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Effect of aqueous extracts of the leaves of *Senna siamea* Lam. and *Pinus carribaea* (Jacq.) ex. Walp. on the germination and seedling growth of *Euphorbia heterophylla* L.

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ABSTRACT

The herbicidal potentials of the aqueous extracts of the leaves of *Senna siamea* and *Pinus carribaea* on the germination and seedling growth of *Euphorbia heterophylla* were investigated. The results showed that the leaf aqueous extracts of the two plants brought about considerable inhibition on the germination of *E. heterophylla* seeds and reduction in the radicle and plumule lengths of *E. heterophylla* seedlings. The degree of inhibition increased with increase in the concentration of the extracts, thus suggesting that the effect of the extracts were concentration dependent. The results obtained revealed that the inhibition was pronounced in both extracts such that no germination of *E. heterophylla* seeds and plumule occurred until 48hrs and 96hrs of the experiment respectively. It was apparent that *Senna siamea* extracts had more inhibitory effect on *E. heterophylla* seeds than those of *Pinus carribaea* treated seeds as no radicle growth was clearly observed in the *Senna siamea* treated seeds until 72hours experimental time whereas in *Pinus carribaea* treated seeds, radicle growth was observed at 48hours of the experiment. Statistical analysis ($P < 0.05$) revealed that significant differences were observed in the germination, radicle and plumule lengths of the two extracts treated seeds when compared to the control experiments. The inhibitory effects of the aqueous extracts derived from the leaves of *Senna siamea* and *Pinus carribaea* might be useful in the control of *E. heterophylla* in farmlands. Further studies should be carried out to ascertain the bio- herbicidal potentials of these plants.

***Keywords*:** Bio-herbicides, *Senna siamea*, *Pinus carribaea*, *Euphorbia heterophylla*, radicle, plumule

1. INTRODUCTION

Interference of weeds with crops on farmlands reduces the growth and yield of agricultural crops thereby resulted in competition between weeds and crops for light, water and other essential nutrients (Bhatt *et. al.*, 2010). Weeds in croplands resulted in economic loss, increasing cost of production and reduce quality of crops (Bhuler *et al*., 1998). Ahmed *et al*. (2007) reported weeds also reduce the soil fertility by absorbing nutrients particularly nitrogen.

There are several methods used in management of weeds such as manual and the use of synthetic herbicides. Manual control of weeds is slower and time consuming for the labourers whereas synthetic herbicides work faster than manual. Chuahan *et al*. (2015) reported that the unavailability of farm labourers and its high wage bill resulted in manual weeding being replaced with synthetic herbicides. Synthetic herbicides have negative effects on the environment causing autotoxicity, soil sickness or biological invasion. Overuse of herbicides cause development of herbicide- resistant weeds (Holethi *et al.*, 2008).

Considering the negative effects of synthetic herbicides, alternative methods of weed management that are cheaper, non- toxic to the environment should be considered. This involves the use of allelopathic plants. Allelopathy is a natural phenomenon by which plants produced and released allelochemicals which can stimulate or inhibit plant germination and growth and permit the development of crops with low phytotoxic residue amounts in water and soil, thus facilitating wastewater treatment and recycling (Macias *et al.*, 2003). Singh *et al*. (2006) referred to allelopathy as any direct or indirect effect of plant on other plants through the release of chemicals and play an important role in many agro- ecosystems (Kohli *et al*., 2001).

The allelochemicals are released from the leaves, flowers, seeds, stems and roots of living or decomposing materials (Weston, 1996; Inderjit, 1996; Kruse *et al*., 2000 and Bertin *et al.*,(2003) through the process of volatilization, leaching, root exudation and decay of plant residues (Chon *et. al.*, 2006), although the efficacy and specificity of many allelochemicals are limited(Bhadoria, 2011).

Allelopathic plants used in agriculture is currently being realised as compounds released from plant rotation, for intercropping , as cover crops or as green manure (Reeves *et al.*, 2005; Igbal *et al*., 2007; Wortman *et al*., 2013; Silva *et al.*, 2014; Haider *et al*., 2015). Cheng and Cheng (2015) noted that the sustainable application of allelopathy toward the improvement of crop productivity and environmental protection through environmental friendly control of weeds, insect pests, crop diseases, conservation of nitrogen in croplands and the synthesis of novel agrochemicals based allelochemicals has attracted much attention from scientific engaged in allelopathic research.

The ability of plants to suppress weeds can be determined by allelopathy and competitiveness. John *et al*. (2010) and Andrew *et al*. (2015) noted that the crop allelopathy can be effectively used to control weeds in the field. Bhowmik and Inderjit (2003) noted the effects of one plant on another may be either both stimulatory and inhibitory depends on the concentration of the released compounds. Ahmed *et al*. (2007) reported that the chemicals at lower concentration that inhibit the growth of different species, at higher concentration may be stimulating the growth of different species.

Kruse *et al*. (2000) and Chon *et al*. (2006) defined a situation when a given species produces and releases allelochemicals that can cause damage to a different plant species; this is called heterotoxicity whereas when its own germination and development is affected, thus the allelopathy is called autotoxicity.

Various researchers had reported on allelopathy of crops on crops (Queslati, 2003); crops on weeds (Om *et al*., 2002 and Ayeni *et al*., 2010); weeds on weeds (Bogatek *et al*., 2006 and Mengal *et al*., 2015); trees on crops (Sundara-moorthy *et al*. 1995; Ibrahim *et al*., 1999); weeds on crops (Singh *et al*.,2001 and Usuah *et al*., 2013) and trees on weeds (William and Hongland, 2007; Wu *et al*., 2011).

Among the plants that have allelopathic potentials in suppressing weeds include *Gliricidia sepium* and *Senna siamea* that inhibit the growth and development of several weeds. Husaain *et al*. (2007) reported that extracts of *C. siamea* affected the germination and seedling growth of weed species such as *Avena fatua, D. aegyptium, Echinocloacolona, Phalaris minor* and *Sorghum halepense*.

Linhares *et al*. (2009) noted that soil mulching with *G. sepium* branches does not have allelopathic effect on corn by decreases weed population. Similarly, Kamara (1995) had earlier noted that *G. sepium* mulch had no allelopathic effect on maize and beans but significantly decrease the population of some weed species.

Euphorbia heterophylla is a weed that is capable of growing on wastelands or disturbed grounds. It belongs to the Family Euphorbiaceae. It is an annual herb. Its seeds are dispersed by explosive mechanism (Water house, 1994) thereby causing large population of this weed on croplands by competing with crops on farmlands. This has resultant effect on providing foods for increasing population. This study is designed to evaluate the allelopathic potentials of *S. siamea* and *G. sepium* on the germination and seedling growth of *E. heterophylla*.

2. MATERIALS AND METHODS

The experiment was conducted in the laboratory of the Department of Plant Science and Biotechnology, Ekiti State University, Ado-Ekiti, Nigeria in August 2016 to examine the allelopathic potentials of the aqueous extracts of the leaves of *S. siamea* and *G. sepium* on the germination and seedling growth of *E. heterophylla*.

The leaves of *S. siamea* and *G. sepium* were obtained on campus of Ekiti State University, Ado-Ekiti. The samples were air- dried for three weeks and later chopped into pieces. Likewise, the seeds of *E. heterophylla* were obtained on campus of Ekiti State University, Ado- Ekiti.

Extract Preparation

Portions of 4 g, 8 g, 12 g, 16 g and 20 g each were measured out using G& G Electronic Top Loading Digital Balance JJ300Y China. Each portion was dispersed in 200 mL distilled water in 500 mL conical flasks. The extracts were shaken intermittently and left for 24 hrs. at 25 °C ±1 °C. The extracts were filtered with Whatman No 1 filter paper and the filtrates were used for the experiment. For each experiment, two layers of Whatman No 1. Filter (9 cm) were put in each petri dish. Ten seeds each of *E. heterophylla* were sown in the petri dishes and were replicated 5 times for each extract concentration.

The filter papers were moistened with 5ml each of the extract concentration using syringe and needle. Control experiments were set up whose filter papers were moistened daily with distilled water and were also replicated 5 times. All the petri dishes were arranged on germination tables at room temperature between 25 °C - 30 °C.

The seeds were considered germinated upon radicle emergence. The number of seed that germinated were counted and recorded. Also, the radicle and plumule growth were recorded for 7 days.

Data Analysis

The data obtained from the experiments were subjected to one- way Analysis of Variance (ANOVA, P< 0.05) and compared statistically to those obtained from the control experiments. Duncan Multiple Range Test (DMRT) was used as a follow up test to separate the means.

3. RESULTS AND DISCUSSIONS

% Seed Germination

The effect of aqueous leaf extracts of *S. siamea* on the % germination of *E. heterophylla* was shown in Table 1. It was observed that 5% of the seeds germinated in the control experiment and 4 g/ 200 mL at 48 hrs experimental time. At 168hrs experimental time, 27.50% of the seeds germinated in 4 g/ 200 mL concentration, those of 8, 12, 16 and 20 g/ 200 mL were 25%, 17.5%, 15% and 15% respectively. This showed that the effect of the extracts were concentration dependent. Statistical analysis (ANOVA, P < 0.05) revealed that significant differences were observed in the extract treated seeds when compared to the control experiment.

Table 2 shows the effect of the aqueous extracts from *P. carribae* leaves on the % germination of the seed of *E. heterophylla*. It was observed that at 48hrs experimental time, higher concentration of the extracts inhibited the germination of *E. heterophylla* seeds. At 168hrs, 40%germinated in the control experiment. 4 g/ 200 mL has 20% germination; those of 8, 12, 16 and 2 0g/ 200 mL had 20%, 15%, 12.50% and 2.50% respectively.

The results showed that the effect of the aqueous leaf extracts of *S. siamea and P. carribae* were concentration dependent on the germination of *E. heterophylla*. This result corroborated Sisodia and Siddiqui (2008, 2009) who noted that the inhibition effect of *Croton bonplandium* was found to increase with increase in the concentration of the aqueous extracts. Also, the result of this study was in accordance with the work of Wafaa (2014) who reported that the extracts from helotrope root and leaf solutions showed inhibitory effect on seed germination of two tested plant species.

Also Soltani *et al*. (2006) reported that aqueous extracts of *Thymus kotschyanus* had considerable inhibitory effects on the germination of *Bromusto mentellus* and *Trifolium repens*. Ayeni and Olabode (2016) reported that the aqueous extracts from the leaves and barks of *Azadirachta indica* reduced the germination of *B. pilosa*. Likewise Ayeni and Kayode (2013) noted that the germination of *E. heterophylla* seeds was inhibited with aqueous extracts of *Sorghum bicolor* stem and maize inflorescence respectively.

Table 1. Effects of Aqueous extracts of *Senna siamea* leaves on the % germination of seeds of *Euphorbia heterophylla*

Extracts/ 200 mL	Hours					
	48	72	96	120	144	168
0	5.00[a]	15.00[a]	30.00[a]	32.50[a]	37.50[a]	40.00[a]
4	5.00[a]	12.50[a]	22.50[a]	22.50[a]	25.00[a]	27.50[ab]
8	0.00[a]	12.50[a]	17.50[ab]	25.00[a]	25.00[a]	25.00[ab]
12	0.00[a]	12.50[a]	15.00[ab]	15.00[b]	15.00[b]	15.00[b]
16	0.00[a]	10.00[b]	15.00[ab]	15.00[b]	15.00[b]	15.00[b]
20	0.00[a]	7.50[b]	12.50[b]	15.00[b]	15.00[b]	15.00[b]

Means followed by the same letter within the column are not significantly different at (P<0.05)

Table 2. Effects of *Pinus carribae* leaves on the % germination of *Euphorbia heterophylla.*

Extracts/ 200 mL	Hours					
	48	72	96	120	144	168
0	5.00[a]	15.00[a]	30.00[a]	32.50[a]	37.50[a]	40.00[a]
4	5.00[a]	12.50[a]	15.00[a]	17.50[ab]	20.00[a]	20.00[ab]
8	2.50[a]	12.50[a]	12.50[a]	15.00[ab]	15.00[ab]	20.00[ab]
12	0.00[a]	7.50[ab]	12.50[a]	12.50[ab]	15.00[ab]	15.00[ab]
16	0.00[a]	5.00[b]	10.00[b]	10.00[ab]	10.00[ab]	12.50[ab]
20	0.00[a]	2.50[b]	2.50[c]	2.50b	2.50[b]	2.50[b]

Means followed by the same letter within the column are not significantly different at (P< 0.05)

Radicle length

The effect of aqueous extracts of the leaves of *S. siamea* on the radicle length (cm) of *E. heterophylla* was shown in Table 3. It was revealed that no radicle germination occurred in extract treated seeds until 72 hrs experimental time. At 168 hrs, control experiment had radicle length of 6.31 cm, those of 4, 8, 12, 16 and 20 g/200 mL had 4.67 cm, 4.54 cm, 4.38 cm, 4.36 cm and 3.48 cm respectively. The radicle growth reduced with increase in the concentration of the extracts.

Table 3. Effects of Aqueous extracts of *Senna siamea* leaves on the Radicle Length (cm) of *Euphorbia heterophylla*

Extracts/ 200 mL	Hours				
	72	96	120	144	168
0	0.60[a]	1.99[a]	3.87[a]	5.51[a]	6.31[a]
4	0.56[a]	1.33[b]	3.53[a]	4.59[a]	4.67[a]
8	0.28[b]	1.14[bc]	3.39[a]	4.26[a]	4.54[a]
12	0.15[c]	0.83[bc]	2.99[a]	4.26[a]	4.38[ab]
16	0.13[c]	0.76[c]	2.85[b]	3.88[b]	4.36[ab]
20	0.10[c]	0.73[c]	1.19[c]	2.95[b]	3.48[b]

Means followed by the same letter within the column are not significantly different at (P< 0.05)

Table 4. Effects of *Pinus carribae* leaves on the radicle Length of *Euphorbia heterophylla*

Extracts/ 200 mL	Hours					
	48	72	96	120	144	168
0	0.13[a]	0.60[a]	1.99[a]	3.87[a]	5.51[a]	6.31[a]
4	0.10[a]	0.55[a]	1.44[b]	3.60[a]	5.26[a]	5.98[a]
8	0.03[b]	0.50[a]	1.39[b]	3.54[a]	4.64[a]	5.60[a]
12	0.00[b]	0.35[bc]	1.11[bc]	3.16[b]	4.36[b]	5.49[a]
16	0.00[b]	0.35[bc]	1.10[bc]	1.45[c]	1.81[c]	2.10[b]
20	0.00[b]	0.20[c]	0.60[c]	1.36[c]	1.71[c]	1.85[b]

Means followed by the same letter within the column are not significantly different at (P<0.05)

The effect of aqueous extracts of *P. carribae* was shown in Table 4. Radicle germination of extract treated seeds occurred at 48 hrs in 4 g/ 200 mL. At 168 hrs experimental time, the radicle length of treated seeds decreased from 5.98 cm to 1.85 cm in 20 g/200 mL, thus indicating that the effects of the extracts were concentration dependent. It was also observed that *S. siamea* might inhibited the radicle length of *E. heterophylla* mostly as no radicle germination occurred until 72 hrs experimental time than those of *P. carribae* treated seeds that germinated early at 48 hrs experimental time. The results corroborated the work of Gulzar *et al.* (2014) who found that the root and shoot length of weed species decreased significantly when plants were exposed to increasing aqueous concentration *of Cassia sophera*.

Plumule length

The effects of aqueous extracts of *S. siamea* and *P. carribaea* on the plumule length of *E. heterophylla* were shown in Tables 5 and 6. The result showed that both extracts inhibited the plumule length of *E. heterophylla*. No germination of plumule occurred until 96 hrs experimental time. It was observed that extracts of *P. carribaea* inhibited the plumule length more than that of *S. siamea* having 0.73 cm in 4 g/ 200 mL which decreased to 0.23 cm in 20 g/ 200 mL at 168 hrs experimental time (Table 6). For *S. siamea* treated seeds, the plumule length decreased from 0.85 in 4 g/ 200 mL to 0.52 cm in 20 g/ 200 mL (Table 5). The results also suggested that the effects of the extracts were concentration dependent. This research lend credence to the work of Azza (2016) who also found that the leaves extracts of *R. stricta* inhibited the plumule lengths of *Viciafaba, Triticum aestivum* and *Hordeum vulgare*. Also Ogbe *et al*. (1994) had earlier noted that water extracts of allelopathic plants have more pronounced effects on the radicle than the plumule lengths. Previous researchers such as Tefera (2002); Yawer *et al*. (2007); Sisodia and Siddiqui (2009) and Khan *et al*. (2011) found that higher degree of inhibition occurred with the leaves extracts at higher concentrations.

The results indicated that aqueous extracts of the leaves of *S. siamea* and *P. carribaea* contained growth inhibitors that were capable of reducing the germination and growth of *E. heterophylla*. *S. siamea* leaves was reported to contain allelochemicals such as tannins, saponin, steroids (Ahmed- Alizaga and Olayanju, 2007; Ali-Smith, 2009 and Bukar *et al.* (2009). Similarly, Pine has been reported to contain terpenes (Asensio *et al.*, 2008), turpentine (Ormerio *et al*., 2008) and phenolic compounds (Mirov and Iloff, 1995; Fernandez *et al.*, 2009). These compounds might be responsible for the reduction in the growth *E. heterophylla* exhibited in this study.

Table 5. Effects of Aqueous extracts of *Senna siamea* leaves on the Plumule Length (cm) of *Euphorbia heterophylla*

Extracts/ 200 mL	Hours			
	96	120	144	168
0	0.41^{a}	0.56^{a}	0.81^{a}	0.89^{a}
4	0.35^{a}	0.54^{a}	0.81^{a}	0.85^{a}
8	0.23^{ab}	0.50^{a}	0.74^{a}	0.80^{a}
12	0.19^{b}	0.45^{ab}	0.66^{ab}	0.71^{ab}
16	0.15^{b}	0.38^{b}	0.64^{ab}	0.69^{ab}
20	0.10^{b}	0.30^{b}	0.38^{b}	0.52^{b}

Means followed by the same letter within the column are not significantly different at (P< 0.05)

Table 6. Effects of Aqueous extracts of *Pinus carribaea* leaves on the Plumule Length (cm) of *Euphorbia heterophylla*

Extracts/ 200 mL	Hours			
	96	120	144	168
0	0.41[a]	0.56[a]	0.81[a]	0.89[a]
4	0.35a	0.53[a]	0.64[ab]	0.73[ab]
8	0.30[a]	0.50[a]	0.54[ab]	0.69[ab]
12	0.25[b]	0.38[b]	0.50[ab]	0.64[ab]
16	0.25[b]	0.33[b]	0.34[b]	0.44[b]
20	0.10[c]	0.13[c]	0.15[c]	0.23[c]

Means followed by the same letter within the column are not significantly different at (P< 0.05)

4. CONCLUSION

The results showed that the leaf aqueous extracts of *S. siamea* and *P. carribaea* contained inhibitory substances that might be responsible for the germination and growth of *E. heterophylla*. It is suggested that aqueous extracts of the leaves of *S. siamea* and *P. carribae* could be used as bio-herbicides of weeds, most especially in the control of *E. heterophylla*. It is recommended that further studies should be carried out on the extracts to ascertain their allelopathic potentials.

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