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Effect of foliar application of humic acid levels and nano-fertilizer application on some quantitative and qualitative traits of pumpkin (*Cucurbita pepo* L.) in climatic conditions of Khorramabad area, Iran

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ABSTRACT

The present study was carried out to evaluate the effect of spraying different levels of humic acid and nano-fertilizer in Khorramabad region in a temperate climate during 2017-2018 cropping year. It was carried out in a factorial arrangement based on completely randomized block design with three replications. The examined factors included humic acid in three levels (control, seed coating with humic acid and humic acid foliar application as H1, H2 and H3 respectively) and foliar application of nano-fertilizer in four levels (control, one, two, and three times nano-fertilizer spraying as N1, N2, N3 and N4 respectively). The results of mean comparison showed that non-application of humic acid and nano-fertilizer treatments had the lowest fruit yield (28400 kg ha^{-1}). Also, by increasing the number of nano-fertilizer application, the fruit yield of pumpkin was elevated. The results showed that there was no significant difference between treatments of one, two and three times the application of nano fertilizer, however, the highest amount of protein and oil yield were 182.7 and 384.9 kg ha^{-1} in three treatments which obtained in nano-fertilizer application and the least amount of oil and protein was obtained in control treatment (distilled water). Overall, the results showed that three-time application of nano-fertilizer with humic acid foliar application as well as seed coating with humic acid could significantly improve the quality and quantity of pumpkin seeds. Therefore, using these fertilizer in the climatic conditions of Khorramabad is recommended.

Keywords: Growth promoting, Nutrition, Fertilizer, Biological fertilizer, Nano.
Article type: Research Article.

INTRODUCTION

Increasing demand for herbs and its economical aspect are developing. During recent decades, WHO, through holding various conventions, gave precious instructions to the member countries about traditional medicines and pharmaceutical products. Pumpkin, is a herb belonging to Cucubitaceae order (Jellin *et al.* 2000). The fruit of Cucurbitaceae is yellow-orangish and its seeds are olive-green. One of the most famous features of this herb is that it does not have skin (Mitra 2001). Its produced oil contains lots of nutrients such as unsaturated fatty acids, vitamin A, vitamin E, minerals, phytosterols, cartonoids and Protochlorophyll. The most important fatty acids which approximately constitute 90% of oil are linoleic acids, oleic acids, and palmitic acids. Linoleic acids have made up 50% of fatty acids (Fruhwirth & Hermetter 2008). These fatty acids play a role in the treatment of intestinal worms, prostate hypotrophy, and urinary tract diseases (Omidbeygi 2012). In recent years, farmers pay more attention to soil quality and health, so that in the industrialized countries using natural, intra-farm, and non-chemical inputs is increasingly taken in consideration. On the other side, increase in the global energy carrier prices has encouraged farmers to apply alternative approaches in order to reduce in inputs consumption (Jahan *et al.* 2013). Soil fertility play a key role in the Cucubitaceae order yield for such a fertilizer application is necessary for those soils lack essential nutrients. Using fertilizers is the quickest way to supply nutrients required for the

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herbs. However, the high cost of fertilizer consumption, its pollution and environmental and also soil degradation are pressing concern. Development of plant resources and biological resources instead of chemical resources can have an important role in fertility and preservation of biological activity, soil organic matter, agro-ecosystems health and enhancement of crops quality (Hosseini *et al.* 2016). Crop management like managing different fertilizers impacts quantitative and qualitative performances of pumpkin. Although fertilization system focuses on provision of a few number of widely consumed elements, it is scientifically has been proven that this plant at least needs 13 minerals and that is one of the main reasons of the loss of balance of the plant (Atiyeh *et al.* 2000). Consequently, crop various measures like nano-fertilizers consumption that contain required micronutrients for plant growth and organic fertilizers such as humic acid are able to overcome the competition between vegetative and reproductive organs and enhance performance potential. Unlike available fertilizers, a little investigation has been conducted on the impact of nano-fertilizers and organic fertilizers such as humic acid on the quantitative and qualitative performances of herbs such as pumpkin. However, available investigations displayed that organic fertilizers have various effects on quantitative and qualitative performances of herbs and even according to some reports, organic fertilizers have no or adverse effects on herbs (Razaghi *et al.* 2016). Razaghifard *et al.* (2016) through examining performance components of pumpkin under influence of mycorrhiza, vermicompost and nano-fertilizer showed that the majority of traits, including grain weight, grain yield, total dry weight were in the maximum amount when vermicompost and nano-fertilizer were applied together, so that, by increased amount of vermicompost to 20 tons per acre, in this combination the amount of above-mentioned traits enhanced significantly. Investigations have illustrated that using nano-fertilizers increases the function of nutrient consumption, decreases soil toxicity and negative side effects of the fertilizers over-consumption and also drops the number of time-period used. Using nano-fertilizers, the time and speed of the elements release correspond to the plants requirement for nutrients, so the plant can absorb the most nutrients and besides decline in leaching elements, the function of the crop will be elevated (Derosa *et al.* 2010). Considering that organic and nano-fertilizers usage is an efficient method to access the stable agriculture, the examination has been done to study the effect of foliar application of humic acid levels and nano-fertilizer application on some quantitative and qualitative traits of pumpkin (*Cucurbita pepo* L.) in Khorramabad area, Lorestan Province, Iran.

MATERIALS AND METHODS

The present study was carried out to assess the effect of spraying different levels of humic acid and nano-fertilizer in Khorramabad City, Lorestan Province, Iran during 2017-2018. Khorramabad is located in altitude of 11478 a.s.l with longitude of 33º and 29′ north latitude and 48º and 21′ east longitude, with average temperature of 17 °F and 52506 mm precipitation annually. The study was conducted in the form of a factorial design based on total random blocks in tree iterations. Examined factors included humic acid (H) in tree levels with controls: H1: foliar spray with pure water; H2: topdress with humic acid; H3: foliar application of humic acid; as well as N: foliar application of nano-fertilizer including in four control levels as N1: foliar application with water; N2: foliar application for one time; N3: foliar application for two times; and N4: foliar application for tree times. Before plowing, a soil sample was provided from the farm, then its physical and chemical features were measured (Table 1). Spring plowing was performed in spring; disk was applied to break the clods; the land was flattened by trowels and then it was furrowed, so that streams were made. Finally, the farm was irrigated one time. Grains were soaked in lukewarm water for 24 h, then put between wet fabric and soaked in benomyl fungicide. Each terrace had 6 planting lines of 5 m with 1-m gap and were planted at a depth of 3-5 cm. Bush distance on planting line was 50 cm. To prevent intermixture of treatments for each 4 terrace 2 unplanted line of 100 cm were considered. According to the environmental temperature, pumpkin was planted on the second half of May and immediately was irrigated.

Table 1. The results of physical and chemical analysis of the soil used in the present study.

Soil texture	Farm humidity	Lime (0/0)	Organic Carbon (0/0)	Total Nitrogen (0/0)	Absorbable phosphorus (mg kg^{-1})	Electrical conductivity (mg kg^{-1})	Electrical conductivity (dS m^{-1})	Acidity
Clay-soil	22.5	15.9	0.98	0.01	6..9	355	0.55	7.9

In harvest time quantitative and qualitative traits were measured including grain yield, grain weight, fruit yield, number of fruit, numbers of grain, protein yield (Hooker *et al.* 2008), oil yield (Jahan *et al.* 2007), and essential yield (ISIRI 2003). Obtained data was analyzed and evaluated using SAS 9.0 software and Excel was used for creating the charts.

RESULTS AND DISCUSSION

Seed yield

Analysis of variance showed that humic acid treatments and foliar application of nano-fertilizers significantly effected seed yield of pumpkin ($p<0.01$), while their interactions did not have a significant impact on the seed yield (Table 2). Mean comparison results showed that spraying humic acid improved the yield of the pumpkin seed, while there was not a significant difference between foliar application (H1) and humic acid topdress (H2) treatments. Foliar application of distilled water (H1) caused the least value of seed yield (522 kg per acre; Fig. 1). Also results showed that there was not a difference between treatments of one, two or three times foliar application of nano-fertilizer (N2, N3 and N4). However, N4 enhanced the yield of the pumpkin seed. The minimum seed yield was 581 kg per acre obtained in N1 (Fig. 2). Zhang *et al.* (2006) declared that using fertilizers having nanoparticles, compared to the same amount of NPK enhanced the height and yield of the plant. After decomposition of plants, they delivered that by using this fertilizer the treated plants had more elements, especially nitrogen. Salehi & Tamaskani (2008) reported that nano silver treatment (50 mg L^{-1}) increased the length of wheat straws. The plants responded to nanoparticles variously. Investigations have shown that the zinc-oxide nanoparticles increased biomass in pea plant (Burman 2013), as well as the length and the number of peanuts branches (Prasad *et al.* 2012). This response may be related to the less production of ROS due to the zinc nano oxide by which lipid peroxidation decreases (Burman 2013). The size of particles impacts how zinc fertilizers get influenced. To do so, the reduced number of particles makes the increased weight unit of nano-fertilizer and enhances specific area as well as the solubility strength of fertilizer, hence upraising the level of fertilizer absorption (Prasad *et al.* 2012) and finally, the yield of plant enhances. Padem *et al.* (1999) by weighing the effect of foliar application of humic acid on traits such as straw, number of leaves, weight and dry weight of straw and root, as well as collection of NPK in the leaves of eggplant and pepper, found that straw diameter, number of leaves, wet weight of straw, dry weight of straw and root significantly increased by applying acid humic on pepper and eggplant. Through a controlled experiment it was shown that by applying dry weight humic materials, particle yield of *Avena ludoviciana* increased significantly (Sharif *et al.* 2002). By the plant and its straw growing, humic acid upraises the yield of dry material.

Table 2. Results of analysis of variance of qualitative and quantitative traits in pumpkin

Sources of Changes	Degrees of freedom	Seed yield	Weight of 1000 seed	Fruit yield	Fruit number	Seed number per fruit	Protein yield	Oil yield	Essence yield
Repitation	2	397902^{ns}	1285.8ns	106941111ns	0.76ns	4374430^{ns}	27486.6^{ns}	106200^{ns}	25.7ns
Humic	2	255580**	13491.6**	806221111**	0.40**	472451**	16677.9**	67145**	16.4**
Solution	3	52385.7**	1246.7**	146967037**	0.02**	50000*	3254.1**	14082**	4.3**
H*N	6	5106.6^{ns}	257.6**	29933704**	0.006	12597^{ns}	336.1^{ns}	1383^{ns}	0.46^{ns}
Error	22	5352.3	15.1	6550808	0.002	13300	317.2	1316	0.47
Multiplier	--	10.6	2.4	6.6	1.9	14.2	10.8	10.4	14.0

Note: *, ** and ns are significant at 5%, 1% and non-significant, respectively.

Weight of 1000 seed

According to the ANOVA test results, humic acid treatments and foliar application of nano-fertilizer and their interactions significantly affect weight of 1000-seed pumpkin ($P < 0.01$; Table 2). Among humic acid treatments, the maximum weight of 1000-seed was observed in H3 followed by H2. In spite of having significant differences, the more the nano-fertilizer applied, the higher the weight of 1000-seed; and also foliar application of humic acid (N4) maximized it up to 194 g (Fig. 3). In a study conducted by Singh *et al.* (2003), employing nano chelate fertilizer and zinc increased the growth of plant shoots, afterwards elevated its yield and the weight of dry shoot. It can be affirmed that while these combinations provide nutrients for the plant, they enhance herbage yield and biomass production (Singh *et al.* 2003). The result of the present study is in accordance with that of Shahsawan & Chamani (2014) who reported that iron sulfate spraying on sunflower cultivars in two common and nanoparticle forms was an effective treatment, which made the dry weight of shoot increased. Foliar application of iron sulfate in form of nanoparticle got dry weight of shoot by %7 increased compared to its common foliar application. Humic acid increases the growth of the plant through hormonal effects (Samavat & Malakoti 2005) and influences on cellular metabolism and also chelating power and absorption of nutrients (Nardi *et al*. 2002). Ayas & Gulser (2005) reported that humic acid makes the growth and height and also yield of the plant raised through elevating nitrogen.

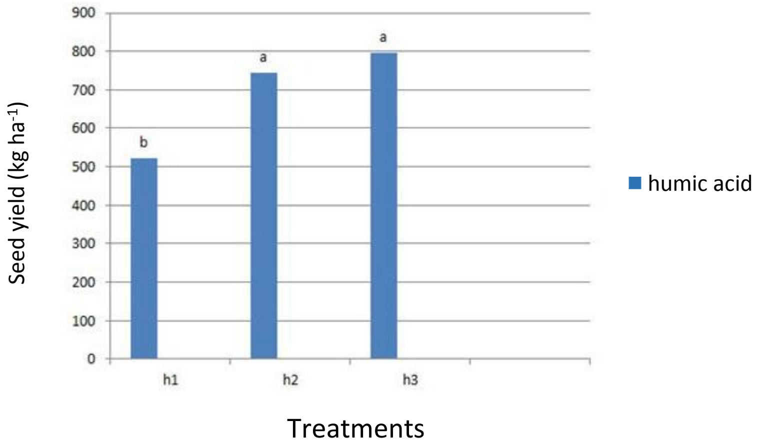

Fig. 1. Mean comparison results of the effects of humic acid on seed yield of pumpkin (means having similar letters have no significant difference at $p < 0.05$).

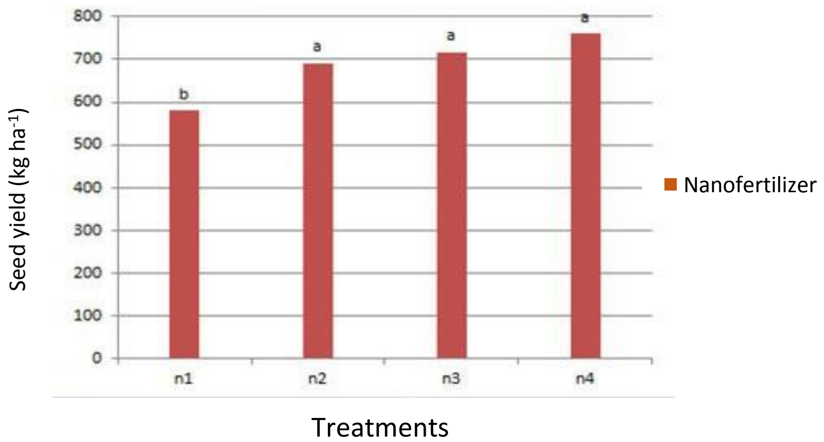

Fig. 2. Mean comparison results of the effects of foliar application of nano-fertilizer on seed yield of pumpkin (means having similar letters have no significant difference at 5%).

Fruit yield

Humic acid treatments and foliar application of nano-fertilizer and their interactive effects significantly affected fruit yield of pumpkin ($P<0.01$; Table 2). Mean comparison results showed that treatments using humic acid and nano-fertilizer exhibited the minimum fruit yield (26267 kg per acre). Increased number of spraying nano-fertilizer led to elevation in the fruit yield. The maximum yields of pumpkin were found in treatments in which foliar application of nano-fertilizer was tree times and humic acid was at amount of 50267 kg per acre. In the non-foliar application of nano-fertilizer treatments, humic acid treatments increased fruit yield as well. In this treatment nano-fertilizer, humic acid spray led to the maximum yield of fruit and humic topdress was placed at the second order (Fig. 4). Nano-fertilizers, due to the smooth and controlled release of nutrients, by elevating the nutrient availability and due to the ability to release nutrients related to the plant, need to make the plant absorbing maximum nutrients and growth (Derosa *et al.* 2010). Fathi & Zahedi (2014) reported that foliar application of iron oxide in the form of nano, compared to their normal form has led to a positive effect on dry matter in maize plants.

Increased dry matter and the plant yield due to the use of humic acid may be due to: speed of photosynthesis, elevated in biomass root, absorption of nutrients and nitrate, ATPase enzyme activity in membranes, plasma of root cells and phosphatase enzyme activity (Shah Hosseini *et al.* 2012). Positive effects of humic acid on cell membrane and longitudinal growth of cells and finally stimulating the shoots and stems to grow have been argued in the previous reports (Movahdpur *et al.* 2014). Stimulating membrane and longitudinal growth of cells makes the plant taller over time (Daneshvar *et al.* 2012).

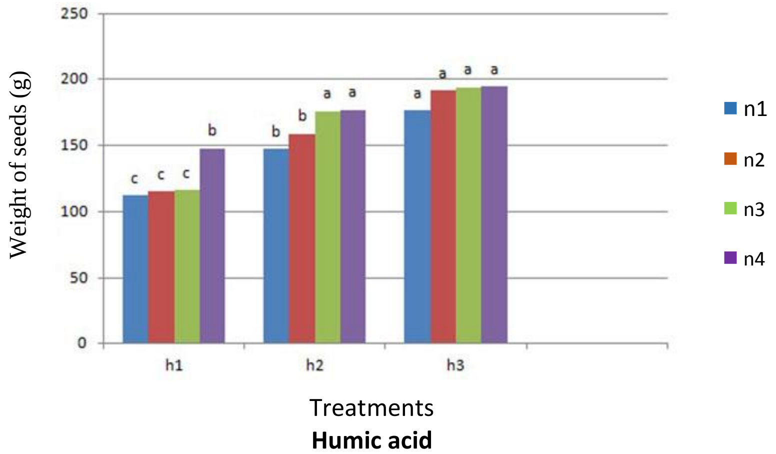

Fig. 3. Mean comparison results of the interaction effects of humic acid and foliar application of nano-fertilizer on 1000-grain weight of pumpkin (means having similar letters have no significant difference at $p < 0.05$).

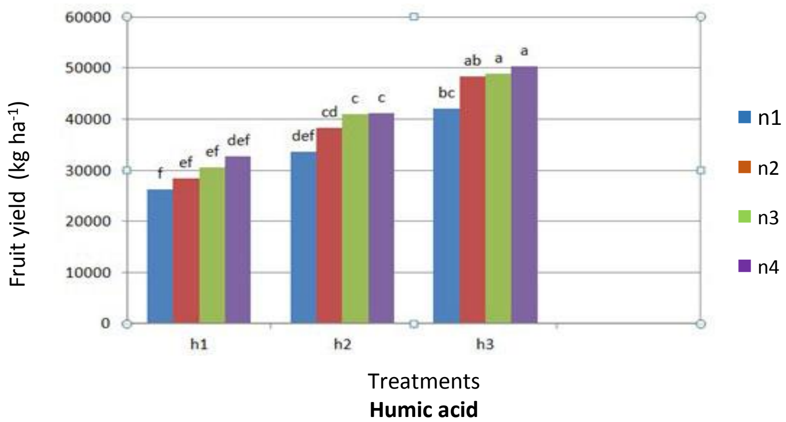

Fig. 4. Mean comparison results of the interaction effects of humic acid and foliar application of nano-fertilizer on fruit yield of pumpkin (means having similar letters have no significant difference at 5%).

Fruit number and seed number per fruit

Based on analysis of variance, hunic acid treatments and foliar application of nano-fertilizer and their interplays efficiently effected fruit number ($P < 0.01$; Table 2). It also showed that the seed number per fruit in pumpkin was significantly under influence of humic acid treatments ($P < 0.01$) and nano-fertilizer ($P < 0.05$). Mean comparison results illustrated that treatments without using humic acid and nano-fertilizer had the minimum number of fruit (2/3) and foliar or topdress with humic acid cause a significant upraise in the fruit number of pumpkin. In all humic acid treatments, there was not an obvious difference between N2, N3 and N4, however, in H1, three foliar application of nano-fertilizer (N2, N3 and N4) displayed the maximum fruit, while there was not a significant difference between N2 and N3 (Fig. 4). The results also showed that the maximum number of seed was in treatment of humic acid spraying at amount of 1000. Among treatments of foliar of nano-fertilizer, foliar exhibited the most (904) number of fruit (Fig. 5). Ahmadian *et al.* (2009), Arazmjoo *et al.* (2010) and Rahmati *et al.* (2008)

showed that consumption of nano-fertilizers compered to controls increased the yield. They concluded that fertilizers increase the flower yield through optimizing access of chamomile to nutrients in relative treatments. The plant absorbs nano-fertilizers quickly and fulfils its needs appropriately (Zhang *et al.* 2006). Also, nano-fertilizers speed up photosynthesis so that the number of flowers in the plant rises (Zhang *et al.* 2005). Peyvandi *et al.* (2011) suggested that because nano-fertilizer dimensions are small, so they are absorbed higher and better, hence fertilizers with nano structure are more efficient. It seems that nano-fertilizers due to the controlled release of nutrients during entire growing season, increase water and absorption availability of more nutrients, upraise photosynthesis and dry matter, so that it gives rise to flowering, generative growth and also improve the yield and its component. Chen & Aviad (1990) reported that growth acceleration and nutrient absorption will happen by application of humic matters. Defline *et al.* (2005) reported the influence of supplemental foliar application of nitrogen and humic acid on growth and yield of particles. Positive influence of humic acid on growth and flowering of a lot of plants, including family Poaceae (Gramineae), was documented well (Shahsavan & Chamani 2014).

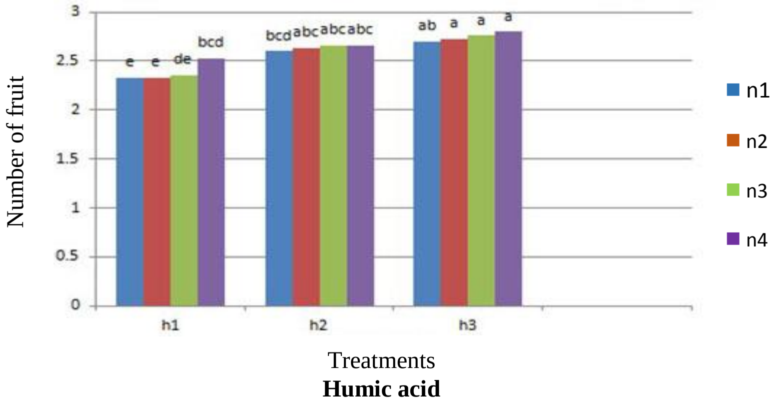

Fig. 5. Mean comparison results of the interaction effects of humic acid and foliar application of nano-fertilizer on fruit number of pumpkin (means having similar letters have no significant difference at 5%).

Table 3. Mean comparison results of the effects of humic acid and foliar application of nano-fertilizer on seed number of fruit and essence yield of pumpkin.

Change Sources	Seed number of fruit	Essence yield (kg ha^{-1})
Humic Acid		
Foliar Application of distilled water	605.2c	3.20b
Topdress	829.1b	4.67a
Foliar Application	1000.9a	5.53 a
Foliar Application of Nano-fertilizers		
Foliar Application of distilled water	730.7b	3.91b
Once	778.9b	5.02a
Twice	833.1b	5.17a
Tree times	904.3a	5.52a

Note: Means having similar letters have no significant difference at $p < 0.05$.

Protein, oil and essence yield

The results of the ANOVA showed that influences of humic acid treatments and foliar application of nano-fertilizers on protein, oil and essence yield of pumpkin were significant and their interaction did not exhibit a significant influence over these indexes ($P < 0.01$; Table 2). Mean comparison results of the effects of humic acid and foliar application of nano-fertilizer on grain number per plant and essence yield of pumpkin showed that foliar application of humic acid treatments increased its yield significantly and these treatments, compared to foliar application of distilled water, upraised the protein and oil yield as 402.9 and 193.5 respectively (Fig. 6). Also, researches displayed that there was not a significant difference among N2, N3 and N4, however, maximum yield of protein and oil were 384.9 and 182.7 kg ha^{-1} in these treatments respectively (Fig. 6). Mean comparison results illustrated that oil and protein in H1 had the least yield (Fig. 6). Mean comparison results displayed that foliar application of humic acid (H2) had the maximum yield of pumpkin essence (5.53 kg ha^{-1}), while H1 caused the minimum yield. Among foliar application of nano-fertilizer treatments, a significant difference was not observed and N4 displayed the maximum yield of essence (5.52 kg ha^{-1}, Table 3). It seems that availability of the most nutrients for the plant in fertilizer treatments has increased photosynthesis production, so in turn, influences on increased secondary metabolites. Proper nutrition in most different fertilizers strengthens involved paths in production of secondary metabolites. Fertilizer treatments in structure of enzymes, involving in biochemical paths impact on synthesis of plant active compounds. As nutrient shortage decreases yield and amount of active compounds, imbalance in applying fertilizers tends to the same result, declining yield (Gleiter 1989). Ayas & Gulser (2005) reported that humic acid, through the elevated nitrogen content, causes growth, height and amount of protein. Positive influence of foliar application of humic acid over side chain in time of flowering can be attributed to stimulate of lateral shoots and growth of side chains (Khan *et al.* 2012). Elevated amount of chlorophyll likely is due to upraised absorption of macro and micro elements. Sabzevari & Khazaee (2009) reported that humic acid increases the number of chlorophyll in surface with 95% probability in wheat plant. Application of humic acid on tobacco and herbs leads to elevation of alkaloids in leaves. It also increases glucose transmitting in cell membranes of onion, sugar beet, and sunflower, and also upraises carbohydrates in potato, sugar beet, carrot and tomato (Tan 2003). Foliar application of humic acid on wheat upraised protein yield to 24%. In another investigation, applying 1000 mg humic acid on 1 kg soil elevated macro and micronutrients in tomato (Turkmen *et al.* 2004).

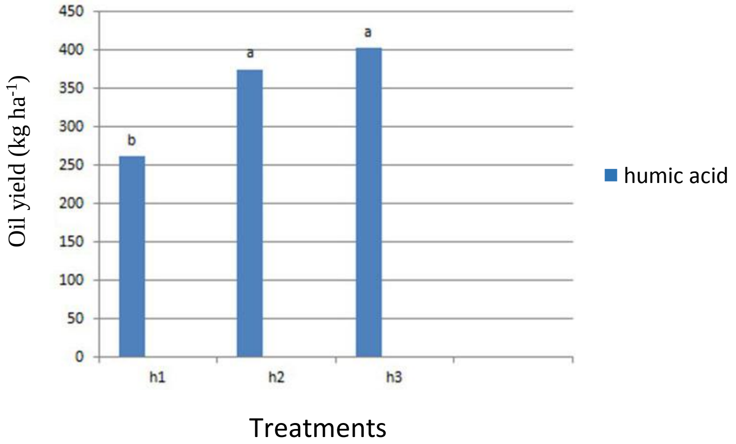

Fig. 6-A. Mean comparison results of the effects of humic acid on oil yield of pumpkin (means having similar letters have no significant difference at 5%).

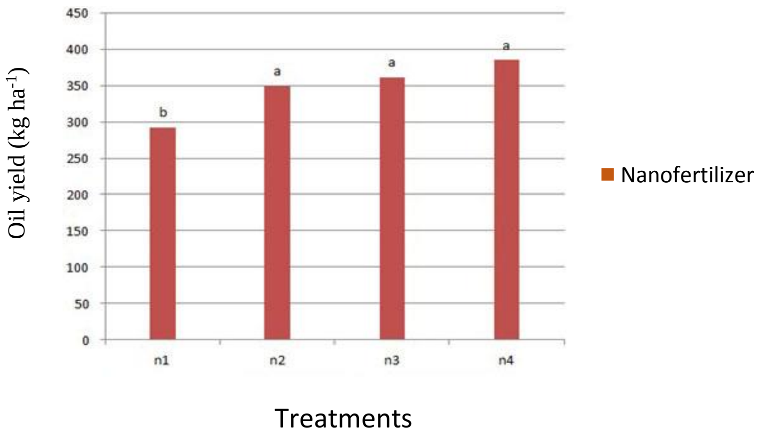

Fig. 6-B. Mean comparison results of the effects of foliar application of nano-fertilizer on oil yield of pumpkin (means having similar letters have no significant difference at 5%).

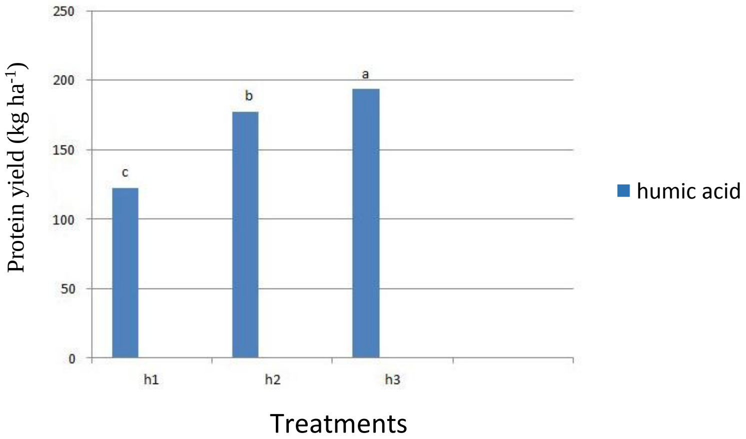

Fig. 6-C. Mean comparison results of the effects of humic acid on protein yield of pumpkin (means having similar letters have no significant difference at 5%).

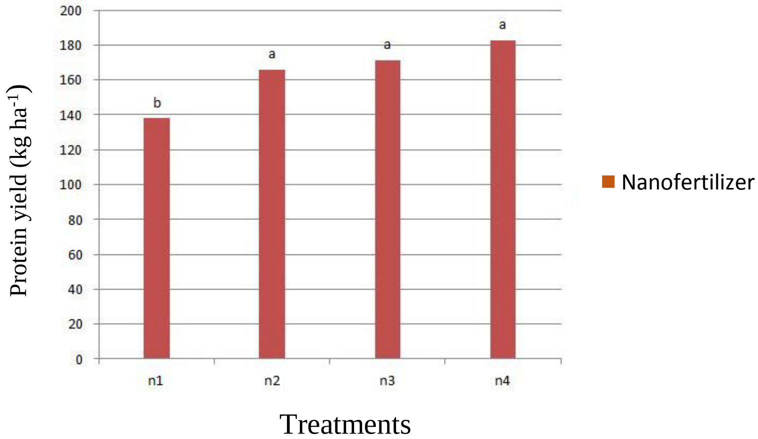

Fig. 6-D. Mean comparison results of the effects of foliar application of nano-fertilizer on protein yield of pumpkin (means having similar letters have no significant difference at 5%).

CONCUSION

Humic acid is one of the best biological fertilizers obtained from microbial, biological and chemical organic matter decomposition. Humic acid increases soil fertility and improves its physical and chemical characteristics such as permeability, aeration, modified gradation, water holding capacity of soil, mobility and nutrient availability. Increase in nitrogen, phosphorus, potassium, calcium, magnesium absorptions via elevation in mobility capacity of these elements has been reported. The aim of the present study was to examine influence of number of applying times of humic acid and nano-fertilizer on pumpkin quantitative and qualitative indicators in climate condition of Khorramabad. It was also found that there was no difference between one, two or three times of foliar application made pumpkin in the highest level of yielding, while the minimum seed yield was found in treatment of foliar application with distilled water as 581 kg per acre. The mean comparison results displayed that in all treatments with no humic acid and nano-fertilizer, the minimum fruit yield (28400 kg per acre) was obtained. Also, elevating in the number of foliar application of nano-fertilizers upraised pumpkin yield. The results showed that there was no significant difference between one, two or three times of foliar application of nano-fertilizer. However, maximum protein and oil yield were respectively 182.7 and 384.9 kg per acre obtained in three times treatment of foliar application of nano-fertilizer, while foliar application with distilled water also had the least oil and protein yield. Altogether, two times foliar application of nano-fertilizer with foliar application of humic acid and topdressing with humic acid could improve quantitative and qualitative traits of pumpkin, so that use of these treatments in climatic conditions of Khorramabad area is recommended.

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