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Impact of allelochemicals from shade trees bark on the performance of cocoa seedlings

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Abstract

Shade trees are important in cocoa agroforestry systems; however, they release allelochemicals from various parts that affect understory plants. Unfortunately, information on allelochemicals produced by shade tree bark in cocoa plantation remain scarce. This study investigates the effect of allelochemicals from bark of shade trees on cocoa seedlings growth. The experiment was a 4 × 11 factorial study, and the treatments were four different concentrations from each of the 11 tree species. The treatments were laid out in a completely randomized design with four replicates. Data were collected at 30, 60, 90, 120, and 150 days after treatment applications. The tree species alone and bark extract concentrations alone significantly impacted plant height from 90 to 150 days after application. *Albizia ferruginea* (Guill. & Perr.) Benth, *Celtis mildbraedii* Engl., and *Triplochiton scleroxylon* K. Schum produced the highest cocoa seedling heights. All concentrations also influenced stem diameter of cocoa seedlings. *Albizia ferruginea* enhanced stem diameter significantly among tree species and the control. Tree species and bark extract concentrations interacted to increase fresh root weights and dry plant biomass. *Albizia ferruginea* consistently increased dry plant biomass, while *C. mildbraedii* produced the highest enhancement for fresh roots. All concentrations enhanced plant biomass, with the 75 mg mL^{-1} concentration consistently producing the highest plant fresh and dry weights. *Albizia ferruginea* and *C. mildbraedii* can be potential tree species in the cocoa agroforestry when 2-month-old cocoa seedlings are to be transplanted on the field. Bark extract of 75 mg mL^{-1} concentration can be used as a growth stimulant on cocoa seedlings.

1 | INTRODUCTION

Cocoa farms are usually intercropped with tree species that provide an overstorey shade for cocoa trees (Hosseini-Bai et al., 2019). The forest shade trees in cocoa cropping systems contribute to carbon (C) sequestration, nutrient recycling, accumulation of soil organic matter, and conservation of biodiversity (Yao et al., 2021). Additionally, shade trees in cocoa plantations also help reduce evapotranspiration and influence microclimatic stability to protect cocoa trees from environmental stress and other harsh climatic conditions (Anim-kwapong, 2003).

Abbreviations: CRIG, Cocoa Research Institute of Ghana; DAA, days after continuous application; IAA, indole-3-acetic acid; LSD, least significant difference; POD, peroxidase.

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Despite all these advantages, there are concerns about the adverse effects of some of these shade trees in the cocoa cropping systems. Some shade species are known to affect soil biota, chemical, physical, and other biological properties. They also serve as alternative hosts for some common pests and diseases that affect cocoa growth and yield (Andres et al., 2018). Some shade trees have also been noticed to reduce biodiversity and the productivity of the understory plants and subsequently cause death to the tree crops, while others do vice versa through a phenomenon called allelopathy (Scavo et al., 2019).

The allelochemicals are mainly found in the leaves, bark, and roots of tree species (Kato-Noguchi & Kurniadie, 2020). These chemicals may impart inhibitory or stimulatory effects on receptor plants (Giuliani et al., 2020; Rizvi et al., 1999) depending on their presence and concentrations in the tree parts.

Forest shade trees in cocoa agroforestry are made up of distinct compositions, architecture, and diverse functions in the agroforestry system. Due to these unique attributes and functions, these allelochemicals released in the environment are diverse. This situation has resulted in an ongoing debate that different tree types may also have a different organ for allelopathic evaluation due to the variation and concentration of allelochemicals and their derivatives that occur in the parts (leaves, stem, flowers, roots, and pollen) of forest shade trees growing in agroforestry (Gatti et al., 2010).

In a study, two month-old cocoa seedlings at the nursery were subjected to undecomposed aqueous extracts of healthy matured leaves from some common cocoa shade trees and later assessed the effect on growth and development. The growth and some physiological parameters of the cocoa seedlings assessed were adversely affected at high concentrations (100 mg mL^{-1}) of the extracts applied, whereas at the low concentrations, their growth parameters were enhanced in the early stages. Similar allelopathic effects have been reported in other crops. For instance, Saha et al. (2018) reported that coumarin (6, 7, 8-trimethoxycoumarin), a secondary metabolite from the bark and roots of Chinese tallow, significantly reduced the growth of weed species. Additionally, Sarmin et al. (2020) observed that the bark and stem exudates of *Eucalyptus camaldulensis* Dehnh. significantly inhibited the germination of maize, country bean, and bottle gourd seeds. However, there has been no study on the potential allelochemicals from the stem bark of common shade trees found in cocoa agroforestry. There is thus the need to investigate if there is a positive or negative effect of the allelochemicals from the bark of these shade trees on cocoa plants in the cocoa agroforestry system. Hence, the study was undertaken to assess the performance of cocoa seedlings using an extract from the stem bark of 11 common shade trees in cocoa agroforestry at nursery settings.

Core Ideas

- *Albizia ferruginea*, *Celtis mildbraedii*, and *Triplochiton scleroxylon* were associated with the highest seedlings.
- All bark extract concentrations had an impact on the stem diameter of cocoa seedlings.
- *Albizia ferruginea* stood out by significantly enhancing stem diameter compared to the control.
- *Albizia ferruginea* produce higher dry plant biomass, and *Celtis mildbraedii* enhanced fresh roots and height.
- Note that 75 mg mL^{-1} concentration of bark extract consistently resulted in the highest fresh and dry plant weights.

2 | MATERIALS AND METHODS

2.1 | Experimental site

The experiments were carried out at Cocoa Research Institute of Ghana (CRIG) main nursery (latitude 60 13′ N, longitude 00 22′ W, elevation 222 m above sea level). The location is in the wet semi-equatorial zone. The area's natural vegetation is humid deciduous forest.

2.2 | Climatic conditions of the experimental site

Temperatures in the area during the experiment ranged from 26°C in August to 30°C in March. The relative humidity ranged from 60% to 70%. The rainfall pattern at the research site was bimodal. The cropping season runs from August to March, and the average monthly rainfall is approximately 123.16 mm.

2.3 | Soil sampling, preparation, and initial analysis

Soil was collected from newly grubbed cocoa farms, heaped, and thoroughly mixed. Soil samples were taken, air-dried, and then sieved through 2-mm mesh screens to remove debris and stored in ziplock plastic bags for physical and chemical analysis. The soil samples were sent to the Soil Science Laboratory of the CRIG, Akim-Tafo, for analysis. Soil pH was determined in a 1:2.5 water ratio using the electrometric method (Page et al., 1982); organic carbon was determined using the Walkley–Black wet oxidation method (Nelson &

TABLE 1 List of plant species used for the study.

S No.	Tree species
1	*Albizia ferruginea* (Guill. & Perr.) Benth
2	*Antiaris toxicaria* Lesch.
3	*Cedrela odorata* L.
4	*Celtis mildbraedii* Engl.
5	*Ficus exasperata* Vahl
6	*Gliricidia sepium* (Jacq.) Kunth ex Walp.
7	*Milicia excelsa* (Welw.) C.C. Berg
8	*Spathodea campanulata* P. Beauv
9	*Terminalia ivorensis* A.Chev
10	*Terminalia superba* Engl. & Diels
11	*Triplochiton scleroxylon* K. Schum

TABLE 2 Chemical and chemical properties of the soil used for raising the cocoa seedlings.

Soil properties	Values
pH (1:1 H_2O)	6.43
Total nitrogen (%)	0.09
Organic carbon (%)	1.40
Available P (mg kg^{-1})	21.9
Exchangeable K (mg kg^{-1} soil)	23.46
Electrical conductivity (μS cm^{-1})	413.60
Textural class	Sandy clay loam

Sommers, 1982); available phosphorus (P) was determined using the Bray No. 1 method (Bray & Kurtz, 1945); total nitrogen (N) was determined using the Kjeldahl distillation method (Bremner & Mulvaney, 1982); exchangeable potassium (K) was determined using the flame photometer.

2.4 | The aqueous bark extracts preparation

The experiment used aqueous stem bark extracts from each of 11 different tree species (Table 1). The stem bark aqueous extracts were prepared using the method of Karamenderes et al. (2006). The stem bark from each of the tree species (Table 1) was harvested to a depth of 3 cm. This harvesting was performed at a height ranging from 140 to 210 cm above the ground level. The stem bark samples were collected from shade tree species within CRIG cocoa farms in July 2021, dried separately under shade for 7 days, and finally ground into powder.

Five hundred grams of each powdered sample was mixed with 5000 mL of water and stored at room temperature (20–25°C) for 72 h. The mixture was shaken vigorously for 10 min and screened through 300 muslin cloth to obtain an undiluted solution. The undiluted extracts (100 mg mL^{-1}) were then further diluted with water to obtain concentrations of 75, 50, and 0 mg mL^{-1} (control) and stored at a cool place until use.

2.5 | Experimental design and treatments

The study used a cross-factorial design with 44 treatment combinations. There were 11 different shade tree species and four different aqueous stem bark extract concentrations (Table 1). The concentrations of aqueous stem bark extract were 100, 75, 50, and 0 mg mL^{-1} (control). The design was a complete randomized design with four replications. The experiment was carried out under a shade net that allowed 40% of the sunlight penetration into the test crop.

2.6 | Physical and chemical properties of soils used for raising cocoa seedlings

The soil used to raise the cocoa seedlings had a pH of 6.43, total nitrogen of 0.09%, organic carbon of 1.40%, available P of 21.90 mg kg^{-1}, exchangeable K of 23.46 mg kg^{-1}, electrical conductivity of 413.60 (Table 2), and has a textural class of sandy clay loam. The soil classification was performed following Landon (2014). Overall, soil fertility was low for the experiment; however, such soil has been described as suitable for cocoa cultivation by Ahenkorah et al. (1987), Afrifa et al. (2009) and Snoeck et al. (2010), and was therefore ideal for this study.

2.7 | Raising of cocoa seedlings and treatment application

Nursery bags with size of 18 cm × 25 cm were first perforated at the bottom with a perforator to create a 4-cm^2 hole to facilitate drainage of excess water. The analyzed soil was used to fill 7040 cocoa nursery bags. Mixed hybrid cocoa seeds obtained from CRIG were sown directly into each bag to raise a total of 7040 cocoa seedlings. Seedlings were arranged on a plastic-lined sheet to protect against soil diseases and limit weed growth. Forty cocoa seedlings (two-month-old) were used per treatment, and 70 mL of each aqueous stem bark extract from the 11 shade trees was applied on each nursed cocoa seedling per week until the experiment was completed. The experiment lasted for 8 months at the nursery.

2.8 | Measured plant parameters

Plant height, stem diameter, and chlorophyll content were measured at 30, 60, 90, 120, and 150 days after continuous

application (DAA) for the experiments. Plant height was determined by measuring from the base of the soil surface to the tip of the apical leaf using a metallic ruler. The stem diameter was measured 5 cm above the soil surface using a digital caliper. The chlorophyll content of the adjacent fully expanded leaves on the fourth position from the apex per plant was determined using the SPAD 502 Plus chlorophyll meter. Leaf chlorophyll content was measured between 8 and 10 a.m. GMT because plants are not stressed and there is no dew on the leaves at that time. The plant fresh and dry biomass of the cocoa seedlings were also evaluated.

2.9 | Biomass assessment

The plant biomass was assessed at the end of the experiment. Ten selected cocoa seedlings were destructively sampled from the nursery bags. The stems and roots were cut with sharp scissors, and the leaves were picked up with the hand. The leaves, stems, and roots were all collected separately and washed in clean water to remove any attached soil. The leaves, stem, and roots fresh weights were determined separately using an electronic weighing balance. The samples were oven-dried at 60°C for 72 h to a constant weight. The dried samples were weighed and used to estimate plant biomass.

2.10 | Cultural practices

Weeding was done by handpicking the weeds in the nursery bags every 3 months and "Attack" insecticide (Emamectin Benzoate 5%) was also sprayed to control pests and diseases at a rate of 16 gL^{-1} of water using a Knapsack sprayer. The plants were watered every 3 days till the end of the experiment.

2.11 | Statistical analyses

The data collected were analyzed using the GenStat statistical software version 12. Significant differences among treatments were determined using the least significant difference procedure at 5% probability. Normality and homogeneity of variance of the data were checked for all analyses.

2.12 | Allelopathy index

Bruce Williamson and Richardson (1988) model was used to calculate the allelopathy index (RI) from the means using Equations (1) and (2):

$$\mathrm{RI} = 1 - \frac{C}{T} \; (T \geq C) \tag{1}$$

$$\mathrm{RI} = \frac{T}{C} - 1 \; (T < C) \tag{2}$$

where T is the treatment value and C is the control value. A positive RI value indicated a promotional and/or stimulating of allelochemicals on cocoa growth parameters, whereas a negative value indicated inhibition and/or suppression or reduction in the growth indicators.

3 | RESULTS

3.1 | Plant height

The interaction effects between tree species and aqueous bark concentrations were not significant for all the measurement periods (Table 3). The shade tree species alone and the bark extract concentration alone significantly increased plant height from 90 to 150 days after application (Table 3). The 50 mg mL^{-1} aqueous bark extracts consistently produced significantly taller cocoa seedlings than the control, 75, and 100 mg mL^{-1} concentrations from 90 to 150 days after application. The 50 mg mL^{-1} bark extract concentrations produced a higher positive allelopathy index on plant height compared to the other concentrations from 90 to 150 days after application (Table 3). All the tree species produced promotional effect on plant height from 90 to 150 DAA except *Antiaris toxicaria*, *Cedrela odorata*, and *Spathodea campanulata*. The measurement periods affected the impact of the bark extract of the tree species on the height of the cocoa seedlings. For instance, *Albizia ferruginea* produced the highest plant height of 47 cm at 90 DAA; *Celtis mildbraedii* had the highest plant height of 52 cm at 120 DAA; and *Triplochiton scleroxylon* had the highest plant height of 58 cm and 14% allelopathy index on cocoa seedlings at 150 DAA (Table 3).

3.2 | Stem diameter

The interaction effects between tree species and aqueous back extract concentrations were not significant at any measurement period. The bark extract concentration alone had a significant effect at 30, 120, and 150 DAA. At 30 DAA, only the 50 mg mL^{-1} concentration showed a higher stem diameter than the control. The 100 mg mL^{-1} concentration had the highest allelopathy index of 7.7. However, at 120 and 150 DAA, the 75 mg mL^{-1} concentration had the highest allelopathy index of 10.53% and 8.58%, respectively. The 100 mg mL^{-1} concentration had the lowest allelopathy index at 120 and 150 DAA. Shade tree species alone significantly

TABLE 3 Effect of aqueous bark extract concentrations and different shade tree types on plant height of cocoa seedlings.

Treatments	Plant height (cm)				
	30 DAA	60 DAA	90 DAA	120 DAA	150 DAA
Concentration (mg mL^{-1})					
Control	22.20 (0.00)	31.18 (0.00)	37.66 (0.00)	42.72 (0.00)	49.88 (0.00)
50	23.75 (6.52)	33.81 (7.78)	46.06 (18.24)	51.28 (8.56)	55.66 (10.38)
75	22.22 (0.09)	33.01 (5.54)	41.96 (10.25)	47.74 (5.02)	53.15 (6.15)
100	21.68 (−2.39)	32.02 (2.62)	42.33 (11.03)	49.15 (6.43)	52.14 (4.33)
Tree species					
Albizia ferruginea	24.87 (10.73)	34.61 (9.91)	47.26 (20.31)	51.15 (8.43)	52.25 (4.54)
Antiaris toxicaria	21.83 (−1.69)	30.18 (−3.31)	35.94 (−4.79)	40.93 (−1.79)	46.11 (−8.18)
Cedrela odorata	21.60 (−2.78)	29.67 (−5.08)	37.65 (−0.02)	43.63 (0.91)	48.60 (−2.63)
Celtis mildbraedii	22.91 (3.09)	33.84 (7.86)	46.10 (18.30)	52.55(9.83)	56.86(12.28)
Terminalia ivorensis	23.26 (4.55)	36.98 (15.68)	45.12 (16.53)	51.09 (8.37)	56.31 (11.42)
Ficus exasperata	21.85 (−1.60)	30.07 (−3.69)	41.15 (8.48)	46.36 (3.64)	51.15 (2.48)
Gliricidia sepium	23.21 (4.35)	32.65 (4.50)	44.48 (15.33)	49.44 (6.72)	54.63 (8.69)
Milicia excelsa	20.14 (−10.22)	30.01 (−3.89)	41.05 (8.25)	50.64 (7.92)	56.56 (11.81)
Terminalia superba	22.88 (2.97)	36.07 (13.55)	41.61 (9.49)	46.64 (3.92)	52.41 (4.83)
Spathodea campanulata	21.66 (−2.49)	29.67 (−5.09)	35.88 (−4.96)	40.46 (2.26)	46.88 (−6.46)
Triplochiton scleroxylon	22.55 (1.55)	33.78 (7.70)	45.78 (17.74)	52.06 (9.34)	58.03 (14.04)
***p*-values (0.05)**					
Concentration (C)	0.150	0.630	0.002	0.001	0.002
Trees (T)	0.296	0.306	0.005	0.001	0.040
T × C	0.416	0.500	0.362	0.337	0.888
SED values					
Concentration (C)	0.947	2.126	2.106	2.006	1.989
Trees (T)	1.571	3.526	3.493	3.326	3.299
T × C	3.142	7.052	6.986	6.653	6.597

Note: Values (%) in parenthesis are allelopathy indicies: minus sign (−) indicates suppression; those without a minus sign indicate promotion.
Abbreviations: DAA, days after continuous application; SED, standard error of difference.

increased the stem diameter of cocoa seedlings at 120 and 150 DAA. *Celtis mildbraedii* and *T. scleroxylon* had the highest positive allelopathy index of 17.32% and 17.3%, respectively, at 120 DAA. Only *T. scleroxylon* had the highest positive allelopathy index on the stem diameter of cocoa at 150 DAA. *Antiaris toxicaria* had the highest negative allelopathy index on stem diameter at 120 DAA, and *A. toxicaria* had a negative allelopathy index at 150 DAA (Table 4).

3.3 | Chlorophyll content

The interaction between aqueous bark extract concentration and shade tree did not significantly affect the chlorophyll content of cocoa seedlings at all the measurement periods (Table 5). Similarly, tree species effect alone did not significantly influence chlorophyll content of cocoa seedlings at all measurement dates; however, the effect of aqueous bark extract concentration was significant. The significant effect of aqueous bark extract concentrations occurred between 30 and 60 DAA (Table 5.). At 30 DAA, only the effect of 100 mg mL^{-1} was comparable to the effect of control; all the aqueous bark concentrations had a suppression effect on chlorophyll content. At 60 and 90 DAA, the aqueous bark concentrations produced more chlorophyll than the control, leading to positive allelopathy effect. The 100 mg mL^{-1} consistently recorded the highest positive allelopathy index of 11.34% and 11.29%, respectively, on 60 and 90 DAA (Table 5).

3.4 | Fresh and dry weights of leaves, stems, and root of seedlings

The interaction effect of tree species and aqueous bark extract concentrations did not significantly affect the fresh leaves and stem weight of cocoa seedlings. However, tree species and aqueous bark extract concentrations interacted to significantly increase fresh root weights and dry weights of

TABLE 4 Effect of aqueous bark extract concentrations and different shade tree types on stem diameter on cocoa seedlings.

	Stem diameter (mm)				
Treatments	**30 DAA**	**60 DAA**	**90 DAA**	**120 DAA**	**150 DAA**
Concentration (mg mL^{-1})					
Control	4.05 (0.00)	5.25 (0.00)	6.41 (0.00)	6.97 (0.00)	9.48 (0.00)
50	4.16 (2.72)	5.32 (1.33)	7.05 (9.08)	7.79 (10.52)	10.22 (7.24)
75	3.88 (−4.19)	5.04 (−4.00)	6.90 (7.10)	7.79 (10.53)	10.37 (8.58)
100	3.74 (7.65)	4.86−7.43)	6.49 (1.23)	7.40 (5.81)	9.90 (4.24)
Tree species					
Albizia ferruginea	4.43 (9.38)	5.50 (4.76)	7.45 (13.95)	7.68 (9.24)	9.73 (2.56)
Antiaris toxicaria	3.91 (−3.46)	5.11 (−2.67)	6.36 (−0.78)	6.06 (−15.01)	9.35 (−1.39)
Cedrela odorata	3.94 (−2.71)	4.92 (−6.28)	6.14 (−4.39)	6.78 (−2.80)	9.49 (0.10)
Celtis mildbraedii	3.99 (−1.48)	5.13 (−2.28)	6.76 (5.17)	8.43 (17.32)	10.05 (5.67)
Terminalia ivorensis	4.04 (−0.25)	5.21 (−0.76)	6.99 (8.29)	8.12 (14.16)	10.28 (7.78)
Ficus exasperata	3.75 (−7.40)	5.15 (−1.90)	6.70 (4.32)	7.82 (10.87)	10.19 (6.97)
Gliricidia sepium	3.97 (−1.98)	5.09 (−3.04)	6.66 (3.75)	7.52 (7.31)	9.73 (2.57)
Milicia excelsa	3.56 (−12.09)	4.73 (−9.90)	6.24 (−2.72)	7.65 (8.88)	10.21 (7.15)
Terminalia superba	4.02 (−0.74)	5.23 (−0.38)	6.77 (5.32)	7.78 (10.41)	10.08 (5.95)
Spathodea campanulata	4.03 (−0.49)	4.99 (−4.95)	6.56 (2.29)	7.29 (4.38)	9.48 (0.00)
Triplochiton scleroxylon	3.92 (−3.20)	5.23 (−0.38)	7.21 (11.09)	8.43 (17.31)	11.34 (16.40)
***p*-values (0.05)**					
Concentration (C)	0.044	0.057	0.059	0.002	0.002
Trees (T)	0.247	0.567	0.140	0.001	0.007
T × C	0.854	0.687	0.286	0.731	0.391
SED values					
Concentration (C)	0.157	0.181	0.274	0.294	0.269
Trees (T)	0.261	0.300	0.454	0.488	0.446
T × C	0.522	0.599	0.908	0.976	0.891

Note: Values (%) in parenthesis are allelopathy indicies: minus sign (−) indicates suppression; those without a minus sign indicate promotion.
Abbreviations: DAA, days after continuous application; SED, standard error of difference.

leaves, stems, and root (Table 6). The effect of tree species alone significantly influenced fresh and dry weights of leaves, stem, and root of cocoa seedlings. *Albizia ferruginea* consistently increased dry weights of leaves, stem, and root of cocoa seedlings, leading to the highest promotional effect of 42%, 44%, and 39%, respectively. *Albizia ferruginea* also increased the fresh weight of leaves and stems but not root, producing the highest positive allelopathy index of 38% and 28%, respectively, for fresh leaf and stem weight. *Celtis mildbraedii*, however, produced the highest allelopathy index of 33% for fresh root of cocoa seedlings. Only a few tree species, such as *A. toxicaria* and *C. odorata*, had negative allelopathy index on both fresh and dry weights of leaves, stems, and root (Table 6). All the aqueous bark concentrations had promotional effect on both fresh and dry weights of leaves, stems, and roots. The 75 mg mL^{-1} consistently produced highest fresh and dry weights of leaves, stems, and roots, leading to highest positive allelopathy index (Table 6).

4 | DISCUSSION

Unlike allelochemicals obtained from matured leaves from different shade trees, allelochemicals released from the stem bark of shade trees may be influenced by the tree type; thus, their concentration, composition, structure, and activity might be extremely different. The important circumstances in which allelopathic effects occur are when allelochemicals reach the recipient plant in their active structure and at their effective concentration, thus extracts of stem bark often show different allelopathic effects. For example, in this study, the interactive effect of bark aqueous and shade tree types did not have significant influences on the plant height of cocoa seedlings; however, the application of aqueous stem bark and the shade tree species alone significantly influenced the plant height of the cocoa seedlings.

The study also indicated that the potency of allelochemicals found in aqueous stem bark concentrations and shade tree species increased from 90 to 150 days after application.

TABLE 5 Effect of aqueous bark extract concentrations and different shade tree types on chlorophyll on cocoa seedlings.

Treatments	Chlorophyll (μmol/cm²)				
	30 DAA	60 DAA	90 DAA	120 DAA	150 DAA
Concentration (mg mL⁻¹)					
Control	26.94 (0.00)	24.60 (0.00)	24.72 (0.00)	32.76 (0.00)	31.29 (0.00)
50	25.56 (−5.39)	26.83 (9.07)	26.95 (9.02)	31.73 (−3.25)	30.26 (−3.40)
75	24.63 (−9.38)	26.87 (9.23)	26.99 (9.18)	32.55 (−0.65)	31.08 (−0.68)
100	26.17 (−2.94)	27.39 (11.34)	27.51 (11.29)	31.44 (−4.20)	29.97 (−4.40)
Tree species					
Albizia ferruginea	25.75 (−4.62)	28.15 (14.43)	28.27 (14.36)	32.65 (−0.34)	31.18 (−0.35)
Antiaris toxicaria	26.79 (−0.55)	26.68 (8.45)	26.80 (8.41)	31.29 (−4.69)	29.82 (−4.93)
Cedrela odorata	25.80 (−4.42)	27.49 (11.74)	27.61 (11.69)	32.96 (0.61)	31.49 (0.63)
Celtis mildbraedii	25.01 (−7.72)	27.53 (11.91)	27.64 (11.81)	32.77 (0.03)	31.30 (0.03)
Terminalia ivorensis	25.95 (−3.82)	27.26 (10.81)	27.38 (10.76)	33.57 (2.41)	32.10 (2.52)
Ficus exasperata	26.91 (−0.11)	27.28 (10.89)	27.40 (10.84)	32.31 (−1.39)	30.84 (−1.45)
Gliricidia sepium	23.91 (−12.67)	25.55 (3.86)	25.67 (3.84)	32.31 (−1.39)	30.84 (−1.46)
Milicia excelsa	26.80 (−0.52)	24.56 (−0.16)	24.68 (−0.16)	31.96 (−2.50)	30.49 (−2.62)
Terminalia superba	25.03 (−7.63)	25.18 (2.35)	25.30 (2.34)	30.38 (−7.83)	28.91 (−8.23)
Spathodea campanulata	25.33 (−6.35)	24.81 (0.85)	24.93 (0.85)	30.85 (−6.19)	29.38 (−6.50)
Triplochiton scleroxylon	26.79 (−0.56)	26.15 (6.30)	26.27 (6.27)	32.27 (−1.52)	30.80 (−1.59)
***p*-values (0.05)**					
Concentration (C)	0.007	0.004	0.004	0.090	0.071
Trees (T)	0.147	0.084	0.084	0.071	0.090
T × C	0.604	0.207	0.207	0.632	0.632
SED values					
Concentration (C)	0.664	0.798	0.796	0.600	0.990
Trees (T)	1.101	1.324	1.323	0.994	0.598
T × C	2.203	2.647	2.646	1.988	1.986

Note: Values (%) in parenthesis are allelopathy indicies: minus sign (−) indicates suppression; those without a minus sign indicate promotion.
Abbreviations: DAA, days after continuous application; SED, standard error of difference.

Note that 50 mg mL^{-1} of aqueous stem bark consistently promoted the plant height of the cocoa seedlings compared with other concentrations. It was observed that the percentage promotion declined as the aqueous stem bark concentration increased. This result indicates that the extracts of stem bark from shade tree species often showed higher promotional effects on cocoa seedlings at low concentrations and lesser promotional effects at high concentrations, which is contrary to what has been found in other investigations (Ghani et al., 2023; Z. Zhang et al., 2021). These effects of allelopathy depend on the concentration of the allelochemicals (R. Wang et al., 2009; Z. Zhang et al., 2021). One possible explanation for this result may also be that the allelochemicals were rapidly made available by soil microbes (Lankau, 2010), which makes the tropism (chemotropism) of the aqueous stem bark more pronounced at the lesser concentrations (Qin et al., 2018). Furthermore, allelochemicals at low concentrations may induce compensatory plant growth, which is similar to what has been shown as a result of low-intensity environmental stresses (C. Wang et al., 2016, 2022).

Except for *A. toxicaria*, *C. odorata*, and *S. campanulata*, which had no stimulatory effect on the plant height of the cocoa seedlings; all other shade tree species influenced the plant height of the cocoa seedlings. Allelochemicals as well as many natural chemical compounds (Ebrahimi et al., 2021; Souri & Hatamian, 2019) can alter the contents of plant growth regulators or induce imbalances in various phytohormones, which inhibit the growth and development of plants, for example, concerning seedling growth, especially plant height. Allelochemicals, primarily phenolic allelochemicals, can stimulate indole-3-acetic acid (IAA) oxidase activity and inhibit the reaction of peroxidase (POD) with IAA, bound gibberellins, or IAA to influence endogenous hormone levels (Yang et al., 2014).

According to the findings of this study, all of the shade trees that had a significant stimulatory effect on plant height had potential allelochemicals that positively altered the plant

TABLE 6 Effect of aqueous bark extract concentrations and different shade tree types on leaf, stem and root fresh and dry weight.

	Fresh weight			Dry weight		
Treatments	**Leaves (g)**	**Stem (g)**	**Root (g)**	**Leaves (g)**	**Stem (g)**	**Root (g)**
Concentration (mg mL^{-1})						
0	9.81 (0.00)	12.55 (0.00)	7.82 (0.00)	3.73 (0.00)	3.51 (0.00)	2.78 (0.00)
50	13.09 (25.06)	13.79 (8.99)	9.49 (17.59)	4.76 (21.63)	4.48 (21.65)	3.31 (16.01)
75	14.50 (32.34)	15.39 (18.45)	11.05 (29.23)	5.41 (31.05)	5.67 (38.09)	4.32 (35.65)
100	14.13 (30.57)	14.12 (11.12)	9.61 (18.62)	5.09 (26.72)	4.95 (29.09)	3.81 (27.03)
Tree species						
Albizia ferruginea	15.96 (38.53)	17.64 (28.85)	10.76 (27.32)	6.47 (42.34)	6.29 (44.19)	4.60 (39.56)
Antiaris toxicaria	9.87 (0.60)	10.47 (−19.87)	7.12 (−9.83)	3.30 (−13.03)	3.05 (−15.08)	2.55 (−9.02)
Cedrela odorata	10.23 (4.10)	10.59 (−18.51)	6.93 (−12.84)	4.02 (7.21)	3.70 (5.13)	2.70 (−2.96)
Celtis mildbraedii	15.46 (36.54)	16.76 (25.12)	11.68 (33.04)	6.10 (38.85)	5.85 (40.00)	4.51 (38.35)
Terminalia ivorensis	14.83 (33.85)	15.57 (19.39)	10.73 (27.12)	5.26 (29.08)	5.06 (30.63)	3.83 (27.42)
Ficus exasperata	12.59 (22.08)	13.64 (7.99)	8.84 (11.53)	4.18 (10.76)	4.09 (14.18)	3.24 (14.19)
Gliricidia sepium	13.14 (25.34)	14.64 (14.27)	10.49 (25.45)	5.41 (31.05)	5.24 (33.01)	4.07 (31.69)
Milicia excelsa	13.83 (29.06)	14.01 (14.27)	8.89 (12.03)	4.79 (22.12)	4.24 (17.22)	3.18 (12.57)
Terminalia superba	11.97 (18.04)	13.21 (10.42)	9.80 (20.20)	4.52 (17.47)	4.53 (22.52)	3.49 (20.34)
Spathodea campanulata	10.43 (5.94)	11.18 (4.99)	9.33 (16.18)	3.62 (−3.03)	3.87 (9.30)	3.13 (11.18)
Triplochiton scleroxylon	13.38 (26.68)	15.88 (−12.25)	9.86 (20.68)	4.54 (17.84)	5.27 (33.39)	3.85 (27.79)
***p*-values (0.05)**						
Concentration (C)	0.001	0.132	0.001	0.001	0.001	0.001
Trees (T)	0.001	0.002	0.001	0.001	0.001	0.001
T × C	0.416	0.481	0.01	0.001	0.002	0.003
SED values						
Concentration (C)	1.969	1.192	0.608	0.273	0.320	0.268
Trees	3.265	1.977	1.008	0.452	0.530	0.444
T × C	6.529	3.955	2.015	0.905	1.061	0.888

Note: Values (%) in parenthesis are allelopathy indicies: minus sign (−) indicates suppression; those without a minus sign indicate promotion.
Abbreviation: SED, standard error of difference.

growth regulators. This might have resulted in a sufficient amount of gibberellic acid levels, which might have accelerated the number and length of cells in the cocoa seedling, causing the plants to grow taller. These findings support the findings of Kong et al. (2019).

This study revealed that the stimulation of the stem diameter was more pronounced when the cocoa seedlings were treated with the highest concentration of the aqueous bark extract (100 mg mL^{-1}) at the initial stage, but when the concentrations of the bark aqueous were reduced to 75 mg mL^{-1}, stimulation of the stem diameter of the cocoa seedlings was more severe at the 120 and 150 evaluation periods. This may be attributed to the uncharacterized allelochemicals in the bark aqueous extract altering the auxin levels in the cocoa seedlings to a more stable state (Yang et al., 2014). When the auxin levels are stable, it results in an increase in cell expansion, which subsequently causes the shoot to increase in size (Yang et al., 2014). This positive effect of the aqueous stem bark concentrations has also been reported in other crops. For instance, extracts of *Euphorbia serpens* Kunth. stimulated growth of the aerial parts and roots of *Lactuca sativa* L. (Dana et al., 2010), and leaf and stem bark extracts of *Phytolacca americana* L. stimulated growth of the aerial parts and roots of *Cassia mimosoides* (L.) Greene. (Kim et al., 2005). It has been shown that different parts of some tropical grasses (Souri, 2017; Souri & Neumann, 2018) and palm tree (*Acrocomia totai* [toh-TAH-ee]) has strong nitrification inhibition properties in soil that finally positively affect other or companion plant growth. However, these findings contradict the report of Abrahim et al. (2000). They discovered that stem bark aqueous extracts from *Cinnamomum camphora* (L.) J.Presl. and citrus fruit trees (limonene) inhibited corn stem and root growth and that this was due to changes in mitochondrial metabolism caused by allelochemicals altering various physiological and metabolic processes associated with plant growth and development. Corn seedlings had a lower mitotic index when the bark of *Leucaena leucocephala* (Lam.) de Wit. was used as mulch; the authors discovered that the decrease in

cell division, stem thickening, and root thickening was caused by increased activity of the enzyme POD in these seedlings (Gonçalves et al., 2016).

In this study, *C. mildbraedii* and *T. scleroxylon* shade trees increased the stem diameter of cocoa seedlings by 16%–18%, whereas *A. toxicaria* stunted the stem diameter. The findings of this study suggest that different tree species have different allelochemicals and these allelochemicals influence the stem thickness of cocoa seedlings in a variety of ways. Among the potential allelochemicals found in the aforementioned trees, *A. toxicaria* has allelochemicals that can inhibit stem thickness, and this inhibition may be due to dormant allelochemicals that cause an imbalance in the hormonal levels in cocoa seedlings. *Antiaris toxicaria* species may release allelochemicals into cocoa plants through the bark, explaining the higher inhibitory effect (2%–18%) with the bark.

Chlorophylls are the predominant pigment in the photosynthetic process. It has been found that the higher their concentration, the better the rate of CO_2 sequestration and thus the higher the growth rate (Rathore & Jasrai, 2013). Under the present experimental conditions, aqueous stem bark concentrations significantly decreased chlorophyll concentration in the cocoa seedlings evaluated at 30 DAA. This could be a sign of allelopathic effect, as leaf greenness and its chlorophyll index are very sensitive to many unfavorable conditions (Serri et al., 2021). On the contrary, at 60 and 90 DAA, the concentration of chlorophyll content was affected positively by all the aqueous stem bark concentrations (Table 5).

According to Blunden et al. (1996), the application of 0.34–0.96 mg L^{-1} of seaweed aqueous extract on food crops (tomato, dwarf French bean, wheat, barley, and maize) increased the concentrations of chlorophyll. This response was explained by the fact that some allelochemicals in the stem bark aqueous concentrations may alter the pH of the soil medium into more acidic and subsequently enhanced the absorption of nutrients such as manganese during the formation of more chlorophyll molecules (Hou et al., 2019), and therefore moderate atoms for the formation of the chlorophyll molecule could be available in the growth medium, thus causing the promotional effect. Furthermore, allelochemicals within the aqueous stem bark may change the morphology of the external membrane of the chloroplast, which improved the structure of the organelle and leads to a higher concentration of functional nutrients such as N in the chloroplast (Gonçalves et al., 2016). Importantly, N is also a key component in the structure of the chlorophyll molecule.

In this study, it was observed that stem bark aqueous extracts and tree species types may exert different effects on plant biomass. The presence of allelochemicals in the stem bark aqueous solution and shade trees influenced fresh leaf and dry plant biomass in cocoa seedlings. The influence of these attributes suggests that allelochemicals in stem bark aqueous extract concentrations and shade tree types or their combination may enhance photosynthate buildup in leaves and stem and as well as improve the length of the primary and lateral roots and subsequently enhanced fresh and dry biomass (Yang et al., 2014). This study corroborates work done by Lariguet et al. (2013), who reported enhanced plant biomass in *Arabidopsis*. Many workers have also observed that promoted plant growth, further facilitates the uptake of nutrients from the rhizosphere, thus enabling the plants to accumulate incremented doses of water and soluble nutrients, subsequently improving the plants' biomass (Yang et al., 2014). Calvo et al. (2014) also reported that a stimulated photosynthesis system reflects an enhanced food factory, which might have influenced the height and root length consequently in all the tested plants. Interestingly, root length was significantly influenced due to the aqueous garlic extract application. Conversely, Danso et al. (in press) found reduced fresh leaves and dry root and leaves biomass in cocoa seedlings treated with 50–100 mg mL^{-1} leaf aqueous concentrations. Hussain et al. (2020) reported that the effects of water-soluble allelochemicals appear to alter a variety of morpho-physiological functions and significantly reduced the leaf fresh and dry biomass. S. Zhang et al. (2020) showed that allelochemicals (isoliquiritigenin) in concentrations ranging between 0.2 and 1.0 mM exhibited toxic impacts that decreased the lettuce seedling growth and plant biomass.

5 | CONCLUSION

The stem bark extracts of common cocoa shade tree species contained uncharacterized allelochemicals that affected the early growth and development of cocoa seedlings. While there were no significant interactions between tree species and extract concentrations on seedling height or stem diameter, tree species and extract concentrations individually had significant impacts on plant height from 90 to 150 days after application. *Albizia ferruginea*, *C. mildbraedii*, and *T. scleroxylon* had the highest plant heights. *Albizia ferruginea* also had the highest stem diameter among tree species and the control. The chlorophyll content of cocoa seedlings was not significantly influenced by the interaction between bark extract concentration and shade tree or by tree species alone. However, the interaction between tree species and bark extract concentrations increased fresh root weights and the dry plant biomass. *Albizia ferruginea* consistently increased dry plant biomass, while *C. mildbraedii* had the highest allelopathy index for fresh roots. Aqueous bark concentrations promoted dry weight, with the 75 mg mL^{-1} concentration consistently producing the highest fresh and dry plant biomass. The study recommends that *A. ferruginea* and *C. mildbraedii* can be potential tree species for cocoa agroforestry when transplanting two-month-old cocoa seedlings. Additionally, the bark extract at a concentration of 75 mg mL^{-1} can be used

as a growth stimulant for young cocoa seedlings in cocoa plantation establishment.

AUTHOR CONTRIBUTIONS

Jacob Danso: Conceptualization; formal analysis; methodology; writing—original draft. **Isaac Kankam Boadu**: Supervision; writing—review and editing. **Joseph Sarkodie-Addo**: Supervision; writing—review and editing. **Michael O. Opoku-Agyeman**: Conceptualization; writing—review and editing. **Francis Padi**: Conceptualization. **Jacob Ulzen**: Writing—review and editing. **Alfred Arthur**: Conceptualization; supervision.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data belong to Cocoa Research Institute of Ghana and are available from the corresponding author upon reasonable request.

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