Application of humic substances with zeolite as carrier to increase the production of water spinach (*Ipomoea reptans* Poir)

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Abstract. Humic substances, which are stable complex compounds that come from the decomposition of organic matter, can be extracted from organic materials such as weathered coal, compost, and soils. They can improve the physical, chemical, and biological properties of soils and, more importantly, can function as plant growth stimulants. However, when applied in liquid form into the soil, these humic substances are readily leached, so that a binding agent that is used as their carrier is needed to make them stay longer in the soil, so that plants can absorb them. In this regard, zeolite, which is a porous mineral with a high cation exchange capacity (CEC), is a promising material to serve the said purpose. Thus, this study aimed to determine the effects of humic substances and zeolite (used as humic substance carrier) on the soil properties, nutrient uptake, and growth and yield of water spinach. Using a randomized block design with 10 treatments and 2 replications, the treatments consisted of (a) control (b) zeolite at 200 kg/ha (Z20), (c) four treatments with two humic substances (Type A and Type B) administered at dosages of 5 and 10 liters/ha (HA5, HA10, HB5, and HB10), and (d) four treatment combinations of humic substances and zeolite (HA5Z20, HA10Z20, HB5Z20, and HB10Z20). Water spinach (*Ipomoea reptans* Poir) was planted in Latosol soil on plots measuring 3 m x 0.7 m in treatment unit. The results showed that the application of humic substance with zeolite raised the soil CEC and exchangeable Ca and increased the uptake of N, P, K, Ca, Mg, Na, Cu, Mn, Fe, and Zn by the water spinach. The highest production of water spinach was achieved in Treatment HA10Z20 (humic substance at 10 liter/ha mixed with zeolite at 20 kg/liter humic substance), with a yield of almost 2.5 times that of control.

Keywords: Humic substances, Nutrient uptake, Plant production, Zeolite

1. Introduction
The organic matter content of soils in the humid tropics is generally low, and this is due to the decomposition of organic matter at a rate much faster than the natural addition of new organic matter. For example, approximately 73% of all agricultural lands in Indonesia has C-organic content of less than 2% (Las and Setyorini 2010). Thus, adding new organic material, on a regular basis and in sufficient quantities, is needed to accumulate it at a higher and stable level. However, adding organic material is quite difficult for farmers, because its source is increasingly difficult to obtain. For this reason, it is necessary to find a faster way of augmenting organic matter into the soil, like, by adding humic substances, which can be extracted from some natural sources.
A humic substance is a natural organic compound that has the characteristics of yellow to black colour and a high molecular weight (Hayes et al. 1989). Arianto et al. (2011) also reported that zeolites have a high cation exchange capacity (CEC) of 120-180 me/100g, and serves as a good adsorbent, molecular filter, and ion exchanger. Further, it has been found that the application of humic substance can increase the soil cation exchange capacity (CEC), increase C-organic (Anwar and Sudadi 2013), and increase the availability of phosphorus and reduce Al saturation (Busyra 1992). However, when applied into the soil in liquid form, a humic substance is easily leached by rainwater, so that it is easily lost from the plant rooting area. For this reason, a carrier agent is required to slow down the release of humic substance in the soil. One potential material for use as carrier of humic substance is zeolite (Suwardi 2012).

Zeolites are hydrated aluminosicates of minerals that have channels and cavities containing alkali and alkali-earth cations and water molecules. Zeolite has a hollow structure that is able to absorb and hold humic substance, and release it slowly back into the soil solution. It is, therefore, expected that the application of humic substance, mixed with zeolite, will regulate the release of humic substance in the soil. According to Sastiono (2004), a single application of zeolite into the soil can improve soil aggregates, control the release of NH$_4^+$ ions, and maintain soil moisture. In terms of its effect on planted crops, Hermawan and Dewi (2012) have found that the application of humic substance mixed with zeolite could increase the production of corn by 19%, and rice by 13%.

Hence, using water spinach (Ipomoea reptans Poir) as indicator plant, this study aimed to determine the effects of applying a humic substance with zeolite used as carrier on the (1) chemical properties of soils and nutrient uptake by plants and (2) plant growth and yield.

2. Materials and Method
This study was conducted in March to June 2017 at Teaching Farm, Faculty of Agriculture, IPB University. The analysis of soils and plants specimens were conducted at the Laboratory of the Department of Soil Science and Land Resources. The humic substance was extracted from weathered charcoal, and zeolite was taken from Tasikmalaya. Two kinds of humic substances Type A and B were extracted from different sources of charcoal and extraction method. Basic fertilizers used were chicken manure and NPK (15-15-15).

This study used a randomized block experimental design with 10 treatments and 2 blocks as replication. The treatments consisted of control, zeolite at 200 kg/ha (Z20), four combinations of humic substance Type A and B, each with dosage of 5 and 10 liters/ha (HA5, HA10, HB5, and HB10), and four combinations of humic substance and zeolite at 1:20 ratio (HA5Z20, HA10Z20, HB5Z20, and HB10Z20). Data were collected and processed by statistical analysis of variance (ANOVA) method with Duncan’s Multiple Range Test (DMRT) at 5% level of significance. The experimental treatments are summarized in Table 1, as follows:
Table 1. Treatment combinations of humic substance and zeolite.

| Treatment | Humic Substance | Zeolite |
|-----------|-----------------|---------|
|           | liter/ha | ml/plot | kg/ha | g/plot |
| Control   | 0        | 0       | 0      | 0      |
| Z20       | 0        | 0       | 20     | 4.2    |
| HA5       | 5        | 1.05    | 0      | 0      |
| HA10      | 10       | 2.10    | 0      | 0      |
| HB5       | 5        | 1.05    | 0      | 0      |
| HB10      | 10       | 2.10    | 0      | 0      |
| HA5Z20    | 5        | 1.05    | 20     | 4.2    |
| HA10Z20   | 10       | 2.10    | 20     | 4.2    |
| HB5Z20    | 5        | 1.05    | 20     | 4.2    |
| HB10Z20   | 10       | 2.10    | 20     | 4.2    |

Description: HA5, HB5, HA10, HB10 denoting treatment of humic substance type A and B corresponding to 5 and 10 liter/ha, and Z20 = zeolite treatment equivalent to 200 kg/ha.

2.1. Field Experiment
In the experimental area, 2 blocks containing 20 plots were constructed. The size of each plot was 3.0 m x 0.7 m with a distance between plots of 30 cm. Chicken manure was applied at a dose of 3.15 kg/plot (15 tons/ha), while lime obtained from agricultural shop was applied at 420 grams (2 ton/ha) as basic treatments.

One week after basic treatments, water spinach was planted at a spacing of 20 cm x 20 cm at three seeds/hole for each plot. One week after planting, NPK fertilizer (15-15-15) was deposited at a dose of 94.5 g/plot (150 kg/ha), at ± 5 cm from the plant. Simultaneously, the treatments of humic substances with zeolite were done. The humic substance with zeolites was diluted at the ratio of 1 liter humic substance to 20 kg of zeolites, mixed, and then placed into the soil.

For the treatment of humic substance without zeolite, the concentrated liquid humic substance was diluted with water at 100 times dilution, and then poured evenly near the roots of the plants.

The maintenance of the experimental plants consisted of regular weeding, pest and disease control, and watering. Measurements on plant height, leaf length, and number of leaves were carried out at 1, 2, and 3 weeks after planting (WAP1, WAP2, and WAP3, respectively). After 22 days, the plants were harvested, and the weights of the harvested plant were recorded.

2.2. Soil Analysis and Plant Nutrient Absorption
Post-harvesting analysis on pertinent soil parameters such as pH, N-Total, P-available, CEC, and exchangeable bases (Ca, Mg, K, Na) was then undertaken to determine the changes in the chemical properties of the soil specimens.

Plant analysis was done by extracting plant tissues through wet washing using H$_2$SO$_4$ and H$_2$O$_2$, and the extractants were used to determine Na and K by the flame photometer method, while the other elements (Ca, Mg, Fe, Cu, Zn, and Mn) were measured by atomic absorption photometer (AAS) method. N and P contents were also determined. Nutrient uptake rates by the plants were calculated by multiplying the nutrient content with the dry weight of the harvested plants.

3. Results and Discussion

3.1. Effect of Humic Material with Zeolite on Soil Chemical Properties
As shown in Table 2, the application of humic substance with zeolite (as carrier) increased significantly the exchangeable Ca and CEC, but it did not significantly affect the levels of pH, total N, available P, exchangeable K, Na, and Mg in the soil. Like the observation of Dewi et al. (2016), the treatment in this study also tended to exchange more cations of Ca than of K, Na, and Mg. Applying zeolite also significantly increased soil CEC. This finding is consistent with that of Mawardi (2014) that demonstrated that the application of humic substance, either singly/alone or combined with zeolite, can increase the soil CEC.

The combination humic substance-zeolite tended to make the soil less acidic, as compared to control. This rise in soil pH is due to the fact that humic substance and zeolite materials contain bases which are able to neutralize acid cations (H\(^+\), Al\(^{3+}\) and Fe\(^{3+}\)), so that the pH goes up (Dewi et al. 2016). This treatment combination also increased Total-N, when compared to control. This can be attributed to the capacity of the humic substance with zeolite to keep the N in the soil, instead of being washed away by rainwater. Further, the humic substance-zeolite combination tended to raise the available P, as compared to control. This increase can be explained by the fact that the humic substance contain carboxyl and phenolic groups that are able to bind ions, such as Fe and Al, from the soil solution, resulting into a lower concentration of these ions, while available P, in the soil solution, goes up.

Table 2. Effect of humic substance with zeolite on exchangeable bases and CEC of soils.

| Treatment | K\(_{\text{ex}}\) | Na\(_{\text{ex}}\) | Ca\(_{\text{ex}}\) | Mg\(_{\text{ex}}\) | CEC |
|-----------|-----------------|-----------------|-----------------|-----------------|-----|
| Control   | 0.74 a          | 0.42 a          | 12.77 a         | 1.55 a          | 19.81 a |
| Z20       | 0.78 a          | 0.43 a          | 17.86 ab        | 1.36 a          | 22.64 d  |
| HA5       | 0.74 a          | 0.44 a          | 19.69 ab        | 1.51 a          | 21.93 bcd |
| HA10      | 0.78 a          | 0.44 a          | 21.35 b         | 1.56 a          | 21.82 abcd |
| HB5       | 0.74 a          | 0.42 a          | 21.52 b         | 1.42 a          | 20.17 ab  |
| HB10      | 0.78 a          | 0.44 a          | 24.55 b         | 1.46 a          | 21.74 ed  |
| HA5Z20    | 0.87 a          | 0.44 a          | 19.27 ab        | 1.55 a          | 22.55 d   |
| HA10Z20   | 0.85 a          | 0.44 a          | 34.59 c         | 1.51 a          | 22.54 d   |
| HBSZ20    | 0.78 a          | 0.42 a          | 25.10 b         | 1.38 a          | 21.74 abcd |
| HB10Z20   | 0.81 a          | 0.42 a          | 24.92 b         | 1.45 a          | 20.45 abc  |

Note: HA5, HB5, HA10, HB10 = treatment humic substance A and B with dose of 5, 10 liters/ha. Z20 = treatment of zeolite 200 kg/ha. Numbers followed by the same letters in the same column denote no significant effect, according to the 5% DMRT.

3.2. Effect of Humic Substance with Zeolite on Macro- and Micro-Nutrient Content and Uptake by Plant

The application of humic substance with zeolite did not significantly affect the level of macro-nutrients in the water spinach, but it significantly influenced the uptake of macro-nutrients N, P, K, Ca, Mg, K, and Na by the plant (Table 3). This increase in macro-nutrient uptake was manifested by the increased plant yield. The same pattern of results was shown in the micronutrients Fe, Cu, Zn, and Mn (Table 4).
Table 3. Effect of humic substance with zeolite on macro-nutrient uptake in plants

| Treatments  | N (kg/ha) | P (kg/ha) | K (kg/ha) | Ca (kg/ha) | Mg (kg/ha) | Na (kg/ha) |
|-------------|-----------|-----------|-----------|------------|------------|------------|
| Control     | 73.37 a   | 6.28 a    | 48.72 a   | 3.81 a     | 3.20 a     | 26.99 a    |
| Z20         | 123.81 c  | 11.19 bc  | 84.68 bc  | 7.22 ab    | 6.21 bc    | 49.27 c    |
| HA5         | 128.40 c  | 11.17 bc  | 83.31 bc  | 6.72 ab    | 5.93 b     | 46.00 bc   |
| HA10        | 104.16 b  | 9.07 b    | 70.70 ab  | 5.79 ab    | 4.79 ab    | 37.20 b    |
| HB5         | 114.36 bc | 11.16 bc  | 74.33 ab  | 5.68 ab    | 5.57 ab    | 43.98 bc   |
| HB10        | 134.91 c  | 11.77 c   | 87.74 bc  | 6.97 ab    | 6.36 ab    | 45.05 bc   |
| HA5Z20      | 173.37 d  | 17.92 e   | 131.24 d  | 10.90 bc   | 8.80 d     | 66.40 d    |
| HA10Z20     | 265.69 e  | 24.40 f   | 170.19 e  | 14.68 c    | 11.87 e    | 92.74 d    |
| HB5Z20      | 183.22 d  | 18.00 e   | 129.63 d  | 10.59 bc   | 8.98 d     | 70.59 e    |
| HB10Z20     | 175.10 d  | 15.44 d   | 111.93 d  | 9.96 bc    | 7.86 d     | 62.80 d    |

Note: HA5, HB5, HA10, HB10 = treatment humic substance A and B with the dose of 5, 10 liters/ha. Z20 = treatment of zeolite 200 kg/ha. Numbers followed by the same letters in the same column denote no significant effect, according to the 5% DMRT.

Table 4. Effect of humic substance with zeolite on micro-nutrient uptake in plants

| Treatment  | Fe (g/ha) | Zn (g/ha) | Cu (g/ha) | Mn (g/ha) |
|------------|-----------|-----------|-----------|-----------|
| Control    | 2.55 A    | 1.00 a    | 0.15 a    | 4.67 a    |
| Z20        | 5.35 Ab   | 1.71 a    | 0.33 a    | 7.59 bc   |
| HA5        | 4.33 Ab   | 1.64 a    | 0.17 a    | 8.86 bcd  |
| HA10       | 4.19 Ab   | 1.50 a    | 0.26 a    | 7.53 bc   |
| HB5        | 3.80 A    | 1.47 a    | 0.29 a    | 6.81 ab   |
| HB10       | 5.02 Ab   | 1.77 a    | 0.32 a    | 9.36 cd   |
| HA5Z20     | 11.00 Bc  | 2.73 ab   | 0.57 b    | 12.61 e   |
| HA10Z20    | 14.92 C   | 4.94 b    | 0.76 c    | 18.11 f   |
| HB5Z20     | 7.00 Ab   | 3.34 ab   | 0.59 bc   | 11.38 e   |
| HB10Z20    | 8.26 Ab   | 2.79 ab   | 0.50 b    | 9.97 de   |

Note: HA5, HB5, HA10, HB10 = treatment humic substance A and B with the dose of 5, 10 liters/ha. Z20 = treatment of zeolite 200 kg/ha. Numbers followed by the same letters in the same column denote no significant effect, according to the 5% DMRT.

3.3. Effect of Humic Substance with Zeolite on Plant Growth and Yield

Based on the ANOVA results, the application of humic substance with zeolite increased significantly the height and the number of leaves of the water spinach, as compared with the control and with single treatment of humic substance or zeolite (Table 5).
Table 5. Effect of humic substance with zeolite on height and number of leaves of plant

| Treatment     | Plant Height (cm) |  | Number of Leaf |  |  |
|---------------|-------------------|---|----------------|---|---|
|               | WAP 1 | WAP 2 | WAP 3 |  | WAP 1 | WAP 2 | WAP 3 |
| Control       | 5.1   | 11.3  | 22.3  a | 2 | 5 | 9 a |
| Z20           | 5.6   | 12.4  | 24.3  a | 3 | 6 | 11 a |
| HA5           | 5.8   | 11.1  | 24.2  a | 3 | 6 | 11 a |
| HA10          | 5.5   | 11.3  | 23.0  a | 2 | 5 | 10 a |
| HB5           | 5.5   | 12.6  | 26.3  ab | 3 | 5 | 13 b |
| HB10          | 5.5   | 13.4  | 23.9  ab | 3 | 6 | 10 a |
| HA5Z20        | 5.8   | 14.7  | 29.3  d | 2 | 7 | 16 d |
| HA10Z20       | 5.9   | 15.8  | 30.3  d | 3 | 7 | 16 d |
| HBSZ20        | 6.5   | 14.8  | 29.3  d | 3 | 6 | 14 d |
| HB10Z20       | 5.7   | 13.5  | 28.0  cd | 2 | 6 | 15 d |

Notes: HA5, HB5, HA10, HB10 = treatment humic substance A and B with the dose of 5, 10 liters/ha. Z20 = treatment of zeolite 200 kg/ha. WAP = Weeks After Planting. Numbers followed by the same letters in the same column denote no significant effect, according to the 5% DMRT.

The application of humic substance with zeolite significantly increased both plant height and number of leaves (Table 5), which indicated that the plant uptake of nutrient, especially N, considerably rose. It has been a long-recognized phenomenon that nitrogen boosts the vegetative growth of plants by stimulating height growth and the development of new plant tissues (Leiwakabessy et al. 2003). Moreover, the growth mechanism in plants is not activated solely by the improvement in soil nutrient content. Humic substances are also known to contain various compounds such as growth-triggering hormones like auxin and gibberellins (Tan 2003). Likewise, as Gardiner and Miller (2004) have pointed out, there are numerous compounds found in humic substances that can stimulate plant growth, such as vitamins, amino acids, auxin, and indole acetic acid. Zeolites, that may hold such growth compounds, can also contribute similar growth-triggering effects.

Figure 1. Comparison of the growth and vigor of water spinach (a) control, (b) zeolite, (c) humic substance, (d) humic substance with zeolite

Figure 1 above shows the comparative growth and vigor of water spinach when treated with zeolite, humic substance, and humic substance with zeolite, and as compared with control. It is plainly obvious that the humic substance-zeolite treatment produced the best plant growth and vigor effects.
This result corroborates the research findings of Dewi (2012) and Hermawan (2012) that humic substance with zeolite can improve plant growth and vigor as a result of increased N uptake and hormonal activity. In addition, humic substances and zeolite encourage the absorption of more soil nutrient that, ultimately, boosts production yields.

3.4. Effect of Humic Substance with Zeolite on Plant Yield

Figure 2 below demonstrates that treatment with humic substance and zeolite, either singly or in combination, can increase the production of water spinach plants, with the latter (combination treatment) logically producing the more pronounced effect than single (either humic substance only or zeolite only) treatment. The highest production of water spinach was obtained in Treatment HA10Z20 (10 liters of humic substance with 20 kg zeolite/liter humic substance), which yielded almost 2.5 times more water spinach than that of control. This positive result was the direct effect of conducive soil and root conditions favoring the availability of nutrients N, P, K, Mg, Na, Ca, Fe, Zn, Cu, and Mn, and their ready assimilation by plants.

![Figure 2. Effect of humic substance with zeolite on plant yield/production](image)

The beneficial effects of applying humic substances with zeolite on crop production can be explained by various mechanisms. The mechanism of nutrient absorption in the soil begins with the absorption of the organic compounds by plants through their roots, stomata and lenticels. These compounds affect, physiologically, plant growth and various metabolisms that take place during the plant’s life (Trevisan et al. 2010). The significant plant response to the administration of humic material in terms of plant height and leaf length emphasizes its role in cell elongation. Such characteristics are exhibited by the hormones auxin and gibberellins. Therefore, the activity of humic compounds within plant tissues is similar to that of the hormone, so that it can increase plant growth (Trevisan et al. 2010). In addition to its role in the metabolic process, the influence of humic substances is also found in the development of plant roots which are thought to be related to increased plant production (Suwardi 2012).

4. Conclusion

The application of humic substances with zeolite can significantly increase the levels of exchangeable Ca and CEC in the soil. It can also increase significantly the uptake of N, P, K, Ca, Mg, Na, Cu, Mn, Fe, Zn by water spinach. In particular, the best treatment is the combination of humic substance and zeolite at a dosage of 10 liters/ha with zeolites 20 kg/liter humic substance (HA10Z20), that yields almost 2.5 times water spinach.
There is, however, an important point to consider. The result of this study showed that the humic substance A is better than B, which implies that different types of humic substance have different characteristics that, in turn, produce different outcomes. Therefore, an analysis of humic substances is required before applying the best type into the soil.

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