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Enhancing Rice Production on Acid Sulfate Soil Using Bio-fertilizer in Combination with Ground Magnesium Limestone or Biochar

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ABSTRACT

Low pH acid sulfate soils are usually nutrient-deficient. They contain toxic metals that affect rice plants negatively. A field experiment was carried out to alleviate acidity in an acid sulfate soil for rice cultivation. In the study, rice seedlings (variety MR219) were planted in the experimental plots treated with bio-fertilizer, ground magnesium limestone (GML) and biochar, either alone or in combination. pH of the untreated topsoil was 3.56, while the exchangeable calcium and magnesium in the topsoil was 4.13 $cmol_c kg^{-1}$, below the requirement to sustain growth and/or production of rice. Addition of bio-fertilizer slightly increased soil pH. The highest soil pH of 5.34 was observed in the plot treated with bio-fertilizer plus GML. Iron in the control plot was believed to exist in the form of Fe^{3+} (pKa 3). Due to treatment with bio-fertilizer plus GML or biochar, soil pH increased from 3.56 to a level >5. As the soil pH was approaching 4.58 (i.e., the pKa of Fe^{2+}), Fe^{3+} was slowly converted to Fe^{2+}. The form of iron causing toxicity to rice in the treated plots was most likely to be Fe^{2+}, rather than Fe^{3+}. At soil pH >5, both Fe^{2+} and Al^{3+} (the pKa is 5) were precipitated as inert hydroxides, thus, no longer causing toxicity to the rice plants. Beneficial microbes present in the bio-fertilizer helped produce growth hormones and organic acids that eventually increased nutrient uptake by rice which in turn enhanced its growth. The organic acids fixed some Fe^{2+} and Al^{3+} in the soil via chelation process. This phenomenon further reduced their toxicity to the rice plants. Application of bio-fertilizer plus GML or biochar improved soil fertility that resulted in higher rice yield. This notion is supported by the enhancement of the rice yield parameters, i.e., plant height (92.21 cm), tiller numbers (6), leaf chlorophyll content (38.14) and the number of filled grains. The use of bio-fertilizer plus GML or biochar is recommended for rice cultivation on acid sulfate soils in order to increase rice self-sufficiency level (SSL) and sustain food security in country in the long run.

Key words: Biochar, bio-fertilizer, ground magnesium limestone, organic acids, plant growth, rice

INTRODUCTION

Sustaining food security throughout the world is one of the main concerns of humans living on earth. Food should be available to anyone who needs it. Notwithstanding, fertile agricultural lands across the globe are slowly being converted to areas not meant for the purposes of food production. If this goes on unchecked, food production to feed the increasing world population will be endangered. We are now seeing farmers starting to utilize available marginal lands in their countries to make a living via agriculture. Attention is currently focused on the vast areas of acid sulfate soils, sporadically distributed along the low-lying coastal regions of the tropics. This very infertile soils found abundantly in Thailand, Vietnam, Indonesia and Malaysia are being cropped to rice, but the yield is lower than that of their national average (Shamshuddin *et al*. 2014).

Acid sulfate soils contain pyrite (FeS_2) which on draining releases sulfuric acid due to its oxidation. In the end, Fe and Al are available in the soils at a toxic level, causing problems to plants and other living organisms. Moore *et al*. (1990) and Shamshuddin *et al*. (2014) found that the most significant restraints to crops growing on acid sulfate are: (i) Acidity (comprising the collective influences of pH, Al-toxicity and nutrient deficiencies), and (ii) Fe-stress (which might be due to the impacts of Fe^{2+}-toxicity and insufficiency of divalent cations)). Application of organic-based nutrient sources on acid sulfate soils could boost the reduction process (Muhrizal *et al.* 2006), leading to the release of Fe^{2+} which is toxic to rice plants (Tran and Vo 2004).

A normal practice to reduce toxicity due to the presence of Al^{3+} and Fe^{2+} is to raise soil pH to a level > 5 via lime application (Haby 2002; Anda *et al*. 2009). The capability of lime to upsurge soil pH and reduce Al and Fe solubility to enhance crop yield is widely known (Brown *et al,* 2008). Application of biochar can increase soil organic carbon, soil pH, and cation exchange capacity (CEC). Application of biochar and compost has been shown to improve soil fertility significantly, including increased soil pH and CEC (Kuzyakov *et al.* 2017). However, biochar application not only enhances soil fertility, but also results in C sequestration (Cooper *et al.* 2020). For biochar, the pyrolysis process, feed-stock and time period are the major components of the controlling factors, which directly affect soil physical and chemical properties (Weber and Quicker 2018). According to Sun *et al*. (2022), the improvement in soil properties through adding biochar depends a lot on soil environmental factors, particularly soil pH and soil texture.

Other agronomic practices to ameliorate acidity problems in acid sulfate soils are submergence, leaching and applying MnO_2 (Sobouti *et al.*, 2020), application of basalt (Jayalath *et al*. 2016) or using bio-fertilizer (Panhwar *et al*. 2014). For rice plants, a better option to overcome low pH stress (acidic condition) and Al or Fe toxicity is application of ground magnesium limestone or ground basalt in combination with a bio-fertilizer, fortified with beneficial microbes (Panhwar *et al.* 2014). Therefore, a field study was designed and conducted to enhance the fertility of an acid sulfate soil in Peninsular Malaysia to increase rice yield through applying bio-fertilizer in combination with ground magnesium limestone or biochar.

MATERIALS AND METHODS

Experimental Site

The field study was conducted on an acid sulfate soil in Semerak-Kelantan, Malaysia, using rice variety MR219. The soil was classified as Typic Sulfaquepts based on Soil Taxonomy (Soil Survey Staff 2014). Soil samples were taken from the experimental site at a depth of 0-15 cm using an auger for soil characterization. The experimental site, about five meters above sea level, is located at latitude of 5°52ʹ 208”N and longitude of 102°28ʹ 501”E.

Experimental Details

Bio-fertilizer with or without GML and biochar were applied in the experimental plots 15 days before transplanting rice seedlings. The experiment comprised: (1) Control; (2) Bio-fertilizer; (3) Bio-fertilizer+GML; and (4) Bio-fertilizer+biochar, applied with the amendments at 4 ton ha^{-1} in each of the 5×5m size experimental plot. Nitrogen (using urea), phosphorus (using TSP) and potash (using MOP) were given at the rate of 120, 30 and 60 kg ha^{-1}, respectively. The initial pH of the untreated soil was 3.56. pH data were recorded monthly till harvest. The experimental design was randomized complete block design (RCBD), with four replications.

Chemical properties of bio-fertilizer, GML and biochar

The bio-fertilizer (with pH 7.30) used in the study contained N (1.19%), P (0.13%) and K (0.63%), while the Al and Fe contents were low (<0.01) (Table 1). The bio-fertilizer contained phosphate-solubilizing and N_2-fixing bacteria (1×10^{-8} CFU g^{-1}). GML was taken from an authorized supplier in Malaysia. Table 1 shows that the pH of the GML was high (9.75). Note that the GML used in the study contained some P (1.71 %), but with high amounts of Ca (19.47 %) and Mg (8.67 %). The biochar used was alkaline in nature with low content of N, P and K (0.54, 0.14 and 3.49%, respectively) with some Fe, Al, Ca and Mg (Table 1).

TABLE 1
Chemical properties of the bio-fertilizer, GML and biochar used in the study

Source	pH	EC (dS m^{-1})	N	P	K	Fe	Al	Ca	Mg	N_2 fixing bacterial population (CFU g^{-1})
			(%)							
Bio-fertilizer	7.30	3.64	1.19	0.13	0.63	< 0.01	0.01	< 0.01	< 0.01	1×10^{-8}
GML	9.75	-	na	1.71	0.33	< 0.01	< 0.01	19.47	8.67	na
Biochar	9.85	1.48	0.54	0.14	3.49	< 0.01	< 0.01	0.13	0.078	na

*na = not available, GML = Ground magnesium limestone

Rice Seedlings and Transplanting

MR 219 rice seeds were surface-sterilized following the method of Amin *et al.* (2004). Twenty-one-day old rice seedlings were transplanted (4×4 cm) in the experimental plots (4×4 m) at a distance of 20×20 cm plant to plant and row to row. The experiment was continued till crop harvest - rice agronomic and yield data were taken at harvest.

Soil Analysis

Soil pH was analyzed using a pH meter (Benton 2001). Exchangeable Ca, Mg, and K in the soil were determined by the method of Schollenberger and Simon (1945) using atomic absorption spectrometry (Perkin Elmer, 5100 PC, USA). Exchangeable Al extracted by 1 M KCl (Barnhisel and Bertsch 1982) was analyzed by inductively coupled plasma atomic - emission spectroscopy (ICPAES). Organic carbon was determined by the Walkley-Black method, and total N was analysed by Kjeldahl digestion method (Bremner and Mulvaney 1982). Available P was determined by the Bray and Kurtz II method (Bray and Kurtz 1945). Extractable Fe in the soil was analyzed by the double acid method.

Agronomical Parameters and Rice Yield

Rice in the experimental plots was harvested at crop maturity and plots (one-meter square) were selected for the rice grain and straw yield calculations. For the calculation, rice filled grains were unglued from the unfilled grains following the method of Seizo (1980). The chlorophyll (SPAD) values were determined using a MINOLTATM, SPAD-502 meter (Konica Minolta, Tokyo, Japan), while plant height, root length, tiller plant^{-1}, number of panicles plant^{-1} and panicles size were determined by the Dobermann and Fairhurst (2000) method.

Statistical Analysis

All data were statistically examined for analysis of variance (ANOVA) using SAS-Software Program Version-9.3 (SAS 2003). The experimental design was randomized complete block using four replications. The means were separated by Tukey's test (5 %) level of confidence.

RESULTS AND DISCUSSION

Chemical Properties of the Untreated Soil

Soil used in the present study was a real acid sulfate soil based on the data presented in Table 2. It was identified as an acid sulfate soil because of the very low soil pH and the occurrence of the yellowish jarosite mottles in the top 50 cm of the soil profile. The initial soil pH in the experimental plot was 3.56, while the EC was 0.34 dS m^{-1}. The amount of N, K, Ca and Mg in the topsoil was insuffucient to support or sustain rice production. On the other hand, exchangeable Al in the topsoil was very high (4.13 cmol$_c$kg^{-1}). This could cause toxicity to the rice growing in the field plots without undergoing amelioration process through agronomic means. However, the soil contained an adequate quantity of organic matter which to, a certain extent, helped remove Al and/or Fe via the chelation process. Addition of the bio-fertilizer with microbes is likely to reduce Al^{3+} and/or Fe^{2+} toxicity and eventually enhance nutrient uptake by the rice plants. This notion is supported by a study conducted earlier by Johan *et al.* (2021).

TABLE 2
Chemical characteristics of the acid sulfate soil

Soil pH	EC	CEC	Soil OC content	Total N content	Av.P	Exchangeable cations			
						K	Al	Ca	Mg
	(dS m^{-1})	($cmol_c kg^{-1}$)	(%)	(%)	(mg kg^{-1})	--------------- ($cmol_c kg^{-1}$) ----------------			
3.56	0.34	5.92	2.03	0.12	18.73	0.05	4.13	0.49	0.71

CEC = Cation exchange capacity, OC= organic carbon

Effect of Treatments on Soil pH

The acid sulfate soil under study contained pyrite, which on oxidation altered to yellowish jarosite (Shamshuddin *et al*, 2014). During the process of pyrite oxidation a high amount of Fe or even Al was released into the soil solution and environment. The hydrolysis of the acid metals intensified the acidification of the soil, reducing its pH to lower than 4 or close to 3. Addition of the bio-fertilizer in combination with the amendments increased soil pH to a value of >4.0 (Table 3), and it stayed at that level till the rice crop was harvested. It appears that the application of bio-fertilizer alone did not increase soil pH much. However, treating the soil with the bio-fertilizer combined with GML or biochar augmented soil pH upto above 5. The maximum soil pH of 5.34 was attained by treating the soil with the bio-fertilizer in combination with GML (Table 3).

The form of iron and aluminum existing in the soil (or soil solution) hinges on soil pH. The pKa of Fe^{3+} and Fe^{2+} are respectively 3.00 and 4.58, while the pKa of Al^{3+} is 5.00. Thus, iron in the water of the untreated acid sulfate soil with pH 3.56 was most likely to be in the form of Fe^{3+}. Due to treatment with the bio-fertilizer in combination with the amendments, the pH of water was initially slightly increased to above 4. From then onwards, slowly but surely, Fe^{2+} started to form in high amounts. When the pH of water was close to 4.58, most of the iron was in the form of Fe^{2+}. The form of toxic iron that caused a reduction in rice growth and/or production was most likely to be Fe^{2+}, not Fe^{3+}. When pH of the water increased up to >5 (Table 3), Fe^{2+} and Al^{3+} were precipitated as inert hydroxides; thus, no longer being toxic to the rice growing in the field plots.

Treating the soil with the bio-fertilizer in combination with either GML or biochar augmented the soil pH significantly. This resulted in a reduction of exchangeable Al (Shamshuddin *et al.* 2014). At the soil pH >5, Al precipitates as Al-hydroxides. In this study, it was noted that applying GML or biochar in combination with the bio-fertilizer increased soil pH to that critical level. Therefore, this agronomic practice helped improve soil fertility. Eventually, the treated acid sulfate soil can be productively used for rice cultivation with yields comparable to that of the normal soils. The results of the current study were in agreement with those obtained by Rahman *et al.* (2018).

We are of the opinion that in the presence of bio-fertilizers, fortified with beneficial microbes, rice roots were able to produce growth hormones and/or organic acids. The organic acids so produced are known to fix and reduce Al^{3+} and Fe^{2+} concentration in the acid sulfate via the process of chelation (Panhwar *et al.* 2014; Panhwar *et al.* 2020). Thus, bio-fertilizers can play an important role in the alleviation Al^{3+} and Fe^{2+} toxicity for rice production on acid sulfate soils, not only in Malaysia, but also throughout the ASEAN region.

Syazana *et al*. (2013) and Syazana *et al*. (2014) stated that GML application increased soil pH leading to improved rice yield grown on an acid sulfate soil in Malaysia. Soil pH, total C and the CEC of the spodic layer of a Spodosol were increased by biochar treatment (Syuhada *et al*. 2022). According to these researchers, the CEC increase due to biochar treatment was positively correlated with the C content in the soil. It is plausible that a similar phenomenon can occur in an acid sulfate soil treated with biochar.

TABLE 3
Effect of treatments on soil pH under field conditions

	Soil pH				
	Initial	30DAS	60DAS	90DAS	At harvest
Control	3.56a	3.81d	4.07c	4.12d	4.03c
Bio-fertilizer	3.56a	4.17c	4.35b	4.31c	4.25b
Bio-fertilizer +GML	3.56a	5.34a	5.15a	5.20b	5.17a
Bio-fertilizer +biochar	3.56a	5.26b	5.29a	5.32a	5.11a

DAS= days after sowing. Means within the similar columns followed by similar letters are not significantly different (P<0.05)

Effects of Treatments on Nutrients in Soil

The highest macronutrient content was found in the bio-fertilizer treatments combined with GML or biochar. The significantly (P<0.05) highest N (0.19 %), P (31.02 mg kg^{-1}), Ca (2.57 $cmol_c$ kg^{-1}) and Mg (2.39 $cmol_c$ kg^{-1}) were observed in the bio-fertilizer plus GML treatment. As expected, the highest K (0.29 $cmol_c$ kg^{-1}) and Al (4.04 $cmol_c$ kg^{-1}) were observed in the bio-fertilizer plus biochar and the control treatments, respectively (Table 4). Microbes in the bio-fertilizer had the capability to fix N_2 and were also able to solubilize unavailable P increasing the macronutrients in the soil (Panhwar *et al*. 2014). GML addition significantly increased Ca and Mg contents in the soil. Upon dissolution of GML, soil pH readily increased to a higher level. When the pH reaches above 5, Al in the water would precipitate as inert Al-hydroxides and become inaccessible to the rice plants. It is believed that the main concerns and the most noticeable Al toxicity symptoms shown by rice plants are root growth inhibition (Frankowski 2016).

The effects of acidification have been critically noticed in the soil. The exchange of base cations (Ca^{2+}, Mg^{2+} and K^+) by H^+ and/or Al^{3+}, and the dissolution of Al, Fe and Mn minerals are the most important mechanisms for soil acidification (Goulding *et al*, 2016). Toxicity due to Fe^{2+} and/or Al^{3+} and inequity of nutrients such as P are believed to occur in acid sulfate soils. Toxicity of Al^{3+} is one of the major risk for the survival of plants grown on acidic soils (Bojórquez-Quintal *et al*, 2017). A significant portion of the nutrients initially present in the biochar in soluble form is susceptible to leaching losses. Panhwar *et al*. (2020) reported the positive impact of applying bio-fertilizer plus GML or biochar on the biochemical properties of acid sulfate in Malaysia in terms of increased rice yield.

TABLE 4
Effects of applying bio-fertilizer plus GML or biochar on macronutrients in soil.

Treatments	Total N	Av. P	Exchangeable cations			
			K	Al	Ca	Mg
	(%)	(mg/kg)	------------ ($cmol_c$/kg) ------------			
Control	0.12c	20.73c	0.16d	4.04a	0.64d	0.61d
Biofertilizer	0.15b	27.27b	0.27c	2.10b	0.97c	0.91c
Biofertilizer +GML	0.19a	31.02a	0.32b	0.87c	2.57a	2.39a
Biofertilizer + biochar	0.17a	30.95a	0.39a	0.89c	1.84b	1.65b

GML = ground magnesium limestone. Means within the similar column followed by the similar letters are not significantly different (P<0.05)

Effect of Treatments on Rice Plant Growth

The bio-fertilizer application combined with GML significantly enhanced plant height (92.21 cm), leaf chlorophyll SPAD values (38.14) and number of plant tillers (6) (Table 5). This might be the subsequent effects of increasing soil pH that reduced Fe^{2+}-toxicity (Panhwar *et al*. 2014). Soil pH regulates the chemistry of plants nutrient colloidal solutions. At certain pH levels, various stresses, i.e., H^+ toxicity and mineral imbalance and their deficiencies can occur in plants (Msimbira and Smith 2020).

The bio-fertilizer comprising a consortium of N_2-fixing and P-solubilizing bacteria produced some organic acids and growth promoting phytohormones, whichchelated Al^{3+} and Fe^{2+} in the soil and eventually enhanced rice growth. Phosphate-solubilizing bacteria converted the insoluble P into soluble form and this was made available to the rice plants. Similar findings were reported in an earlier study by Panhwar *et al*. (2014) that the production of phytohormones by beneficial bacterial present in the bio-fertilizer proliferated root growth and augmented nutrient uptake, which subsequently increased rice yield.

TABLE 5
Effects of treatments on rice growth and leaf chlorophyll content

Treatments	Plant height (cm)	Chlorophyll (SPAD value)	Tillers plant^{-1}
Control	79.34c	32.12c	4c
Bio-fertilizer	88.32b	36.67b	5b
Bio-fertilizer + GML	92.21a	38.14a	6a
Bio-fertilizer + biochar	90.65a	37.34a	6a

GML = ground magnesium limestone. Means within the similar column followed by the similar letters are not significantly different (P<0.05)

Effects of treatments on yield and yield contributing characters of rice

Bio-fertilizer, GML or biochar have been found to have a positive impact on crop growth. In this study, application of bio-fertilizer combined with GML or biochar significantly enhanced the yield and yield subsidizing characters of rice. The highest filled grain (83%), number of panicles (17), size of panicles (23.27 cm), rice grain (5.67 t ha^{-1}) and straw yield (10.39 t ha^{-1}) were observed in the bio-fertilizer in combination with GML, followed by bio-fertilizer plus biochar treatment (Table 6). Similar results were reported by earlier studies that biochar treatment improved soil quality. The new environment results in increasing soil microbial activities which enhance the growth and yield contributing parameters of crops (Yao *et al.* 2017; Wong *et al.* 2019).

TABLE 6
Effects of treatments on yield and yield contributing characters of rice

Treatments	Number of panicles $plant^{-1}$	Size of panicles $^{-1}$ (cm)	Number of filled grains (%)	Grain yield (t ha^{-1})	Straw yield (t ha^{-1})
Control	10c	19.41d	69c	3.45e	8.56d
Bio-fertilizer	14b	23.08c	78b	4.84c	9.65b
Bio-fertilizer + GML	17a	23.27a	83a	5.67a	10.39a
Bio-ferti.+biochar	16a	22.94b	81a	5.23b	9.83a

GML = ground magnesium limestone. Means within the similar column followed by the similar letters are not significantly different (P<0.05)

It is evident that bio-fertilizer plus GML or biochar had a substantial impact on the growth and yield of rice. Amending the acid sulfate soil with the bio-fertilizer in combination with GML or biochar improved soil fertility, which resulted in an upsurge of plant dry biomass and ultimately grain yield of rice. Syuhada *et al*. (2022) reported that the use of biochar combined with NPK fertilizers on a Spodosol sustained corn growth/production in long run. Similarly, the use of rice husk biochar with inorganic fertilizers improved nutrient availability and enhanced the growth and yield of rice in the acidic soils (Oladele *et al,* 2019). On other hand, GML alone could only supply additional calcium and magnesium to the acid sulfate soil. This is a good agronomic practice as the soil under this study had insufficient amounts of calcium and magnesium for healthy rice growth. Hence, the use of bio-fertilizer in combination with GML or biochar is an appropriate agronomic practice that not only reduced soil acidity and Al^{3+} and/or Fe^{2+} toxicity, but also increased soil calcium and magnesium to sustain crop production (Jones *et al.* 2003).

CONCLUSIONS

Application of the bio-fertilizer in combination with ground magnesium limestone or biochar improved fertility of the acid sulfate soil under investigation significantly and subsequently augmented rice yield. This notion is indicated or supported by the improvement of rice yield parameters such as plant height, tiller numbers, leaf chlorophyll content and the number of filled grains. Therefore, bio-fertilizers fortified with beneficial microbes applied together with GML or biochar is an excellent agronomic practice, which is recommended for rice cultivation on acid sulfate soils. The adoption of this agro-tech in Malaysia is likely to result in an increasing level of self-sufficiency in rice and lead towards sustaining food security for the country in the long run.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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