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Eco-physiological manipulation by using humic acid and micronutrient for improving soil biological quality and rice yield in coastal agricultural land

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Abstract Coastal agricultural land generally has some characteristics such as water stress and lack of nutrients, low organic matter content, high salinity, high temperature and strong wind, thereby causing low rice yield. Besides, humic acid and micronutrients' application ameliorates soils for a better rice yield. The importance of humic acid and micronutrients compound mixed with biological fertilizers to soil biological quality and rice yield in an agricultural coastal land were recorded from July to December 2020 in Beringin Raya, Muara Bangkahulu sub-district, Bengkulu City. The result showed the *Inpago 10* was the highest productive variety compared to *Red* and *White* varieties, while the coastal land ameliorated with humic acid produced the highest *Azotobacter* population, PSB, AMF colonization in the root system, and rice yield. *Inpago 10* and humic acid treatment combination makes soil more basic and produced the highest weight of 1000-grain and milled dry rice.

Keywords: Humic acid, Micronutrient, Upland rice, Aagricultural coastal land

Introduction

Agricultural development in coastal areas encounters some constraints from inherent soil characteristics such as structural destabilization, high evapotranspiration, low moisture, high salt and low soil organic matter content, hence causing low cation exchange capacity. Soil salinity stress in the rhizosphere affects all crop yield components (Shereen *et al.*, 2005; Kanawapee *et al.*, 2013), due to osmotic change in soil solution and salt ion toxicity (Kronzucker *et al.,* 2013; Fahad *et al.,* 2014). Furthermore, osmotic pressure within plant cell influences water and nutrient absorption from soil solution through the root cell (Debez *et al.,* 2004). Soil salinity stress causes equilibrium disturbance between plant cell and soil solution osmotic pressure

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(Xu *et al.*, 2013) which is higher in the soil solution than plant cell due to high salt ion concentration. Therefore, causing inability to absorb water and nutrients such as K^+ and Ca^{2+} (Joseph and Mohanan, 2013).

Coastal lands categorized as suboptimum agro-ecosystem for intensive agricultural activities tend to become an alternative for agricultural development in the future when a suitable technological package is applied. A potential agricultural commodity often planted on a drained coastal land is upland rice. Bertham *et al.* (2019) stated by biological technology and organic matter implementation, the drained coastal land tends to be an area for intensive cultivation, even though the current rice yield is still below the potential production target. The coastal land has low micronutrient contents such as Si, Zn, Cu, Cl, Mn and B content which cause low rice yield.

Although micronutrients are needed in small amounts, however, they play a significant role in rice yield especially determining grain development. Si applied in coastal land decreases the amount of empty grain per panicle, increases rice grain weight (Mauad *et al.,* 2003), and improves plant resistance to disease attacks (Ranganathan *et al.,* 2006). Meanwhile, Mn acts on plant physiological processes as an enzyme activator and also catalyzes water ionization in photosynthesis (Aref, 2012). Fe is involved in chlorophyll, while its deficiency causes chlorosis on leaf veins and the symptoms appear initially on young leaves (Zu *et al.,* 2012). Furthermore, Cu promotes Fe role along with chlorophyll synthesis, while Mo is an important nitrate reductase enzyme component in plant and it also plays an important role in Fe translocation in plants (Graham and Welch, 2002). Bo functions in precipitating excessive cations or acts as a buffering anion, and regulates other plant nutrition, as well as promotes cell development in meristem tissue, starch translocation, phosphor mobilization in plant tissue, and cell wall development (Zu *et al.,* 2012).

Along with micronutrients, other components which improve rice yield in agricultural coastal land are humic acids. These acids ameliorate soil physical, chemical and biological properties. Due to the soil fertilities amelioration, nutrients uptake by plant increases as well as optimal growth and yield. The humic acids have a direct effect on plant metabolism such as improving photosynthesis (Heil, 2005) because of the increase in leaves chlorophyll content (Ferrara and Brunetti, 2010). They also promote phosphorus (P) uptake by the plant as well as increases root weight and rice yield to about 31% (Suwardi and Wijaya, 2013). Application of humic acids incubated with phosphorus solubilizer bacteria makes soil more basic and available P (Winarso *et al*., 2011). Humic acid also decreases NH_3 loss and improves NH_4 availability (Ahmed *et al*., 2006). Therefore, the research aimed to evaluate the importance of micronutrients compound and humic acid inoculated with biological fertilizer to soil biological quality and rice yield cultivated with a dryland system in agricultural coastal areas.

Materials and methods

This research was conducted from July to December 2020 in Beringin Raya, Muara Bangkahulu sub-district, Bengkulu City. Biological fertilizer inoculant formulation and engineering, as well as soil biological analysis, were carried out in Soil Science Laboratory, Faculty of Agriculture, University of Bengkulu. A split-plot design was used in which three rice varieties namely *Inpago 10, Merah,* and *Putih* were planted on the main plot, while three treatments namely humic acid, micronutrient compound, and recommended inorganic fertilizers were applied to the subplot. The humic acid treatment was 8 L ha^{-1} dose, dissolved in distilled water with a ratio of 1L: 40L to obtain a humic solution of about 328 L ha^{-1} or 147.6 mL $plot^{-1}$. The micronutrients contained 2.5 % Fe, 7% Mn, 5% Zn, 2% Cu, 2% Boron, and 0.1% Mo, while the recommended inorganic fertilizer was 200 kg Urea ha^{-1}, 100 kg SP36 ha^{-1}, and 100 kg KCl ha^{-1}, and each treatment combination unit was replicated 4 times. Data were analyzed using Analysis of variance and Duncan Multi Range Test with 5% significance level, respectively.

Land preparation was initially carried out by getting rid of weeds, followed by soil tillage and making trial plots with each plot unit of 1.5 m x 3.0 m, while the plots distance was 50 cm, and the distance between replication plots was100 cm. To all the experiment plots, equal 10 ton ha^{-1}composted leather coffee beans and 200 kg ha^{-1} dolomite were added, which was then incubated for 2 weeks. Afterwards, planting holes with 5 cm depth were dug, and then 3 rice seeds were put in each. The distance among planting holes was 30 cm x 30 cm ensuring each plot composed of 50 planting holes. The seeds planted were previously treated with biological fertilizer inoculant namely AMF, phosphorus solubilizer bacteria (PSB), and *Azotobacter*. Inorganic fertilizer was applied at the same time of seed planting. This includes applying urea twice, the first was during seed planting together with SP36 and KCl and the second was after 1 month. To maintain optimal rice growth, the growing media was treated with watering, followed by weeds and diseases control, as well as fenced the land using bamboo. The micronutrient mixture was previously dissolved with distilled water of 1:8 ratio, then sprayed to plant leaves in the amount of 135 ml $plot^{-1}$ at flowering age and 135 ml $plot^{-1}$ after 2 weeks. Humic acid application on the plot surface was conducted 2 days before seed planting.

Assessment of soil biological properties, pH (1: 2.5 with the electrometric method), and C-organic with Black and Walkey method was carried out at the end of vegetative growth. The biological properties included *Azotobacter* population in the Asbhy's planting media, phosphorus solubilizer bacteria population in the Alexandrov planting media and AMF root colonization, which were identified with the coloring method. Meanwhile, the rice yield components observed were the number of pithy rice grain per panicle, percentage of pithy rice grain per panicle, 1000-grain weight, milled dry grain weight plant^{-1} and milled dry grain plot^{-1}.

Results

The soil ameliorant significantly affected the soil acidity in *Merah* and *Putih* rice varieties planting media but had no significant effect on *Inpago 10* planting media. Humic acid application caused soil more basic in *Merah* and *Putih* rice planting media to pH 5.53 and 5.52, respectively (Table 1). Soil acidity showed a significant difference among the rice varieties planting media when humic acid, micronutrient compound, and recommended inorganic fertilizer mixture were applied (Table 2).

Table 1. Effect of rice variety and soil ameliorant on soil pH

	Rice variety		
Ameliorant	*Inpago 10*	*Merah*	*Putih*
Micronutrient compound	5.44 a	5.47 a	5.55 a
	A	AB	A
Humic acid	5.62 a	5.53 a	5.52 a
	A	A	A
Inorganic fertilizers	5.53 a	5.27 a	5.15 a
	A	B	B

Note: A number followed by the same capital/small letter in the same column/line means no significant difference

The rice varieties planted with upland systems yielded a significantly different 1000-grain weight when micronutrient compound and recommended inorganic fertilizers were applied to the planting media. In contrast, there was no significant effect when humic acid was applied. The *Inpago* 10 had the highest 1000-grain weight compared to other varieties, which yielded 23.83 g and 23.80 g respectively when the micronutrients and recommended inorganic fertilizers were applied. However, the humic acid applied produced a significantly different 1000-grain weight in the *Merah* rice variety, namely 22.20 g compared to the other two compounds (Table 2).

Table 2. Effect of rice variety and ameliorant interaction on 1000-rice grain weight (g)

	Rice variety		
Ameliorant	***Inpago 10***	***Merah***	***Putih***
Micronutrient compound	23.83 a A	19.33 b A	20.28 b A
Humic acid	24.25 a A	22.20 a A	22.03 a A
Inorganic fertilizers	23.80 a A	15.48 b B	20.85 a A

Note: A number followed by the same capital/small letter in the same column/line means no significant difference

Rice grain dry weight showed a significant difference among the rice varieties when soil ameliorants were applied, and *Inpago 10* produced the highest yield for all the ameliorants. Humic acid produced the highest values for *Inpago 10* and *Putih* rice varieties in which *Inpago 10* had a rice grain dry weight of 2,310.36 g plot^{-1} or about 5.13 ton ha^{-1}. In contrast, the weight decreased to 2,090.31 g plot^{-1} or 4.65 ton ha^{-1} when micronutrient compound was applied, and then to 1,846.00 g plot^{-1} or 4.10 ton ha^{-1} when the recommend inorganic fertilizer mixture was applied (Table 3).

Soil biological characteristics, number of pithy per panicle, and rice grain weight per plant in the planting media showed a significant difference as represented in Table 4.

Table 3. Effect of rice variety and ameliorant on rice grain dry weight plot^{-1} (g)

	Rice variety		
Ameliorant	***Inpago 10***	***Merah***	***Putih***
Micronutrient compound	2,090.31 a A	1,713.41 b A	1,607.90 b B
Humic acid	2,310.36 a A	1,762.01 b A	1,680.92 b A
Inorganic fertilizers	1,846.00 a B	1,650.60ab A	1,596.49 b B

Note: A number followed by the same capital/small letter in the same column/line means no significant difference

The highest *Azotobacter* and PSB population growth, namely 71.59 10^{5} CPU g^{-1} and 594.50 10^{5} CPU g^{-1} respectively, as well as AMF root colonization, namely 92.50%, occurred in *Inpago 10* rhizosphere. Besides, this rice variety also produced the highest yield for the number of pithy rice grain per panicle and rice grain dry weight, namely 143.43 grain and 40.71 g, respectively.

Table 4. Soil biological characteristics and yield performances from three rice varieties

Variety	*Azotobacter* population (10^5 CPU g^{-1})	PSB population (10^5 CPU g^{-1})	AMF root colonization (%)	Number of pithy per panicle	Rice grain weight per plant (g)
Inpago	71.59 a	594.50 a	92.50 a	143.45 a	40.71 a
Merah	47.04 b	463.54 b	90.00 a	93.34 b	33.67 b
Putih	45.92 b	473.97 b	84.17 b	88.05 b	32.55 b

Note: number followed by the same capital/small letter in the same column/line means no significant difference

Humic acid application on the rice planting media promoted the highest *Azotobacter* and PSB population, as well as AMF root colonization, namely 71.91 10^5 CPU g^{-1}, 638.461^5 CPU g^{-1}, and 95.83% respectively. Besides, this also produced the highest number of pithy rice grain per panicle and rice grain weight per plant, namely 114.79 grain and 36.42 g (Table 5).

Table 5. Effect of ameliorant on Soil biological characteristics and rice yield

Ameliorant	Azotobakter population (10^5 CPU g^{-1})	PSB population (10^5 CPU g^{-1})	Root colonizatio n (%)	Number of pithy rice grain	Rice grain weight per plant (g)
Micronutri ent	49.71 b	479.11 b	90.83 b	109.64ab	36.08 a
Humic acid	71.91 a	638.46 a	95.83 a	114.79 a	36.42 a
An-organic fertilizers	42.93 b	414.44 a	80.00 c	100.42 b	34.44 b

Note: A number followed by the same capital/small letter in the same column/line means no significant difference

Discussion

The results showed humic acid application increased the soil pH due to precipitation of soil H^+ ion by OH^- activities released from carboxyl (-COOH) and hydroxyl (-OH) groups. This was also reported by Ifansyah (2013) and Mindari *et al.* (2014). Increasing soil acidity increases nutrient uptake and plants yield. It has been proven that humic acid also produced higher 1000-rice grain weight and rice grain dry weight $plot^{-1}$ in all varieties. This is in line with Saha *et al.* (2013) which stated 1000-grain weight was affected by the humic acid application. Furthermore, Osman *et al.* (2013) stated humic acid sprayed on leaves increased 1000-grain weight and N, P, K content in rice grain and straw.

The results also showed humic acid application improved *Inpago 10* variety adaptation capability on agricultural coastal land with the projected potential rice yield reaching 5.13 ton ha^{-1}. Compared to the rice variety description released, the *Inpago 10* yield was higher than the average harvest of 4.0 ton ha^{-1}. However, this result was below the potential yield of 7.3 ton ha^{-1}. Kumar *et al.* (2014) stated humic acid application tends to improve rice grain dry weight in which the higher the humic acid added, the higher the rice grain dry weight.

Soil biological characteristics in the rice variety planting media showed a significant difference in which the highest *Azotobacter* and PSB population growth, as well as AMF root colonization occurred in *Inpago 10* rhizosphere. Furthermore, *Inpago 10* produced the highest number of pithy rice grain and rice grain dry weight. It also had high quality root exudate compared to *Merah* and *Putih*, leading to optimal microorganism growth in the root zone. Oyewole and Kalejaiye (2012) stated another factor affecting microorganism activities was crop cultivar. Walker *et al.* (2003) stated soil microorganism population and development was promoted by energy resources in soil causing plant root metabolism to release exudates.

Inpago 10 variety had *Azotobacter* and PSB population in the highest level, and dense AMF root colonization, therefore N, P, and K availability increased promoting high rice yield. The inherent genetic factor also affected all varieties' yield including the number of pithy rice grain and rice grain weight (Xing and Zhang, 2010; Anyaoham *et al.,* 2018), which is equally influenced by environmental condition (Huang *et al.,* 2010; Ikeda *et al.,* 2013). Herawati *et al.* (2019) reported the number of pithy rice grain per panicle and rice grain weight was significantly affected by genetic factors.

The results also showed humic acid application on rice planting media promoted the highest *Azotobacter* and PSB population, as well as AMF root colonization. The humic acid applied ameliorated the rhizosphere ecosystems, leading to a suitable root zone for soil microorganism development. In line with previous research, humic acid promoted soil health especially by improving microorganism growth (Tikhonov *et al.,* 2010; Canellas and Olivares 2014). Inorganic fertilizers application in different perspective from this research indicated reduced soil microorganism activities. Yunus *et al.* (2017) reported synthetic fertilizers applied decreased soil microorganism population. Triyono *et al.* (2013) stated continuous synthetic fertilizers application for long periods makes soil more acidic, causing unhealthy microorganism activities.

Humic acid application increased soil microorganism activities and rice yield. This also produced the highest number of pithy rice grain per panicle and rice grain weight per plant. An increase in *Azotobacter* and PSB population, as well as AMF root colonization, lead to increasing N, P, and K plant uptake which promoted high rice yield. Eshwar *et al.* (2017) stated humic acid increased N, P, and K uptake by the rice planted with a dryland system. Also, Ahmed *et al.* (2013) stated humic acid forms chelate with micronutrients to ensure a slow rate release during plant growth, hence avoiding micronutrients precipitation, fixation, leaching, and oxidation in soil.

It concluded that the *Inpago 10* rice variety planted on agricultural coastal land had the highest yield performance. Furthermore, humic acid application promoted the highest *Azotobacter* and PSB population, as well as AMF root colonization and rice yield. *Inpago 10* rice variety combined with humic acid treatment gave the highest soil pH, 1000-rice grain weight, and rice grain dry weight. Also, the estimated yield in the planting media reached 5.13 ton ha^{-1}, which was higher than the average harvest of 4.0 ton ha^{-1}. This result is still below the potential yield of 7.3 ton ha^{-1} for the provided rice description.

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