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ENHANCING THE FERTILITY OF AN ACID SULFATE SOIL FOR RICE CULTIVATION USING LIME IN COMBINATION WITH BIO-ORGANIC FERTILIZER

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Abstract

The acid sulfate soils contain pyrite (FeS_2) which is due to oxidation results in the production of high amount of acidity, aluminum and iron significantly affecting rice growth. A glasshouse study was arranged to determine the effect of ground magnesium limestone (GML) in combination with bio-organic fertilizer (JITU^{TM}) application on the chemical properties of soils and rice yield. Three rice seedlings were transplanted in pots which were previously amended with 0, 2, 4, 6 and 8 t/ha GML with or without bio-organic fertilizer. The common rice varieties (MR 219 and MR 253) were cultivated for two seasons in the same pots. The critical Fe^{2+} and Al^{3+} activities for MR 219 were 14.45 and 4.23 µM, while for MR 253 were 7.45 and 5.53 µM, respectively. However, without applying the amendments, rice grown on the soils was affected severely by the high acidity (Fe^{2+} and Al^{3+} toxicity). The soil pH increased to 5 and the higher grain yield of MR 219 (99.77 and 121.38 g/pot) and MR253 (98.63 and 112.60 g/pot) was in first and second season with the application of 2 t GML application combined with 0.25 t JITU^{TM}/ha respectively. In addition, 1000 grain weight, number of panicle, number of spikelets panicle^{-1} and the percentage of filled spikelet, were also higher than without the soil amendments. Hence, the infertility of acid sulfate soils for sustainable rice cultivation in Malaysia can be improved by applying 2 t GML/ha combined with 0.25 t JITU^{TM}/ha for two seasons in long run.

Key words: Acid sulfate soil, Aluminum and iron toxicity, Lime, Bio-organic fertilizer and rice.

Introduction

Global demand for rice is increasing by the years. Of late, however, more than 15 million people depend on the rice for their income generation and/or survival. Considering the significance of rice and its economic role in affected countries of the world, rice production should be improved significantly due to fast increasing world population. Unfortunately, to open up new land areas for rice cultivation in most Asian countries is not possible or practical due scarcity of good or fertile land. Therefore, using the available but less productive soils in these countries should be one of best options to increase rice production. Past studies had shown that acid sulfate soils which are sporadically disseminated in the coastal plains of Malaysia can be alleviated for sustainable crop production, especially for oil palm and rice (Shamshuddin & Auxtero, 1991; Shamshuddin *et al*., 2014). After ameliorating these soils using appropriate amendments, the productivity of the soils is expected to be increased to the level suitable for rice cultivation. There is a big challenge in using acid sulfate soils though because using farmers' practice the yield of rice obtained is < 2 t/ha/season, way below (3.8 t/ha/season) the national average yield.

Acid sulfate soils are categorized by the existence of pyrite. Under anaerobic conditions, pyrite forms when sulfate (SO_4^{2-}) from seawater and Iron (Fe^{3+}) from coastal sediments are reduced to S^{2-} and Fe^{2+} ions, respectively. These types of reactions are stimulated by the microorganisms which feed on the organic matter present in the sediments. If this pyrite is exposed to atmosphere due to land development, it will be oxidized, resulting in the release of sulfuric acid (Shamshuddin *et al*., 2004; Shamshuddin, 2006).

The Al toxicity is attached with P deficiency as in soil with high Al will lessen P availability due to Al-Fe-phosphate interaction (Liao *et al*., 2006; Dent, 1986). The Al at low pH in solution could reach at the critical level of 1-2 mg/L which could damage plant parts (Catling, 1992). However, the Fe toxicity is reported as one of the important limits for the growth of rice, like Al (Neue *et al*., 1998). In soil with low pH, presence of Fe is increased, which causes a higher Fe accumulation especially in the rice leaves. The bronzing leaves are the external symptom of Fe toxicity in rice plants and Fe can enhance the panicle sterility that may cause in the reduction of rice yield (Joseph & Albano 1996). The soluble Al and Fe accumulated in rice root because of their absorption by the negatively charged cells wall that in the end prevented cell division and significantly elongation of root (Elisa *et al*., 2011). Rice roots quickly absorb Al^{3+} and Fe^{2+}, causing a reduction in root length and inhibited root growth that reduces nutrients uptake (Gupta & Toole, 1986; Elisa *et al*., 2011).

The growth of crops grown on acid sulfate soils is not only exaggerated by the high acidity as well as Al and/or Fe, but also by the lack of P, Ca and Mg (Suswanto *et al*., 2007; Dent, 1986). Thus, by applying lime alone is not enough to improve the infertility of acid sulfate soils. It has been found that P (normally being fixed by Al and Fe) in the acidic soils could be made more available for rice growth than otherwise is by applying bio-organic fertilizer fortified with phosphate-solubilizing bacteria (Panhwar *et al*., 2014a; Panhwar *et al*., 2014b). Also the bio-organic fertilizer so applied is expected to produce balanced nutrition in the crops growing on the soils, especially macronutrients which positively affect their yield (Miller, 2007).

Liming appears to be the most common approach to raise soil pH, inactivate soluble Fe^{2+} and/or Al^{3+}, thereby reduces their toxicity. Moreover, increase in pH, lime (GML) can supply Ca and Mg to the soils that are needed in large amount for rice growth. It was reported that the yield of rice cultivated on acid sulfate could reach up to 4.5 t/ha application of GML at 2 t/ha/year (Suswanto *et al*., 2007). Under flooded conditions, pH increases, depending on the type of organic matter present (Muhrizal *et al*., 2006), but it is still below the critical level of 5. That means a lot of Al is still present in the water. According to Suswanto *et al*., (2007), application of GML in combination with bio-organic fertilizer on acid sulfate soils in the Kelantan Plains, Malaysia, could produce up to 7.5 t/ha of rice. It is therefore worthwhile to investigate further the use of lime in combination with bio organic fertilizer to amend the infertility of acid sulfate soils for rice cultivation.

According to Anon., (2010), high yielding rice variety (MR 219) although its non-acid tolerant and it is widely grown throughout Peninsular Malaysia. Recently, however, Anon., (2010) has released a new variety called MR 253, which was claimed to achieve better than MR 219 in the marginal soils (peat or organic soils). Hence, it is believed that MR 253 is considered as acid tolerant species by some. So far no study has been lead to test whether MR 253 is really acid tolerant or can be grown on acid sulfate soils smoothly. The objectives of the study was to determine the effects of applying ground magnesium limestone (GML) with or without bio-organic fertilizer (JITU™) on the chemical properties of acid sulfate soils and growth of rice varieties MR 219 and MR 253.

Materials and Methods

Location of soil sampling and background: The soil was taken from a rice field in the Integrated Agricultural Development Project (IADP), Kelantan situated in the northeastern part of Peninsular Malaysia (5.86009 N, 102.44119 E). The experimental soil was analysed first and it was found real acid sulfate soil and classified as Typic Sulfaquept and the common rice varieties i.e. MR 219 and MR 253 were selected for the experiment.

Experimental layout: Pot experiment was conducted in a glasshouse in Field 10, Universiti Putra Malaysia, Serdang, Malaysia. Three seedlings were transplanted in each pot containing 20 kg of topsoil (0-15cm depth). The soil in the pot was amended with 0, 2, 4, 6 and 8 t/ha GML with or without 0.25 t/ha bio-organic fertilizer (Table 1). The treated soils in the pots were mixed thoroughly before15 days of transplanting. N, P and K were applied at 120, 30 and 60 kg/ha, respectively. The experiment was laid out in Randomized Complete Block Design (RCBD) in 3 replications. The same experiment was conducted for another season in the same pot without applying the amendments.

Table 1. Description of the treatments in experimental pots.

Treatment	Description
T1	Control
T2	0.25 t/ha JITU
T3	2 t/ha GML
T4	2 t/ha GML + 0.25 t/ha JITU
T5	4 t/ha GML
T6	4 t/ha GML + 0.25 t/ha JITU
T7	6 t/ha GML
T8	6 t/ha GML + 0.25 t/ha JITU
T9	8 t/ha GML
T10	8 t/ha GML + 0.25 t/ha JITU

GML – Ground Magnesium Limestone, JITU – Sugar cane-based bio-organic fertilizer

Soil and water analysis: Soil sample collected from each pot was air-dried and passed through a 2 mm sieve while water samples for the analysis was taken 15 days after planting from the each pot. Soil pH was taken in water (1:2.5 soil: water) using a PHM210 Standard pH meter (Benton, 2001). Electrical conductivity (EC) was measured by an EC meter (Benton, 2001). Exchangeable bases (Ca, Mg and K) and cation exchange capacity (CEC) were analysed by the ammonium acetate buffered at pH 7 (Benton, 2001). Total carbon was analysed by the Carbon Analyzer Leco CR-412 (Leo Corporation, St. Joseph, MI). Exchangeable Al was extracted by 1 M KCl at 1:10 soil: soil solution ratio by shaking for 30 minutes (Barnhisel & Bertsch, 1982) and the Al in the extract was analysed by Optima 8300 ICP OES Spectrometer (Perkin Elmer, Massachusetts, USA). Total N was analysed by Kjeldahl digestion method (Bremner & Mulvaney, 1982). Available P was analysed by the method of Bray and Kurtz (1945) with auto-analyzer (QuikChem 8000 Series FIA System, Lachat Instruments, Loveland, USA). The Ex. Fe from soil sample was extracted using 0.05 M HCl in 0.0125 M $H_2SO_4$by double acid method.

Ca, Mg, K, Al and Fe concentrations were determined by Optima 8300 ICP OES Spectrometer (Perkin Elmer, Massachusetts, USA), while the anions (sulfate, phosphate, nitrate and chloride) were determined by Ion Chromatography (Metrohm, Herisau, Switzerland) using Rezex ROA-Organic Acid H+ (8%) with a flow rate 0.5 of mL/min and pressure at 7.9 MPa and then converted into activities using GEOCHEM-EZ as described by Shaff *et al*. (2010).

Chemical analysis of bio-organic fertilizer: The pH of bio-organic fertilizer was measured in water (1:2.5). Total N, P, K, Fe and Al were analysed using Lefortaqua regia solution (3:1 v/v nitric acid to hydrochloric acid). The concentration of K, Fe and Al in the solution was determined by ICP-OES. The N and P concentrations were analysed by using auto-analyzer (QuikChem 8000 Series FIA System, Lachat Instruments, Loveland, USA).

Determination of Al^{3+} and Fe^{2+} activities: The water containing cations and anions was sampled 15 days after planting to allow maximal reduction of ferric to ferrous ions. Al and Fe speciation was carried out using GEOCHEM-EZ program as explained by Shaff *et al*. (2010).

Tissue analysis: For this study, the N, P, K, Ca, Mg, Fe and Al in plant tissues were determined by wet digestion method. This solution was then diluted with distilled water. K, in solution were analysed by using ICP-OES. N and P were analysed by using auto-analyzer (QuikChem 8000 Series FIA System, Lachat Instruments, Loveland, USA).

Statistical analysis: The data were analyzed by factorial and for mean comparison Tukey test was using SAS version 9.2 (SAS Institute, Inc., Cary, N.C., USA). However, all diagrams in the study were prepared using Excel Program in the Microsoft.

Table 2a. Chemical properties of the acid sulfate soil used for pot experiments.

Depth (cm)	pH	EC (mS/cm)	Total N (%)	Avail. P (mg/kg)	Exchangeable cations				Fe (mg/kg)	CEC ($cmol_c$/kg)
					Ca	Mg	K	Al		
					------------- ($cmol_c$/kg) -------------					
0-15	3.64	0.69	0.003	4.72	0.02	0.15	0.04	5.53	135.4	10.39
15-30	3.51	0.57	0.004	4.28	0.05	0.10	0.02	6.43	208.5	11.50
30-45	3.25	0.59	0.003	5.38	0.05	0.25	0.01	6.04	286.1	7.78
45-60	3.24	0.59	0.003	40.46	0.04	0.26	0.03	5.82	483.1	5.18
60-75	3.13	0.63	0.002	41.09	0.03	0.29	0.03	6.15	368.2	8.06

EC = Electrical conductivity, Avail.= Available, CEC = Cation exchange capacity

Table 2b. Chemical characteristics of JITU™ and GML used in the study.

	pH	N	P	K	Fe	Al	Ca	Mg
		--------------------------------- (%) ---------------------------------						
JITU™	7.35	5.02	0.25	0.35	< 0.01	0.01	< 0.01	< 0.01
GML	9.75	na	na	na	< 0.01	< 0.01	19.55	9.40

*na = Not available

Results

Initial chemical properties of the soil: The soil of the experimental used was acid sulfate. The topsoil (0-15 cm) pH was 3.64 and at deeper depth it became lower (3.13). The exchangeable K, Ca and Mg in the soil were lower than the adequacy level for the rice growth however, exchangeable Al was above the critical level (> 5 $cmol_c$ kg^{-1}). The available P in the topsoil was < 5 mg kg^{-1}, a very low value indeed. The chemical characteristic of soil collected for this study was shown in Table 2a. The content of Al and Fe in soil was very high, way above the toxic level for the growth of rice. Hence, the fertility of the soil needs to be alleviated before rice is cultivated.

Chemical analysis of bio-organic fertilizer and GML: The bio-organic fertilizer used in this study contained 5.02, 0.25 and 0.35% N, K and P respectively, while its pH was 7.35. On the other hand, it did not contain much Al and/or Fe content. The GML has high pH (9.75) and have lower contents of Fe and Al. However, the Ca and Mg were in high contents (Table 2b).

Effects of treatment on chemical properties of the soil: The chemical properties of acid sulfate soil sampled before planting influenced by GML and/or bio-organic fertilizer application are shown in Table 3a. The soil pH, exchangeable Ca and Mg increased with rate of GML while exchangeable Al and Fe decreased. Addition of bio-organic fertilizer to the soil showed a slight increase in soil pH, CEC and soil nutrients (Ca, Mg, K, N and P). However, addition of bio-organic fertilizer does not significantly reduced extractable Fe in the soil as the organic fertilizer took longer time to react with the soil (Table 3a).

The chemical properties of soil sampled after harvest Season 1 as affected by GML and/or bio-organic fertilizer (Table 3b). The pH, CEC and soil nutrients (Ca, Mg, K, N and P) increased with rate of GML while exchangeable Al and Fe decreased. Addition of bio-organic fertilizer to the soil significantly reduced the exchangeable Al and extractable Fe. It also showed a minor increase in soil pH, CEC, total N and available P. However, addition of bio-organic fertilizer does not significantly increase exchangeable Ca, Mg and K in the soil (Table 3b).

The chemical properties of acid sulfate soil sampled after harvest Season 2 as influenced by GML and/or bio-organic fertilizer application (Table 3c). The soil pH, CEC and soil nutrients (Ca, Mg, K, N and P) were higher with the rate of GML while exchangeable Al and Fe decreased. Adding bio-organic fertilizer resulted in a slight reduction in exchangeable Al and extractable Fe. Additionally, there was a slight increase in soil pH, CEC, total N and available P. However, there's no interaction between GML and bio-organic fertilizer in soil exchangeable Ca, Mg and K (Table 3c). At harvest season 2, T8 (6 t/ha GML + 0.25 t/ha JITU) and T10 (8 t/ha GML + 0.25 t/ha JITU) showed the highest soil pH and the lowest Al and Fe.

Effects of treatment on rice growth: The application of GML with or without bio-organic fertilizer had positively affected yield of rice in Season 1 (Table 4). The grain yield, 1000 grain weight, number of panicle, number of spikelets $panicle^{-1}$ and the percentage of filled spikelet, increased with rate of GML application. However, adding bio-organic fertilizer did not have significant effects on the 1000 grain weight, number of panicle, number of spikelet $panicle^{-1}$ and the percentage of filled spikelet.

Table 4 shows the rice yield for Season 2. The grain yield, 1000 grain weight, number of panicle, number of spikelet $panicle^{-1}$ and the percentage of filled spikelet, increased with rate of GML application. The result revealed that there were difference in grain yield, number of panicle, number of spikelet $panicle^{-1}$ and the percentage of filled spikelet for addition of bio-organic fertilizer. However, addition of bio-organic fertilizer showed no significant different for 1000 grain weight. T6, T8 and T10 showed high grain yield for Season 2. But, it is too costly for farmers to apply the amendments at this rate; hence, it is not practical and not suitable. For both season, MR 219 and MR 253 showed no significant difference in grain yield, grain weight (1000 grains), number of panicles, number of spikelets $panicle^{-1}$ and the percentage of filled spikelets.

Table 3a. Soil chemical characteristics before rice planting.

Treatments	pH	Exchangeable cations				Extr. Fe (mg/kg)	Total N (%)	Avail. P (mg/kg)	CEC ($cmol_c$/kg)
		Ca	Mg	K	Al				
		----------------- ($cmol_c$/kg) -----------------							
T1	3.20^{c}	0.15^{d}	0.16^{d}	0.01^{d}	4.89^{a}	308.50^{a}	0.13^{d}	0.09^{c}	10.62^{c}
T2	3.35^{d}	0.25^{d}	0.47^{d}	0.04^{d}	4.68^{a}	367.66^{a}	0.40^{d}	0.46^{d}	10.58^{c}
T3	3.32^{c}	0.31^{c}	0.53^{c}	0.04^{cd}	4.06^{b}	291.73^{b}	0.52^{c}	1.13^{c}	10.68^{bc}
T4	3.51^{c}	0.32^{d}	0.84^{c}	0.08^{cd}	2.82^{b}	254.71^{b}	0.62^{c}	2.77^{cd}	10.69^{c}
T5	3.48^{b}	1.04^{b}	1.24^{b}	0.06^{c}	2.22^{c}	234.46^{c}	0.63^{b}	3.16^{b}	10.70^{bc}
T6	3.61^{c}	1.17^{c}	1.38^{b}	0.20^{c}	1.67^{c}	177.91^{c}	0.68^{c}	4.51^{bc}	10.74^{c}
T7	3.58^{b}	1.12^{b}	1.45^{ab}	0.13^{b}	2.00^{c}	172.85^{cd}	0.73^{b}	4.21^{b}	10.90^{b}
T8	4.01^{b}	1.55^{b}	1.62^{b}	0.70^{b}	1.28^{d}	155.83^{cd}	0.96^{b}	6.65^{b}	11.42^{b}
T9	3.74^{a}	1.33^{a}	1.59^{a}	0.48^{a}	1.15^{d}	156.76^{d}	1.23^{a}	7.11^{a}	11.92^{a}
T10	4.26^{a}	1.82^{a}	2.12^{a}	0.93^{a}	1.02^{d}	135.25^{d}	1.42^{a}	14.94^{a}	12.65^{a}

Means followed by the same letter within a column are not significantly different (Tukey's test, $p>0.05$)

Table 3b. Soil chemical characteristics at harvest of 1st season of rice.

Treatments	pH	Exchangeable cations				Extr. Fe (mg/kg)	Total N (%)	Avail. P (mg/kg)	CEC ($cmol_c$/kg)
		Ca	Mg	K	Al				
		----------------- ($cmol_c$/kg) -----------------							
T1	3.14^{c}	0.11^{c}	0.15c	0.02^{d}	4.85^{a}	398.34^{a}	0.10^{d}	0.09^{d}	10.35^{d}
T2	3.85^{c}	0.36^{c}	0.24^{d}	0.03^{c}	4.50^{a}	375.48^{a}	0.43^{d}	0.61^{d}	10.65^{d}
T3	4.01^{b}	3.67^{b}	1.27^{b}	0.04^{c}	4.43^{a}	300.04^{b}	0.84^{c}	1.40^{d}	10.68^{c}
T4	4.07^{c}	3.85^{b}	1.32^{c}	0.05^{bc}	2.99^{b}	284.83^{b}	0.96^{d}	2.91^{cd}	10.87^{cd}
T5	4.20^{ab}	6.73^{a}	1.50^{b}	0.06^{c}	2.38^{b}	208.97^{c}	1.13^{b}	2.96^{c}	10.80^{bc}
T6	4.23^{bc}	6.79^{a}	1.76^{b}	0.08^{ab}	1.64^{c}	199.27^{c}	1.34^{c}	4.79^{bc}	11.20^{c}
T7	4.34^{ab}	7.03^{a}	2.11^{a}	0.08^{b}	1.93^{b}	185.36^{d}	1.17^{b}	4.33^{b}	11.02^{b}
T8	4.49^{b}	7.15^{a}	2.24^{a}	0.09^{ab}	1.18^{d}	149.37^{d}	1.67^{b}	7.63^{b}	12.75^{b}
T9	4.58^{a}	7.22^{a}	2.31^{a}	0.11^{a}	1.15^{c}	141.83^{d}	1.95^{a}	7.93^{a}	12.16^{a}
T10	5.00^{a}	7.59^{a}	2.38^{a}	0.12^{a}	1.08^{d}	131.44^{d}	2.25^{a}	15.59^{a}	13.59^{a}

Means followed by the same letter within a column are not significantly different (Tukey's test, $p<0.05$)

Table 3c. Soil chemical characteristics at harvest of 2nd season of rice.

Treatments	pH	Exchangeable cations				Extr. Fe (mg/kg)	Total N (%)	Avail. P (mg/kg)	CEC ($cmol_c$/kg)
		Ca	Mg	K	Al				
		----------------- ($cmol_c$/kg) -----------------							
T1	3.13^{d}	0.04d	0.07d	0.03c	5.32a	418.64a	0.12d	0.09d	10.13d
T2	4.04d	0.46d	0.24d	0.05b	3.70a	354.32a	0.36d	3.57b	10.80d
T3	4.22^{c}	4.30c	1.11c	0.06bc	2.26b	303.53b	0.89c	3.63c	11.54c
T4	4.78c	4.75^{c}	1.47c	0.08b	1.71b	260.71b	1.03c	4.33b	12.03c
T5	5.28^{b}	5.29b	1.73b	0.09b	1.21c	210.54c	1.16b	4.14bc	12.31b
T6	5.73b	5.96b	2.02b	0.12a	0.67c	167.28c	1.45b	4.63b	12.52dc
T7	5.98^{a}	6.14a	1.97ab	0.12a	0.85cd	175.47cd	1.47b	4.29b	12.69b
T8	6.61a	6.60a	2.40a	0.14a	0.31cd	149.14cd	1.76a	5.87b	13.55b
T9	6.41^{a}	6.32a	2.07a	0.15a	0.49d	152.80d	2.20a	7.40a	14.01a
T10	6.83a	6.81a	2.47a	0.18a	0.18d	133.93d	2.43a	14.04a	14.98a

Means followed by the same letter within a column are not significantly different (Tukey's test, $p<0.05$)

Effects of treatment on the nutrient contents in the rice straw and root: Table 5 and Table 6 showed that application of GML with or without bio-organic fertilizer had positively affected the N, P and K content in straw and root of rice. The N, P and K content increased with rate of GML. The results revealed that there was difference in root N and K content due to addition of bio-organic fertilizer. But, it did not increase P content by the addition of bio-organic fertilizer although it did not affect the N and K content. There was no difference in the N, P, and K content of the rice straw and root between the two rice varieties.

Relationship between Fe^{2+} or Al^{3+} activities and water pH: As shown in Fig. 1a, Fe^{2+} activities reduced exponentially with increasing pH of the water. Likewise, Al^{3+} activities decreased exponentially with increasing pH (Fig. 1b). The relationship for both phenomena are given by $Y = 11362e^{-1.40x}$ ($R^2 = 0.89$) and $Y = 1E + 11e^{-5.75x}$ ($R^2 = 0.92$), respectively.

Relationship between relative rice yield and Fe^{2+} or Al^{3+} activities: Fig. 2a shows that the relative yield of MR 219 and MR 253 has negative relationship with Fe^{2+} activity and equations are showing the relationships are presented by $Y = 98.15- 0.56x$ ($R^2 = 0.96$) and $Y = 92.39 - 0.32x$ ($R^2 = 0.83$), respectively. The critical Fe^{2+} activity (the Fe^{2+} at 90% relative yield) for MR 219 was 14.55 µM, while that of MR 253 was 7.45 µM, indicating that the former was more tolerant to Fe^{2+} than that of the latter. This is not consistent with the belief that MR 253 can be grown successfully on acidic soils with high Fe content.

Table 4. The agronomic parameters at harvest of rice varieties.

Treatment	Variety	Yield (g/pot)		Spikelet (no/panicle)		Filled spikelet (%)		Panicle (No/pot)		1000 grain weight (g)	
		Season 1	Season 2	Season 1	Season 2	Season 1	Season 2	Season 1	Season 2	Season 1	Season 2
T1	MR219	46.17^{d}	34.67^{a}	80^{c}	75^{c}	38.57^{c}	21.70d	23b	17b	20.10b	20.53b
T2		51.36^{c}	66.15^{b}	97^{c}	118^{c}	45.53c	43.97c	28c	32d	20.80b	21.33c
T3		65.87^{c}	74.09^{ab}	109^{bc}	118^{b}	54.18^{b}	54.47c	31b	35a	22.10ab	22.60ab
T4		79.70^{b}	84.53^{ab}	126^{b}	129^{c}	61.31b	64.03b	39b	41cd	23.60a	24.57b
T5		75.35^{bc}	85.63^{b}	133^{ab}	136^{ab}	69.13^{ab}	63.67bc	41a	43a	23.40ab	24.27ab
T6		94.00^{a}	99.13^{ab}	154^{a}	153^{b}	80.30a	72.90b	45ab	46bc	23.80a	24.83b
T7		84.96^{ab}	91.57^{c}	150^{a}	151^{a}	79.17^{a}	75.73ab	42a	44a	22.67ab	23.67ab
T8		96.66^{a}	112.42^{a}	157^{a}	161^{b}	83.63a	85.63a	46ab	55ab	24.40a	26.37ab
T9		89.67^{a}	97.99^{d}	156^{a}	157^{a}	80.73^{a}	84.93a	45a	46a	23.93a	25.37a
T10		99.77^{a}	121.38^{a}	164^{a}	182^{a}	84.10a	90.07a	50a	60a	25.80a	27.23a
T1	MR253	48.03d	33.07c	89c	72d	37.50c	23.93d	26b	13b	21.57c	20.97b
T2		55.43c	70.32d	97c	116c	48.00b	63.63b	34a	33c	22.80b	21.20c
T3		64.57c	71.78b	122b	122c	57.70b	63.87c	40ab	33a	23.87b	21.77b
T4		79.40b	83.21c	127b	128c	60.70b	68.33b	43a	39bc	25.27a	23.07bc
T5		74.50b	83.43ab	129ab	136bc	68.37ab	67.90bc	44ab	39a	25.13ab	23.37ab
T6		92.80a	98.19b	142ab	155b	77.10a	75.00b	46a	45abc	25.07ab	24.10ab
T7		80.83ab	92.52^{ab}	135ab	150ab	68.90ab	79.57ab	46a	47a	26.07a	24.53^{cab}
T8		95.40a	103.06ab	156a	159ab	80.40a	93.13a	48a	50ab	25.57a	25.80a
T9		88.37a	99.41a	145a	161a	79.60a	83.50a	47a	47a	25.80a	25.77a
T10		98.63a	112.60a	156a	173a	81.00a	91.23a	49a	53a	25.10ab	26.27a

Means followed by the same letter within a column are not significantly different (Tukey's test, $p<0.05$)

Table 5. Elemental composition of the rice straw and root of MR 219.

Plant part	Treatments	N	P	K	Al	Fe	Ca	Mg
		(%)						
Rice straw	T1	0.71d	0.05c	0.12c	0.84a	1.83a	0.10b	0.10b
	T2	1.26c	0.10b	1.74b	0.68a	1.34a	0.10b	0.10b
	T3	1.24c	0.10b	1.55b	0.68ab	0.92b	0.10b	0.10b
	T4	1.31bc	0.14ab	1.79b	0.67a	1.33a	0.11ab	0.11b
	T5	1.33b	0.12b	2.09a	0.65b	0.57c	0.11ab	0.11b
	T6	1.42ab	0.13ab	2.17b	0.30b	0.34b	0.11ab	0.13ab
	T7	1.38b	0.13b	2.24a	0.33c	0.44cd	0.10ab	0.12b
	T8	1.43ab	0.19ab	2.25b	0.23b	0.25b	0.11a	0.15a
	T9	1.50a	0.17a	2.39a	0.12d	0.24d	0.11a	0.13a
	T10	1.57a	0.20a	3.20a	0.03c	0.07b	0.11a	0.16a
Rice root	T1	0.11c	0.02c	0.05c	0.98a	1.57a	0.01a	0.03a
	T2	0.14c	0.04c	0.08b	0.78a	1.15a	0.01b	0.02b
	T3	0.15b	0.06b	0.08b	0.65b	1.03b	0.01a	0.03a
	T4	0.18b	0.07b	0.09b	0.56b	0.85ab	0.01b	0.02b
	T5	0.17ab	0.07b	0.09b	0.51b	0.87b	0.01a	0.03b
	T6	0.21ab	0.07b	0.11ab	0.46bc	0.63b	0.01b	0.02b
	T7	0.20ab	0.07ab	0.10b	0.44bc	0.75b	0.01a	0.03ab
	T8	0.22ab	0.09b	0.12ab	0.33c	0.48b	0.01b	0.02b
	T9	0.21a	0.09a	0.12a	0.26c	0.35c	0.01a	0.02b
	T10	0.24a	0.11a	0.15a	0.09d	0.05c	0.02a	0.05a

Means followed by the same letter within a column are not significantly different (Tukey's test, $p<0.05$)

In Fig. 2b it is also shown that the relative rice yield of MR 219 and MR 253 has a negative relationship with Al^{3+} activity and the equations are representing the relationship given by $Y = 94.83 - 1.14x$ ($R^2 = 0.90$) and $Y = 93.98 - 0.72x$ ($R^2 = 0.82$), respectively. Using Fig. 2b, the critical Al^{3+} activity for both rice varieties were determined and the respective values were 4.23 and 5.53 µM. This means that there was little difference in terms of their sensitivity Al^{3+} between the two rice varieties.

Relationship between relative yield and pH: Relative rice yield of MR 219 and MR 253 was positively correlated with the pH of water and equations shows the relationship given by $Y = 10.97 + 12.88x$ ($R^2 = 0.82$) and $Y = 41.09 + 7.85x$ ($R^2 = 0.80$), respectively (Fig. 3). The critical pH (the pH at 90% relative yield) for rice varieties MR 219 and MR 253 was about 6. It shows that both MR 219 and MR 253 rice varieties were mostly comparable in order to their tolerance to low pH stress.

Table 6. Elemental composition of the rice straw and root of MR 253.

Plant part	Treatments	N	P	K	Al	Fe	Ca	Mg
		(%)						
Rice straw	T1	0.82b	0.05c	0.12d	0.89a	1.68a	0.11a	0.10a
	T2	1.16b	0.10c	1.63c	0.67a	1.27a	0.11a	0.10c
	T3	1.18a	0.11b	1.66c	0.66b	0.94b	0.11a	0.10a
	T4	1.23b	0.14b	1.89bc	0.67a	1.18a	0.10a	0.10bc
	T5	1.27a	0.13ab	2.07b	0.65b	0.61c	0.10a	0.11a
	T6	1.41a	0.15b	2.42b	0.29b	0.35b	0.14a	0.12abc
	T7	1.37a	0.16ab	2.28ab	0.34c	0.49c	0.12a	0.13a
	T8	1.49a	0.17b	2.42b	0.26b	0.24bc	0.11a	0.15ab
	T9	1.45a	0.18a	2.52a	0.10d	0.17d	0.12a	0.11a
	T10	1.51a	0.20a	3.21a	0.04c	0.07c	0.11a	0.16a
Rice root	T1	0.10c	0.03c	0.06d	1.01a	1.54a	0.01a	0.02b
	T2	0.15b	0.05c	0.09b	0.75a	1.19a	0.01a	0.02a
	T3	0.16bc	0.06b	0.08c	0.63b	1.07b	0.01a	0.02b
	T4	0.16b	0.07b	0.09b	0.66b	0.83ab	0.01a	0.02a
	T5	0.16ab	0.06b	0.09bc	0.52bc	0.90bc	0.01a	0.02b
	T6	0.33a	0.07b	0.12ab	0.48	0.64ab	0.01a	0.02a
	T7	0.20ab	0.07b	0.10b	0.41cd	0.71cd	0.01a	0.03ab
	T8	0.22a	0.06b	0.13ab	0.37b	0.52b	0.01a	0.03a
	T9	0.23a	0.06a	0.13a	0.28d	0.45d	0.01a	0.03a
	T10	0.25a	0.11a	0.14a	0.11c	0.06c	0.05a	0.05a

Means followed by the same letter within a column are not significantly different (Tukey's test, $p<0.05$)

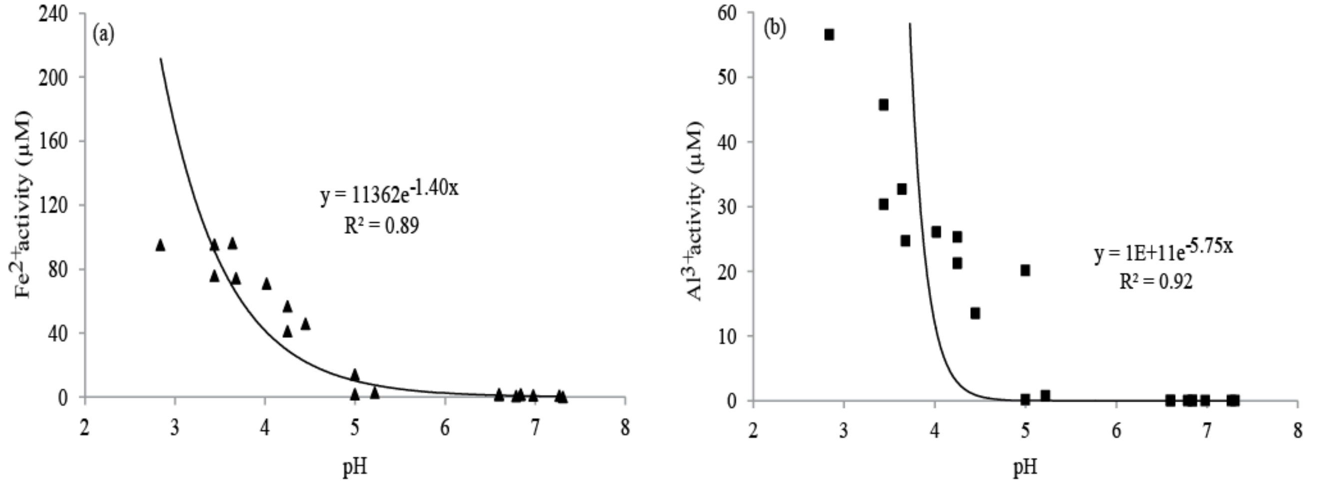

Fig. 1. Relationship between (a) Fe^{2+} activities and (b) Al^{3+} activities and pH of water.

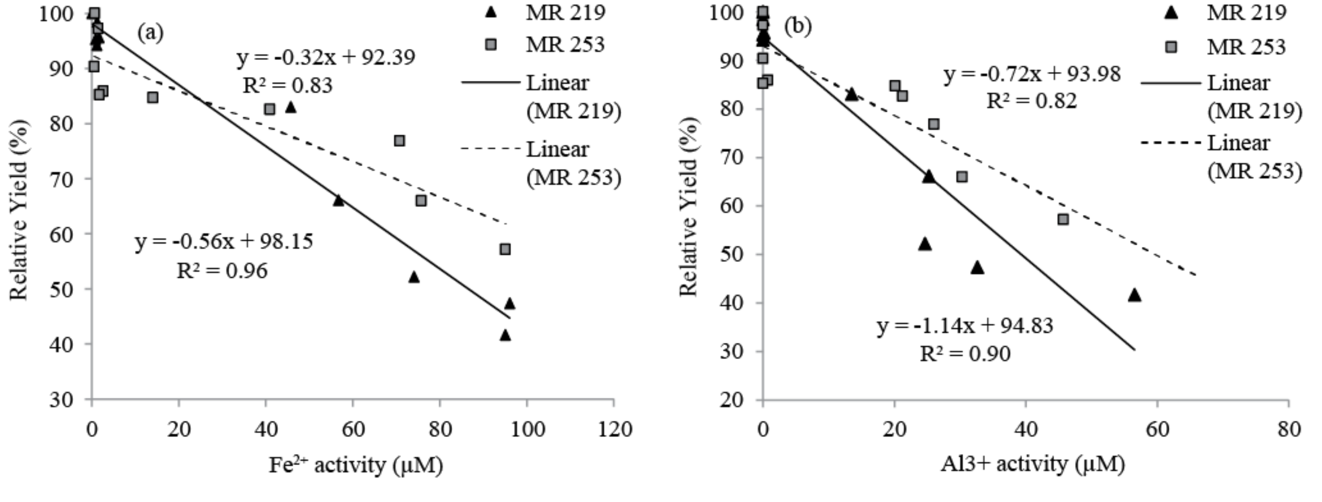

Fig. 2. Relationship between (a) relative rice yield and Fe^{2+} activities and (b) relative rice yield and Al^{3+}activities.

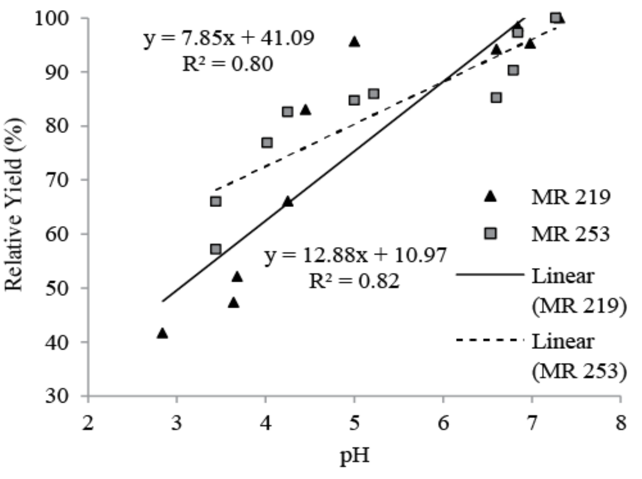

Fig. 3 Relationship between relative rice yield of MR 219 and MR 253 rice varieties and pH of water.

Discussion

The experiment was conducted in the acid sulfate soil and from the preliminary analysis it was shown that the topsoil exchangeable Al was higher, while the availability of P was low. It has been known that Al toxicity is frequently associated with P deficiency due to soil with high Al concentrations would reduce the availability of P because of Al-phosphate interaction (Dent, 1986). It was possible that some of the insoluble P had been made available by the effective microbes which might be present in the bio-organic fertilizer when it was applied into the soil during the experiment.

The rice plant under study was not only exposed to extreme acidity and Al^{3+} and/or Fe^{2+} toxicity, but also nutrient deficiency, such as N, Ca and Mg. The total N, exchangeable Ca and Mg are below the levels which are required for the healthy growth of rice 2%, 2 and 1 $cmol_c$ kg^{-1} soil, respectively (Palhares, 2000; Dobermann & Fairhurst, 2000). The deficiencies of Ca and Mg could be improved by applying (GML) at the proper rate (Shamshuddin, 2006).

Iron's form of Fe^{2+} is toxic to the rice plants (Shamshuddin *et al*., 2014). So, it is this form of Fe in the water where rice is grown should be presented instead of total. In many parts of the world, paddy fields are irrigated for rice cultivation. While flooding the paddy fields in those areas which are occupied by acid sulfate soils, reduction process takes place soon after that consumes protons along the way (Carla *et al.*, 1994; Muhrizal.*et al.*, 2006). The following reaction shows that pH increases in tandem with proton and at this stage of reduction process, Fe^{2+} ions occur at the maximal concentration.

$$Fe(OH)_3 + ¼CH_2O + 2H^+ \rightarrow Fe^{2+} + ¼CO_2 + 11/4H_2O$$

The pyrite oxidation occurring in acid sulfate soils generates a lot of acidity that lowers soil pH which can affect rice growth. However, rice plant required pH of 5.0-7.5 for optimum growth. Elisa *et al*. (2011) and Shamshsuddin *et al*. (2014) revealed that rice required the pH of 6 for its maximal growth. Applying GML to alleviate acidity in acid sulfate soil is among the most common agronomic practice (Shamshuddin, 2006) which can neutralize the soil acidity, but also furnishes Ca and Mg for the rice growth in the field.

The addition of bio-organic fertilizer helped to supply some N, P and K to soil. On the other hand pH of bio-organic fertilizer was 7.35 hence, its application would increase soil pH slightly. The organic matter from the bio-organic fertilizer was able to inactivate Al and Fe by chelation process (Shamshuddin, 2014). Further explanation on this matter is given by the study of Patcharee and Hidenori (1993). Due to this reaction, the soil treated with bio-organic fertilizer contained less Al and/or Fe compared to without bio-organic fertilizer. Furthermore, bio-organic fertilizer would accelerate the reduction of Fe that resulted for the quicker increase in soil pH (Muhrizal *et al*., 2003; Muhrizal *et al*., 2006).

The statistical analysis presented that there were no significant difference between variety MR 219 and MR 253 for season 1 and 2. However, there was highly significant interaction between bio-organic fertilizer and GML in grain yield for both seasons. This means that the application of GML and/or in combination with bio-organic fertilizer on acid sulfate soil had significant influence on the yield of rice.

The grain yield of rice for the untreated soil was critically low for both seasons. This was because of both rice genotypes were significantly influenced by Al^{3+} and Fe^{2+} toxicity; hence, their growth was suppressed. Several studies were lead in the past to elucidate the reasons of Al inhibiting the of crop root growth. It is believed that Al can decrease root cell division; therefore, it causes disturbance of root cap processes, preventing root elongation (Barker & Pilbeam, 2007). When its root is disrupted due to Al toxicity, the available nutrients in soil cannot be taken up by the plant. Thus, it can reduce the yield. This explains why rice plants in the pots of the treated soils contained higher nutrients in the tissues compared to control. In the Season 2, grain yield and percentage of filled spikelet were higher treated with bio-organic fertilizer. This was because of the application of bio-organic fertilizer could improve nutrient availability, especially N and P which cell division and root development. We know that good root development would increase the translocation of carbohydrates from source to growing points which in the end increased rice yield significantly. This finding is supported by the study of Mirza *et al*. (2010).

Rice has the potential to decrease the uptake of Fe^{2+} by a certain mechanism. In the current study, we understood that rice plant pushed in O_2 downwards via roots, by creating an oxidized zone. The Fe^{2+} around the surface of root surface would be oxidized to $Fe^{3+}$by forming ferric hydroxide that was precipitated as in brown crust. This created coating is not suitable for rice as it decreases the uptake of the other needed plant nutrients (Dobermann & Fairhurst, 2000). Furthermore, rice has the trend to uptake more Fe than any other plant. This is clearly shown in this study where relative rice yield of MR 219 and MR 253 in Season 1 was negatively correlated with Fe^{2+} activity in the water. Under anaerobic conditions, Fe^{2+} was available and could easily be taken up at the root cortex in the plant and later, this reduced form of Fe get entered in the xylem via the Casparian strip and when the Fe^{2+} is rich within the cell, it may catalyze active oxygen generation, like superoxide, hydroxyl-radical and H_2O_2 (Marschner, 1995).

In the study it was observed that more Al^{3+} was occurred in the root apex particularly at elongation zone, root cap and meristem, as compared to other like mature root tissues. Similar results were reported by Elisa *et al*. (2011). The root apex quickly bound Al^{3+} in the apoplast due to the existence of pectic matrix that contain carboxylic groups which were negatively-charged and had great affinity for Al^{3+} (Chang *et al*., 1999). The binding between pectic matrix and Al affected loosening of enzymes in the cell wall, which results in the loss of its functioning. Therefore, it reduced by physiologically cell wall extensibility (Wehr *et al*., 2004) and decreased root length with curtailed root growth because of less amount of nutrients had been taken up.

The critical water pH for the two rice varieties was estimated to be pH 6.1 and pH 6. This shows that MR 219 and MR 253 genotypes were more or less similar in terms of their tolerance to soil acidity; hence, the claim that MR 253 is acid tolerant is unfounded. Under normal circumstances, water pH in the paddy fields of acid sulfate soils in Malaysia is 3-4 (Shamshuddin, 2006; Shamshuddin *et al*., 2014). Thus, the soil water pH in the field has to be raised to about 5, above which Al^{3+} becomes no longer a threat to rice production. Above pH 5, the ions are precipitated as inert Al-hydroxides. Fortunately, this study showed that the two rice varieties under study were able to grow even at pH below 5. This is because rice has a different ability to defend itself towards soil acidity and the toxicity related to Fe^{2+} and Al^{3+} (Shamshuddin *et al*., 2014; Panhwar *et al*., 2014a).

Conclusion

Rice cultivation on acid sulfate soils in Malaysia was severely influenced by the prevalent high acidity and Fe^{2+}as well as Al^{3+} toxicity. However, both varieties were able to grow quite well even at pH below 5 under higher Fe^{2+} and Al^{3+} activities. The ameliorative effects of applying these amendments had last at least 2 seasons. Furthermore, the bio-organic fertilizer supplied some NPK which can improve rice growth and yield. This study showed that the infertility of acid sulfate soils in Malaysia could be ameliorated by applying 2 t GML/ha combined with 0.25 t bio-organic fertilizer/ha for sustainable rice cultivation.

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