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Evaluation of the Effect of Various Amounts of Humic Acid on Yield, Yield Components and Protein of Chickpea Cultivars (*Cicer Arietinum L.*)

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ABSTRACT

Objective: In addition to the role of organic matter in plant nutrition, this material improves soil quality, physical structure, ventilation and water supply, and ease of penetration and expansion is rooted deep in the soil surface. Effects of organic matter due to climatic factors, soil characteristics may vary from region to region, resulting in organic materials management must be given to all factors affecting. **Methods:** Effect of various amounts of Humic acid on yield, Yield Components and Protein of Chickpea Cultivar s an experimental was conducted on farms of Raan Agricultural Company in spring 2013 at the city of Firouzkouh in a factorial based on randomized complete block design format with three replication In this test, Humic acid was studied in four levels of nil (control), 1.5, 3 and 4.5 kg in each ha on three species of chickpea named "ILC482", "Hashem" and "Arman". Humic acid solution spraying was conducted at three stages of 2-4 leaves, 6-8 leaves and early flowering stage. **Results:** The Delete results showed that use of Humic acid has had significant effect on most of studied traits and at the level of 1% and 5% probability. Use of 3 kg of Humic acid in each hectare showed more effect in most studied traits. The maximum percentage of protein was obtained in use of 3 kg of Humic acid in each hectare as much as 20.48 percent while the minimum amount (11.41 percent) related to the control group. The results showed that significant difference was observed between species of chickpea in terms of studied traits. The chickpea species "ILC482" stood at superior rank than the others species in terms of yield, Yield production with its yield rate of 2,386 kg in each hectare.

INTRODUCTION

Based on prediction made in this regard, world population will hit 8 billion people in 2025, expandable to 8.9 million people in 2050. It should be noted that approx. 80 million people is added to world total population every year and 97 percent of population growth is observed in developing countries. Therefore, it can be concluded that dire need to production of food will be doubled by 2025. In the same direction, rapid

trend of burgeoning population in developing countries will follow unpleasant consequences and outcomes. Shortage of food and malnutrition are of the problems that world community is facing it and poverty and famine are very serious and dangerous in many regions and countries (Qorbani et al. 2011). It should be noted that a chickpea with its scientific name of (*Cicer arietinum L.*) (Banaei, 1997) is regarded as the most important plant from subcategory of cereals in the country (Sabaghpour, et al. 2010). The said chickpea species named "(Cicer

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arietinum L.) has specific significance than other agricultural plants due to its various and diversified usages and also ability of this plant for growth in different agricultural systems with low basic raw materials under unfavorable conditions in terms of soil and dry environments (Pirdadeh et al. 2011).

This plant has more compatibility with various climatic conditions of the country than the other species of cereals. Moreover, this chickpea is able to meet a part of required protein due to the limitations existing in supply of animal proteins (Sabbaghpour, 2003). Presently, agricultural land areas in the Earth are not enough for people of the world and proper nutrition of population, who are increasing day by day, is a very difficult task. In other words, world is facing burgeoning population and nutrition of a huge number of people in world in a very difficult task (Mazaheri and Majnoon Hosseini, 2002). Burgeoning population, destruction of environment and low production output in each acre, etc. are regarded as one of the most important and major concerns of human community (Timothy et al. 2000). Increasing performance in each acre is considered as proper solution for increasing agricultural produce. In agriculture, higher performance is obtained when there is proper combination of water, soil, air and plant factors (Taylor and Smith, 1992). In other words, a plant for better growth needs proper water, soil, and air. Vast and widespread publicities have been taken after for use of organic and animal fertilizers in recent years due to the environmental problems caused by application of chemical fertilizers, energy used for their production and adverse effects which leave on bio ecosystems of farming systems and physical property of the soil. It is believed that these fertilizers organic and animal manures are environment friendly, healthy, harmless and without any adverse effects. Therefore, observing rules and standards in all management dimensions of organic fertilizers is a basic and fundamental principle in safeguarding environment and health of human. In other words, organic and animal fertilizers observe requirements of international standards (Fat'hi et al. 2010). Moreover significant role of organic materials in nutrition of plants, these materials have other fruitful and useful effects as well. Organic materials improves quality of soil, corrects physical structure, stores and ventilates water properly, corrects PH, and facilitates penetration and expansion of root in depth and surface of soil. On the other hand, adding organic fertilizers causes increased capability of storing food elements (Cation-Exchange Capacity, CEC), reducing runoff and controlling soil erosion. Humus part of organic materials has a partial amount of phosphorus that is available to the plant gradually. Unlike mineral phosphorus (inorganic), humus part of the plant is free from stabilization and non-absorbing risk in calcareous soils which has almost covered more agricultural land areas of the country (Malakouti, 2006). Humic acid is a natural organic polymeric compound (Salt et al. 2001) that is the most important component of soil humus

(Sparks, 2003), tasked with controlling pathogenic factors and increases soil health, improves better absorption of food elements and nutrients by the plant and increases accessibility to minerals and finally boosts quality of product to a great extent. (Moromikal et al. 2011). In addition, Humic Acid can be applied in liquid or powder form in soil or one plant leaves (Olkan, et al. 2008). Humic acid has several advantages and benefits and all farmers across the world have come to this conclusion that humic acid is considered as an inseparable and integral part of fertilization program and soil fertility (El-Ghamri 2009). Hereunder are regarded as salient biological advantages of Humic acid in plants: instigating plant growth by increased meiosis, accelerating root growth speed, increasing performance of dry materials, enhancing generation of seed, increasing vitamin value in plants, increasing permeability of plant membrane, accelerating absorption of food elements and nutrients (Burdick 1965). Aerial growth and plant root are instigated by Humic acid but the acid affects more on plant root. Moreover, Humic acid increases root volume and caused effectiveness of root system. Sabzevari et al. (2010)

In their studies, El Basoni et al. (2010) stated that germination growth of green bean such as leaf, branch, plant wet and dry weight, height, pods' green yield and their quality such as length and weight of pod, and content of green leaf chlorophyll of green bean was increased due to the application of Humic acid. The studies showed that application of Humic acid on tobacco and medicinal herbs cause increase of amount of alkaloids at leaves. Also, Humic acid caused increase of transfer of glucose from among cellular membranes in sunflower, sugarcane and onion plants and also increase of degree of carbohydrate in potato, sugarcane, carrot and tomato (Tan, 2003). In another study, Verlinden et al. (2010) studied effect of Humic acid on some grasses and found out that application of Humic acid caused remarkable increase in number and weight of plant's leaf and branch in pastureland. Results of studies conducted by Rasaei, et al. (2012) with regard to the physiological effects of application of Humic acid on green peas indicated that use of Humic acid showed significant effect on value of chlorophyll a and b and content of relative water of leaf as well as value of solution sugars in leaf than those plants that did not use Humic acid. In other words, Humic acid showed significant effect on plants while those plants without Humic acid did not grow better. Results of studies conducted by Sibi and Mirzakhani (2012) with regard to the index of harvest of chickpea as affected by the consumption of salicylic acid of seaweed extract and Humic acid in dry-farming land condition showed that use of Humic acid was significant on weight of 100 seeds in 5% level. In their studies on potato plant, Haj Mohammad Hosseini et al. (2011) stated that application of Humic acid in irrigation water showed significant effect on both dry- and wet weight of plant's aerial organs and also weight of glands. Bulent

Asik et al. (2011) tested use of Humic acid on a species entitled "**Triticum durum Salihli**". The results showed that Humic acid increases absorption of phosphor, potassium, magnesium, sodium, copper and zinc in plant. In another study, Chris, et al. (2005) reported that spraying solution and earthen application of Humic acid increased content of oil of mustard plant significantly. In their studies on chickpea plant, Rasaei et al. (2013) concluded that effect of Humic acid was significant on leaf surface and plant's solution sugar. Reviewing effect of various values of application of Humic acid on yield and components of yield of chickpea cultivar in Firouzkouh area was the main objective of this study. Moreover, finding the best response of cultivar to different concentrations of Humic acid was the other objective of the study.

2. MATERIALS AND METHODS

This experiment was conducted in 2013 on the land areas of Agricultural Company, located at Firouzkouh, with longitudinal specifications (eastern 52 degrees and 44 minutes) and latitudinal specifications (northern 35 degrees and 45 minutes) as high as 1,930 meter from sea level, in a factorial based on randomized complete block design format with three replication. The soil of farm of the study was of loamy clay, with 0.55 percent soil azotes (nitrogen) and soil absorbable Phosphorus as much as 10 mg/kg. In the same direction, ammonia sulfate fertilizers (20 kg/ha) and triple super phosphate (90 kg/ha) were consumed simultaneous with planting based on results of soil test in order to retrieve shortage of soil phosphor and nitrogen. The land area of furrow was measured as large as six square meters. With considering distance of two repetitions from each other as large as 1.5m, six rows of planting were created inside each furrow. Therefore, cultivation of seed was conducted manually with considering distance of bushes on 37.5cm row and inside 8cm row. Factors of test

included three chickpea cultivars i.e. "Hashem", "Arman" and "ILC482". Moreover, Humic acid was used in four levels of "nil", "1.5", "3" and "4.5" kilogram/ha. That is to say that nil, 1.5, 3 and 4.5 kilogram/ha of Humic acid was used in four levels and these amounts were sprayed at 2-4, 6-8 leaf stages and early flowering stage. Spraying solution was conducted by 20-liter sprayer with pressure gauge. 3-kg Diazinone Pesticide was used in each ha land area at the early stage of formation of pod in order to fight with chickpea cocoon-eater worm. Fighting with weed was conducted manually at vegetative, growth, flowering stages and formation of pod. Sampling was conducted randomly for measurement of traits from each furrow. Two lateral-cultivation lines were removed. In addition, 0.5 percent meter was removed from first and last line of each furrow. Traits such as height of plant length of main root, diameter of stem (using digital caliper), stem and leaf's dry and wet weight (using digital scale), number of pod in plant, number of filled pod, weight of one hundred seeds, yield and yield components, value of chlorophyll using spectrophotometer and percentage of protein were measured through evaluation of percentage of seed nitrogen. After collecting and sorting data, analysis of variance was performed using SAS statistical software and comparison of means by Duncan test at 5% probability level was used and the charts from Excel software was used. In the same direction, PATH74 software package was used for studying analysis of causality of remaining effective traits.

3. RESULTS AND DISCUSSION

The results of variance analysis (ANOVA) (Table 1) showed that use of Humic acid showed significant effect on traits of number of pod in bush, number of pod per plant, weight of hundred seeds and yield of seed in 5 and 1 percent probability levels.

Table1-Results of Analysis of Variance for measured triats.

s.o.v	df	Pod numbers per plant	Number of Solid Pod in Bush	Weight of 100 seeds	Seed yield	Protein Percentage
Rep	2	31.37ns	63652.31ns	5.36ns	0.59ns	0.67ns
Humic acid	3	1568.43**	159600.25**	21.55*	0.82*	128.92**
Cultivars of chick pea	2	973.45**	144526.20*	80.19**	1.71**	89.45**
Humic acid× Cultivars	6	628.34**	85978.48*	7.19ns	0.47*	135.9**
error	22	137.22	32289.37	5.66	0.18	3.11
CV%		21.57	26.01	10.81	31.1	10.66

* and **: non-significant and significant at 5% and 1% of probability levels, respectively.

3.1. Number of Pod per plant

Results of variance analysis (ANOVA) showed that use of different values of Humic acid on Number of Pod per plant was significant at 1% probability level. Also, effects (chickpea cultivar × Humic acid) was significant in 1% probability level for trait of number of pod in plant as well. In the same direction, chickpea cultivars have turned significant in 1% probability level with relation to the number of pod in plant. The results of average and mean comparisons showed that maximum number of pod in plant (62.76) was obtained in use of 3 kg of Humic acid per hectare while the minimum value (34.64) related to lack of use of Humic acid (control group). It should be noted that use of 1.5 and 3 kg of Humic acid per ha did not show any significant difference with each other. Based on results of comparison of means, the maximum number of pod in plant was observed in "Hashem" chickpea cultivar (60.53) while the minimum number of pod in plant related to "Arman" chickpea cultivar (43.95). The mutual effects of Humic acid and chickpea cultivars turned significant in 1% level. Accordingly, maximum number of pod in plant (58.07) was observed in "ILC482" cultivar using 3kg Humic acid

in each ha while minimum number of pod in plant (52.40) was observed in "Arman" cultivar. Also, "Hashem" chickpea cultivar showed maximum number of pod in plant (86.20) with use of 4.5 kg of Humic acid in each ha land area while "Arman" chickpea cultivar showed minimum number of pod in plant (33.13).

It seems that number of pod in plant in broad bean family is one of the functional components which have been affected by genotype and environmental factors and final performance in this family is mainly dependant on this part of performance. Similar results have been reported with regard to the increased number of pod in soya bush (Farnia, 2006). Increased number of pod in bush has also been reported by Kaya et al. (2005) in green bean plant due to the use of Humic acid. In other words, number of pod in plant has been increased in green bean upon using Humic acid. Also, it is reported that pre-care chickpea seeds with use of Humic acid led to the increased number of pod in bush (16.7) than non-pre cared seeds (10.2) (Ulukan, et al. 2012). In a study conducted by Hagh-Parast et al. (2012), application of Humic acid on chickpea cultivar caused noticeable increase in number of pod in chickpea bush.

Table 2- Mean comparison for humic acid effects on measured triats.

humic acid	Pod numbers per plant	Number of Solid Pod in plant	Weight of 100 seeds	Seed yield	Protein%
0	34.64b	493.11b	19.89b	890a	11.41c
1.50	61.17a	760.33a	23.22a	1520ab	16.68bc
3.00	62.76a	778.11a	22.22ab	1510ab	20.48a
4.50	58.52ab	731.56ab	23.11a	1630b	17.62ab

In each column, means with similar letter (s) are not significantly different at 0.05 of probability level according to Duncan test.

Table 3- Mean comparison for cultivar effects on measured triats.

Cultivar	Pod numbers per plant	Number of Solid Pod in plant	Weight of 100 seeds	Seed yield	Protein%
ILC482	58.33ab	817.5a	24.75a	1.78a	13.68b
Hashem	60.53a	627.33b	22ab	1.35ab	16.84ab
Arman	43.95b	627.5b	19.58b	1.04b	19.12a

In each column, means with similar letter (s) are not significantly different at 0.05 of probability level according to Duncan test.

Table 4- Mean comparison for intraction effects of tratments on measured triats.

humic acid	Cultivar	Pod numbers per plant	Number of Solid Pod in plant	Seed yield	Protein%
0	ILC482	39.50	673.00	1.31	11.67
0	Hashem	38.63	379.33	0.65	9.63
0	Arman	25.80	427.00	0.70	12.95
1.5	ILC482	59.80	720.00	1.63	14.53ab
1.5	Hashem	59.23	631.33	1.22	10.5b
1.5	Arman	64.47	929.67	1.72	25.03a
3	ILC482	77.8a	1075.67a	2.39a	11.91b
3	Hashem	58.07b	694.67ab	1.34ab	31.5a
3	Arman	52.4b	564b	0.81b	18.03ab
4.5	ILC482	56.23ab	801.33	1.79ab	16.63b
4.5	Hashem	86.2a	804.00	2.18a	15.75b
4.5	Arman	33.13b	589.33	0.91b	20.48a

In each column, means with similar letter (s) are not significantly different at 0.05 of probability level according to Duncan test.

3.2. Number of Pod per plant

Results of variance analysis (ANOVA) showed that different values of Humic acid has turned significant on Number of Pod per plant in 1% probability level. Also, chickpea cultivar and mutual interactions (chickpea cultivar \times Humic acid) also have shown significant effect in 5% probability level with regard to the trait of Number of Pod per plant bush. Results of mean comparisons showed that maximum number of solid pod in plant (778.11) was observed in use of 3 kg of Humic acid in each ha land area while the minimum number of pod was related to the lack of consumption (control) of Humic acid. Accordingly, chickpea cultivar "ILC482" has produced the maximum number of pod in plant (817.50) while the minimum quantity is related to "Hashem" chickpea cultivar (627.33). It should be noted that two chickpea cultivars named "Arman" and "Hashem" did not show any significant difference in this trait. According to Table 2 on number of solid pod in the plant, slicing of reciprocal effects showed that chickpea cultivars in 3-kg use of Humic acid on each ha had significant difference in 1% probability level. Therefore, according to Table 4, maximum number of solid pod in the plant (1075.67) was observed in "ILC482" chickpea cultivar with using 3 kg of Humic acid on each ha while the least number of pod in the plant was observed in "Arman" chickpea cultivar (564). At the outset of its flowering stage, chickpea plant has rapid and fast growth. If available humidity is provided for chickpea, the length of growth period and its current photosynthesis is increased. In the same direction, if current photosynthesis is increased, more flowers will be formed in chickpea plant which is effective on formation of fertile pod and seed production (Goldani and Moghadam, 2007). With the increased activity of Robisco enzyme, Humic acid will cause remarkable increase in plant photosynthesis activity and consequently, performance and yield of chickpea is increased to a great extent (Delphin, et al. 2005). In solution-spraying test of different value of Humic acid on chickpea, Sadeghi Moghaddam et al. (2013) proved significant effect of Humic acid on the number of seed in pod. Also, in another study conducted by Qorbani, et al. (2010) on corn, they observed remarkable growth in number of seed in the row due to the use of Humic acid.

3.3. Seed Weight

The results of variance analysis (ANOVA) showed that different values of Humic acid had significant

effect on weight of grain seed in 5% probability level. In the same direction, chickpea cultivars had significant effect on trait of weight of grain in 1% probability level. The results of mean comparison, according to Fig. 6, showed that maximum weight of grain stood at 23.22 gr with use of 1.5 kg of Humic acid on each ha land area while minimum weight of hundred seeds stood at 19.89 gr with lack of use of Humic acid (control) in each ha land area. It should be noted that 1.5 and 4.5 kg of Humic acid did not show any significant difference on weight of grain on each ha land area. The Table 3 showed that "ILC482" chickpea cultivar produced maximum weight of seed (24.75 gr) while "Arman" chickpea cultivar produced minimum value (19.58 gr). It seems that use of Humic acid leads to the increased rate of photosynthesis in plant and consequently, production of photosynthesis materials have increased in plant with use of Humic acid as well. In general, use of Humic acid has increased length of growth period, rate of carbohydrates, amino acids and proteins in plant. In the same direction, rate of retransfer of photosynthesis materials is done to a great extent from growth parts and consequently, weight of seed will be increased (Farnia and Nasrallahi, 2010).

Saeini and West Gate (2000) announced that seed weight is due to the increased number of endosperm, amiloplast cells and photosynthesis materials that is increased probably as a result of effect of growth hormones on meiosis. The result of study made by Haghparast et al. (2012) showed that application of Humic acid in foliar form has had significant effect on grain weight of chickpea and has caused remarkable increase in grain weight.

3.4. Weight of 100 seeds

Based on Table 1, results of variance analysis (ANOVA) showed that different values of Humic acid showed significant difference on yield of seed in 5% level. Chickpea cultivars and interactional effects (chickpea cultivars \times Humic acid) was significant in 5% probability level. The results of mean comparisons showed that maximum seed yield (1,630 kg/ha) was obtained in use of 4.5 kg of Humic acid on each ha while the minimum amount (890 kg/ha) related to the lack of use of Humic acid (control). In the same direction, 1.5 and 4.5 kg use of Humic acid did not show any significant difference. Slicing of interactional effects showed that chickpea cultivars showed significant difference with use of 3 and 4.5 kg of Humic acid on each ha with 1% probability level.

The maximum seed yield (238.66 kg/ha) was obtained with use of 3 kg of Humic acid in "ILC482 chickpea cultivar" while the minimum amount (813.33 kg/ha) related to "Arman" chickpea cultivar. Maximum seed yield (2,180 kg/ha) related to "Hashem chickpea cultivar" with use of 4.5 kg of Humic acid while "Arman chickpea cultivar" accounted for minimum amount of 906.66 kg/ha. At the outset of reproductive growth, formation of seeds and grain filling period will cause increased seed yield using transfer of photosynthesis materials from vegetative organs to reproductive organs (Rahmani et al. 2010). Humic acid will causes noticeable increase of yield of plants using positive physiological effects such as effect of metabolism of plant cells and increased concentration of leaf chlorophyll (Nardi et.al 2002). In a study of spraying Humic acid at the stage of development of wheat branch, seed yield was increased by 7 to 8 percent than treatment (Exodan, 1986). In another study, Humic acid caused remarkable increase of seed yield in barley (Ayosou, et al. 1996).

3.5. Protein Percentage

Based on Table 1, results of variance analysis showed that various values of Humic acid have shown significant difference on chickpea cultivars and interactional effects (chickpea cultivar × Humic acid) on trait of protein yield in 1% probability level. The mean comparisons showed that maximum protein value (20.48%) was obtained in use of 3 kg of Humic acid in each ha while the minimum value (11.41%) was obtained due to lack of use of Humic acid in control group. Also, according to the abovementioned Table 3 maximum protein yield (19.12%) is related to "Arman" chickpea cultivar while the minimum value (13.68%) was obtained from "ILC482" chickpea cultivar. The interactional effects showed that chickpea cultivars had significant difference in use of 1.5, 3 and 4.5 kg of Humic acid on each ha with 1% probability level especially on seed protein. According to Table 4, maximum percentage of protein (25.03%) on each ha related to "Arman" chickpea cultivar while the minimum value (9.63%) related to "Hashem" chickpea cultivar. In the same direction, maximum protein percentage (31.50%) was obtained in "Hashem" chickpea cultivar with use of 3 kg of Humic acid in each ha while minimum (11.91%) belonged to "ILC482" chickpea cultivar. With use of 4.5 kg of Humic acid, "Arman" chickpea cultivar accounted for maximum protein value while "Hashem" chickpea cultivar obtained minimum value

(15.75%). Meanwhile, two chickpea cultivar "ILC482" and "Hashem" did not show any significant difference. Kaya et al. (2005) reported that Humic acid spraying solution (foliar) has increased protein value at 3-6 leaf stage in green bean plant.

Conclusion:

The results indicated that use of Humic acid showed significant effect on most studied traits and in 1% and 5% probability level. In most traits, use of 3 kg of Humic acid on each ha land area showed significant effect. Generally speaking, "ILC482" chickpea cultivar showed priority to the other species of chickpea cultivar in terms of production of seed yield. In comparing with values of consumption, the results showed that 4.5 kg use of Humic acid on each ha showed significant effect on yield with 1630 kg mean on each ha. With due observance to the more effect of use of 3 kg of Humic acid on most traits, this level is more acceptable for the climatic conditions in area of Firouzkouh with regard to the chickpea.

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