Research Article

Application of humic acid supplemented with micronutrient increase rice production

Valdi Muhamad Rafiansyah Siregar ¹, Soekarno Mismana Putra ¹, Muhamad Abdul Aziz ¹, Hana Fadila ¹, Poppy Arisandy ¹, Sri Wahyuni ¹, Priyono ¹, Insyiah Meida Luktyansyah ², Sulastri ², Rizky Nugraha ¹, Mira Maulidina ¹, and Siswanto ^{1,*}

- ¹ Indonesia Oil Palm Research Institute, Jl. Taman Kencana No.1, Bogor 16128, West Java, INDONESIA
- ² PT Pupuk Kalimantan Timur, Jl. James Simandjuntak No.1, Bontang 75313, East Kalimantan, INDONESIA
- * Corresponding author (siswanto99@yahoo.com)

ABSTRACT

Fertilizer is one of the crucial inputs to maximize nutrients needed by plants, especially rice. However, insufficient ameliorant application may affect the fertilizers' effectiveness. Humic acid is believed as one of the ameliorants to improve soil conditions resulting in higher nutrient availability. The study aimed to evaluate the effect of combining humic acid and micronutrients on growth, production, and nutrient contents in rice. Three treatments were applied, i.e., control (without humic acid), humic acid, and humic acid + micronutrients. The result showed that both humic acid treatments produced the highest number of tillers, humic acid application solely stimulated the highest root length, and humic acid + micronutrients stimulated the highest plant height, and fresh and dry biomass weight. Grain weight was not affected by treatments, but humic acid + micronutrients gave a higher weight of milled grain and number of filled grains. Humic acid application solely resulted in plant biomass and grains having higher N, P, and K contents. Overall, the application of humic acid + micronutrients is recommended to increase rice production.

Keywords: ameliorant; fertilizer; nutrient availability; nutrient uptake

INTRODUCTION

Rice is the leading commodity in Indonesia, and its annual production is 54.42 million tons of unhusked rice with an average productivity of 5.23 tons ha⁻¹ (Statistics Indonesia, 2022). The General Secretariat of the Agriculture Ministry of Indonesia (2021) recorded that national rice consumption in 2021 reached 30.03 million tons with an average annual consumption of 92.5 kg per capita, leaving a small amount of rice surplus of 1.33 million tons. Therefore, increasing rice production to produce a larger amount of surplus is important.

One of the efforts to increase rice production is through increasing productivity by improving organic material as an ameliorant. Organic material is important for soil because it increases soil fertility (Hardjowigeno, 2015). To some extent, organic materials become an important soil supplement because they will stabilize soil conditions when farmers apply inorganic fertilizers (Irawan & Antriyandarti, 2020).

In Indonesia, generally, rice farmers apply some popular single-analysis fertilizers such as urea (nitrogen source), SP-36 (phosphorus source), KCl (potassium source), and compound fertilizers such as NPK (15:15:15). Especially N-based fertilizers, effectivity is low (approximately 60%) due to high nutrient loss in soil by volatilization, leaching, and runoff (Fageria et al., 2014). Phosphorus might be less available in the soil because of binding by Fe and Al, erosion, and runoff (Muktamar et al., 2020; Reid et al., 2018).

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Recently, the application of humic acid as an organic source has been increasing for stimulating plant production (Kailola et al., 2023), because of its role as the soil ameliorant, bio-stimulant, and improves soil fertility. It can add more cation exchange capacity (CEC) and metals ion chelation such as Fe and Al because their material contains -COOH and -OH groups which can bind cations and heavy metals in soil (Ampong et al., 2022). Moreover, humic acid also tends to increase phosphorus availability for plants (Yang et al., 2019). In addition, humic acid also stimulates nutrient uptake especially N, due to the induction of H⁺-ATPase in the root membrane (Canellas et al., 2015; Khaled & Fawy, 2011). It is evident that application of humic acid increases rice production by around 18% and spray application of humic acid increases yield by around 3.5-7.0% (Saha et al., 2013; Sivakumar et al., 2007). According to Suhardjadinata et al. (2015), supplements of 3 kg ha⁻¹ humic acid with 67.5 kg N ha⁻¹ resulted in the highest agronomic efficiency in rice.

Micronutrients such as Zinc (Zn), Boron (B), Copper (Cu), and Manganese (Mn) are often less available in the soil for particular plants including rice (Das, 2014). The micronutrients commonly play important roles in plant metabolism such as Zn for enzyme activation, membrane protection, and chlorophyll formation; Mn for enzyme activator and photosynthesis catalysator; Cu for lignin synthesis and enzyme activation, and Boron (B) for carbohydrate metabolism and panicle formation (Das, 2014; Nadeem & Farooq, 2019). The study aimed to evaluate the effect of combining humic acid and micronutrients on growth, production, and nutrient contents in rice.

MATERIALS AND METHODS

The research was conducted from August 2022 to December 2022 in the irrigation rice field of West Karawang District, Karawang Regency, West Java Province, Indonesia. The 25-day-old seedlings were planted on 1st August 2022. Soil and plant analysis was observed in The Biochemical and Food Analysis Laboratory, Indonesian Oil Palm Research Institute, Bogor, Indonesia.

The field used for this research was around 9,000 m². Materials used 'Ketan putih' rice variety, urea and NPK (15:15:15) fertilizers, and humic acid powder and solution from NPK waste. The experiment used a randomized complete block design with 3 repetitions. There were three treatments, i.e., P0: control (without humic acid), P1: humic acid, and humic acid + micronutrients containing Mn, Zn, Cu, and Boron (mixing humic acid powder and micronutrient powder such as Mn, Zn, Cu, and Boron). One repetition had ten plant samples, and all total samples that were observed were 90 samples.

Two forms of humic acid were applied: powder (solid) and solution (liquid). The solid humic acid was mixed and applied together with fertilizers application. Fertilizer was applied at 3 and 5 weeks after planting (WAP) with a dosage of around 125 kg ha⁻¹ urea and 125 kg ha⁻¹ NPK (15:15:15) per one application. The humic acid powder was applied at a rate of 5% of the total fertilizer through soil application. Total fertilizer was calculated from total NPK + urea. The liquid humic acid was applied one day after fertilizer application with a dosage of around 10 cc L⁻¹ with a spray volume of 200 L ha⁻¹ based on a recommendation from the manufacturer. Micronutrients were incorporated in both powder and solution forms of humic acid. In each humic acid form, the micronutrient level was 1.1% Mn, 0.4% Zn, 0.2% B, and 0.8% Cu. In total, the micronutrients applied were 333 g Mn ha⁻¹, 123 g Zn ha⁻¹, 54 g B ha⁻¹, and 243 g Cu ha⁻¹.

Soil analysis was observed before planting and after harvesting by measuring: soil pH, cation exchange capacity (CEC), total N, available P, exchangeable K, organic-C, and micronutrients (B, Zn, Cu, Mn). Vegetative growth was observed at 28, 43, and 52 days after planting (DAP) to measure: number of tillers, plant height, and leaf color. Root lengths were observed on five samples per replication at 60 DAP. The number of productive tillers (panicle) was observed at 60, 79, and 105 DAP. Fresh and dry weight biomass were observed after harvesting. At harvest, parameters observed were:

harvested grain weight, milled grain weight (water content ca 14%), filled grain weight, number of filled grains, and 1,000 grains index. Biomass and grain analysis with tree repetition per treatment were observed to measure N, P, and K contents to determine the nutrient uptake (NU) with the formula as follows: NU (Px) = NC(Px) x DBG (Px); NU: N, P, or K nutrient uptake of grains or biomass; NC: N, P, or K nutrient contents of grains or biomass; DBG: Dry biomass or grains; Px: Treatment (P0, P1, or P2). Biomass was taken to analysis with destruction at 52 DAP and measured after sun-drying for seven days.

Data were analyzed statistically using ANOVA, and any significant effect was further evaluated using the Duncan Multiple Range Test (DMRT) at α =0.3 or 70% level of confidence for field variables especially in the milled dry grains variable. Further DMRT test used α =0.05, especially for nutrient contents and its uptake.

RESULTS AND DISCUSSION

Humic acid contents

Original humic acid in the form of liquid and powder had different contents (Table 1). The high pH content of both humic acid sources was due to the humic acid being extracted from lignite with alkaline extraction. Liquid NPK waste from a fertilizer factory was the replacement of water for the process of humic acid extraction in order to add the value by increasing the content of N, P, and K in that humic acid. NPK waste causes environmental pollution around their surrounding environment and after many processes, their residue is dumped into the sea (Azalia & Hendrasarie, 2022). These wastes contain 0.04-1.57 ppm nitrogen, 5-64 ppm phosphate, and 18-55 ppm potassium (Uba & Ekundayo, 1995).

Daramotor	Humi	c acid
Falalletel	Solution	Powder
Humic content	18.65%	38.23%
Solubility	-	82.51%
Water content	-	10.66%
Natrium content	0.92%	0.77%
pН	9.20	9.50

Table 1. Humic acid content of powder and solution forms.

Chemical characteristics of soil

Soil analysis before planting is shown in Table 2. It was revealed that the soil of this experiment was acidic. There were several value changes in soil parameters after harvesting (Table 3). Three treatments showed a pH increase after harvesting. The finding is in line with research by Wulandari et al. (2019). The increase in pH in the humic acid-treated soil indicates the functionality of humic acid as a pH buffer (Xu et al., 2021). Moreover, the roles of humic acid can decrease Al and Fe solution in ultisols from 4.02 to 4.19 (Irfansyah, 2013).

Humic acid application for both treatments with or without micronutrients increased cation exchange capacity (CEC) (Table 3). Increasing CEC after a humic acid application is also noted in other experiments (Harada & Inoko, 2012; Xu et al., 2021). Increasing 1% of humic acid level in soil increases CEC by two times higher than without humic acid application (Xu et al., 2021). Furthermore, the CEC value of humic acid was also higher than average soil, which is around 100 to 500 me 100 g⁻¹ (Harada & Inoko, 2012).

Table 2. Chemical characteristics of the soil before planting.

Parameters	Value	Level
pH (H ₂ O)	4.86	Strongly acidic
Cation exchange capacity (CEC) (cmol kg ⁻¹)	21.80	Medium
Total N (%)	0.15	Low
Available P (ppm)	13.40	Low
Exchangeable K (cmol kg ⁻¹)	3.67	Very high
Soil organic-C (%)	0.84	Very low
B (ppm)	0.03	Very low
Zn (ppm)	60.40	Very high
Mn (ppm)	20.49	Very high
Cu (ppm)	36.62	Very high

Note: The level is based on Hardjowigeno (2015) and Khokhar (2019).

Total N increased for all three treatments because of adequate nitrogen fertilizer application by using urea and NPK (Table 3). However, treatment from humic acid with micronutrients gave higher results. Humic acid will reduce N losses in soil by around 23-25% from leaching, NH_3 volatilization, and N_2O emission (Kong et al., 2022). The available P in control was zero but its level significantly increased with both humic acid treatments. Humic acid is known to increase available P by adsorbing heavy metal ions such as Fe and Al and increase P movement in the soil (Zhu et al., 2018).

Exchangeable K in soil seems to have the same value from the beginning until harvest (Table 2 and Table 3). It is probable that medium CEC might have a role in retaining K cations in the soil, but higher CEC might increase K retaining. Such a mechanism might exist to minimize K lost from leaching (Ketterings et al., 2007). Organic-C soil increased in all treatments, but a substantial increase was shown in soil with humic acid applications. Table 3 shows that micronutrient contents in P2 soil were significantly higher than in other treatments which could be a consequence of humic acid + micronutrients application.

Number of tillers

The effect of humic acid in rice crops had significant differences between each treatment (Table 4). Rice crops at 28 DAP from humic acid + micronutrients application showed the highest number of tillers, followed by control and humic acid treatments. However, at 43 DAP, there was a significant increment from humic acid without micronutrients application. Eventually, at 52 DAP, both humic acids with or without micronutrients gave a higher number of tillers rather than the control treatment. Micronutrients especially boron have the ability to form new cells in meristematic cells (Das, 2014).

Table 3. Chemical characteristics of soils after harvesting.

Devenuetore	Value ^z				
Parameters	P0		P1	P2	
рН	6.36	Ac	6.57 N	6.34	Ac
Cation exchange capacity (CEC) (cmol kg ⁻¹)	22.53	Μ	33.25 H	33.98	Н
Total N (%)	0.91	VH	1.32 VH	2.45	VH
Available P (ppm)	0.00	VL	173.43 VH	175.49	VH
Exchangeable K (cmol kg ⁻¹)	5.28	VH	5.41 VH	5.21	VH
Soil organic-C (%)	1.57	L	1.93 L	2.35	М
B (ppm)	0.011	VL	0.032 VL	1168.86	VH
Zn (ppm)	59.739	VH	99.128 VH	1691.467	VH
Mn (ppm)	45.991	VH	48.239 VH	2258.847	VH
Cu (ppm)	22.031	VH	30.564 VH	1982.324	VH

Note: ²The level is based on Hardjowigeno (2015) and Khokhar (2019). Ac: acidic; N: neutral; VL: very low; L: low; M: medium; H: high; VH: very high. P0: control; P1: humic acid; P2: humic acid + micronutrients.

Nur	nber of tillers	
28 DAP	43 DAP	52 DAP
38b	48b	28b
34c	69a	34a
42a	50b	34a
	Nur 28 DAP 38b 34c 42a	Number of tillers28 DAP43 DAP38b48b34c69a42a50b

Table 4. Number of tillers of rice per plant on humic acid with or without micronutrients.

Note: The column represents the average value of the parameter. A significant value is indicated by the letter from highest to lowest result inside each column (α =0.3). P0: control, P1: humic acid, P2: humic acid + micronutrient.

Leaf color

A leaf color chart is one indicator to measure color in the leaves which is related to nitrogen sufficiency. Leaf color ranged from 3 to 4 (Table 5). The best value in leaf color is 4 which means the nitrogen supply for plants is sufficient, a value below 4 indicates the necessary to add nitrogen, and >4 indicates nitrogen toxicity (Paryoto, 2020). Table 5 shows no significant difference among treatments on leaf color. Moreover, at 52 DAP, the leaf's color indicates less greenish in the generative phase. This is because more nutrients in the leaves or sources will be transported into generative organs such as flower buds or panicles (Taiz et al., 2022).

Table 5. Average leaf color of rice crops on humic acid with or without micronutrients.

Treatmonte	Leaf color chart			
	28 DAP	43 DAP	52 DAP	
P0	4	4	3	
P1	4	4	3	
P2	4	4	3	

Note: P0: control, P1: humic acid, P2: humic acid + micronutrient.

Plant height

There were significant differences in plant height at four observation days (Table 6). First, at 28 DAP, control, and humic acid gave the highest result. Second, there was the most considerable height increment from humic acid + micronutrient at 43 DAP, but still lower than humic acid without micronutrients. Subsequently, at 52 DAP, humic acid + micronutrient gave the highest result averaging around 104.1 cm. Finally at 105 DAP, after yield harvesting, the highest plant height was given by humic acid + micronutrients. It was also revealed by Al-Bourky et al (2021), that application of humic acid 4 mL L⁻¹ produces the highest plant height than the control. According to a trial by Lahijani et al (2020), micronutrients such as B, Zn, Cu, Mn and Fe applied by a two-times foliar spray technique showed the highest result of plant height than the control.

Table 6. Average tiller height of rice on humic acid with or without micronutrients

Treatmonte		Plant heig	ht (cm)	
Treatments	28 DAP	43 DAP	52 DAP	105 DAP
PO	59.2a	88.7c	99.2b	111.0b
P1	58.3a	93.0a	100.1b	111.3b
P2	55.9b	91.0b	104.1a	116.1a

Note: A significant value is indicated by the letter from highest to lowest result inside each column (α =0.3); P0: control, P1: humic acid, P2: humic acid + micronutrient.

Root length

According to data shown in Figure 1, humic acid without micronutrients gave the highest result of root length around 25.87 cm while the control gave around 24 cm, and humic acid + micronutrient, around 22.67 cm. According to a review made by Canellas et

al. (2014), humic acid increases root architecture particularly, root size and root hair because there is a stimulation of H⁺ ATPase activity in the cell membrane. It was revealed by Shahzad & Amtmann (2017), that root length will grow more prominent when the micronutrients supply is inadequate, which is why root length in P2 was lower because plants were given adequate micronutrients, by means more nutrient source was supplied into aboveground parts.



Figure 1. The effect of humic acid on average rice root length at 60 DAP (n=5); A significant value is indicated by the letter on top of each bar (α =0.3); P0: control, P1: humic acid, P2: humic acid + micronutrient.

Fresh and dry biomass of aboveground and roots

Biomass without grains was harvested after harvesting their grains (Figure 2). In Figure 2a, fresh aboveground biomass was the highest after humic acid + micronutrient treatment, i.e., 443.9 g, followed by the control which was 373.2 g, and humic acid without micronutrients was 355.7 g. Most micronutrients such as zinc and boron help plants by increasing stem elongation, leaf extension, and cell dividers in developing tissue (Yadav et al., 2022). Conversely, the highest result for fresh root weight was given by only humic acid treatment around 165.7 g, while humic acid + micronutrient and control were around 158.5 g and 152 g, respectively. According to research by Büyükkeskin et al. (2015), humic acid with a concentration of around 10 mL L⁻¹ increases the fresh roots' weight by around 24-35%.



Figure 2. Rice biomass from different humic acid treatments. (A) fresh biomass of aboveground and roots, (B) dry biomass of aboveground and roots. A significant value is indicated by the letter on top of each bar (α=0.3).
P0: control, P1: humic acid, P2: humic acid + micronutrient.

Figure 2b shows that both humic acid with micronutrients and control gave the highest dry biomass, around 131.7 and 125.7 g, respectively. However, humic acid treatment gave the lowest result around 100.1 g. There were no significant differences between all three treatments with results for P0, P1, and P2 around 61.1 g, 61.3 g, and 59

g, respectively. De Hita et al. (2020) stated that humic acid as foliar spray could stimulate the production of lateral roots and shoot, resulting in higher biomass.

Number of productive tillers

Number of productive tillers was only significant at 60 DAP with the highest value for humic acid + micronutrient application (Figure 3). However, it was insignificant with the application of humic acid only. At 79 DAP and 105 DAP (harvesting time), there were no significant differences between all three treatments. Whereas, the effect of humic acid gave better results than control. Boron is one of the crucial elements for flowering. Boron deficiency will reduce flowering or panicle formation and eventually induce flower bud abortion (Nadeem & Farooq, 2019).



Figure 3. Average number of productive tillers of humic acid treatment. A significant value is indicated by the letter on top of each bar (α =0.3). P0: control, P1: humic acid, P2: humic acid + micronutrient.

Grain yield and yield components

There was no significant difference in the weight harvest dry grains (Table 7), although, the data showed that P2 (humic acid+ micronutrient) had the highest result. Equally, that treatment has better results on milled dry grains per plant but has no significant difference with an application of humic acid only. Humic acid increases grain weight by around 5.6% than without humic acid (Zheng et al., 2022). Humic acid derived from cow manure of 1,125 g increases grains by around 9.5% in a pot experiment (Hindersah et al., 2022).

Table 7. Yield and yield component of rice crop on humic acid treatment at 105 DAP.

Treatmonte	Harvest dry grains	Milled dry grains	Weight of filled	Number of filled	Weights of
Treatments	per plant (g)	per plant (g)	grains (g)	grains	1,000 grains (g)
P0	84.70	48.93b	45.16c	1672b	28.28
P1	85.85	51.60ab	48.46b	1830a	28.44
P2	88.14	56.11a	52.87a	1876a	28.70

Note: A significant value is indicated by the letter from highest to lowest result inside each column (α =0.3). P0: control, P1: humic acid, P2: humic acid + micronutrient.

The highest weight of filled grains per plant was given by humic acid + micronutrient application treatment, followed by only humic acid and control (Table 7). However, humic acid treatments with or without micronutrients had no significant differences in the number of filled grains. According to Kumar & Singh (2017), the application of NPK, zinc sulfate, and potassium humate on rice around 10 ppm stimulates the highest result by around 35% higher than NPK alone. Moreover, Boron application on wheat increases grain by around 64%, while Boron deficiency induces flower sterility, and low formation of grains (Iqbal et al., 2016; Nadeem & Farooq, 2019). In the present experiment, there was no significant difference in 1,000 grain weights from all three treatments (Table 7).

Grain productivity per hectare showed no significant effect among treatments (Table 8). Nevertheless, for practical in farmers' fields, humic acid + micronutrient application had the highest increment of their yield around 10.2% over the control treatment. The application of humic acid without micronutrient produced an incremental yield of 9.5% as compared to the control. The research finding is lower than the available reports. According to Suntari et al. (2015), humic acid application with urea in rice gave 22.1% increment over the control treatment. Moreover, Senthilkumar et al. (2021) noted that micronutrients application such as B and Zn stimulate increment yield on rice by 12.5-13.5% in Tanzania. Therefore, it is probable that the effect of micronutrient application on rice productivity might be different from site to site.

Treatments	Harvest dry grains (ton ha ⁻¹)	Increment over control (P0) (%)
PO	5.8	-
P1	6.3	9.5
P2	6.4	10.2

Table 8. Average yield of rice crop on humic acid with or without micronutrients.

Nutrient contents

Humic acid + micronutrient (P2) application gave the highest results on N, P, and K contents among other treatments (Table 9). The N and P contents of grain were higher in humic acid + micronutrient treated plant than control but the N content of that treatment was similar statistically to humic acid treatments without micronutrient. The high P level in soil supplemented with humic acid is in line with soil data shown in Table 3. It is interesting for K level in grain that exhibited similar levels from different treatments, although the level of biomass showed variation. Factors affecting K absorption in rice gran need further evaluation.

	Nutrient contents		
Treatments	N (%)	P (ppm)	K (ppm)
		Biomass	
PO	2.723b	25.33c	123.33b
P1	1.910b	131.00b	146.66b
P2	3.738a	156.33a	310.00 a
		Rice grains	
PO	1.351b	14.27c	31.57
P1	1.451ab	199.80a	34.63
P2	1.560a	186.97b	33.37

Table 9. Nutrient contents of biomass and rice grains from different humic acid treatments.

Note: A significant value is indicated by the letter from highest to lowest result inside each column (α =0.05). P0: control, P1: humic acid, P2: humic acid + micronutrient.

Total N was higher in humic acid + micronutrient treatment in both biomass and rice grains (Table 9). It could be the role of humic acid that retains more nitrogen especially NH_{4^+} in the soil (Suntari et al., 2015). The explanation why nitrogen contents from plants treated with humic acid + micronutrient was the highest in the present experiment is because micronutrients such as boron and zinc have the ability to increase nitrogen metabolism and protein quality according to Saquee et al. (2023).

P availability in the soil was also higher in both humic acid applications, while it was lowest in the control (Table 9). However, exchangeable K in the soil had the same value for all treatments (Table 3), resulting in K level in grains was not significant in all treatments. However, the K content of biomass in humic acid + micronutrient treatment was the highest (Table 9). According to Raza et al. (2021), the combination of K and Zn improves the K contents in maize, because of an increase in K₂O fixation and

photosynthesis activity. Moreover, P contents in grains were higher in only humic acid application because P at high concentration might inhibit Zn and Cu uptakes (Hardjowigeno, 2015).

Nutrient uptakes

Overall, humic acid + micronutrient gave the highest result of nutrient uptake of N, P, and K since that treatment gave the highest dry biomass weight (Table 10). Grains absorbed the highest N in humic acid + micronutrient treatment. Moreover, there was no significant difference in P and K uptake by grain for humic acid treatments with or without micronutrient although humic acid-treated plants uptake higher PK than the control treatment.

Table 10. Nutrient uptakes of biomass and grains rice crops on humic acid with or without micronutrients.

	Nutrient uptake		
Treatments	N (g)	P (mg)	K (mg)
		Biomass	
P0	2.724b	3.184c	15.500b
P1	1.918c	13.112b	19.310b
P2	4.921a	20.582a	31.029a
		Grains	
P0	0.662b	0.700b	1.548b
P1	0.750b	10.324a	1.790a
P2	0.879a	10.533a	1.881a

Note: A significant value is indicated by the letter from highest to lowest result inside each column (α =0.05). P0: control, P1: humic acid, P2: humic acid + micronutrient.

In the present experiment, the role of humic acid on the growth and yield of rice is apparent. It is probable that the ability of humic acid to increase plant nutrient adsorption by increasing the permeability of the cell membrane (Tan, 2014). Additionally, the reason behind the highest uptake in biomass of humic acid + treatment was the highest result of dry biomass weight as shown in Figure 2b. Humic acid as foliar spray increased N and P uptake in radish due to stimulating plasma membrane H⁺-ATPase activity (Sayed et al., 2014). Humic acid also increases P uptake because of increasing available P in the soil as a result of the hydrolyzation of organic P into soluble P (Purwanto et al., 2021).

CONCLUSIONS

Humic acid application improves rice crop yield and nutrient uptake, especially with the combination of micronutrients. However, the combination treatment gave better results for biomass aboveground while humic acid without micronutrients gave the highest root performances. Humic acid improves soil chemical characteristics especially cation exchange capacity and P, thus improving nutrient contents of P and K in rice.

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