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The Effects of Humic Acid and Ammonium Sulfate Foliar Spraying and Their Interaction Effects on the Qualitative and Quantitative Yield of Native Garlic (*Allium sativum* L.)

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ABSTRACT

In order to investigate the effects of ammonium sulfate and humic acid foliar spraying and their interaction effects on the yield and components yield of native garlic (Allium sativum L.), an experiment as a factorial form in a randomized complete block design in the farming year 2013-2014 was conducted. The considered treatments were included ammonium sulfate at four levels of zero (control), 100, 150 and 200 kg.ha-1 and humic acid at four levels zero (control), 1000, 2000 and 3000 mg.lit-1. According to the variance analyze results, ammonium sulfate and humic acid on the measured characteristics at one percent probable has significant effect, also the interaction, ammonium sulfate with humic acid at one percent probable on the clove number, chlorophyll and allicin at five percent probable on the leaves number and bulb yield ha significant effect but on the clove weight and essential oil percent has not significant effect. The obtained results of the mean comparison showed that the using of ammonium sulfate caused the increasing of yield and yield components except clove weight, so that the most positive effect was observed in the 150 kg per hectare of ammonium sulfate treatment, also the using of humic acid by increasing yield and yield components except the clove weight was observed and the 3000 mg.lit-1of humic acid treatment has the most measured characteristics amounts.

KEYWORDS: allicin, ammonium sulfate, clove, essential oil percent, humic acid.

INTRODUCTION

Garlic (*Allium sativum L.*) is the second most important crop after onion and as vegetables, spices and drugs are used. Garlic world production is 12 million tons which has doubled from 1986 to 2006. The most area under cultivation and production belongs to china country with 489200 hectare and 6.6 million tons (Anon., 2007). Some researchers the source of this medicinal plant associates with central Asia, near Mongolia or Afghanistan which then by early immigrants moved to East Europe and Asia (Mollafilabi et al., 2005). Garlic has properties such as disinfectant power, insecticide, antibacterial, antifungal, anticancer and lowering sugar and blood lipids that is due to the presence of substances such as protein, fiber, fat, Vitamins A, B, C and natural sugars and lots of micronutrients (Cu, Fe, Sn, Ca) is observed (Bayat and Nosrati, 2001). Although various commercial varieties of this medicinal plants in all around the world is produced, but the results of some studies due to the flexibility of biological phenomena, but the planting of this plant has recommended by using of local varieties (Stern, 2001). Nitrogen in plant nutrition is an essential element and plays multiple roles in plants. Nitrogen to amino acids synthesis that are necessary to components proteins, are required, these amino acids in formation of protoplasm, cell division and plant growth are used. If available nitrogen to plants were low, the plant is not able to make proteins for metabolic processes, structure and maintain the desired level of growth (Barker and Pilbeam, 2006). Nitrogen in the vital functions such as photosynthesis and enzymatic reactions plays a pivotal role and are the components of several essential vitamins such as biotin, niacin, thiamin and riboflavin and is part of the nucleic acids (Barker and Pilbeam, 2006). Onion bulb weight and leaves number were increased by decreasing of soil nitrogen, the additional nitrogen can also encourage leaf growth and reduce the onion growth (Nasree et al., 2007). Researchers (Nasreen et al., 2007) in the investigate the effect of nitrogen and sulfate fertilizers concluded that due to the antagonistic effect of nitrogen, the most yield and the highest amount of sulfur and nitrogen and by onion during the using high doses of nitrogen (160 kg per hectare) and moderate amounts of sulfur (40 $kg.ha^{-1}$) are obtained. As the results of some

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studies (Rafi, 2007) also found that the using of nitrogen by stimulating the vegetative growth increases the yield. Since the penetration depth of onion plants roots such as garlic in soil is relatively low (Hutchinson and Mc Giffen, 200) and according to the positive effect of nitrogen on the rooting process (Edwards et al., 1992), it appears that That the addition of nitrogen in the soil, in addition to stimulating growth by increasing nutrient uptake influenced the development of more root system, increasing of garlic cloves weight was followed that it would be effective in improving the yield of this species industries. It seems that the using of nitrogen In addition to improving the vegetative growth, with an increase in cell division (Gaviola and Lipinski, 2008) increases the clove number that it would be have a positive role in improving the yield of this worthwhile plant. Researchers study (Lurie, 2005) is an Argentine survey showed that the five varieties of garlic the maximum average yield 12.2 ton.ha^{-1} with 225 kg.ha^{-1} of nitrogen treatment was obtained.

The using of nitrogen fertilizer was increased the rate of photosynthesis in the leaves, thus increasing of the rate, the more seeds was created (Gosh et al., 1981). Researchers (Arefi et al., 2012) by investigating the effect of nitrogen different levels on shallot yield stated that by increasing of nitrogen, leaf photosynthesis and consequently yield significantly improved. However, the using of nitrogen as a widely used element has a significant effect on the growth and yields of this medicinal plant therefore the appropriate strategies to maintain the proper functioning of this cashing plant should be used. The conducted investigations results in the field of nitrogen using in garlic agriculture, the using of 100 kg.ha^{-1} nitrogen is recommended to this plant but it should be carefully considered the time using of it because nitrogen fertilizer using from mid-April to later causes a delay in the garlic formation (Gaviola and Lipinski, 2008; Panchal et al., 1992).

Researchers (Baghalian et al., 2005) by surveying of 24 Iranian garlic masses reported that genetic factors had more effect than environmental factors on the garlic pungency. The using of humic acid as a foliar spraying and using in the soil increase the absorption of nutrients from the soil and plant nutrient efficiency (Adani et al., 1998). In the study of humic acid on Bent grass plant showed that humic acid significantly increases photosynthetic rate, root biomass development and nutrient content that this increasing in the concentration of 400 mg.lit^{-1} humic acid was observed (Pinton et al., 2008). Humic acids can directly cause the positive effects on plant growth. Shoot and root growth is stimulated by humic acid, but its effect is more prominent on the roots. Humic acid increased root content and caused the root system effectiveness. Also the humic acid increases the absorption of nitrogen, potassium, calcium, magnesium and phosphorus by plants. (Sabzevari et al., 2009). The researcher by investigating the effect of different levels of humic acid on wheat concluded that humic acid levels had significant differences between stem weight and plant height and the amount of nitrogen in wheat growth (Tahir et al., 2011). In a study the effect of humic acid on some grass was studied and was found that using of humic acid increased the rangeland herb (Verlinden et al., 2010).

The purpose of this experiment, was investigated the qualitative and quantitative yield of native garlic that influenced by ammonium sulfate and humic acid foliar spraying.

MATERIALS AND METHODS

This experiment in the farming year of 2013-2014 as a factorial form in the randomized complete block design with 3 replications in the promotional research of Dastjerd Qom farm was conducted. The Used treatments in this experiment were included the ammonium sulfate at four levels: zero (control), 100, 150 and 200 kg.ha^{-1} as well as humic acid foliar spraying at four levels: zero (control), 1000, 2000, and 3000 mg.lit^{-1}. To cultivate the product of Hamedan white garlic mass was used that has bulb diameter (55.6 mm), clove, and 5.24 gr of clove weight and 18.32 mm diameter of clove. Before planting, to prepare the land was used from plowing operation, disc and leveler. According to the soil test of 40 ton.ha^{-1} in each plot from farmyard manure was used. The examined Ammonium sulfate in each plot was used from split application and half of it in the planting time was mixed with soil and then the planting operation was done. And the other half were given as a slippage at the 2014.3.25 to the product. Clove in each plot at a distance of 20 cm between row and 10 cm on the rows at depth of 5 cm of soil were planted, so that the tip part of cloves was up and then carefully their soft soil was poured around them so that clove was surrounded very well. Humic acid treatments as a foliar spraying at concentrations of zero, 1000, 2000, 3000 mg.lit^{-1} at the

2014.4.4, 2014.4.25, 2014.5.26 were done three times. To applying the same conditions to all plants, the control plots also with distilled water were sprayed. After finishing of planting at the 2013.11.8, the first irrigation was done and then after the finishing of winter rains and snow and the begging of spring was irrigated regularly. The planting operation in the first of summer 2014 was conducted.

For evaluate the chlorophyll content 0.2 g samples prepared from middle part of the fourth leaf and were completely worn. Then 10 mL acetone (90%) was added and at the end, the absorbance was read at 470, 663 and 645 nm wavelengths by spectrophotometer (Optizen 3220UV) and chlorophyll content calculated by following formula (Arnon, 1967):

V= 10
W= 0.2
Chlorophyll a = [(12/7 ×A663)-(2/69 ×A645)]V/(1000×W)
Chlorophyll b = [(22/9 ×A645)-(4/69 ×A663)]V/(1000×W)
Total Chlorophyll = [(20.2 ×A645) + (8.02 ×A663)]V/(1000×W)

Essential oil percentage was evaluated by Clevenger apparatus. The samples essential oil was extracted by distillation with water for 4 h method. Extracted essential oil was dehumidifier by dry sodium sulfate and then essential oil percentage was calculated. To analysis of essential oil and accurate measurement of its ingredients gas chromatography was used. For this purpose, gas chromatograph (Hewlett-Packard 6890) with Splitless injector and capillary column with 30 m length, 0.25 mm internal diameter and 25 mm thickness (Agilent/J and W Scientific, Folsom, Ca, USA) was used. Detector of its ionizing and its radiation was with 210˚C, in which hydrogen and air was passed at 40 ml per minute rate. The firs temperature was 80˚C for 2 minutes and the increased 10˚C per minutes to 140˚C. After 1 minutes temperature changes 4˚C per minute to 190˚C and kept for 2 minutes and the temperature changes 2˚C per minutes to 210˚C. Ultra-pure helium was used as the carrier gas with flow rate of 1 ml per minutes. Output peaks based on retention times compared with standard samples ad determined the identity and determined the concentration based on the area under the curve (Young-Cheol et al., 2005). Analysis of variance was performed with Spss software and mean comparison was conducted by Duncan's multiple range tests at 5% probably.

RESULTS

Number of leaf and clove: According to the analysis of variance (Table 1) ammonium sulfate and humic acid affected significantly ($P<0.01$) number of leaf and clove. Interaction of ammonium sulfate with humic acid had significant effect ($P<0.05$) on number of leaf and clove. Ammonium sulfate increased number of leaf and clove. Number of leaf and clove increased from 9.33 and 8 at without ammonium sulfate to 15.42 and 12.08 at 150 kg/ha ammonium sulfates treatment. Also, application of humic acid increased number of leaf and clove. The highest number of leaf (9.92 leaves) and clove (12.58 cloves) obtained at 3000 mg/l humic acid treatment (Table 2 and 3). Mean comparison of interaction of ammonium sulfate and humic acid showed that the highest number of leaf and clove was 18.67 leave and 15.67 cloves at 150 mg/ha ammonium sulfate with 3000 mg/l humic acid (Table 4).

Clove weight and bulb yield: according to the analysis of variance ammonium sulfate and humic acid had significant effect ($P<0.01$) on clove weight and bulb yield and also interaction of them had significant effect ($P<0.05$) on bulb yield but did not significant on clove yield (Table 1).

Ammonium sulfate decreased clove weight and increased bulb yield. Clove weight decreased from 5.48 g at control treatment to 4.22 g at 150 kg/ha ammonium sulfate and bulb yield increased from 15078.61 kg/ha at control treatment to 17490.83 kg/ha at 150 kg/ha ammonium sulfate treatment (Table 2). Also observed that the application of humic acid decreased clover weight and increased bulb yield (Table 3). The mean comparison results showed that (Table 4) that the highest amount of clove weight was at control and the highest bulb weight was at 150 kg/ha ammonium sulfate with 3000 mg/l humic acid and the lowest amount of bulb yield obtained at without humic acid and 1000 mg/l humic acid without ammonium sulfate.

Total Chlorophyll: the analysis of variance results showed that ammonium sulfate, humic acid and interaction of the had significant effect ($P<0.01$) on total chlorophyll (Table 1). Ammonium sulfate significantly increased total chlorophyll and by using ammonium sulfate, total chlorophyll increased from 0.73 mg/ g fresh weight at control

treatment to 1.01 mg/ g fresh weight at 150 mg/ha ammonium sulfate treatment (Table 2). Application of humic acid has significant effect on total chlorophyll and increased total chlorophyll from 0.74 mg/g fresh weight at control treatment to 1.01 mg/g fresh weight at 3000 mg/l humic acid (Table 3). According to the mean comparison (Table 4) the highest total chlorophyll was 1.22 mg/g fresh weight at 3000 mg/l humic acid with 150 kg/ha ammonium sulfate and the lowest amount of that was 0.66 mg/g fresh weight at control treatment.

Essential oil percentage and amount of allicin: analysis of variance showed that ammonium sulfate and humic acid had significant effect ($P<0.01$) on essential oil and allicin percentage. The interaction of ammonium sulfate and humic acid had significant effect ($P<0.01$) on allicin but did not significant effect on essential oil (Table 1). The levels of ammonium sulfate significantly increased essential oil and allicin. The essential oil increased from 0.53% at control treatment to 0.64% at 150 kg/ha ammonium sulfates and also amount of allicin increased from 4.79 mg/g at control treatment to 5.41 mg/g at 200 kg/ha ammonium sulfates treatment. Application of humic acid significantly increased essential oil and allicin percentage. The essential oil and allicin percentage increased from 0.55% and 4.91 mg/g at control treatment to 0.64% and 5.31 mg/g at 3000 mg/l humic acid, respectively (Table 2 and 3). According to the mean comparison of interaction of ammonium sulfate and humic acid the highest percentage of essential oil was 0.7% at 150 mg/ha ammonium sulfate with 3000 mg/l humic acid and the highest amount of allicin was 5.51 and 5.61 mg/g at 200 kg/ha ammonium sulfate with 2000 and 3000 mg/l humic acid, respectively (Table 4).

Table 1. The variance analysis results of ammonium sulfate and humic acid effects on qualitative and quantitative yield of native garlic

S.O.V	df	Leaves number	Clove number	Clove weight	Bulb yield	Total Chlorophyll	Essential oil percentage	Amount of allicin
Block	2	36.33^{**}	19.56^{**}	0.34^{*}	116086.3^{**}	0.02^{**}	0.002^{**}	0.05^{**}
Ammonium sulfate (a)	3	75.58^{**}	34.92^{**}	3.37^{**}	11748525.85^{**}	0.16^{**}	0.02^{**}	0.83^{**}
Humic acid (b)	3	60.03^{**}	53.64^{**}	3.49^{**}	10629668.23^{**}	0.17^{**}	0.02^{**}	0.39^{**}
a*b	9	2.49^{*}	1.73^{**}	0.11^{ns}	374101.13^{*}	0.005^{**}	0.0001^{**}	0.1^{**}
Error	30	0.93	0.5	0.06	146219.65	0.001	0.001	0.004
CV%		8.16	7.13	5.06	2.34	3.67	5.39	1.24

**, * and ns are significantly at 1%, 5% and not significant, respectively

Table 2. The mean comparison of ammonium sulfate different levels effect on the quantitative and qualitative characteristics of native garlic

Ammonium sulfate ($kg.ha^{-1}$)	Leaves number	Clove number	Clove weight (g)	Bulb yield ($kg.ha^{-1}$)	Total Chlorophyll	Essential oil percentage	Amount of allicin ($mg.g^{-1}$)
0	9.32^{c}	8^{d}	5.48^{a}	15078.61^{c}	0.73^{c}	0.53^{c}	4.79^{d}
100	11.67^{b}	9.33^{c}	5.06^{b}	16482.5^{b}	0.85^{b}	0.58^{b}	5.01^{c}
150	15.42^{a}	12.08^{a}	4.22^{c}	17490.83^{a}	1.01^{a}	0.64^{a}	5.22^{b}
200	12.42^{b}	10.08^{b}	5.07^{b}	16397.5^{b}	0.87^{b}	0.6^{b}	5.41^{a}

Each value is the mean of three replicates. Values followed by different letters in each column are significantly different at $P\leq5$

Table 3. The mean comparison of humic acid effect on the quantitative and qualitative characteristics of native garlic

Humic acid ($mg.Lit^{-1}$)	Leaves number	Clove number	Clove weight (g)	Bulb yield ($kg.ha^{-1}$)	Total Chlorophyll	Essential oil percentage	Amount of allicin ($mg.g^{-1}$)
0	9.92^{d}	7.75^{d}	5.61^{a}	15387.22^{d}	0.74^{d}	0.55^{c}	4.91^{d}
1000	11^{c}	8.75^{c}	5.14^{b}	15895.28^{c}	0.8^{c}	0.56^{c}	5.01^{c}
2000	12.92^{b}	10.42^{b}	4.72^{c}	16610^{b}	0.9^{b}	0.6^{b}	5.2^{b}
3000	15^{a}	12.58^{a}	4.36^{d}	17556.94^{a}	1.01^{a}	0.64^{a}	5.31^{a}

Each value is the mean of three replicates. Values followed by different letters in each column are significantly different at $P\leq5$

Table 4. The mean comparison of Ammonium sulfate and humic acid effects on the quantitative and qualitative characteristics of native garlic

Ammonium sulfate ($kg.ha^{-1}$)	Humic acid ($mg.Lit^{-1}$)	Leaves number	Clove number	Clove weight (g)	Bulb yield ($kg.ha^{-1}$)	Total Chlorophyll	Essential oil percentage	Amount of allicin ($mg.g^{-1}$)
0	**0**	8.33^{h}	6.67^{g}	43.64^{h}	14547.78^{h}	0.66^{j}	0.51^{i}	4.67^{i}
0	**1000**	8.67^{h}	7.33^{efg}	44.29^{h}	14763.33^{h}	0.69^{ij}	0.52^{hi}	4.75^{ij}
0	**2000**	9.33^{gh}	8.67^{defg}	45.6^{fgh}	15201.11^{fgh}	0.75^{fghi}	0.54^{fghi}	4.84^{ghi}
0	**3000**	11^{efgh}	9.33^{def}	47.41^{efg}	15802.22^{efg}	0.81^{fg}	0.56^{efgh}	4.92^{gh}
100	**0**	8.37^{h}	7^{fg}	45.19^{gh}	15062.22^{gh}	0.72^{hij}	0.54^{ghi}	4.82^{hi}
100	**1000**	10^{fgh}	8^{defg}	47.92^{ef}	15974.44^{ef}	0.79^{fgh}	0.56^{efgh}	4.9^{ghi}
100	**2000**	12.33^{defg}	9.67^{de}	50.6^{cd}	16865.56^{cd}	0.89^{de}	0.6^{cde}	5.09^{ef}
100	**3000**	15.67^{abc}	12.67^{bc}	54.08^{ab}	18027.78^{ab}	1^{bc}	0.63^{bc}	5.25^{cd}
150	**0**	12.33^{defg}	9.33^{def}	48.64^{de}	162112.22^{de}	0.82^{ef}	0.59^{def}	4.98^{fg}
150	**1000**	14^{bcde}	10.33^{cd}	50.97^{cd}	16991.11^{cd}	0.93^{cd}	0.62^{bcd}	5.06^{ef}
150	**2000**	16.67^{ab}	13^{b}	53.94^{ab}	17980^{ab}	1.06^{ab}	0.65^{b}	5.36^{bc}
150	**3000**	18.67^{a}	15.67^{a}	56.34^{a}	18780^{a}	1.22^{a}	0.7^{a}	5.47^{ab}
200	**0**	10.33^{fgh}	$8d^{defg}$	47.18^{efg}	15726.67^{efg}	0.74^{ghi}	0.56^{efgh}	5.19^{de}
200	**1000**	11.33^{efgh}	9.33^{def}	47.56^{efg}	15852.22^{efg}	0.79^{fgh}	0.57^{efg}	5.32^{cd}
200	**2000**	13.33^{cdef}	10.33^{cd}	49.18^{de}	16393.33^{de}	0.92^{d}	0.61^{bcd}	5.51^{a}
200	**3000**	14.67^{bcd}	12.67^{bc}	52.85^{bc}	17617.11^{bc}	1.01^{b}	0.64^{bc}	5.61^{a}

Each value is the mean of three replicates. Values followed by different letters in each column are significantly different at P≤5

DISCUSSION

The results of some studies showed that response of plant to nitrogen application as an essential element for growth depend to soil condition, plant species and amount of soil nutrients (Panchal et al., 1992). Nitrogen stimulate plant growth and leaf area production (Sarmadnia and Koochaki, 2001). Therefore, with increasing nitrogen application plant height and number of leaf increases. Also, since the shoots of this plant are used, thus application of nitrogen fertilizer increasing plant growth and yield. The results of some studies have shown that clove is one way to increases of garlic (Pelter et al., 2000). Also, it seems that trait is more influenced by genetic factors, but environmental factors can also have positive effect on the growth development of this medicinal plant. In addition to this, it seems that one of the reasons for the increasing the number of clove in garlic by increasing of nitrogen application is limitations of clove size as a storage of assimilates. If appropriate environmental condition for growth, the assimilates increases in per clove, and if the plant in terms limited from storage, plant increases number of clover and increases bulb diameter (Pelter et al., 2000). The garlic yield depend on shoot drowth and application of nitrogen improve plant growth (Marschner, 2011). Therefore, improvement of garlic yield with increasing of nitrogen application during periods of active growth of the plant seems logical. Increasing of yield and bulb weight by application of ammonium sulfate may be due to increases of absorption of nutrients needed by plants and consequently to an increase in the photosynthesis-related process. Similar results obtained by Hanan et al. (2008) and Nasser and El-Grizawy (2009). Nitrogen fertilizer application increased essential oil production, because application of nitrogen in early stages of growth can be improve vegetative growth and increases number of flowers in reproductive stages and thereby increases the active ingredient (Franz et al., 1983). The reaserches by evaluation the different amounts of ammonium sulfate found that sulfur fertilizer can increases growth and organic compounds of plant and resulting increases yield and food value (El-Sayed et al., 2000). Research results showed that the use of sulfur lead to an increase in chlorophyll (Marschner, 1995). According to the research reports (Wang et al., 2003) most of these compounds containing nitrogen, sulfur increases the nitrogen use efficiency and increase the absorption of other nutrients in plants. Therefore, the application of sulfur may be partly due to the increases of chlorophyll amount in plants. Foliar and soil application of humic acid in plant increases auxin, cytokinin and GA hormones in plant (Abdel-Mawgoud et al., 2001). Humic acid increases root and shoot development which are associated with increases of leaf area and yield (Adami et al., 1998). Improvement of yield and yield component by humic acid application can the effect of increasing of nutrients, especially phosphor and hormone-like effect of humic acid (Autio, 200). The researchers reported that the humic acid by increasing the nitrogen content increases growth, height, number of leaves, and yield (Ayas and Gulser, 2005). Humic acid maintaince photosynthetic tissues

and increase the yield by positive effects on cellular metabolisms and increases of leaf chlorophyll (Naderi et al., 2002). Humic acid has hormone-like activity and increases mineral such as phosphor and potassium absorption and improve photosynthesis, increase chlorophyll and essential oil percentage (Naderi et al., 2002).

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