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Effect of humic acid application on accumulation of mineral nutrition and pungency in garlic (*Allium sativum* L.)

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Humic acid promote the conservation of mineral nutritions and as well as stimulate the pungency usually being more prominent in the cloves of garlic bulb. The effect of humic acid application on accumulation of mineral nutritions and pungency in garlic (*Allium sativum* L.) cv. 'Gangajali' was studied. Here, we observed that the maximum concentration of Ca, Fe and S were shown when plants were treated with 400 ppm humic acid. While, in the case of Mg, P and Zn content, the maximum values were observed by application of 200 ppm humic acid. The maximum concentration of K, Cu, Mn were observed when plants were treated with 300 ppm humic acid. Regarding, pungency, content was determined as pyruvic acid development significantly increased with increase in concentration of humic acid applications. Our results also showed significant positive correlations between Mg and P, Mg and Zn, P and Zn, Cu and PAD, and S and PAD, respectively. Based on average values and ANOVA of overall variables, the highest result was observed by application of 300 ppm of humic acid followed by 400 and 200 ppm of humic acid, which may be the proper value addition in garlic bulb by enhancing the mineral nutritions and pyruvic acid development that needs to be studied in the future.

Key words: *Allium sativum* L., humic acid, mineral nutritions, pungency.

INTRODUCTION

Currently, the major challenges of plant scientists and agronomists are to enhance crop yields in more resource, efficient and environmentally friendly cropping systems. One of the potential systems involved is creation of innovative means for plant nutrition and growth promotion. The urgency to emphasize the importance of humic substances (HS) and their value as fertilizer ingredients has never been more important than at

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present, as the content of organic matter in agricultural soils has reached drastically low levels (Loveland and Webb, 2003). The value of HS in soil fertility relates to the many functions these complex organic compounds perform. It is well established that HS improve the physical, chemical and biological properties of soil and favourably influence plant growth (Nardi et al., 2002). Although seed treatment and foliar application of HS is increasingly used in agricultural practice, the mechanism of possible growth promoting effect is usually attributed to hormone-like impact, activation of photosynthesis and improved nutrient uptake (Chen and Aviad, 1990; Fernandez et al., 1996; Kulikova et al., 2005). Humic acids (HA) are characterized as a heterogeneous natural resource, ranging in color from yellow to black, having high molecular weight, and resistance to decay. Humic acid, as a commercial product contains 44-58% C, 42-46% O, 6-8% H and 0.5-4% N, as well as many other elements (Larcher, 2003; Lee and Bartlette, 1976). It improves soil fertility and increases the availability of nutrient elements by holding them on mineral surfaces. The humic substances are mostly used to remove or decrease the negative effects of chemical fertilizers from the soil and have a major effect on plant growth, as shown by many scientists (Linchan, 1978; Ghabbour and Davies, 2001; Pal and Sengupta, 1985).

As per literature survey many researchers reported that the humic substances stimulated root development and enhanced nitrogen, K^+, Cu^{2+} and Mn^{2+} content (Bidegain et al., 2000) in ryegrass. According to Adani et al. (1998), commercial humic acid affected tomato root fresh and dry weights of tomato as well as iron content, depending on the source of the humic acid. The two concentrations (20 and 50 mg/L) of humic acid, resourced from fertilizer, caused iron to increase to 113 and 123%, whereas humic substance derived from leonardite increased iron content to 135 and 161% in tomato roots. Fernández-Escobar et al. (1999) found that application of HA stimulated chlorophyll content and accumulation of K, B, Mg, Ca and Fe in leaves of olive trees. The foliar application of humic substances becomes effective in promoting uptake and accumulation of nutrients in leaves of olive trees (Fernandez et al., 1996). The foliar application of humic substances exerted positive effect on nutrient status of *Thuja orientalis* L. as reported by Zaghloul et al. (2009). The humic acid application can also greatly improve plant growth and nutrient uptake as reported by Dursun et al. (2002) and Paksoy et al. (2010).

Often called the “stinking rose,” garlic may be known for its odor as much as its flavor, but it is actually odorless until its cells are ruptured by being “bruised, cut or crushed” (Simon et al., 1984; Tuckar et al., 2000; Woodward, 1996). Garlic’s scent comes primarily from sulfur compounds. When a garlic clove is cut, alliin, an “odorless, sulfur-containing amino acid derivative” (Small, 1997) reacts with the enzyme alliinase to form allicin and other sulfur compounds (Koch et al., 1996; Tuckar et al., 2000; Woodward, 1996). Allicin breaks down into diallyl disulfide, which is largely responsible for garlic’s odor (Koch et al., 1996; Tuckar et al., 2000). In addition to scent, allicin is also responsible for many of garlic’s health benefits including its antioxidant, anti-microbial, cholesterol-lowering and blood-thinning properties (Blumenthal, 2000) and is likely to play a role in garlic’s anti-cancer effects (Koch et al., 1996).

The goal of the present study was to evaluate the effect of foliar application of commercially produced humic acid on accumulation of mineral nutritions and pungency in cloves obtained from bulb of garlic (*Allium sativum* L.) grown in the field experiment. The research findings are based on the key parameters necessary for evaluation of mineral nutrients (Ca, Fe, Mg, P, K, Zn, Cu, Mn and S) and pungency (PAD) quality as victual food and hoped to obtain information for patients and researchers.

MATERIALS AND METHODS

Field experiment

Sandy loam soil was plowed twice and 50 MT•ha^{-1} of well-rotted cow manure incorporated. Synthetic fertilizer at 40 N:60 P:40 K kg•ha^{-1} was applied as preplant to the soil. Nitrogen was from urea, phosphate was from single superphosphate, and potash was from muriate of potash. Garlic cloves obtained from bulbs (weighing about 35 g each) of cv. Gangajali was planted 3 cm deep in furrows with 15-cm spacing between rows and 10 cm between plants in plots that were 1.5 × 1.5 m^2. The experiment was established in the last week of October 2012 at the Research Farm, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, and West Bengal, India. Additional nitrogen at 40 kg•ha^{-1} was applied 30 days after planting. The experiment consisting of five treatments including control (no humic acid applied) were arranged in a randomized complete block design with three replications of each treatment. The detail treatments are summarized in Table 1.

Because garlic is shallow-rooted, supplementary irrigation was required and nine irrigation events providing approximately 5 cm of water each at 15 day intervals were applied, with the first immediately after planting. There were four hand weedings within 9 weeks of planting. The insecticide malathion at 1 ml•L^{-1} (Rallis India Ltd., Mumbai, Maharashtra, India) was applied to control thrips and the fungicide Dithane M-45 at 2.5 g•L^{-1} (Indofil Industries Ltd., Andheri, Maharashtra, India) was applied to control purple blotch disease caused by *Alternaria porri* (Ellis) cif. Three applications of both pesticides were started 30 days after planting and at 15 day interval. After harvesting, ten bulbs were collected from each treatment. Fresh cloves, separated from bulbs, were washed, dried with a soft tissue, peeled, and finely chopped before analysis of mineral nutritions and pungency (PAD).

Preparation of plant samples

Digestion of plant samples

Wet digestion by A.O.A.C. (1970) was used.

Procedure:

1. 1.0 g of ground material was weighed into a 250 ml conical flask.

Table 1. Preparation of humic acid solution for spray on garlic (*Allium cepa* L.) plant.

Standard stock solution (ml)	Diluted with distilled water (ml)	Solution made (ppm)
20	1000	100
40	1000	200
60	1000	300
80	1000	400

Extraction solution: In a 500 ml volumetric flask with 200 ml of distilled water, was added 80g NaOH, 8 ml ethanol and brought to volume 500 ml with distilled water. Standard stock solution (5000 ppm): Weighted out 1.075 g of humic acid and diluted to 200 ml with extraction solution and shaken for 1 h. The source of humic acid was granular, peat, brown coal (Maharashtra Chemicals and Fertilizers, Nageshwar Nagar, Malegoan, Pune-413115 and Maharashtra, India). The spraying was done three times with sticker starting from 25 days after planting and subsequent ones at an interval of 15 days during vegetative stage.

2. 4 ml of $HClO_4$, 25 ml of concentrated HNO_3 and 2 ml of concentrated H_2SO_4 were added to the sample in the conical flask.
3. The content were mixed and heated on a hot plate.
4. The heating continued until dense white fumes appeared.
5. 2 ml of concentrated HNO_3 was added and heated for a minute.
6. It was allowed to cool and 40 ml of distilled water was added and boiled for half a minute on a hot plate.
7. It was cooled and filtered with Whatman No. 42 filter paper into a 100 ml Pyrex volumetric flask and made up to the mark with distilled water.
8. The solution was preserved for the determination of mineral nutritions.

Amount of Cu, Fe, Mn and Zn were determined with an atomic absorption spectrophotometer (model 2380, Perkin-Elmer, Shelton, Conn.) and potassium content was analyzed with a flame photometer (model 1020, Chemito, Mumbai, India). Calcium and Mg were determined by titration using eriochrome black T and calcon indicators (Jackson, 1973). Phosphorus was determined calorimetrically by the phospho-vanado molybdate yellow color method (Jackson, 1973). Sulfur was determined by turbidimetry using the method of Butters et al. (1959). Pungency of garlic bulbs was determined as pyruvic acid (Anthon and Barrett, 2003; Schwimmer and Weston, 1961). Pyruvic acid concentration was determined with 2, 4-dinitrophenylhydrazine using the method of Schwimmer and Weston (1961).

Statistical analysis

Data were analyzed statistically by using Daniel's XL Toolbox 6.52 software for analysis of variance.

RESULTS AND DISCUSSIONS

Calcium (Ca)

In our present study (Table 2), calcium is known to show remarkable variation in its effects among treatments. In garlic, calcium content significantly increased positively with increase of concentration of humic acid applications as compared to the control. The increase was ~1.36 (100 ppm), ~1.88 (200 ppm), ~2.30 (300 ppm) and ~2.77 (400 ppm) fold, respectively. Our results are in agreement with that of Akinci et al. (2009), they also reported that the humic acid applications increased calcium concentration in broad been roots, and same was reported in tomato by David et al. (1994).

Iron (Fe)

The iron content (Table 2), in response to humic acid applications did show significantly increased effect in all treatments except in 100 ppm of humic acid only as compared to the control, which (HA_1) was also increased over the control, though not significantly. Our results support that of Akinci et al. (2009), who found that the Fe^{3+} increased in humic acid treated broad bean roots as compared to the control. In tomato plants grown in green house conditions, applying humic acid increased the Fe^{3+} content in its roots (David et al., 1994). The maximum value was observed by application of 400 ppm of humic acid (~2.13 fold).

Magnesium (Mg)

Increase in the concentration of magnesium of maize fodder in response to humic acid application was reported by Daur and Bakhashwain (2013). Our results also showed that (Table 2) the magnesium concentration significantly increased in all treatments except 400 ppm of humic acid only as compared to the control treatment, in which (HA_4) also increased over the control, though not significantly. The highest magnesium concentration was observed by application of 200 ppm (~3.10 fold) followed by 100 ppm (~2.33 fold) of humic acid.

Phosphorus (P)

Phosphorus is a major mineral nutrient which is essential to all plant life mostly in terrestrial plants. It also activates crucial enzymatic reactions and contributes to the osmotic pressure of the vacuole, which helps to maintain structural rigidity. In the present study (Table 2), the concentration of phosphorus, with significantly positive

Table 2. Effect of humic acid applications on accumulation of mineral nutrient ($mg.100^{-1}g$) and pungency ($mmol.100^{-1}g$) in garlic (*Allium sativum* L.).

Treatment	Ca	Fe	Mg	P	K	Zn	Cu	Mn	S	PAD
HA_0 (control)	177.09^n	1.69	27.56	160.00	400.77	1.11	0.33	1.69	0.68	4.39
HA_1 (100 ppm)	241.76 ±9.23	1.97 ±0.18	69.00 ±3.55	233.59 ±1.61	679.06 ±25.96	1.46 ±0.27	0.37 ±0.07	1.95 ±0.04	0.87 ±0.06	5.77 ±2.19
HA_2 (200 ppm)	333.00 ±14.33	3.07 ±0.16	91.67 ±3.18	309.11 ±3.44	933.09 ±43.92	1.60 ±0.11	0.44 ±0.11	2.22 ±0.15	1.00 ±0.07	7.96 ±0.68
HA_3 (300 ppm)	407.95 ±13.21	3.05 ±0.05	53.33 ±3.23	153.79 ±3.30	1179.11 ±69.88	1.25 ±0.04	0.52 ±0.03	2.29 ±0.08	1.20 ±0.1	8.60 ±1.43
HA_4 (400 ppm)	489.99 ±12.00	3.60 ±0.15	33.05 ±1.95	141.06 ±1.46	1013.23 ±13.12	1.18 ±0.04	0.49 ±0.03	2.00 ±0.15	1.56 ±0.1	10.03 ±1.04

Data are mean ± standard deviation values (n=3).

influences were seen in a limited range of humic acid application from 0.00 to 200 ppm. While, 300 and 400 ppm of humic acid applications did not show negative effect on concentration of phosphorus as compared to the control. However, Abdel-Rezzak and EI-Sharkawy (2013) reported that the humic acid, significantly increased in concentration of phosphorus in garlic cloves during 2008/2009.

Potassium (K)

As per literature survey, many researchers reported that the humic substances provoked a better efficiency of plant water uptake and improved the potassium concentration (Delfine et al., 2005; Morard et al., 2011; Daur and Bakhashwain, 2013). However, humic acid significantly increased in the potassium concentration in cucumber at 20 and 40 ppm of humic acid applications as compared to the control (Mohsen Kazemi, 2013).

Our results also revealed that (Table 2) the humic acid applications exerted significantly positive influence on concentration of potassium content as compared to the control. The highest concentration was observed by application of 300 (~2.94 fold) followed by 400 (~2.53 fold) and 200 ppm (~2.33 fold) of humic acid, respectively. While, according to Samson and Visser (1989) humic acid induced increase in permeability of bio-membranes for electrolytes accounting for increased uptake of potassium.

Zinc (Zn)

The beneficial effect of humic acid in soil might have prevented the formation of insoluble complexes of Zn and facilitating their uptake by plants (Tenshia and Singaram, 1992). In our present experiment, with respect to Table 2, it was shown that the concentration of zinc significantly increased in 200 ppm (~1.44 fold) of humic acid application only, while remaining treatments that is, 100, 300 and 400 ppm of humic acid also increased, apparently, though not significantly. However Akinci et al. (2009) reported that the zinc content decreased in humic acid treated plants. On the other hand, Zn^{2+} with decrease in broad bean root may be related with the Fe^{3+} causing the absorption of Zn^{2+} and its toxicity (Olsen, 1972).

Copper (Cu)

In the present study, application of humic acid through different treatments could produce no significant differences in the content of copper (Table 2). Therefore, no significant effects could be observed by application of humic acid on the content of copper. However, copper content in *Vicia faba* L. treated with HA decreased by 27% but did differ significantly from controls (Akinci et al., 2009).

Eyheraguibel et al. (2008) also found that the Cu^{2+} increased significantly in HA treated maize plant roots by 14% as compared to control. According to Mackowiak et al. (2001), in wheat plants grown with HEDTA, Cu^{2+} cannot freely enter its root since HEDTA can make a stronger bond with Cu^{2+} as compared to humic acid Cu^{2+} bonds. David et al. (1994) stated that in tomato plants grown under low nutrient media, addition of humic acid causes increase of Cu^{2+} in its roots while Cu^{2+}concentration increased in tomato stems, under high nutrient treatment.

Manganese (Mn)

In the present experiment, with respect to Table 2, it is shown that garlic : manganese concentration exerted significantly positive effects in 200 (~1.31 fold) and 300 ppm (~1.35 fold) of humic acid applications, while, other applications of humic

Table 3. Pearson's correlation matrix of all variables.

	Ca	Fe	Mg	P	K	Zn	Cu	Mn	S
Ca									
Fe	0.964*								
Mg	-0.068	0.104							
P	-0.268	-0.057	0.933*						
K	0.887*	0.881*	0.252	-0.041					
Zn	-0.071	0.095	0.984*	0.950*	0.192				
Cu	0.932*	0.907*	0.073	-0.204	0.981*	0.011			
Mn	0.629	0.696	0.598	0.316	0.905*	0.512	0.821		
S	0.981*	0.917*	-0.184	-0.351	0.793	-0.158	0.851	0.482	
PAD	0.990*	0.988*	0.063	-0.131	0.907*	0.059	0.932*	0.695	0.955*

*Significant at 5%, Student's t-test.

acid like 100 and 400 ppm, did not show any significant effects over the control. The result also seems to be related to the antagonistic effect of Ca^{2+} on Mn^{2+} uptake (Bozcuk, 2000).

Sulfur (S)

Sulfur is an essential component of important metabolic and structural compounds in plants (Marschner, 1995). Garlic is known as a S-demanding crop, as it is a component of secondary compounds, that is, allicin, cycloallicin and thiopropanol, which not only control the taste, pungency and medicinal properties of garlic, but also are important for resistance against pests and diseases (Schnug, 1993; Brown and Morra, 1997; Raina and Jaggi, 2008). Our present study shows that the sulfur content (Table 2), positively increased with increased concentration of humic acid applications, which significantly increased in 200 (~1.47 fold) 300 (~1.76 fold) and 400 ppm (~2.29 fold) humic acid applications, respectively as compared to the control.

Pyruvic acid development (PAD)

A common assessment of pungency is made by measuring pyruvate, formed as a stable primary compound from the enzymatic decomposition of flavor precursors and formed in a mole-for-mole relationship with the flavor precursors. We indicated that, concentration of pyruvic acid development (Table 2) had positive increment among all the treatments, which significantly increased in 300 (~1.96 fold) and 400 ppm (~2.28 fold) humic acid applications, respectively as compared to the control.

Correlation among variables

There are correlations between pairs of variables (Table 3). Most of the mineral nutrient like Ca had significant positive correlation with Fe, K, Cu, S and PAD. There are also positive significant correlations of Fe with K, Cu, S and PAD. Here also significant positive correlations between Mg with P, Mg with Zn, P with Zn, Cu with PAD and S with PAD were seen, respectively. There are significant positive correlations of K with Cu, Mn and PAD.

These relationships indicate that improving the primary (P and K) and secondary nutrients (Ca, Mg and S) to enhance micronutrients (Fe, Zn, Cu and Mn) concentration in garlic (*Allium sativum* L.) bulbs could accompany improvement of quality as well as improve pungency (PAD).

Conclusions

In the present study, remarkable variable effects were seen on accumulation of mineral nutritions and pungency content in response to humic acid application in clove of garlic (*A.sativum* L.) bulb. Based on average values and ANOVA results, the highest result was observed by application of 300 followed by 400 and 200 ppm humic acid, which may be the proper value addition in cloves of garlic bulb by enhancing the mineral nutritions and pyruvic acid development that needs to be studied in the future.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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