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Research Article

Assessment of Allelopathy Effects of Agroforestry Trees Leachates on Seed Germination and Growth of *Vigna unguiculata L.Walp*

Sobola, O.O.,[1] Maiguru, A.A.,[1] Olusola, J.A.[2] and Mch-mcha, B.D.[1]

[1]Department of Forestry and Wildlife Management, Federal University of Wukari, P.M.B. 1020, Taraba State Nigeria.
[2]Department of Forestry Technology, Federal College of Agriculture, Akure, Nigeria

Abstract

Agroforestry is an approach to sustainable land management that integrates trees with crops and/or animals. Sensitivity of understory crops to allelochemicals produced by woody perennials required careful evaluation of which species can be effectively incorporated into *Vigna unguiculata L* models. This study aimed at investigating the allelopathic potentials of *Eucalyptus camaldulensis, Albizia lebbeck*, and *Gmelina arborea* on germination and growth behavior of *Vigna unguiculata L.* Fresh leaves of healthy and mature plants were collected from the mother trees, and were used in the preparation of aqueous extract using distilled water. Germination and growth rate experiments were laid in a completely randomized design (CRD). The germination experiment lasted for 14 days under partial shade while the growth rate was monitored for 45days to examine the effect of the aqueous extract on germination of *Vigna unguiculata L*. The species leaves extract were tested for the presence of allelochemicals such as phenolic, alkaloid, and flavonoid using standard laboratory techniques. Findings from this research showed 98% germination for seeds administered with *Albizia lebbeck* leachates and the control (water), while the least 90% was observed in seeds treated with *Eucalyptus camaldulensis* leachate. The highest stimulatory effect (+1.02) was observed in *Albizia lebbeck* leaf leachates, neutral effect was observed in the control while *Eucalyptus camaldulensis* leave leachates had inhibitory effect (-7.77) on germination. *Albizia lebbeck* proved to be an excellent species that could be incorporated in agroforestry system owing to its stimulatory effect on the test crop. It is recommended that the use of *Albizia lebbeck* should be encouraged in agroforestry system while *Eucalyptus* and *Gmelina* should only be considered for plantation establishment or restoration of the degraded forest landscape.

Keywords: *Allelopathy, agroforestry, germination, phenolic, stimulatory.*

Introduction

Allelopathy has been described as any direct or indirect harmful or beneficial effect of one plant on another through the production of chemicals (allelochemicals) which may be phytotoxic substances that can influence the growth, survival, development, and reproduction of other organisms (Cheng and Cheng, 2015; Abdullahi. *et al.*, 2017). These allelochemicals are bio-substances such as phenolic, terpenoids, alkaloids, and their derivatives which can have promotion effects (positive allelopathy) or detrimental (negative allelopathy) effects on the targeted organism and the community (Stephen, 2018). Studies have shown that certain plants can favorably or unfavorably affect other plants through release of chemicals into the environment, such substances were named allelochemicals (Inderjit and Weston, 2003; Stephen, 2018). Activities of allelochemicals have been seen to influence germination rate, shoot/root growth, and nutrient uptake of other plants. These effects could be a reduction in the yield of various crops as well as plays an

***Corresponding Author:**
Sobola, O.O;

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appreciable role in the distribution of vegetation under and around (weed inhibition) the allelopathic tree (Ahmed. *et al*., 2008; Uddin. *et al.,* 2003; Sahoo. *et al*., 2010).

Biochemical substances are released by plant leaching, root exudation, volatilization, residue decomposition, and other processes in both natural and agricultural systems (Kruse. *et al*., 2000; Stephen, 2018). Studies have shown the allelopathic substances of the different parts of plant species can affect different plant organs in different ways. The basic model applied in allelopathic research for crops has been to screen for both crop plants and trees for their capacity to suppress weeds (Scavo. *et al.,* 2018). In the laboratory, plant extract and leachates are usually screened for their effects on seed germination and seedling growth. This is followed by the isolation and identification of the active allelochemical compounds from greenhouse tests and field soil to confirm the observed data of the laboratory experiment (Hussain. *et al.,* 2021). However, the interaction of plants and trees through chemical signals 'allelopathy' has many agricultural and ecological applications. Studies have shown possible strategic uses of allelopathic trees in forest restoration models to improve restoration in areas where invasive weeds inhibit restoration success (Cummings. *et al*., 2012). In addition, allelopathy may contribute to invasion resistance of native communities through the release of allelochemicals which could impede invasion of introduced plants or weeds (Weidenhamer and Romeo, 2005). Previous works (Hussain and Reigosa, 2014; Ali. *et al*., 2017; Macias. *et al*., 2019; Farooq. *et al*., 2020) have suggested that allelopathy holds great prospects for finding alternative strategies for weed management. Thereby, the reliance on traditional herbicides in crop production can be reduced.

However, allelopathy effects of agroforestry trees have always been considered as one of the main factors for the poor performance of associated companion crops (Stephen, 2018). Different plant parts, including flowers, leaves, leaf litter, and leaf mulch, stems, bark, roots, soil, and soil leachates and their derived compounds, can have allelopathy activity that varies over a growing season, as well as their effect on soil and neighboring plants. Recently in Nigeria, many farmers have found suitable the use of exotic species such as *Eucalyptus camaldulensis, Gmelina arborea, Tectona grandis* in agroforestry landscape without adequate knowledge of their allelopathic effects on the surrounding crops. Since the major aim of agroforestry system is crop productivity there is need to certify the suitability of the various component of the system for maximum productivity The present species of study; *Eucalyptus camaldulensis, Albizia lebbeck, Gmelina arborea* have characteristic chemical compounds of significance that need to be researched, to certify their suitability in agroforestry system. *This research* gap on the *Eucalyptus camaldulensis*, *Albizia lebbeck*, and *Gmelina arborea* on crops, especially on *Vigna unguiculata* needs to be bridged. Hence, the present study seeks to determine the allelopathic effects of Agroforestry tree leachate on seed germination and seedlings' growth of *Vigna unguiculata*.

Materials and Methods

Location of study

This study was carried out in Taraba State, Nigeria and the State lies roughly between latitude $6^0$30′ and $9^0$30′N, and longitude $9^0$10′ to $11^0$5′E. It is bounded on the north by Bauchi State and Gombe State in the north-east, Adamawa State in the East, by Plateau State in the north-west. The State is further bounded to the west by both Nasarawa and Benue State, while it shares an international boundary with the Republic of Cameroon to the south and south-east (Sobola. *et al*., 2021; Adebayo and Orunoye, 2013). The State comprises four agro-ecological zones; Guinea savannah, Sudan savannah, mountain regions as well as rain forest eco-system (Sobola. *et al*., 2021). The location of Taraba State makes it strategic for agricultural activities of diverse dimensions due to the abundant resources in the area.

Collection and Preparation of Aqueous Leachate from Leaves

Leaves of field-grown, healthy and mature plant were collected separately from *Albizia lebbeck, Eucalyptus camaldulensis*, and *Gmelina arboreas'* mother tree on the filed, from their natural occurrence in the vicinity of Federal University Wukari, Taraba State. Leaf leachate of the each tree species was prepared using distilled water. The leaves leachate was filtered using ordinary Whatman filter paper (NO.1) and the freshly prepared leachates were stored in a dark condition at room temperature until it was used for the study using Cowpea seeds in a bioassay until maximum germination was achieved. Determination of concentration of allelochemicals such as alkaloid, phenol, and flavonoid present in the leaves was done using standard laboratory techniques.

Bioassay Experiment

Twenty seeds of Cowpea were sown in trays filled with topsoil, watering of the sowing media was done with 200mls of leaf leachates for seven days before sowing for each of the treatments. 100mls of leaf leachates from the test species (treatments) were applied to the seeds on alternate days, while the control experiment were moistened with 100mls of ordinary water. All the trays were kept under partial shade in the nursery. The Seeds were directly sown in well-labeled germination trays arranged in a completely randomized block design. Data on daily emergence were taken to assess the germination and growth rate. Germination percentage as well as speed of germination were calculated using Equation i and ii).

$$\% \text{ of germination} = \frac{\text{No. of seed germinated}}{\text{No. of seed planted}} \times 100 \text{ ----------------------Eqn. i}$$

$$\text{Germination Speed} = \sum_{i=1}^{n} \frac{n}{t} \text{ --Eqn. ii}$$

Where n= number of newly germinated seed at time t

t = number of days since sowing

The Magnitude of Inhibition and Stimulation in the Bioassay

The magnitude of inhibition and stimulation in the bioassay were studied using the response index (R.I) described by Williamson and Richardson (1988) and R.I. was determined as follows.

$$I = 100 - \frac{C}{T} \times 100$$

Where I the % inhibition

C is the control

T is the treatment

T > C then RI = 1 - (C/T) ---Eqn. iii

T= C then RI = 0 --Eqn. iv

T < C then RI = (T/C)-1 --Eqn. v

C = Control

T= Treatment

Experimental Design

The experiment was laid out in a completely randomized design (CRD) with three replicates.

Experimental Model

Yij = u + Tj + Eij

Yij = Individual Observation

U = General Mean

Tj = Effect of Treatments

Eij = Experimental error containing All uncontrolled sources of variation

Data Analysis

The data was analysed using SPSS Statistical package (version 20). One-way ANOVA was used to compare the seed germination and early growth characteristics of seedlings under the various treatments. Treatments means found to differ significantly were separated using Duncan multiple Range Test.

Results

The germination response of Cowpea (*Vigna unguiculataL.* Walp) administered with the leaf leachates of *Eucalyptus camaldulensis, Gmelina arborea, Albizia lebbeck, and* control are shown in figure 1 Germination commenced on the fifth day (5th day) for all three treatments used for this research including control. However, the cumulative germination result revealed that the highest (98%) cumulative germination percentage was recorded under the seeds treated with the leaf leachates of *Albizia lebbeck* and the control. This was followed by seeds treated with *Gmelina arborea* (95%) and the least (90%) was recorded in seeds administered with *Eucalyptus camaldulensis leachate*. The speed of germination clearly shows that seeds subjected to *Albizia lebbeck* leachates have the highest (5.02) germination speed, followed by *Eucalyptus camaldulensis* and *Gmelina arborea* simultaneously with (3.01) the least was recorded under control (figure 2). Although there were variations in the cumulative germination percentage under the various treatments in this study, statistically, there were no significant difference (P > 0.05) in the germination of cowpea under the treatments (table 3). The Inhibitory and stimulatory effects of leaf leachates on the germination of cowpea seeds were determined from the response index calculation as described by Williams and Richardson (1988). The results shows that *Eucalyptus camaldulensis* leave leachates had inhibitory effect on the germination of cowpea seeds in the experiment with a response index of (-7.77) while response index of (-2.11) was obtained for *Gmelina arborea.* However, *Albizia lebbeck* leaf leachates had a stimulatory effect of (+1.02) on the seeds germination. The cowpea seeds administered with ordinary water (control) had a neutral effect on the germination of cowpea *(Vigna unguiculata)* seeds as shown in Table1.

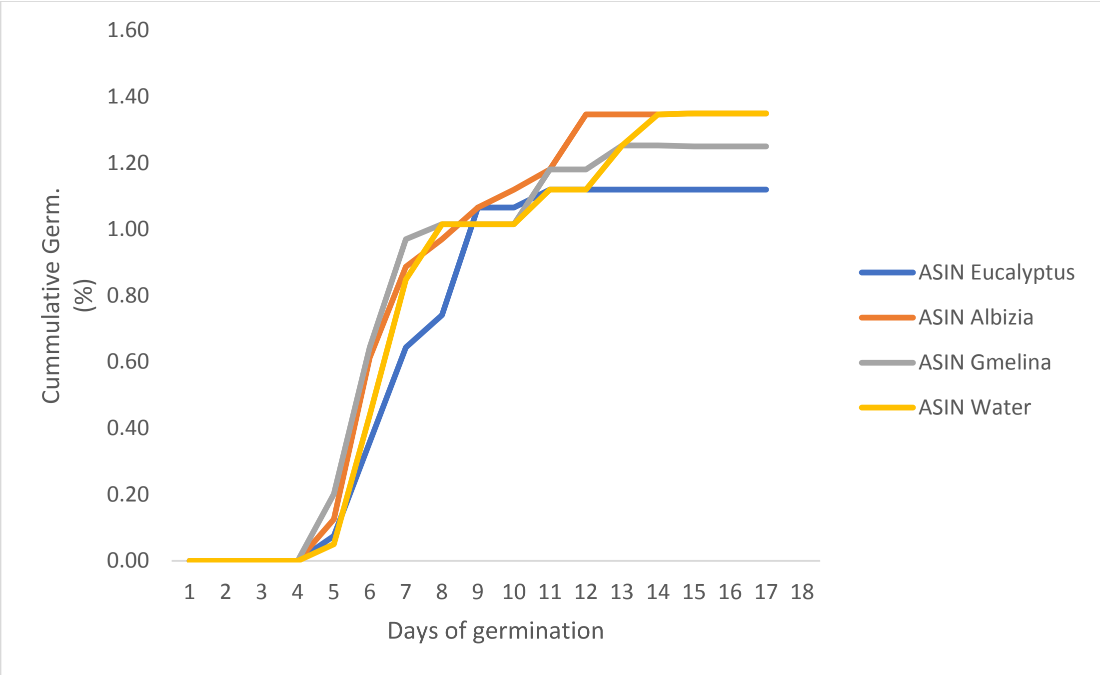

Figure 1: Cumulative Germination of Cowpea as affected by trees leave leachates

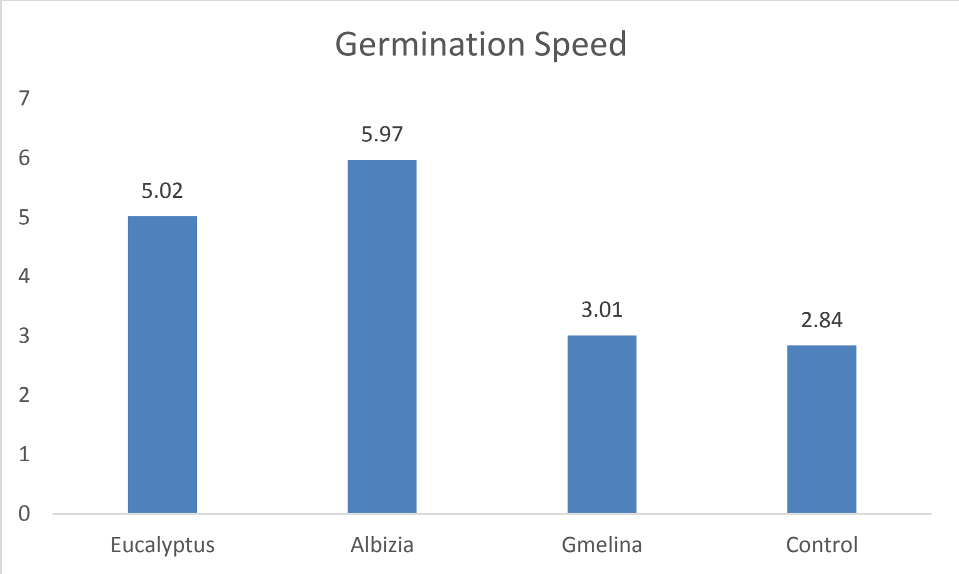

Figure 2: Germination speed of Cowpea (*Vigna unguiculata L. Walp*)

Table 1: Inhibitory and Stimulatory Effect of Leaf Leachates on Germination of Cowpea Seeds

Treatments	Seed Germination (%)	Response index (RI)	Effects
Eucalyptus	90	-7.77	Inhibitory
Albizia	98	+1.02	Stimulatory
Gmelina	95	-2.11	Inhibitory
Water (Control)	97	0	Neutral

Effect of Leaf Leachates on the Growth rate of Cowpea Seedlings

The effect of leave leachates on the growth rate of Cowpea is shown in Figures 3, 4, and 5. A significant effect was observed in the plumule length from the three treatments. Plumule length was 56,17cm, 54.32cm, 53.22cm, 49.21cm, and 44.48 for control, *Albizia lebbeck, Gmelina arborea*, and *Eucalyptus camaldulensis*. However, there was no significant difference (p<0.05) in the plumule length of the cowpea seedlings under the control, *Albizia lebbeck* and *Gmelina arborea* treatments but was significantly higher than those treated with leaf leachates of *Eucalyptus camaldulensis* (Table 1). Radicle length ranged from 8.1cm to 9.38cm. *Eucalyptus camaldulensis* had the highest radicle length of (9.38), followed by control (8.6) and the least 8.1cm was recorded under *Gmelina arborea*. Statistical analysis revealed that the effect of leave leachate was significantly higher (p>0.05) in *Eucalyptus camaldulensis* treatment than other treatments. Seedlings administered with leachate of *Albizia lebbeck*, control, *Gmelina arborea* had similar branching characteristics (4) but were better than that of *Eucalyptus camaldulensi* that had just (2). The number of leaves were not significantly different (p>0.05) for treatments with *Albizia lebbeck*, *Eucalyptus camaldulensis*, and control but was significantly higher than seedling treated with *Gmelina arborea* leachates.

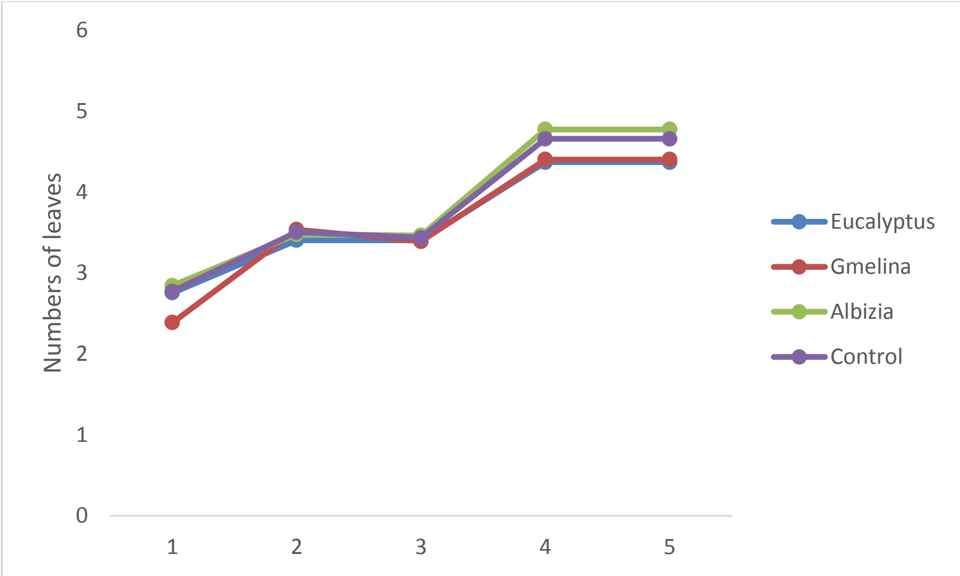

Figure 3: Effect of leave leachates on the number of leaves of Cowpea seedlings

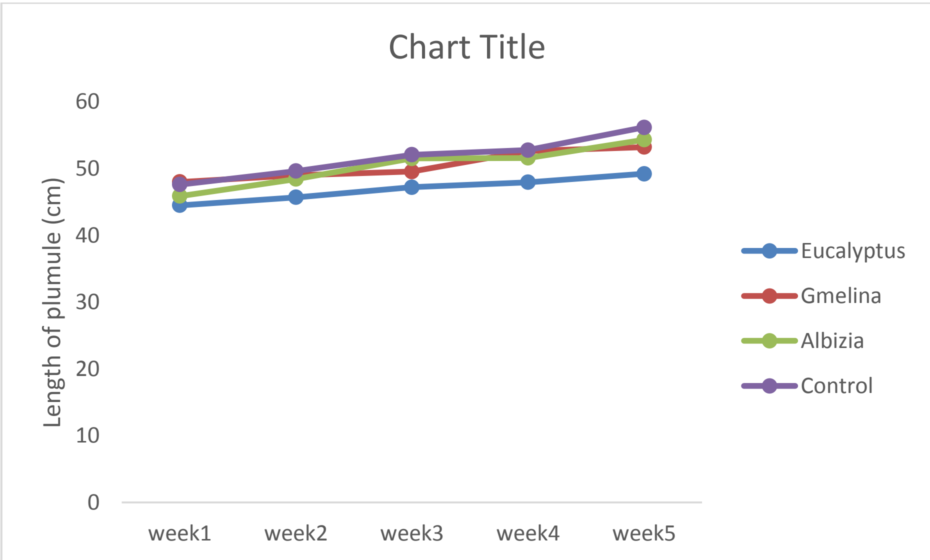

Figure 4: Effect of leave leachates on the plumule length of Cowpea seedlings

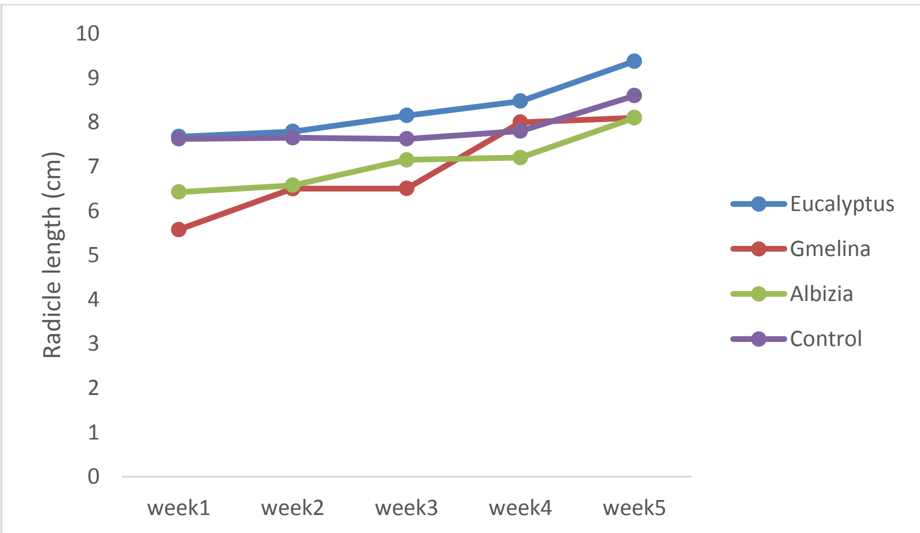

Figure 5: Effect of tree species leave leachate on radicle length of cowpea seedlings

Table 2: Effect of the tree leave leachates on the Germination and growth characteristics of cowpea

Parameters	Eucalyptus leachate	Gmelina leachate	Albizia leachate	Control
Germination%	1.59a	1.58a	1.77a	1.60a
Plumule length	44.48b	49.55a	51.58a	52.07a
Radicle length	9.15a	6.53c	7.24bc	7.87b
Number of branches	2.20b	3.50a	3.7a	3.60a
Number of leaves	7.60a	5.70b	8.10a	7.70a

Value: in the same column with the same superscript shows there is no significant difference ($p>0.5$)

Effect of leave leachates on early growth of the tree species as affected by Phytochemical Composition

The magnitude of the inhibitory and stimulatory effect of the leaf leachates on the early growth performance of cowpea seedlings from the germinated seed is presented in table 3, the result shows that both *Eucalyptus camaldulensis* and *Gmelina aborea* inhibited the growth characteristic of cowpea while *Albizia lebbeck*, majorly stimulated the early growth of the test crop i.e radicle length, number of branches, number of leave and germination (%) were stimulated. However, the inhibitory and stimulatory effect of leaf leachates on *Vigna unguiculata* under *Eucalyptus camaldulensis* shows variation in the level of inhibition; the inhibitory effect of (-1.32) was recorded in the number of leaves, % Germination (-7.77), radicle length (- 8.70) and plumule length (-17.06). With leachate from *Gmelina arborea,* inhibition of the number of leaves was (-35.09) and radicle length was inhibited by (-20.52). However, under *Albizia lebbeck* leachate treatment the radicle length was stimulated (+13.99), followed by the number of leaves (+4.90) and the least was the germination percentage (+1.02), and number of branches (+2.70). Meanwhile, the Plumule length was neutral (± 0.95).

The phytochemical compositions of the leaves were presented in Table 3. The three species were tested for the presence of allelochemicals as well as their concentration. The three selected tree species were tested for, alkaloid, phenol, and flavonoid which are known to be associated with allelopathic interactions. The result in Table 6 revealed that *Eucalyptus camaldulensis* had the highest percentage of alkaloid and flavonoid followed by *Albizia lebbeck* while *Gmelina arborea* was high in phenol, and there is a significant difference among the mean values ($p<0.05$).

Table 3: Inhibitory and Stimulatory Effects of Leaf Leachates *Eucalyptus*, *Gmelina*, and *Albizia* on *Vigna unguiculata*

	Germination (%)	Plumule length	Radicle length	Number of branches	Number of leaves
Eucalyptus	-7.77	-17.06	- 8.70	-63.64	-1.32
Gmelina	-2.11	-5.09	- 20.52	-2.86	-35.09
Albizia	+1.02	±0.95	+13.99	+2.70	+4.90
Control water)	±0				

Note: values with a negative sign (-) shows inhibitory effect and values with + sign shows stimulatory effect while values with (±) show the neutral effect on the crop

Table 4: Leaf Phytochemical Composition of the Three Selected Tree Species

Phytochemical Content			
Tree Species	Alkaloid (%)	Phenol (%)	Flavonoid (%)
Gmelina arborea	50.0[c]	71.36[a]	11.20[c]
Albizia lebbeck	61.60[ab]	11.41[b]	13.27[b]
Eucalyptus camaldulensis	74.40[a]	1.37[c]	15.20[a]

Source: Laboratory Analysis (2021)

Discussion

Cowpea is an economically important legume grown in the savanna and semi-arid tropics, mostly by small-scale farmers. It is well known and appreciated for its many useful values and nutritional composition (Odendo. *et al*., 2011; Kebede and Bekeko, 2020). It is a rich source of proteins and calories, as well as minerals and vitamin C. A cowpea seed consists of 25% protein and is low in anti-nutritional factors (Range. *et al.*, 2003). In recent times farmers have been interested in intercropping cowpea with perennial trees in the savanna region of Nigeria, hence the need to understand the allelochemical effect of possible tree species for productive cowpea agroforestry. The fact that the germination of the test crop commenced on the same day 5th day, shows the presence of appropriate environmental and edaphic conditions suitable for seed germination.

The result revealed variations in the germination behavior of the cowpea seed administered with leaf leachates from the three tree species.

The higher germination rate and stimulatory effects observed for cowpea seeds administered with *Albizia lebbeck* leachate show that the level of toxic allelochemicals present in *Albizia lebbeck* is very low and could stimulate germination and growth of crops. This agrees with the findings of Abdullahi. *et al*., (2017) who demonstrated that at a low level of toxins there is little or no inhibition on growth. The result also corroborates the findings of Oyebamiji. *et al*., (2017) that maximum production of crops was observed in agroforestry plots with *Albizia lebbeck* as the perennial component. *Albizia lebbeck* is known for soil fertility improvement through soil moisture conservation and increased nitrogen and organic matter composition of the soil (Kareem, 2017). On the other hand, the lower germination rate and inhibitory effects observed for cowpea seed administered with leachates of *Eucalyptus camaldulensis, Gmelina arborea* compared to the control have been reported in existing literature (Madhan. *et al.*, 2014; Madhan, 2014; Muhammad. *et al*., 2018) and is attributed to the presence of bioactive compounds such as tannins, alkaloid, polyphenols, saponins and terpenoids in the leaf extract which work to inhibit the expression and activity of the enzymes required for efficient seed germination.

The inhibitory effect of *Gmelina arborea* and *Eucalyptus camaldulensis* in this study may be due to the presence of secondary compounds such as phenols, benzoic, and cenamic acids derivatives, flavonoids, tannins, terpenoids, alkaloids, and polyacetylene, in the leaf extract which work to inhibit the expression and activity of the enzymes required for efficient seed germination. Studies have shown that as the concentration of toxins increases the effect becomes dominant and increases until the plant dies, or is highly inhibited in growth (Kruse and Strandberg 2000; Grover, 2008; Fikreyesus. *et al.,* 2011). The phenolic composition of *Gmelina arborea* in this study was very high, an indication that incorporating the species with crops could be detrimental to plant growth and subsequent reduction in crop productivity. Studies have shown that a high concentration of the phenolic compound in plants could have a negative allelopathic effect on the growth of neighboring crops or trees (John and Sarada, 2012). Another report by Hegab (2016), on seedling growth of wheat and maize, shows that it was reduced with treatment of *Eucalyptus rostrata* leaf leachates and subsequently growth parameter was generally reduced. Patil. *et al*., (2003) also reported the allelopathic effect of *Eucalyptus camaldulensis* on a Cowpeas (*Vigna unguiculata*), seed germination, and survival of germinated seedlings were also inhibited at closer *Eucalyptus* spacing.

Phytochemical Composition of the Leaf of the Three Selected Tree Species

Allelopathic inhibition is complex and can involve the interaction of different classes of chemicals like phenolic compounds, flavonoids, terpenoids, alkaloids, steroids, carbohydrates, and amino acids, with mixtures of different compounds sometimes having a greater allelopathic effect than individual compounds alone. Allelopathic chemicals can also persist in soil, affecting both neighboring plants as well as those foliar and leaf litter leachates of *Eucalyptus* species (Sasikumar. *et al*., 2002 and Stephen, 2018). Previous research on leaf essential oil of *Eucalyptus camaldulensis* species has shown that it is dominated by a-pinene (24.40%) and 8-cineole (24.26%), while that of *Gmelina arborea* is characterized by the abundance of (z)-3-hexenol (17.9%), 1-octen-3-ol (8.4%) and hexenol (6.1%), *albizia lebbeck* are naturally 100% extracts. These plants contains phenolic acids released from the leaves, bark, and roots that have deleterious effects on other plant species (Sasikumar. *et al*., 2002). The allelochemicals released include phenols, terpenoids, alkaloids, and their derivatives. They may inhibit shoot and root growth, nutrient uptake or may attack the naturally occurring symbiotic relationship thereby destroying the plant's usable source of the nutrient. Detrimental effects of allelochemicals from *Eucalyptus camaldulensis* species on plant germination and growth have been reported (Bogatek and Gniazdowska, 2006) The result obtained in this study agrees with the fact that the inhibitory effect on seedling growth is due to the release of different kinds of phytotoxic compounds such as; phenolics, sesquiterpenes, p-cymene, ascaridole, aritazone, and lactones, from the vegetative part of living plants as reported by Belz. *et al*., (2007).

Conclusion and Recommendation

Eucalyptus camaldulensis and Gmelina arborea leave leachates had inhibitory effect on the germination of cowpea seeds while stimulatory effect was recorded with the application of *Albizia lebbeck* leaf leachates. *Albizia lebbeck* is therefore recommended for incorporation in agroforestry system, due to the stimulatory effect recorded in this study. The species will be a suitable component for incorporation with cowpea in an agroforetry system because it results in the promotion of seed germination and growth rate of cowpea seedlings in this study. *Eucalyptus camaldulensis* could also be used with a suitable micro-zonal arrangement such as life fencing as the level of Phenolic compound is relatively low, which is one of the major allelochemicals involved in the inhibition of plant growth, However, further field investigation and laboratory work should be carried out to examine the effect of different concentration of this extract on cowpea and other agricultural crops which are of economic importance.

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