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Characterization and Effects of Plant Derived Humic Acid on the Growth of Pepper under Glasshouse Conditions

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ABSTRACT

A glasshouse study was conducted to evaluate the effects of plant derived humic acid (HA), previously characterised along with coal derived HA using HPLC and UV-VIS techniques on pepper growth, cholorophyll contents, P and K concentration in soil and plant. Characterization results classified coal, sunflower and maize derived HA as type A, B and Rp respectively. Plant growth parameters such as branches/plant, root length and weight, were significantly improved with HA, maximum branches (11) were found by HA application at highest rate (150 mg/kg). While, at lower application rate (30 mg/kg) maximum fruit weight was obtained. Similarly, longest roots were obtained with HA application at same rate. Flower and fruit chlorophyll contents significantly improved with HA application, yielding maximum flower cholorophyll contents by application of 90 mg/kg, whereas, in case of fruit maximum cholorophyll contents were found by application of HA at the rate of 30 mg/kg. Despite, plant growth improvement, fruit K concentration were also significantly improved with HA application. These results suggest that HA application at the optimum rate (30 mg/kg) improved plant growth parameters, however soil nutrients concentration only responded to HA application at the higher rate side.

Keywords: Humic acid, pepper yield, soil and plant P, Humic acid types, cholorophyll content

1. INTRODUCTION

The low soil organic matter (<1.0 %) in Pakistan coupled with high demand for agricultural produce triggers the need for commercially available inorganic fertiliser; urea, dia-ammonium phosphate (DAP) or triple superphosphate (TSP) and sulphate or muriate of Potassium (K_2SO_4, KCl) respectively. Currently, over 5 million tons (urea), around 1 million tons DAP and 27’000 tons SOP are used in Pakistan both for Rabi and Kharif crops (NFDC, 2012)[1]. It is seen that appropriate integration of organic with inorganic fertilisers could possibly improve plant growth, as well as reduce excessive use of inorganic fertiliser.

As synonym for soil organic matter, humic matter (humic acid and fulvic acid) acid[2, 3] can directly and indirectly benefit plant growth[4]. The HA plays significant role in nutrient acquisition[5] by chelating metallic cation[6] and through improving root growth and soil physical properties. Many researchers reported positive effect of HA on plant biomass weight of different crops; maize, oats seedlings and teak[7,8,9,10]. However, some studies showed that HA improved P availability without increasing lettuce yield[11]. The HA application increases nutrients absorption[12], in addition to their transport and distribution of [2,6] increased Fe bioavailability due to Fe-HA complexing behaviour and enhancing plant Fe and Zn nutrition have been reported[15,16]. Improved plant dry weight, nutrients of Fe, Na ($p < 0.05$) K, Mg, Cu, Zn and Mn ($p < 0.01$) were reported in glasshouse study of soil applied HA in maize crop[17]. The HA application has been linked to improve nutrients composition, plant photosynthesis pigments and total sugar contents of pea plant[18].

Chemically, HA composed of several aromatic rings interact with other aliphatic compounds giving rise to macromolecules[19]. Classical approaches involve understanding of HA composition based on elemental composition. In last few years various spectophotometric methods were used for this purpose. Some these techniques; Ultraviolet and Visible (UV-VIS) spectroscopy, Nuclear magnetic resonance (NMR), Electron paramagnetic resonance (EPR), Fourier transform infrared (FTIR) fluorescence and High performance liquid chromatography (HPLC), are employed to identify functional groups and understand macromolecular structure of HA. Such characterization not only helps in better understanding of organic matter decomposition but also suggests how management practices induce alteration in HA structure [20, 21].

In UV-VIS spectroscopy HA yield uncharacteristic spectra in the UV and visible region electromagnetic spectrum[22], and the spectra recorded both in wave length of 400 and 600 (E_4/E_6) provides information about HA characterization. There has been reported inverse relation between (E_4/E_6) and particle size or molecular weight of HA[23].

The current study aimed at characterization of plant derived HA and quantify K, and P concentration in plant and soil.

2. RESULTS AND DISCUSSION

2.1 *Ultraviolet and visible (UV-VIS) spectroscopy*

Results of UV-VIS spectroscopy showed spectra in the UV-VIS region. Decrease in absorption intensity with increase in wave length both in maize (*Zea mays*. L), sunflower (*Helianthus annus*. L) along with coal (Fig 3a; Fig 3b).

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However, in comparison to maize and sunflower, spectra of coal derived HA showed least steepness (Fig 3b). While, comparing maize derived HA with sunflower derived HA, the latter showed sharp decline (as wave length increases from 300 nm to 400 nm). The curve remained flatten in sunflower derived HA as wave length exceeds 423 nm.

The shoulder noticed around 370 nm could be ascribed to chromophore, absorbing light in the whole analysing region. Such behaviour could be associated to aromacity in HA[24]. The value of Δ log K (difference between log K at 600 nm and 400 nm) (Table 2) were found to be 0.96, 0.88 and 0.41 for maize, sunflower and coal respectively. Based on Δ log K, Kumada, (1987)[23] classified HA into 4 different types; A, B Rp and P. Type A lacks characteristics absorption having Δ log K ranges < 0.6, type B having value of Δ log K (0.6-0.8), while type Rp having similar spectrum as B, but possess Δ log K between 0.8 -1.1. Having characteristic absorption spectrum in 615, 570, and 450 nm visible regions HA are type P.

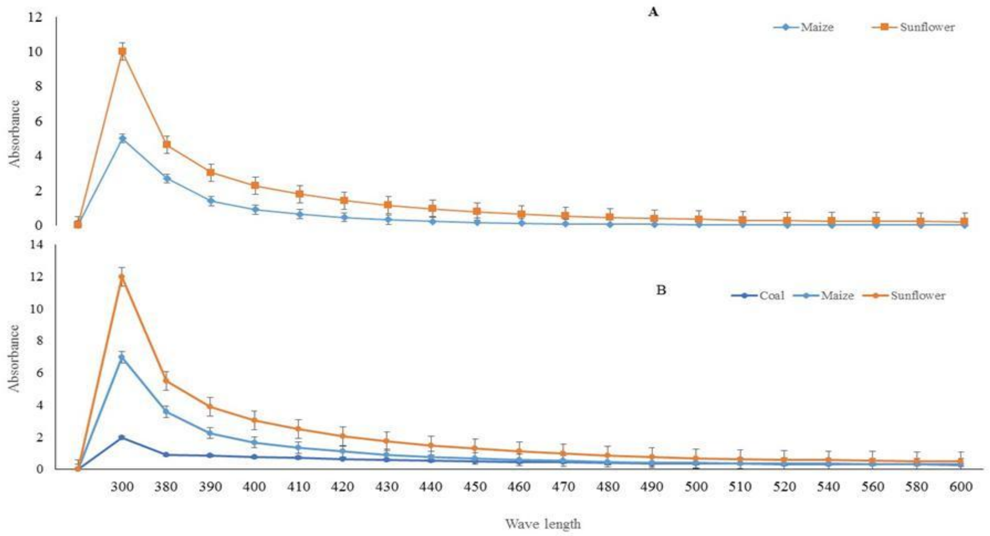

Fig-3: UV-VIS spectra of maize and sunflower derived HA substances (A), and UV-VIS spectra comparison of plants (maize and sunflower) with coal derived HA substances (B)

Looking to Δ log K values (Table 2) in the context of above classification, coal derived HA was type A, and sunflower derived HA with Δ log K (0.88) was type B, whereas maize derived HA showed resemblance to Rp type.

Table-2: Difference in the optical properties of HA derived from plant and coal materials

Plant materials	E4/E6	Δ log K
Maize	9.6	0.96
Sunflower	7.6	0.88
Coal	2.6	0.41

The HPLC chromatograms of both maize and sunflower derived HA (Fig 4; Fig 5) were separated by acetonitrile/water solution. The first peak appeared between t_R =1.5-3.5 corresponds the excluded fraction. Both maize and sunflower chromatograms showed sharp differences in the peak area. The fraction peak at t_R = 2-2.4 (Fig 4) and t_R = 2 (Fig 5) comprised the largest contributions of hydrophilic end. While, fractions in t_R = 14-16.5 ascribed to hydrophobic character of HA. The differences in chromatograms of HA showed structural differences of HA derived separately from maize and sunflower.

2.2 AGRONOMIC MEASUREMENTS

2.2.1 *Number of branches per plant*

Significant Increase in branches number/plant was observed HA application rate increases (Fig 6). It was observed that maximum branches (11) per plant were found by HA application @ of 150 mg/kg. Though, statistically non-significant number of branches increased beyond 0 mg/kg till maximum application of HA @ 150 mg/kg.

2.2.2 *Fruit weight, root length and root weight*

Soil application of HA has significant effect (p< 0.05) in fruit weight measured at the harvest. It is evident that means values of fruit weight in all treatments were significantly higher in comparison to control treatment where no HA was applied (Fig 7). Heaviest fruit (158.6 g) was obtained in treatment where HA was applied @ 30 kg/ha followed by 142.3 g HA application at the rate of 30 kg/ha. Results from this study showed overall improvement of 16%-45% in HA application at different levels. Root growth in term of length was also significantly improved with HA application in this study; showing longest root of 29.3 cm by HA application at @ 30 kg/ha, whereas 20 cm was obtained in control treatment where no HA was given (Fig 8). The response of root weight (Fig 9) also confirms that HA application at the rate of 30 kg/ha caused heavier root of the plant as compared to other treatment where HA were applied either in higher of lower rates.

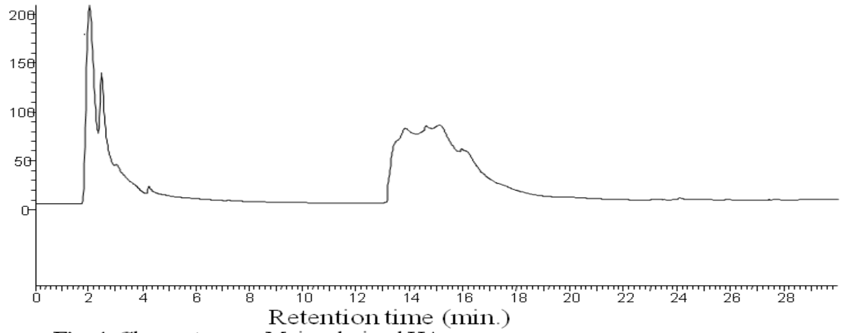

Fig-4: Chromatogram Maize derived HA

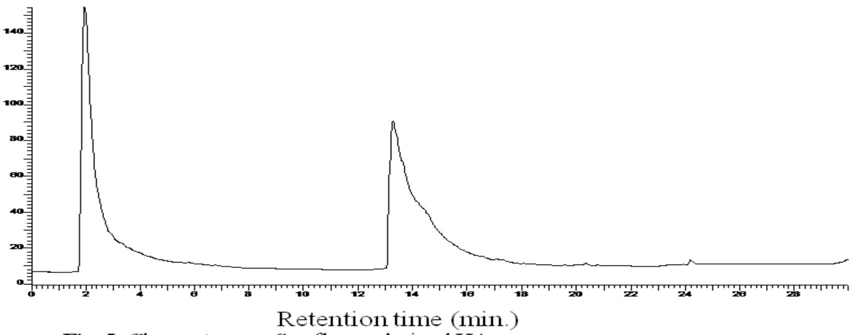

Fig-5: Chromatogram Sunflower derived HA

Results from this study showed that HA application at the higher rate is not as much effective as in lower to optimum rate. Earlier research[25] also reported effectiveness of HA at optimum rate both in plant root and shoot parts.

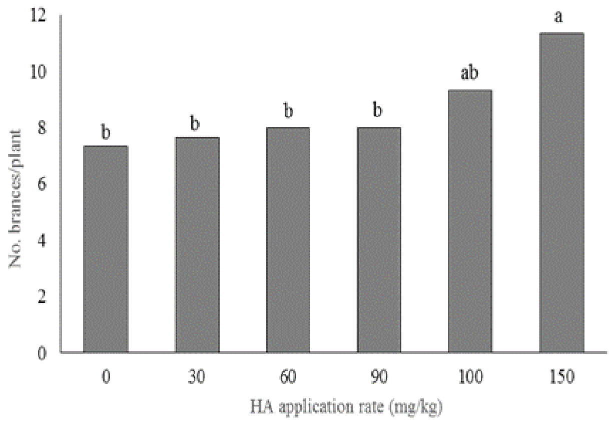

Fig-6: Branches per plant as affected by different rate of HA application

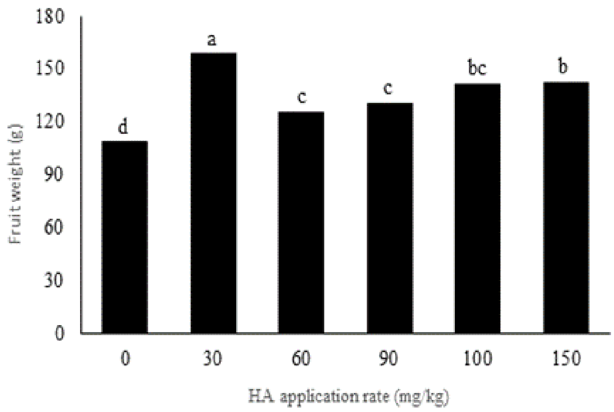

Fig-7:Fruit weight as affected by rate of HA application

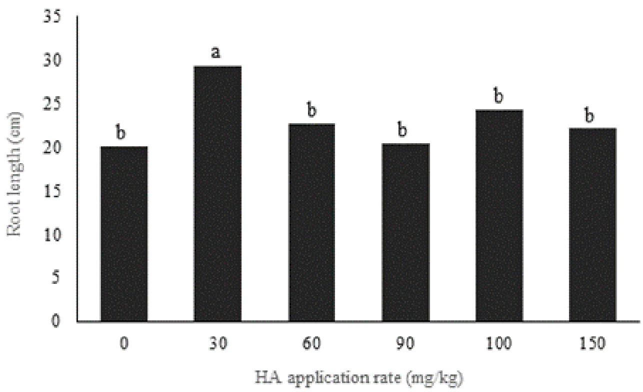

Fig-8: Response of root length to different rate of HA application

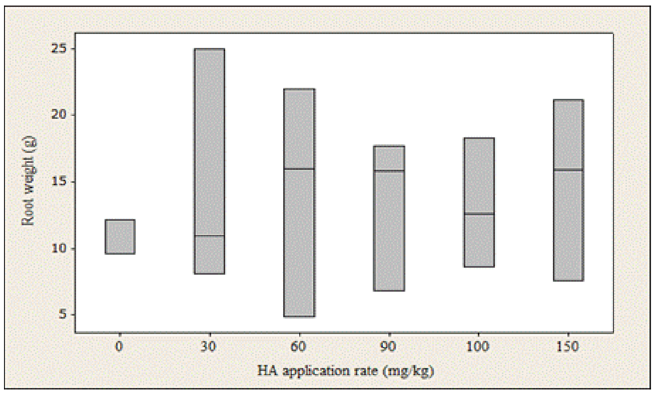

Fig-9:Response of root weight to HA application

2.2.3 ***Fruit, flower chlorophyll contents and plant P and K concentration***

Results suggest that HA application results in improving chlorophyll contents both fruit and flower in pepper plant (Fig 10; Fig 11). Lightbourn et al., (2006)[26] reported significance of fruit chlorophyll contents by linking it to fruit quality characteristics. General green appearance of pepper fruit likely to influence consumer acceptance and market value. The plant P concentration increased as HA acid application increased (Fig 12) however remained statistically non-significant till HA application up to 100 mg/kg and then statistically significant from rest of application levels by HA application at 150 mg/kg. The plant K concentration also significantly responded to HA applied at different rates (Fig 13). Maximum K concentration of around 3% was obtained by HA application at the highest rate of 150 kg/ha.

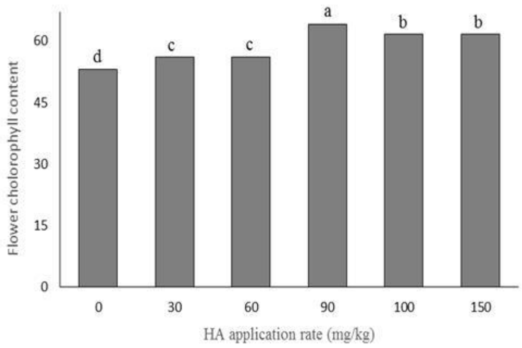

Fig-10: Flower chlorophyll contents as affected by rate of HA application

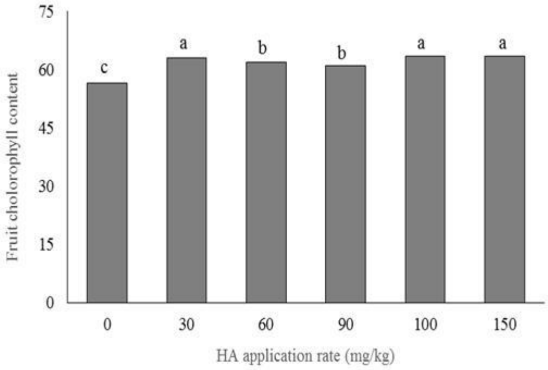

Fig-11: Fruit chlorophyll contents as affected by rate of HA application

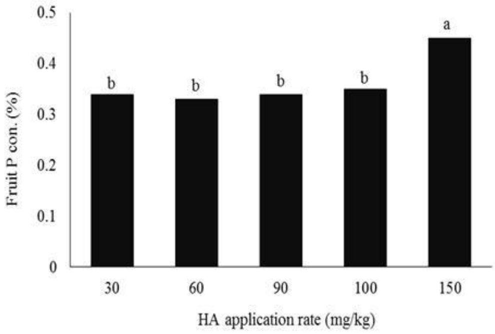

Fig-12: Effect of different rate of HA on P concentration in fruit

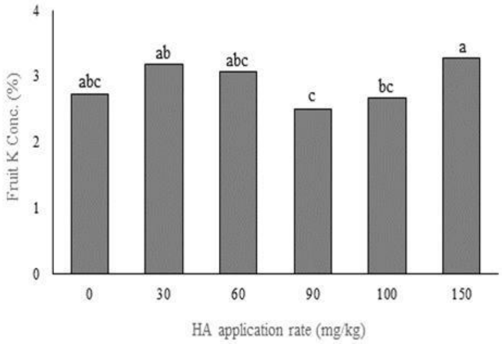

Fig-13: Effect of different rate of HA on K concentration in fruit

3. EXPERIMENTAL

3.1 ***Humic acid extraction from plant based waste materials (Maize, Sunflower)***

The HA was extracted from waste plant materials; maize (*Zea mays*. L) and sunflower (*Helianthus annuas*. L), soaked separately overnight into 2000 ml KOH solution (0.1 N) (Fig 1); stirred, sieved (Mesh No 115) and diluted to 2000 ml (with distilled water), washed again, followed by sieving and then stored into label container (Fig 2). The pH of HA was adjusted (7-8) with conc. H_2SO_4. The elemental analysis showed that HA contains 0.032 % N (kjeldahl), 0.89 % P (spectrophotometer method), 2. 12 % K (flame photometer), 0.41 mg/kg Fe and 0.45 mg/kg Zn (atomic absorption spectrophotometer).

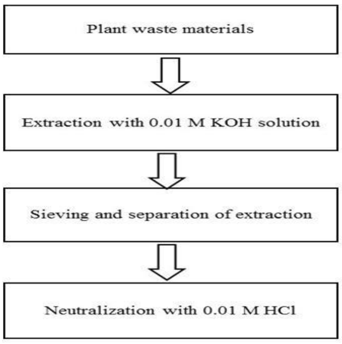

Fig-1: Schematic diagram showing extraction of HA from plant waste materials

Fig-2: Humic acid extraction from waste plant materials

Agronomic data pertaining to fresh fruit weight, leaf weight, root length and weight at harvest stage. Each fruit picking was carried out as the fruit get mature and noted for fresh weight.

3.2 ***Setting up trial***

A pot experiment was conducted at Land Resources Research Institute (LRRI), National Agricultural Research Centre (NARC) Islamabad under glasshouse conditions to evaluate the effects of different plant derived HA on the growth and nutrient uptake of hybrid pepper. Five levels of HA (control no HA applied), 30 mg/kg, 60 mg/kg, 90 mg/kg 100 mg/kg and 150 mg/kg along with basal dose of NPK applied as urea, DAP and KCl respectively. Pepper seedlings collected from vegetable program were transplanted into plastic pots having 6 kg of Rawal soil series having detailed chemical properties are listed (Table 1)[24]. The soil was air-dried and passed through <2 mm sieve after homogenizing. It is well drained, moderately fine texture calcarious soil having brown to dark brown colour.

All experimental pots were arranged in completely randomized (RCB) design, replicated three times. Pots were irrigated periodically to maintain at filed capacity. Plant growth data on biomass weight, root length and number of branches per plant were collected before harvesting at 150^{th} day of the experiment setup.

Plant parts (leave, fruit and shoot) were washed with tap water following by with deionised water, dried at 56-70 ^{o}C; and process for nutrient concentration. Bulk soil sample collected after plant harvest and ground; sieved and used for analysing various nutrients such as P, N and micronutrients following standard analytical procedures.

Table-1: Selected Chemical properties of soil

Parameter	Unit	Value
pH	ds/m	8.0
EC		0.24
$CaCO_3$	%	2.8
Organic matter		1.2
P ($NaHCO_3$)	mg/kg	6.5
K(exchangeable)		84
Zn (DTPA. ext.)		0.38
Cu (DTPA. ext.)		0.92
Fe (DTPA. ext.)		9.8
Mn (DTPA. ext.)		9.6

Source: Rashid et al.(1994)[24]

3.3 ***Chemical Analysis of soil and plant samples***

Leaf and fruit samples were process for wet digestion using mixed acid digestion [(HNO_3: $HCOl_4$ (2:1)], for micronutrients (Mn, Zn, Cu and Zn) analysed by atomic absorption. Potassium (K) and phosphorus (P) were determined in leaf tissues by flame photometer and spectrophotometer (Genyses 5) respectively. Chlorophyll contents in 3^{rd} fully matured leaf and pepper fruit was determined using cholorophyll, SPAD-502 (Spectrum technology, Plainfield, IL, USA).

3.4 ***Characterization of humic substances***

Following available techniques were used to characterize plant derived HA in this study:

3.4.1 ***High performance liquid chromatography (HPLC)***

The 20 µl HA solutions (0.05-0.2 g/l) was injected into Machery Nagel RP C18 column,
(200 mm×4 mm), particle size of 7 µm and 1000 A pore size. Sample of the purified and dialysed HA was dissolved in 5 ml NaOH (0.1 M) using ultrasonic bath for 15 min., diluted and neutralized with 0.1 M HCl (pH 7). All chromatograms were detected in the UV-VIS in 254 and 280 nm range, and the fluorescence emissions were monitored by 470 and 375 nm.

3.4.2 ***Ultraviolet and visible spectroscopy (UV-VIS)***

One mg HA sample was dissolved in 5 ml of 0.1 M KOH to measure optical density at series of wave length (300 nm-600 nm) with UV-VIS spectrophotometer (Perkin Elmer Lamda 3B). All measurements were carried out at 25 C $\pm$ with a constant light path of 1 cm. Optical density was plotted against wave length for HA derived for coal, sunflower and maize derived HA (Fig 3a and Fig 3b). The E_4/E_6 ratio related to absorbance coefficients (E) at 400 and 600 nm designated as E_4 and E_6 respectively. The difference between log of E_4 and E_6 defined as Δlog K (Kumada, 1987)[23] as shown (Equation 1)

$$\Delta \log K = \log K_{400} - \log K_{600} \qquad \text{(Equation 1)}$$

3.5 ***Statistical Analysis***

All data collected on soil and plant parameters were analysed using statistical analysis system (SAS, 9.1.2, SAS Institute, Cary, N.C)[25]. One way analysis of variance (ANOVA) was used for all variables (fresh fruit weight, leaf weight, root length and root weight) to test the HA application rates. Duncan multiple range (DMR) test explained difference in treatments mean. Bar charts and selected diagrams were drawn on Excel 2013 and sigmaplot 9 respectively.

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