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Combining humic acid with NPK fertilizer improved growth and yield of chili pepper in dry season

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All relevant data are within the paper and its Supporting Information files.

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The authors declare no competing interests.

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Abstract: This research aimed to study the effect of humic acid and NPK fertilizer (15:15:15) on growth and yield of red chili, and to obtain the most suitable composition of humic acid and NPK fertilizer which gave the best growth and yield. The study used a randomized block design with five replications. The treatments tested were the composition of humic acid and NPK fertilizer: 100% humic acid; 75% humic acid + 25% NPK; 50% humic acid + 50% NPK; 25% humic acid + 75% NPK; and 100% NPK. Data on plant growth and yield were processed by Analysis of Variance, and means were compared using Fisher's Least Significant Difference test. In addition, data on plant biochemical and soil chemical parameters were determined compositely by mixing leaves taken from sample plants or soil samples into one homogenous sample. Results showed that there was no significant difference in growth and yield of plants treated with 100% humic acid in comparison with those plants treated with 100% NPK. However, in comparison with 100% humic acid, the application of different ratios of humic acid/NPK increased plant chlorophyll contents by 65% - 82% and total sugar by 28% - 71%. The application of humic acid/NPK increased soil fertility by improving soil pH as well as N, P and K. In the combination of humic acid/NPK, the best growth and yield were obtained with the application of 25% humic acid + 75% NPK fertilizers. Therefore, for the sustainability of chili cultivation, the use of humic acid needs to be accompanied with NPK fertilizers at a reduced amount, along with the increase in the dose of humic acid.

1. Introduction

In Indonesia Chili (*Capsicum annuum* L.) is one of vegetable crops with high economic value due to its large domestic and export demaind and, from time to time, price fluctuations may cause consumer unrest. The national inflation rate is significantly influenced by the increase in chili prices in certain seasons. To control the price fluctuations the Indonesian government is increasing the planting area in rainy season, and controlling the planting area as well as production during dry season.

Increasing production in the dry season normally is managed by application of inorganic fertilizers and use of chemicals to control pests and diseases. However, this practice has a negative impact on agricultural ecosystem and the environment. Additionally, implementing principles of sustainable agriculture such as the application of humic acid, a soil conditioner, has the potential to improve this conventional agricultural practice. Humic acid has a complex molecular structure and capability to stimulate and activate biological and physiological processes in soil organisms.

The study of Abdellatif *et al.* (2017) showed that the application of 14.4 kg ha^{-1} humic acid to chili plants grown under heat stress could increase plant's average height, number of flower buds, number of flowers, number of fruits per plant, as well as fruit weight. The application of 7.5-12 mL of humic acid into 10 kg of sterile soil increased the population of soil microorganisms *Azotobacter beijerinckii* and *Aspergillus niger*. Foliar spray of 50 mL L^{-1} humic acid significantly increased leaf area, yield and total soluble solids in grapes (Popescu and Popescu, 2018). Onion plants sprayed with 1000 mg L^{-1} humic acid produced the highest growth rate and yield (Al-Fraihat *et al.*, 2018).

In addition to the improvement in cultivation, chili production can be increased by growing them during the dry season. Whilst a lot of land is available for cropping during the dry season the production is constrained by the limited availability of water. Application of humic acid as a soil conditioner could remediate water shortage in plants due to increasing leaf water content, increasing photosynthesis, antioxidant metabolism and enzyme activity; thus improving plant tolerance to stress (Al-Shareef *et al.*, 2018).

Previous studies have demonstrated that humic acid improved plant growth and yield in a number of plant species. Hermanto *et al.* (2013) reported that application of 20 kg ha^{-1} humic acid along with 100% dose of NPK fertilizer resulted in maximum growth and yield as well as increased the availability and uptake of N, P, K, Zn and Fe in maize. Furthermore, Moraditochaee (2012) showed that the application of humic acid at 40 mg L^{-1} and 75 kg ha^{-1} N produced the highest harvest index in peanut.

Sustainable production of plants can be achieved by reducing the use of inorganic fertilizers. Application of humic acid could help achieve this important goal. Whilst humic acid has been widely applied to various plant species; the search of literature showed that no research has focused on the effect of humic acid in combination with NPK fertilizer on growth and yield of chili pepper. Therefore, this study aimed at assessing the effect of humic acid in combination with NPK fertilizer on growth and yield of red chili, and to obtain the most suitable ratio of humic acid/NPK for best growth and yield in chili plants grown in dry season in Indonesia.

2. Materials and Methods

The trial was conducted at the Teaching and Research Farm within the Faculty of Agriculture at the University of Jambi in Indonesia during the 2020 dry season. The study used commercially available humic acid (AH-90, with 90% humic acid content) and inorganic NPK fertilizer (15:15:15). There were 5 combinations tested: 100% humic acid; 75% humic acid + 25% NPK; 50% humic acid + 50% NPK; 25% humic acid + 75% NPK and 100% NPK. A randomized block design with 5 replications was used in this study. Humic acid was applied at the recommended dose (5 kg ha^{-1}) and so was NPK fertilizer (700 kg ha^{-1}). The humic acid and NPK were applied one week before planting in between rows.

Seeds were sown on a standard seedling medium, then transferred to nursery at 7 days after sowing and left in the nursery for 21 days. The medium used during the nursery growth was a mixture of soil, compost, and sand (2:1:1). Seedlings were sprayed every 7 days with foliar fertilizer Bayfolan-D at 2 mL L^{-1} and insecticide (active ingredient Amabektin and Mesurol) at 2 mL L^{-1}.

The one-month-old seedlings were transplanted in the field onto the growing media supplemented with humic acid and NPK fertilizer at different rates as per treatments described above. A silvercoated black plastic was placed on the beds before transplanting the seedlings. Prior to planting, a fungicide (Carbofuran) was applied into planting holes at 1.5 g per plant. The planting space was 50 cm x 50 cm. A liquid NPK fertilizer (15:15:15) was applied at a concentration of 2 g L^{-1} with a volume of 100 mL per plant at 6, 8 and 10 weeks after transplanting.

Harvesting was carried out during the first flowering period (10 to 13 weeks after transplanting), with the criterion that 80% of fruits becoming red. Observation of vegetative growth was carried out during fruit enlargement period (9 weeks after transplanting) on the following parameters: plant height, number of productive branches, leaf area, and plant total dry weight. The leaf area was measured using gravimetric method with the following formulae (Sitompul and Guritno, 1995):

$$\text{Leaf area (cm}^2\text{)} = (x/y) \cdot z$$

where x = total leaf dry weight, y = sub sample leaf dry weight, and z = sub sample leaf area.

Observation of plant biochemical measurements were made on chlorophyll content, total sugar, and relative leaf water content (RLWC). Composite leaf samples were made by physically mixing individual leaves taken from 3 sample plants of 3 replicates into one homogenous sample. Compositing reduced the number of analyses to be performed and was designed to provide a representative sample of the treatment. Ten youngest mature leaves on main stem were collected at 9 weeks after transplanting (WAT). Clean and dry leaf samples were placed in a sample bag prior to laboratory analyses. Chlorophyll analysis was carried out using Acetone method (Arnon, 1949), and total sugar was analyzed using Anthrone method (Yoshida *et al.*, 1976). Relative Leaf Water Content (RLWC) was determined according to González and González-Vilar (2001) method.

Data on yields in terms of number of the fruits and fruit weight per plant were collected at harvest time. In addition, soil chemical analyses were performed before transplanting and after the last harvest. Soil analyses included pH, moisture content, organic C based on Walkley-Black method (Walkley and Black, 1934), total N using Kjeldahl method (Kjeldahl, 1883), available P applying Bray-I method (Bray and Kurtz, 1945) and exchangeable K (NH_4OAc 1N extraction at pH 7) using method as described in (Warncke and Brown, 1998).

The data were scrutinized employing an analysis of variance (ANOVA) from Microsoft Excel application (Version 16.63.1, 2022, Microsoft Corporation, USA). Fisher's Least Significant Difference (FLSD) at 5% level of probability was used to compare means of treatments.

3. Results

Analysis of variance showed that the application of humic acid (HA) and NPK fertilizer did not significantly affect plant height, number of branches, total leaf area, and dry weight. However, application of 25% HA in conjunction with 75% NPK fertilizer resulted in enhanced growth compared to other treatments (Fig. 1). In addition, the application of 25% HA + 75% NPK improved growth compared with 100% HA application whereby plant height, number of branches, leaf area and plant dry matter increased by 29.46%, 21.96%, 23.59% and 28.42% respectively.

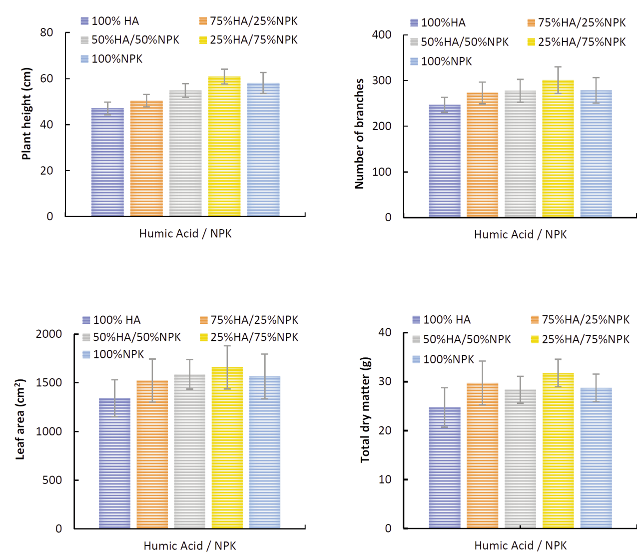


Fig. 1 - Growth of chili on various ratios of humic acid (HA)/NPK at 9 weeks after transplantation (A = plant height, B = number of branches, C = leaf area, D = total dry weight). Error bars indicate the Standard Error (n=5).

The measurement of biochemical parameters showed that the application of 100% HA resulted in the lowest chlorophyll content (2.40 mg L^{-1}) and total sugar (0.07%). Furthermore, the lowest relative leaf water content (RLWC) was found on 100% NPK application (Table 1).

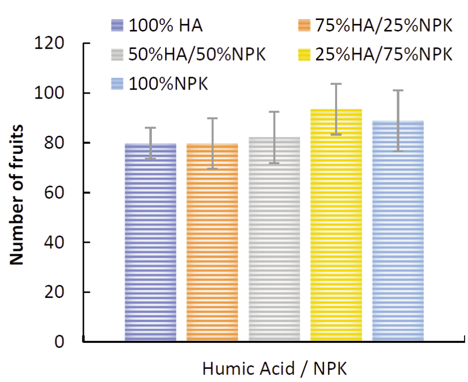
Table 1 - Effect of different ratios of humic acid) HA / NPK on chlorophyll content (*), total sugar, and relative leaf water content (RLWC) at 9 weeks after transplantation

Humic acid/NPK	Chlorophyll content (mg·L^{-1})	Total sugar (%)	RLWC (%)
100% HA	2.40	0.07	92.6
75% HA/25% NPK	3.97	0.12	93.3
50% HA/50% NPK	4.15	0.12	93.7
25% HA/75% NPK	4.37	0.09	93.0
100% NPK	4.30	0.11	91.3

(*) Chlorophyll content and total sugar were determined compositely by physically mixing individual leaves taken from each 3 sample plants of 3 replicates into one homogenous sample.

Analysis of variance showed that the application of different ratios of HA/NPK had no significant effect on the number and weight of fruits per plant. However, among the combinations tested the 25% HA + 75% NPK resulted in the highest number of fruits, and the 50% HA + 50% NPK produced the largest fruit weight (Fig. 2).

The results of soil chemical analyses (Table 2) showed that the application of various ratios of HA/NPK had altered soil chemical properties such as pH, organic C, total N, P, K, and soil water content. Generally, the application of HA along with NPK fertilizer improved N, P and K content. Whereas soil pH and organic carbon are highest when 100% HA is applied (Table 2).



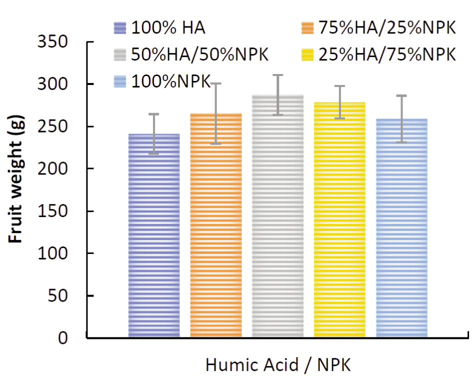


Fig. 2 - Effect of different ratios of humic acid (HA)/NPK on number of fruits (A) and fruit weight (B). Error bars indicate the Standard Error (n=5).

4. Discussion and Conclusions

The application of different humic acid/NPK ratios had no significant effect on growth and yield of chili as measured by plant height, total leaf area, number of branches and total dry weight, as well as number of fruits and average fruit weight. This means that humic acid can substitute NPK fertilizer. However, when compared to the use of only humic acid or NPK fertilizer, the application of different ratios of humic acid/NPK improved plant height, total leaf area, number of branches, total dry weight, fruit numbers and fruit weight.

NPK is widely used to improve plant growth and yield of many horticultural crops (Nafiu *et al.*, 2011; Agbede *et al.*, 2017; Achiri *et al.*, 2018; Adekiya *et al.*, 2019; Nwokwu *et al.*, 2020). However, the use of inorganic fertilizers such as NPK to help increase plant growth and yield need to be carefully considered, due to their detrimental impact on soil structure and soil biota. According to Adekiya *et al.* (2019) NPK fertilizer did not increase soil organic matter, nor reduced soil bulk density. Krestini *et al.* (2020) claimed that NPK fertilizer did not provide carbon compounds that contributed to the improvement of soil physical and biological properties. In addition, Agbede *et al.* (2017) reported that continued application of inorganic fertilizers can increase soil acidity and soil degradation because inorganic fertilizers release nutrients more quickly. Therefore, balanced fertilization is recommended to increase the efficiency of fertilizer use and help improve physical, chemical, and biological properties of soil.

The results obtained here show that application of 100% humic acid provided less growth and yield in

Table 2 - The effect of different ratios of humic acid (HA)/NPK on soil chemical properties

Humic acid/NPK	pH	C-organic (%)	N-total (%)	P (ppm)	K (cmol$^+$/kg)	Water content (%)
100% HA	6.09	1.55	0.22	104.50	0.06	7.70
75% HA/25% NPK	6.02	1.45	0.29	124.80	0.19	7.56
50% HA/50% NPK	5.47	1.28	0.28	152.30	0.47	7.61
25% HA/75% NPK	5.52	1.20	0.24	144.70	0.62	7.67
100% NPK	5.32	1.22	0.23	227.30	1.07	7.50

chili plants compared with mixture of humic acid/NPK during the one growth season of this research. However in a longitudinal experiment on peanut Li *et al.* (2019) demonstrated that significant increase in N, P and K content of soil was found after three years continuous application of humic acid.

The reason for this increase is due to humic compounds high stability rate, and therefore the mineralization process can occur very slowly. As a consequence, humic acid cannot be used as a source of plant nutrition (Canellas *et al.*, 2015). In research reported here the chemical analyses indicated soil that was treated with 100% humic acid was lower in N, P and K content, but higher in organic carbon and soil moisture content in comparison with those treated with different humic acid/NPK ratios (Table 2). This was in accordance with (Li *et al.*, 2019; 2020) findings that the application of humic acid-based fertilizer could increase soil nutrient contents, including the total soil nitrogen, phosphorus, and potassium.

Increase in soil water content was aligned with increase in relative leaf water content (RLWC) in chili plants (Table 1). Al-Shareef *et al.* (2018) reported that the application of humic acid as a soil enhancer could overcome water shortage in plants by increasing RLWC, thereby increasing plant tolerance to stress. Further, Xu *et al.* (2015) stated that the application of soil enhancers such as humic acid could increase plant growth by retaining available water and nutrients, therefore reducing the impact of drought stress and nutrient loss.

The application of 100% humic acid also increased soil pH from 4.43 to 6.09, resulting in better plant growth and development due to improved availability of various nutrients in the soil at pH of 6.09. Wahyudi (2007) reported that the application of humic acid might reduce the amount of exchangeable aluminum and increase chelating aluminum in soil, thereby increasing soil pH. Hasanudin (2003) suggested that the reduction in exchangeable aluminum was due to the formation of complex organometal compound (Al-chelate). Increase in soil pH will increase the availability of soil nutrients needed by plants (Gardner *et al.*, 1985). In peanut, Li *et al.* (2019) found that compared with control, humic acid significantly increased the yield and quality of peanut in continuous cropping. Their results showed that application of humid acid significantly increased the soil total nitrogen, total phosphorus, total potassium, available N, available P, available K, and organic matter from second year, with maximum effect displayed in third year.

Chili plants treated with different humic acid/NPK ratios showed better growth and yields in comparison to those plants treated with humic acid or NPK alone. The composition of 25% humic acid + 75% NPK fertilizer produced the highest growth (plant height, leaf area, number of branches and plant dry matter), as well as the highest yield (number of fruits) compared to other compositions. Furthermore, application of 50% humic acid + 50% NPK fertilizer resulted in the highest fruit weight. This result is in line with the increase in plant chlorophyll content, where the composition of 25% humic acid + 75% NPK fertilizer caused higher plant chlorophyll content. An increase in chlorophyll content intensifies the ability of plants to carry out photosynthesis, thereby increasing plant growth (Ferrara and Brunetti, 2010).

Increase in soil K content due to humic acid / NPK application caused better K uptake, increasing plant growth and yield in potato (Xu *et al.*, 2015). Potassium is crucial in plant photosynthetic activity (Marschner, 2012) and assimilate translocation efficiency (Sawan, 2018). This is in line with our results where application of humic acid and NPK (75% HA and 25% NPK or 50% HA and 50% NPK) increased sugar content in plant tissue (Table 1).

The composition of 50% humic acid + 50% NPK and 25% humic acid + 75% NPK in this study provided better growth and yield of chili plants than 100% humic acid or 100% NPK applications. This indicates a positive effect of the use of humic acid in partially replacing the role of NPK fertilizer. The ability of humic acid to replace NPK fertilizer is probably due to the ability of humic acid to modify the plant root system architecture as well as improving the capacity of soil to bind water, thus enabling plants to acquire water and nutrients more easily (Suwahyono, 2011; Canellas and Olivares, 2014), reducing plant stress in dry land cultivation during periods of drought (Xu *et al.*, 2015).

The analysis of soil water content for different ratios of humic acid/NPK showed medium treated with humic acid has a better water content than those treated with 100% NPK (7.56-7.7 versus 7.5). In addition, the relative leaf water content (RLWC) at various ratios of humic acid / NPK was higher than that of 100% NPK application (Table 1).

Our findings are consistent with a number of previous studies indicating that the application of humic acid needs to be accompanied by NPK fertilizer to help improve plant productivity (For example in peanut (Moraditochaee, 2012), tomato (Sarno *et al.*, 2015) and cocoa (Santi, 2016)). The slow rate of humic acid mineralization needs to be followed by the application of NPK fertilizers. This is aimed to increase the availability of nutrients for plants, especially in relation to the relatively short lifespan of the chili plant which is only 3 - 4 months. In addition, it is also necessary to consider the amount of humic acid and the time of application, so that the use of NPK fertilizer as a source of plant nutrition can be further reduced.

Data presented here indicate that the application of different ratios of humic acid / NPK could greatly increase the growth and yield of chili pepper grown in dry land cultivating, where the composition of 25% humic acid + 75% NPK fertilizer resulted in improved plant growth and yield. It was found that the application of humic acid could reduce the use of NPK fertilizers by up to 50%. Thus, for sustainable chili production in dry land cultivation, application of humic acid in conjunction with NPK fertilizer is recommended at a reduced amount.

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