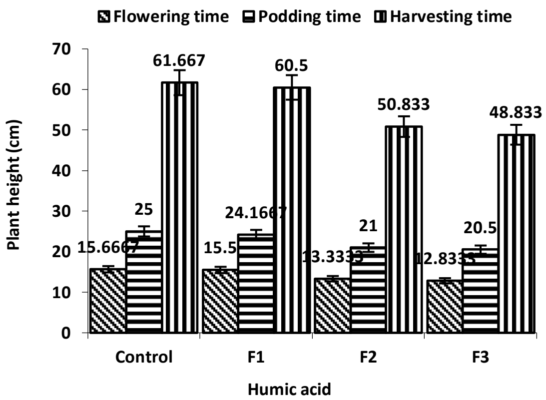

Fig. 6. Effect of humic acid on plant height in guar plant.

Seed yield

The results showed that humic acid had a significant effect on seed yield of guar plant (Table 1). The highest amount of seed yield was related to F3 treatment (585.11) and the lowest amount was related to control treatment (471.43) and they had a significant difference. However, there was no significant difference between treatments F2 (563/7) and F3, and also control treatment and F1 (474.25) (Fig. 7). It was reported that the application of humic acid during flowering had the greatest effect on seed yield in chickpea (Armin and Moslehi, 2012).

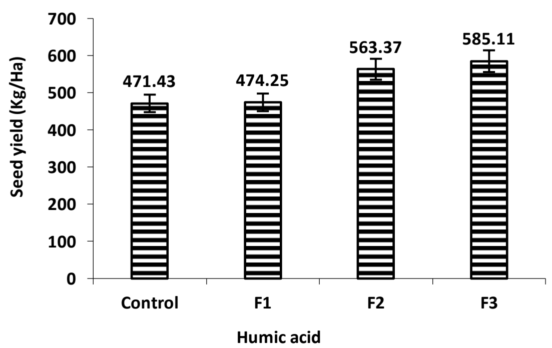

Fig. 7. Effect of humic acid on seed yield in guar plant.

Number of pods per plant

The results showed that humic acid had a significant effect on Number of pods per plant (Table 1). The highest amount of pods per plant was related to F3 treatment (77.667) and the lowest amount was related to control treatment (60.500) and they had a significant difference. However, there was no significant difference between treatments F2 (76.333) and F3, and also control treatment and F1 (61.833) (Fig. 8). With the use of humic acid during reproductive and flowering stages, the plant gains great power in converting raw materials into cultured fruits and by doing so, it increases its resistance and by preventing the flower from falling, it results in more flowers and more fertile pods. It was reported that the application of humic acid during flowering had the greatest effect on number of pods per plant in chickpea (Armin and Moslehi, 2012).

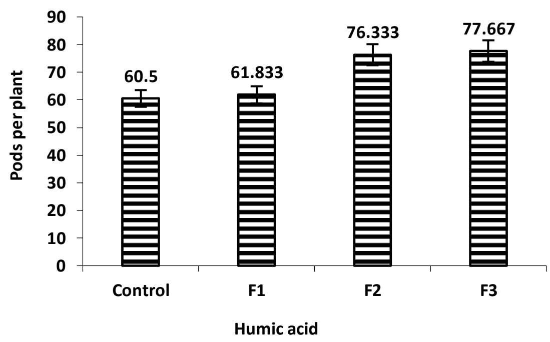

Fig. 8. Effect of humic acid on number of pods per plant in guar plant.

Number of seeds per pod

The results showed that humic acid had a significant effect on Number of seeds per pod (Table 1). Control and F3 treatments respectively with the mean of 6.0000 and 7.8333 seeds per pod, had the lowest and the highest number of seeds per pod and were placed in different statistical groups (Fig. 9). In guar, at the beginning of plant production phase, humic acid has an effect on accelerating vegetative growth and in case of availability of environmental conditions such as moisture and available food elements, the period of reproductive growth increases and it leads to the formation of more flowers in the plant, which affects the formation of fertile pods and grain production (Goldani and Rezvani Moghaddam, 2007). This report is consistent with the result of this test. It has been reported that the application of humic acid during flowering had the greatest effect on number of pods per plant, number of seeds per pod, grain yield and biological yield of chickpea (Armin and Moslehi, 2012).

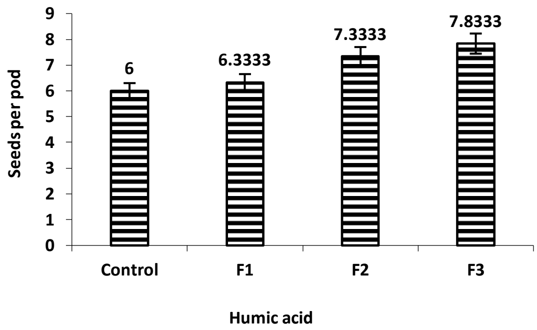

Fig. 9. Effect of humic acid on number of seeds per pod in guar plant.

Conclusion

The results of this study showed that fertilization had a significant effect on all morphological traits except the number of lateral shoots and weight of 100 seeds. The reason for the different effects of humic acid on measured indices in this study can be that the effect of humic acid is strongly influenced by environmental conditions. One of the reasons for the lack of positive effects of humic acid on some of the studied indices, such as plant height, can be the appropriate and controlled environmental conditions where Guar is grown. Given that the seeds and gum obtained from it, is the most practical part of this plant for humans and also considering the use of this plant for the animal as a forage, seed yield, number of lateral shoots, stem diameter, leaf number and leaf area index were considered as the most effective traits. Analysis of variance showed that fertilization treatment of 6 liters per hectare had a significant effect on seed yield, stem diameter, leaf number and leaf area index and increased them, but on number of lateral shoots was ineffective. On the other hand, there was no significant difference between the fertilization treatment of 6 liters per hectare and fertilization treatments of 3 liters per hectare. Therefore, Guar's fertilizer treatment of 3 liters per hectare is considered as the most suitable treatment for reducing costs and harvesting the most crops in the shortest possible time.

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