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BIO- HERBICIDAL POTENTIALS OF THE AQUEOUS EXTRACTS OF THE LEAVES AND BARKS OF *AZADIRACHTA INDICA* A. JUSS ON THE GERMINATION AND SEEDLING GROWTH OF *BIDENS PILOSA* L

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ABSTRACT: *The bio-herbicidal potentials of aqueous extracts of the leaves and barks of Azadirachta indica (A. Juss.) were investigated. The extracts from leaf and bark of A. indica 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 more pronounced in the extracts derived from A. indica leaves, as no germination and radicle length emerged until 96hrs experimental time. Similarly, the radicle length in A. indica leaf extract treated seeds reduced from 0.17cm in 3g/200mL concentration to 0.07cm in 15g/200mL extract concentration at 168hrs experimental time. In A. indica bark treated seeds, the radicle length was 0.48cm in 3g/200mL which reduced to 0.17cm in 15mL/200g concentration at 168 hrs. Statistical analysis (ANOVA, P < 0.05) revealed that there were significant differences in the germination, growth of radicle and plumule lengths of the extracts treated seeds when compared to the control experiments. Results obtained on plumule growth were similar to those of radicle in both extracts. This tends to suggest that extracts from A. indica leaves and barks might be useful in the bio-eradication of B. pilosa. Further studies should be carried out to ascertain the potentials of the extracts as bio- herbicides.*

KEYWORDS: Azadirachta indica, Gliricidia sepium, herbicidal potentials, Bidens pilosa, aqueous extracts.

INTRODUCTION

Weeds have detrimental effects on agricultural crops. They compete with crops for light, nutrients and moisture, causing losses in grain yield and quality. Their invasion on croplands reduces the food security for the ever-growing population of this millennium thereby hindering the possibility of achieving desired goal of food for all. This had forced farmers to use synthetic herbicides in controlling weeds. The synthetic herbicides had been reported to affect the environment negatively. Mackinon and Freedman (1993) and Stephenson (2000) noted that most agricultural systems collectively use three million tonnes of herbicides per year.

Synthetic herbicides have also proved difficult to be procured by rural and resource poor farmers who are major stakeholders in agriculture. Quite often the synthetic herbicides are scarce and unavailable. Khalaj *et al*. (2013) noted that the continuous use of herbicides for weed control has created many problems including their persistence in soil, contamination of environment, crop injury, and increase in herbicide- resistant weed population among others. Abdullahi (2004) noted that weeds and weeds control have become major loss factors determining the economic profitability of crop production.

The need for weed control method that would be cheaper and readily available to farmers are advocated. This involves the use of plant extracts to control the menace of weeds. Belz (2007)

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and Kruse *et al*. (2000) suggested that the application of the allelopathic properties of some crops for weed management was desirable and thus enhance the possibility of reducing the application of expensive and pollutant synthetic herbicides.

Bidens pilosa L. is a tropical weed of the family Asteraceae commonly called beggar's stick. It is a notorious weed on farmlands and grows aggressively on disturbed land in the study area. The study being reported here aimed at determining the herbicidal potentials of extracts from leaf and bark of *Azadirachta indica* on the growth of *Bidens pilosa*.

MATERIALS AND METHODS

Study Area

The experiment was conducted in the laboratory of the Department of Plant Science, Ekiti State University, Ado – Ekiti, Nigeria in January 2014. The leaves and barks of *Azadirachta indica* were obtained on the campus of Ekiti State University, Ado- Ekiti. The samples were chopped into pieces and were air dried for three weeks and later ground to fine powders. *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 ground powders 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 extracts were 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.

The seeds were considered germinated upon radicle emergence and the number that germinated were counted and recorded for 168hours. The radicle and plumule growth elongations were recorded at 24 hours interval for 7 days. The data obtained from the experiments were compared statistically to those obtained from the control experiment, using Analysis of variance (ANOVA, $P<0.05$) with Duncan Multiple Range Test (DMRT) to separate the means.

RESULTS AND DISCUSSION

% Seed Germination

The effects of aqueous extracts from the leaves and barks of *Azadirachta indica* on the germination of the seeds of *Bidens pilosa* are shown in Tables 1and 2 respectively. It was observed that the % germination of *Bidens pilosa* extract treated seeds in the four treatments

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were retarded (Tables 1 and 2). No germination occurred until 96 hours experimental time (Tables 1 and 2). The effects of the extracts on the % germination increased with increase in the concentration of the extracts. The % germination of seeds of *Bidens pilosa* from the bark extracts of *Azadirachta indica* at 168 hours experimental time was 58.33% in the control experiment, those of 3, 6, 9, 12, and 15g/200ml extracts were 36.67%, 33.33%, 25%, 25% and 25% respectively (Table 2). Likewise the germination of seeds of *B. pilosa* in *A. indica* leaves extracts reduced from 30% in 3g/200ml to 13.33% in 15g/200ml

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 in the control experiment.

The study corroborated the earlier assertion of Seerjana *et al.* (2007) that the leaves aqueous extracts of *Parthenium hysterophorus* L exhibited significant inhibitory effects on seed germination and seedling growth of all test species in cruciferous plants. Similarly, Oyun (2006) reported that germination of maize seeds was significantly lowered by leachates from *Gliricidia sepium* and *Acacia aurilicuformis*. Ayeni and Kayode (2013) noted that maize root and rice husk extracts reduced the germination of *Bidens pilosa.* Previous study by Ashrafi *et al*. (2008) revealed that the greatest inhibition of the germination of lettuce seeds was observed in the water soluble fraction of extracts obtained from shoot of *Azadirachta indica*.

Radicle Length

The effects of the aqueous extracts from the leaves and barks of *Azadirachta indica* on the radicle lengths of *B. pilosa* are shown in Tables (3 and 4). The results showed that the extracts retarded the radicle length of *Bidens pilosa* seedlings.

The radicle length of *Bidens pilosa* seedlings were mostly retarded with *Azadirachta indica* leaf extracts (Table 3). At 168 hours experimental time, the radicle length in the control experiment was 1.01cm, those of 3, 6, 9, 12, and 15g/200mL were 0.17, 0.17, 0.14, 0.07, and 0.07cm respectively. Statistical analyses (ANOVA $P<0.05$) revealed that significant differences were observed in the radicle lengths of the extract treated seeds compared to the control experiment.

Plumule Length

The effects of aqueous extracts from the leaves and barks of *Azadirachta indica* on the plumule length of *B. pilosa* are shown in Tables (5 and 6). The results showed that the

Table 1: Effects of aqueous extracts from *A .indica* (A.Juss) Leaves on the Germination of the seeds of *B. pilosa* L.

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Extracts g/200ml	Time(hours)						
	24	48	72	96	120	144	168
0	7.53a	8.00a	13.33a	18.33a	40.00a	43.33a	58.33a
3	0.00b	0.00b	0.00b	1.67b	5.00b	16.67b	30.00b
6	0.00b	0.00b	0.00b	1.67b	1.67b	10.00b	21.67bc
9	0.00b	0.00b	0.00b	0.00b	1.67b	10.00b	18.33bc
12	0.00b	0.00b	0.00b	0.00b	1.67b	1.67c	15.00c
15	0.00b	0.00b	0.00b	0.00b	0.00b	1.67c	13.33c

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

Table 2. Effects of aqueous from *A.indica* (A.Juss) Barks on the Germination of the seeds of *B. pilosa* L.

Extracts g/200ml	Time(hours)						
	24	48	72	96	120	144	168
0	7.53a	8.00a	13.33a	18.33a	40.00a	43.33a	58.33a
3	0.00b	0.00b	0.00b	11.67ab	28.33b	33.33ab	36.67b
6	0.00b	0.00b	0.00b	11.67ab	18.33bc	23.33bc	33.33b
9	0.00b	0.00b	0.00b	5.00bc	11.67cd	16.67cd	25.00b
12	0.00b	0.00b	0.00b	3.33bc	6.67d	11.67cd	25.00b
15	0.00b	0.00b	0.00b	0.00b	3.33d	8.33d	25.00b

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

Table3. Effects of aqueous extracts from *A. indica* leaves on the radicle length (cm) of *B.pilosa* L.

Extracts g/200ml	Time(hours)						
	24	48	72	96	120	144	168
0	0.03a	0.05a	0.12a	0.23a	0.53a	0.72a	1.01a
3	0.00b	0.00b	0.00b	0.02b	0.04a	0.08b	0.17b
6	0.00b	0.00b	0.00b	0.01b	0.02b	0.06b	0.17b
9	0.00b	0.00b	0.00b	0.00b	0.01b	0.06b	0.14b
12	0.00b	0.00b	0.00b	0.00b	0.01b	0.02b	0.07b
15	0.00b	0.00b	0.00b	0.00b	0.01b	0.01b	0.07b

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

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Table 4. Effects of aqueous extracts from *A. indica* leaves on the radicle length (cm) of *B.pilosa* L

Extracts g/200mL	Time(hours)						
	24	48	72	96	120	144	168
0	0.03a	0.05a	0.12a	0.23a	0.53a	0.72a	1.01a
3	0.00b	0.00b	0.00b	0.08b	0.30b	0.36b	0.48b
6	0.00b	0.00b	0.00b	0.07b	0.17bc	0.24bc	0.37b
9	0.00b	0.00b	0.00b	0.04b	0.07bc	0.15c	0.25b
12	0.00b	0.00b	0.00b	0.03b	0.07bc	0.11c	0.22b
15	0.00b	0.00b	0.00b	0.00b	0.05c	0.08c	0.17b

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

Table 5. Effects of aqueous extracts from *A. indica* leaves on the Plumule Length (cm) of *Bidens pilosa* L.

Extracts g/200ml	Time(hours)						
	24	48	72	96	120	144	168
0	0.00a	0.03a	0.09a	0.20a	0.28a	0.43a	0.57a
3	0.00a	0.00b	0.00b	0.00b	0.00b	0.02b	0.07b
6	0.00a	0.00b	0.00b	0.00b	0.00b	0.02b	0.07b
9	0.00a	0.00b	0.00b	0.00b	0.00b	0.01b	0.05b
12	0.00a	0.00b	0.00b	0.00b	0.00b	0.01b	0.04b
15	0.00a	0.00b	0.00b	0.00b	0.00b	0.00b	0.03b

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

Table 6. Effects of aqueous extracts from *A. indica* bark on the plumule length (cm) of *Bidens pilosa* L.

Extracts g/200ml	Time(hours)						
	24	48	72	96	120	144	168
0	0.00a	0.03a	0.09a	0.20a	0.28a	0.43a	0.57a
3	0.00a	0.00b	0.00b	0.08b	0.23ab	0.42b	0.51a
6	0.00a	0.00b	0.00b	0.07ab	0.23ab	0.35b	0.41b
9	0.00a	0.00b	0.00b	0.02b	0.05b	0.11b	0.17b
12	0.00a	0.00b	0.00b	0.02b	0.05b	0.07b	0.13b
15	0.00a	0.00b	0.00b	0.00b	0.03b	0.07b	0.10b

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

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plumule lengths of *Bidens pilosa* were also retarded by the extracts. The results suggested that *Azadirachta indica* leaves extract mostly retarded the plumule length of *Bidens pilosa* as no germination occurred until 144hours experimental time. Plumule length from *A. indica* leaves treated seeds at 168hours in the control experiment was 0.57cm, those 3, 6, 9, 12, and 15g/200mL were 0.07cm, 0.07cm, 0.05cm, 0.04cm, and 0.03cm respectively. Similarly, the control experiment from *A. indica* barks extract treated seeds at 168hours experimental time was 0.57cm, those of 3, 6, 9, 12, and 15g/200mL were 0.51cm, 0.41cm, 0.17cm, 0.13cm, and 0.10cm respectively.

The results corroborated the earlier report of Sisodia and Siddiqui (2010) who noted that radicle length, plumule length and dry weights of seedlings of test species were reduced significantly in response to varying concentrations of *C. bonplandianum* extracts. Singh and Sangeeta (1991) noted that foliar leachates of *Parthium hysterophorus* reduced root and shoot elongations of *Orza sativa* and wheat. Salam *et al*. (2009) reported that rice extracts contained growth inhibitory substances that limit the root and shoot growth of *E. crus-galli.*

The findings corroborated the work of Aisha *et al.* (2010) and Monica *et al*. (2011) who reported that the aqueous extracts of *Ascarum europaeum* L. inhibited the germination and growth of *Lycopersicum esculentum*. Abu – Romman (2010) noted that allelochemicals released to the surrounding might inhibit or retard root or radicle and shoot coleoptile of plants. The leaves of *A. indica* inhibited mostly the germination of radicle and plumule length of *B. pilosa* seeds. This is in accordance to the work of Tefera (2002) who reported that the inhibitory allelopathic impact of leaf was more powerful than other vegetative parts. Also, Sissodia and Siddiqui (2010) asserted that among the different plants parts, the leaves contained more allelopathic substances while the stems have the least. This might suggest the reason why *Bidens pilosa* seeds were more retarded by leaves extracts of *Azadirachta indica* than the other treatment.

CONCLUSION

The leaves and bark of *Azadirachta indica* contain some allelochemicals responsible for the inhibition exhibited on *Bidens pilosa* seeds in this study. According to Lale and Abdulrahman (1999), chemical compounds in seeds and leaves of *A. indica* included nimbin, nimbidin, salannin and Azadirachtin. These chemical compounds present in these plants might be responsible for the inhibition of *Bidens pilosa* L seeds in this study.

FURTHER RESEARCH

It is hereby recommended that further studies should be carried out to ascertain the potentials of the extracts from *A. indica* leaves and barks as bio- herbicides.

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