DonnishJournals

Donnish Journal of Agricultural Research
Vol 3(3) pp. 017-021 August, 2016.
http://www.donnishjournals.org/djar
ISSN: 2984-8938

Original Research Paper

Bio-herbicidal Potential of the Aqueous Extracts of the Leaves and Barks of *Gliricidia sepium* (Jacq) Kunth Ex Walp on the Germination and Seedling Growth of *Bidens pilosa* L.

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Accepted 10th August, 2016.

The herbicidal potentials of aqueous extracts of the leaves and barks of *Gliricidia sepium* (Jacq.) Kunth ex Walp on *Bidens pilosa* were investigated. The extracts from leaf and bark residues of the tree inhibited the germination and seedling growth of *Bidens pilosa* L. The degree of inhibition demonstrated in both extracts was concentration dependent. However, the results obtained revealed that the inhibition was pronounced in both extracts derived from *G. sepium,* no germination and radicle length emerged until 72hrs experimental time. Similarly, the extracts retarded the plumule length of *B. pilosa*, no germination and radicle length emerged until 96hrs experimental time. The *G. sepium* bark extracts retarded the radicle length of *B. pilosa* at 168hrs experimental time, the control experiment had 1.08cm which reduced to 0.09cm in 15g/200ml concentration. In leaf extracts treated seeds, the radicle was 1.08 cm in the control experiment which reduced to 0.11cm in 15g/200ml at 168hrs experimental time. Statistical analysis (AVOVA, P<0.05) revealed that significant differences were observed in the germination, radicle and plumule lengths of the extract treated seeds when compared to the control experiments. The inhibitory effects of the aqueous extracts derived from leaves and barks of *G. sepium* might justify the usefulness of this plant in the eradication of *B. pilosa* on farmlands. Further research should be carried out to ascertain the potentials of the extracts as bio-herbicides.

Keywords: *Bidens pilosa*, *Gliricidia sepium*, Bio-herbicides, Aqueous extract, Inhibitory.

INTRODUCTION

The invasion of weeds on farmlands reduces crop yield and thus poses a major threat to food security to meet the increasing global population thereby hindering the millennium goal of food for all. Weed populations in fields had led farmers to the use of synthetic herbicides. Unfortunately, the synthetic herbicides and pesticides contaminate the soil, water and also affect soil microorganisms (Shequnoval *et al*., 2007; Ferencz and Balog, 2010; Anasco *et al*; 2010; Woudneh *et al*., 2009; Lang and Cai, 2009 and Evans *et al*., 2010). Gupta (2011) reported that the world consumption of herbicides is about 48% of the total manufactured pesticides in the world. Herbicides cause great damage to the environment if compared with other crops (Hozayn *et al*., 2015).

An alternative method of controlling weeds is now being advocated. This might involve the use of allelopathic plants which offers natural control of weeds. Patil (2007) refers to allelopathy as the inhibitory or stimulatory effects of one plant species on the other plant species in terms of germination, growth and development. Singh *et al*. (2006) noted that allelopathy is any direct or indirect effects of plants on other plants through the release of chemicals. Chemical compounds mostly secondary metabolites (Faroog *et al*., 2011a) called allelochemicals are released from the donor plants through leaves, seeds, flowers, rhizomes etc. into the surrounding through leachates, root exudates, volatilization and decomposition of plant residues (Rice, 1984).

Ahmed and Wardle (1994) noted that accumulation of allelochemicals causes toxicity affecting growth and final yield of crops, thereby influencing the growth and development of agricultural and biological ecosystem excluding mammals (Kruse *et al*., 2000, Weston, 2005). Duke (2015) noted that allelochemicals are generally weak phytotoxins that exert their effects at low but constant or increasing concentration over a long period. However, Hiradate (2006) and Hiradate *et al*.

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(2010) reported that whether a compound is involved in allelopathy is dependent on many things, including the level of phytotoxicity in soil and the amount produced by allelopathic plants.

Anwar *et al*. (2003) and Cheema *et al*. (2012) noted that allelopathic water application at lower concentration stimulates germination and growth of different crops. Many researchers such as Mattner (2006), Soyler *et al*. (2012), Jamil (2009), Cheema (2004) and Igbal *et al*. (2007) have reported on the inhibitory effects of allelopathic crops and weeds for weed management. Various aspects of allelopathy have been studied by many researchers such as effects of crop on weeds (Akemo *et al*., 2000; Khaliq *et al*. 2010), Weeds on weeds (Kohli *et al*., 1993), Jefferson and Bennacchio (2003), weeds on crops (Sisodia and Siddiqui, 2010; Ogbe *et al.*,1994) and microorganisms affect crops (Mandava, 1985) and trees on crops (Oyun, 2006). Allelopathy is a pragmatic substitute of synthetic herbicides as allelochemicals do not have residual toxic effects (Bhadoria, 2011). It is cost effective and eco-friendly

B. pilosa is a notorious weed commonly found on croplands and disturbed areas in south western Nigerian. It belongs to the Family Asteraceae. It is commonly called beggar's stick. It reduces crop yield on farmlands causing failure of agricultural crops to meet food demand of the growing population of the study area. The study being reported is designed to examine the herbicidal potentials of extracts from leaf and bark of *Azadirachta indica* on the growth of *Bidens pilosa*.

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 March 2014 to examine different concentrations of aqueous extracts of leaves and barks of *Gliricidia sepium* on the germination and seedling growth of *Bidens pilosa*. The leaves and barks of *Gliricidia sepium* were obtained on the campus of Ekiti State University, Ado-Ekiti. The samples were chopped into pieces and were air dried for two weeks and later ground to small particles. *Bidens pilosa* seeds were also obtained from Iworoko Ekiti in Irepodun/Ifelodun Local Government area of Ekiti State, a town located at about 1km from the University campus.

Extract Preparation

Portions of 3g, 6g, 9g, 12g, and 15g each of the powdered extracts from the leaves and barks were measured out using G and G Electric Top loading Digital Balance JJ 300Y China. Each portion was dispersed in 200mL distilled water in 500mL conical flasks. The mixtures were shaken intermittently and left for 24hours at 25˚C±1˚C. The extract of each plant residue was filtered with Whatman No 1 filter paper and the filtrates were used for the experiment.

In each treatment, two layers of Whatman No 1 filter paper (9cm) were put in each petri dish. Fifteen seeds each of the weeds were sown in the petri dish and were replicated four times for each extract concentration. The filter papers were moistened with 5mL each of the extract concentrations using syringe and needle. Control experiments were set up whose filter papers were moistened daily with distilled water and were also replicated four times. All the petri dishes were arranged on germination tables at room temperature between 25-30˚C.

RESULTS

% Seed Germination

The effects of aqueous extracts of the leaves and barks of *Gliricidia sepium* on the germination of the seeds of *Bidens pilosa* are shown in Tables 1 and 2 respectively. It was observed that the % germination of *Bidens pilosa* in the leaves and barks of extract treated seeds in the four treatments were retarded as no germination of the seeds of *Bidens pilosa* occurred until 72 hours experimental time (Tables 1 and 2). The effects of the extracts on the % germination (Tables 1 and 2) increased with increase in the concentration of the extracts.

The % germination of the seeds of *Bidens pilosa* seeds in *Gliricidia sepium* leaves extract treated seeds at 168 hours experimental time in control experiment was 63.33%, those of 3, 6, 9, 12, and 15g/200mL were 28.33%, 23.33%, 21.67.00%, 21.67% and 20% respectively. Likewise, the *G. sepium* barks inhibited the germination of *B. pilosa*. 15g concentration inhibited the germination of *B. pilosa* seeds mostly as no germination occurred until 144hrs experimental time. In *G. sepium* bark extract treated seeds, percentage germination at 168hrs experimental time in the control experiment was 63.33%, those of 3,6,9,12 and 15g/200mL were 25%, 25%, 21.67%, 21.67% and 15% respectively.

Statistical analyses (ANOVA $P<0.05$) revealed that there were significant differences in the % germination of extract treated seeds when compared to those of the untreated seeds of the control in all the treatments.

Radicle Length

The effects of aqueous extracts of the leaves and barks of *G. sepium* on the radicle length of *B. pilosa* are shown in Tables 3 and 4. It was revealed that both extracts had inhibitory effects on the germination of radicle as no radicle emerged until 72hrs experimental time in the extract treated seeds. The radicle length reduced with increase in the concentration of the extracts.

For the leaf extracts treated seed, the radicle length at 168hrs experimental time in the control experiment was 1.80cm; those of 3, 6,9,12 and 15g/200mL were 0.23cm, 0.22cm, 0.19cm, 0.13cm and 0.11cm respectively. These showed similar results to those of *G. sepium* bark extract treated seeds. Statistical analyses showed that significant differences were observed when the extract treated seeds in both treatments were compared to the control experiments.

Plumule Length

The effects of the aqueous extracts of leaves and barks of *G. sepium* on the plumule length of *B. pilosa* are shown in Tables 5 and 6 respectively. It was observed that both extracts inhibited the plumule growth. The degree of retardation was concentration dependent. No plumule emerged in extract treated seeds until 96hrs experimental time. The retardation of the plumule growth was more pronounced in the leaf extracts.

At 168hrs experimental time, the plumule length in the control experiment was 0.87cm. Those of 3g, 6g, 9g, 12g and 15g/200mL were 0.15cm, 0.14cm, 0.09cm, 0.06cm and 0.05cm respectively. Those of *G. sepium* bark treated seed reduced from 0.87cm in the control experiment to 0.08cm in 15g/200mL. Statistical analysis (ANOVA, $P < 0.05$) showed that there were significant differences in the extract treated seeds compared to the control experiments.

Table 1: Effects of aqueous extracts from *Gliricidia sepium* Leaves on the Germination of the seeds of *B. pilosa* L.

Extracts g/200ml	Time(hours)						
	24	48	72	96	120	144	168
0	6.67a	16.00a	28.33a	41.67a	51.67a	60.00a	63.33a
3	0.00b	0.00b	5.00b	11.67b	15.00b	21.67b	28.33b
6	0.00b	0.00b	5.00b	10.00b	11.67bc	18.33bc	23.33b
9	0.00b	0.00b	3.33b	8.33b	11.67bc	15.00bc	21.67b
12	0.00b	0.00b	0.00b	3.33cd	3.33c	11.67bc	21.67b
15	0.00b	0.00b	0.00b	0.00d	3.33c	10.00bc	20.00b

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

Table 2: Effects of aqueous extracts from *Gliricidia sepium* bark on the Germination of the seeds of *B. pilosa* L.

Extracts g/200ml	Time(hours)						
	24	48	72	96	120	144	168
0	6.67a	16.00a	28.33a	41.67a	51.67a	60.00a	63.33a
3	00.00b	0.00b	8.33b	11.67b	15.00b	20.00b	25.00b
6	0.00b	0.00b	5.00b	6.67bc	13.33b	18.33b	25.00b
9	0.00b	0.00b	0.00b	6.67bc	8.33bc	16.67b	21.67b
12	0.00b	0.00b	0.00b	5.00bc	6.67bc	13.33b	21.67b
15	0.00b	0.00b	0.00b	0.00c	0.0bc	11.67b	15.0b

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

Table 3: Effects of aqueous extracts from *Gliricidia sepium* leaves on the Radicle Length (cm) of *B. pilosa* L.

Extracts g/200ml	Time(hours)						
	24	48	72	96	120	144	168
0	0.07a	0.09a	0.20a	0.30a	0.56a	0.80a	1.80a
3	0.00b	0.00b	0.02b	0.04b	0.09b	0.17b	0.23b
6	0.00b	0.00b	0.02b	0.04b	0.08bc	0.15bc	0.22b
9	0.00b	0.00b	0.01b	0.03b	0.06bc	0.11bcd	0.19bc
12	0.00b	0.00b	0.00b	0.01b	0.03bc	0.07cd	0.13bc
15	0.00b	0.00b	0.00b	0.01b	0.01c	0.04d	0.11d

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

Table 4: Effects of aqueous extracts from *Gliricidia sepium* Barks on the Radicle Length (cm) of *B. pilosa* L.

Extracts g/200ml	Time(hours)						
	24	48	72	96	120	144	168
0	0.07a	0.09b	0.20a	0.30a	0.56a	0.80a	1.08a
3	0.00b	0.00b	0.02b	0.04b	0.09b	0.14b	0.24b
6	0.00b	0.00b	0.02b	0.04b	0.06b	0.12b	0.22b
9	0.00b	0.00b	0.01b	0.03b	0.05b	0.12b	0.17bc
12	0.00b	0.00b	0.00b	0.01b	0.03c	0.08bc	0.15bc
15	0.00b	0.00b	0.00b	0.00b	0.01c	0.03c	0.09c

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

Table 5: Effects of aqueous extracts from *Gliricidia sepium* leaves on the Plumule Length (cm) of *B. pilosa* L.

Extracts g/200ml	Time(hours)						
	24	48	72	96	120	144	168
0	0.01a	0.02a	0.09a	0.21a	0.33a	0.53a	0.87a
3	0.00b	0.00b	0.00b	0.01b	0.03b	0.07b	0.15b
6	0.00b	0.00b	0.00b	0.01b	0.02b	0.07b	0.14b
9	0.00b	0.00b	0.00b	0.00b	0.02b	0.05b	0.09b
12	0.00b	0.00b	0.00b	0.00b	0.01b	0.03b	0.06b
15	0.00b	0.00b	0.00b	0.00b	0.00b	0.02b	0.05b

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

Table 6: Effects of aqueous extracts from *Gliricidia sepium* bark on the Plumule Length (cm) of *B. pilosa* L.

Extracts g/200ml	Time(hours)						
	24	48	72	96	120	144	168
0	0.01a	0.02a	0.09a	0.21a	0.33a	0.53a	0.87a
3	0.00b	0.00b	0.00b	0.01b	0.02b	0.07b	0.13b
6	0.00b	0.00b	0.00b	0.01b	0.02b	0.05b	0.09b
9	0.00b	0.00b	0.00b	0.00b	0.00b	0.04b	0.08b
12	0.00b	0.00b	0.00b	0.00b	0.00b	0.01b	0.08b
15	0.00b	0.00b	0.00b	0.00b	0.00b	0.01b	0.08b

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

DISCUSSION

The aqueous extracts from the leaves and barks of *G. sepium* inhibited the germination of *Bidens pilosa*. The study corroborated the earlier assertion of (Alizera and Asaadi, 2010) who noted that seed germination, root and shoot lengths of weeds exhibited different degrees of inhibition according to the concentration of the aqueous extracts. Bisal *et al.* (1992) reported that Eucalyptus have harmful effects on germination and seedling growth of wheat, barley, Chickpea, mustard and many weeds. Likewise, Hollander *et al*. (2007) reported that germination of weed seeds may be inhibited by secretion of allelochemicals suppressing mechanism. Silva *et al*. (2009) reported that the *Gliricidia sepium* mulch has no allelopathic effect on corns or beans but significantly decrease the population of some weed species.

The reduction in the radicle length by the extracts might be as a result of allelochemicals released from the leaves and barks of *G. sepium* in this study. The result corroborated the work of (Oyun, 2006) who noted that *G. sepium* had inhibitory effects than *A*. *auriculiformis* thus decreased the seedling vigour of maize. Also (Salam *et al.*, 2009) noted that rice extracts of many crops contain growth inhibitory substances that limit the root and shoot growths of *E. crus-galli.* Ayeni *et al.* (2010) noted that the germination and growth of *B. pilosa* was inhibited by aqueous extracts of rice husk and sorghum stem respectively.

The aqueous extracts from the leaves and barks of *G. sepium* also reduced the plumule length of *B. pilosa* in this study. This lends credence to the work of (Sisodia and Sidiqui, 2010) who had earlier asserted that among the plant parts, leaves contained more allelopathic substances than the stem. Abu-Romman (2010) noted allelochemicals released to the surrounding might inhibit or retard root and coleoptile of plants. Also, Salam *et al.* (2009) noted that inhibitory effect of *G. sepium* and *A. auriculiformis* on the germination and seedling vigour of maize might be as a result of allelochemicals such as tannins, flavonoids and phenolic acids. These allelochemical might also be applicable to the inhibitory effects of the leaves and barks of *G. sepium* reported in this study.

CONCLUSION

The reduction in the germination and growth of *B. pilosa* might be as a result of allelochemicals present in the leaves and barks of *Gliricidia sepium*. Ramamurthy and Paliwal (1993) reported that *G. sepium* contains protocatechuic acid, gallic acid, vallinic acid, myricetin, ferulic acid and coumarin. Also, the inhibitory effect might be due to synergistic effects rather than single one (Menger, 1987). *G. sepium* might be used as mulch to the associated crops to suppress weed growth. Further studies should be carried out to ascertain the potentials of these extracts as bio-herbicides.

ACKNOWLEDGEMENTS

The author acknowledges the effort of Olabode Funmibi Victoria for collection of plant samples and experimental analysis.

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