EVALUATION OF ACIDIFIED BIOCHAR AND FARMYARD MANURE AS SUSTAINABLE SOIL MANAGEMENT AND MAIZE CULTIVATION IN ALKALINE CALCAREOUS SOILS

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Abstract

Poor soil organic matter is a significant problem in arid and semi-arid zones. Shifting trends towards the use of inorganic fertilizers over organic fertilizers have played a massive role in the depletion of soil organic pools. It also induced negative impacts on soil health and fertility status. Conventionally, farmyard manure (FYM) and recently activated carbon biochar are practical solutions. Growers usually discourage using alkaline nature biochar, especially when soil is high in pH. That is why a knowledge gap exists regarding using combined acidified biochar (AB) and FYM. To overcome this, a pot study was conducted. There were three levels of FYM (0M, 15M and 30M) and three levels of acidified biochar (0AB, 0.5AB and 1.0AB). Results confirmed that 30M and 15M remained significantly better than 0M under 1.0AB for improving different soil attributes. A significant increase in total soil N, available soil P, extractable soil K, plant height, 1000 grains weight, grains yield, plant N, P and K were observed where 30M and 1.0AB were applied compared to 0M. Treatment 30M with 1.0AB also played an imperative role in decreasing soil pH and increasing soil organic matter and EC compared to 0M with 1.0AB. In conclusion, 15M and 30M application rates are best when 1.0AB is applied in the soil to improve soil characteristics. To declare 15M and 30M with 1.0AB under different agroclimatic, more investigations are suggested under different soil textures.

Key words: Activated carbon; Growth attributes; Macronutrients; Organic fertilizer; Soil chemical attributes; Yield.

Introduction

Agricultural soils in dry and semi-arid areas are usually skeletal, have low organic matter levels (1%) and are lacking in nutrients like nitrogen (N), potassium (K), and phosphorus (P) (Brahim et al., 2014). Arid soils have a sandy texture, a weak soil structure, and a restricted water-holding capacity. As a result, plant growth and development in these arid zones are unfavorable and hostile. Inorganic fertilizers are traditionally used to improve soil fertility and plant productivity. Chemical fertilizer provides nutrients to crops in conveniently accessible forms, which results in quicker crop development and yields. However, because these fertilizers do not increase soil organic matter content, they do not appreciably improve several soil properties important to soil health (Garcia et al., 2017). Furthermore, mineral fertilizer leaks below the root zone due to irrigation or rainwater input, which can lead to more serious difficulties such as surface and groundwater contamination (Chen, 2006; Liang et al., 2013).

Organic additions improve the soil's physical, chemical, and microbiological characteristics, improving soil fertility and crop production (Eid *et al.*, 2017; Chen *et al.*, 2018). Farm manure has historically been applied to enhance soil organic matter content and related features (soil microbial activity, structure, water content, nutrient retention and cycling) (Lakhdar *et al.*, 2010). At the same time, the world's increasing urbanization and thriving economy have resulted in massive amounts of organic garbage. Organic waste (including municipal solid waste compost and sewage sludge compost) is increasingly used as fertilizer. It is a potentially beneficial way of recycling

and a possible alternative to landfill or incineration (Blanco-Canqui & Ruis, 2018).

Another essential organic amendment that has become the center of attention for many scientists worldwide is activated carbon, named biochar (Woods and Denevan, 2009). When combined with soil, biochar can alter the texture, pore size distribution, and bulk density, improving aeration and water-holding capacity (Verheijen et al., 2019; Werner et al., 2019; Hussain et al., 2020). In clay soils, for example, a bigger pore size (greater than the primary clay pores, like 10 nm in diameter) might be advised to promote the aeration of these very compacted soils. In soils with significant sand concentration, biochar can increase waterholding capacity and nutrient adsorption rather than aeration (Rawal et al., 2016). As a result, the heterogeneity of the porous structure of biochar can enhance the quantity of water held and the size and distribution of minerals as soil formers (Rawal et al., 2016).

Biochar has a liming impact (Feigl *et al.*, 2012). It improves soil cation exchange capacity by increasing pH, altering nutrient availability, and avoiding leaching (Fidel *et al.*, 2017). For example, high pH has been demonstrated to reduce the availability of phosphate, iron, boron, zinc, and manganese (Cheng *et al.*, 2018). As pH rises, so does microbial nitrification, resulting in nitrate losses and decreased availability of ammonium, the preferred nitrogen source for plants (Xiao and Meng, 2020). In this approach, the pH-raising impact of biochar might generate adverse circumstances for plants in some situations, especially in calcareous soils, resulting in yield losses (Bachmann *et al.*, 2016). That's why considering the importance of biochar and farmyard manure, the current study was planned to examine their combined impact on soil characteristics. This study covers the knowledge gap regarding using acidified biochar and farmyard manure variable combination application in the soil to evaluate their possible impacts on soil attributes. It is hypothesized that the combined use of farmyard manure and biochar could be more beneficial for improving soil characteristics than their sole application in soil. The novel aspect of this study is the use of acidified biochar with farmyard manure, while in old literature, most of the utilized biochar was alkaline in pH.

Material and Methods

Experimental site: The research experiment was conducted in the research area of Pesticide Quality Control Laboratory Multan, Punjab, Pakistan. The soil was mixed river alluvium, moderate calcareous, ochric epipedon and cambic sub-surface horizon. The physiochemical attributes of the soil are provided in (Table 1).

Experimental design and treatment plan: There were nine treatments, including three levels of acidified biochar and e application rates of farmyard manure. Treatments were applied in three replications following a completely randomized design (CRD). The treatments include control (no FYM and no AB = 0M+0AB), FYM 15 t/ha and no acidified biochar (15M+0AB), FYM 30 t/ha and no acidified biochar (30M+0AB), 0.5% acidified biochar and no FYM (0.5AB), 15M+0.5AB, 30M+0.5AB, 1.0% acidified biochar and no FYM (1.0AB), 15M+1.0AB and 30M+110AB.

Acidified biochar and Farmyard manure: Vegetable waste was collected from the local fruits and vegetable market (30.191821N; 71.479667E). After sun drying

collected material was pyrolyzed at 350°C for 3 hours to make biochar. After manufacturing, biochar was ground and put through a 2mm sieve. Finally, powder biochar was sprayed with a 60% H_2SO_4 solution to create acidity. After that, solar drying was completed before storage. The farmyard manure was obtained from a nearby dairy farm. Well-rotten manure was ground to pass through a 2mm sieve. Finally, manure was air dried and stored in plastic jars.

Seeds collection, sowing and incubation: Seeds of maize (Pioneer 30y87 Hybrid Corn) were purchased from the certified seed dealer of the industrial estate area of Multan. Initially, damaged seeds were screened out manually. After that 5 healthy seeds were separated for each pot for sowing. Plastic pots with 6-inch diameter and 12-inch-deep dimensions were used for incubation. One kg of experimental site soil was placed in each container. All therapies were administered following the treatment plan. Finally, the experiment maintained a temperature of $25 \pm 3^{\circ}$ C and a moisture content of 60% of the field capacity (w/w basis). Tap water is used for the maintenance of soil moisture. The attributes of tap water are provided in (Table 1).

Data collection and soil analysis: The soil was collected for examination after 28 days of incubation. Soil pH, EC organic matter, soil bulk density, N, P, and K levels were measured in samples. A 1:1 soil-deionized water paste was prepared for pH analysis. However, for determining soil EC, a soil extract was taken from a 1:10 soil-deionized water combination (Rhoades, 1996; Thomas, 1996). Potassium di-chromate was used with concentrated sulfuric acid to determine soil organic matter. Finally, titration was done with 0.5M ferrous ammonium sulphate (Nelson & Sommers, 1982). Analyses of bulk density (Blake, 1965), phosphorus (Kuo, 1996) and potassium (Pratt, 2016) were done as per standard protocols.

Soil	Units	Values	Reference	Water	Units	Values	Reference	
Sand		40		pН	-	7.11		
Silt	%	35	(Goo and Paudor 1086)	EC	μS/cm	432		
Clay		25	(Gee and Bauder, 1986)	TDS	mg/L	276		
Texture	Silt l	Loam		Carbonates		0.00		
Soil pH	-	8.04	(Page et al., 1982)	Bicarbonates		5.12	(Estefan et al., 2013)	
Soil EC	dS/m 1.61		(Rhoades, 1996) Chlorides meq./L 0		0.12	(Esteran et al., 2015)		
Soil OM	%	0.55	(Nelson and Sommers, 1982)	Ca+Mg		2.33		
Total N	%	0.05	(Bremner and Mulvaney, 1982)	Na		3.16		
Available P	ua/a	3.42	(Kuo, 1996)	SAR	-	2.93		
Extractable K	µg/g	151	(Pratt, 2016)	RSC	meq./L	2.79		
Biochar	Units	Values	Reference	Farmyard	Units	Values	Reference	
Biochar	Units	values	Kelefence	Manure	Units	values		
pН							(Bremner & Mulvaney,	
	-	5.21	(Page et al., 1982)	C:N	-	20:1	1982; Nelson &	
							Sommers, 1982)	
EC	dS/m	4.56	(Rhoades, 1996)	CEC	meq/100g	200	(Chapman, 1965)	
Volatile matter	%	20	(Danish and Zafar-ul-Hye,	Organic matter	%	25	(Nelson and Sommers,	
			(Dumbh and Zahar ar 1190, 2019)	0			1982)	
Ash contents	%	49	,	Total P	%	2.51	(Kuo, 1996)	
Fixed Carbon	%	31	(Noor <i>et al.</i> , 2012)	Total K	%	1.20	(Pratt, 2016)	
Total N	%	0.36	(Bremner & Mulvaney, 1982)					
Total P	%	0.16	(Kuo, 1996)					
Total K	%	0.80	(Pratt, 2016)					

Table 1. Physiochemical attributes of soil, water, acidified biochar and farmyard manure.

Antioxidants: To determine SOD activity, the inhibition of nitro blue tetrazolium (NBT) reduction in the presence of riboflavin was measured. The reaction mixture, containing enzyme extract, NBT, riboflavin, and phosphate buffer, was illuminated, and the absorbance change at 560 nm was monitored (Dhindsa et al., 1982). POD activity was assessed by monitoring the oxidation of a suitable substrate, such as guaiacol or o-dianisidine. The increase in absorbance resulting from substrate oxidation was measured at a specific wavelength (Hori et al., 1997). CAT activity was determined by monitoring the enzyme's decomposition of hydrogen peroxide (H₂O₂). The decrease in absorbance at 240 nm resulting from H₂O₂ decomposition was measured (Aebi, 1984). For ascorbate oxidation (APX) activity, in the presence of H₂O₂ was monitored (Nakano & Asada, 1981). The decrease in absorbance at a specific wavelength was measured over time. The level of MDA, an indicator of lipid peroxidation, was assessed by reacting the sample extract with thiobarbituric acid (TBA) to form a colored complex. The absorbance of the complex was measured, and the MDA content was calculated.

Statistical analyses

All the statistical analysis was done as per standard methods (Steel *et al.*, 1997). Paired comparisons and chord graphs were made by using the OriginPro 2021 software. Parallel plots also provided data ranges for all the studied attributes (OriginLab Corporation, 2021).

Results

Soil pH: Effect of acidified biochar (AB) and farmyard manure (M) variable application rates was significant on soil pH. The addition of 15M with 0AB showed no significant alteration in soil pH compared to 0M with 0AB. The application of 30M caused a significant decrease in soil pH over 0M and 15M under 0AB. In the case of 0.5AB, 0M, 15M and 30M were statistically similar for soil pH. However, on average, soil pH was less in 0M, 15M and 30M with 0.5AB compared to 0M, 15M and 30M with 0AB. A significant change in soil pH was noted where 15M and 30M were applied as treatments over 0M with 1.0AB. Both 15M and 30M showed no significant change in soil pH among each other under 1.0AB. The soil pH data showed that the combination of 0AB and 0M resulted in a pH value of 8.67. Compared to this control treatment, adding 0.5% AB resulted in a decrease in soil pH by 1.69% at 0M, while the increase in AB to 1.0% led to a decrease of 2.24%. At the same time, the application of M at a rate of 15M resulted in an increase in pH by 0.33%, whereas an application rate of 30M decreased pH by 1.06% compared to the control. When both AB and M were applied simultaneously, the results varied. The application of 0.5% AB and 15M increased soil pH by 0.26% compared to the control, while an increase in AB to 1.0% reduced the pH by 2.64% when combined with 30M. Interestingly, the combination of 0.5% AB and 30M decreased soil pH by 2.03% while increasing AB to 1.0% reduced 2.62% (Fig. 1A).

The chord diagram showed that the influence of 1.0AB was most prominent in decreasing the soil pH under 0M, 15M and 30M (Fig. 1B). It was observed that contribution of 1.0AB (32.89%) was higher than 0.5AB (33.27%) and 0AB (33.84%) for decrease in soil pH. Values also showed that 0.5AB caused more soil pH decline than 0AB.

Soil EC: The influence of AB and M variable application rates also remained significant on alteration in soil EC. It was observed that soil EC did not differ significantly for 0M and 15M treatment applications under 0AB. However, 30M caused a significant increase in soil EC over 0M under 0AB. At 0.5AB, 0M, 15M and 30M remained statistically alike for soil EC. However, 15M and 30M differed significantly for an increase in soil EC at 1.0AB. No significant alteration was observed between 15M and 30M under 1.0AB for soil EC. The data revealed that applying 0M and 0AB resulted in an EC of 0.817. Compared to this control treatment, adding 0.5% AB led to an increase in EC by 6.49%, while an increase in AB to 1.0% resulted in a further increase of 13.46%. The application of M at a rate of 15M decreased EC by 3.22% compared to the control. In contrast, an application rate of 30M led to a reduction of 7.36%. When both AB and M were applied together, the results were also affected by combining the two factors. The combination of 0.5% AB and 15M led to an increase in EC by 0.49% compared to the control, while the addition of 1.0% AB with 30M led to an increase in EC by 4.70%. On the other hand, the combination of 0.5% AB and 30M decreased EC by 2.94%, while an increase in AB to 1.0% led to a reduction of 3.43%. (Fig. 2A).

The chord diagram showed that the influence of 1.0AB was most prominent in decreasing the soil EC under 0M, 15M and 30M (Fig. 2B). It was observed that the contribution of 1.0AB (34.06%) was higher than 0.5AB (33.25%) and 0AB (32.69%) for increase in soil EC. Values also showed that 0.5AB caused more increase in soil EC compared to 0AB.

Soil organic matter: Results showed that soil organic was significantly changed by applying variable levels of AB and M. A significant improvement in soil organic matter was observed in 30M under 0AB over 0M. The addition of 15M also caused a significant increase in soil organic matter compared to 0M under 0AB. Similar improvements in soil organic matter were also noted in 15M and 30M over 0M, where 0.5AB was applied. However, 15M and 30M remained statistically alike for soil organic matter under 0.5AB. A significant improvement in soil organic matter was noted in 30M compared to 15M and 0M under 1.0AB. However, 15M also differed significantly better than 0M under 1.0AB for soil organic matter (Fig. 3A). According to the results of this study, the addition of acidified biochar (AB) and farmyard manure (M) had a significant impact on soil organic matter (OM) content. Adding 0.5% AB led to an increase in OM by 5.94% compared to the control treatment. Increasing the AB level to 1.0% resulted in a further increase of 6.69%. Similarly, the application of M at a rate of 15M resulted in a significant increase in OM by 46.29% compared to the control, whereas an application rate of 30M led to an even higher increase of 56.98%. However, when both AB and M were applied together, the results were affected by the combination of the two factors. The combination of 0.5% AB and 15M led to an increase in OM by 41.55% compared to the control, while the addition of 1.0% AB with 30M led to an even higher increase in OM by 54.21%. On the other hand, the combination of 0.5%AB and 30M decreased OM by 11.26%, while an increase in AB to 1.0% led to a reduction of 5.22%.

The chord diagram showed that the influence of 1.0AB was most prominent in decreasing the soil OM under 0M, 15M and 30M (Fig. 3B). It was observed that the contribution of 1.0AB (33.62%) was higher than 0.5AB (32.85%) and 0AB (33.53%) for an increase in soil OM.

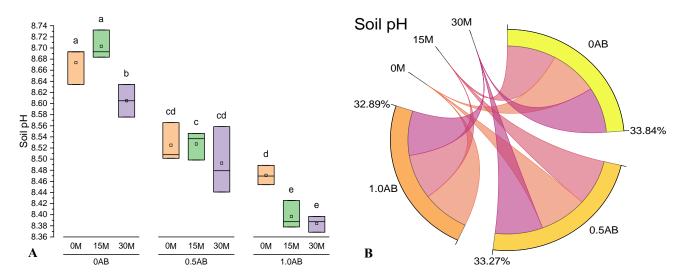


Fig. 1. Effect of farmyard manure and acidified biochar application rates on soil pH. Different bars are an average of 3 replicates \pm SE. Variable letters on the bars show significant changes at $p \le 0.05$ (A). The chord diagram shows the percentage contribution of each acidified biochar level for altering soil pH under different levels of farmyard manure (B).

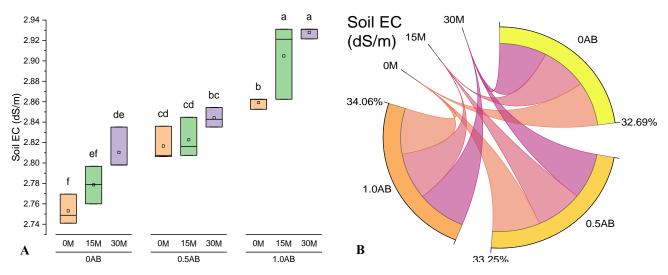


Fig. 2. Effect of farmyard manure and acidified biochar application rates on soil EC. Different bars are an average of 3 replicates \pm SE. Variable letters on the bars show significant changes at $p \le 0.05$ (A). The chord diagram shows the percentage contribution of each acidified biochar level for altering soil EC under different levels of farmyard manure (B).

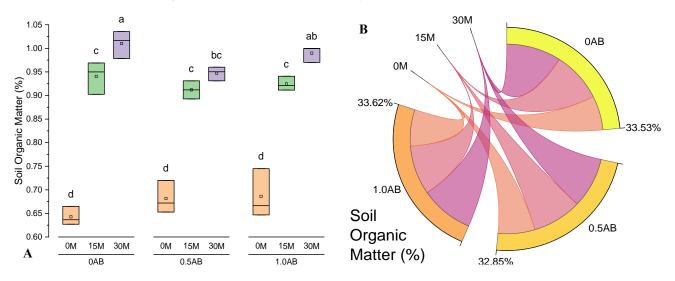


Fig. 3. Effect of farmyard manure and acidified biochar application rates on soil organic matter. Different bars are an average of 3 replicates \pm SE. Variable letters on the bars show significant changes at $p \le 0.05$ (A). Chord diagram shows the percentage contribution of each acidified biochar level for altering soil organic matter under different levels of farmyard manure (B).

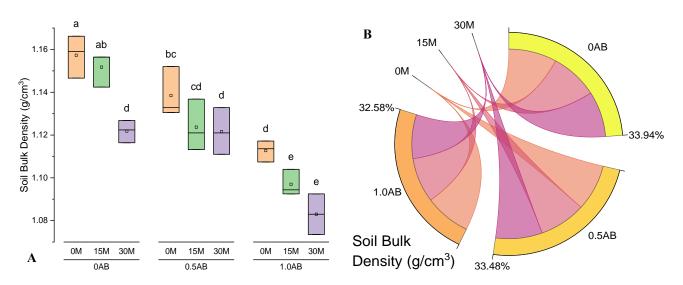


Fig. 4. Effect of farmyard manure and acidified biochar application rates on soil bulk density. Different bars are an average of 3 replicates \pm SE. Variable letters on the bars show significant changes at $p \le 0.05$ (A). Chord diagram shows the percentage contribution of each acidified biochar level for altering soil bulk density under different levels of farmyard manure (B).

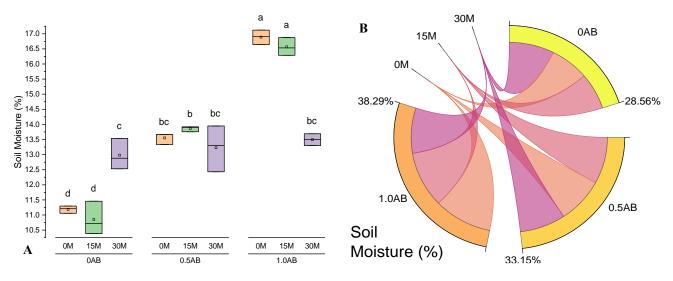


Fig. 5. Effect of farmyard manure and acidified biochar application rates on soil moisture. Different bars are an average of 3 replicates \pm SE. Variable letters on the bars show significant changes at $p \le 0.05$ (A). The chord diagram shows the percentage contribution of each acidified biochar level for altering soil moisture under different levels of farmyard manure (B).

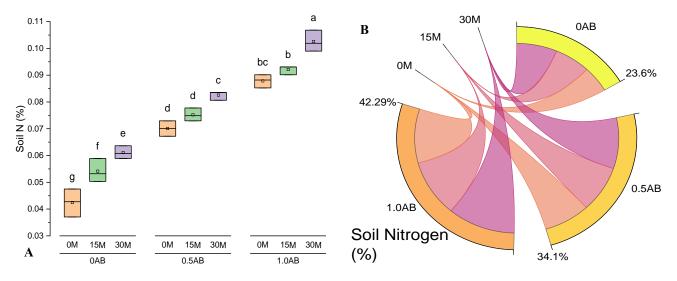


Fig. 6. Effect of farmyard manure and acidified biochar application rates on soil nitrogen. Different bars are an average of 3 replicates \pm SE. Variable letters on the bars show significant changes at $p \le 0.05$ (A). The chord diagram shows the percentage contribution of each acidified biochar level for altering soil nitrogen under different levels of farmyard manure (B).

Soil bulk density: The impacts of treatments, i.e., AB and M, were significant for change in soil bulk density. Statistical analysis confirmed that 0M and 15M did not cause any significant change in soil bulk density with each other under 0AB and 0.5AB. A significant decrease in soil bulk density was observed due to adding 30M under 0AB and 0.5AB compared to 0M. However, applying 15M under 1.0AB caused a significant decrease in soil bulk density over 0M. In addition to the above, 30M also caused a significant decrease in soil bulk density compared to 0M under 1.0AB. Both 15M and 30M remained statistically similar for soil bulk density when applied with 1.0AB (Fig. 4A). Compared to the control treatment, the addition of 0.5% AB resulted in a decrease in bulk density by 1.52%, while an increase in AB to 1.0% resulted in a further reduction of 5.85%. However, the application of M at a rate of 15M and 30M led to an increase in bulk density by 0.39% and 3.07%, respectively, compared to the control. When both AB and M were applied together, the results were also affected by combining the two factors. The combination of 0.5% AB and 15M led to a decrease in bulk density by 0.41% compared to the control, while the addition of 1.0% AB with 30M resulted in a decrease in bulk density by 6.32%. On the other hand, the combination of 0.5% AB and 30M resulted in an increase in bulk density by 2.98%. In comparison, an increase in AB to 1.0% led to an increase in bulk density by 2.29%.

The chord diagram showed that the influence of 1.0AB was most prominent in decreasing the soil bulk density under 0M, 15M and 30M (Fig. 4B). It was observed that the contribution of 1.0AB (32.58%) was higher than 0.5AB (33.48%) and 0AB (33.94%) for the decrease in soil bulk density. Values also showed that 0.5AB caused more decrease in soil bulk density compared to 0AB.

Soil moisture: Soil moisture was significantly affected by adding variable application rates of AB and M. Treatment 30M was significantly better compared to 15M and 0M for the improvement in soil moisture contents under OAB. No significant variation in soil moisture was observed where 15M was applied over 0M under 0AB. It was observed that 0M, 15M and 30M remained statistically alike under 0.5AB for soil moisture contents. Furthermore, 0M and 15M were significantly better than 30M for the enhancement in soil moisture under 1.0AB. However, no significant change was observed in soil moisture where 0M and 15M were applied as treatments under 1.0AB (Fig. 5A). Adding AB and M at different levels had varying effects on soil moisture content compared to the control treatment. The addition of 0.5% AB resulted in a significant increase in soil moisture by 21.10%, while an increase in AB to 1.0% resulted in a further increase of 50.59%. The application of M at a rate of 30M also resulted in a significant increase in soil moisture by 16.16% compared to the control. In contrast, applying 15M led to a decrease in soil moisture by 2.94%. When both AB and M were applied together, the results were also affected by combining the two factors. The combination of 0.5% AB and 15M resulted in an increase in soil moisture by 23.47% compared to the control, while the addition of 1.0% AB with 30M led to a decrease in soil moisture by 17.08%. On

the other hand, the combination of 0.5% AB and 30M increased soil moisture by 18.26%, while an increase in AB to 1.0% led to a decrease in soil moisture by 20.01%. The chord diagram showed that the influence of 1.0AB was most prominent in improving the soil moisture contents under 0M, 15M and 30M (Fig. 5B).

The chord diagram showed that the influence of 1.0AB was most prominent in decreasing the soil moisture under 0M, 15M and 30M (Fig. 5B). It was observed that the contribution of 1.0AB (38.29%) was higher than 0.5AB (33.15%) and 0AB (28.56%) for an increase in soil moisture. Values also showed that 0.5AB caused more increase in soil moisture compared to 0AB.

Soil nitrogen: The effects of treatments also remained significant for improvement in soil nitrogen concentration. A significant enhancement in soil nitrogen concentration was noted where 15M and 30M were applied over 0M under 0AB. In the case of 0.5AB, 30M performed significantly better compared to 15M and 0M for the enhancement in soil nitrogen concentration. However, 15M and 0M remained statistically similar for soil nitrogen concentration under 0.5AB. Similar results were also observed under 1.0AB where 30M showed significantly higher soil nitrogen concentration than 15M and 0M (Fig. 6A). In comparison to the control treatment (0AB and 0M), the introduction of 0.5% AB resulted in a 65.13% increase in soil N concentration, while increasing AB to 1.0% led to a further 106.47% increase. Additionally, applying M at 15M and 30M rates led to a notable rise in soil N concentration by 27.86% and 142.16%, respectively, relative to the control. When both AB and M were utilized concurrently, the combination of the two factors significantly influenced the outcomes. The combination of 0.5% AB and 15M resulted in a significant 77.02% increase in soil N concentration when compared to the control, whereas the application of 1.0% AB with 30M led to an even higher increase of 182.57%. Conversely, the combination of 0.5% AB and 30M decreased soil N concentration by 22.57%, while increasing AB to 1.0% led to a reduction of 14.27%. The chord diagram showed that the influence of 1.0AB was most prominent in enhancing the soil nitrogen concentration under 0M, 15M and 30M (Fig. 6B).

The chord diagram showed that the influence of 1.0AB was most prominent in decreasing the soil N under 0M, 15M and 30M (Fig. 6B). It was observed that the contribution of 1.0AB (42.29%) was higher than 0.5AB (34.10%) and 0AB (23.60%) for increase in soil N. Values also showed that 0.5AB caused more increase in soil N compared to 0AB.

Soil phosphorus: The influence of applied AB and M variable levels was significant on soil phosphorus concentration. No significant change in soil phosphorus concentration was noted among 0M, 15M and 30M under 0AB and 0.5AB. However, significant enhancement in soil phosphorous concentration was noted in 1.0AB when 30M and 15M were applied as treatments. No significant change in soil phosphorus was noted when 15M and 30M were compared under 1.0AB (Fig. 7A). Over control treatment (0AB and 0M), adding 0.5% AB resulted in a modest increase in soil P concentration by 11.55%, while

increasing AB to 1.0% led to a further increase of 41.05%. Additionally, the application of M at 15M and 30M rates also led to significant increases in soil P concentration by 7.31% and 12.21%, respectively, compared to the control. The interaction between AB and M further influenced the results. The combination of 0.5% AB and 15M led to a slight increase in soil P concentration by 12.91% compared to the control, while the addition of 1.0% AB with 30M resulted in a higher increase of 25.63%. However, the combination of 0.5% AB and 30M decreased soil P concentration by 2.63%, while increasing AB to 1.0%

resulted in a reduction of 3.34%. The chord diagram showed that the influence of 1.0AB was most prominent in enhancing the soil phosphorus concentration under 0M, 15M and 30M (Fig. 7B).

The chord diagram showed that the influence of 1.0AB was most prominent in decreasing the soil P under 0M, 15M and 30M (Fig. 7B). It was observed that the contribution of 1.0AB (36.74%) was higher than 0.5AB (33.33%) and 0AB (29.93%) for increase in soil P. Values also showed that 0.5AB caused more increase in soil P compared to 0AB.

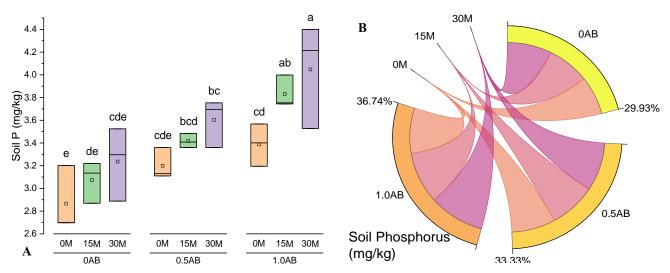


Fig. 7. Effect of farmyard manure and acidified biochar application rates on soil phosphorus. Different bars are an average of 3 replicates \pm SE. Variable letters on the bars show significant changes at $p \le 0.05$ (A). The chord diagram shows the percentage contribution of each acidified biochar level for altering soil phosphorus under different levels of farmyard manure (B).

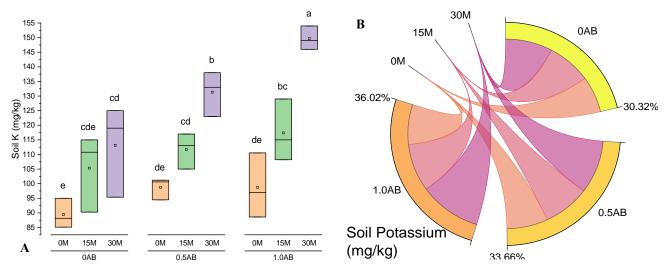


Fig. 8. Effect of farmyard manure and acidified biochar application rates on soil potassium. Different bars are an average of 3 replicates \pm SE. Variable letters on the bars show significant changes at $p \le 0.05$ (A). Chord diagram shows the percentage contribution of each acidified biochar level for altering soil potassium under different levels of farmyard manure (B).

Soil potassium: In the case of soil potassium concentration, the impact of treatments was significant. The addition of 30M performed significantly better to enhance soil potassium concentration over 0M under 0AB and 0.5AB. No significant change in soil potassium concentration was noted where 15M and 0M were applied under 0AB and 0.5AB. However, 30M was significantly better than 15M under 0.5AB but showed no significant variation under 0AB for

soil potassium concentration. It was noted that both 15M and 30M performed significantly better than 0M under 1.0AB for the improvement in soil potassium concentration. The application of 30M also differed significantly better than 15M to improve soil potassium concentration under 1.0AB (Fig. 8A). Results showed that compared to the control treatment (0AB and 0M), adding 0.5% AB resulted in a moderate increase in soil K concentration by 10.47%, while

24.98% compared to the control, while the addition of 1.0% AB with 30M resulted in an even higher increase of 66.74%. However, the combination of 0.5% AB and 30M resulted in a decrease in soil K concentration by 13.51%, while an increase in AB to 1.0% led to a reduction of 13.19%. The chord diagram showed that the influence of 1.0AB was most prominent in enhancing the soil potassium concentration under 0M, 15M and 30M (Fig. 8B).

The chord diagram showed that the influence of 1.0AB was most prominent in decreasing the soil K to under 0M, 15M and 30M (Fig. 7B). It was observed that the contribution of 1.0AB (36.02%) was higher than 0.5AB (33.66%) and 0AB (30.32%) for increase in soil K. Values also showed that 0.5AB caused more increase in soil K compared to 0AB.

Plant height, 1000 grains weight and grains yield: Impact of variable application rates of AB and M was significant on plant height, 1000 grains weight and grains yield of maize. It was observed that 15M and 30 significantly improved maize plants height compared to 0M under 0AB and 0.5AB. Both 15M and 30M remained statistically alike for plant height under OAB. Furthermore, 30M remained significantly better than 15M for improvement in maize plant height under 0.5AB. No significant change in plant height was observed where 0M and 15M were applied under 1.0AB. However, the addition 30M was significantly better than 15M and 0M under 1.0AB. Compared to the control treatment (0AB and 0M), the addition of 0.5% AB resulted in a slight increase in plant height by 3.36%, while an increase in AB to 1.0% led to a further increase of 18.28%. Moreover, the application of M at a rate of 15M and 30M also resulted in significant increases in plant height by 8.61% and 5.73%, respectively, compared to the control. When both AB and M were applied together, the results were also influenced by the combination of the two factors. The combination of 0.5% AB and 15M led to a significant increase in plant height by 8.51% compared to the control, while the addition of 1.0% AB with 30M resulted in an even higher increase of 18.19%. On the other hand, the combination of 0.5% AB and 30M decreased plant height by 4.21%, while an increase in AB to 1.0% led to a reduction of 11.68%. Maximum increase of 18.20% was observed in plant height where 30M+1.0AB was applied compared to 0M+0AB.

For 1000 grains weight, 15M and 30M differed significantly for enhancement compared to 0M under 0AB. No significant change in 1000 grains weight was noted where 15M and 0M were applied under 0.5AB and 1.0AB. However, significant enhancement in 1000 grains weight was noted in 30M compared to 0M under 0.5AB and 1.0AB. Based on the data presented, compared to the control treatment of 0AB and 0M, the addition of 0.5% AB resulted in a slight increase in plant height by 3.38%, while an increase in AB to 1.0% led to a further increase of

18.12%. Moreover, the application of M at a rate of 15M and 30M also resulted in significant increases in plant height by 2.56% and 6.08%, respectively, compared to the control. When both AB and M were applied together, the results were also influenced by combining the two factors. The combination of 0.5% AB and 15M led to a significant increase in plant height by 8.78% compared to the control, while the addition of 1.0% AB with 30M resulted in an even higher increase of 18.20%. On the other hand, the combination of 0.5% AB and 30M resulted in a slight decrease in plant height by 0.63%, while an increase in AB to 1.0% led to a reduction of 2.50%. Regarding the 1000 grains weight, compared to the control treatment, the addition of 0.5% AB led to a moderate increase in 1000 grains weight by 8.16%, while an increase in AB to 1.0% led to a further increase of 25.40%. Additionally, the application of M at a rate of 15M and 30M resulted in significant increases in 1000 grains weight by 8.89% and 21.35%, respectively, compared to the control.

In the case of grain yield, the addition of 15M and 30M induced significant enhancement compared to control under 0AB. Treatment 15M did not differ significantly over 0M under 0.5AB for grain yield. However, 30M remained significantly better for improvement in grain yield from 0M under 0.5AB. It was observed that 0M, 15M and 30M showed no significant change for enhancement in grain yield under 1.0AB. The results showed that the application of 0.5% AB resulted in a moderate increase in grains yield by 7.76%, while an increase in AB to 1.0% led to a further increase of 32.90%. Similarly, the application of M at a rate of 15M and 30M resulted in significant increases in grains yield by 7.86% and 18.60%, respectively, compared to the control. When both AB and M were applied together, the results were also influenced by the combination of the two factors. The combination of 0.5% AB and 15M led to a significant increase in grains yield by 7.89% compared to the control, while the addition of 1.0% AB with 30M resulted in an even higher increase of 32.77%. However, the combination of 0.5% AB and 30M resulted in a decrease in grains yield of 2.65%, while an increase in AB to 1.0% led to a reduction of 2.06% (Table 2).

POD, SOD, CAT, APx, H₂O₂ and MDA activity: For the control condition with 0AB and 0M, the mean POD activity was 32.13 U/mg Protein. When 0AB and 15M were introduced, there was a slight decrease in POD activity of 1.25%. Similarly, when 0AB was combined with 30M, the POD activity decreased by 1.51%. Moving to the treatment with 0.5AB and 0M, there was a significant decrease in POD activity, representing a substantial decrease of 37.42% compared to the control, while 0.5AB was combined with 15M, the POD activity further decreased by 3.76%. In comparison to the control the most substantial reduction in POD activity occurred when 0.5AB was combined with 30M, resulting in a remarkable 7.87% decrease. The highest level of AB at 1.0AB and 0M showed a significant decrease in POD activity, representing a substantial 61.04% reduction in contrast to the control. When 1.0AB was combined with 15M, the POD activity decreased by 5.70%. The most significant decrease in POD activity occurred with 1.0AB and 30M, showing a remarkable 10.36% reduction compared to the control (Table 3).

Acidified biochar	Farmyard manure	Plant height	1000 grains weight	Grains yield
(%)	(t/ha)	(cm)	(g)	(kg/ha)
0AB	0M	132.37e	204.88e	6872f
0AB	15M	137.12d	222.93d	7406e
0AB	30M	140.60cd	229.27bc	8160cd
0.5AB	0M	136.96d	231.04cd	7955d
0.5AB	15M	143.68c	229.12cd	8363b-d
0.5AB	30M	149.76b	248.32ab	8669a-c
1.0AB	0M	148.96b	241.73bc	8611a-c
1.0AB	15M	151.25b	248.92ab	8699ab
1.0AB	30M	156.47a	256.11a	9125a

Table 2. Effect of farmyard manure and acidified biochar application rates on growth attributes of maize. Different values are an average of 3 replicates. Variable letters show significant changes at *p*<0.05.

AB = Acidified biochar; M = Farmyard manure

Table 3. Effect of farmyard manure and acidified biochar application rates on antioxidant attributes (POD (Peroxidase), SOD (Superoxide Dismutase), CAT (Catalase), APx (Ascorbate Peroxidase), H₂O₂ (Waer content), and MDA (Malondialdehyde)) of maize. Different values are an average of 3 replicates. Variable letters show significant changes at *p*<0.05.

maize. Different values are an average of 5 replicates. variable letters show significant changes at $p \ge 0.05$.							
Acidified	Farmyard	POD (U/mg	SOD (U/mg	CAT (U/mg	APx (U/mg	H ₂ O ₂	MDA (nmol/mg
biochar (%)	manure (t/ha)	protein)	protein)	protein)	Protein)	(n mol/g FW)	protein)
0AB	0M	32.13a	21.85a	56.20a	4.95a	45.02a	1.24a
0AB	15M	31.74a	20.56ab	55.17a	4.80ab	41.70a	1.20a
0AB	30M	31.66ab	18.77ab	53.36ab	4.70abc	40.74ab	1.15ab
0.5AB	0M	23.38bc	16.41bc	42.26abc	3.84abc	27.07bc	0.88bc
0.5AB	15M	22.54cd	15.62bcd	40.09bc	3.70bcd	25.58cd	0.84bc
0.5AB	30M	21.68cde	10.83de	37.95cd	3.59cd	23.94cde	0.79cd
1.0AB	0M	14.52def	11.45cde	25.44de	2.51de	13.67cde	0.51de
1.0AB	15M	13.74ef	9.37e	24.34de	2.37e	11.96de	0.47de
1.0AB	30M	13.16f	8.15e	23.36e	2.10e	10.99e	0.45e
AD = A + J + f + J	$1 \cdot 1 \cdot$						

AB = Acidified biochar; M = Farmyard manure

Table 4. Effect of farmyard manure and acidified biochar application rates on maize plants' N, P and K. Different values are an average of 3 replicates. Variable letters show significant changes at p≤0.05.

Acidified biochar	Farmyard manure	Plant nitrogen	Plant phosphorus	Plant potassium
(%)	(t/ha)	(%)	(%)	(%)
0AB	0M	0.98g	0.117f	1.58e
0AB	15M	1.25f	0.117f	1.64de
0AB	30M	1.36df	0.124ef	1.67c-e
0.5AB	0M	1.51e	0.141de	1.67с-е
0.5AB	15M	1.85d	0.147d	1.69cd
0.5AB	30M	2.05c	0.170bc	1.72b-d
1.0AB	0M	2.19b	0.154cd	1.75a-c
1.0AB	15M	2.46a	0.176ab	1.80ab
1.0AB	30M	2.56a	0.189a	1.83a

AB = Acidified biochar; M = Farmyard manure

Without AB (0AB) and M (0M), the SOD activity was measured at 21.85 U/mg Protein. When 15M was introduced under the 0AB conditions, there was a 6.27% reduction in SOD activity over the control, while the addition of 30M under 0AB conditions led to a more pronounced decrease in SOD activity, with a 16.43% reduction. Under 0.5AB conditions without M (0M), the SOD activity decreased significantly, indicating a 33.18% reduction compared to the control and when 15M was introduced under the same 0.5AB conditions, there was a 5.01% reduction in SOD activity. Notably, the addition of 30M under 0.5AB conditions led to a remarkable 73.28% decrease in SOD activity over the control. Finally, under 1.0AB conditions without M (0M), representing a 43.25% decrease in SOD activity compared to the control. When 15M was introduced under the same 1.0AB conditions, there was a 22.19% reduction in SOD activity over the

control. The addition of 30M under 1.0AB conditions led to a 40.53% decrease in SOD activity (Table 3).

Under 0AB conditions, adding 15M resulted in a slight decrease of 1.87% in CAT levels, while applying 30M led to a more substantial decrease of 5.34%. Moving on to 0.5AB conditions, CAT activity exhibited a significant decrease of 32.99% when no M was added over the control (0AB). When 15M was introduced, CAT activity decreased by 37.62% and the highest decrease was observed when 30M was added, resulting in a 40.61% reduction in CAT levels compared to the control. Finally, under 1.0AB conditions, CAT levels showed a remarkable increase of 66.09% when no M was present. The addition of 15M led to a slightly lower but still substantial decrease of 64.71% over the control. The inclusion of 30M resulted in a noteworthy 62.42% decrease in CAT levels compared to the control (Table 3).

The results for APx (U/mg Protein) showed significant variations in response to different combinations of Acidified Biochar (AB) and Farmyard Manure (FYM). Under 0AB conditions, adding 15M led to a 3.19% decrease in APx activity, while 30M resulted in a 5.46% decrease. Furthermore, under 0.5AB conditions, APx activity exhibited a 29.10% decrease without M, whereas 15M and 30M resulted in 3.60% and 6.87% decreases. respectively over the control (OAB). In comparison to the control (0AB), at 1.0AB treatment APx activity showed a substantial decrease of 52.65% without M, while 15M and 30M led to decreases of 6.20% and 19.68%, respectively. The presence of 15M under 0AB conditions resulted in a 29.61% decrease in APx activity, whereas 30M led to a 30.83% decrease related to the control 0AB. Under 0.5AB conditions, 15M and 30M caused decreases of 3.60% and 6.87%, over the control and at 1.0AB treatment, the addition of 15M and 30M resulted in increases of 6.20% and 19.68%, respectively, compared to the control group with AB and M (Table 3).

The results for H_2O_2 (n mol/g FW) demonstrated notable variations in response to different combinations of Acidified Biochar (AB) and Farmyard Manure (FYM) levels.

Under 0AB conditions, the application of 15M led to a 7.97% decrease in H₂O₂ levels compared to the control AB with M, while 30M resulted in a more substantial reduction of 10.50%. Moving to 0.5AB conditions, the absence of M (0M) resulted in a remarkable 66.31% decrease in H₂O₂ levels compared to the control. When 15M was added under the same conditions, there was a significant 5.84% reduction in H₂O₂ levels. The most prominent change occurred with 30M, which led to a substantial 13.09% decrease in H₂O₂ levels compared to the control AB with M. Under 1.0AB conditions, the absence of M (0M) resulted in an impressive 98.02% reduction in H₂O₂ levels compared to the control. The addition of 15M led to a substantial 14.30% increase in H₂O₂ levels, while 30M resulted in an even more remarkable 24.35% increase compared to the control AB with M (Table 3).

Under 0AB conditions, adding 15M and 30M exhibited MDA levels of 1.20 and 1.15 nmol/mg Protein, respectively. This indicated a 3.33% decrease for 15M and an 8.14% decrease for 30M compared to the control AB with M. When the acidified biochar (AB) level was increased to 0.5AB, represented a 4.37% decrease for 15M and an 11.44% decrease for 30M when compared to the control AB with M. Under the highest AB level of 1.0AB, resulted an 8.45% decrease for 15M and a 14.07% decrease for 30M related to the control AB with M. Overall, it can be observed that increasing the AB level generally led to a decrease in MDA levels, with the greatest reductions occurring in the presence of both 0.5AB and 1.0AB (Table 3).

Plants N, P and K: Results showed that 15M and 30 performed significantly better than 0M for an increase in N under 0AB, 0.5AB and 1.0AB. Both 15M and 30 did not differ significantly for plant nitrogen under 0AB and 1.0AB. However, 30M remained significantly better than 15M for enhancement in plant nitrogen under 0.5AB. Specifically, compared to the control treatment (0AB and

0M), the application of 0.5% AB led to a moderate increase in plant nitrogen content by 54.08%, while an increase in AB to 1.0% and 1.5% led to a further increase of 141.84% and 262.24%, respectively. Similarly, the application of M at a rate of 15M and 30M resulted in significant increases in plant nitrogen content by 27.55% and 38.78%, respectively, compared to the control. When both AB and M were applied together, the results were also influenced by combining the two factors. The combination of 0.5% AB and 15M led to a significant increase in plant nitrogen content by 89.79%, while the addition of 1.0% AB with 30M resulted in an even higher increase of 175.51%. On the other hand, the combination of 0.5% AB and 30M resulted in a decrease in plant nitrogen content by 26.47%, while an increase in AB to 1.0% led to a reduction of 24.49%. Maximum increase of 16.89% was observed in plant nitrogen where 30M+1.0AB was applied compared to 0M+1.0AB (Table 4).

In the case of plant phosphorus, 0M,15M, and 30M remained statistically alike under OAB. No significant change in plant phosphorus was noted between 0M and 15M, but 30M remained significantly better for improvement in plant phosphorus under 0.5AB (Table 3). It was noted that 30M and 15M performance was significantly better than 0M under 1.0AB for enhancement in plant phosphorus. The addition of 0.5% AB resulted in a moderate increase in plant phosphorus content by 5.13%, while increasing AB to 1.0% led to a further increase of 26.50%. Similarly, the application of M at a rate of 15M and 30M resulted in significant increases in plant phosphorus content by 6.84% and 25.64%, respectively, compared to the control. When both AB and M were applied together, the results were also influenced by the combination of the two factors. The combination of 0.5% AB and 15M led to a significant increase in plant phosphorus content by 15.38% compared to the control, while the addition of 1.0% AB with 30M resulted in an even higher increase of 61.54%. On the other hand, the combination of 0.5% AB and 30M resulted in a decrease in plant phosphorus content by 14.53%, while an increase in AB to 1.0% led to a reduction of 10.26%. Maximum increase of 22.72% was observed in plant phosphorus where 30M+1.0AB was applied compared to 0M+1.0AB (Table 4).

Compared to the control treatment (0AB and 0M), the application of 0.5% AB resulted in a moderate increase in plant potassium content by 3.80%, while an increase in AB to 1.0% led to a further increase of 15.19%. Similarly, the application of M at a rate of 15M and 30M resulted in significant increases in plant potassium content by 3.80% and 5.70%, respectively, compared to the control. When both AB and M were applied together, the results were also influenced by combining the two factors. The combination of 0.5% AB and 15M led to a significant increase in plant potassium content by 5.06% compared to the control, while the addition of 1.0% AB with 30M resulted in an even higher increase of 10.76%. On the other hand, the combination of 0.5% AB and 30M decreased plant potassium content by 1.90%. In comparison, an increase in AB to 1.0% led to a reduction of 2.55% (Table 4).

The principal component analysis (PCA) results showed that soil pH was the most important factor in

explaining the variation in soil properties, with an eigenvalue of 4.0669 accounting for 50.84% of the total variance. The second most important factor was soil organic matter (SOM), with an eigenvalue of 2.75221, explaining 34.40% of the variance. Soil electrical conductivity (EC) and bulk density had eigenvalues of 0.41153 and 0.25488, respectively, and accounted for 5.14% and 3.19% of the variance, respectively. Soil moisture and nitrogen (N) content had eigenvalues of 0.21588 and 0.18239, respectively, explaining 2.70% and 2.28% of the variance, respectively. Soil phosphorus (P) and potassium (K) had lower eigenvalues of 0.0771 and 0.03912, respectively, and accounted for 0.96% and 0.49% of the variance, respectively. The cumulative percentage of variance explained by the first four principal components was 93.57%, indicating that these factors accounted for the majority of the variation in soil properties (Fig. 9).

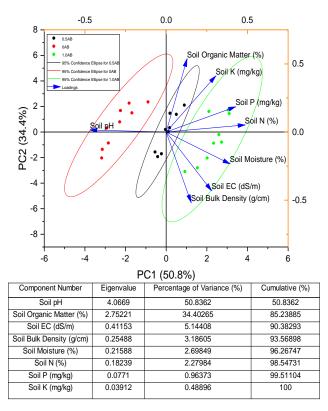


Fig. 9. Principal component analysis for the studied soil attributes.

Discussion

Biochar is a carbon-rich substance that is introduced into the soil. It is composed of organic matter, a nutrient-rich substance with a highly porous structure. Once introduced into the soil, biochar can remain there for decades, providing long-term benefits to the ecosystem. The structure of biochar is highly fragrant, which can attract soil microbes to colonize its surface(Lehmann *et al.*, 2011; Farrell *et al.*, 2014). The porous structure of biochar enhances soil porosity, which improves the soil's water-holding capacity, aeration, and drainage. The soil benefits from the nutrient-rich qualities of biochar, enhancing fertility through the supply of essential nutrients that foster plant growth. Moreover, biochar serves as a soil-building substance, imparting a durable structure that encourages the development of water-stable soil

aggregates. This can result in improved soil structure, reduced erosion, and increased plant growth (Lehmann et al., 2011; Farrell et al., 2014). Furthermore, adding organic matter and enhancing the physicochemical characteristics of the soil are two additional benefits of biochar (Ahmed et al., 2021). Through a gradual oxidation process, carboxylic functional groups are developed on the surface of biochar. The development of these acidic functional groups played an imperative role in neutralizing alkalinity, resulting in a decrease in soil pH (Cheng et al., 2008). The ash concentration and composition of biochar, as well as the ionic makeup of the soil, are the critical determinants of electrical conductivity (Ahmed et al., 2021). Higher ash contents in acidified biochar in the current study were responsible for increased soil EC (Lentz and Ippolito, 2012). Acidified biochar has the potential to increase the solubility of phosphorus (P) in calcareous soils, which are characterized by high pH and the presence of calcium (Ca) and magnesium (Mg). This is because acidified biochar has a lower pH than the soil, which can cause a decrease in the concentration of Ca and Mg ions in the soil solution. This reduction in Ca and Mg ions can increase the solubility of P in the soil, making it more available for plant uptake (Bashir et al., 2018). Furthermore, acidified biochar can also provide additional P to the soil by releasing P in the biochar. This P release can be facilitated by the lower pH of the acidified biochar, promoting the dissolution of P-containing minerals in the biochar (DeLuca et al., 2015). Soil organic matter contributes to the preservation of an optimal physical surroundings in soils by affecting and improving physical soil characteristics such as void fraction (or porosity), aggregation, bulk density, and water ability (Jenkinson, 1981). Organic matter disintegration is a natural process that plays a crucial role in soil quality enhancement. This process has been shown to have positive effects on soil porosity and water-stable aggregation, which is attributed to the synthesis of a complex chain of polysaccharides and secretory byproducts by soil bacteria. These materials help to improve soil structure and provide a more favorable environment for plant growth. However, the breakdown of organic matter can also lead to a significant drop in soil pH, which can affect soil fertility negatively (Tiemann & Billings, 2011). Fortunately, organic fertilizer inputs can counteract these negative effects by promoting the production of CO₂ and organic acids during the microbial process of decomposition. These substances help to neutralize the acidity and promote microbial growth, resulting in microbial activation as a result of the addition of organic manures as a substrate amount. The overall result is improved soil quality and enhanced plant growth, highlighting the importance of organic matter disintegration in sustainable agriculture (Tiemann & Billings, 2011). When carbon dioxide (CO₂) dissolves in water (H₂O), it forms carbonic acid (H₂CO₃), which can react with natural calcium carbonate (CaCO₃) in the soil. This reaction releases Ca into the soil solution and also produces bicarbonate ions (HCO₃⁻). However, the addition of Ca to the soil solution can lead to the displacement of other cations such as sodium (Na⁺), which can lower the soil pH due to microbial decomposition (Nannipieri et al., 2018). Nitrogen is recognized to be functional in forming amino acids and chlorophyll, which can influence plant growth and development by influencing photosynthesis and mineral absorption (Santachiara et al., 2017; Wu et al., 2019). The increased N absorption in the

plant resulted in a considerable rise in seedling height and root collar diameter (Cuesta et al., 2010; Andivia et al., 2011). Grain filling is a physiological mechanism that governs grain development. Suitable N rate and time influence grain filling rate and time, thus affecting grain weight (Wei et al., 2019). Phosphorus is essential for crop productivity and is involved in plant energy transfer. Plants cannot repair CO₂ without the presence of phosphorus. Phosphorus is required for several plant physiological activities such as sugar and starch consumption, photosynthesis, energy storage and transport. It is also found in the nucleus of cells and is required for cell division and the formation of meristematic tissues (Magalhaes et al., 2017). It also stimulates quicker shoot and root growth and accelerates leaf development. Phosphorus supplementation promotes normal plant development, resulting in an increase in hundred grain weight (Ahmed et al., 2018).

Conclusions

In conclusion, the application rates of 15M and 30M with 1.0AB effectively improve soil attributes. Additionally, treatment with 0.5AB also showed improvements in soil attributes, but 1.0AB proved more effective than 0.5AB and 0AB. Of the two application rates, 30M was the most effective in regulation of antioxidants with 1.0AB. However, growers can also use 15M to achieve potential benefits when using 1.0AB. Both 1.0AB and 30M effectively improved soil moisture contents and fertility (N, P, and K). Further research is necessary under various soil textures, temperature zones, and soil alkaline conditions to confirm that 1.0AB and 30M are the most effective treatment for the achievement of better mize growth and yield.

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