

Original Research Article

The use of sawdust biochar as soil amendments to improve allelochemical-laden soils in the landscape

ABSTRACT

This study was conducted to find out the ameliorative effect of biochar in allelochemical-laden soils to improve upon soil physicochemical properties. The study was conducted at the Department of Horticulture, KNUST-Ghana. Some trees in the landscape suppress the growth of any other plant species beneath them. This is reported to be caused by the presence of allelochemicals which are released into the soil by the plants, a mechanism known as allelopathy. Soil amendment is therefore needed to curb the effects of these allelochemicals and make nutrients in the soil available to other plant species that may be planted beneath the allelopathic trees. Biochar, a pyrolised biomass, is a fine-grained, highly-porous charcoal substance that is used as a soil amendment. Biochar produced from three different types of sawdust (*Tectona grandis*, *Celtis mildbraedii*, and *Entandrophragma cylindricum*) and absolute control were the treatments used. The treatments were applied to the soil at a depth of 3 inches at a ratio of 1:1. Data collected included; allelochemicals in the tree and in the soil, rate of growth of grass, percentage coverage of grass, soil water-holding capacity, soil pH and soil nutrient analysis. The study was carried out beneath a bamboo stand—a tree species suspected to be allelopathic. *Stenotaphrum secundatum* (St. Augustine's grass) was used for the study because it prefers shaded growing environmental conditions. Data collected over a period of twelve weeks included presence of allelochemicals in the soil and in the tree species, soil physicochemical analysis, rate of growth, and percentage coverage of grass. The results of the study indicated that the bamboo species is allelopathic. Biochar was able to nullify the effects of the allelochemicals and hence allowed the grass to grow well.

Keywords: sequestration, compact, allelochemicals, phytochemical, environment

1. INTRODUCTION

Trees have developed in ecological systems filled with many other organisms. The environment, shared by all, contains limited resources and less-than-ideal growth conditions. All living things have strategies to thrive in this intense struggle for life. Allelopathy is one such strategy of life [1]. The term "allelopathy" is from Greek literally meaning "to suffer from each other." It was first detected by Davis (1928) in black walnut tree (*Juglans nigra*) whose foliar leachate containing juglone was found to damage germination and seedling growth of crops beneath the tree [2]. Allelochemicals are released into the environment via leachates and volatiles from live or dead plant roots and leaves. In most cases, the release of these chemicals results in more resources available to the allelopathic plant for uptake [3]. To allow other plants to survive in allelochemical-laden soils it is important to amend such soils to nullify the effects of the allelochemicals and boost the resistance of the plant. Davis and Wilson (2005) defines soil amendment as any material added to a soil to improve its physical properties [4]. To do its work, an amendment must be thoroughly mixed into the soil. There is evidence from thousands of years of traditional use of charcoal as amendment in soils, the most well-known example is the fertile *Terra Preta* soils in Brazil. In more recent times however, charcoal has remained a technologically important material, primarily as a result of its adsorptive properties. Biochar is a name for charcoal when it is used for particular purposes, especially as soil amendment. Like all charcoal, biochar is created by pyrolysis of biomass mostly from organic matter [5]. 'Biochar' is however much broader than traditional charcoal. It encompasses black carbon produced from any biomass feedstock [6]. Studies have shown that biochar amendment can enhance the growth and quality of certain crop plants, although the mechanisms by which it does so are far from clear [4]. Like other pyrolysis products biochar is expected to be highly surface active materials that strongly adsorb organic compounds. Joyakumar *et al.* (1978a, 1978b); Melkania (1987), Eyini *et al.* (1989); Bansal *et al.* (1992) and Bora *et al.* (1999) have studied and reported on the allelopathic effects of eucalyptus, acacia, bamboo and teak on germination and seedling growth of certain food crops [7-12]. The addition of strong adsorbents such as biochar to soil may disrupt the function of allelochemicals. It is therefore reasonable to assume

that these amendments could become a useful management option for landscape designers. The objectives of the study are to find out the specific allelochemicals present in the allelopathic plants and also to find out if biochar and charcoal can improve the physicochemical properties of the soil for better plant performance.

2. MATERIAL AND METHODS

2.1 Study Site

The field experiment was carried out at the Department of Horticulture, Kwame Nkrumah University of Science and Technology (KNUST), Ghana. The site lies on latitude 6° 43' and 6°45'N and longitude 1°25" and 1°36"W. It is within the moist semi-deciduous belt. The soil type is sandy loam with a pH range of 6.65–7.21. It is of the series of Auroso Orchrosols. The land **aea** is characterized by a bi-modal rainfall distribution with peaks in June and September. The first and second growing seasons typically last from late March to mid-July and from mid-August to the end of November, respectively, separated by a short dry spell of about four weeks in July. The major dry season starts mid-November and lasts till the end of February or mid-March.

2.2 Experimental design and procedure

Randomized Complete Block Design (RCBD) layout with four (4) treatments and three (3) replicates were employed in the study. The plot sizes were 0.4m x 2m. Distance between the treatments was 0.2m. The first replicate was laid 2m away from the bamboo stand, the second replicate was laid 6m away from the stand and the third replicate was laid between 3m and 5m away from the stand. The feedstock which was used in the production of biochar was sawdust. Sawdust of *Tectona grandis* (Teak), *Entandrophragma cylindricum* (Sapele), and *Celtis mildbraedii* (Esa) was collected and charred in a Biochar Reactor at Chirepatre, in Kumasi (Ashanti Region), Ghana. The leaves and roots of *Bambusa sp* (Bamboo) – a landscape tree suspected to be allelopathic – were collected, oven-dried at a temperature of 40°C for about 24 hours and blended into a powdered form. Phytochemical screening was carried out on the powdered leaves and roots to test for the presence of any or all of the following allelochemicals: flavonoids, tannins, glycosides, triterpenoids, saponins, ferulic acids and alkaloids. The presence of any of these chemicals in large quantities suggests that the tree is allelopathic. Based on the results from the laboratory analysis, soils from beneath the bamboo stand was used as a medium to grow grass. Soils beneath the bamboo stand were also collected 1m away from the base of the stand and analyzed to find out if the allelochemicals from the leaves and roots had been released into the soil. Initial analysis on total nitrogen, available phosphorous, soil potassium, soil organic carbon, soil pH and water-holding capacity of the soils were also analyzed. Chemical analyses were carried out on each of the biochar samples to determine the pH, total nitrogen, potassium and available phosphorous levels before application. The treatments were then applied and mixed into the soil at a depth of 3 inches in a 1:1 ratio. The control plots had no soil amendments.

2.3 Data Collected

Initial analyses were carried out before planting, a second analysis was carried during the experiment and a final analysis at the end of the experiment. The following parameters were studied:

2.3.1 Rate of growth of grass

The growth parameters that were measured were leaf count and the length of grass. A 30cm rule was used to measure the length of grass once every week. The number of leaves on each stolon was also counted once every week.

2.3.2 Percentage coverage of grass

The percentage coverage of grass was recorded every two weeks after planting. A 30cm² quadrant was used for measuring.

2.3.3 Soil water-holding capacity

A filter paper was placed on the screen inside a Hilgard cup. The cup was gently filled with 20 g of air-dried biochar and placed in a shallow pan of water allowing only the bottom few centimetres of the cup to become wet. The biochar was allowed to become saturated from the bottom of the cup to the surface. The cup was removed from the pan of water and placed in humid enclosure till drainage was completed. The total weight of moist biochar and moisture container was taken after the biochar was carefully removed from the Hilgard cup, put in a pre-weighed container. The biochar was then dried in an oven at 105 °C until no further water loss occurred, and reweighed to record the oven-dried **sample The** WHC was then determined for all the media.

2.3.4 Soil pH

The pH of the biochar was determined according to the procedures recommended by McLaughlin (2010) for the settlement of floating biochar particles [13]. The pH was measured with Metrohm 827 Lab pH meter.

2.3.5 Soil nutrient analysis

Soil Organic Carbon was done based on the Walkley and Black method-Nelson and Sommers, 1982 [14]. The total Nitrogen was carried out using the Kjeldahl method [15]. Available Phosphorus was determined using the procedures by Bray and Kurtz, 1945 [16]. Potassium (K) was determined following the procedures of Black, 1986 [17].

2.3.6 Quantitative determination of phytochemicals in the tree and in the soil

The following analytical procedures were followed in the determination of phytochemicals;

1. Test for Alkaloids-Mayer's test

To a few ml of plant sample extract, two drops of Mayer's reagent are added along the sides of test tube. Appearance of white creamy precipitate indicates the presence of alkaloids [18].

2. Test for Glycosides

For 50 mg of extract is hydrolysed with concentrated hydrochloric acid for 2 hours on a water bath, filtered and the hydrolysate is subjected to the Legal's test where 50 mg of extract is dissolved in pyridine, sodium nitroprusside solution is added and made alkaline using 10% NaOH. Presence of glycoside is indicated by pink colour.

3. Test for Phenolic compounds and Tannins

a. Ferric Chloride test

The extract (50 mg) is dissolved in 5 ml of distilled water. To this few drops of neutral 5% ferric chloride solution are added. A dark green colour indicates the presence of phenolic compound [19].

c. Lead acetate test

The extract (50 mg) is dissolved in of distilled water and to this 3 ml of 10% lead acetate solution is added. A bulky white precipitate indicates the presence of phenolic compounds.

d. Alkaline reagent test

An aqueous solution of the extract is treated with 10% ammonium hydroxide solution. Yellow fluorescence indicates the presence of flavonoids.

4. Test for Saponins The extract (50 mg) is diluted with distilled water and made up to 20 ml. The suspension is shaken in a graduated cylinder for 15 minutes. A two cm layer of foam indicates the presence of saponins [20].

2.4 Data analysis

All data gathered in the research were recorded and classified in the Microsoft Office Excel 2010. Data obtained were subjected to analysis of variance (ANOVA using Statistix version 9. The treatments means were separated using the Tukey's Multiple Comparison Test at 5%.

3. RESULTS AND DISCUSSION

3.1 Phytochemical Screening

Phytochemical screening was carried out on the powdered leaves and roots of the bamboo before the research was started. Analysis was also carried out on the soil samples collected beneath the bamboo stand to find out if any allelochemicals have been released into the soil. The results are shown in Table 1.

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Table 1: Results of Initial Phytochemical Screening

TEST: ALKALOIDS – MAYER’S (DRAGENDORFF’S REAGENT)	
Sample	Concentration
Bamboo leaves	++
Bamboo roots	++
TEST: TANNINS (1% LEAD ACETATE)	
Bamboo leaves	++
Bamboo roots	++
TEST: TANNINS (1% FERRIC CHLORIDE)	
Bamboo leaves	++
Bamboo roots	++
TEST: GLYCOSIDES (GENERAL TESTS)	
Bamboo leaves	++
Bamboo roots	++
TEST: SAPONINS	
Bamboo leaves	++
Bamboo roots	++
TEST: FLAVONOINDS	
Bamboo leaves	++
Bamboo roots	++
TEST: TRITERPERNOIDS	
Bamboo leaves	++
Bamboo roots	++

Inference: ++ means Allelochemicals are present in high concentrations for the samples

The results of the initial phytochemical screening revealed that the following allelochemicals were present in the leaves and roots of the bamboo: alkaloids, tannins, glycosides, saponins, flavonoids and triterpenoids. These allelochemicals are secondary metabolites which are found in many plants. Secondary metabolites are classified as allelochemicals based on their concentrations [21]. Due to the high concentrations of the allelochemicals found in the leaves and roots of the bamboo species it was classified as allelopathic.

The release of the allelochemicals in the leaves and roots however gave no indications of their release into the surrounding soils. Initial phytochemical screening carried out on the soil collected beneath the bamboo stand revealed that the allelochemicals were not released into the soil. Coder (1999) reported that once an allelochemical is outside its producer (conveyer) the chemical is easily modified, torn-apart, reassembled and or used by other organisms [1]. Hence it could be suggested that the allelochemicals may have been released into the soil but because there was no undergrowth competing with the bamboo stand the allelochemicals may rather have been modified or used up by soil microorganisms. Many organisms respond quickly to allelopathic attack by breaking-up the chemicals or transforming them into non-damaging forms [1]. Two phytochemical screenings were therefore carried out during the research and also at the end of the research to find out if the allelochemicals were released into the soil after planting. Table 2 shows the results of the analyses.

Table 2: Phytochemical Screening for Soil Samples Collected beneath the Bamboo Stand at 0WAP, 4WAP and 12WAP of *S. secundatum*

TEST: ALKALOIDS – MAYER’S (DRAGENDORFF’S REAGENT)			
Sample	Concentration at 0WAP	Concentration at 4WAP	Concentration at 12WAP
Teak Biochar	-	++	-
Esa Biochar	-	+	-
Sapele Biochar	-	++	-
Control	-	++	++
TEST: TANNINS (1% LEAD ACETATE)			
Teak Biochar	-	++	+
Esa Biochar	-	+	+
Sapele Biochar	-	++	+
Control	-	++	+
TEST: TANNINS (1% FERRIC CHLORIDE)			
Teak Biochar	-	++	-
Esa Biochar	-	++	-
Sapele Biochar	-	++	+
Control	-	+	+
TEST: GLYCOSIDES (GENERAL TESTS)			
Teak Biochar	-	+	-
Esa Biochar	-	++	+
Sapele Biochar	-	+	-
Control	-	++	+
TEST: GLYCOSIDES (CYNOGENIC GLYCOSIDES)			
Teak Biochar	-	+	-
Esa Biochar	-	+	-
Sapele Biochar	-	+	+
Control	-	+	-
TEST: SAPONINS			
Teak Biochar	-	++	-
Esa Biochar	-	++	+
Sapele Biochar	-	++	+
Control	-	++	+
TEST: FLAVONOIDS			
Teak Biochar	-	-	-
Esa Biochar	-	-	-
Sapele Biochar	-	-	-
Control	-	-	-
TEST: TRITERPERNOIDS			
Teak Biochar	-	++	+
Esa Biochar	-	+	+
Sapele Biochar	-	+	-
Control	-	++	++

Inferences: ++: Allelochemicals are present in high concentrations in the sample.

+: Allelochemicals are present in low concentrations in the sample.

- : Allelochemicals are absent in the sample.

3.2 Biochar Samples Analysis before Application

Analyses were carried out on the biochar samples before application to the soils. The initial levels of nutrients in the samples are based on the type of feedstock that was used and the method of production. Table 3 shows the results of the analyses.

Table 3: Results of Biochar Samples Analysis

SAMPLE ID	Total N%	AVAIL P(mg/kg)	K (cmol/kg)	pH
Esa biochar	0.535	0.17	1.63	9.2
Sapele biochar	0.34	0.085	1.73	7.5
Teak biochar	0.145	0.195	1.62	6.7

Different nutrient compositions were recorded between the biochar samples analyzed. These differences may arise, even within the same biomass feedstock type, due to distinct growing environmental conditions and those relating to the time of harvest [22]. Again, the method of biochar production as well as the temperature at which pyrolysis occurred also affects the nutrient composition and structure of the biochar produced.

3.3 Soil Physicochemical Properties

3.3.1 Soil Physicochemical Properties beneath Bamboo Stand 0WAP

Initial soil analysis carried out indicated that Soil Organic Carbon, total Nitrogen and available Phosphorus were all moderate whereas soil K was low. The pH was also slightly alkaline (Table 4).

Table 4: Soil physicochemical properties beneath bamboo stand 0WAP

Physicochemical Properties						
SOC (%)	Total N (%)	Available (mg/kg)	P	Soil K (cmol/kg)	Soil pH	Soil Water-Holding capacity
1.52	0.15	10.47		0.16	7.2	7.02

Many recent studies have been carried out to show the effect that biochar and charcoal have on soil physical, biological and chemical properties. These properties altogether affect plant growth and its response in the soil.

Soil Physicochemical Properties beneath Bamboo Stand 8WAP. Significant differences at $P \leq 0.05$ were noted in available P with teak biochar performing best with a means of 22.03. The observed increase in available P by the eighth week due to application of biochar could be due to the presence of high P in the feedstock used in the biochar production. This affirms findings by Nigussie et al. (2012) that P was made available in the soil due to the presence of high P in the feedstock [23]. Again significant differences at $P \leq 0.05$ were recorded in soil K as well as in the water-holding capacity with Esa biochar performing best in both cases 8WAP. Soil water-holding capacity was increased in all treatments 8WAP. Dempster et al. (2012) and Kammann et al. (2011) have reported on the remarkable water-holding capacity of soils amended with biochar [24,25]. The high surface area of biochar as reported by Glaser et al. (2002) can lead to increased water retention. pH was reduced in all the biochar amended plots but no significant differences were recorded [26]. Even though no significant differences were recorded in the SOC it was increased in the biochar amended plots from low to moderate 8WAP. Shenbagavalli and Mahimairaja (2012) reported similar results after biochar application to soil. It was reported that the application of different rates of biochar had significant effect on SOC content [27]. Again no significant differences were recorded for the total N. Esa biochar however performed best with a means of 0.16 in increasing total N (Table 5).

Table 5: Soil Physicochemical Properties beneath Bamboo Stand 8WAP

Treatments	Physicochemical Properties		
	Available P (mg/kg)	Soil K (cmol/kg)	Soil water-holding capacity (%)
Teak biochar	22.03a	0.09b	5.59b
Esa biochar	10.48d	0.20a	6.76a
Sapele biochar	18.27b	0.17ab	5.43b
Control	16.46c	0.16ab	4.36c
HSD (0.05)	0.56	0.09	0.44

3.3.2 Soil Physicochemical Properties beneath Bamboo Stand 12WAP

After 12 weeks of planting, SOC was decreased in the biochar amended plots and there were no significant differences between the treatment means. Fontaine et al. (2004) reported that biochar has a priming effect when it is applied to soil [28]. The priming effect is defined as “the acceleration of soil carbon decomposition by fresh carbon input to soil” and is generally considered to be short-term changes in the turnover of soil micro-organisms [29]. The priming effect is thought to be a function of changes in microbial community composition upon fresh carbon input into soil [28]. This means that addition of a ‘new’ source of carbon (such as biochar or charcoal) into the soil system can potentially lead to a priming effect whereby SOC is reduced. This could be the reason why the SOC was reducing by the twelfth week by all the treatments.

There were also no significant differences for total N and soil K however esa biochar performed better in both cases. The decrease in N content is in agreement with findings by Shenbagavalli and Mahimairaja (2012) where the addition of biochar to soil resulted in marked changes in the N (NH_4^+ , N^- and NO_3^-) content of the soil. The reduction might be due to adsorption of NH_4^+ onto biochar particles [27]. Even though there were no significant differences in the soil K it was increased in the biochar amended plots. The increase in the soil K was due to the high content of K in the sapele biochar before application to the soil. Lehmann et al. (2003) also reported an increase in soil K due to the high content of K in biochar prior to its use as soil amendment [30]. Their work concluded that high concentrations of biochar are likely to increase the soil K considerably and this can be beneficial in K deficient soils.

Soil water-holding capacity also did not show any significant differences 12WAP but esa biochar performed well in increasing the water-holding capacity of the soil. Glaser et al. (2002) reported that water retention capacity was 18% higher in soils amended with charcoal as compared to adjacent soils where there was no amendment. Piccolo et al. (1997) also reported that the presence of small pores in the charcoal residues and charred biomass increases soil water-holding capacity of the soil [31].

There were however significant differences in the available P between all the treatments. Significant differences were also recorded for the treatment means 12WAP for soil pH (Table 6).

Table 6: Soil Physicochemical Properties beneath Bamboo Stand 12WAP

Treatments	Physicochemical Properties	
	Available P (mg/kg)	Soil pH
Teak biochar	18.27a	6.8ab
Esa biochar	8.84d	7.0a
Sapele biochar	9.65c	6.5ab
Control	12.13b	6.4b
HSD (0.05)	0.73	0.59

3.4 Rate of Growth of *S. secundatum* 4WAP

There were no significant differences between the treatments applied to the soil underneath the bamboo stand 4WAP (Plate 1).



Plate 1: From left – Sapele Biochar, Esa Biochar, Teak Biochar and Control plots beneath bamboo stand 4WAP

3.5 Rate of growth of *S. secundatum* at 8WAP

Again there were no significant differences between the treatments 8WAP nevertheless Esa biochar performed better with a mean of 16.0 as compared to the other treatments (Plate 2).



Plate 2: Teak Biochar (left) and Sapele Biochar (right) plots beneath the bamboo stand 8WAP

There were no significant differences between the treatments applied to the soil underneath the bamboo stand 4WAP (Plate 1). The results indicated that alkaloids, tannins, glycosides, saponins, and triterpenoids were released into the soil after planting – 4 weeks after planting (4WAP). Coder (1999) stated that allelochemicals are produced when a plant is under stress. Nitrogen, phosphorus, water, and temperature extremes can all accelerate allelopathic chemical production [1]. As stress between an allelopathic plant and a neighbouring plant species becomes great, allelopathy increases in importance. At 12WAP the concentrations of most of the allelochemicals were minimal in the soil. Flavonoids were tested for but due to the unstable nature of the compound it was not detected in any of the samples. The control plot had most of the allelochemicals still present after twelve weeks.

3.6 Rate of growth of *S. secundatum* 12WAP

By the end of the twelfth week (12WAP), Esa biochar appeared to be performing better than all the other treatments with a mean of 29.1 even though there were no significant differences between the treatments (Plates 3 and 4).



Plate 3: Sapele Biochar (left) and Esa Biochar (right) plots beneath bamboo stand 12WAP



Plate 4: Control plot beneath bamboo stand 12WAP

3.7 Percentage coverage of *S. secundatum* beneath Bamboo Stand

There were no significant differences between the treatments with respect to the percentage coverage of *S. secundatum*. There was a slow rate of spread from the second week through to the sixth week for all the treatments. However from the eighth week through to the twelfth week the rate of spread of the *S. secundatum* rose steadily in all the treatments but control plots. The rate of spread for the control started declining from the eighth week through to the twelfth week (Plate 5).



Plate 5: Control plot (left) as compared to Esa Biochar plot (right) at 12WAP

4. CONCLUSION

Bambusa sp (Bamboo) was found to be allelopathic. The biochar amendments used were able to nullify the effects of the allelochemicals in the soil and enabled the *S. secundatum* planted beneath the bamboo stand to do well. The biochar amendments were also able to increase the available nutrients in the soil thereby making the nutrients available for plant

use. The amendments also improved upon the soil pH, increased soil carbon as well as improved upon the water-holding capacity of the soil. Biochar prepared from *Celtis mildbraedii* (Esa) however performed best in improving the soil's ability to support plant growth amidst the presence of allelochemicals in the soil beneath the bamboo stand.

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