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*Corresponding author

Anthony Ojewumi Wale

anthony.ojewumi@lasu.edu.ng

RECEIVED :17 /05/ 2024

ACCEPTED :09/11/ 2024

PUBLISHED :31/ 12/ 2024

KEYWORDS:

Solid biochar, Liquid biochar, biochemical responses, pyrolysis, vegetable production.

Bio-fortification of Agronomic Attributes and Biochemical Molecules in Black Night Shade as Influenced by Two Forms of Guinea Grass Biochar

Anthony Wale Ojewumi^{1*}, Samuel Ayodotun Ayoola²,
Musa Hussaini Abdullahi³, Zubedat Badru Adeola¹,
Sunday Makinde Olubunmi¹

1Department of Botany, Lagos State University, Badagry Express Way, Ojo Lagos

2Department of Pure and Applied Botany, Federal University of Agriculture, Abeokuta, Nigeria

3Department of Biological Sciences, Federal University of Education, Kano, Nigeria

ABSTRACT

This experiment was carried out in the greenhouse of Department of Botany, Lagos State University, Ojo, campus, South West, Nigeria in 2024. This study evaluated effects of guinea grass biochar (liquid and solid) on growth and nutritional profile in Black Night Shade (BNS). BNS seeds treated with 150% liquid biochar (LB) emerged 4days earlier, produced highest number of seedlings (8.44) and germination (84.38%) as well as plumule length (4.65 cm) in the seeds treated with 120% LB. Contribution (153.63%) and yield (27.41%) were substantially higher in the seeds treated with 150% LB. Plant height (42.33 cm) of the vegetable treated with 15g solid biochar (SB) as well as number of leaves (24.67) in the vegetable treated with 15g/L LB showed significant increase. In addition, Leaf area (51.91 cm²), Specific leaf area (167.81(m² kg⁻¹), leaf area index (0.13 m²/m²), and leaf area ratio (0.25(m² kg⁻¹) were improved in BNS treated with 15g/L LB. Relative growth rate (0.24(mgg⁻¹ day⁻¹) and net assimilation rate (0.08 (gm⁻² day⁻¹) showed similar significant increase in the vegetable treated with 15g SB. Dry matter fat, ash, crude fiber and carbohydrate were improved by 15 g SB as well as most of the vitamins and minerals. In conclusion, 150% LB enhanced seedling emergence while 15g SB improved morphological traits and nutritional profile of BNS, therefore incorporation of the biochar at 15 g SB and 150% LB are suggested for production of BNS.

1. Introduction

Solanum nigrum L. Black night shade (BNS) is an annual vegetable grown as a weed in moist tropical and subtropical agroclimatic regions (Xu *et al.* 2012; Rani *et al.*, 2017). Due to poor soil nutritional uniformity, unfavorable soil and meteorological conditions under which vegetables including BNS are cultivated need to be fortified with organically rich nutrient sources such as biochar in other to boost food production and attain food security (Khan *et al.*, 2024).

According to Lehmann and Joseph (2015), biochar is a carbon-rich by-product of pyrolysis of carbo-containing biomass. The use of the product has gained attention recently owing to its unique physical and chemical properties (Farah *et al.*, 2023). It enhances carbon sequestration, waste management, maintain population of microorganism, reduce leaching of macronutrients, mitigate salt or drought stress and improve production of reactive oxygen species scavenging (Yuan *et al.*, 2023). Studies of Ahmad *et al.*, (2020) showed that due to high contents of essential nutrients, biochar is an ideal choice for improving soil chemical, physical and biological properties, seedling emergence, yield improvement and amelioration of water stress outbreak in plants (Ahmad *et al.*, 2020).

Biochar can be generated from virtually all forms of plants including grasses. Typical examples of plant whose biochar can be of great benefit for soil improvement is *Panicum maximum* L. (jacq) (guinea grass). The grass is one of the most economically important grasses which contain of high forage quality used to feed livestock. Its seeds attract many seed-eating birds and serve as source of animal nutrient (Döndü, 2021). The grass grows profusely on drained soils used to control erosion.

The grass has fibrous root system used to survive bush burning without harming their underground roots (Döndü, 2021). After fire outbreak, ashes produced by the grass usually enhance massive emergence of diverse species of plants as important physiological and ecological processes such as succession, regeneration and speciation (Bashirzadeh *et al.*, 2024).

Although, the fire outbreak or bush burning often destroy ecological zones of fauna and flora yet

the incidence is often preceded by regeneration and biodiversity of plant species possibly due to nutritional components of the grass. This attribute may be regarded as positive influence of biochar or ashes generated by the fire-outbreak from plant on new emergence of new seedlings or speciation as influenced by the ecological phenomenon. On the other perspective, biochar or ashes of such grass may be used to enhance survival of plant experiencing quite long drought periods, its physiological and biochemical effects on vegetables including BNS. Seedling emergence due to application of guinea grass biochar may have broader implications on the developmental strategies to mitigate the adverse effects of poor soil condition on vegetable production. In order to provide information on cheap and organic methods of improving growth and nutritional quality of BNS, this study elucidated effects of two forms of guinea grass biochar on agronomic attributes and biochemical molecules in BNS.

2. Materials and Methods

2.1 Materials and experimental design.

A pot experiment was conducted in the greenhouse of Department of Botany, Lagos State University, Ojo, campus. Nigeria. Seeds of Black Night Shade (BNS) were collected from local farmers.

Top soil was collected between 0-6 cm depths from the open field of Botanical Garden of the institution using soil probe (Ojewumi *et al.*, 2023). The soil was mixed and sieved using a 4mm sieve to remove debris and pebbles. One thousand (1000g) of the soil were poured into perforated planting buckets and the buckets were divided into two based on the forms of the biochar (solid and liquid). Distilled water served as control. The planting buckets were arranged in a completely randomized design of five replications.

2.2 Biochar preparation

Guinea grass straws were obtained, air-dried for three weeks and used for biochar production (Hann *et al.*, 2021 and Edirisinghe *et al.*, 2022). Two (2%) (20g) powdered biochar was regarded as 100 % liquid biochar (LB) soaked in 100mL of distilled water for 24hrs, filtered and the filtrate produced was used for germination experiment (Agboola 1996, Ajiboye and Agboola 2014, Ojo *et*

al., 2022). Briefly, 10 seeds of BNS were placed in 25 petri-dishes lined with cotton wool after which 1mL of 30, 60, 90, 120, and 150% of the LB was applied at every other day (Ojo et al., 2022). The Petri dishes were covered, arranged in a completely randomized design of five replicates and monitored for four days for number of seed germinated, percentage germination plumule length, contribution and yield.

2.3 Analysis of Yield loss potential of seeds

Total yield loss potential (%) of the seeds was calculated using the contribution% of the seeds and their respective vigor index-2 values as shown below:

$$\text{Contribution\%} = \frac{\text{Number of seeds germinated}}{\text{Number of days after the seeds were plated to germinate in the nth month}} \times 100 \dots \text{eq. 1}$$

The yield loss potential (%) for the seed was calculated as:

$$\text{Yield potential \%} = \frac{\text{€ contribution \%}}{\text{Maximum VI}} \times \text{mean VI} \dots \text{eq.2}$$

$$\text{Yield loss potential \%} = 100 - \text{yield potential \%} \dots \text{eq.3}$$

Where, VI is the vigor index-2 (VI-1) value of the seed, and the € contribution (%) is 100.

$$\text{Contribution to yield loss \%} = \text{contribution \%} \times \text{yield loss potential \%} \dots \text{eq. 4}$$

Potential yield loss (%) was determined by addition of contributions to yield loss (%) for the seeds soaked in different concentrations.

$$\text{Potential yield loss \%} = 100 - \text{€ contribution to yield loss \%} \dots \text{eq.5}$$

$$\text{VI-2} = \text{Seedling Length} \times \dots \text{eq. 6}$$

$$\text{Germination \% and VI-2} = \text{Seedling Dry Weight} \times \text{Germination \%} \dots \text{eq. 7}$$

The vigor formula stated of Abdul-Baki (1973) in Hossain et al. (2014) was adopted

2.4 Analysis of physical and Chemical Properties of Soil:

The soil was analyzed for soil pH, Organic carbon, total nitrogen and phosphorus (Huang et al. 2022). Soil size was determined as outlined in Hardie et al., (2014). Major macro elements were also assayed (Huang et al. 2022).

2.5 Nursery preparation

Seedlings of BNS were grown in the Greenhouse for 21 days during dry season (February-March).

Seedlings whose heights ranged between 8-9cm were selected and transplanted into 48 planting buckets. The field experiment adopted two factors (Two forms of biochar-Liquid and solid) at four levels of inclusion of each form). The buckets were arranged in a randomized completely block design of five replicates in 2 x 4 factorial arrangement. The seedlings were further watered daily with 100 mL distilled water using foliar application for another one week to facilitate establishment of the seedlings in the planting buckets (Ojewumi et al., 2023).

Furthermore, 0.5%, 1%, 1.5 % and 2% (5, 10, 15 and 20g) of the powdered biochar were determined in relation to 1000g soil, regarded as solid biochar (SB) and applied once throughout the period of the experiment (Ojewumi et al., 2020). In addition, 100 mL of 5, 10, 15 and 20g/L liquid biochar (LB) was applied once daily throughout the season on another set of the vegetable using foliar application. 100 mL distilled water served as control experiment. The experiment was monitored for four weeks

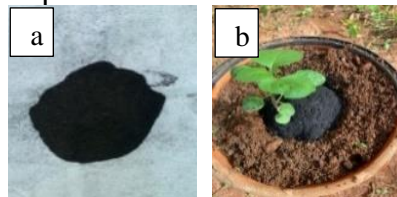


Figure 1: Biochar form (a) and its method of application (b)

2.6 Analysis of morphological trait and growth components

Morphological traits (plant height, number of leaves and weights) of the vegetable were determined according to Ojo et al. (2022). Total leaf area was measured using LI-3000 C Portable Leaf Area Meter (Ojewumi et al., 2022) while other leaf growth related attributes stated below were also determined (Ojewumi et al 2023)

$$\text{Specific leaf area} = \frac{\text{leaf area}}{\text{corresponding weight of leaf}} \dots \text{eq.8}$$

$$\text{Leaf area index} = \frac{\text{leaf area}}{\text{Area of litter fall}} \dots \text{eq.9}$$

$$\text{Relative growth rate} = \frac{\text{Log } w_2 - \text{Log } w_1}{\text{t}_2 - \text{t}_1} \dots \text{eq. 10}$$

$$\text{Net assimilation Rate} = \frac{w_2 - w_1}{A_2 - A_1} \cdot \frac{\text{Log } e w_2 - \text{Log } e w_1}{\text{t}_2 - \text{t}_1} \dots \text{eq.11}$$

$$\text{Leaf area ration} = \frac{\text{Log } A_2 - \text{Log } A_1}{w_2 - w_1} \dots \text{eq.12}$$

Where A1 = Area of leaf at t1, A2 = Area of leaf at t2, W1 = first measured weight (g), W2 = second measured weight (g), T1 = initial time (weeks), T2

= final time (weeks), e= exponential

2.7 Analysis of nutritional metabolites:

At the flowering stage (post vegetative stage), leaves of vegetable were collected and assayed for proximate contents following the procedure of AOAC (2000) using the formula; Moisture was determined using hot air oven technique for 1hour at 72°C

Moisture=

$$\frac{\text{weight of sample before drying} - \text{weight of sample after drying}}{\text{Weight of sample before drying}} \times 100 \dots \text{eq.13}$$

Dry mater (%)=100-Moisturecontent.....eq. 14

Crude fibre was assayed as outlined in AOAC (2000)

crude fiber=

$$\frac{\text{Weight of spoutless beaker containing crude fibre} - \text{Weight of spoutless beaker}}{\text{Weight of the sample}} \dots \text{eq. 15}$$

The total carbohydrate in the sample was determined as shown below.

Total carbohydrate =100 – (% moisture + % fat + % protein + %fibre) eq. 16

Micro-Kjeldahl technique described in (Ojewumi and Oyebanji (2020) was used to determine total nitrogen

Protein(%)=

$$\frac{\text{Titter value} \times 1.4 \times 6.25 \times 0.1 \text{NHCLXvol used}}{\text{Weight of sample} \times \text{Aliquot digested sample used for distillation}} \times 100 \dots \text{eq.17}$$

Crude fat and ash were estimated using the formula stated below

Crude fat (%)=

$$\frac{\text{Weight of flask with fat} - \text{weight of empty flask}}{\text{Weight of original sample}} \times 100 \dots \text{eq. 18}$$

Ash (%) =
$$\frac{\text{weight of ash}}{\text{Weight of samples}} \dots \text{eq. 19}$$

2.8 Analysis of nutritional elements

Atomic Absorption Spectrophotometer (Perkin – Elmer Model 2280) was used to assay calcium, magnesium, zinc and sodium in the sample. Phosphorus was determined using Calorimetric methods (Sahaptin and Bassiri 1975)

Rretinol and Niacin were assayed using spectrophotometer (Metrohm Spectronic 21D Model) AOAC (2000).

Vitamin B6 =

$$\frac{\text{Absorbance of sample} \times \text{gradient factor} \times \text{gradient factor}}{\text{weight of sample}} \dots \text{eq. 20}$$

Vitamin C (Ascorbic acid) was determined using UV/visible spectrophotometer.

Vitamin E and Vitamin K (ug) were assayed according to AOAC, (2000)

VitaminE(ug/100g)
 =

$$\frac{\text{Absorbances of sample} \times \text{Gradient factor} \times \text{Dilution.Factor} \times \text{Weight of sample}}{\text{weight of samples}} \dots \text{eq. 21}$$

Vitamin K (ug) =

$$\frac{\text{Absorbance of sample} \times \text{average gradient factor} \times \text{gradient factor}}{\text{Weight of sample}} \dots \text{eq.22}$$

2.9 Statistical analysis

The data collected were subjected to a two-way analysis of variance procedure of the General Linear Model of Statistical Analysis System (SAS) 2013). The means were separated using Tukey’s HSD test at p ≤ 0.05

3. RESULTS AND DISCUSSION

Biochar has widely been used has not only in industries or for domestic activities as sources of energy but also in environmental protection, improvement of soil quality, climate change mitigation, remediation of contaminated soil and crop production for sustaining food security (Sun *et al.*, 2014; Lehmann and Joseph, 2015). The soil used for growing BNS investigated in this present study characterized sandy loam texture with (830g kg⁻¹), (39 g kg⁻¹) clay m and (127 g kg⁻¹) silt as shown in table (1). The soil was neutral (pH; 7.2) with considerable quantities of organic carbon (16.4 g kg⁻¹), total nitrogen (3.3 g kg⁻¹) and phosphorus (20.64mg kg⁻¹) (Table 1). The outcome of assessment of the soil is a clear indication that although the soil contained appreciable quantity of the nutrients, however, it needs further enhancement with organically rich substances such biochar to boost the nutrients.

Table 1: The physical and chemical properties of the soil used for the experiment

| Parameters | Quantities determined |
|---|-----------------------|
| Textural class | Sandy Loam |
| pH (H ₂ O) (1.1) | 7.2±1.03 |
| Particle size distribution (g kg⁻¹) | |
| Sand | 830±2.70 |
| Clay | 39.0±1.41 |
| Silt | 127±2.58 |
| Organic Carbon (g kg ⁻¹) | 16.4±1.45 |
| Total N (g kg ⁻¹) | 3.3±0.56 |
| Available P (mg kg ⁻¹) | 20.64±1.56 |
| Exchangeable Bases (cmol kg⁻¹) | |
| Potassium | 1.50±0.02 |
| Calcium | 5.41±1.45 |
| Sodium | 1.70±0.04 |
| Magnesium | 1.46±0.03 |

Application of liquid biochar (LB) of the guinea grass produced substantial effects on emergence and yield components of the BNS seeds. Seeds of the vegetable treated with 150% LB emerged 4 days earlier compared with other levels of inclusion of LB and control. Highest number of seeds germinated (8.44) and germination (84.38%) were also observed in the seeds of BNS treated with the treatment as well as plumule length (4.65 cm) in the seeds treated with 120% LB. Furthermore, contribution (153.63%) and yield (27.41%) of the seeds treated with 150% LB was significantly higher compared with other levels of inclusion of the LB (Table 2).

Early seed emergence recorded in the seeds treated with 150% LB informs that germination and sprouting of the seeds were activated by the concentration of the treatment, hence the early seeds sprouting observed 4 days earlier (Mentges et al., 2016, Macedo et al., 2017; De Souza Moraes et al., 2020; Ojewumi et al., 2023). The observation may also presume that the treatment enhanced higher imbibition or hydration in the seeds (Kuntal et al., 2022).

These processes might have softened the seeds coat of the seeds, allowing inflow of the treatment and oxygen into the seeds for activation of hydrolytic (hydroxyl enzymes) or proteolytic enzymes (amylase and proteinase) required for degrading cell walls of the seeds. The imbibition might have stimulated activities of hydroxyl enzymes for conversion of metabolites needed by plumule or radicle after sprouting of embryo through the seed coat. This assertion corroborates reports of Ojo *et al.* (2022) who reported higher seedling emergence at 150m/L botanicals on *Citrullus lanatus* and *Citrullus colocynthis* seeds. On the other view, the imbibition might have activated production and actions of some growth hormones to regulate cell division, proliferation and development in the seeds (Wolny et al., 2018). Furthermore, higher germination and number of seed germinated recorded in seeds treated with 150% LB or longer plumule length produced by seedlings of the vegetable treated with 120% LB suggested that the parameters might have been influenced by the constituents of the treatments enhancing

activities such as cell division and elongation required for growth and development of the seedlings (Paz-Ferreiro *et al.* 2016; Amoakwah *et al.* 2022). Beyond physiological processes such as germination and seedling emergence, the influence of plant-derived organic fertilizer (Biochar) on seedling growth explored in the present study showed notable variations in the emergence and yield of BNS as influence by various levels of liquid biochar. This observation could imply that level of inclusion of liquid biochar of the grass can promote enzymatic activities on the seeds, improve translocation of nutrients to growing points of the seeds and seedling emergence of BNS (Duan et al., 2024). Higher yield loss (97.33%) contribution, yield loss (11041.67%) and potential yield loss (12224.73%) were recorded in the seedlings of the vegetable treated with control (Table 2). This observation may depict significant influence or involvement of the water (control) to the total outputs of the seeds compared with level of the treatments on the morphological characters and total production of the seeds (Rao et al., 2017).

Table 3 and 4 revealed that both SB and LB produced significant improvement in plant height and number of leaves of BNS from 1 week after treatment through 4 weeks. Highest plant height (42.33 cm) was observed in the vegetable treated with 15g SB while number of leaves (24.67) was counted in the vegetable treated with 15g/L LB compared with other values of the parameters in the vegetables grown under other levels of inclusion of the LB.

Apart from seedling germination and emergence, guinea grass biochar produced other notable effects on morphological traits and leaf related growth components of BNS. Higher plant height and number of leaves recorded in the vegetable grown under 15g SB may suggest that the treatment contained maximum nutritional contents that can enhance physiological activities such as tissue formation and elongation, bud and leaf formation and general proliferation of cells which can improve plant growth (Kumari et al., 2022). Higher effects of 15 g SB biochar on the vegetable as indicated by relative reduction in the parameters may preempt that excessive amendment of soil with

biochar may cause growth retardation and other physiological processes (Hamzah and Shuhaimi, 2018).

The effects of varying levels of solid and liquid biochar on growth components in BNS revealed that LA (51.91 cm²), SLA (167.81 (m² kg⁻¹), LAI (0.13 m²/m²), and LAR (0.25 (m² kg⁻¹) were significantly higher (p<0.05) the BNS grown under 15g/L LB. Similar substantial increase was noticed in RGR (0.24 (m² kg⁻¹ day⁻¹) and NAR (0.08 (gm⁻² day⁻¹) in the BNS treated with 15g SB (Table 5). In addition, the two forms of biochar at various levels of inclusion produced significant effects on proximate contents of the vegetable. Highest moisture (91.74%) was noticed in the leaves of BNS treated with 15 g LB. However, dry matter (8.53%), fat (1.68%), ash (1.98%) and carbohydrate (3.02%) showed substantial improvement in the vegetable grown under 15g SB. Similar trend was noticed in crude fibre (2.71%) in 5g LB and crude protein (3.09%) in the vegetable treated with 20g LB (Table 6). In addition, higher leaf related growth components observed in the vegetable grown under 15g/L may indicate that the treatment has better ability due to its forms to supply higher nutritional content to the vegetable as demonstrated in wider leaf area incurred as precursor of photosynthetic activities, metabolites assimilation and utilization established in the growth rate of the vegetable.

Furthermore, retinol (121.68mg/g), niacin (3.58 mg/100g), pantothenic (0.86 mg/100g), ascorbic acid (15.44 mg/100g) and phyloquinone (74.52 mg/100g) were considerably increased in BNS treated with 15g/L of SB. Similar significant increase was noticed in sodium (17.33 mg/100g), calcium (61.33 m/100g) and zinc (6.00 mg/100g) in the leaves of the vegetable grown under 15g of SB as well as magnesium (51.00 mg/g) and phosphorus (121.33 mg/g) in the vegetable sprayed with 15 g /L of LB (Table 7).

Results of this study also demonstrated that inclusion of the biochar has potential influence on nutritional status of the vegetable as indicated by appreciable qualities of nutritional indices such as dry matter, fat, ash, carbohydrate,

calcium, magnesium and zinc as well as higher proportion of vitamins recorded in 15g SB. Thus, the observation suggests clearly that 15g SB can be adopted for production of the vegetable.

CONCLUSION

Result revealed that guinea grass biochar contains appreciable amount of nutritional value suitable for sustainable cultivation of BNS. Also, all the levels of guinea grass biochar influenced growth of BNS, however 150% and 120% of guinea grass in germination showed better performance on the morphological and physiological attributes of the vegetable. Therefore, 15 g /L and 20 g/L liquid biochar are recommended for BNS cultivation.

Table 2: Effects of varying levels of liquid biochar on emergence and yield of Black Night Shade seeds

| Level of biochar (%) | DBG | NS | %Germination | PL | Contribution | Yield | Yield Loss | Contribution. yield loss | Potential yield loss |
|----------------------|-------------------------|------------------------|--------------------------|-------------------------|----------------------------|--------------------------|---------------------------|--------------------------------|--------------------------------|
| 30 LB | 5.75±0.21 ^{bc} | 4.88±0.77 ^b | 47.50±8.141 ^b | 2.05±0.52 ^{bc} | 84.08±14.75 ^c | 20.61±2.18 ^c | 90.45±4.18 ^{abc} | 9210.84±1337.34 ^b | 9418.84±1178.89 ^{ab} |
| 60 LB | 6.00±0.00 ^b | 5.69±0.71 ^b | 56.88±7.11 ^b | 4.65±0.79 ^a | 94.79±11.86 ^{bc} | 19.22±2.69 ^c | 95.14±2.69 ^{ab} | 9430.99±1118.50 ^b | 9330.99±1118.50 ^{ab} |
| 90 LB | 6.50±0.13 ^a | 5.00±0.66 ^b | 50.00±6.58 ^b | 3.29±0.56 ^{ab} | 76.93±10.06 ^c | 15.84±0.92 ^d | 97.43±0.92 ^a | 7835.61±951.72 ^c | 7735.61±951.72 ^b |
| 120 LB | 4.20±0.22 ^d | 5.70±0.65 ^b | 57.50±6.49 ^b | 4.96±0.66 ^a | 106.07±11.93 ^{bc} | 15.96±0.92 ^d | 75.08±13.82 ^{ab} | 10283.10±1144.28 ^{ab} | 10183.10±1144.28 ^{ab} |
| 150 LB | 4.00±0.13 ^{cd} | 8.44±0.13 ^a | 84.38±1.28 ^a | 2.58±0.68 ^{bc} | 153.96±1.86 ^a | 27.40±10.94 ^a | 72.59±10.94 ^c | 11041.67±1651.99 ^a | 10679.07±1773.06 ^{ab} |
| Tap water | 5.25±0.11 ^d | 6.31±0.63 ^b | 63.13±6.31 ^b | 1.21±0.33 ^c | 123.13±12.39 ^{ab} | 24.93±13.82 ^b | 97.33±0.92 ^b | 10830.29±2003.67 ^{ab} | 12224.73±946.61 ^a |

Means± standard error followed by different superscripts on the same columns are significantly different (p< 0.05) according to Tukey's HSD test at p <0.05.LB= liquid biochar, DBG= Days before germination, NS= Number of seedlings, PL=Plumule length

Table 3: Effects of varying levels of solid and liquid biochar on height (cm) of Black Night Shade

| Quantity (g) | Forms | Weeks after treatment | | | | Quantity (g) | Forms | Weeks after treatment | | | |
|--------------|-------|---------------------------|--------------------------|--------------------------|--------------------------|--------------|------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| | | 1 | 2 | 3 | 4 | | | 1 | 2 | 3 | 4 |
| 5 | SB | 13.67±1.17 ^{ab} | 23.17±2.09 ^b | 31.00±3.51 ^{ab} | 25.00±1.00 ^{cd} | 5 | SB | 8.00±1.16 ^{ab} | 11.00±2.09 ^{ab} | 12.33±0.88 ^{ab} | 19.67±3.48 ^{ab} |
| | LB | 7.50±1.44 ^b | 10.50±2.26 | 10.50±2.25 ^f | 14.00±3.06 ^e | | LB | 5.67±0.67 ^b | 7.33±2.25 ^b | 7.33±1.33 ^c | 13.33±2.33 ^c |
| 10 | SB | 12.83±1.74 ^{ab} | 20.33±3.38 ^{bc} | 27.00±5.03 ^b | 26.00±2.03 ^c | 10 | SB | 9.00±1.52 ^{ab} | 11.33±3.38 ^{ab} | 11.33±2.40 ^{ab} | 14.67±1.45 ^c |
| | LB | 11.33±2.33 ^{ab} | 16.33±4.91 | 16.33±4.91 ^e | 20.00±3.23 ^d | | LB | 7.67±1.202 | 8.00±4.91 ^b | 8.00±2.64 ^c | 14.00±5.13 ^c |
| 15 | SB | 14.00±2.04 ^a | 26.17±2.09 ^a | 33.33±7.13 ^a | 42.33±2.86 ^a | 15 | SB | 9.00±2.65 ^{ab} | 11.67±3.09 ^{ab} | 13.33±0.88 ^{ab} | 20.67±2.40 ^{ab} |
| | LB | 12.33±2.33 ^{aab} | 19.83±3.98 ^{bc} | 24.00±3.00 ^c | 32.33±3.93 ^b | | LB | 9.33±1.20 ^a | 12.00±3.98 ^a | 14.00±1.52 ^a | 24.67±5.18 ^a |
| 20 | SB | 11.50±1.26 ^{ab} | 20.33±2.72 ^{bc} | 28.33±3.84 ^b | 30.67±3.19 ^{bc} | 20 | SB | 7.33±1.20 ^b | 11.67±2.73 ^{ab} | 12.33±1.45 ^{ab} | 17.67±0.88 ^b |
| | LB | 12.50±3.12 ^{ab} | 19.00±4.00 ^{bc} | 19.00±4.00 ^d | 27.33±2.91 ^c | | LB | 9.03±1.33 ^{ab} | 10.00±4.00 ^{ab} | 10.00±1.00 ^b | 14.33±0.88 ^c |
| Tap water | | 8.17±0.44 ^b | 15.67±2.03 | 24.00±3.00 ^c | 27.33±1.45 ^c | Tap water | 6.00±1.00 ^b | 10.67±2.03 ^{ab} | 12.33±0.33 ^{ab} | 16.33±3.84 ^b | |

Means± standard error followed by different superscripts on the same columns are significantly different (p< 0.05) according to Tukey's HSD test at p <0.05. SB= Solid biochar, LB= Liquid biochar

Table 4: Effects of varying levels of solid and liquid biochar on number of leaves of Black Night Shade

Means± standard error followed by different superscripts on the same columns are significantly different (p< 0.05) according to Tukey's HSD test at p <0.05. SB= Solid biochar, LB= Liquid biochar

Table 5: Effects of varying levels of solid and liquid biochar on growth component of Black Night Shade.

| Quantity (g) | Forms | LA (cm ²) | SLA (m ² kg ⁻¹) | LAI (m ² m ⁻²) | LAR (m ² kg ⁻¹) | RGR(mgg ⁻¹ day ⁻¹) | NAR(mgg ⁻¹ day ⁻¹) |
|--------------|-------|-------------------------|--|---------------------------------------|--|---|---|
| 5 | SB | 28.64±1.46 ^c | 85.33±2.13 ^d | 0.07±0.01 ^{ab} | 0.03±0.02 ^c | 0.18±0.03 ^{ab} | 0.01±0.01 ^b |
| | LB | 11.00±0.56 ^f | 53.79±2.21 | 0.03±0.01 ^{ab} | 0.12±0.05 ^b | 0.22±0.05 ^{ab} | 0.02±0.00 ^b |
| 10 | SB | 30.65±1.03 ^b | 82.24±2.28 ^d | 0.07±0.02 ^{ab} | 0.01±0.00 ^c | 0.11±0.07 ^b | 0.02±0.00 ^b |
| | LB | 13.60±3.22 ^f | 57.67±1.32 ^e | 0.03±0.01 ^{ab} | 0.08±0.04 ^b | 0.11±0.07 ^b | 0.01±0.00 |
| 15 | SB | 33.70±3.19 ^b | 56.54±1.55 ^e | 0.08±0.03 ^{ab} | 0.02±0.01 ^c | 0.24±0.17 ^a | 0.08±0.00 ^a |
| | LB | 51.91±3.67 ^a | 167.81±1.31 ^a | 0.13±0.08 ^a | 0.25±0.17 ^a | 0.05±0.01 ^c | 0.01±0.00 ^b |
| 20 | SB | 20.61±3.820 | 91.36±3.31 ^c | 0.05±0.01 ^{ab} | 0.01±0.02 ^c | 0.17±0.08 ^{ab} | 0.01±0.00 ^b |
| | LB | 16.07±0.53 ^e | 44.76±3.31 ^f | 0.04±0.00 ^{ab} | 0.08±0.02 ^b | 0.08±0.04 ^c | 0.01±0.00 ^b |
| Tap water | | 21.39±1.08 ^d | 100.63±2.08 ^b | 0.05±0.00 ^{ab} | 0.05±0.02 ^b | 0.10±0.05 ^b | 0.02±0.00 ^b |

Means± standard error followed by different superscripts on the same columns are significantly different (p< 0.05) according to Tukey's HSD test at p <0.05, SB= Solid Biochar, LB= Liquid biochar, LA=Leaf area, SLA= Specific leaf area, LAI= Leaf area index, RGR= Relative growth rate, NAR= Net assimilation rate, LAR= Leaf area ratio

Table 6: Effect of varying levels of solid and liquid biochar on proximate compositions in black night shade

| Quantity (g) | Forms | Moisture (%) | Dry Matter (%) | Fat (%) | Ash (%) | Crude Fiber (%) | Crude Protein (%) | Carbohydrate (%) |
|--------------|-------|---------------------------|------------------------|------------------------|--------------------------|-------------------------|-------------------------|--------------------------|
| 5 | SB | 91.59±0.30 ^{ab} | 6.45±0.28 ^b | 1.28±0.06 ^b | 1.63±0.06 ^b | 1.75±0.34 ^d | 2.29±0.01 ^e | 1.86±0.06 ^c |
| | LB | 89.79±0.29 ^{bcd} | 7.69±0.02 ^a | 1.23±0.02 ^b | 1.81±0.029 ^{ab} | 2.71±0.11 ^a | 2.89±0.04 ^{bc} | 2.65±0.14 ^{ab} |
| 10 | SB | 89.64±0.32 ^{cd} | 6.61±0.23 ^b | 1.30±0.10 ^b | 1.85±0.03 ^{ab} | 2.31±0.08 ^c | 2.89±0.03 ^{bc} | 2.55±0.09 ^b |
| | LB | 89.36±0.30 ^{de} | 8.22±0.03 ^a | 1.21±0.03 ^b | 1.72±0.09 ^{ab} | 2.69±0.035 ^a | 2.93±0.03 ^b | 1.88±0.06 ^c |
| 15 | SB | 91.39±0.58 ^{abc} | 8.53±0.29 ^a | 1.68±0.50 ^a | 1.96±0.01 ^a | 2.63±0.01 ^{ad} | 2.44±0.04 ^d | 3.02±0.295 ^a |
| | LB | 91.74±0.54 ^a | 7.69±0.04 ^a | 1.27±0.06 ^b | 1.76±0.01 ^{ab} | 2.54±0.03 ^b | 2.89±0.05 ^{bc} | 2.43±0.02 ^b |
| 20 | SB | 91.33±0.58 ^{abc} | 6.75±0.08 ^b | 1.23±0.06 ^b | 1.80±0.09 ^{ab} | 2.45±0.13 ^{ab} | 2.49±0.01 ^d | 2.55±0.18 ^b |
| | LB | 89.49±0.48 ^{de} | 8.44±0.26 ^a | 1.22±0.02 ^b | 1.83±0.04 ^{ab} | 2.69±0.12 ^{ab} | 3.09±0.08 ^a | 2.69±0.079 ^{ab} |
| Tap water | | 87.78±1.15 ^e | 8.29±0.59 ^a | 1.23±0.05 ^b | 1.85±0.08 ^{ab} | 2.53±0.06 ^b | 2.78±0.03 ^c | 2.35±0.09 ^b |

Means± standard error followed by different superscripts on the same columns are significantly different ($p < 0.05$) according Tukey's HSD test at $p < 0.05$, SB= Solid biochar, LB= liquid biochar

Table 7: Effect of varying levels of solid and liquid biochar on vitamin compositions in black night shade.

| Quantity(g) | Forms | Retinol (mg/100g) | Niacin (mg/100g) | Pyridoxine((μ g/100g) | Ascorbic acid (mg/100g) | Tocopherol (μ g/100g) | Phylloquinone (μ g/100g) |
|-------------|-------|--------------------------|------------------------|----------------------------|--------------------------|----------------------------|-------------------------------|
| 5 | SB | 92.57±0.12 ^b | 1.67±0.07 ^b | 1.24±0.12 ^{ab} | 11.34±0.123 ^b | 1.73±0.09 ^a | 71.68±0.65 ^c |
| | LB | 85.73±0.03 ^e | 1.54±0.01 ^b | 1.12±0.06 ^{ab} | 10.60±0.29 ^b | 1.51±0.01 ^b | 69.35±0.06 ^{ef} |
| 10 | SB | 91.77±0.76 ^b | 1.73±0.09 ^b | 1.22±0.09 ^{ab} | 11.32±0.06 ^b | 1.74±0.03 ^a | 71.76±0.07 ^c |
| | LB | 87.93±0.03 ^c | 1.51±0.01 ^b | 1.06±0.03 ^b | 10.61±0.01 ^b | 1.64±0.06 ^{ab} | 70.52±0.29 ^d |
| 15 | SB | 121.68±0.06 ^a | 3.58±0.03 ^a | 1.13±0.06 ^{ab} | 15.44±0.29 ^a | 1.61±0.04 ^{ab} | 74.52±0.09 ^a |
| | LB | 86.95±0.02 ^d | 1.60±0.04 ^b | 1.07±0.03 ^b | 10.69±0.03 ^b | 1.60±0.04 ^{ab} | 70.13±0.23 ^{de} |
| 20 | SB | 85.45±0.19 ^e | 1.70±0.12 ^b | 1.10±0.06 ^{ab} | 11.00±0.29 ^{ab} | 1.49±0.01 ^b | 68.84±0.02 ^{fg} |
| | LB | 76.90±0.08 ^f | 1.54±0.05 ^b | 1.00±0.01 ^b | 10.49±0.37 ^b | 1.65±0.15 ^{ab} | 67.85±1.00 ^g |
| Tap water | | 92.29±0.17 ^b | 1.66±0.08 ^b | 1.44±0.24 ^a | 11.28±0.05 ^b | 1.67±0.05 ^{ab} | 73.41±0.21 ^b |

Means± standard error followed by different superscripts on the same columns are significantly different ($p < 0.05$) according to Tukey's HSD test at $p < 0.05$, SB= solid biochar, LB= liquid biochar.

Table 8: Effect of varying levels of solid and liquid biochar on mineral composition of black night shade leaves

| Quantity(g) | Forms | Sodium(mg/100g) | Calcium(mg/100g) | Magnesium(mg/100g) | Phosphorous(mg/100g) | Zinc(mg/100g) |
|-------------|-------|--------------------------|-------------------------|-------------------------|--------------------------|------------------------|
| 5 | SB | 14.00±0.00 ^b | 51.33±0.33 ^b | 46.00±0.00 ^b | 112.00±0.00 ^b | 4.00±0.00 ^b |
| | LB | 13.00±0.00 ^{bc} | 49.67±0.33 ^c | 44.00±0.00 ^c | 103.67±0.82 ^e | 3.33±0.33 ^c |
| 10 | SB | 14.00±0.00 ^b | 51.00±0.00 ^b | 46.00±0.00 ^b | 111.33±0.33 ^b | 4.00±0.00 ^b |
| | LB | 13.00±0.00 ^{bc} | 50.00±0.00 ^b | 45.00±0.00 ^b | 106.00±0.00 ^d | 4.00±0.00 ^b |
| 15 | SB | 17.33±0.33 ^a | 61.33±0.33 ^b | 46.00±0.00 ^b | 110.67±0.33 ^c | 6.00±0.00 ^a |
| | LB | 13.00±0.00 ^{bc} | 50.00±0.00 ^b | 51.00±0.00 ^a | 121.33±0.33 ^a | 4.00±0.00 ^b |
| 20 | SB | 13.33±0.33 ^{bc} | 49.00±0.00 ^c | 44.00±0.00 ^c | 105.00±0.00 ^d | 4.00±0.00 ^b |
| | LB | 11.00±0.00 ^c | 46.00±0.00 ^d | 42.00±0.00 ^d | 95.00±0.00 ^f | 3.33±0.33 ^c |
| Tap water | | 13.33±0.33 ^{bc} | 51.00±0.00 ^b | 45.67±0.33 ^b | 112.00±0.00 ^b | 4.00±0.00 ^b |

Means± standard error followed by different superscripts on the same columns are significantly different ($p < 0.05$) according to Tukey's HSD test at $p < 0.05$. SB= solid biochar, LB= Liquid biochar

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