Effect of Humic acid and phosphate sources on nutrient composition and yield of Radish grown in calcareous soil

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ABSTRACT
Two field experiments were conducted at farmer's field, Ebshway, El-Fayoum Governorate, Egypt during two seasons (2012-2013 and 2013-2014). The objective of this study was to determine the effect of foliar applications of humic acid and different source of phosphorus fertilization on dry matter, some nutrient elements and uptake by radish grown under calcareous soil conditions. Humic acid sprayed on the leaves at 0.05 and 0.1 % doses at 15 and 30 days after sowing. Phosphorus fertilization was applied to the soil at 100 and 200 kg P_2O_5 fed^{-1} from two source Calcium super phosphate (15.5% P_2O_5) and super Bio-phosphate (19.0% P_2O_5). Foliar application of humic acid at the rate 0.1 % combined with super Bio-phosphate at a rate 100 kg P_2O_5 fed^{-1} had statistically significant effect on fresh and dry weight of root and shoot, root length and diameter as well as nutrient content and uptake. Moreover, the highest values of chlorophyll a, b, a+b and carotenoids were obtained from the application of humic acid at the rate 0.1 % with super Bio-phosphate. As well as, nitrogen, phosphorus and potassium content recorded highest significant values in Radish plants with same treatment. Nitrate accumulation reduced significantly by application of both phosphate forms. Also, sodium content of root and shoot tissues decreased significantly with increasing phosphorus applied rate.

Key words: Calcareous soil-Humic acid-Phosphorus fertilization-Radish plant

INTRODUCTION

Radish (*Raphanus sativus* L.) is an important vegetable crop grown extensively in Egypt. Recently, excessive amounts of fertilizers have been used on many soils for commercial vegetable production in Egypt. Soil health is a crucial factor for realizing higher yield of vegetables. Excessive application of chemical fertilizers may affect soil health and sustainable productivity. It is imperative to search for possible alternate organic source that can sustain soil health and crop production. In calcareous soils, precipitation of insoluble Ca phosphates is believed to be a major factor in the loss of availability of applied P **Sample *et al*., (1980)**, although the relative contribution of adsorption and precipitation processes to P fixation in calcareous soils seems to depend on P application rate **Afif *et al*.,** (**1993**); **(Castro and Torrent, 1995)**. An initial low soil P status may constrain agricultural use, especially in calcareous soils, where a sizeable fraction of applied P can precipitate as poorly soluble Ca phosphates **(Delgado and Torrent, 2000).**

Phosphorus (P) is an essential nutrient required by plants for normal growth and development. The availability of P to plants for uptake and utilization is impaired in alkaline and calcareous soil due to the formation of poorly soluble calcium phosphate minerals **(Bryan and Ellsworth 2005).** Adsorption and precipitation processes in soils result in only part of applied P fertilizer remaining available to plants. The apparent recovery of applied fertilizer is usually low in the first cropping year following application, and residual P plays a major role in agricultural soils by supplying P to plants **Matar *et al*., (1992)**.

Humic acid derived from lignite is the most concentrated form of organic material and it is a ready source for carbon and nitrogen. Humic acid improves the physical, chemical and biological properties of the soil and influences plant growth **(Chen *et al*., 2001)**. Humic substances have a very profound influence on the growth of plant roots. When humic acids and fulvic acids are applied to the soil, enhancement of root initiation and increased root growth was observed **(Pettit, 2004).** The stimulatory effects of humic substances have been directly correlated with enhanced uptake of macronutrients, such as nitrogen, phosphorus; sulfur **Chen *et al*., (1999).**

Humic acids (HA) represent the fraction of humic substances insoluble in water under acidic conditions, which becomes soluble and extractable at higher soil pH. Molecules of HA are characterised by acidic groups such as carboxyl and phenol OH functional groups **(Hofrichter and Steinb€uchel, 2001)**, It has been demonstrated that HA contribute to reducing the physical mobility (diffusion, mass flow) of various metal species micronutrient in the soil, and thus reduce the consequent risk of lateral or vertical contamination of water bodies, as acetic acid extraction of metals is generally reduced with HA **Halim *et al*., (2003).** The effects of humic compounds on root growth and morphology have not been well documented so far, and there is a need to gain more insight on the relationship between root characteristics and HA application, especially for phytoremediation purposes. **Zaky *et al*. (2006)** found that the number of shoots/plant, average leaf area, total yield, average pod fresh weight and P content were increased by application of humic acid as a foliar fertilizer at a rate of 1 g/L.

The aim of the study was to investigate the effect of Humic acid and phosphate forms on increasing the availability of phosphorus under calcareous soil conditions.

MATERIALS AND METHODS

A field experiments were conducted at El-Fayoum Governorate, to study the role of foliar application Humic acid and phosphorus application to maximize the phosphorus availability for increasing radish production under calcareous soil condition. Humic acid (HA) applied as foliar spray at a rate of (0.05 and 0.01 %). Humic acid contains (Humic 15%, Fulvic 10%, P 4%, K 6% and N 1%) and phosphorus applied at rates of 100 and 200 kg P_2O_5 fed^{-1} from two sources calcium super phosphate (15.5% P_2O_5) and super Bio-phosphate (19% P_2O_5). The experimental treatments were arranged in a factorial experiment and laid out in randomized block design with three replicates. Treatment details as follows: **T1:** Control of no applied P (P0), **T2:** Ca Super phosphate at 100 kg P_2O_5 fed^{-1} (Ca Super P 1), **T3:** Ca-Super phosphate at 200 kg P_2O_5 fed^{-1} (Ca Super P 2), **T4:** Super Bio phosphate at 100 kg P_2O_5 fed^{-1} (Super Bio P 1), **T5:** Super Bio phosphate at 200 kg P_2O_5 fed^{-1} (Super Bio P 2), **T6:** P0 + Humic acid (HA) 0.05 %, **T7:** Ca Super P (1)+ HA 0.05 %, **T8:** Ca Super P (2)+ HA 0.05 %, **T9:** Super Bio P (1)+ HA 0.05 %, **T10:** Super Bio P (2) + HA

0.05 %, **T11:** P0 + Humic acid (HA) 0.1 %, **T12:** Ca Super P (1)+ HA 0.1 %, **T13:** Ca Super P (2)+ HA 0.1 %, **T14:** Super Bio P (1)+ HA 0.1 %, **T15:** Super Bio P (2)+ HA 0.1 %.

Some physical and chemical properties of a representative soil sample used in the experimental soil were determined before preparation according to **(Rebecca, 2004)** and presented in (Table 1).

Table 1: Some physical and chemical properties of soil (Mean of two seasons)

pH (1:2.5)	**EC dSm^{-1}**	**OM %**	**$CaCO_3$ %**	**Particle size distribution**			**Texture class**
				Sand %	**Silt %**	**Clay %**	
8.39	1.22	0.79	13.04	21.20	30.20	48.55	**Clay**

Available nutrients (mg/100 g soil)			**Cation and Anion (mg / 100 g soil)**							
N	**P**	**K**	**Na^+**	**K^+**	**Ca^{++}**	**Mg^{++}**	**CO_3^{--}**	**HCO_3^-**	**CL^-**	**SO_4^{--}**
8.17	1.19	17.96	27.2	0.91	42.88	26.3	1.13	94.76	72.64	181

Nitrogen fertilizer in the form of ammonium nitrate (33.5%N) at the rate of 80 kg N fed^{-1} was added in two equal doses. The first one was applied after germination and the other one 10 days later. Potassium fertilizer in the form of potassium sulphate (48% K_2O) with the above-mentioned levels was applied in one dose after thinning. The other cultural practices were carried out as recommended.

At harvest stage in the two seasons, root dimensions cm (length and diameter) and fresh root and shoot weight (g) were determined. Chlorophyll and carotene was also estimated according to **(Lichtenthalar and Wellburn 1983)**.Then, fresh radish samples were oven dried ground and digested for the determination of N, P, K and Na contents as described by **(Motsara and Roy 2008)**.

The analysis of variance was carried out according to **(Gomez and Gomez 1984)** using MSTAT computer software, after testing the homogeneity of the error according to Bartlett's test. Means of the different treatments were compared using the least significant difference (LSD) test at $P<0.05$.

RESULTS AND DISCUSSION

Growth parameters

Data in Table (2) showed that fresh and dry root and shoot of radish plant at harvest a remarkable increased as affected by Humic acid application with different source of phosphorus fertilization particularly with application of super bio-phosphate at a rate of 100 kg P_2O_5 fed^{-1} and foliar application Humic acid (HA) at a rate 0.1% as compared with control of no applied P. Data also revealed that the same treatment significantly increased of root length and root diameter by 31.3 % and 48.1% respectively as compared with super bio-phosphate solo applied at the rate 100 kg P_2O_5 fed^{-1}. Obtained results confirmed that the humic acid can increase uptake of certain elements and stimulate the dry matter production of root and shoot.

Table (2): Yield parameters of radish as affected by Humic acid and phosphorus source (Data mean of two seasons).

Treatments	Fresh weight (g plant^{-1})		Dry weight (g plant^{-1})		Root length (cm plant^{-1})	Root diameter (cm plant^{-1})
	Root	Shoot	Root	Shoot		
P0 (Control)	21.9	22.8	2.11	5.04	8.40	1.19
Ca Super P (1)	23.5	32.9	3.64	6.12	9.43	1.39
Ca Super P (2)	24.6	37.3	4.11	6.83	10.63	1.66
Super Bio P (1)	24.2	33.9	3.72	6.28	10.10	1.53
Super Bio P (2)	25.6	39.5	4.47	7.09	10.73	1.73
P0 + HA 0.05 %	22.8	27.2	2.90	5.35	8.80	1.26
Ca Super P (1)+ HA 0.05 %	26.1	40.0	4.87	7.39	11.30	1.94
Ca Super P (2)+ HA 0.05 %	27.4	43.2	5.83	8.13	11.83	2.33
Super Bio P (1)+ HA 0.05 %	26.7	42.9	5.24	7.84	11.50	2.12
Super Bio P (2) + HA 0.05 %	28.2	44.7	5.85	9.11	12.13	2.42
P0 + HA 0.1 %	23.2	30.6	3.07	5.95	9.10	1.34
Ca Super P (1)+ HA 0.1 %	27.8	43.9	6.12	8.63	12.10	2.34
Ca Super P (2)+ HA 0.1 %	28.6	49.9	7.65	9.34	13.33	2.62
Super Bio P (1)+ HA 0.1 %	29.8	58.0	8.34	9.91	14.70	2.95
Super Bio P (2)+ HA 0.1 %	29.2	54.3	8.13	9.82	14.40	2.85
LSD 0.05 %	0.71	1.79	0.38	0.34	0.39	0.16

The plants take more mineral elements due to the better-developed root systems. Besides, the stimulation of ion uptake by treatments of humic materials led many investigators to propose that these materials effect to membrane permeability **(Zientara, 1983).** It is related to the surface activity of humic substances resulting from the presence of both hydrophilic and hydrophobic sites. These results were also consistent with the conclusions drawn by **Chen *et al.* (2004)** in their recent review of the use of humic substances (HS) in agriculture. They concluded that although HS can affect plant productivity through a variety of mechanisms, soil application of commercial humic products at typical use rates is unlikely to elicit a significant agronomic response. **Abdel Fatah *et al.*, (2008)**, who observed that application of humic acid improved growth parameters and K promotes photosynthesis and transport assimilates of the carbohydrates to the storage organs.

Photosynthetic pigments

The presented results in Table (3) showed that, humic acid with different source of phosphorus fertilization increases the content of photosynthetic pigments (chlorophyll a, b, a+b and carotenoids). The highest values were obtained from the application of using super bio-phosphate at a rate of 200 kg P_2O_5 fed^{-1} and foliar application humic acid (HA) at a rate 0.1%. The increment effect of chlorophyll a, b, a + b and carotenoids by 1.270, 0.241, 1.511 and 0.582, respectively.

Table (3): Chlorophyll (Chl), carotene and nitrite contents as affected by humic acid and phosphorus sources (Data mean of two seasons).

Treatments	Chl. a	Chl. b	Chl. a + b	Carotene	NO_3
	(mg/g fresh weight)				(ppm)
P0 (Control)	0.353	0.058	0.411	0.278	196.4
Ca Super P (1)	0.753	0.101	0.855	0.418	186.1
Ca Super P (2)	1.410	0.214	1.624	0.662	165.9
Super Bio P (1)	1.020	0.158	1.178	0.466	162.9
Super Bio P (2)	1.203	0.258	1.461	0.433	159.0
P0 + HA 0.05 %	0.420	0.094	0.514	0.296	187.4
Ca Super P (1)+ HA 0.05 %	0.607	0.107	0.714	0.297	179.0
Ca Super P (2)+ HA 0.05 %	0.727	0.132	0.858	0.421	171.5
Super Bio P (1)+ HA 0.05 %	1.021	0.172	1.194	0.491	161.3
Super Bio P (2) + HA 0.05 %	1.119	0.213	1.332	0.493	153.6
P0 + HA 0.1 %	0.460	0.141	0.601	0.263	174.2
Ca Super P (1)+ HA 0.1 %	0.760	0.085	0.844	0.444	166.0
Ca Super P (2)+ HA 0.1 %	0.863	0.139	1.002	0.448	155.2
Super Bio P (1)+ HA 0.1 %	0.933	0.146	1.079	0.482	137.3
Super Bio P (2)+ HA 0.1 %	1.270	0.241	1.511	0.582	141.4
LSD 0.05 %	0.061	0.008		0.026	12.18

These results are confirmed by those recorded by **Kamari-Shahmaleki *et al.* (2012), (Ferrara and Brunetti 2010) and Pouzeshi *et al.* (2011).** Formation of complex between humic acid and mineral ions, catalysis of humic acid by the enzymes in plant, influence of humic acid on respiration and photosynthesis, stimulation of nucleic acid metabolism and hormonal activity of humic acid are amongst effective assumptions that has been expressed to describe the effect of humic acid on plants growth parameters

Turkmen *et al*., (2004).

Cangi *et al*. (2006) indicated that foliar spraying of humic acid and amino acids on Asparagus plants increase uptake of macro and micro elements in shoot and rhizome has increased carbohydrates production, chlorophyll and carotenoids in edible stems. Enhancing the quantitative and qualitative characteristics as a result of increased respiration, photosynthesis and total protein in the plants, due to humic acid and folic acid application has also been reported by **Nardi *et al*. (2002)**. **Fernández-Escobar *et al.*, (1999)** found that application of HA stimulated chlorophyll content and accumulation of K, B, Mg, Ca and Fe in leaves. **(Ayas and Gulser 2005)** reported that HA application was the main reason of enhancing nitrogen uptake in spinach.

Data in table (3) indicate that NO3 concentration was significant affected by humic acid and bio-phosphorus fertilizers. The lowest contents were obtained by 18.65 % as affected by fertilization radish plants with 100 kg P2O5 fed-1 and foliar application of humic acid at the rate 0.1 %. as compared with super bio-phosphate individual at a rate of 100 kg P2O5 fed-1 may be attributed to role of humic acid in decrease of nitrate accumulation. These results were also consistent with the conclusions drawn by **Chen and Aviad (1990) and Nardi et al. (2002)**. **Suchhanda and Nad (2012)** indicated that spinach maintained a very high level of NO3-N in its tissue throughout the growing period. NO3-N was increased with increasing nitrogen level and was reduced with phosphorus and sulfur application and also with advancement in growth.

Nutrient content

Nitrogen and phosphorus

In the regard of nitrogen and phosphorus uptake, data in Fig 1,2 represented N and P uptake in Radish tissues that increased by using humic acid with different source of phosphorus fertilization .

The highest values of N and P uptake were recorded with plants received humic foliar application at a rate 0.1% with super bio-phosphate at a rate of 100 kg P_2O_5 fed^{-1} as compared with control. Also, observed a rise in values with the addition of calcium super phosphate but less than super bio-phosphate and order to contain microorganisms that availability of elements. These results may be due to the role of humic acid in the modulation of nutrient uptake via an interaction with plasma membrane H+ -ATP. In their study the contemporary presence of humic substances caused stimulation of the nutrient uptake capacity and of the plasma membrane H+ -ATP activity with the same pattern observed for nutrient uptake. The stimulation of plasma membrane H+ -ATP activity was also reported by several other authors **Canellas *et al*., (2002)** and is considered as an important action of humic substances on plant nutrient acquisition. The enhanced uptake of phosphorous in plants with application of humic substances is mainly due to the increased

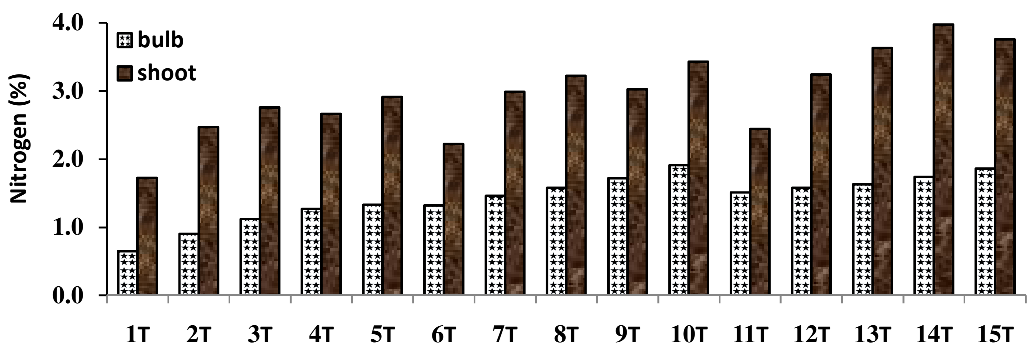

Fig (1): Nitrogen content as affected by Humic acid and phosphorus sources

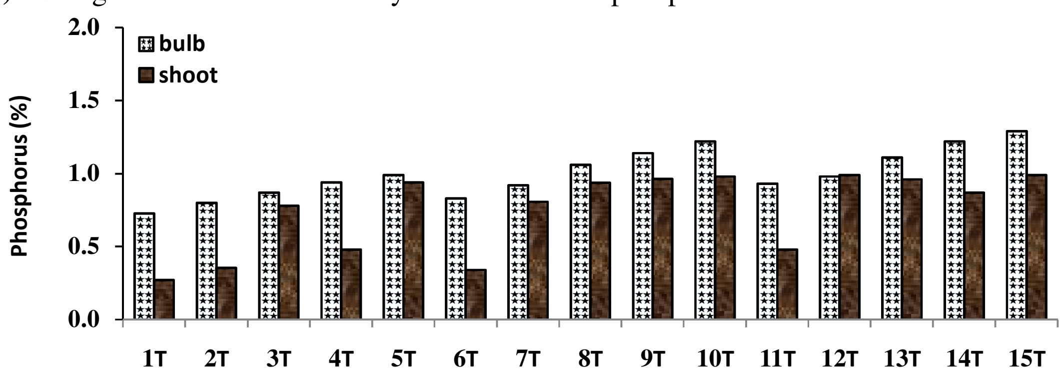

Fig (2): Phosphorus content as affected by Humic acid and phosphorus sources

availability of phosphate in the soil **(Zalba and Peinemann, 2002)**. In many soils a large part of total phosphorous is insoluble (calcium phosphate precipitation) and thus unavailable to the plants. The major mechanism involved in the effect of humic acid increasing phosphorus recovery is the interference on calcium phosphate precipitation **Delgado *et al*., (2002); (Satisha and Devarajan, 2005).** The effect of HA on the availability of P and micronutrients has been given particular attention because of observed increases in uptake rates of these nutrients following application of HA **Ayuso *et al*., (1996). (Cimrin and Yilmaz 2005)** stated that application of humic acid increased head weight of lettuce (*Lactuca sativa* L. *var.*

longifolia) by increasing the availability of phosphorus and nitrogen. **(Ayas and Gulser 2005)** concluded that increased nitrogen uptake caused by humic acid application was the main reason of enhanced vegetation growth of spinach. The positive effects of the humic substances were also observed on the studies such as dry matter yield increases on corn and oat seedling. **Manas Denre**, ***et al*** **(2014)** reported that the phosphorus concentration was significantly positive; 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 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.

Potassium and sodium

It is clear from Table 4 that, potassium content was significantly enhanced by the humic foliar application at a rate 0.1% with different source of phosphorus fertilization particularly with application of super bio-phosphate at a rate of 100 kg P_2O_5 fed^{-1}. The increments were 52 % and 33 % of root and shoot respectively as compared with the super bio-phosphate the same levels individual. While sodium content, data in Table 4 represented Na content percentage in radish tissues that gradually decreased by using humic foliar application at a rate 0.1% with different source of phosphorus fertilization particularly with application of super bio-phosphate at a rate of 100 kg P_2O_5 fed^{-1}.

Table (4): **Sodium and potassium content (%) as affected by Humic acid and of phosphorus sources (Data mean of two seasons).**

Treatments	Sodium %		Potassium %		Na/K ratio	
	Root	Shoot	Root	Shoot	Root	Shoot
P0 (Control)	2.72	4.06	0.23	0.65	11.79	6.24
Ca Super P (1)	2.61	3.99	0.76	1.18	3.44	3.38
Ca Super P (2)	2.41	3.91	0.89	1.25	2.70	3.13
Super Bio P (1)	2.21	3.66	1.25	1.23	1.77	2.97
Super Bio P (2)	0.75	2.65	1.30	1.33	0.58	1.99
P0 + HA 0.05 %	2.27	3.75	0.23	1.08	9.93	3.47
Ca Super P (1)+ HA 0.05 %	1.78	3.46	1.31	1.40	1.36	2.47
Ca Super P (2)+ HA 0.05 %	1.54	3.40	1.67	1.42	0.92	2.40
Super Bio P (1)+ HA 0.05 %	1.23	2.98	1.40	1.40	0.88	2.13
Super Bio P (2) + HA 0.05 %	0.62	2.56	2.25	1.46	0.28	1.75
P0 + HA 0.1 %	1.25	2.91	0.60	1.12	2.06	2.60
Ca Super P (1)+ HA 0.1 %	1.23	2.91	2.02	1.45	0.61	2.01
Ca Super P (2)+ HA 0.1 %	1.14	2.76	2.27	1.61	0.50	1.71
Super Bio P (1)+ HA 0.1 %	0.76	2.70	2.63	1.85	0.29	1.46
Super Bio P (2)+ HA 0.1 %	0.45	2.37	2.47	1.77	0.18	1.34
LSD 0.05 %	0.138	0.14	0.16	0.044		

Obtained results show that the concentration of the most important macronutrients was enhanced with application of humic acid foliar application. Increased concentration of macronutrients has been reported before when humic substances were applied to the soil as simple solution **Cooper** ***et al.*****, (1998); Sharif** ***et al.*****, (2002)** and even more pronounced when they were mixed into the nutrient solution **Ayuso** ***et al.*****, (1996);**

Pinton *et al*., (1999), suggesting the existence of a synergistic effect of combined applications of mineral nutrients and humic substances. As humic substances behave like weak acid polyelectrolytes, the occurrence of anionic charged sites accounts for the ability to retain cations like K^+ and Mg^{2+} and the cation exchange capacity of the soil will be increased.

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