



Mycorrhizae and a soil ameliorant on improving the characteristics of sandy soil

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ARTICLE INFO	ABSTRACT
<p>Keywords: Cow dung Biochar Rock phosphate SOC Glomalin</p> <p>Article history Submitted: 2020-08-12 Accepted: 2021-06-3 Available online: 2021-06-30 Published regularly: June 2021</p> <p>* Corresponding Author Email address: aktavia_h@staff.uns.ac.id</p>	<p>Agricultural constraints on sandy soil are poor chemical characteristics and low biological activity resulting in the soil becoming less productive to be planted. One of the efforts to improve the quality of sandy soil is an application of mycorrhizae and a soil ameliorant. The purpose of this study was to determine the effect of mycorrhizae and a soil ameliorant on soil chemical characteristics and soil biological activity. The experiment was arranged using a Complete Randomized Design with two factors. The first factor (1) was mycorrhizae dose, without mycorrhizae (M0) and six spores of mycorrhizae/plant (M1), and the second factor (2) was types of soil ameliorant, without ameliorant (S0), cow dung (S1), rock phosphate (S2), biochar (S3), cow dung–rock phosphate (S4), cow dung–biochar (S5), and rock phosphate–biochar (S6). The results indicated that combination of six spores mycorrhizae/plant–cow dung 60 tons ha⁻¹–biochar 25 tons ha⁻¹ (M1S5) increased soil organic carbon (SOC) (235%), available P (675%), cation exchange capacity (CEC) (216%), total glomalin (101%), and easily extracted glomalin (69%), decreased exchangeable sodium percentage (66%), and increased absolutely for root infection and spore density than without mycorrhizae and a soil ameliorant (control). The lowest SOC, total glomalin and easily extracted glomalin were found on non-mycorrhizae-rock phosphate, but available P, CEC, root infection, spore density were found on the control. The application of mycorrhizae, cow dung, and biochar improved the sandy soil characteristics.</p>
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1. Introduction

The decrease in agricultural land becomes a problem because it leads to a decrease in land productivity and food production, whereas demand for food keeps increasing. Thus, agricultural extensification is required and could be done by utilizing coastal sandy soil. This soil possesses poor chemical and biological quality and becomes the limiting factor for plant growth. Sandy soil has low organic matter content, total nitrogen, and cation exchange capacity (CEC) (Rahayu, Saidi, et al., 2019; Zulkoni et al., 2020), which will lead to low fertility, water retention, and macro and micronutrient content (Yost & Hartemink, 2019). Its properties also do not provide support for microorganisms' life (Huang & Hartemink, 2020).

There have been studies demonstrating that mycorrhizae and a soil ameliorant positively affect soil and plant growth.

The contribution of subsoil arbuscular mycorrhizae fungi was to enhance soil formation, reduction of nutrient leaching, access to deep nutrient and water pools in the suboptimal conditions that prevail in the topsoil, and improvement of the soil structure, leading to aggregate-protected organic matter, competition with saprotrophic bacteria and fungi, thus reducing decomposition rates, increased carbon input in subsoil via mycelial exudates and turnover and formation of highly stable mineral-associated organic matter fractions (Sosa-Hernández et al., 2019) and increasing the P availability in the desertification soils in the Karst area (Li et al., 2019). The mineral status of plants in potassium was enhanced by mycorrhizae treatment up to 0.53 mg g⁻¹ dry weight for bean plants and 0.50 mg g⁻¹ for wheat plants in Mediterranean semi-arid areas (Raklami et al., 2019).

Research on soil ameliorants has been previously carried out. Application of cow dung in sandy soils increases soil cation base, moisture content, soil C-organic content (Han et al., 2016; Xie et al., 2015), and total chili production (Putra et al., 2020). The addition of biochar increased nutrient availability and water retention while also providing favorable habitats for symbiotic microorganisms such as mycorrhizae (Zhang et al., 2017). Kocsis et al. (2020) also reported that appropriate biochar doses are needed for the improvement of soil biological activities and increased yield of maize compared to the control. In addition, bacterial inoculation in biochar-based rock phosphate enhanced available P in soil (de Amaral Leite et al., 2020). Mycorrhizae colonization was significantly decreased fertilizer input by 100 mg P kg⁻¹; and a combination of mycorrhizae fungi, biochar and P fertilizer significantly increased shoot biomass in polluted soils (Xiao et al., 2020) and the growth of chili in sandy soil (Putra et al., 2020). Liu et al. (2020) reported that in cadmium (Cd)-polluted soils, treatment with no biochar and P input had a positive mycorrhizae response, but treatment with biochar or high-P input had a negative mycorrhizae response.

Various studies using mycorrhizae and soil ameliorants have been successfully carried out in various soils, but nothing is specific to sandy soils. The research on mycorrhizae and soil ameliorants in sandy soil is needed because the chances of success and novelty from this research are very high. This research aimed to determine the effect of mycorrhizae and a soil ameliorant on soil chemical characteristics and soil biological activity in sandy soil.

2. Materials and Methods

This experiment was conducted from May to September 2019 at the Screen House of the Faculty of Agriculture of Sebelas Maret University. The experiment was a polybag experiment with 10 kg of soil per polybag, arranged using complete a randomized design consisting of two factors. The first factor was the dosage of mycorrhizae, without mycorrhizae (M0) and with six spores mycorrhizae/plant (M1). The mycorrhizae used are an endomycorrhizal biofertilizer with 30 spores in 50 grams of zeolite carriers, which means that at 10 grams of zeolite carriers, there are six mycorrhizae spores. The determination of the dose of six mycorrhizae spores plants was based on a previous study by Syamsiyah et al. (2014), which demonstrated that the mycorrhizae dose of 5 g plant⁻¹ showed the best results in paddy rice. The second factor was a soil ameliorant, without an ameliorant (S0), cow dung 60 tons ha⁻¹ (S1) (Prasetyo, 2014), rock phosphate 150 kg ha⁻¹ (S2), biochar 25 tons ha⁻¹ (S3) (Rahayu, Saidi, et al., 2019), cow dung 60 tons ha⁻¹ + rock phosphate 150 kg ha⁻¹ (S4), cow dung 60 tons ha⁻¹ + biochar 25 tons ha⁻¹ (S5), and rock phosphate 150 kg ha⁻¹ + biochar 25 tons ha⁻¹ (S6). Each unit of the experiment was repeated three times so that there were 42 total polybags experimental units.

The sandy soil used in this study was taken from Bantul Regency of Yogyakarta Province, Indonesia, characterized by

neutral soil pH H₂O (1:2.5); very low total nitrogen, C-organic, and cation exchange capacity (CEC); low available P and K (Table 1). Application of mycorrhizae was carried out during the transplanting of chili seedlings. At harvesting (110 days after planting), tissue samples were taken by cutting the stem of the plant and leaving the roots planted in the soil. Then, the remaining roots were stressed for 7 days, after which the soil samples and root samples were taken for laboratory analysis.

Parameters of soil characteristics include pH of the soil using a potential metric method; available P was analyzed using the Olsen method; soil organic carbon (SOC) was analyzed using the Walkley and Black method; CEC, base saturation, and exchangeable sodium percentage (ESP) were analyzed using the Ammonium Acetate pH 7.0 saturation method. The parameters of soil biological activity include mycorrhizae infection using the Tryphan blue staining method and spore density analyzed using the wet sieved method, and glomalin levels were analyzed using the glomalin extraction method. The data obtained were analyzed by using Analysis of Variance with a confidence level of 95%; then, Tukey HSD was conducted with a confidence level of 95%. To find the relationship between parameters, the Pearson correlation test was used.

3. Results

3.1. Effect of mycorrhizae and soil ameliorants on soil chemical characteristics

The combination of mycorrhizae and soil ameliorants has a significant effect on available P, SOC, CEC, base saturation and ESP but not significant on the soil pH (Table 2). The range of pH in all treatments of mycorrhizae and ameliorant combinations was classified as neutral (7.33–7.51). Even without the application of mycorrhizae and soil ameliorants, soil pH in the control treatment (MOS0) and initial soil pH were classified as neutral. Application of mycorrhizae–rock phosphate 150 kg ha⁻¹ resulted in the highest of available P (115.17 ppm) but was not significantly different from the application of mycorrhizae–cow dung and mycorrhizae–cow dung–biochar (Table 3). The treatment of mycorrhizae–rock phosphate presented the highest available P (115.17 ppm), which was 12.8 times higher than that of the control treatment (9.99 ppm). Application of mycorrhizae and types of soil ameliorant significantly affected SOC (Table 3).

Table 1. Initial sandy soil characteristics

No	Characteristic (unit)	Method	Value
1.	pH H ₂ O	Potentiometric	7.12
2.	Soil organic C (%)	Walkley and Black	0.18
3.	Total N (%)	Kjeldahl	0.015
4.	Available P (ppm)	Olsen	7.34
5.	Available K (me/100g)	Saturation of NH ₄ OAc 1 N	0.27
6.	CEC (cmol/kg)	Saturation NH ₄ OAc 1 N	4.43
7.	Spore density 100 g ⁻¹	Wet sieving	ud

Remark: ud = undefined

Table 2. P-value from ANOVA test on soil chemical characteristic ($\alpha=0.05$)

Parameter	pH	Available P	SOC	CEC	Base saturation	ESP
Mycorrhizae (M)	0.717 ^{ns}	<0.01**	0.990 ^{ns}	<0.01**	<0.01**	0.284 ^{ns}
Soil Ameliorant (S)	0.984 ^{ns}	<0.01**	<0.01**	<0.01**	<0.01**	<0.01**
M * S	0.937 ^{ns}	<0.01**	<0.01**	<0.01**	<0.01**	<0.01**

Remarks: SOC= Soil Organic Carbon; CEC= Cation Exchangeable Capacity; ESP= Exchangeable Sodium Percentage; ns= no significant difference; **= highly significant difference

Table 3. Effect of mycorrhizae and soil ameliorant on soil chemical properties

Treatment	pH	Available P (ppm)	SOC (%)	CEC (cmol kg ⁻¹)	Base saturation (%)	ESP (%)
S0	7.48 ± 0.09	9.99 ± 0.28 c	0.17 ± 0.01 d	4.57 ± 0.34 d	43.46 ± 1.91 bc	28.73 ± 0.22 a
S1	7.51 ± 0.17	30.01 ± 3.50 bc	0.32 ± 0.02 bcd	6.58 ± 1.56 cd	35.60 ± 8.10 bcde	15.83 ± 3.28 bcde
S2	7.39 ± 0.22	25.25 ± 2.38 bc	0.11 ± 0.12 d	9.50 ± 0.99 bc	43.19 ± 2.96 bc	18.59 ± 0.65 bcde
M0 S3	7.45 ± 0.11	29.28 ± 6.03 bc	0.22 ± 0.06 cd	8.76 ± 0.19 c	30.21 ± 1.89 cde	14.81 ± 1.51 cde
S4	7.44 ± 0.24	55.26 ± 34.54 bc	0.45 ± 0.01 bc	6.74 ± 0.74 cd	81.79 ± 9.43 a	19.34 ± 3.84 bcd
S5	7.50 ± 0.12	23.53 ± 3.37 bc	0.80 ± 0.13 a	8.19 ± 0.59 cd	42.53 ± 3.04 bc	14.55 ± 1.88 cde
S6	7.40 ± 0.29	18.12 ± 5.23 c	0.17 ± 0.15 d	9.58 ± 0.34 bc	34.82 ± 2.45 cde	16.38 ± 1.73 bcde
S0	7.55 ± 0.23	21.01 ± 3.27 c	0.12 ± 0.15 d	7.31 ± 0.32 cd	35.88 ± 0.44 bcde	21.70 ± 0.70 abc
S1	7.33 ± 0.48	77.47 ± 34.16 ab	0.56 ± 0.18 ab	8.65 ± 0.63 c	40.73 ± 3.96 bcd	13.62 ± 1.80 cde
S2	7.48 ± 0.25	115.17 ± 45.70 a	0.07 ± 0.02 d	6.08 ± 1.00 cd	51.95 ± 11.10 b	24.16 ± 7.02 ab
M1 S3	7.41 ± 0.37	23.99 ± 8.68 bc	0.32 ± 0.12 bcd	8.79 ± 0.42 c	29.16 ± 2.48 cde	19.49 ± 2.33 bcd
S4	7.49 ± 0.31	27.33 ± 3.93 bc	0.27 ± 0.01 cd	12.99 ± 1.92 ab	22.43 ± 5.50 e	11.81 ± 4.14 de
S5	7.33 ± 0.02	77.45 ± 13.93 ab	0.57 ± 0.13 ab	14.43 ± 3.68 a	24.95 ± 5.90 de	9.80 ± 2.11 e
S6	7.39 ± 0.09	20.38 ± 5.80 c	0.33 ± 0.10 bcd	8.82 ± 1.20 c	29.01 ± 5.33 cde	20.44 ± 4.00 abcd

Remarks: The numbers after the ± sign are standard deviation, the means followed by the same letters in the same column indicate no significant difference according to Tukey HSD ($\alpha=5\%$); SOC= Soil Organic Carbon CEC= Cation Exchangeable Capacity; ESP= Exchangeable Sodium Percentage; M0= without mycorrhizae; M1= mycorrhizae 6 spores/plant; S0= without ameliorant; S1= cow dung 60 tons ha⁻¹, S2= rock phosphate 150 kg ha⁻¹; S3= biochar 25 tons ha⁻¹; S4= cow dung 60 tons ha⁻¹ + rock phosphate 150 kg ha⁻¹; S5= cow dung 60 tons ha⁻¹ + biochar 25 tons ha⁻¹; S6= rock phosphate 150 kg ha⁻¹ + biochar 25 tons ha⁻¹

The combination of cow dung–biochar without mycorrhizae (M0S5) had the highest of soil organic C (0.8%), which increased up to 4.7 times compared to the control (0.17%). Application of mycorrhizae–rock phosphate 150 kg ha⁻¹ was the lowest of soil organic C (0.07%).

The combination of mycorrhizae and soil ameliorant significantly affected CEC (Table 3). The highest of CEC (14.43 cmol kg⁻¹) was found in the combination of mycorrhizae–cow dung 60 tons ha⁻¹ with biochar 25 tons ha⁻¹ (M1S5), increasing up to 3.16 times than that in the control (4.57 cmol kg⁻¹). Interaction of mycorrhizae and soil ameliorants had a significant effect on base saturation. The highest base saturation was found on the treatment without mycorrhizae–cow dung–rock phosphate (81.79%) (M0S4) and was significantly different from that due to other treatments (Table 3). The combination of mycorrhizae–cow dung–rock phosphate contributed to increasing base saturation in the soil by 1.88 times compared to the control (43.46%). The combination of mycorrhizae and soil ameliorants has a significant effect on decreasing the ESP value (Table 3). The lowest ESP was found on the combination of mycorrhizae–cow dung–biochar (9.80%), lower up to 2.99 times than that on the control (28.73%). The highest ESP was found in the control.

3.2. Effect of mycorrhizae and soil ameliorants on soil biological characteristics

The application of mycorrhizae combined with soil ameliorants had a significant effect on root infection, spore

density, easily extracted glomalin (EEG), and total glomalin (TG) in soil, as presented in Table 4. Application of mycorrhizae without soil ameliorants (M1S0) had the highest root infection (98.33%), spore density (54 spores/100 grams of soil), and TG (0.300 mg g⁻¹), but application of mycorrhizae–cow dung 60 tons ha⁻¹ (M1S1) had the highest EEG (0.290 mg g⁻¹) (Table 5). Root infection on the only mycorrhizae was not significantly different with a combination of mycorrhizae–cow dung–rock phosphate (M1S4). On mycorrhizae treatment, application of biochar 25 tons ha⁻¹ resulted in the lowest root infection (70%) and spore density (20/100-gram soil). All types of soil ameliorants without mycorrhizae prevented root infection and spore density.

Application of mycorrhizae–cow dung 60 tons ha⁻¹ (M1S1) was the best for EEG (0.290 mg g⁻¹), and application of only rock phosphate 150 kg ha⁻¹ was the lowest of EEG (0.038 mg g⁻¹). The application of only mycorrhizae (M1S0) and mycorrhizae–cow dung 60 ton ha⁻¹ (M1S1) were the best on TG up to 0.300 mg g⁻¹ but not significantly different with the application of mycorrhizae–cow dung (M0S1) (0.299 mg g⁻¹) and mycorrhizae–rock phosphate (0.298 mg g⁻¹) (Table 5). Application of only 150 kg ha⁻¹ of rock phosphate was the lowest of TG (0.044 mg g⁻¹).

The available P had a positive correlation with root infection ($r = 0.364$, $P < 0.05$), spore density ($r = 0.363$, $P < 0.05$), TG ($r = 0.318$, $P < 0.05$) but a negative correlation with EEG ($r = -0.411$, $P < 0.01$), as presented in Table 6. Cation exchange capacity had a positive correlation with root

infection ($r = 0.378$, $P < 0.05$) and spore density ($r = 0.307$, $P < 0.05$). Root infection had a strong positive correlation with spore density ($r = 0.946$, $P < 0.01$), TG ($r = 0.485$, $P < 0.01$) and easily extracted glomalin ($r = 0.378$, $P < 0.05$); spore density had a strong positive correlation with TG ($r = 0.490$, $P < 0.01$); and TG had a strong positive correlation with EEG ($r = 0.476$, $P < 0.01$).

4. Discussion

The interaction of mycorrhizae–cow dung–biochar (M1S5) significantly increased SOC (0.57%), CEC (14.43 cmol kg⁻¹), root infection (90%), and spore density (44/100 grams of soil) and reduce the ESP value (9.80%) in sandy soils (Table 3 and Table 5). Application of mycorrhizae–rock phosphate significantly increased P availability up to 115.17 ppm (Table 3). By contrast, the application of mycorrhizae–cow dung (M1S1) significantly increased EEG (0.290 mg g⁻¹) and TG 0.300 mg g⁻¹ (Table 5). In line with Wu et al. (2015), mycorrhizae significantly increased the production of EE-glomalin-related soil protein (GRSP), T-GRSP, and SOC, production of EE-GRSP and T-GRSP significantly positively correlated with SOC.

An increase of SOC increased CEC. SOC and CEC on mycorrhizae–cow dung–biochar increased by 235% and 216%, respectively, compared to control. Ramos et al. (2018) reported that organic carbon influenced the sign and the

magnitude of the net charge of the soils and contributed approximately 40 times more to the CEC than the clay fraction, the increase in CEC due to the increase of the organic carbon content. CEC was strongly linked to SOC, especially in the top mineral soil with the larger presence of organic matter (Solly et al., 2019). Minardi et al. (2020) reported that adding cow manure to the soil resulted in the highest soil organic matter in Alfisols, due to the decomposing organic matter from cow manure. Adding mycorrhizae–rock phosphate increased available phosphor. Sosa-Hernández et al. (2019) reported that P uptake increased significantly with the addition of mycorrhizae through increased water-holding capacity, reduction of the risk of leaching, and intercept P that has migrated down the soil profile and delivered P to the plant. The role of mycorrhizae on P availability through solubilization and mineralization reactions, and immobilization of P into microbial biomass and/or formation of sparingly available forms of inorganic and organic soil P, or increased root growth through either an extension of existing root systems (e.g., mycorrhizal associations) or hormonal stimulation of root growth, branching, or root hair development (Richardson & Simpson, 2011). Mycorrhizae inoculants combination with rock phosphate can be used as a suitable biofertilizer for improving the P status and growth of plants in alkaline soils (Wahid et al., 2019).

Table 4. P value from ANOVA test on soil biological characteristic ($\alpha = 0.05$)

Treatment	Root infection	Spore density 100 g ⁻¹ of soil	Easily extracted Glomalin	Total Glomalin
Mycorrhizae (M)	<0.01**	<0.01**	<0.01**	<0.01**
Soil Ameliorant (S)	<0.01**	<0.01**	<0.01**	<0.01**
M * S	<0.01**	<0.01**	<0.01*	<0.01**

Remarks: * = significant difference; ** = highly significant difference

Table 5. Effect of mycorrhizae and soil ameliorant on soil biological properties

Treatment	Root infection (%)	Spore density 100 g ⁻¹ of soil	EEG (mg g ⁻¹)	TG (mg g ⁻¹)	
M0	S0	0 ± 0.00 g	0 ± 0.00 f	0.067 ± 0.06 cde	0.094 ± 0.03 bc
	S1	0 ± 0.00 g	0 ± 0.00 f	0.138 ± 0.00 cd	0.299 ± 0.05 a
	S2	0 ± 0.00 g	0 ± 0.00 f	0.038 ± 0.01 e	0.044 ± 0.01 c
	S3	0 ± 0.00 g	0 ± 0.00 f	0.056 ± 0.09 de	0.192 ± 0.03 abc
	S4	0 ± 0.00 g	0 ± 0.00 f	0.164 ± 0.04 bc	0.176 ± 0.05 abc
	S5	0 ± 0.00 g	0 ± 0.00 f	0.070 ± 0.05 cde	0.09 ± 0.02 bc
	S6	0 ± 0.00 g	0 ± 0.00 f	0.096 ± 0.01 cde	0.100 ± 0.01 bc
M1	S0	98.33 ± 1.53 a	54 ± 2.00 a	0.099 ± 0.07 cde	0.300 ± 0.03 a
	S1	80.00 ± 1.00 d	28 ± 2.00 d	0.290 ± 0.09 a	0.300 ± 0.01 a
	S2	85.00 ± 3.00 c	36 ± 2.00 c	0.073 ± 0.03 cde	0.298 ± 0.01 a
	S3	70.00 ± 3.00 f	20 ± 2.00 e	0.074 ± 0.00 cde	0.088 ± 0.01 c
	S4	98.00 ± 2.65 a	28 ± 2.00 d	0.243 ± 0.04 ab	0.269 ± 0.05 ab
	S5	90.00 ± 2.00 b	44 ± 4.00 b	0.113 ± 0.11 cde	0.189 ± 0.06 abc
	S6	75.00 ± 1.00 e	30 ± 4.00 d	0.107 ± 0.08 cde	0.178 ± 0.01 abc

Remarks: The numbers after the ± sign are standard deviation, the numbers followed by the same letters indicate no difference according to Tukey HSD ($\alpha = 0.05$); TG= Total Glomalin; EEG= Easily extracted Glomalin; M0= without mycorrhizae; M1= mycorrhizae 6 spores/plant; S0= without ameliorant; S1= cow dung 60 tons ha⁻¹, S2= rock phosphate 150 kg ha⁻¹; S3= biochar 25 tons ha⁻¹; S4= cow dung 60 tons ha⁻¹ + rock phosphate 150 kg ha⁻¹; S5= cow dung 60 tons ha⁻¹ + biochar 25 tons ha⁻¹; S6= rock phosphate 150 kg ha⁻¹ + biochar 25 tons ha⁻¹.

Table 6. Correlation (r) analysis of soil characteristics

Soil Characteristics	Available P	CEC	Root Infection	Spore Density	Total Glomalin
Soil Organic Carbon	0.112	0.221			
Root infection	0.364*	0.378*			
Spore density	0.363*	0.307*	0.946**		
Total Glomalin	0.318*	0.050	0.485**	0.490**	
Easily Extracted Glomalin	-0.411**	0.233	0.378*	0.239	0.476**

Remarks: *=significant at $\alpha=0.05$; **= significant at $\alpha=0.01$

The application of cow dung and biochar had a significant effect on increasing SOC, P availability, and CEC (Haliru et al., 2018). This is in line with Alvernia et al. (2017), who observed an increase in SOC by 26% after the application of cow dung of 5 tons ha⁻¹ on Alfisols. The addition of biochar to sandy soil will increase the concentration of humic and fluvic acids, followed by an increase in SOC (Amoakwah et al., 2020; Gtąb et al., 2020), and increased available P averages of 7.9% in paddy soil (Jing et al., 2020). The interaction of organic matter and biochar may work in both directions because the addition of easily degradable substrates or the presence of labile SOM in biochar-amended soils can increase biochar decomposition (Ameloot et al., 2013). Application of cow dung and biochar can provide the highest organic C in sandy soil.

TG and EEG with mycorrhizae and cow dung increased 213% and 333%, respectively, compared to those without mycorrhizae and soil ameliorants (Table 5). Wu et al. (2014) reported that root colonization significantly positively correlated with EEG, difficultly-extractable GRSP (DE-GRSP), and TG. In more detail, Wu et al. (2015) said that GRSP concentrations are often positively related to root colonization and soil hyphae. Greater root systems could provide more chances to be colonized by AMF, thereby increasing the production of hyphae, spore density and more GRSP production. The external root of mycorrhizae produces compounds in the form of glomalin glycoproteins and organic acids that can increase SOC (Syamsiyah et al., 2014). Adding cow dung and biochar with high carbon will increase nutrients and soil biological activity. Mycorrhizae are obligate biotrophs that depend on host cells for their C supply, up to 85% of the carbon is transferred to the mycorrhizae and then used to form glomalin (Vlček & Pohanka, 2020). Mycorrhizae will produce a protein, glomalin, which is called GRSP, and increase production of glomalin due to increasing atmospheric CO₂ because of the symbiotic association that exists between plants and mycorrhizae (Singh et al., 2013).

The increasing doses of biochar can increase enzyme activities and improve soil microbial biomass, thus suggesting a selection pressure of biochar to microbial population in soil (Kocsis et al., 2020). According to Xie et al. (2015), giving cow dung can increase levels of EEG, and TG due to increased nutrients for soil biological activity will be followed by the release of growth-promoting substances. Glomalin is a component resulting from mycorrhizal symbiotic activity with root plants. This substance is a constituent of the walls of hyphae and spores produced by mycorrhizae during nutrient and water transport activities. After the activity of the hyphae stops, glomalin will be released with the hyphae and unite with minerals in the soil. Even though glomalin from the soil is difficult to extract, mycorrhizae inoculation could extract it

and then increase the concentration of EEG. This is in line with Syamsiyah et al. (2014), who found that there was a significant difference effect between no inoculation and mycorrhizae inoculation with EEG and TG. Increasing the TG was followed by root infection and spores that were produced by mycorrhizae (Birhane et al., 2020). The evidence is the very strong correlation between the root infection and the spore density ($r = 0.946$, $P < 0.01$) and TG ($r = 0.485$, $P < 0.01$) and EEG ($r = 0.378$, $P < 0.05$) (Table 6).

Application of mycorrhizae and cow dung–biochar significantly increased CEC up to 216% and available P up to 675% compared to those without mycorrhizae (control) (Table 3). There was positive correlation between CEC and root infection ($r = 0.378$, $P < 0.05$) and spore density ($r = 0.307$, $P < 0.05$) (Table 6). This is because mycorrhizae play an active role in the process of organic C decomposition, releasing organic acids to increase soil CEC and the availability of P (Syibli et al., 2013). Leaching of soil nutrients was not easy to occur because of the wider absorption area due to the high content of soil organic colloids (Bruun et al., 2012). The high soil organic matter content will be followed by an increase in nutrient absorption due to the CEC value (Sithole & Magwaza, 2019). Maftu'ah & Nursyamsi (2019) reported that biochar application correlated positively with P availability and increased P uptake in maize plants compared to control treatment. The increase in P availability is in line with the increase in root infection and spore density. This is evidenced by the positive correlation between P availability with root infection ($r = 0.364$, $P < 0.05$), spore density ($r = 0.363$, $P < 0.05$), TG ($r = 0.318$, $P < 0.05$) (Table 6).

The application of mycorrhizae and rock phosphate can increase the availability of phosphorus in the soil. Mycorrhizae of six spores/plant and rock phosphate 150 kg ha⁻¹ showed the highest available P-value, 115.17 ppm, an increase of 1,053% compared to control (Table 3). Mycorrhizae inoculation can accelerate mycorrhizae colonization of plant roots when added with rock phosphate as a source of phosphorus. Mycorrhizae inoculation combines with rock phosphate to increase soil P availability and accelerate the solubility of P through organic acids released (Li et al., 2019; Sosa-Hernández et al., 2019).

The combination of cow dung 60 tons ha⁻¹ and rock phosphate 150 kg ha⁻¹ increased base cations in the soil. During the decomposition process of organic matter, nutrients are released, including base cations (Cai et al., 2019). In addition, rock phosphate also contains elements of Ca and Mg (Kasno & Sutriadi, 2012); when P elements are released, Ca and Mg elements are also released. An increase in the elements of Ca and Mg will be followed by a decrease in Na, evidenced by the decreasing ESP value. The application

of mycorrhizae and cow dung–biochar significantly reduced the ESP value by a decrease of 66% compared to the control (Table 3). This is in line with the finding of (Rahayu, Syamsiyah, et al., 2019), that biochar and compost can reduce soil salinity levels. Mycorrhizae play an important role in reducing the stress of their host plants in stressful environments, including high levels of soil salinity (Barin et al., 2013). A high concentration of the Na element will affect the salinity of the soil, disruption of growth, and decrease productivity in plants. In addition, plants with Na toxicity can reduce N-P-K uptake (Yun et al., 2018) and increasing Na⁺, Cl⁻ and SO₄²⁻ causes ion imbalance in plants (Syamsiyah et al., 2020).

In this study, mycorrhizae six spores/plant and cow dung 60 tons ha⁻¹–biochar 25 tons ha⁻¹ (M1S5) affected to increase of CEC and decrease of ESP, and mycorrhizae–rock phosphate increased available P (Table 3); application of only mycorrhizae increased root infection, spore density and TG, but mycorrhizae with cow dung was the best for EEG (Table 5). The lowest SOC was in non-mycorrhizae and rock phosphate; available P, CEC, root infection, and spore density were found in the control (Tables 3 and Table 5), but the lowest of TG and EEG were found in non-mycorrhizae–rock phosphate (M0S2) (Table 5).

5. Conclusion

Addition of mycorrhizae up to six spores/plant, cow dung and biochar was the best ameliorant combination for improving soil chemical and biological characteristics in sandy soil. Application of mycorrhizae six spores/plant and cow dung 60 tons ha⁻¹–biochar 25 tons ha⁻¹ increased SOC (235%), available P (675%), CEC (216%), TG (101%), EEG (69%) and decreased ESP (66%). It also increased root infection and spore density than control (without mycorrhizae and soil ameliorants). The lowest of SOC, TG and EEG were found in non-mycorrhizae–rock phosphate (M0S2); but available P, CEC, root infection, spore density were found in the control. Further research is required regarding the long-term effects of soil ameliorants on sandy soil.

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Declaration of Competing Interest

The authors declare no competing financial or personal interests that may appear and influence the work reported in this paper.

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