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EFFECT OF RICE HUSK BIOCHAR AND CHICKEN MANURE AS A SOIL AMENDMENT ON YIELD, HEAVY METALS AND NUTRIENT UPTAKE OF *Phyllanthus niruri*

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ABSTRACT

Aim: To determine the effects of rice husk biochar and chicken manure on yield, heavy metals concentration and nutrient uptake of *Phyllanthus niruri*.

Methods: Rice husk biochar (0, 5, 10 t/ha) and chicken manure (0, 2.5, 5 t/ha) were applied as combination treatment. Plants were harvested at 45 days after transplanting for analysis of heavy metals (Cd, Pb, Hg, As) and nutrients (N, P, K, Ca, Mg) determination while soil was collected for soil chemical analysis (pH, Total C, Total N, available P, cation exchange capacity and exchangeable bases).

Results: Shoot dry weight biomass was significantly affected by rice husk biochar (BC) and chicken manure (CM) rates. Cadmium (Cd) and arsenic (As) concentrations were not significantly affected by BC x CM, while mercury (Hg) and lead (Pb) were not detected in the plant samples. Plant nutrients uptake (N, P, K) showed a linear response for all BC rates as the rates of CM increases. Application of BC and CM has significant effects on soil chemical properties.

Conclusion: Application of soil amendment, rice husk biochar and chicken manure influenced the yield, heavy metals concentration and nutrients uptake of *Phyllanthus niruri*. The selected rates for the BC was 10 t/ha with combination 2.5 t/ha CM due to optimum yield and low heavy metals concentration.

Keywords: Rice husk biochar, Chicken manure, *Phyllanthus niruri*, Heavy metals, Nutrient

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uptake

1. INTRODUCTION

Currently, herbal industry are gaining attention and flourishing all over the world. Since Malaysia is rich in biodiversity, so Malaysian has identified the herbal industry as one of the entry point project (EPP) under the agriculture national key economic areas (NKEA) in the economic transformation programme (ETP) [1]. Due to the rapid increase in the use of medicinal plants and herbal remedies all over the world, demand for these plants has significantly increased in recent years. *Phyllanthus niruri,* known as “Dukung Anak” is common weeds in Malaysia [2]. For centuries, the plants were used as a natural medicine to treat disease [3]. It can treat jaundice, syphilis, against constipation, gonorhoea, kidney disorders [4, 5], jaundice, skin ulcer and diabetes [6]. Whole plant of dukung anak can possesses hypoglycemic, antibacterial, antifungal and antiviral [7], antinociceptive [8], antitumor [9], antimutagenic [10] and anti-inflammatory [11] properties. However, the production of herbs in Malaysia is limited due to low soil fertility.

Malaysian soil mainly, Oxisols and Ultisols and most agricultural land including fertile soil are planted with oil palm. Thus, available land for herb cultivation would most like be on acidic highly weathered infertile soil. This means the soil needs an organic amendment when starting or opening land for cultivation of *Phyllanthus niruri* and other herbs, even in an organic cultivation production. The qualities of herbs tend to vary when grown under different ecological conditions. Thus, good cultivation practices and use of organic amendment may be a solution to improve the bioactive compounds concentration [12]. Organic amendments able to improve the soil physical and chemical properties, increase the soil pH, and activate the microbial activity in the soil. So, application of organic soil amendment should be applied to improve the soil fertility.

Chicken manure has been used since centuries as fertilizers because it has high nitrogen content [13] and can act as soil amendments by adding organic matter [14]. Chicken manure also helps in improving the physical properties of the soil and soil fertility by adding essential nutrients which improve moisture and nutrient retention [15]. Dry matter weight will improved due to better root development that increase nutrient uptake and water translocation to plant part [16]. However, chicken manure cannot be applied continuously as it contains heavy metals that may accumulate in the soil and absorbed by plants. But applying it as an amendment in the beginning of crop cultivation may boost the crop establishment. Thus, co-application of biochar with chicken manure may result in better growth than apply chicken manure alone.

Biochar is a product from agricultural wastes that undergo pyrolysis process under high temperature and low oxygen [17, 18]. There are many benefits of biochar application includes reduce disease incidence in crops [19, 20], improve of soil water-holding capacity, boost plant

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growth [21], chelates the heavy metals [22], reducing soil N_2O emission [23] and hold nutrients from leaching. Thus, the objective for this study is to determine the optimum rates of chicken manure and rice husk biochar for improvement in biomass yield, plant nutrient uptake and low heavy metals concentration of *Phyllanthus niruri*.

2. MATERIALS AND METHODS

2.1 Media preparation and seedling transplanting

Polybag (12" x 12") was filled with 4 kg of soil (Bungor Series, clay type) obtained from Organic Unit Ladang 15, Universiti Putra Malaysia (UPM), Malaysia. The experiment was conducted in the glasshouse at Ladang 2. Three rates of rice husk biochar (BC) (0, 5, 10 t/ha) with combination of three rates of chicken manure (CM) (0, 2.5, 5 t/ha) were used in this study. The polybag was arranged with distance 30 x 30 cm and was laid out in a randomized complete block design (RCBD) with 4 replications. BC and CM were applied into the soil a week before transplanting. A total of 200 kg N/ha equivalent of organic fertilizer (Total Nitrogen: 2.52%) was incorporated for all treatments a day before transplanting. Two months old seedlings of *P. niruri* were transplanted into the polybag. The plants were watered manually twice a day and organic pesticide was sprayed once a week. Plant growth parameters such as plant height and number of branches were recorded every two weeks after transplanting.

2.2 Harvesting, drying and analysis

The plants were harvested 45 days after transplanting by cutting the basal stem at 5 cm from the soil level. Plant tissues were analyzed after oven-dried at 45 °C (3 days) for total nitrogen (TN), total phosphorus (TP), total potassium (TK), total calcium (TCa), and total magnesium (TMg) using wet digestion method while heavy metals concentrations (Cd, Pb, As, Hg) were determined using dry ashing method. Soil samples were taken from the polybag after harvesting for the determination of pH_{H_2O} (1:2.5 ratio soil: water), available phosphorus (Avai. P) according to Bray II Method, cation exchange capacity (CEC) and total exchangeable bases (K, Ca, Mg) using ammonium acetate at pH 7.0 method, and total carbon (TC) and total nitrogen (TN) using a CNS analyzer.

2.3 Statistical analysis

The data was analyzed by using two ways ANOVA followed by LSD (Least Significance Difference) test through ANOVA. The difference was considered significant at $p<0.05$ when mean treatments compared with control. Regression analysis was conducted to study the relationship between the independent and dependent variables when interactions between treatments existed.

3. RESULTS

3.1 Dry weight shoots biomass and plant growth parameter (plant height, no. of branches)

The dry weight shoot biomass was significantly affected by interaction effects between BC x CM (**Table 1**). However, BC and CM has no significant effects on plant height and number of branches. There were significant linear and quadratic responses between dry weight of *P. niruri* and rates of CM produced at three rates of BC (**Figure 1**). From the quadratic equation (**Figure 1**), the optimum dry weight was obtained when 10 t ha^{-1} BC with 3.99 t ha^{-1} CM were applied where the dry weight shoot biomass obtained are 1820.94 mg $plant^{-1}$. However, after comparing with the application of 2.5 t ha^{-1} CM with combination of 10 t ha^{-1} BC, there were only 5% increases in dry weight with the application of 3.99 t ha^{-1} CM with dry weight shoots biomass of 1720.8 mg $plant^{-1}$. Application of 0 t ha^{-1} and 5 t ha^{-1} BC with all rates of CM produced in slightly lower dry weight of *P. niruri* compared to the application of 10 t ha^{-1} BC.

Table 1. Output of two-way ANOVA for the effects of rice husk biochar and chicken manure on dry weight shoot biomass of *Phyllanthus niruri*.

Treatment	Dry weight (mg $plant^{-1}$)	Plant height (cm)	No. of branches
Biochar (BC)	**	NS	NS
Chicken manure (CM)	**	NS	NS
BC x CM	*	NS	NS

Asterisk symbols are significant at **P*<0.05, ***P*<0.01, ****P*<0.001, respectively; NS = not significant.

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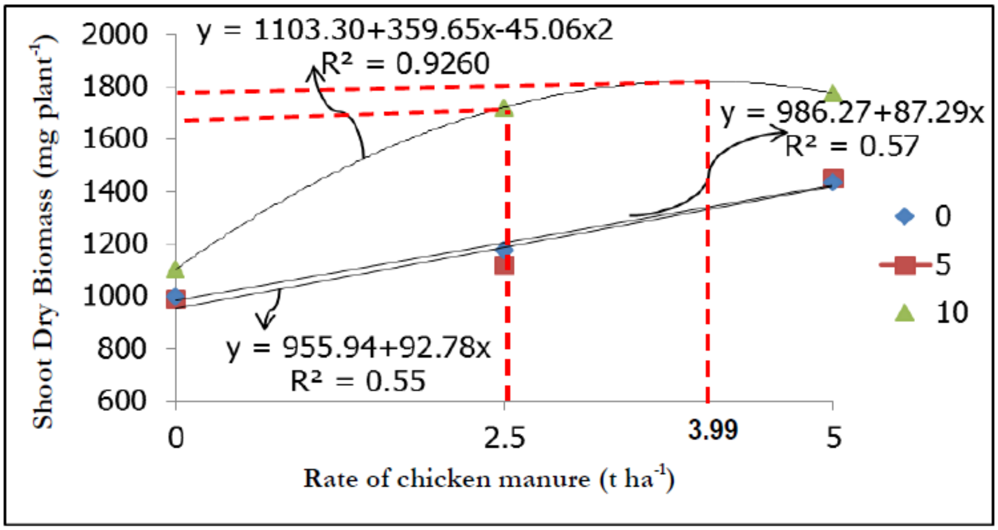


Figure 1. Relationships between dry weight shoot biomass of *Phyllanthus niruri* and rates of chicken manure (0, 2.5, 5 t ha^{-1}) produced at three rates of rice husk biochar (0, 5, 10 t ha^{-1}).

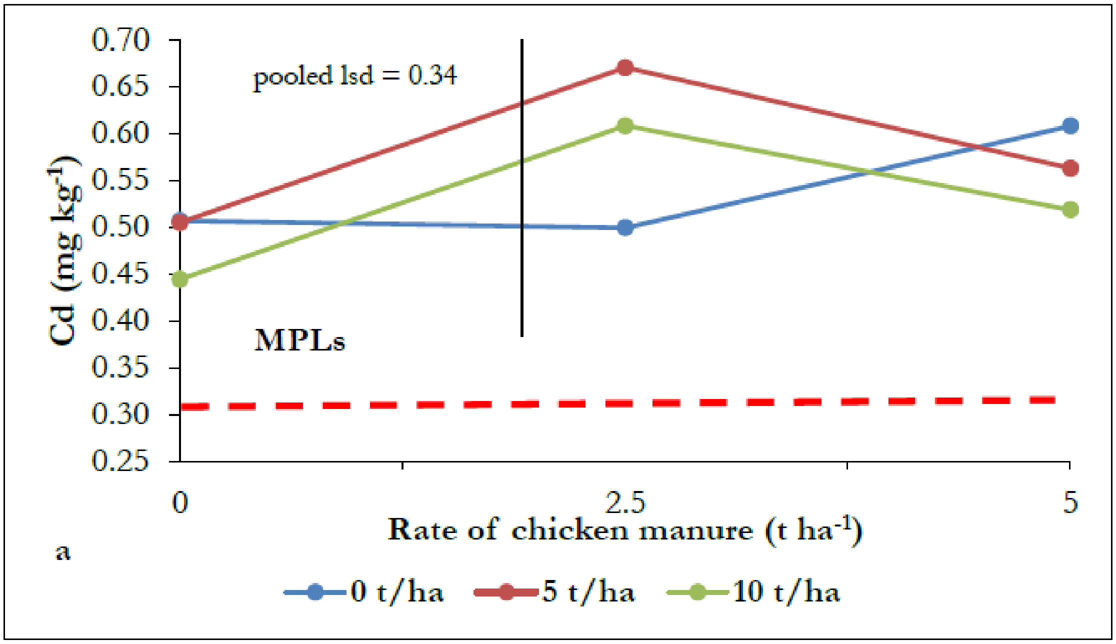
3.2 Heavy metals content

Cadmium (Cd) was affected by main factor (BC and CM) while there were no significant interaction effect between BC x CM on Cd and arsenic (As) (Table 2). Lead (Pb) and mercury (Hg) were not detected in *P. niruri* indicating that the plants were not contaminated by the two heavy metals. However for Cd, the content was higher than Maximum Permissible Limits (MPLs) according to Malaysian Herbal Monograph whereby only 0.3 mg kg^{-1} is allowed to be in herbs (Figure 2). The concentration of arsenic in plant tissue is below the MPLs with treatment of 5 t ha^{-1} and 10 t ha^{-1} BC at all rates of CM. Figure 2a showed that application of 10 t ha^{-1} BC and 0 t ha^{-1} CM has lower Cd concentration compared to non-amended treatment. However, treatment 10 t ha^{-1} BC with 2.5 t ha^{-1} CM had produced slightly higher Cd concentrations compared to 0 t ha^{-1} CM with same rate of BC. When 10 t ha^{-1} BC combined with 5 t ha^{-1} CM, the Cd concentrations was reduced while the 0 t ha^{-1} BC with 5 t ha^{-1} produced high Cd concentrations. This showed that Cd comes from CM and presence of BC help to reduce the concentrations in plant. Same situation goes to As concentrations where 0 t ha^{-1} BC and 5 t ha^{-1} has high As in plant.

Table 2. Output of two-way ANOVA for the effects of rice husk biochar and chicken manure on heavy metals concentration of *Phyllanthus niruri*.

Treatment	mg kg^{-1}			
	Cd	As	Pb	Hg
Biochar (BC)	*	NS	ND	ND
Chicken manure (CM)	*	NS	ND	ND
BC x CM	NS	NS	ND	ND

Asterisk symbols are significant at *P<0.05, **P<0.01, ***P<0.001, respectively; NS: not significant; ND = not detected.



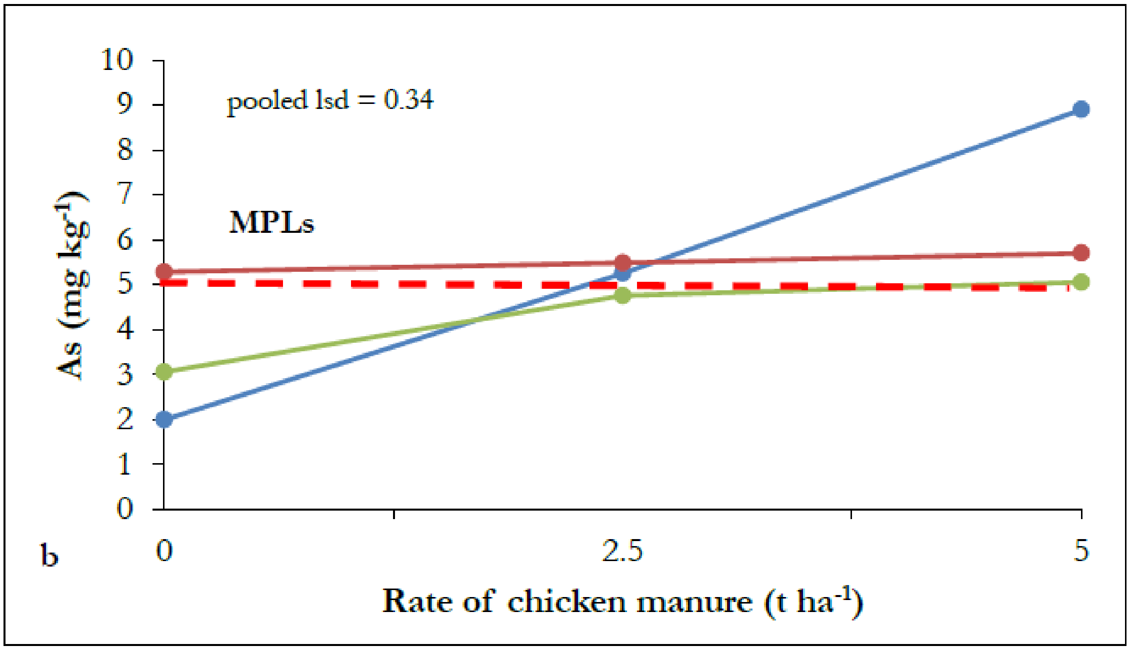


Figure 2. Relationships between cadmium (a) and arsenic (b) concentration of *Phyllanthus niruri* and rates of chicken manure (0, 2.5, 5 t ha^{-1}) produced at three rates of rice husk biochar (0, 5, 10 t ha^{-1}) (Maximum Permissible Limits (MPLs) by Malaysian Herbal Monograph, As: 5.0 mg kg^{-1} and Cd: 0.3mg kg^{-1})

3.3 Plant nutrients uptake

The N, P, K, Ca and Mg uptake of *P. niruri* was significantly affected by the main effects of BC, CM and interaction effects between BC x CM (Table 3). There was a significant linear response between N uptake of *P. niruri* with application of 10 t ha^{-1} BC, significant linear response between P uptakes of *P. niruri* with application of 0 t ha^{-1} BC, and significant linear response between K uptakes of *P. niruri* with three rates of BC (Figure 3). Application of 10 t ha^{-1} BC at all rates of CM contributes to higher N and K uptake in plants.

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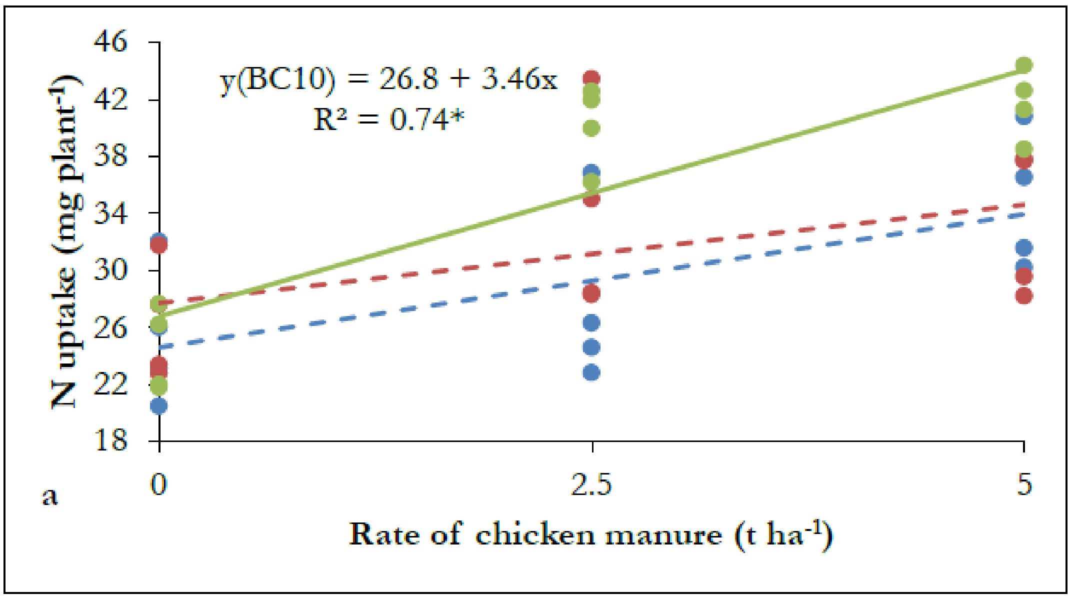
Table 3. Output of two-way ANOVA for the effects of rice husk biochar and chicken manure on plant nutrients uptake of *Phyllanthus niruri*.

Treatment	mg plant^{-1}				
	N	P	K	Ca	Mg
Biochar (BC)	**	**	**	**	*
Chicken manure (CM)	**	**	**	**	**
BC x CM	**	*	*	**	*

Asterisk symbols are significant at *P<0.05, **P<0.01, ***P<0.001, respectively.



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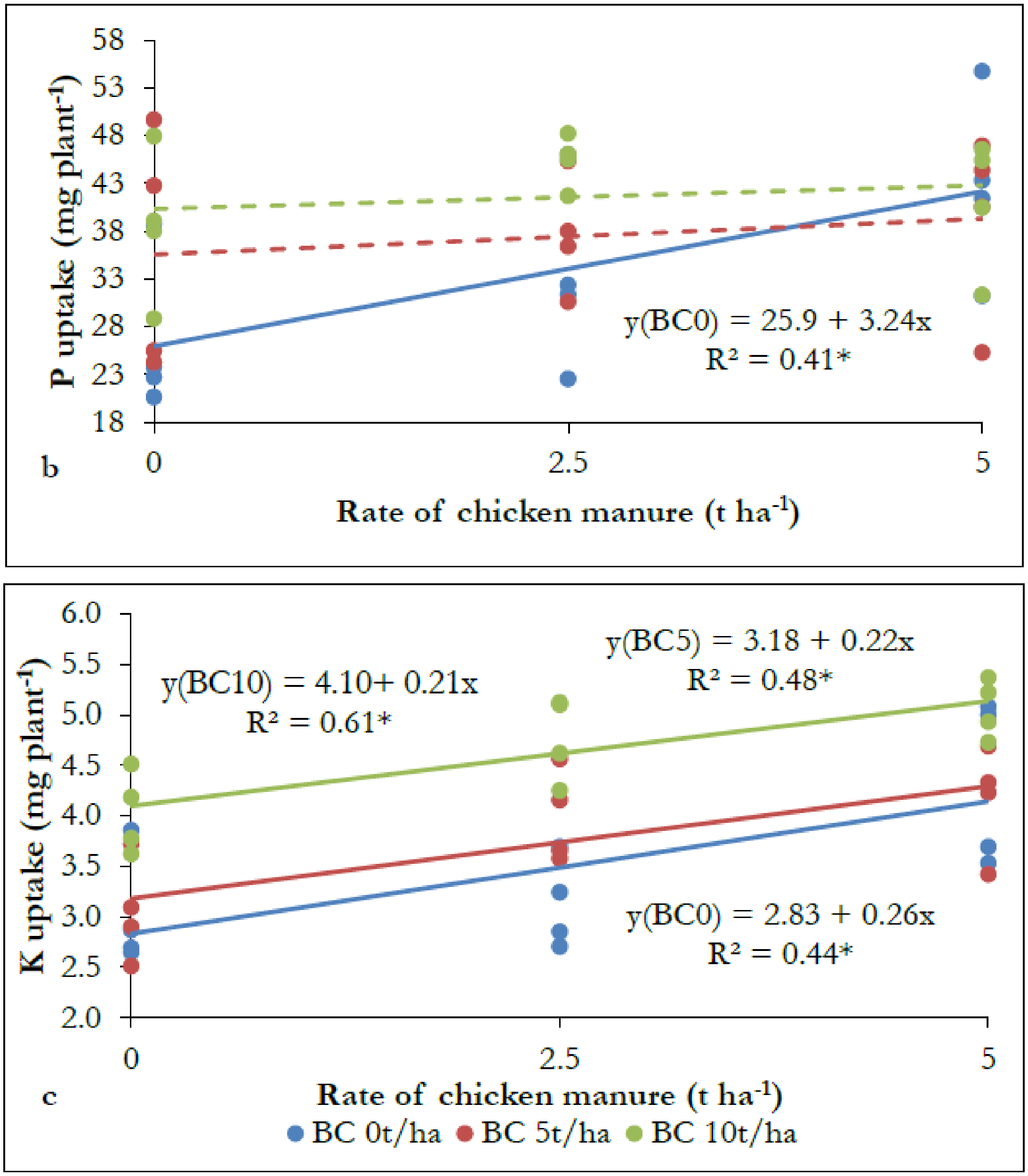

Figure 3. Relationships between nitrogen (a), phosphorus (b) and potassium (c) uptake and rates of chicken manure (0, 2.5, 5 t ha^{-1}) of *Phyllanthus niruri* produced at three rates of rice husk biochar (0, 5, 10 t ha^{-1}).

3.4 Soil chemical properties

Available P, CEC and exchangeable Mg was strongly affected by interaction factor BC x CM (Table 4). Soil pH, total N, available P, exchangeable K, Ca and Mg were affected by main factor of CM while for total C, available P, CEC, exchangeable K and Mg were affected by main factor BC. There were linear response between BC at rates 0 and 5 t ha^{-1} for available P and exchangeable Mg and quadratic response at rates 10 t ha^{-1} with increasing rates of CM. For CEC, there were quadratic response at 0 and 10 t ha^{-1} BC at increasing rates of CM.

Table 4. Output of two-way ANOVA for the effects of rice husk biochar and chicken manure on soil chemical properties where *Phyllanthus niruri* were planted.

Treatment	pH	N (%)	C (%)	Avai. P (mg/kg)	CEC (cmol(+)/kg)	Exch. K (cmol(+)/kg)	Exch. Ca (cmol(+)/kg)	Exch. Mg (cmol(+)/kg)
Biochar (BC)	NS	NS	*	**	**	NS	**	**
Chicken manure (CM)	***	*	NS	**	**	**	**	NS
BC x CM	NS	NS	NS	**	NS	NS	*	**

Asterisk symbols are significant at *P<0.05, **P<0.01, ***P<0.001, respectively; NS: not significant; Avai. P = available phosphorus; CEC = cation exchange capacity; Exch. = exchangeable.

DISCUSSIONS

There were significant (p<0.05) differences in dry weight shoot biomass of *P. niruri* after harvest. Plants which had been amended with 10 t ha^{-1} BC and 5 t ha^{-1} CM exhibited higher yields than those applied with 0 t ha^{-1} of and 5 t ha^{-1} at all rates of CM. This could be attributed to large quantities of available P and K that are present in CM and BC. The low performance of the 0 t ha^{-1} which is control treatment was as a result of nutrient stress by the plants and serious attack by pest during planting. The increase in yield recorded by the manures and combination with biochar might have been due to the improvement of the physical structure of the soil and the nutrients supplied as stated by [24]. Increase in dry weight shoot biomass related to nutrient uptake by plants. From this study, there were an increase in plant nutrients uptake in the soil treated with BC and CM (**Figure 3**). The increase in nutrient N, P and K could be due to the increase in soil pH treated with BC and CM. Besides, biochar are able to hold the availability of nutrients that comes from chicken manure and reduce leaching losses in soil [25]. According to [26], application of biochar and chicken manure in soil could potentially improve P availability and affects P uptake by ryegrass and maize plant. Biochar can act as liming effect which then decreased the P fixation by Al and Fe oxide [27] and organic P was released due to decomposition of organic matter [28].

The accumulation of heavy metals is one of the great concerns in agricultural products especially herbs because it can become threat to human. It is apparent that BC treatments reduced metal concentrations in shoot of *P. niruri*. In general, arsenic and cadmium concentrations decreased with the increasing rates of amendments application. Results showed that application of CM alone produced high concentration of arsenic and cadmium in shoot of *P. niruri*. This indicates that chicken manure contains high amount of cadmium and arsenic concentration. However, when CM treatment was combined with BC, the cadmium and arsenic decreased. Presence of biochar helps to precipitate the heavy metals in soil so shoot of *P. niruri* cannot uptake the metals. In this study, Pb and Hg were not detected in shoot of *P. niruri*. One of the reasons is that CM and BC has low plumbum concentrations. Besides, Pb was the metal that least extracted by plants. According to [29], applications of biochar in maize crop decreased the concentrations of Cd, As and Pb in shoot. Arsenic act differently compared to other metals because arsenic response with increasing soil pH and this has been proved [30] that arsenic in *Miscanthus* was reduced when amended with hardwood biochar. The reduction of heavy metals in plants could also be due to immobilization caused by amendments and leaching due to watering the plants daily.

The effect of BC and CM on the soil chemical properties was presented in **Table 4**. In general, application of organic soil amendments significantly improved the chemical properties of the soil planted with *Phyllanthus niruri*. There was an increase in available P, CEC and exchangeable bases. The results in **Table 4** also show that application of BC and CM did not significantly influence the soil pH, total N and total C. The highest CEC, available P and exchangeable Mg were observed in soil treated with 10 t ha^{-1} BC and 5 t ha^{-1} CM. The increased in soil pH treated with BC and CM compared to the soil pH before planting might be due to their high pH. This result shows that BC and CM can be used as lime materials to increase the pH of the acidic soil. The increase in CEC with BC treatment might be due to the negative charge that is present from the carboxyl groups and this result was supported by [31]. However, treatments BC have no significant effects on soil pH. Properties of biochar that has negative charge surface and porous structure increased the CEC and helps to reduce the leaching of nutrients such as N, P and K into groundwater [32] while chicken manure provides essential nutrients for plants such as K, Ca, Mg, NO^{3-} and NH^{4+} in the soil [33].

CONCLUSION

Application of 10 t ha^{-1} BC and 5 t ha^{-1} CM in this study produced highest shoot biomass dry weight, which were 1555.22 mg, respectively, per plant basis. Increasing rates of CM with 10 t ha^{-1} BC shows better nutrient uptake of *P. niruri*. Combination treatments of CM with BC are able to reduce the heavy metals in plant tissue. Arsenic was lower that MPLs level while and Cd concentration was decreased with the selected rate above. Based on all results obtained, rates of 10 t ha^{-1} BC in combination with 2.5 t ha^{-1} CM were selected as the optimum rates for the soil amendments due to better dry weight shoot biomass, high plant nutrients uptake and lower heavy metal concentrations.

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