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Optimization of upland rice yields inoculated with *Azotobacter*, *Agrobacterium tumefaciens,* and *Mycorrhizae* through humic acid and micro-Fertilizer provision in Coastal Land

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Abstract The result showed that the treatment combination of 16 L ha^{-1} humic acid and 140 g ha^{-1} micro-fertilizer produced the heaviest grain weight of 4.03 kg $plot^{-1}$ or an equivalent of 7.16 tons ha^{-1}. Yield was higher than the average upland rice production of the Inpago 10 variety, i.e. 4.0 tons ha^{-1} and it reached 98.08% of the potential yield of 7.30 tons ha^{-1}. The best dose of humic acid was 16 L ha^{-1} which produced the highest grain weight per plant, pH-KCl, plant tissue N and P content, and seed Zn and Fe. Furthermore, the best dose of micro-fertilizer was 140 g ha^{-1} which generated the greatest number of paddy grains per panicle and grain weight per plant, as well as the highest P content in the tissue, and Cu, Zn, Mn, and Fe in seeds.

Keywords: Humic acid, Coastal area, Micro fertilizer, Upland rice

Introduction

Indonesia is a maritime country with the second longest coastline in the world after Canada. This coastal area has been marginalized from agricultural activities due to unfavorable soil characteristics and properties that are harmful to plants. The main problem with coastal sandy soils is low water and nutrient retention because of the minor clay and organic matter content (Minhal *et al.,* 2020), lesser microelements (Naher *et al.,* 2011), and high salt content which will lead to decreased crop yields (Fogliatto *et al.,* 2019; Machado and Serralheiro, 2017; Rad *et al.,* 2011). However, coastal land can be used for cultivation provided the right technology is applied.

One of the agricultural commodities that have the potential to be cultivated in coastal areas is upland rice. It is important to note that rice is very

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susceptible to rhizosphere salinity compared to other cereal crops (Hussain *et al*., 2017). Salinity stress interferes with rice growth, causing a decreased yield (Dolo *et al.,* 2016; Tahtay *et al.,* 2013). This was reported to reduce the number of productive tillers to 27% and grain yields to 50% (Dramalis *et al.,* 2020). Therefore, appropriate planting technology is needed in increasing the carrying capacity of coastal land to obtain optimal upland rice yield, which includes providing fertilizer in a humic acid and microelement form.

Humic acid is an organic substance with a complex molecular structure and a high molecular weight containing an active group. Moreover, it attracts positive ions and forms chelate with micronutrients, which are released slowly once needed by plants (Ahmed *et al.,* 2013). Humic acid has great potential in increasing soil fertility and improving plant growth (Canellas and Olivares, 2014; Li, 2020), while the application has been shown to increase rice yields (Minarsih *et al.,* 2021).

Micronutrients are as important as macronutrients in plant nutrition (Siddika *et al.,* 2016). Meeting the needs of microelements is necessary for optimal rice yields (Husnain *et al.,* 2019; Lahijani *et al.,* 2020; Liew *et al.,* 2010). In contrast, microelement deficiency is one of the problems encountered in rice cultivation (Nadeem and Farooq, 2019). This is because the lack of even one essential micronutrient only can interfere with plant development and reduce yields (Tripathi *et al.,* 2015). Microelements that can affect rice yields include boron (B), copper (Cu), iron (Fe), manganese (Mn), and zinc (Zu) (Lahijani *et al.,* 2021; Rahman *et al.,* 2020).

The application of micro-fertilizers and humic acid is expected to increase upland rice yields in coastal areas. Therefore, this study aimed to determine the best dose of micro-fertilizer and humic acid and their combination in increasing upland rice yields in coastal areas.

Materials and methods

This study was conducted from July to November 2021 in the experimental field of the Agricultural Faculty, Bengkulu University, Indonesia. Biofertilizer inoculation and the measurement of plant dry weight, initial and final soil analysis, and tissue nutrient content assessment were carried out at the Soil Biology Laboratory of Bengkulu University.

Moreover, a split-plot design was used with humic acid as the main plot consisting of three dosage levels, namely 0 L ha^{-1}, 8 L ha^{-1}, and 16 L ha^{-1}, while the subplots were micro-fertilizers consisting of five dose levels, namely 0 g ha-1, 35 g ha^{-1}, 70 g ha^{-1}, 105 g ha^{-1}, and 140 g ha^{-1}. The treatment combinations were repeated four times to obtain 60 experimental units, each of which contained 50 plants, making the total population 3,000 plants. The data were analyzed using analysis of variance (ANOVA) at a 5% level. Significantly different variables were further separated by DMRT at a 5% level.

Land preparation began with weed clearing, then the soil was processed using a hoe. The experimental plots were made to have a size of 3 m x 1.5 m with the distance between plots being 50 cm, while the distance between replications was 100 cm. Afterward, 10 tons ha^{-1} of coffee husk compost and 200 kg ha^{-1} of dolomite with the active ingredient CaMg(CO3)2 were added to the land surface. The inorganic basic fertilizers were applied at 25% of the dose recommended by Jember Plant Seed Center, i.e. 50, 25, and 25 kg ha^{-1} of Urea, SP-36, and KCl, respectively. Urea fertilizer was applied twice, namely half of the dose during planting and the rest after one month, while TSP and KCl fertilizers were given at the same time of planting.

Humic acid was applied by spraying evenly on the soil surface, 2 days before planting. Each dose was dissolved in distilled water with a ratio of 1 to 40. Microfertilizer application was carried out by spraying after diluting in water with a ratio of 1 to 8000 (w/v). This was conducted twice when the plants began to flower and 2 weeks later. The tested micro-fertilizers contained compound microelements of Fe, Mn, Zn, Cu, B, and Mo of 2.5, 7, 5, 2, 2, and 0.1%, respectively.

The next step was planted for three paddy seeds in holes measuring 5 cm in depth each at spaces of 30 cm x 30 cm. Furthermore, they were inoculated with biofertilizers of Azotobacter (N-fixing bacteria), Agrobacterium tumefaciens (K-solvent bacteria), and arbuscular mycorrhizal fungi (P-solvent) as recommended in the previous report by Bertham *et al.* (2020). The biological fertilizers used had been identified for genomic DNA through the methods of Sambrook and Russel (2001) in the Biology Laboratory of the Mathematics and Natural Sciences Faculty, Bengkulu University.

Plant maintenance was conducted according to cultivation practices such as watering, replanting, weeding, and controlling pests and plant diseases. Watering was performed daily in the afternoon when not rainy, embroidery was carried out for seeds that failed to grow, weeding was done manually, while pests and diseases were controlled organically by spraying the leaf extract of soursop and in case of severe attacks, chemical pesticides were used.

Harvesting was carried out on the matured plants as indicated by 85% of the rice panicles that became golden yellow and 90% of their yellow paddy grains while the panicles were drooping. The grains were hard when pressed by hand and left with no marks. The rice was harvested by cutting the base of the panicles using scissors, then they were put inside an envelope for observation. The study variables observed were pH-KCl (electrometry 1:2.5), N, P, and K content in plant tissue (wet instructions of H2SO4 pa. and H2O), Cu, Zn, Mn, and Fe in the seeds, number of productive tillers, number of pithy grains per panicle, the weight of pithy grain per plant, and weight of pithy grain per plot.

Results

The result showed a significant interaction between the humic acid and micro-fertilizer dose on the weight of grain per plot. The application of humic acid with different doses had significantly affected on pH-KCl, N and P content in plant tissue, grain weight per plant, grain weight per plot, and the content of Zn and Fe in seeds. The application of micro-fertilizer with different doses were significantly affected P content in tissue, the number of grains per panicle, grain weight per plant, grain weight per plot, and the content of Cu, Zn, Mn, and Fe in seeds as presented in Table 1.

Table 1. Summary of F-counts from ANOVA

Variables observed	F-count			CV (%)
	Humic acid	Micro-fertilizer	Interaction	
pH-KCl	8.44*	2.53 ^{ns}	1.76 ^{ns}	4.44
Number of productive tillers	0.37 ^{ns}	1.53 ^{ns}	1.33 ^{ns}	14.31
N-tissue content	6.23*	1.25 ^{ns}	1.29 ^{ns}	12.32
P-tissue content	5.89*	2.98*	0.98 ^{ns}	16.49
K-tissue content	4.26 ^{ns}	2.12 ^{ns}	1.02 ^{ns}	15.75
Cu-seed content	5.09 ^{ns}	3.21*	0.82 ^{ns}	15.82
Zn-seed content	6.78*	12.67*	1.29 ^{ns}	19.21
Mn-seed content	2.19 ^{ns}	8.34*	0.92 ^{ns}	20.13
Fe-seed content	9.23*	16.79*	2.19 ^{ns}	16.32
Number of pithy grains per panicle	1.44 ^{ns}	3.95*	0.81 ^{ns}	22.89
The weight of grains per plant	9.06*	51.96*	1.57 ^{ns}	11.63
The weight of grains per plot	8.49*	26.59*	2.78*	11.78
F-table 5%	5.14	2’63	2.21	-

Note: * = significant effect, ^{ns} = non significant effect, CV = Coefficient of Variation

The weight of grains per plot showed significant differences among the micro-fertilizer doses either once added with humic acid at a dose of 8 and 16 L ha^{-1} or without it. The application of micro fertilizer at 105 g ha^{-1} produced the heaviest grains per plot in the absence of humic acid, but when humic acid was applied at 8 and 16 L ha^{-1}, the heaviest grain per plot was generated by giving micro-fertilizer at 140 g ha^{-1}. On the other hand, the weight of grain per plot was not significantly different between the humic acid doses when not fertilized with microelements. Once micro-fertilizers were applied, the weight of grains per plot was significantly different among the humic acid doses, where a dose of 16 L ha^{-1} consistently produced the heaviest grains per plot as presented in Table 2.

Table 2. The effect of humic acid and micro-fertilizer on the weight of grains per plot (kg)

Humic acid dose (L ha^{-1})	Micro-fertilizer dose (kg ha^{-1})				
	0	35	70	105	140
0	2.57 c A	2.59 c B	3.11 b B	3.34 a B	3.21 a C
8	2.60 c A	3.34 b A	3.72 b A	3.95 a A	3.99 a B
16	2.78 c A	3.22 c A	3.81 b A	3.92 b A	4.03 a A

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

Based on the results, the 16 L ha^{-1} humic acid generated the highest pH-KCl of 4.82, which was not significantly different from the 8 L ha^{-1} dose, but different from the humic acid with the lowest pH-KCl production (i.e. 4.45) as presented in Table 3.

Table 3. Effects of humic acid on tissue N and P content, seed Zn and Fe content, and grain weight per plant (GWPP)

Humic Acid Dose (L ha^{-1})	pH KCl	N-tissue (%)	P- tissue (%)	Zn-seed (ppm)	Fe-seed (ppm)	GWPP (g)
0	4.45 b	1.25 b	0.29 c	16.76 b	29.48 b	50.15 b
8	4.69 ab	1.34 b	0.36 b	21.43 a	34.54 a	67.53 a
16	4.82 a	1.66 a	0.41 a	22.53 a	35.26 a	67.89 a

Note: A number followed by the same letter in the same column means no significant difference

In addition to increasing soil pH, the application of humic acid also elevated the content of N and P in plant tissue as well as Zn and Fe in seeds. The use of humic acid at 16 L ha^{-1} produced the highest contents of tissue N and P as well as seed Zn and Fe, namely 1.66%, 0.41%, 22.53, and 35.36 ppm. The tissue N and P generated by 16 L ha^{-1} humic acid were significantly different from other doses, while the Zn and Fe contents in seeds were not significantly different when using 8 L ha^{-1}, but significantly different from a dose of 0 L ha$^{- 1}$. Furthermore, without humic acid application, the lowest tissue N and P content was observed at 1.25 and 0.29% as displayed in Table 3.

Table 4 showed that the application of140 g ha^{-1} micro-fertilizer produced the highest tissue P content, namely 0.40%, which was not significantly different from the 70 g ha^{-1} and 105 g ha^{-1} doses. However, it was significantly different from using the 35 g ha^{-1} dose and control which produced the lowest tissue P content of 0.24%. The result of 35 g ha^{-1} was not significantly different from the control (0 g ha^{-1}), but both were significantly different from the 70, 105, and 140 g ha^{-1} doses.

Table 4. Effects of micro-fertilizer on tissue P content, Cu, Zn, Mn, and Fe content of seeds, number of grains per panicle (NGPP), and grain weight per plant (GWPP)

Micro-fertilizer dose (g ha^{-1})	P-tissue (%)	Cu-seed (ppm)	Zn-seed (ppm)	Mn-seed (ppm)	Fe-seed (ppm)	NGPP	GWPP (g)
0	0.24 b	4.65 c	17.54 c	29.67 c	23.65 c	116.84 c	39.30 c
35	0.28 b	5.69 b	19.24 b	32.86 c	30.35 b	123.34 c	57.01 b
70	0.36 a	5.89 b	22.19 ab	38.18 b	35.26 a	140.22 b	62.87 b
105	0.39 a	6.02 a	23.82 a	42.42 a	37.43 a	154..70 a	72,24 a
140	0.40 a	6.10 a	24.03 a	42.43 a	37.86 a	157.48 a	77,85 a

Note: A number followed by the same letter in the same column means no significant difference

The application of microelements increased the Cu, Zn, Mn, and Fe content in rice seeds. The 140 g ha^{-1} dose of micro-fertilizer produced the highest rice seed Cu, Zn, Mn, and Fe levels of 6.10, 24.03, 42.43, and 37.86 ppm, respectively. Meanwhile, without the micro-fertilizers, the lowest levels of Cu, Zn, Mn, and Fe were generated in rice seeds. There was no significant difference in seed levels of Cu and Mn between the 105 and 140 g ha^{-1} doses.

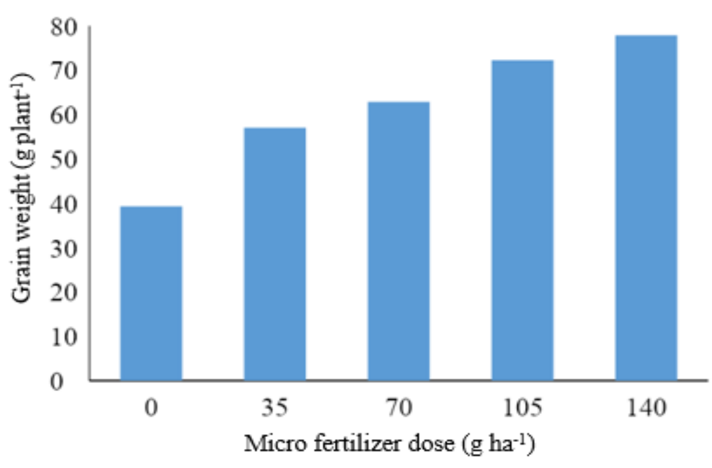

Figure 1. The effect of micro-fertilizer doses on grain weight per plant

Micro-fertilizer application at 140 g ha^{-1} produced the highest number of pithy grains per panicle and the heaviest grain weight per plant, namely 157.48 seeds and 77.85 g, respectively, but not significantly different from 105 g ha^{-1}

and different from other doses. Meanwhile, without micro-fertilizer application, the number of grains per panicle and the lowest grain weight per plant were 116.84 pieces and 29.30 g, respectively, as presented in Table 5. The results indicated that the use of a 105 g ha^{-1} dose was sufficient to augment upland rice yields to ensure increasing the micro-fertilizer dose will not affect the yield as displayed in Figure 1.

Discussion

The results showed that grains weight per plot elevated with increasing doses of humic acid and micro-fertilizers, where the heaviest grains weight per plot was produced by a combination of 16 L ha^{-1} humic acid and 140 g ha^{-1} micro-fertilizer of 4.03 kg $plot^{-1}$ or 7.16 tons ha^{-1}. This yield is higher than the average upland rice production of the Inpago 10 variety, which is 4.0 tons ha^{-1}, and it has reached 98.08% of the potential yield of the Inpago 10 variety, i.e. 7.30 tons ha^{-1}. This is because humic acid can improve soil fertility, thereby increasing nutrient uptake (Noroozisharaf and Kaviani, 2018) which has an impact on elevating rice yields (Suhardjadinata *et al.,* 2015). On the other hand, the micro-fertilizer application can meet the microelement of rice to increase the yields, because microelements are important minerals needed by plants. Microelements have been indicated to increase rice yields in saline soils. The combined application of humic acid and micro-fertilizers was reported to increase maize yield (El-Mekser *et al.,* 2014).

Humic acid application at 16 L ha^{-1} produced the highest pH-KCl. This is because humic acid releases OH- ions from the carboxyl group (-COOH) and hydroxyl group (-OH) causing the soil pH to rise. This is in line with the results of previous studies which also reported an increase in soil pH after humic acid application (Ifansyah, 2013; Zaremanesh *et al.,* 2020).

Humic acid can increase the availability of macro and micronutrients in the soil (Manjeera *et al.,* 2021). This acid produces auxin which is useful for promoting root development, thereby increasing nutrient absorption (Jomhataikool *et al.,* 2019). Moreover, it forms chelates with microelements, which are released gradually once needed by plants and prevent the formation of precipitation, fixation, leaching, and oxidation of micronutrients in the soil (Al-Issawi *et al.,* 2021; Eshwar *et al.,* 2017). Previous studies also reported that humic acid application tends to augment the levels of N, P, Zn, and Fe in rice plant tissue (Chang *et al.,* 2012; Manjeera and Subbaiah, 2021; Palanivell *et al.*, 2015; Saha *et al.,* 2014; Vijayakumar *et al.,* 2021). In addition, the increase in rice seed levels of Zn and Fe levels was due to humic acid containing several microelements which had an impact on elevating the uptake of Zn and Fe in the seeds (Hu *et al.,* 2014).

The result showed that humic acid application at 8 L ha^{-1} was sufficient to augment the yield of upland rice, indicating the increase in the dose of humic acid did not significantly increase the yield. Humic acid increased soil fertility as indicated by the rise in pH-KCl, tissue N and P as well as the seed Zn and Fe contents as demonstrated in Table 3. Increasing soil pH will elevate the availability of nutrients (Ahmad *et al.,* 2022) to ensure the uptake of nutrients such as N and P by plants also increases, which has an impact on augmenting rice yields (Al-Bourky *et al.,* 2021; Hussain *et al.,* 2022).

The tissue phosphorous content is elevated with increasing doses of microelements because micronutrients can increase root development which promotes nutrient absorption from the soil. Furthermore, micronutrients regulate the auxin concentrations and have a simulator effect on most of the physiological and metabolic processes of plants, which possibly assists in the absorption of larger nutrient quantities from the soil (Choudhary *et al.,* 2017). The levels of Zn and Fe in grain seeds were also not significantly different among the 70, 105, and 140 g ha^{-1} doses of micro-fertilizers as presented in Table 4. The content of Cu, Zn, Mn, and Fe in rice seeds elevated along with the increasing dose of micro-fertilizer. This was because the greater the applied dose, the more Cu, Zn, Mn, and Fe elements were absorbed by the plants, therefore, the accumulation in the seeds became higher (Sharifianpour *et al.,* 2014).

The increase in rice yields due to micronutrient application was also reported by Girma and Sisay (2020); Liu *et al.* (2018); and Mahmoodi *et al.* (2020). The elevation of upland rice yields because of micro-fertilizer application is related to the role of each microelement in rice. The Cu element plays a role in nitrogen exchange, protein and hormone formation, respiration, and photosynthesis (Shrestha *et al.,* 2020). Zinc (Zn) participates in growth and development and is involved in many major functions of plants including maintenance of cell membrane structure, photosynthesis, hormone activity, lipid and nucleic acid metabolism, gene expression and regulation, protein synthesis, and defense against drought and diseases (Hafeez *et al.,* 2013). It also induces enzyme activity and auxin metabolism in plants (Ahmad and Khan, 2016) and has been shown to increase rice yields (Fageria and Baligar, 2005; Tuiwong *et al.,* 2021). The Mn element plays a role in activating more than 35 enzymes in plants (Mousavi *et al.,* 2011), catalyzing water molecules breakdown during the photolysis process of photosynthesis (Aref, 2012), and influencing rice production (Yomso and Bhagawan, 2021). Iron (Fe) is an essential micronutrient for plant cell function, which acts as a cofactor in metabolic processes, specifically during photosynthesis (Zheng *et al.,* 2009), and can increase rice yields (Ibrahim *et al.,* 2018).

It is concluded that fertilization using a combination of 16 L ha^{-1} humic acid and 140 g ha^{-1} micro-fertilizers generated the heaviest grain weight per plot of 4.03 kg $plot^{-1}$, equivalent to 7.16 ton ha^{-1}. Yield was higher than the average upland rice production of the Inpago 10 variety, which is 4.0 tons ha^{-1} and it has reached 98.08% of the potential Inpago 10 yield, namely 7.30 tons ha^{-1}. The best dose of humic acid was 16 L ha^{-1} which produced the highest pH-KCl, tissue N and P, seed Zn and Fe content, and grain weight per plant. Furthermore, the best dose of micro-fertilizer was 140 g ha^{-1} which produced the greatest tissue P, seed Cu, Zn, Mn, and Fe, as well as the highest number of pithy grains per panicle and grain weight per plant.

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