



Improving Soil Fertility and Maize Growth in Suboptimal Land Through Application of Humic Acid

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ABSTRACT

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Humic acid (HA) has been reported to increase plant growth and crop yields, as well as improve soil fertility. However, the potential utilization of HA extracted from various organic waste composts as organic amendment in suboptimal soils has not been studied in depth. The experiment used a two-factor Completely Randomized Design (CRD) with three replications. Four types of HA were used, namely bagasse HA (BHA), water hyacinth HA (WHA), market waste HA (MHA), and commercial HA (CHA). It also comprised of four doses HA i.e., 0.05, 0.10, 0.15 and 0.20% (of soil on w/w base). The results revealed that fluctuations in soil pH and nutrient release with the HA application had a variable quadratic response pattern. Organic carbon increased by 17%, while total N and available P decreased by 5% and 38.6% during the last weeks of incubation. The HA application could improve the growth response and nutrient uptake of maize significantly. CHA_{0.20%} was the best interaction treatment which had the highest average value on dry weight and NPK uptake, which were 98.0 g pot⁻¹, 178.8 mg plant⁻¹, 27.4 mg plant⁻¹ and 216.9 mg plant⁻¹, respectively. The scanning electron microscopic (SEM) showed that HA could increase in the length and density of maize root hairs. Furthermore, the HA application significantly increased pH, CEC, C-organic content and availability of soil nutrients.

1. INTRODUCTION

The sustainability of the production system and environments are the main factors in agricultural development in order to realize a country's food sufficiency [1, 2]. Limited land capacity and water resources, widespread degradation and conversion of agricultural land, use of quality seeds that are not yet optimal, and high pest and disease attacks pose a threat to agricultural revitalization. With the availability of productive land resources decreasing, while the need for food continues to increase, then as an alternative option that is expected to increase agricultural productivity is the use of suboptimal lands. Kang et al. [3] stated that suboptimal land is responsible for about 36 percent of the world's agricultural land.

Suboptimal land is land that has low productivity and soil fertility so that it is not able to support plant growth optimally and is not economically profitable, either due to natural causes or due to human activities [4, 5]. Therefore, as an effort to increase land productivity, it is necessary to improve soil fertility and essential nutrient cycles through the addition of organic matter, balanced fertilization, and/ or liming application [6, 7]. Shahid and Al-Shankiti [8] stated that the organic matter addition can increase the efficiency of inorganic fertilizers through positive interactions in improving the quality of soil biological, chemical and physical properties.

Utilization of compost, manure, plant mulch and biochar as sources of organic matter can effectively improve plant physiology and restore soil nutrients [9-11].

Humic acid (HA), a humified organic compound, is a potential natural resource that can be utilized to increase nutrient availability, plant growth and production [12, 13]. This substance is defined as a dispersed colloidal material that is amorphous, brown-black in color, has a relatively high molecular weight with a particle size between 0.01–0.10 µm, and consists of an aromatic carbon skeleton and has functional groups that partially contain oxygen atoms [14]. HA can be extracted from compost, vermicompost, mineral soil, peat, lignite or coal [15-18].

The use of HA, as an environmentally friendly soil amendment, is said to increase the land capacity as a growing medium, while providing nutrient for crops. The indirect effect of HA is associated with physicochemical and biological improvements in soil, including aggregation, aeration, permeability, cation exchange capacity, water and nutrient retention, mineralization of organic matter, as well as population and activity of soil organism [19, 20]. In addition, the role of HA in increasing plant growth and crop yields is directly influenced by the bio stimulant effect of HA on plant improvement which is characterized by structural and physiological changes in plant roots and shoots in increasing the efficiency of nutrient use [21, 22]. Furthermore, HA has

high levels of phenolic acid, which contains auxin-like structures that are effective in increasing enzymatic activity in plant roots and HC-ATPase activity [23]. Nardi et al. [24] added that this compound can increase the rate of photosynthesis and plant respiration, reduce the rate of transpiration, and increase protein synthesis and hormonal activity in plants. The HA application can increase plant tolerance to environmental stressors (such as heavy metals, salinity, drought), including suppressing soil-borne disease infections in root plants [25-28].

Therefore, in order to increase the carrying capacity of suboptimal land for improving plant productivity with a slight negative effect on the environment, the use of HA as a natural soil amendment needs to be studied more deeply. In this study, a greenhouse experiment was conducted to evaluate the effectiveness of HA on soil nutrient release and plant nutrient uptake, as well as its effect on plant physiology and growth of hybrid maize. Another objective of this study was to compare four types of HA with different sources while at the same time getting the best dose of HA application.

2. MATERIALS AND METHODS

2.1 Humic acid preparation

The humic acid used was the result of the compost extraction from bagasse (by-products) of the Kebon Agung sugar mill, water hyacinth biomass in the Selorejo reservoir, and organic waste of the Karangpulo market located in Malang regency, East Java province, Indonesia. HA extraction method was used a modification of Stevenson [29] especially using NaOH as a base solution to extract organic waste compost (alkali extraction method).

The procedure started by weighing 100 grams of compost, then put into a centrifuge bottle and 1,000 mL of 0.1 N NaOH solution was added, and then shaken for 24 hours. After being allowed to stand for a while, the filtrate was separated from the precipitate by centrifugation (at 6,000–10,000 rpm) for 20 minutes and filtered with Whatman 42 paper. Extraction was carried out again on the precipitate obtained in the previous step. The precipitate obtained was known as the humin fraction. Furthermore, the filtrate was acidified with concentrated H_2SO_4 until it reached pH 2.0 and shaken for 2 hours at 130 rpm, then allowed to stand for 24 hours. At this stage, the HA will precipitate. The filtrate was separated from the precipitate by centrifugation (at 6,000–10,000 rpm) for 15 minutes and filtered with Whatman 42 paper. The filtrate obtained called as the fulvic acid (FA) fraction. The precipitate obtained was dried in a freezer-dryer or oven-dryer at a temperature of 40°C for 2x24 hours, to remove the water content before determining the HA functional group using an infrared spectrophotometer.

In addition to HA derived from compost, commercial HA derived from Leonardite was also used as a comparison.

As additional information, differences in types of HA compost were not significantly affected to the content of functional groups. However, the highest yield and C-humic content of HA compost was found in the bagasse compost (7.01% and 0.26%, respectively). Another characteristic of HA compost that used for this research consisted of having infrared spectrum at wave numbers 2927,94-2941,44 cm^{-1} (for aliphatic C-H stretching for $-\text{CH}_2$, $-\text{CH}_3$), 1602.85-1614.42 cm^{-1} (for aromatic C-C groups); 1508.33-1512.19 cm^{-1} (for

COO-symmetric stretching or N-H deformation and $-\text{C}=\text{N}$ stretching), 1452.5 cm^{-1} (for aliphatic C-H groups), and 694.37-819.75 cm^{-1} (for C-H surface deformation and vibration).

2.2 Soil preparation

The soil for the pot experiment was obtained from a field at research farm of Politeknik Pembangunan Pertanian Malang, East Java, Indonesia. Soil texture is clay loam and is classified as Typic Dystrudepts (Soil Survey Staff, USDA, 2014). Based on the physicochemical characteristics of the soil, it showed that the experimental soil was very low C-organic content and deficient in N and P nutrition (Indonesian Soil Research Institute, 2012). The soil having pH 5.97; C-organic 0.70%; total N 0.09%; P-Olsen 9.50 mg kg^{-1} ; total P 98.70 mg 100g^{-1} ; CEC 24.58 $\text{cmol}(+) \text{kg}^{-1}$; extractable K, Ca, Mg and Na 0.36, 6.98, 1.76 and 0.61 $\text{cmol}(+) \text{kg}^{-1}$, respectively. In addition, the soil bulk density was 1.25 g cm^{-1} and the volumetric water content under the field capacity (θ_{2.0}) and permanent wilting point (θ_{4.2}) conditions were 0.42 and 0.29 $\text{cm}^3 \text{cm}^{-3}$, respectively.

For the experiment, bulk surface soil samples (0–20 cm) were collected using a composite soil sampling technique. Then the soil was air-dried and ground to pass through a 2 mm sieve.

2.3 Incubation setup

The pot incubation was carried out at the soil laboratory, Politeknik Pembangunan Pertanian Malang-Indonesia, to observe the effect of various sources and doses of HA application on soil nutrient release to support nutrient recovery and soil chemical properties in suboptimal land. The experiment used a two-factorial completely randomized design (CRD), which each combination treatment with three replicates. The first factor used four sources of HA, namely bagasse humic acid (BHA), water hyacinth humic acid (WHA), market organic waste humic acid (MHA) and commercial humic acid (CHA). And followed by four doses of HA, namely 0.05, 0.10, 0.15 and 0.20% (of soil on w/w base). Before applied to the soil, HA was buffered at a pH of 7.0 (neutral).

In the incubation experiment, air-dried soil sample that passed through a 0.2 mm sieve was mixed evenly with HA based on the treatment. Then it was put into pots, where each pot contains 500 grams of soil, and incubated for eight weeks. During the incubation periods, the soil moisture content was maintained at 80% of the field capacity condition, and the soil temperature was in the range of 25–30°C. Parameters determined in this stage were soil pH using digital pH meter (Ohaus type Starter 3100), C-organic (%) by Walkley and Black method, total N (%) by micro Kjeldahl method, and the availability of P nutrient (mg kg^{-1}) by wet oxidation was using spectrophotometry. Furthermore, observations were carried out periodically every two weeks.

2.4 Greenhouse study

The pot experiment was conducted in a greenhouse at Politeknik Pembangunan Pertanian Malang, to evaluate the potential of HA for improving plant growth and nutrient uptake of hybrid maize in suboptimal land. Meanwhile, the external environmental conditions have a monthly rainfall average of 11–589 mm, an average daily temperature of 22.5–

26.2°C, and average relative humidity of 66–95%. As with the incubation experiment, the calculated amount of HA according to the prescribed dose was thoroughly mixed with the air-dried soil of each pot, separately. Pots were filled with 18 kg soil passed through 2 mm sieve. The experiments were laid out in CRD with three replications of each treatment. After one week of HA application, two pre-soaked healthy seeds of maize hybrid (BISI 2) were planted \pm 4 cm deep in each pot. Thinning of seedlings into one plant pot⁻¹ was done after a week (three leaf stages). Furthermore, basic fertilization was given according to the recommended doses of N, P, and K for maize, namely 300, 175 and 100 kg ha⁻¹, respectively were applied in the form of Urea, SP-36 and KCl [30]. Pots were irrigated using de-ionized water to maintain soil moisture during whole experimental period. Other agronomic practices were carried out as needed. Maize was harvested after 56 days of growth initiation (tasseling stage) or at eight weeks after planting (WAP). This stage takes place when the plant reaches its full height and begins to shed its pollen [31].

2.5 Plant and soil measurements

The plant parameters measured just before harvesting were plant height, number of leaves, and stem diameter using a ruler and digital caliper. Meanwhile, root length and plant fresh weight were measured directly after harvesting using an analytical balance (Matrix type AJ1002B). Furthermore, the third fully expanded leaf from each plant was selected to determine chlorophyll content using spectrophotometry (a destructive method) [32]. The plant samples were air-dried and oven-dried at 65°C for 2x24 hours. After reaching a constant weight, the plant dry weight was recorded.

The oven-dried shoot samples were finely ground with chamber and stainless-steel blades. Plant tissue analysis was carried out to determine nutrient uptake by each sample as the effect of HA treatment. Nitrogen was determined by the wet oxidation method with H₂SO₄ using a Kjeldahl distillation apparatus, while phosphorus and potassium were determined by the wet oxidation method with HNO₃ and HClO₄ using a UV-visible spectrophotometer and flame photometer. Total uptake of N, P and K was calculated separately for each nutrient by using the Eq. (1):

$$\text{Nutrient uptake} = \frac{(\text{NC} \times \text{DM})}{100} \text{ mg} \quad (1)$$

where, *NC* is nutrient concentration (%); *DM* is dry matter (mg plant⁻¹).

Subsequently, the air-dried root samples were randomly selected for electron microscopic examination [33]. Observation of root samples using scanning electron microscopic (SEM) to compare the morphology and density of root hairs in the treatment with and without HA. The density of root hairs was determined visually from each root segment observed with a microscope.

Post-harvest soil analysis began with collecting soil samples from each pot. After air-drying, the soil sample was ground and filtered using a 0.2 mm sieve. The chemical properties analyzed included soil pH, C-organic using the Walkley and Black method, total N using the micro-Kjeldahl method, available P using spectrophotometry, exchangeable K and Na using flame photometry, Ca and Mg by wet oxidation method using AAS, and also CEC by ammonium acetate compulsory displacement method.

2.6 Statistical analysis

Data were analyzed using Variance Analysis Method (ANOVA). The post hoc tests were carried out using methods Duncan's Multiple Range Test (DMRT) at the 95% confidence level ($\alpha=0.05$).

3. RESULTS AND DISCUSSION

3.1 Pattern of soil pH and nutrients release by application of humic acid during incubation period

The effect of HA application from various sources to soil pH at each dose showed the same pattern, which tended to decrease until the fourth to sixth week, and increase again at the end of incubation period slowly (Figure 1). The average pH value was in the range of 5.75 to 6.00 (slightly acid) at the end of the incubation period. Based on the type, CHA had a higher pH value than other types at each given dose. At low doses, pH changes tend to be slower, so that the minimum pH value was achieved longer. Meanwhile at high doses, changes in pH tend to be faster, so that the minimum value was achieved more quickly. Zaremanesh et al. [34] stated that the addition of HA into the soil caused the exchange of [H⁺] with soil colloids, where their position was replaced by single and/or multiple cations, causing a decrease in soil pH. However, along with the increase in the dose of HA and the length of the incubation period, there was an increase in the affinity of the [OH⁻] originating from the carboxylic group (–COOH) and phenolic compounds (–OH). Furthermore, [OH⁻] will neutralize [H⁺] in the soil solution or adsorbed, so that the concentration of exchangeable [H⁺] decrease and pH can increase. In addition, based on the value of the determination coefficient (R²), a fairly high number was obtained which indicated that the addition of HA to soil pH was influenced by incubation time.

Other external factors that may affect the fluctuation of soil pH value include humidity, temperature, texture, buffering capacity and CEC of soil. The effect of temperature and water content indirectly affects the activity of microorganisms and the decomposition process of soil organic matter. Furthermore, along with the increase in soil organic matter, the natural buffering capacity and CEC of the soil also increases, resulting in an increase in pH in the soil which tends to be acidic through the mechanism of releasing carboxyl and hydroxyl groups in humus [35]. Likewise, with soil texture, the indirect effect is related to the content of clay and organic matter in the soil, where clay soils tend to be more resistant to changes in pH than sandy soils because they have a higher buffer capacity.

As with soil pH, the application of various sources and doses of HA to the soil organic carbon content showed the same pattern (except the 0.05% dose), which decreased up to fourth weeks, then increased in the sixth week of incubation (Figure 2). The percentage decrease in C-organic content in the initial phase and an increase in the final phase reached 29.1% and 17.1%, respectively. The average value of C-organic at the end of the incubation period ranged from 0.72 to 1.32%, where the highest content was found in the CHA_{0.10%} treatment. The decrease in organic carbon at the beginning can be caused by the activity of microorganisms in the soil that utilize organic carbon or other substances as substrates in their metabolic processes and release CO₂ and H₂O [36]. However, in general, with increasing doses of HA given, the soil C-organic content tended to increase. This is because carbon is

the main constituent of all organic materials, including the HA. Gaffney et al. [37] stated that humic substances contain 40–60% carbon, 30–50% oxygen, 4–5% hydrogen, 1–4% nitrogen, 1–2% sulfur, and 0–0.3% phosphorus. Added by

Ahmad et al. [38], humic substances (HA and FA) compose 65–70% of soil organic matter. Based on the R^2 value obtained, it showed that the addition of various sources and doses of HA to soil C-organic was affected by incubation time.

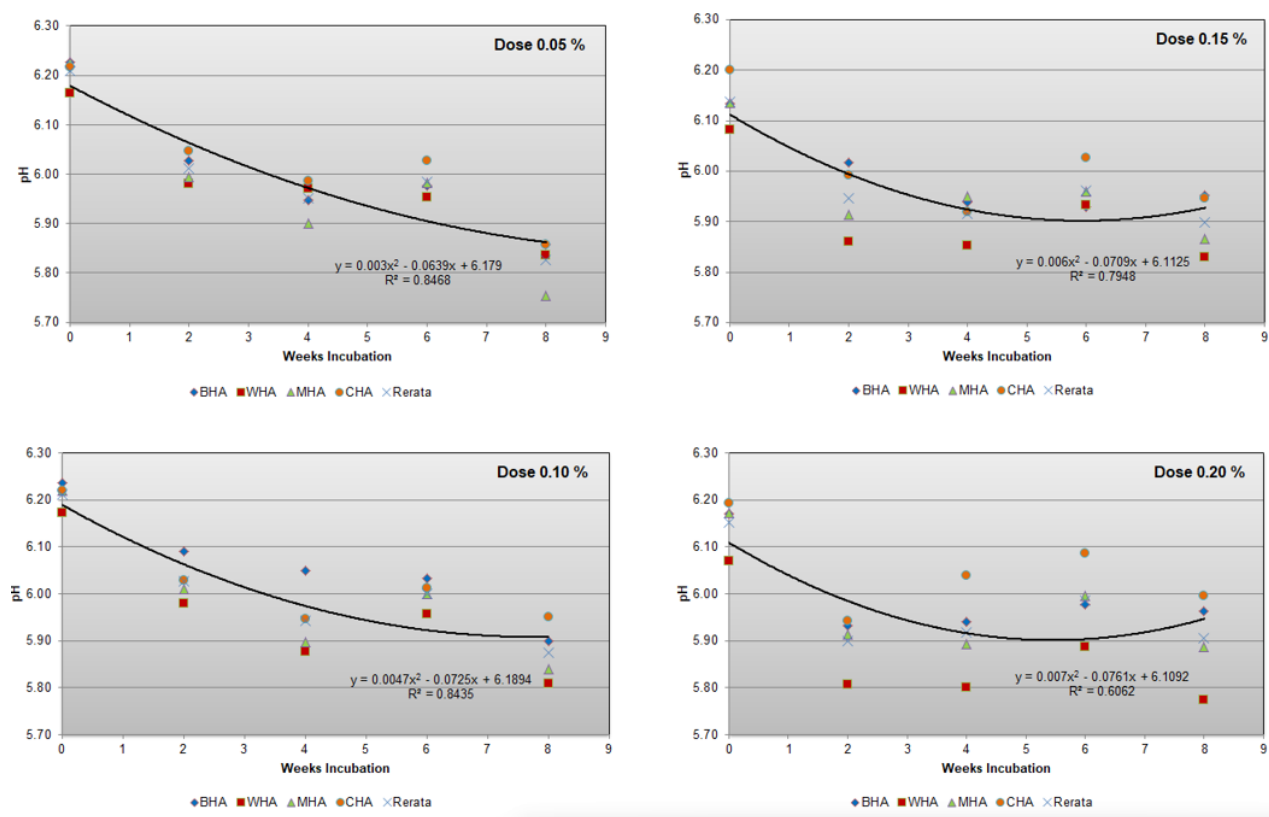


Figure 1. Effect of various sources and doses of humic acid on soil pH fluctuation under incubation periods

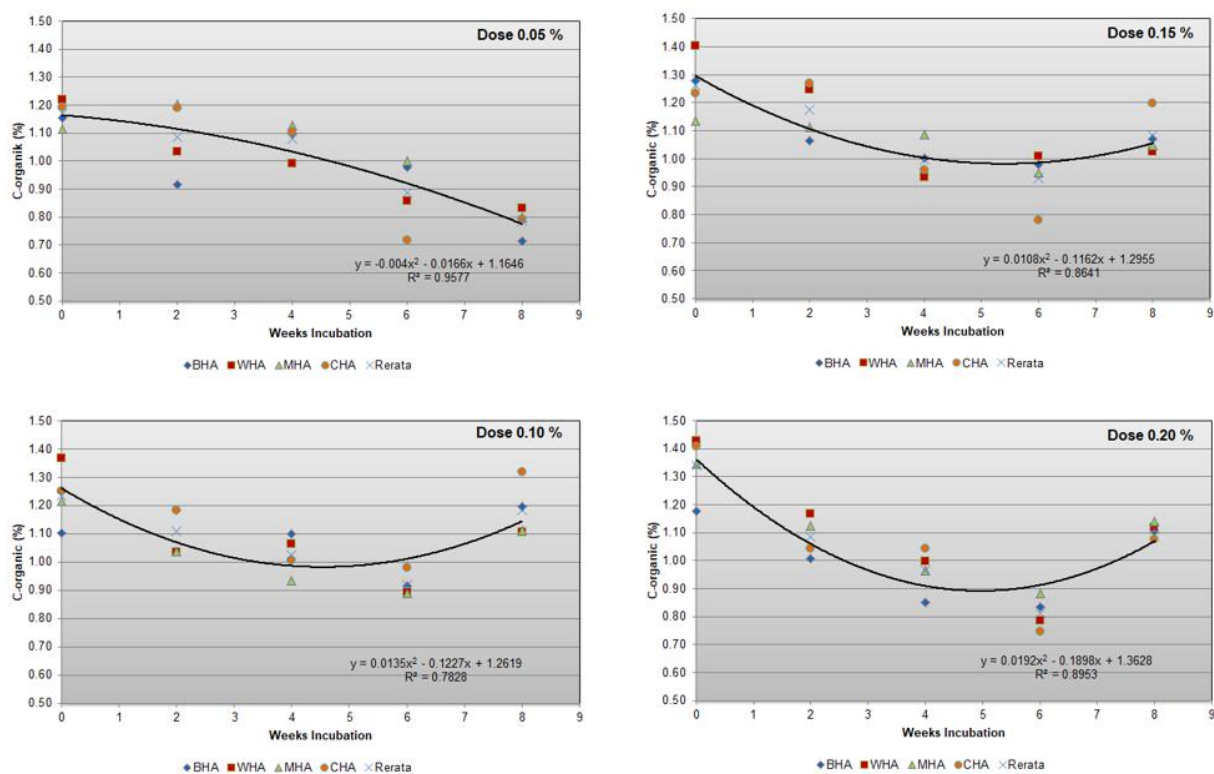


Figure 2. Effect of various sources and doses of humic acid on C-organic content fluctuation under incubation periods

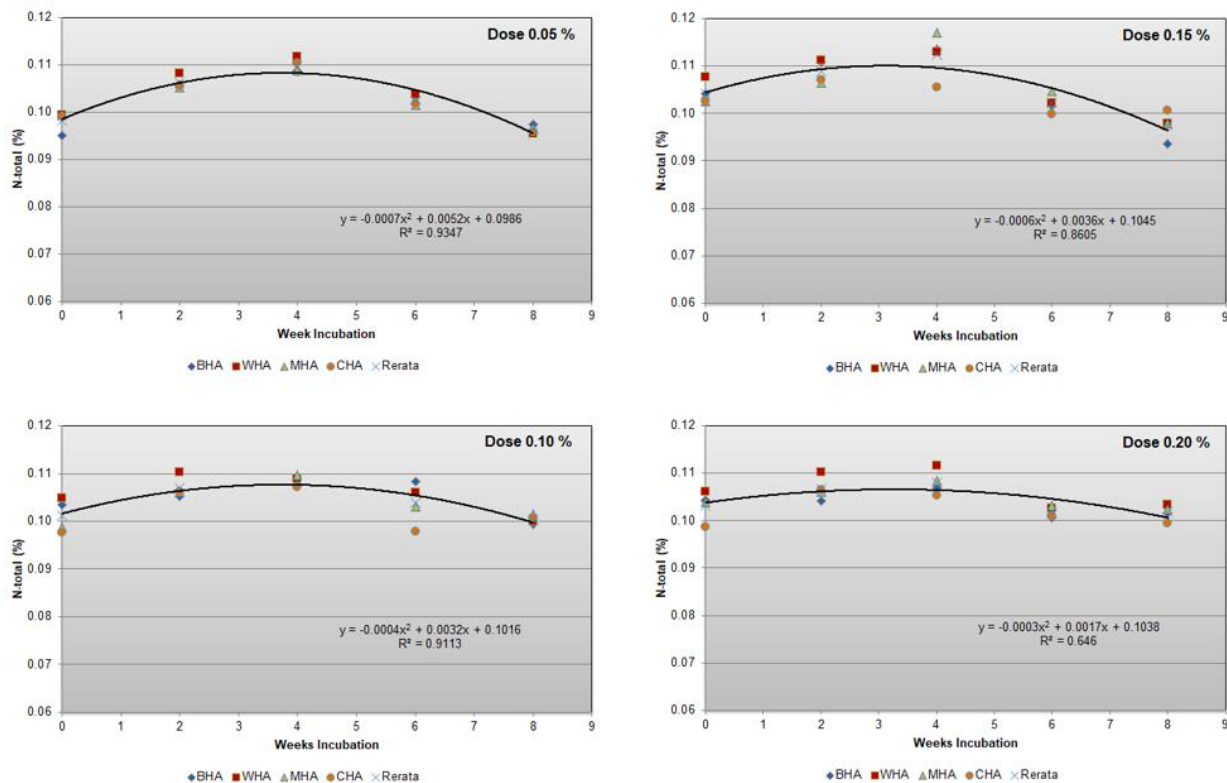


Figure 3. Effect of various sources and doses of humic acid on total Nitrogen fluctuation under incubation periods

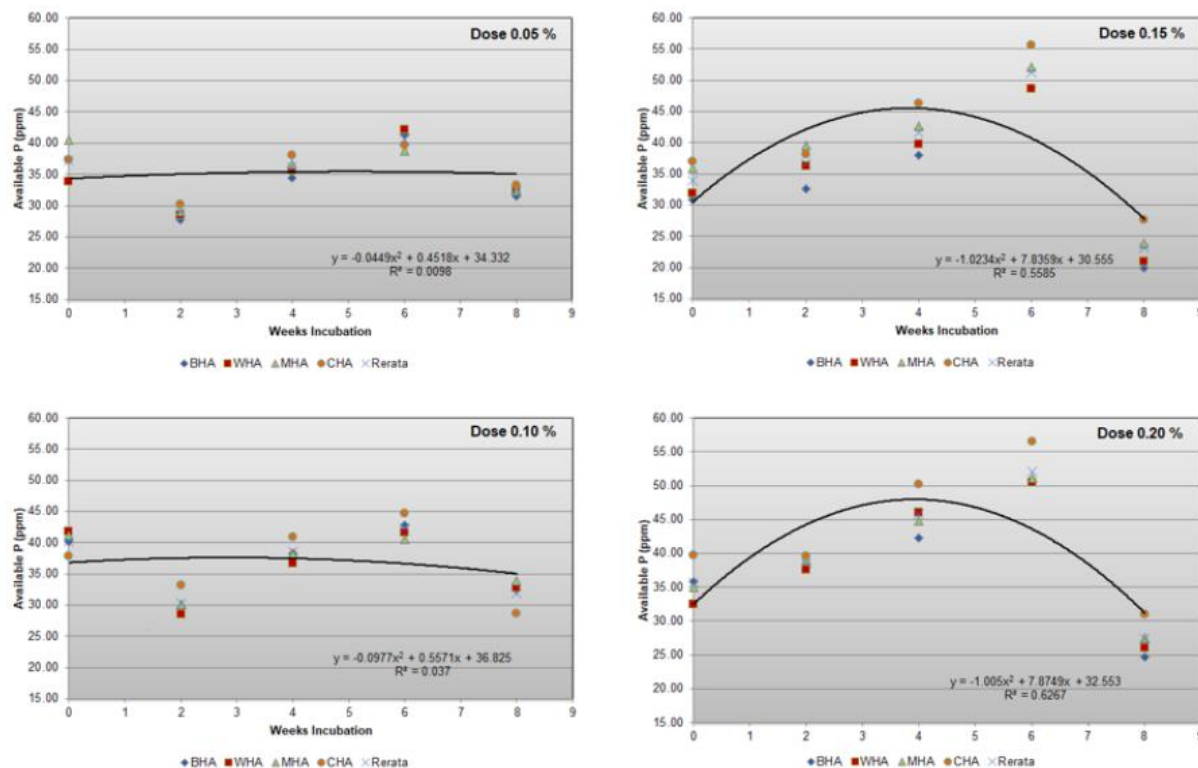


Figure 4. Effect of various sources and doses of humic acid on available Phosphorous fluctuation under incubation periods

The concentration of total nitrogen released during the incubation period was presented in Figure 3. At each dose, all HA treatments had the same trend, namely increasing at the beginning, and tending to decrease after the fourth week to the end of the incubation period. The percentage decrease in total N in all treatments reached 5.4%, while the increase reached 6.4% during four weeks of incubation. The average total N

released at the end of the incubation period was 0.09–0.10%, where the values obtained were not significantly different between HA treatments. The short time span so that these nutrients are available in maximum quantities can be used as a consideration in determining the right time to apply N fertilizer according to the needs of the plant growth stage. The increase in total N at the beginning indicates that organic compounds

release their nutrients gradually through the mineralization process of organic N (amino acids) into inorganic N (ammonium and nitrate) by proteolytic enzymes [39]. However, according to Al-bataina et al. [40] there are other factors that can affect the release of N, namely the content of polyphenols in organic compounds which are considered as immobile N forms. This is related to the toxicity of polyphenolic compounds to microorganisms that play a role in the nitrogen mineralization process of organic compounds, as well as their strong affinity for amide groups and their high protein binding capacity, so that the total N measured decreases. In addition, the use of nutrients by microorganisms to synthesize biomass can also reduce the availability of these nutrients in the soil [41, 42]. In line with our results, Niedzinski et al. [43] found that the percentage of total N released by organic fertilizers from poultry manure and fungal substrates was only 20–32% and 15–20% during an incubation period of 35 days, which was lower than inorganic fertilizers (DAP-diammonium phosphate) which reaches 70%. Furthermore, based on the R^2 value, the total N release pattern in each HA treatment showed the effect of incubation time.

In addition to N, the concentration of available phosphorus released during the incubation period was presented in Figure 4. Based on the pattern of available P release, the highest concentration occurred in the fourth to sixth weeks, which was in the range of 33.43–56.47 mg kg⁻¹, and tended to decrease at the late incubation period, which reached 19.87–33.97 mg kg⁻¹. The graph also showed that the highest available P mean

value was found in the CHA treatment at each given dose. While the available P concentrations were known to increase with increasing doses given to each type. The high value of released P could be caused by the ability of HA to increase soil pH and decrease soil P adsorption capacity [44]. A similar trend was also found by AyanfeOluwa et al. [45] that the highest increase in available P during the incubation period occurred at the sixth to eighth weeks in Alfisols and at the sixth to tenth weeks in Ultisols with 60 kg N ha⁻¹ of conventional compost added, and then decreased until the end of the incubation period. Based on the R^2 value, the pattern of available P release indicates the influence of incubation time (except at a dose of 0.05 and 0.10%).

There are several other factors that may influence fluctuations in the availability of P nutrients in the soil include: (1) soil texture; soil that has a high clay content, has greater P retention power, (2) types of clay minerals; kaolinite clay which contains Fe and Al oxides, fixes P higher than montmorillonite and vermiculite clays, (3) soil pH; on acid soil, P are fixed by free Fe and Al or oxyhydroxides, whereas in alkaline soil, P fixed by Ca or Mg ions into insoluble form. In addition, soil pH affects the form of P availability in the soil solution, where at low pH the dominant form of HPO₄²⁻, while at high pH the dominant form of H₂PO₄⁻, (4) soil moisture; high soil water content can increase the solubility of available P and does not limit movement of P towards the root zone to be absorbed by plants, (5) total soil P content; soils containing very high P tend to release P into the soil solution [46–48].

Table 1. Effect of various sources and doses of humic acid on physiological and agronomical attributes of maize hybrid at eight weeks after planting (WAP)

Treat-ment	Plant height (cm)		Leaves number		Stem diameter (cm)		Root length (cm)		Total Chlorophyll (mg mL ⁻¹)	
<i>Type</i>										
BHA	134.5	a	13	a	1.4	ab	76.3	b	3.2	a
WHA	141.2	b	13	a	1.4	ab	69.3	a	3.5	b
MHA	136.9	ab	13	a	1.4	a	77.7	b	3.5	ab
CHA	150.6	c	13	a	1.5	b	80.2	b	3.3	ab
<i>Doses</i>										
0.05%	136.7	b	13	a	1.4	ab	69.8	a	3.6	b
0.10%	128.6	a	13	a	1.4	a	74.1	b	3.3	a
0.15%	147.6	c	13	a	1.4	b	81.6	c	3.3	a
0.20%	150.2	c	14	b	1.6	b	77.9	c	3.3	a
<i>T x D</i>										
BHA _{0.05%}	126.0	a	12	a	1.3	ab	63.0	a	3.3	abc
WHA _{0.05%}	128.2	a	12	a	1.3	ab	66.3	ab	3.4	abcd
MHA _{0.05%}	136.7	abc	13	ab	1.3	ab	81.0	cd	3.9	d
CHA _{0.05%}	156.0	def	13	abc	1.6	bcd	69.0	b	3.8	cd
BHA _{0.10%}	128.0	a	13	ab	1.3	a	61.7	a	3.3	abcd
WHA _{0.10%}	128.3	a	13	abc	1.3	ab	64.3	ab	3.4	abcd
MHA _{0.10%}	128.8	a	13	abc	1.4	abc	78.7	c	3.4	abcd
CHA _{0.10%}	129.3	a	13	ab	1.4	abcd	91.7	e	3.0	a
BHA _{0.15%}	148.8	cd	13	abc	1.7	bcd	84.3	d	3.2	ab
WHA _{0.15%}	146.0	bcd	13	ab	1.4	ab	81.7	cd	3.7	bcd
MHA _{0.15%}	130.2	a	13	ab	1.3	ab	86.0	d	3.0	a
CHA _{0.15%}	165.3	f	13	abc	1.7	cd	64.7	ab	3.2	ab
BHA _{0.20%}	135.0	ab	14	bc	1.5	abcd	96.3	e	3.0	a
WHA _{0.20%}	162.3	ef	14	bc	1.8	d	64.7	ab	3.5	abcd
MHA _{0.20%}	151.7	de	14	c	1.5	abcd	65.0	ab	3.5	abcd
CHA _{0.20%}	151.7	de	14	c	1.5	abcd	85.7	d	3.0	a
Means	140.8		13		1.5		75.9		3.4	
CV (%)	6.3		4.7		13		4.1		9.2	

Note: The number displayed is the average value; Numbers followed by the same letters in the same column showed no significant differences based on the DMRT Test at $\alpha=0.05$

3.2 Response of growth and nutrient uptake of maize in greenhouse

The effect of HA application from various sources and doses to suboptimal soil on physiological and agromorphological aspects of hybrid maize was performed in Table 1.

Based on the analysis of variance (ANOVA), it was known that interactions between source and dose of the HA application was significantly ($p \leq 0.05$) affected to plant height, leaf number, stem diameter, length roots, as well as the total chlorophyll content of maize. Among the treatments, the average optimum plant height was obtained in the CHA, both at doses of 0.15 and 0.20%, where the highest value of 165.3 cm was found in the CHA_{0.15%}. Furthermore, the average number of leaves reached 12 to 14 strands plant⁻¹, with the highest number found at MHA_{0.20%} and CHA_{0.20%}. The increasing in the dose of HA affected the increase in the number of leaves, although the types of treatment were not significantly different. Meanwhile, the highest average stem diameter and root length of 1.8 cm and 96.3 cm were found in WHA_{0.20%} and BHA_{0.20%}, respectively. Similar results were also obtained by Aziz et al. [49] which states that the addition of HA at a dose of 4 kg ha⁻¹ can increase maize growth more optimally with an average plant height 127.1 cm, leaves number 15.5 strands and stem girth 10.2 cm (Ø3.2 cm).

Additionally, the results showed that the highest total chlorophyll content was found in the MHA_{0.05%}, which was 3.88 mg mL⁻¹. Chlorophyll is an important photosynthetic pigment for plants, and greatly determines the photosynthetic capacity. According to Meganid et al. [50], the addition of HA can increase the chlorophyll content and leaf area. It was further explained that humic substances could increase chlorophyll synthesis and/or delay the degradation of chlorophyll in leaves, as well as facilitate the process of respiration and photosynthesis through modification of mitochondrial and chloroplast functions [51].

Improvements in the physiological and growth characteristics of maize were assumed to be a positive response of plants to the use of HA. It can be seen that statistically the difference in the dose given significantly affects the observed parameters, and numerically shows that the higher the dose given, the higher the value obtained (except for the total leaf chlorophyll). Trevisan et al. [52] stated that these organic compounds interact with root cells which in turn affect plant physiology and growth. It was further explained that it is possible that in the structure of humic substances there are hormone-like substances, which are directly involved in photosynthesis, respiration, protein synthesis, and other enzymatic reactions [53]. Application of HA into the soil can also increase the population and activity of microorganisms, as well as improve the rhizosphere, which is accelerated by the presence of root exudates [54, 55]. This allows plant roots to explore a wider volume of soil, while supporting plant adaptation to suboptimal soil conditions that have low nutrient content and lack of water. Stimulation of root hairs and increased root initiation by HA can optimize soil nutrient uptake which in turn improves plant growth characteristics, and increases the role of roots as anchoring plants [56, 57]. Furthermore, based on SEM micrograph results (Figure 5), the HA application showed an increase in the length and density of root hairs compared to the control (no HA). Schmidt et al. [58] stated that the addition of HA caused various changes in root morphology, such as an increase in the length and density

of root hairs, the formation of ectopic root hairs, and an increase in cell proliferation in the root base tissue. This is because these compounds affect genes in epidermal cells, which specifically play a role in the early stages of root cell differentiation, accompanied by changes in proteins involved in energy metabolism and protein transport [59].

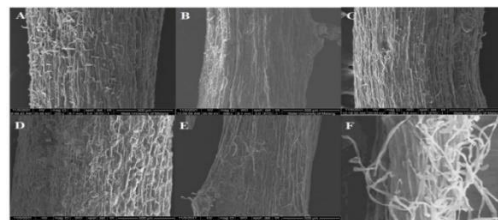


Figure 5. SEM of maize root treated with and without humic acids: (A) control (no HA), (B) with BHA, (C) with WHA, (D) with MHA, and (E) with CHA. Bars 500 µm. (F) another SEM of maize root hair treated with humic-like acids from vermicompost [60]

The response of the HA application to wet weight, dry weight and nutrient uptake of hybrid maize was presented in Table 2. In terms of plant wet and dry weight parameters (shoot and root), the results showed a significant effect of HA addition to those parameters ($p \leq 0.05$). Generally, the highest average wet and dry plant weights were found in CHA at each dose given. The highest average values were obtained at CHA_{0.15%} and CHA_{0.20%}, which were 424.3 and 98.0 gram pot⁻¹ for wet and dry plant weights, respectively. This value was higher than the maize dry weight obtained by Khaled and Fawy [61], which was 20.2 grams pot⁻¹, in the application of humic substances through the soil at a dose of 2 grams kg⁻¹ and 0.0 mM NaCl. This showed that the response of plants to the HA application through the soil was very positive, by increasing the role of roots in the mechanism of nutrient absorption from the soil and nutrients transport to plants. In addition to being a biostimulant, HA has phenolic and quinone groups that interact with enzymes in plant cells and stimulate plant metabolism, thereby promoting growth optimization and increasing crop yields [62].

Along with plant dry weight, it was found that HA application had a significant difference ($p \leq 0.05$) on N, P and K nutrient uptake, with the highest average nutrient uptake found in CHA_{0.20%}, which was 178.8 mg N plant⁻¹, 27.4 mg P plant⁻¹ and 216.9 mg K plant⁻¹, respectively. In general, the increase in nutrient concentration and absorption was directly proportional to the levels of HA given. Besides that, CHA gave the best effect than other types of HA on plant nutrient uptake. According to Palanivell et al. [63], increased nutrient absorption and efficiency of nutrient use by plants is the role of humic substances as nutrient chelators. In addition, this organic compound also has a high cation exchange capacity, allowing for the retention and release of nutrients at the right time for plant needs. It was further explained that HA can increase the synthesis and activity of plasma membrane H⁺-ATPase, an enzyme that converts energy for transmembrane transport of nutrients, then energizes secondary ion transporters and promotes plant nutrient uptake [64].

3.3 Effect of various sources and doses of humic acid on post-harvest soil properties

Statistical analysis showed that the HA application from various sources and doses had a significant effect on pH, CEC,

C-organic content and availability of soil nutrients (Table 3). The results exhibited that the average soil pH was in the range of 5.45 to 6.30 (slightly acid), where these values (except BHA_{0.10%}) had met the prerequisites for maize to grow well, namely pH 5.6-7.5 [65]. As one of the main aspects in determining soil quality, the average C-organic content of the soil tends to increase with the addition of HA dose, with the highest value obtained at CHA_{0.20%} of 1.21%. Similar results with the study [66], the value of C-organic increased significantly as the application level increased for each type of HA used. Furthermore, based on the correlation analysis between plant response and soil characteristics, there was a positive correlation between soil pH and C-organic content with root length, dry weight, and plant N, P and K uptake (namely $r=0.41$, $r=0.48$, $r=0.37$, $r=0.49$, and $r=0.43$ for pH and $r=0.39$, $r=0.674$, $r=0.45$, $r=0.59$, and $r=0.49$ for C-organic; r table 1%=0.37). Application of HA through the soil can also increase CEC, where the highest value was found in the BHA_{0.05%}, which was 21.80 cmol(+) kg⁻¹. Although based on the table it was known that the dose treatment did not show a significant difference. However, different results were obtained by Duong et al. [67] which stated that the increase in CEC was in line with the increase in the dose of organic matter given. This is influenced by the number of binding sites owned by humified organic compounds to bind cations and water. Mindari et al. [68] also added that the CEC HA values derived from compost, coal and peat were quite high, namely 80.72, 104.09 and 116.83 cmol(+) kg⁻¹.

N, P and K are essential nutrients needed in large quantities

by plants, so the availability of these nutrients in the soil is very crucial. The highest average values for total N and exchangeable K were obtained by CHA_{0.20%} at 0.18% and 1.31 cmol(+) kg⁻¹, while the highest mean available P was found in WHA_{0.10%} at 65.0 mg kg⁻¹. This nutrient concentration was higher than the results of research by Arjumend et al. [69] which obtained values of 0.35% N, 5.05 mg P kg⁻¹ and 0.36 cmol(+) K kg⁻¹ in the HA application at a dose of 200 mg kg⁻¹. The increase in soil nutrient status was possibly due to the influence of HA which can inhibit urease activity, so that N loss through leaching and volatilization processes can be reduced [70, 71]. The same thing also happened to P, where the HA application was possible to increase the availability and absorption of these nutrients by reducing the deposition rate of calcium phosphate (Ca-P) or aluminum phosphate (Al-P) because of the potential to form metal bridges with HA and/or FA [72, 73]. Likewise the increase in K, Zhang et al. [74] reported that HA stimulates the binding and release of K in the soil by influencing the surface properties of clay minerals and the reaction of K⁺ with clay minerals. Apart from N, P and K, in this study, exchangeable Ca and Mg were also observed. Based on results, the highest Ca content was found in the BHA_{0.20%} at 8.35 cmol(+) kg⁻¹ and the highest Mg was found in the BHA_{0.15%} at 2.59 cmol(+) kg⁻¹. In general, the addition of HA can improve soil nutrient status when compared between low-dose and higher-dose nutrient status. This showed that HA can be used as a soil amendment, because it was able to improve the fertility of sub-optimal agricultural land and increase plant growth and production.

Table 2. Effect of various sources and doses of humic acid on fresh weight, dry weight and nutrient uptake of maize hybrid at eight weeks after planting (WAP)

Treat ment	Fresh weight		Dry weight		Nutrient Uptake				
	---(g pot ⁻¹)---				N	P	K		
<i>Kind</i>					-----(mg plant ⁻¹)----				
BHA	262.2	a	60.9	ab	126.6	a	14.9	a	119.8
WHA	230.2	c	62.4	b	130.4	a	15.8	a	134.6
MHA	224.6	b	43.0	a	131.9	a	16.1	a	125.3
CHA	349.2	d	93.4	c	163.7	b	20.9	b	154.3
<i>Doses</i>									
0.05%	227.6	b	61.8	b	134.4	a	13.7	a	106.2
0.10%	179.5	a	58.9	a	130.0	a	14.0	a	123.9
0.15%	299.8	c	67.5	c	137.5	a	19.4	b	136.7
0.20%	359.3	d	71.4	d	150.8	a	20.7	b	167.2
<i>K x D</i>									
BHA _{0.05%}	225.0	b	48.0	ab	118.9	a	11.1	a	102.9
WHA _{0.05%}	230.7	bc	48.3	ab	119.3	a	11.6	ab	102.2
MHA _{0.05%}	255.0	d	55.3	b	119.0	a	14.1	abc	101.5
CHA _{0.05%}	403.3	h	95.7	e	180.4	b	18.0	bcd	118.0
BHA _{0.10%}	201.0	a	46.0	a	117.7	a	12.3	ab	113.3
WHA _{0.10%}	245.0	cd	50.0	ab	128.8	a	12.3	ab	111.4
MHA _{0.10%}	233.3	bc	50.3	ab	130.1	a	14.3	abc	123.8
CHA _{0.10%}	255.0	d	63.3	c	142.2	ab	17.2	abcd	134.8
BHA _{0.15%}	292.7	e	72.3	d	128.8	a	19.4	cd	137.0
WHA _{0.15%}	258.3	d	56.0	b	129.7	a	19.4	cd	130.2
MHA _{0.15%}	256.7	d	51.3	ab	137.9	ab	17.6	abcd	132.0
CHA _{0.15%}	424.3	i	97.3	e	153.4	ab	22.4	de	147.5
BHA _{0.20%}	330.0	f	77.3	d	141.1	ab	17.0	abcd	125.9
WHA _{0.20%}	403.3	h	95.3	e	142.6	ab	20.0	cd	182.1
MHA _{0.20%}	356.7	g	72.7	d	140.7	ab	18.2	bcd	144.0
CHA _{0.20%}	347.0	g	98.0	e	178.8	b	27.4	e	216.9
Means	266.5		64.9		138.2		16.9		133.5
CV (%)	3.0		6.3		17.2		20.3		22.5

Note: The number displayed is the average value; Numbers followed by the same letters in the same column showed no significant differences based on the DMRT Test at $\alpha=0.05$

Table 3. Effect of various sources and doses of humic acid on soil properties after harvesting

Treatment	pH H ₂ O	C-org -----%-----	N-total	Av. P mg kg ⁻¹	K -----cmol(+) kg ⁻¹ -----	Ca	Mg	CEC
<i>Kind</i>								
BHA	5.7 a	0.84 a	0.13 a	21.0 a	0.27 a	7.73 b	2.21 b	18.9 b
WHA	5.7 a	0.80 a	0.15 c	35.3 b	0.37 b	6.43 a	1.80 ab	18.5 ab
MHA	5.9 b	0.83 a	0.15 bc	30.5 b	0.59 c	7.04 ab	1.70 a	17.0 a
CHA	6.1 c	1.02 b	0.14 ab	28.4 ab	0.85 d	7.20 ab	1.98 ab	18.8 b
<i>Doses</i>								
0.05%	5.7 a	0.82 a	0.14 a	31.0 b	0.53 a	6.74 a	2.04 ab	18.7 a
0.10%	5.8 b	0.86 ab	0.13 a	28.1 ab	0.46 a	6.87 a	1.65 a	17.6 a
0.15%	5.8 b	0.87 b	0.15 a	29.7 ab	0.49 a	7.35 a	1.93 ab	18.2 a
0.20%	6.0 c	0.96 c	0.16 b	26.4 a	0.59 a	7.43 a	2.07 b	18.6 a
<i>K x D</i>								
BHA _{0.05%}	5.6 ab	0.65 a	0.13 a	18.5 abcd	0.18 a	7.22 b	2.08 abcd	21.8 d
WHA _{0.05%}	5.6 ab	0.79 b	0.14 ab	16.0 abc	0.44 cd	4.32 a	1.80 abc	16.8 ab
MHA _{0.05%}	5.8 bcde	0.88 bcd	0.15 abc	58.5 ef	0.90 e	7.88 b	2.25 bcd	18.1 abcd
CHA _{0.05%}	5.8 bcde	0.94 cde	0.13 a	31.0 cdef	0.61 d	7.57 b	2.03 abcd	18.4 bcd
BHA _{0.10%}	5.5 a	0.81 b	0.14 ab	11.5 a	0.24 ab	7.30 b	1.75 abc	16.7 ab
WHA _{0.10%}	5.8 bcd	0.78 b	0.15 abc	65.0 f	0.52 cd	7.31 b	1.99 abcd	20.9 cd
MHA _{0.10%}	6.1 ef	0.84 bc	0.13 a	13.0 ab	0.51 cd	6.45 ab	1.61 ab	14.4 a
CHA _{0.10%}	6.0 def	1.00 e	0.13 a	23.0 abcde	0.56 cd	6.45 ab	1.62 ab	18.4 bcd
BHA _{0.15%}	5.7 abc	0.96 de	0.13 a	29.0 cdef	0.47 cd	8.06 b	2.59 d	20.1 bcd
WHA _{0.15%}	5.6 abc	0.79 b	0.16 bcd	37.0 ef	0.19 a	7.33 b	1.88 abc	17.5 abc
MHA _{0.15%}	5.9 cde	0.78 b	0.16 bcd	22.5 abcde	0.40 bcd	7.29 b	1.42 a	17.5 abc
CHA _{0.15%}	6.1 ef	1.03 e	0.14 ab	34.5 def	0.91 e	6.82 b	1.85 abc	18.9 bcd
BHA _{0.20%}	6.0 def	0.93 cde	0.14 ab	25.0 abcdef	0.19 b	8.35 b	2.41 cd	16.7 ab
WHA _{0.20%}	5.9 cde	0.86 bcd	0.17 cd	23.0 abcde	0.35 abc	6.78 b	1.91 abcd	18.8 bcd
MHA _{0.20%}	5.7 abcd	0.84 bc	0.16 bcd	28.0 bcdef	0.54 cd	6.55 ab	1.52 a	18.0 abcd
CHA _{0.20%}	6.3 f	1.21 f	0.18 d	29.5 cdef	1.31 f	8.06 b	2.44 cd	20.8 cd
Means	5.8	0.87	0.14	28.8	0.52	7.10	1.92	18.3
CV (%)	2.9	6.4	8.7	13.9	25.6	18.7	18.4	11.0

Note: The number displayed is the average value; Numbers followed by the same letters in the same column showed no significant differences based on the DMRT Test at $\alpha=0.05$

4. CONCLUSIONS

The fluctuations of soil pH and soil nutrient release with application of HA from various sources at various doses suggested the varied patterns, where the curves of soil pH and soil organic carbon tended to decrease at the beginning and then increase until the end of the incubation period. Meanwhile the curves of total N and P available increases at the beginning and then decreases until the end of the incubation period. At low doses, pH changes tend to be slower, while at high doses, changes in pH tend to be faster. Organic carbon content increased by 17%, while total N and available P decreased by 5% and 38.6% during the last weeks of incubation. Generally, with increasing doses of HA given, the soil C-organic content and available P concentrations tended to increase to each type of HA applied.

Based on the obtained results the application of humic acid can significantly improve the growth response and nutrient uptake of maize. Commercial humic acid gave the highest increase in plant height, stem diameter, root length, fresh weight, dry weight, and nutrient uptake of N, P and K compared other derived. Along with the increase in the given dose, the use of humic acid from various sources increased the plant growth and nutrient uptake, except for total chlorophyll content and N uptake. Generally, CHA_{0.20%} was the best interaction treatment which had the highest average value on dry weight and NPK uptake, which were 98.0 g pot⁻¹, 178.8 mg plant⁻¹, 27.4 mg plant⁻¹ and 216.9 mg plant⁻¹, respectively.

The application of humic acid increased pH, CEC, C-organic content and availability of soil nutrients significantly. However, the response of soil nutrient status to each type of

humic acid was different. The highest mean value of pH, C-organic, N and K was found in commercial humic acid treatment (namely 6.30, 1.21%, 0.18% and 1.31 cmol(+) kg⁻¹), the highest of available P was in humic acid from water hyacinth compost (namely 65.0 mg kg⁻¹), while the highest of CEC, Ca and Mg was obtained on humic acid from bagasse compost (namely 21.8 cmol(+) kg⁻¹, 8.35 cmol(+) kg⁻¹ and 2.59 cmol(+) kg⁻¹). Furthermore, in soil pH, C-organic content, total N and Mg, the mean value increased significantly as the dose of HA application was increased. This showed that the use of humic acid on suboptimal land can improve soil fertility and maize growth effectively.

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