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Physicochemical property of rubber nursery plants biochar and its effect on the growth of *Hevea brasiliensis* seedlings

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Abstract**—***In order to improve the reutilization rate of rubber seedling-stock shoots waste and minimize the burden on the ecological environment*, *we investigated physicochemical property of rubber nursery plants biochar and its effect on growth of Hevea seedlings. The results showed that sand bed seedlings biochar had the smaller stem diameter, the more N and P content but the less K content and soluble sugar, in comparison with polybag budding biochar and polybag seedling biochar. Stem of polybag budding plants as nutrient medium had less pH value and more electrical conductivity than that of seedling plants biochar. Medium in N and K nutrient were negatively correlated (P<0.05) with plant height, stem diameter and leaf whorls of rubber seedlings. Taken together, stem biochar as nutrient medium was better than leaf tissue powder as nutrient medium for the growth of rubber seedlings.*

Keywords— ***Hevea brasiliensis, Rubber nursery plants, Biochar, Physicochemical property, Growth.***

I. INTRODUCTION

Rubber buddings in polybags nursery with surface soils are the main maintenance of nursery. The seedling stocks are green-budded at six to eight months old. The stocks of successful buddings are cut-back. Rubber nursery production for polybag-buddings normally consume 600 ton surface soil and accompany with 756.8 ton seedling-stock shoots every year in our rubber nursery, and 34,400 ton seedling-stock shoots during the 13th Five-Year (2016-2020) Plan Period in rubber planting areas of China. Those seedling-stock shoots were burn or thrown away in the past, which has caused a burden on the ecological environment. In order to improve the reutilization rate of agricultural waste and minimize the burden on the ecological environment, crop production biochar has been introduced since last two decades (Laird., 2008). Biochar, a carbon rich source application ameliorates drought stress by increasing the plant growth, biomass, nutrient uptake and improves gaseous exchange in drought stress. Application of biochar reduces drought stress by increasing water holding capacity of soil through modification of soil physio-chemical properties that in turn increases water availability to plants and also enhances mineral uptake and regulation of stomatal conductance. Biochar mediates the retention of moisture, nutrients, inhibits harmful bacteria, absorbs heavy metals, pesticides, prevents soil erosion, increases soil pH, improves cationic exchange and boosts soil fertility (Mansoor *et al*., 2021). Biochar addition can significantly improve the growth and physiology of *Phragmites australis*, increase soil organic carbon content and decrease soil $NH4^{+}$-N content due to the N uptake by *Phragmites australis* (Liang *et al*., 2021). The combination of biochar and chemical fertilizer dissolved organic carbon and shaped soil bacterial community by pH, total nitrogen and available potassium for the improvements of tea growth and low nutrients acidic tea orchard soil (Yang et al, 2021). Wood carbonization increased soil pH, soil exchangeable P and

K (Chidumayo,1994).

For the research reported herein, we investigated physicochemical property of rubber nursery plants biochar and its effect on growth of *Hevea* seedlings, and further explore the feasibility of using it as a seedling-raising substrate.

II. MATERIAL AND METHODS

Rubber Nursery Plants Biochar

The experiment was conducted from October 2017 to November 2020 in the nursery base of natural rubber of Rubber Research Institute of Chinese Academy of Tropical Agricultural Sciences(19°49′22″N, 109°49′27″E), Danzhou City, Hainan Province, China. Sand bed seedlings, polybag seedlings (rootstock-plants), polybag buddings (scion-plants) were harvested, with leaves sundried and with stem girth measured, and pyrolyzed at 500℃for 96 hours (Table1), and then ground, respectively. Each treatment contained 50 plants, three replications.

Analytical Methods

The plants biochar volume weight, total porosity, aeration porosity, water-holding porosity and gas-water ratio were measured according to Liu (2001). The plants biochar EC value was determined with a Electrical Conductivity Meter (DDS-307A, Shanghai, China) according to by mixing plants biochar and water to a mass ratio of 1:5. The plants biochar pH was determined with a pH Meter (Mettler Toledo, SevenCompact S210, Zuirich, Switzerland) by mixing plants biochar and water to a mass ratio of 1:25. The plants biochar total nitrogen content was measured by the alkali-diffusion method, the plants biochar total phosphorus was determined with the molybdenum antimony resistance spectrophotometric method, and the plants biochar total potassium was estimated with a flame photometer (Jingke-F6410, Shaihai Yidian Analytical Instrument Co., Shanghai, China). Soluble Sugar content were measured according to Li (2000).

Rubber Seedlings Growth Experiment

Rubber seedlings with one leaf whorl at leaf-expansion stage were transplanted into root-container with 6cm upper diameter, 2cm lower diameter and 37cm high. The nursery medium were rubber leaves tissue powder and stem biochar (Table1), respectively. At 2-3 mature leaf whorls stage of rubber seedling, plant height, stem diameter, and leaf whorls were measured, respectively.

Statistical Analyses

Statistical analyses were performed with Data Processing System (DPS) statistical software package version 16.5 using one-way ANOVA followed by the Duncan's Multiple Range Test (SSR) to evaluate significant difference among seedlings from different rubber plants biochar as nursery medium and seedlings growth parameters. All data were shown in the mean ± SD of three biological replicates (each replication contained 50 plants).

III. RESULT AND DISCUSSION

Characteristic of rubber nursery plants biochar

As shown in Table1, dried leaves and stem pyrolyzed at 500℃ for 96 hours were ground into different colors. Stem girth of stem3 (polybag seedling stem) was 92.10% more ($P<0.01$) and 401% more ($P<0.01$) than that of stem2 (polybag budding stem) and stem1 (sand bed seedling stem), respectively. Stem girth of stem2 (polybag budding stem) was 161% more ($P<0.01$) than stem1 (sand bed seedling stem).

Table1 Characteristic of rubber nursery plants biochar

Samples	Leaves	Stem1	Stem2	Stem3
Stem girth(mm)	-	15.47±2.01cC	40.36±5.15bB	77.53±8.89aA
Dried samples				
Powdered samples				

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Notes: Leaves, dried leaves. Stem1-3, stem pyrolyzed at 500℃ for 96 hours. Stem1, sand bed seedling stem. Stem2, polybag budding stem. Stem3, polybag seedling stem. Data are means and SD, n=3.

Nutrient content of rubber nursery plants biochar As shown in Table2, N content of leaf1, was 22.69% more (P<0.01) and 19.18% more (P<0.01) than that of leaf2 and leaf3, respectively. N content of stem1 was 155% more (P<0.01) and 286% more (P<0.01) than that of stem2 and stem3, respectively. There were no significant difference in N content between leaf 2 and leaf 3, between stem2 and stem3, respectively. P content of leaf1, was 75% more (P<0.01) than that of leaf2. Leaf1 and leaf3 gave no significant difference in P content. P content of stem1 was 200% more (P<0.01) and 300% more (P<0.01) than that of stem2 and stem3, respectively. While P content of stem2 was 33.33% more (P<0.05) than that of stem3. K content of leaf1, was 16.19% less (P<0.01) and 22.12% less (P<0.01) than that of leaf2 and leaf3, respectively. K content of leaf2 was 7.08% less (P<0.01) than that of leaf 3. K content of stem1 was 22.39% more (P<0.01) and 78.26% more (P<0.01) than that of stem2 and stem3, respectively. K content of stem2 was 45.65% less (P<0.01) than that of stem3. Soluble sugar content of leaf1 was 46.73% less (P<0.01) and 36.85% less (P<0.01) than that of leaf2 and leaf3, respectively. There were no significant difference in soluble sugar content between leaf2 and leaf 3, among stem1, stem2 and stem3, respectively. C/N ratio of stem1 was 45.28% less (P<0.01) and 64.33% less (P<0.01) than that of stem2 and stem3, respectively. C/N ratio of stem2 was 34.82% less (P<0.01) than that of stem3. These results showed that leaves had more nutrients than stem, seedling plants had more nutrients than budding plants, and the nutrient of sand bed seedlings had more N than polybag seedling. It is necessary that the reuse of rubber nursery wastes in rubber nursery is an appropriate method for environment management and nutrients provider to raise the rubber nursery seedlings and budding plants.

Table2 Nutrient content of rubber nursery plants biochar

Parameters	Leaf1	Leaf 2	Leaf 3	Stem1	Stem2	Stem3
N%	2.92±0.19aA	2.38±0.04bB	2.45±0.15bB	1.43±0.1cC	0.56±0.06dD	0.37±0.03dD
P%	0.21±0.01bAB	0.12±0.01cC	0.2±0.01bB	0.24±0.02aA	0.08±0dD	0.06±0eD
K%	0.88±0.02cC	1.05±0.02bB	1.13±0.01aA	0.82±0.06dC	0.67±0.01eD	0.46±0.02fE
Soluble sugar content(mg.g^{-1})	144.29±12.39aA	98.34±6.82bB	105.44±2.71bB	27.74±0.97cC	19.9±0.95cC	20.02±1.37cC
C/N ratio	49.65±6.06abAB	41.41±2.93bcBC	43.18±3.84bcABC	19.47±2.01dD	35.58±4.46cC	54.59±6.51aA

Notes: Leaf1, dried leaves of Stem1, Leaf 2, dried leaves of Stem2, Leaf 3, dried leaves of Stem3. Stem1-3, stem pyrolyzed at 500℃ for 96 hours. Stem1, sand bed seedling stem. Stem2, polybag budding stem. Stem3, polybag seedling stem. Data are means and SD, n=3. Lowercase and uppercase indicate significant difference at 0.05 and 0.01 levels, respectively.

Physicochemical property of rubber nursery plants biochar as nutrient medium As shown in Table3, volume weight of leaf3 was 58.97% more (p<0.01) and 58.97% more (p<0.01) than that of leaf1 and leaf2, respectively. There were no significant difference in volume weight between leaf1 and leaf2, among stem1, stem2 and stem3, respectively. Total porosity, aeration porosity, water-holding porosity and gas-water ratio gave no significant difference among leaves and stem. There were no significant difference in pH value among leaf1, leaf2 and leaf3, among stem1, stem2 and stem3, respectively. However, stem1 and stem2 had less pH value than leaves at 0.05 and 0.01 level, respectively. Electrical conductivity of leaf1 was 59.69% more and 56.22% than that of leaf2 and leaf3, respectively. Electrical conductivity of leaf2 and leaf3 had no significant difference. Electrical conductivity of stem1 was 48.35% more and 80.33% more than that of stem2 and stem3, respectively. Electrical conductivity of stem2 and stem3 had no significant difference. The results showed that stem of budding plants as nutrient medium had less pH value and more electrical conductivity than that of seedling plants. Physio-chemical analysis (N, P, K, pH, EC) were best in the growing media comprising biochar + peat moss + leaf compost for flower production

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(Altaf *et al*, 2021), which might indicate that different plants have various nutrient medium with biochar.

Table3 Physicochemical property of rubber nursery plants biochar as nutrient medium

Parameters	Leaf1	Leaf 2	Leaf 3	Stem1	Stem2	Stem3
Volume Weight (g/cm^3)	0.39±0.02cB	0.39±0.05cB	0.62±0.07aA	0.55±0.13abAB	0.49±0.01bcAB	0.44±0bcB
Total porosity(%)	7.93±0.02aA	7.93±0.07aA	8±0.01aA	7.99±0.02aA	7.93±0.04aA	7.96±0.02aA
Aeration porosity %	7.48±0.18aA	7.82±0.07aA	7.82±0.57aA	7.99±0.32aA	7.61±0.35aA	8.08±0.37aA
Water-holding porosity %	6.81±0.14aA	6.45±0.09aA	6.09±0.55aA	6.26±0.28aA	6.53±0.27aA	6.09±0.73aA
Gas-water Ratio	1.1±aA	1.21±aA	1.3±aA	1.28±aA	1.17±aA	1.34±aA
pH value	5.55±0.02abA	5.56±0.3abA	5.63±0.08aA	5.12±0.02cAB	4.94±0.02cB	5.21±0.38bcAB
Electrical conductivity	1342±77.9aA	840.36±178.08bcBC	859.04±132.65bcBC	633.56±174.83cdBC	939.8±60.11bB	521.16±95.63dC

Notes: Leaf1, dried leaves of Stem1, Leaf 2, dried leaves of Stem2, Leaf 3, dried leaves of Stem3. Stem1-3, stem pyrolyzed at 500℃ for 96 hours. Stem1, sand bed seedling stem. Stem2, polybag budding stem. Stem3, polybag seedling stem. Data are means and SD, n=3. Lowercase and uppercase indicate significant difference at 0.05 and 0.01 levels, respectively.

Effect of rubber nursery plants biochar as nutrient medium on rubber seedling growth As shown in Table4, plant height of rubber seedlings grown in stem1 as nutrient medium was 10.83% lower ($p<0.01$) and 13.93% lower ($p<0.01$) than that of stem2 and stem3, respectively. There were no significant difference in plant height between stem2 and stem3, among leaves as nutrient medium, respectively. Stem diameter of rubber seedlings grown in leaf3 as nutrient medium was 7.09% smaller ($p<0.01$) and 4.83% smaller ($p<0.05$) than that of leaf2 and leaf1, respectively. There were no significant difference in stem diameter between leaf1 and leaf2, among stem as nutrient medium, respectively. Leaf whorls of rubber seedlings grown in leaf2 as nutrient medium was 10.68% less ($p<0.05$) and 15.73% less ($p<0.01$) than that of leaf1 and leaf3, respectively. Leaf whorls of rubber seedlings grown in stem1 as nutrient medium was 15.84% less ($p<0.01$) and 15% less ($p<0.01$) than that of stem2 and stem3, respectively. There were no significant difference in leaf whorls between leaf1 and leaf3, between stem2 and stem3 as nutrient medium, respectively. Stem diameter of rubber seedlings grown in leaf3 as nutrient medium was 4.84% lower($p<0.05$) and 7.09% smaller ($p<0.01$) than that of leaf1 and leaf2, respectively. There were no significant difference in stem diameter between leaf1 and leaf2, among stem as nutrient medium, respectively. Leaf whorls of rubber seedlings grown in leaf2 as nutrient medium was 10.68% less ($p<0.05$) and 15.73% lower ($p<0.01$) than that of leaf1 and leaf3, respectively. Leaf whorls of rubber seedlings grown in stem1 as nutrient medium was 15.84% less ($p<0.01$) and 15% lower ($p<0.01$) than that of stem2 and stem3, respectively. There were no significant difference in leaf whorls between leaf1 and leaf3, between stem2 and stem3 as nutrient medium, respectively. These results suggested that stem as nutrient medium was better than leaf as nutrient medium for the growth of rubber seedlings. Considering the cost of river sand, availability and germination capacity, leached coir pith is considered as an ideal seed germination medium for rubber (Joseph and Jessy, 2005), while the tomato seedlings grew on the pure wheat straw decomposed matter showed the worst performance(Yang et al., 2020), which showed that different plants had their own suitable medium.

Table4 Effect of rubber nursery plants biochar as nutrient medium on rubber seedling growth

Medium composition	Plant height (cm)	Stem diameter (mm)	Leaf whorls
Leaf1	35.11±4.22cBC	3.72±0.38bBC	2.34±0.48bBC
Leaf2	35.57±3.69cBC	3.81±0.26bAB	2.09±0.29cC
Leaf3	34.35±4.14cC	3.54±0.31cC	2.48±0.51bB
Stem1	37.87±5.66bB	3.86±0.44abAB	2.55±0.51bB
Stem2	42.47±4.91aA	4.01±0.31aA	3.03±0.45aA
Stem3	44±3.95aA	4.03±0.27aA	3±0.27aA

Notes: Leaf1, dried leaves of Stem1, Leaf 2, dried leaves of Stem2, Leaf 3, dried leaves of Stem3. Stem1-3, stem pyrolyzed at 500℃ for 96 hours. Stem1, sand bed seedling stem. Stem2, polybag budding stem. Stem3, polybag seedling stem. Data are means and SD, n=3. Lowercase and uppercase indicate significant difference at 0.05 and 0.01 levels, respectively.

Correlation analysis of rubber nursery plants biochar as nutrient medium on rubber seedling growth As shown in Table 5, plant height was significantly positive correlation with stem diameter (p= 0.0118), leaf whorls (p= 0.0162) and stem girth of rubber nursery plants as medium nutrient (p= 0.0245), respectively. However, plant height was significantly negative correlation with N (p= 0.0035), K (p= 0.0224), soluble sugar (p= 0.0247) content and pH value (p= 0.0174) of rubber nursery plants biochar as nutrient medium, respectively. Stem diameter was significantly negative correlation with N (p= 0.0298), K (p= 0.0259) content and pH value (p= 0.0349) of rubber nursery plants biochar as nutrient medium, respectively. Leaf whorls were significantly negative correlation with N (p= 0.0158), K (p= 0.037) content and stem girth (p= 0.0208) of rubber nursery plants biochar as nutrient medium, respectively. N content of rubber nursery plants biochar as nutrient medium was significantly positive correlation with K (p= 0.0321), soluble sugar (p= 0.0021) content and pH value (p= 0.0246), but significantly negative correlation with stem girth (p= 0.0122), respectively. K content of rubber nursery plants biochar as nutrient medium was significantly negative correlation with stem girth (p= 0.0069). Soluble sugar content of rubber nursery plants biochar as nutrient medium was significantly positive correlation with pH value (p= 0.0154). These results showed that high nutrients of rubber nursery medium was not good for the growth of rubber seedlings due to the existence of seed nutrient at the initial growth stage.

IV. CONCLUSION

In summary, sand bed seedlings as rubber nursery plants biochar had the smaller stem diameter, the more N and P content but the less K content and soluble sugar, in comparison with polybag budding and polybag seedling. Stem of polybag budding plants as nutrient medium had less pH value and more electrical conductivity than that of seedling plants. Stem as nutrient medium was better than leaf as nutrient medium for the growth of rubber seedlings.

Table5 Correlation analysis of rubber nursery plants biochar as nutrient medium on rubber seedling growth

Factors	Plant height	Stem diameter	Leaf whorls	N%	P%	K%	Soluble sugar	pH	Stem girth
Plant height		0.0118	0.0162	0.0035	0.1053	0.0224	0.0247	0.0174	0.0245
Stem diameter	0.91		0.1487	0.0298	0.1305	0.0259	0.0588	0.0349	0.0622
Leaf whorls	0.8943	0.666		0.0158	0.2632	0.037	0.0557	0.0737	0.0208
N%	-0.9515	-0.8556	-0.8956		0.1507	0.0321	0.0021	0.0246	0.0122
P%	-0.7219	-0.6884	-0.5452	0.6636		0.2279	0.3101	0.4649	0.1026
K%	-0.8751	-0.8655	-0.8384	0.85	0.5797		0.0987	0.0899	0.0069
soluble sugar	-0.8687	-0.7948	-0.8006	0.9627	0.5022	0.7312		0.0154	0.0645

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pH	-0.8903	-0.8433	-0.7693	0.869	0.3742	0.744	0.8969		0.1619
Stem girth	0.8695	0.7888	0.8797	-0.9082	-0.7256	-0.9313	-0.7848	-0.6505	

Notes: Correlation coefficients on the lower left, p-value on the upper right.

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