

## Effect of Humic Acid Isolated by IHSS-N<sub>2</sub>/Mn Method and P Fertilization on Yield of Pepper Plants

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**Abstract:** A field experiment was carried out at Ismailia Agriculture Research Station, Agriculture Research center, during summer season of 2010, to benefit from composting of remnants of food factories and then humic acid (HA) was extracted from mature compost and using it with P fertilization to study their impact on yield and nutrients content of pepper plants. Extraction and purification of humic acid (HA) was based on the IHSS –N<sub>2</sub> method and IHSS –N<sub>2</sub>/Mn method (IHSS, "International Humic Substances Society"). Total acidities and phenolic hydroxyls contents are the highest for HA isolated by modified method (IHSS-N<sub>2</sub>/Mn) which indicates that the HA posse less altered eases oxidisable phenolic hydroxyl groups. Humic acid isolated by IHSS-N<sub>2</sub>/Mn method and applied with drip irrigation water as it was added every two weeks starting from the stage of germination till harvest. Treatments were representing at all the combinations of humic acid (1, 2 and 3 ml L<sup>-1</sup>) and P fertilizer rates 60, 90 and 120 kg P<sub>2</sub>O<sub>5</sub> fed<sup>-1</sup> (fed. Equal 4200 m<sup>2</sup>). Results showed that, increasing the rates of HA and P fertilization increased pepper yield (quality and quantity) compared with that of control (without humic acid or P fertilization). The most promising treatments for production of marketable and unmarketable pepper yield could be: Those of (3 ml L<sup>-1</sup> of HA+ 120 kg P<sub>2</sub>O<sub>5</sub> fed<sup>-1</sup>) which showed an increment of (+10.0%) and (+7.22%), respectively. This high rate could be recommended for obtaining the highest rate of income from the marketable yield of pepper.

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**Key word:** Humic acid (HA), IHSS –N<sub>2</sub>/Mn method, P fertilization, Pepper yield.

### 1. Introduction

Food processing is a major industry that is rapidly growing because of a demand for packaged foods in urban areas. The companies of food processing have large processing facilities where vegetables, such as lettuce, cabbage, onions, peas, artichoke, etc. are cleaned, chopped, mixed and packaged. In a typical operation, the amount of wastes generated equal in quantity (by weight) to the amount of product shipped. Presently, these wastes are land disposed or land filled. Vegetable wastes do not provide any known concerns relating to pathogens or human health issues, however, they are prone to potential odors during decomposition and are expensive to dispose because of their high moisture content leading to high landfill tip fee and transportation cost.

Composting can be defined as being the breakdown of organic materials by large numbers of microorganisms in a moist, warm and aerated environment leading to the production of carbon dioxide, water, minerals and stabilized organic matter. The process generally starts by stacking the organic wastes in piles. The mixture is then composted in the presence of air for a period of 4-12 weeks depending on the type of system used, followed by a maturation phase (curing) of approximately the same duration ( Diaz *et al.*, 2002). Jovi i *et al.*, (2009) showed that the creation of compost has become a more popular option of waste management as a waste and reduce pressure on landfill. Because of the importance of

composting in order to achieve the objectives of waste management in the world. As necessarily, regional composting plants need to be built. Reusing this materials will remarkable reduce final quantity of deposited wastes. This is the main benefit, but compost is very useful product in agricultural business, that's why material retrieves validity. Remarkably, (McGuckin *et al.*, 1999) reported that the sulfur content of lettuce and onion wastes were 0.2 and 0.7 %, respectively. Discarded components of lettuce and onions have an effective carbon to sulfur ratio of 215 and 62, moisture contents of 96.2 and 91.1% and carbon to nitrogen ratios of 10.3 and 11.5, respectively. The low C/S ratio of onions indicates that mixes with high fractions of onions can result in release of odorous sulfur compounds. High water content, most of which is bound within the vegetable fiber, results in significant leachate formation during composting and collapse of the composting matrix from initial height of e.g. 1.5 m to a lower value of e.g. 0.5 m resulting in reduction in air space and oxygen availability within the pile.

Humic acid (HA) is a major fraction of humic substances, forms strong complexes with metal and plays a vital role in metal ion mobilization and transportation in the subsurface soil and aquatic environments. Humic acids capability for binding metal ions is largely due to diverse functionalities in the HA structure (Osman *et al.*, 2009). Humic acid is one of the most active fractions of organic matter, it improves the absorption of nutrients by

plants and soil microorganisms, have a positive effect on the dynamic of N and P in soil, stimulate plant respiration and the photosynthesis process, and favor the formation of soil aggregates, etc. (Hernandez et al., 2001 and Brunetti et al., 2007).

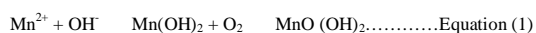
There are several of methods for extraction of HA, using different extraction reagents, which are summarized in Table (1):

Table (1): Methods of HA extraction.

Type of material	Extractant	Organic matter extracted
Humic substances	NaOH	to 80 %
	Mild extractants:	
	Na <sub>4</sub> P <sub>2</sub> O <sub>7</sub> and other Organic chelates: acetyloacetone, cupferron, hydroxyquinoline	to 30 % to 30 %
	Formic acid (HCOOH)	to 55 %

In general, the disadvantages of alkali extraction are as follows: a) alkali solutions dissolve silica from the mineral matter and this silica contaminate the organic fractions separated from the extract; b) alkali solutions dissolve protoplasmic and structural components from fresh organic tissues and these become mixed with the humified organic matter; c) under alkaline conditions, autoxidation of some organic constituents occurs in contact with air both during extraction and when the extracts are allowed to stand; d) in alkaline solution condensation between amino acids and aldehydes or quinines can occur (Andelkovic *et al.*, 2001).

Because of the contact air with humic substances material under alkaline condition, new method like IHSS was established (Sparks, 1996). However, for isolation of even less altered material, the presence of O<sub>2</sub> in the alkaline extraction solution (AES) should not be neglected, although it usually is. Andelkovic *et al.*, (2001) modified standard IHSS procedure by using MnSO<sub>4</sub> for deoxygenation of AES. Dissolved oxygen present in the AES, rapidly oxidizes dispersed divalent manganous hydroxide precipitate to hydroxide of higher valence state, as shown in the equation (1):



This deoxygenized AES is, after filtration in N<sub>2</sub> atmosphere, used for humic acid extraction. Therefore, the aims of this research were: (i) comparison between the two extraction methods of HA isolated from compost to choose the best way of extraction using FT-IR and E4/E6 ratio; (ii) study the effect of humic acid and P fertilization rates on nutrients content and yield of pepper.

## 2. Materials and Methods

A field trial was conducted on a loamy sand soil at Ismailia Agricultural Research Station, by cultivating pepper (*Capsicum annum L.*, cv

Marrkony) at summer season of 2010. Main and interaction effects of different rates of HA and super phosphate (as P source) on yield components and nutrients content of pepper plant were investigated. The experiment was carried out following the randomized complete block design, with three replicates for each experimental unit. Humic acid was added with drip irrigation water as it was added every two weeks starting from the stage of germination until harvest, which was combined with three P<sub>2</sub>O<sub>5</sub> rates of (60, 90 and 120 kg P<sub>2</sub>O<sub>5</sub> fed<sup>-1</sup>) in the form of superphosphate (15 % P<sub>2</sub>O<sub>5</sub>). N fertilization rate (80 kg N fed<sup>-1</sup>) was added in the form of ammonium sulfate (20 % N). K fertilization rate (24 kg K<sub>2</sub>O fed<sup>-1</sup>) was added in the form of potassium sulfate (50 % K<sub>2</sub>O). The N and K fertilization was run entirely through preparing the soil before planting, at the recommended doses of mineral N and K fertilization (ammonium sulfate = 400 kg fed<sup>-1</sup> as source of N and potassium sulfate = 300 kg fed<sup>-1</sup> as source of K) and also without HA addition to act as a control treatment which were compared to the other treatments.

Humic acid were extracted from the compost, which was made from remnants of food factories, where the waste was a peel fruits of pea and artichoke leaves fruit and broccoli plant residues. Composting process was over within two months. Compost has been added to the experience at one of 10 ton/fed<sup>-1</sup>.

Extraction and purification of HA from compost was based on two methods to choose the best of HA, one of those methods was traditional extraction of HA (International humic acid substances society, IHSS- N<sub>2</sub> method), while the other was the modified method of IHSS (IHSS- N<sub>2</sub>/Mn method). It is known that, both of the two methods are the same technique; however, MnSO<sub>4</sub> is added in the modified method. In initial treatments, phosphoric acid was used rather than

HCl, which is recommended by IHSS, because  $H_3PO_4$  would prevent the oxidation of organic substances by the Fe (III) ion. Also, alkaline extraction solution was deoxygenized by dissolving 2.0 g  $MnSO_4$  in 1000 ml KOH solution at pH 11. After 12h, the solution was filtered, under  $N_2$  atmosphere, in order to decrease  $O_2$  level as much as possible. HA was dissolved in 0.1 M KOH (prepared with distal water in which  $N_2$  was purged in order to remove dissolved  $O_2$ ), in a separating funnel of appropriate volume so that no air was left over the solution surface. HA was passed over Dowex and collected in a flask (in which a minute volume of  $H_2SO_4$  was added) connected with  $N_2$ . HA rapidly coagulates in acid solution. The protonated HA was centrifuged, this method was described by Andelkovic *et al.*, (2001).

The experimental soil plots were sampled initially before pepper planting to determine some physical and chemical properties according to the standard procedures outlined by Cottenie (1980) (Table, 2).

Chemical properties of the tested humic acid was measured according to the standard methods described by Cottenie (1980). The infrared spectra (FT-IR) were recorded from pellets containing 2 mg of the dried humic acids with 250 mg of dry KBr. The instrument used was PerkinElmer 1600 FTIR spectrophotometer covering a wave number range of 400-4000  $cm^{-1}$ . The E4/E6 parameter was determined by UV/VIS method on a spectrophotometer by dividing the absorbance of 2 mg dried humic acid in 25 ml 0.025 M  $NaHCO_3$  at 465 nm by absorbance at 665 nm.

Plant samples were collected from mature pepper plants at harvest stage for analysis. Plant samples were dried at 65°C for 48 hrs, ground and wet digested using  $H_2SO_4:H_2O_2$  method (Cottenie, 1980). The digests were then subjected to measurement of N using Micro-Kjeldahl method; P was assayed using molybdenum blue method, while, K was determined by Flame Photometer (Chapman and Pratt, 1961). Ascorbic acid content was assayed using oxalic acid method (Jacobs, 1951).

Table (2): Some physical and chemical properties of the soil used.

Soil property	Value	Soil property	Value
Particle size distribution %		pH (1:2.5 soil suspension)	7.52
Coarse sand	69.9	ECe ( $dS m^{-1}$ )	1.26
Fine sand	14.2	Soluble ions ( $meq L^{-1}$ )	
Silt	5.70	$Ca^{++}$	5.66
Clay	10.2	$Mg^{++}$	4.08
Texture	Loamy sand	$Na^+$	1.94
$CaCO_3$ %	2.10	$K^+$	0.22
Saturation percent	23.3	$CO_3^{--}$	nd*
Organic carbon %	0.02	$HCO_3^-$	1.99
Available N ( $mg kg^{-1}$ )	9.36	$Cl^-$	4.78
Available P ( $mg kg^{-1}$ )	1.81	$SO_4^{--}$	5.13
Available K ( $mg kg^{-1}$ )	65.9	CEC ( $meq 100 g^{-1}$ soil)	6.50

nd : not detected

### 3. Results and Discussion

#### 3.1. Comparison between the two humic acid extraction methods (IHSS- $N_2$ and IHSS- $N_2/Mn$ )

Results in (Fig, 1) indicated that, the main common and different FT-IR features of HA isolated from compost by IHSS- $N_2$  and IHSS- $N_2/Mn$  methods, and their corresponding assignments, according to Stevenson (1994); Plaza *et al.* (2002); Senesi *et al.* (2003) they found: a common, intense broad band at about 3400  $cm^{-1}$  usually attributed to O-H stretching, the intensity of 3373  $cm^{-1}$  with the IHSS- $N_2$  increases in the other way of extraction. This line is observed in spectra of distillate water.

In this situation links in the range of 2923  $cm^{-1}$  are reduced which are related to  $CH_2$  bond. These links are weakened and with some displacement are observed in the range of 2921-2923  $cm^{-1}$ . The bands observed at 2923  $cm^{-1}$  and at 2845  $cm^{-1}$  are due to the asymmetric and symmetric stretching modes of

the methylene chain in the membrane lipids. The band at about 1445  $cm^{-1}$  assigned to the aliphatic C-H deformation, corresponding to HA obtained from IHSS- $N_2/Mn$  method similar for HA obtained from IHSS- $N_2$ . The absorption at about 1700  $cm^{-1}$  due to C=O stretching of COOH and other carbonyl groups are more intense for HA obtained from IHSS- $N_2/Mn$  method than for this HA obtained from IHSS- $N_2$  method, where they appear at 1732 and 1710  $cm^{-1}$  respectively. The band at 1642 and 1632  $cm^{-1}$  are related to c=c aromatic in the HA extracted by IHSS- $N_2$  and IHSS- $N_2/Mn$  respectively. The band at about 1500  $cm^{-1}$ , preferentially ascribed to N-H deformation and C-H stretching of amides, are more evident in FT-IR spectra of HA obtained from IHSS- $N_2/Mn$  method than in spectra of HA obtained from IHSS- $N_2$  where it appears at 1515  $cm^{-1}$ . The band about 1280  $cm^{-1}$  is generally ascribed to C-O stretching and deformation of carboxyl and C-O stretching of

ethers and phenols has medium intensity in the spectra corresponding to HA obtained from IHSS-N<sub>2</sub>/Mn method only, and did not appear in spectra corresponding to HA obtained from IHSS-N<sub>2</sub> method. The absorption at about 1000-1200 cm<sup>-1</sup>, ascribed to C-OH stretching of aliphatic O-H,

corresponding to HA obtained from IHSS-N<sub>2</sub>/Mn method similar for HA obtained from IHSS-N<sub>2</sub>. While, it is common to find P=O group (phosphene oxide) at wavenumber 1172cm<sup>-1</sup> for HA obtained from IHSS-N<sub>2</sub>/Mn.

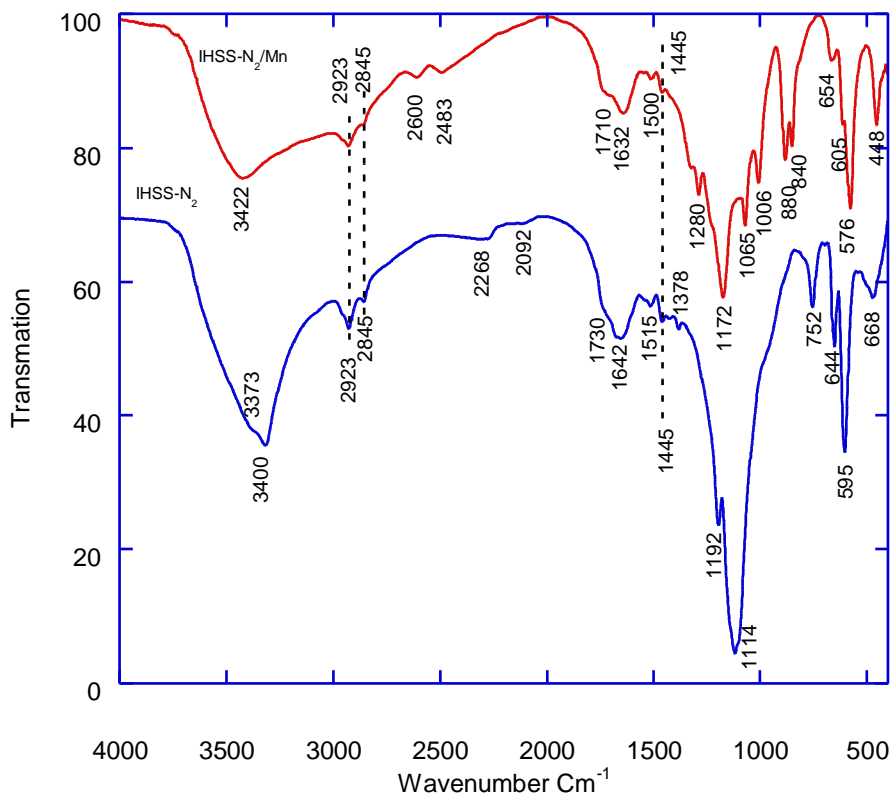


Fig. 1: FT-IR spectra of the humic acid isolated from compost by IHSS-N<sub>2</sub> and IHSS-N<sub>2</sub>/Mn methods

The band in the range from 1580-1650 cm<sup>-1</sup> are related to the group function Si-O-Si which obvious found in the HA extracted from IHSS-N<sub>2</sub>, while, it is not found in IHSS-N<sub>2</sub>/Mn method of extraction. The band ranges below 1000cm<sup>-1</sup> is related to metal ion complex, it is obvious to be more in the HA extracted by IHSS-N<sub>2</sub>/Mn due to the presence of Mn.

Nitrogen, phosphorus and potassium content, C/N ratio and E4/E6 ratio of HA studied are shown in Table 3. Two methods extraction did not differ in N, P and content as well as C/N ratio since this ratio is an indicator of source of humic acid in natural system. Therefore, as long as the source of HA from the same source (compost), the C/N ratio of HA did not differ of two methods (Meyers and Ishiwatari, 1993 and Chai *et al.*, 2007).

From the above mentioned discussion it is obvious that the best HA extraction methods method, is related to the IHSS-N<sub>2</sub>/Mn. This may be due to the ability to make metal complexes with Mn which affect the absorption of the nutrient over the colloids and increase the nutrient transfer to the plant. Furthermore, the total acidities and phenolic hydroxyls contents are the highest for HA isolated

by modified method (IHSS-N<sub>2</sub>/Mn) which indicates that the HA posse less altered eases oxidisable phenolic hydroxyl groups.

E4/E6 ratio of HA extracted from IHSS-N<sub>2</sub>/Mn method was more than E4/E6 ratio of HA extracted from IHSS-N<sub>2</sub> method, so HA extracted from IHSS-N<sub>2</sub>/Mn method is a low content of carbon, a low molecular weight, a high total of oxygen and COOH groups and a high total acidity. Polak *et al.*, (2007) reported that UV/VIS spectroscopy is the source of information on the composition of molecules of HA and on their origin. For this purpose E4/E6 ratio value was determined. The low E4/E6 ratio for HA may be attributed to the absorption by aromatic C=C functional groups. Additionally, the high degree of condensation of the aromatic rings as well as the large molecular weight of HA are believed to contribute to its relatively high absorption in the visible range (Chen *et al.*, 2004). On the other hand, the high value of E4/E6 ratio points to a low molecular weight of HA, a low content of carbon, a high content of oxygen and COOH groups. Moreover it also indicates a high total acidity and a high degree of aromatization of humic substances

(Polak *et al.*, 2011 ). Accordingly, the method of HA extraction by modified method of IHSS (IHSS-N<sub>2</sub>/Mn). Andelkovic *et al.*, (2001) showed that HAs were isolated and purified by three method: by modified method, IHSS-N<sub>2</sub>/Mn method, IHSS method and IHSS-O<sub>2</sub> method. Concerning

differences in the obtained data of all three HAs, it is reasonable to assume that significant alteration occurs in the cases when MnSO<sub>4</sub> is not used in the isolation procedure. So, it is recommended to use this modified, IHSS-N<sub>2</sub>/Mn method in isolation of HA with more preserved structure.

Table (3): Nitrogen, phosphorus and potassium content, C/N ratio and E4/E6 ratio of HA isolated by IHSS-N<sub>2</sub>/Mn and IHSS-N<sub>2</sub> methods.

Source	pH (1:2.5 )	N%	P%	K%	Organic carbon %	C/N	E <sub>4</sub> /E <sub>6</sub> ratio
Humic acid (IHSS -N <sub>2</sub> /Mn method)	7.65	2.11	1.36	3.27	51.8	24.5	2.02
Humic acid (IHSS -N <sub>2</sub> method)	7.66	2.10	1.36	3.27	53.5	25.5	1.87

### 3.2. Effect of humic acid and P fertilization on yield components of pepper plants.

Results in (Table, 4) indicate that increasing P fertilization rate under humic acid (HA) rates significantly increased for yield components. The mostly induced parameters, i.e., marketable, unmarketable yields (unmarketable yield was mean first and second packing), fruit length and diameter, ascorbic acid content, and total chlorophyll all of which under the highest rates of applied HA (3 ml L<sup>-1</sup>) as well as the highest P fertilization rate (120 kg P<sub>2</sub>O<sub>5</sub> fed<sup>-1</sup>).

In other words, the dual synergistic effect probably was mutual for HA and P fertilization rates. However, the average values of yield and yield parameters increased significantly under highest HA compared with the lower HA one. Türkmen *et al.*, (2005) reported that humic acid application increased the plant growth, nutrient uptake and pepper yield and quality. Increasing the rate of both P and organic fertilizer treatments significantly enhanced fresh fruit yield per plant when compared with the control treatment. This also enhanced significantly the yield per hectare and yield components such as the fruit length and diameter (Alabi, 2006). Mesut *et al.*, (2010) reported that high levels of HA and P applications increased the growth and growth parameters, as well as pepper yield components than each separate effect. Results in Table (5) indicate decrements in

both marketable and unmarketable yield of pepper under all treatments as compared with control. The rate of reduction was partially compensated by increasing the added humic acid rate from 60 to 120ml L<sup>-1</sup> and the addition of highest rates of P rates consistently. The high reduction in marketable and unmarketable yield amounted to (-18.42% and -7.222%), respectively, under lower rate of humic acid (1 ml L<sup>-1</sup>) and P fertilization rate (60 kg fed<sup>-1</sup>). This reduction of both yield reduced by increasing humic acid and P fertilization rates.

The most promising treatment (3 ml L<sup>-1</sup> of HA + 120 kg P<sub>2</sub>O<sub>5</sub> fed<sup>-1</sup>) which showed an increments of (+10.0%) and (+7.222%) of marketable and unmarketable yield. Account these values into net income by taking into considerations the price of added fertilizer and expected price of marketable yield, the calculations reveal that the net income for the treatment could be higher than that of control treatment by 400\$. The treatment of (3 ml L<sup>-1</sup> of HA + 120 kg P<sub>2</sub>O<sub>5</sub> fed<sup>-1</sup>) could be recommended for obtaining the highest rate of income from the marketable yield of pepper.

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Table (4): Interaction effect between humic acid and P fertilization rates on yield and yield components of pepper plant.

Humic acid rates ml \L	P <sub>2</sub> O <sub>5</sub> kg fed <sup>-1</sup> (P)							
	60	90	120	mean	60	90	120	mean
	marketable yield ( ton fed <sup>-1</sup> )				Unmarketable yield ton fed <sup>-1</sup>			
1	7.750	7.990	9.270	8.337	1.670	1.750	1.860	1.760
2	8.660	9.380	9.630	9.223	1.680	1.810	1.900	1.797
3	9.000	9.380	10.45	9.610	1.750	1.850	1.930	1.843
Mean	8.470	8.917	9.783		1.700	1.803	1.897	
L.S.D. <sub>0.05</sub> humic acid = 0.243 P = 0.240 Humic acid *P = 0.342 Control = 9.500				L.S.D. <sub>0.05</sub> humic acid = 0.058 P = 0.039 Humic acid *P = 0.055 Control = 1.800				
	Fruit length ( cm)				Fruit diameter (cm)			
1	9.560	9.630	10.00	9.730	8.110	8.310	8.390	8.270
2	10.55	11.49	11.73	11.26	8.410	8.710	8.960	8.693
3	11.60	11.74	11.84	11.73	8.700	8.790	9.120	8.870
Mean	10.57	10.95	11.19		8.407	8.603	8.823	
L.S.D. <sub>0.05</sub> humic acid = 0.284 P = 0.502 Humic acid *P = 0.711 Control = 11.64				L.S.D. <sub>0.05</sub> humic acid = 0.112 P = 0.230 Humic acid *P = 0.326 Control = 8.620				
	Vitamin C (%)				Total chlorophyll			
1	71.52	72.15	72.52	75.77	45.36	48.80	50.60	48.25
2	74.19	79.91	81.38	51.93	53.25	54.60	55.90	54.58
3	81.60	83.04	83.97	82.87	55.38	55.97	56.50	55.95
Mean	75.77	78.37	79.29		48.25	50.12	54.33	
L.S.D. <sub>0.05</sub> humic acid = 2.139 P = 1.227 Humic acid *P = 1.733 Control = 82.10				L.S.D. <sub>0.05</sub> humic acid = 2.113 P = 1.201 Humic acid *P = 1.633 Control = 54.50				

Table (5): Surplus (+) or deficit (-) values for yield relating the different experimental treatments over or under those obtained by the control treatment.

Treatment		Percentage of Yield	
Humic acid ml/L	P <sub>2</sub> O <sub>5</sub> (kg fed <sup>-1</sup> )	Marketable	Unmarketable
1	60	-18.42	-7.222
	90	-15.89	-2.778
	120	-2.421	+3.333
2	60	-8.842	-6.667
	90	-1.263	+0.556
	120	+1.368	+5.556
3	60	-5.263	-2.778
	90	-1.263	+2.778
	120	+10.0	+7.222

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