



RESEARCH PAPER

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The effect of different levels of humic acid fertilizer on components of biological nitrogen fixation in cowpea cultivars in Ahvaz

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Key words: Humic acid, biological nitrogen fixation, cowpea, cultivars, yield.

<http://dx.doi.org/10.12692/ijb/5.2.167-174>

Article published on July 28, 2014

Abstract

Making use of humic acid in agriculture due to different physiological effects not only increases the yield but also decreases the consumption of chemical fertilizers and reduces environment pollution. Humic acid is among the substances which have dual effect on biological nitrogen fixation, i.e. it can develop the roots and has positive effect on activity of soil microorganisms and can increase biological nitrogen fixation in legumes. In order to study the effect of different levels of humic acid on components of biological nitrogen fixation in cowpea cultivars a factorial experiment in the form of randomized complete block design was carried out in Ahvaz in 2011. Three levels of humic acid (H1 = 0, H2 = 75 PPM, H3 = 150 PPM) and two cowpea varieties (V1 = local, V2 = Egyptian) were placed in plots as a combination. Humic acid was once sprayed to crops at the end of vegetative growth. The results showed that humic acid foliar spray fertilizer significantly increased root growth, number of roots, and number of nodules, root dry weight, and nitrogen percentage of crop nodule. The highest percentage of nitrogen fixation belonged to H3 treatment by 6.10%. Moreover, the effect of cowpea cultivar on all traits was significant except on the number of roots and the length of pod. The highest percentage of nitrogen fixation belonged to Egyptian cultivar by 5.81%. In this experiment, Egyptian cultivar was more efficient than local cultivar.

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Introduction

Statistics indicate the increasing importance and daily development of legumes as an important source of food in most countries (Golabi and Lak, 2005). Supplying food for human population of the world mainly depends on capability of green plants to convert solar energy to carbohydrates through photosynthesis process. Later storage of carbohydrates or changing them into other storage or structural materials through biochemical changes will provide a direct source of food supply for human being (Ramezan, 1998). Legumes with high level of energy, protein, different kinds of vitamins, minerals, and medicinal properties are among the most important crops in Iran. Legumes forage also has high nutritional value due to its high percentage of protein and has a good alternative effect and can improve biological and chemical fertility of soil due to its nitrogen fixation ability (Noori *et al.*, 2005; Majnoon Hosseini, 1993).

The importance of nitrogen in the formation, survival, and evolution of life is to the extent that without this element the life absolutely would be quite different from what we see today. Nearly, 78% of atmosphere is made up of N₂. Plants, animals, and microorganisms are all surrounded by nitrogen and in fact all of them live in N₂ environment. However, this great source of nitrogen is unusable for most of the living things except for some bacteria (Abbasi *et al.*, 2005). Molecular nitrogen entrance to biosphere is called nitrogen fixation, a process that changes nitrogen into an accessible form for biological systems. This is done by nitrogenase which is a catalyst in converting N₂ to NH₃.

Nitrogen fixation and changing it into usable form for plants is mainly possible through industrial or biological methods (by some group of bacteria) (Sprent, 1990). Wider use of biological nitrogen fixation is emphasized as a vital necessity for realization of sustainable agricultural systems (Haghighi *et al.*, 2011).

Soil health is one of the key factors in determining

crop yield. Humic acid in soil has several positive effects on its main components (Haghighi *et al.*, 2011). By chelating essential elements, humic acid will increase their absorption, soil fertility, and crops yield (Liu and Cooper, 2000). Moreover, humic acid increases photosynthetic activity of plant by enhancing rubisco activities (Delfine *et al.*, 2005).

Humic acid is an organic acid without environmental effects. Considering positive effects of humic acid on growth and activity of soil microorganisms and the increase of contact area between roots and rhizobium bacteria and development of the roots it can be said that humic acid improves biological nitrogen fixation in legumes (Haghighi *et al.*, 2011).

The main objectives of this research are

1. Investigating the effect of humic acid foliar spray fertilizer on biological nitrogen fixation and measurement of nitrogen percentage of nodule in cowpea.
2. Investigating the effect of humic acid on increase of root volume in cowpea.
3. Investigating the effect of humic acid on increase of number of nodules in the roots of cowpea.

Materials and methods

Characteristics of Experimental plant and Factors

This research was conducted by using factorial experiments as randomized complete block design with four replications.

Studied experimental factors including

- a. Variety treatment (V) including two levels: V1 (local) and V2 (Egyptian)
- b. Humic acid treatment including three levels: H1: control treatment, H2: 75 PPM humic acid, H3: 150 PPM humic acid.

Humic acid which was applied was produced by Caspian Bio-Fertilizer Company under the license of Sweden Jura Forum and as recommended, it was sprayed to plants 40 days after planting.

Sampling Stages

Biological Nitrogen Fixation

In order to study the components of biological nitrogen fixation every 12 days 3 plants were removed from each plot and were profiled in the soil so that the sub roots and nodules were not damaged and then the plant root was removed from the soil in a cylindrical form. After separating the roots from the plants they were washed and the number of nodules on the root, number of sub roots, and root volume were measured. The roots were placed in the oven for 48 hours at 75°F and then the dry weight was measured. After counting side roots and measuring dry weight of the roots, the volume of dry roots was calculated through Archimedes' principle and the difference of volume of cylinder water. Some of the nodules which were intact in each experimental unit were carried to water and soil laboratory to measure the rate of nitrogen and then nitrogen percentage of nodule was calculated through Kjeldahl method.

Results and discussion

Number of Nodules per Plant

The effect of different levels of humic acid on the number of nodules during the growth season was significant at 1% level. The highest number of nodules belonged to H3 treatment by 58.75 and the lowest number of nodules belonged to the control treatment by 40.01. The highest number of nodules in H3

treatment could be due to the effect of microelement in nodulation process. For instance, zinc plays a role in nodulation launch, increase of leg hemoglobin, and nitrogen uptake. Other microelements also have some positive effects on nodulation (Parsa and Bagheri, 2008). High number of nodules in treatment with high level of humic acid is due to the interactions that are made in nodules by humic acid. The results of this part of experiment were consistent with the findings of Haghighi *et al.* (2011). Tan and Tanti Wiramond (1982) stated that application of humic acid and folic acid had a significant effect on total dry weight of roots and nodules, and the number of nodules increased in comparison to control treatment. Bkardwaj and Guar (1972) found that humic acid like sodium humate and folic acid had a stimulatory effect on nodule and growth of rhizobium trifolii. The results were consistent with the findings of Asghari *et al.* (1988). They stated that the number of root nodules significantly increases in the treatment with 150 ppm humic acid in comparison to other treatments. Emergence of primary nodules and production of more nodules in the treatment with 150 ppm humic acid could be due to stimulatory effect of humic acid on nodulation process. Remarkable activity of nitrogenase enzyme is related to the effects of humic acid.

Table 1. The ANOVA of number of nodules, nitrogen percentage of nodule, number of roots, and root volume of cowpea at different levels of cultivar and humic fertilizer.

Sources of variations	Degree of freedom	Mean of squares			
		Number nodules	of Nitrogen percentage of nodule	of Number of roots	Volume of root
Replication	3	179.28	1.32	8.47	0.52
Cultivar	1	196.67**	2.80*	15.04 ^{ns}	1.04*
Humic acid	2	703.17**	4.44**	84.04**	8.73**
Humic acid x cultivar	2	89.17*	2.93*	54.29*	0.67*
Error	15	22.58	0.50	11.79	0.16
Coefficient of variations (CV%)		16.15	12.95	14.71	7.87

ns, *, ** respectively indicate non-significant difference, and significant difference at 55 and 1% levels.

The ANOVA results showed that the effect of cultivar on the number of nodules was significant at 1% level (Table 1). Mean comparison showed that Egyptian cultivar had the highest number of nodules by 52.25

and local cultivar had the lowest number of nodules by 46.58 (Table 2). The cultivar which has more positive reaction to humic acid will have higher number of nodules and in this research the Egyptian

cultivar was so. The results were consistent with the findings of Saki Nejad (2010).

According to table 1, the interactive effect of humic acid and cultivar on the number of nodules was significant at 5% level. As shown in diagram 1, the highest number of nodules belonged to the treatment with Egyptian cultivar and H3 (150 ppm humic acid) by 60.75 and the lowest number of nodules belonged to the control treatment (without humic acid) and

local cultivar by 36.75. The higher number of nodules in treatment with interactive effect of H3 and Egyptian cultivar was associated with genetic potential of plant and the effect of fertilizer on the treatment and also higher growth indices of root in Egyptian cultivar although the macro elements used in humic acid increased the stimulatory effect of humic acid on nodulation. The results were consistent with the findings of Haghighi *et al.* (2011).

Table 2. Mean comparison of simple effects of different levels of cultivar and humic fertilizer on number of nodules, nitrogen percentage of nodule, number of roots, and root volume of Cowpea.

Treatments	Traits mean			
Cultivar	Number of nodules	Nitrogen percentage of nodule	Number of roots	Root volume (CC)
Local	46.58 b	5.12 b	19.75 a	13.48 b
Egyptian	52.25 a	5.81 a	21.33 a	13.97 a
Level of humic fertilizer				
0	40.01 c	4.70 c	18.25 b	12.67 c
75	49.50 b	5.52 b	19.12 b	13.64 b
150	58.75 a	6.19 a	24.25 a	14.76 a

According to Duncan's multi range test, the means of treatments with similar letters were not significantly different from each other at 5% level.

Nitrogen percentage of Nodule

The ANOVA results (Table 1) showed that the effect of different levels of humic acid on nitrogen on the rate of nitrogen of nodule in cowpea was significant at 1% level. The highest rate of nitrogen of nodule was related to the treatment with H3 (150 ppm humic acid) by 6.19 and the lowest rate was related to control treatment by 4.70. Since biological nitrogen fixation requires a lot of energy, some macro-elements such as nitrogen and phosphorus can meet such a need. In fact, humic acid increases the uptake and transport of elements such as Na, K, Ca via increasing permeability of root cell membrane (Pinton and Kasko, 1999). When the plant owns all elements particularly those which are needed for biological nitrogen fixation, it will remarkably increase biological nitrogen fixation (Haghighi *et al.*, 2011). Experiments showed that humic acid would increase the root growth, carbon reservation, photosynthesis, resistance against the diseases, and

biological nitrogen fixation. The importance of Nitrogen is to the extent that it has led to the survival of living things and without nitrogen life would definitely be different from what it is now. Nearly, 78% of atmosphere is covered by nitrogen, but all plants are not able to make use of it. Legumes make this form of nitrogen to usable form by using biological nitrogen fixation. Considering the positive effects of humic acid on growth and activity of soil microorganisms, it could be said that humic acid improves biological nitrogen fixation in legumes (Saki Nejad, 2010). Tan and Tanti Wiramond (1982) showed that stimulatory effects of humic acid on growth might be due to positive pulses of nitrogen in soil. Asghari *et al.* (1988) reported that humic acid had a significant effect on development of nodules and nitrogenase activity and increased nitrogenase activity. Due to its microelements such as Mo, Zn which are effective in nitrogenase activity and have a direct effect on biological nitrogen fixation equation,

humic acid makes these elements available for the plant more easily and fixation is done easily and more N is fixated. The results of this part of experiment were consistent with the findings of Tan and Tanti Wiramond (1982) who stated that the effect of humic acid on legumes growth might be due to improvement of biological nitrogen fixation and increase of nitrogen percentage in plant.

According to the ANOVA results (Table 1) the effect of cultivar on nitrogen percentage of nodule was significant at 5% level. The highest nitrogen percentage of nodule was observed in Egyptian cultivar by 5.81 % and the lowest nitrogen percentage of nodule belonged to local treatment by 5.12% (Table 2). This might be due to genetic effects of the plant. The results were consistent with the findings of Saki Nejad (2010).

Table 3. Mean comparison of interactive effects of different levels of cultivar and humic fertilizer on number of nodules, nitrogen percentage of nodule, number of roots, and root volume of Cowpea.

Treatment			Mean of traits			
Cultivar	Levels of humic fertilizer		Number of nodules	Nitrogen percentage	Number of volumes	Root volume (cc)
Local	0		36.75 e	4.10 c	18.02 c	12.37 c
	75		46.50 cd	5.51 ab	17.50 c	13.60 b
	150		56.75 ab	5.76 ab	23.75 ab	14.47 a
Egyptian	0		43.25 de	5.30 b	18.50 bc	12.97 c
	75		52.75 bc	5.52 ab	20.75 abc	13.67 b
	150		60.75 a	6.61 a	24.75 a	16.05 a

According to Duncan's multi range test, the means of treatments with similar letters were not significantly different from each other at 5% level.

The interactive effect of humic acid and cultivar on nitrogen percentage of nodule was significant at 5% level. According to Table 3, the treatment with H3 and Egyptian cultivar fixated the highest percentage of nodule nitrogen and control treatment and local cultivar fixated the lowest percentage of nodule nitrogen. Tan and Tanti Wiramond (1982) reported that stimulating effect of humic acid on growth of legumes is due to the improvement of biological nitrogen fixation in soil. Humic acid causes the increase of dry matter production, nodulation, and nitrogen content in the nodules of legumes. Haghighi *et al.* (2011) found that humic had a positive effect on activity of soil microorganisms and could increase biological nitrogen fixation in legumes cultivars. According to the results of experiment, since Egyptian cultivar had a more positive reaction to fertilizer treatment it could be said that Egyptian cultivar had a better reaction to fertilizer than local cultivar in terms of nitrogen percentage of nodule. The obtained results were consistent with the findings of Haghighi *et al.* (2011), and Saki Nejad (2010).

Number of Secondary Roots

According to the ANOVA results (Table 1) the effect of application of different levels of humic acid on the number of secondary roots of cowpea is significant at 1% level. Humic acid causes the increase of number of secondary roots (Table 2). The highest and the lowest number of secondary roots in this experiment belonged to H3 and H1 treatments by 24.25 and 18.25 respectively. Application of humic substances significantly increases the concentration of antioxidants in leaves and causes the increase of photosynthesis, respiration, synthesis of nucleic acids and absorption of ions and increase of root growth (Smith and Zhang, 1998). Moreover, Vaughan and Malcom (1985) reported that among the overall effects of humic acid, the increase of root growth is more tangible than increase of stem growth. The results were consistent with the findings of Leila *et al.* (2011), Eakins *et al.* (2003).

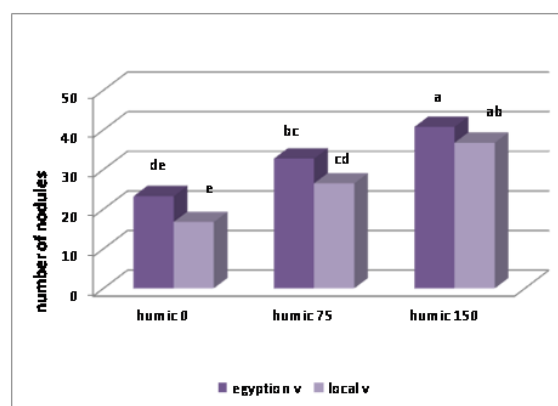


Fig. 1. the interactive effect of cultivar and humic fertilizer on number of nodules in cowpea.

According to Table 2, the effect of cultivar on the number of secondary root was not significant, but the highest number of secondary root belonged to Egyptian cultivar by 21.33 and the lowest one belonged to local cultivar by 19.75.

According to Table 1, the interactive effect of humic acid and cultivar on the number of secondary roots was significant at 5% level. The highest number of secondary roots was related to the interactive effect of H3 and Egyptian cultivar by 24.75 and the lowest one was related to H2 and local cultivar by 17.50 (Table 3). Haghighi *et al.* (2011) in an experiment treatment and the lowest one was related to control treatment examined the effect of humic acid on root of cowpea. Their results showed that the highest and the lowest number of secondary roots belonged to the treatments with macro humic acid and control treatment cultivar. Moreover, the highest dry weight of the root was related to humic acid and it had a positive correlation with biological nitrogen fixation (the rate of nodule nitrogen) (Bartal *et al.*, 1988). In addition, humic acid increased the content of root sodium and potassium, but it didn't have a significant effect on calcium and iron and caused a significant decrease of copper, manganese, and zinc. The effect of different concentrations of humic acid stimulated root system and nodulation in plant and increased the emergence of secondary roots (Asghari *et al.*, 1988).

Root Volume

The ANOVA results (Table 1) show that the effect of

application of humic acid on root volume was significant at 1% level, and the effect of cultivar and the interactive effect of cultivar and humic acid on root volume were significant at 5% level. According to the mean comparison table (Table 2), the highest root volume belonged to H3 treatment by 14.76 CC and the lowest volume belonged to control treatment by 12.67 CC. Application of humic substances significantly increases the concentration of antioxidants in leaves and causes the increase of photosynthesis and absorption of ions and increase of root growth, dry weight of root, and consequently the increase of roots volume (Liu and Cooper, 1988).

Table (2) shows that the effect of cultivar on root volume was significant and the highest volume of root belonged to Egyptian cultivar by 13.90 CC and the lowest one was related to local cultivar by 13.48 CC.

According to the table of mean comparison of integrated effects (Table 3), the highest volume of root was related to Egyptian cultivar and H3 by 16.05 CC and the lowest volume was related to local cultivar and control treatment (H₁) by 12.37 CC. The root volume depends on the number of roots. Since 150 ppm humic acid (H3) could increase the number of roots in Egyptian cultivar, it has dedicated the highest volume of the root to itself. Lakis and Petras (1995), Haghighi *et al.* (1998) stated that humic acid would increase the number of secondary roots in cowpea. Liu *et al.* (1998) did an experiment on Bent Grass Plant and announced that humic acid with concentration of 44 mg/l significantly increased root dry weight and also the activity of nitrogenase enzyme which in turn increased the root respiration and its growth. In this experiment, Egyptian cultivar was more efficient than local cultivar and was more influenced by fertilizer in comparison to local cultivar. The results were consistent with the findings of Haghighi *et al.* (2011).

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