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ALLELOPATHIC EFFECT OF *Bidens Pilosa* ON SEED GERMINATION AND GROWTH OF *Amaranthus Dubius*

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ABSTRACT

Allelopathy is the inhibitory effect of one plant on another by releasing chemicals through leaching, root exudation and residue decomposition. Allelopathic inhibition involves the interaction of different classes of chemicals which affect germination, plant growth and development. Studies have been done showing that *Bidens pilosa* has allelopathic effects on maize, sorghum and lettuce but information on it's effect on *Amaranthus dubius* is lacking. *Amaranthus dubius* has become one of Kenya's most important vegetables. It's famous for its high protein content and medicinal properties. This study was done to investigate allelopathic effects of *B. pilosa* on germination and growth of *A. dubius*. Different concentrations of *B. pilosa* extract were prepared (0%,25%, 50%, 75%, and 100%) using tap water as control . Twenty seeds were germinated in petri dishes lined with filter paper, then soaked with the aqueous extract solutions. The treatments were replicated four times and data was collected for two weeks. Growth tests were carried out in the greenhouse using 24 3Litre pots. Twenty seeds were planted in each pot and leaves of *B.pilosa* of different amounts (0g, 20g, 40g, 60g, 80g, and 100g) were added into the soil. Treatments were replicated four times and data collected for six weeks and subjected to Analysis of Variance using SAS package. Treatment means were compared using Least Significance Difference (P≤0.05). The aqueous extract of *B. pilosa* significantly reduced germination rate. Fresh leaf biomass of *B. pilosa* significantly increased shoot height, leaf number,and leaf area, fresh and dry weights. Leaf biomass of *B. pilosa* had no significant effect on root length and chlorophyll content. The study revealed that *B. pilosa* has potential use as green manure in crop production.

Contribution/ Originality: This study is one of very few studies which have investigated allelopathic effects of *Bidens pilosa* on germination and growth of *Amaranthus dubius*.

1. INTRODUCTION

Leafy vegetables are among the most commonly consumed food in the Kenyan daily diet. Among the various vegetables available to the Kenyan population, African indigenous vegetables are one of many peoples' favorites, also known as 'kienyeji'. These vegetables grew on their own as weeds, but they were later on domesticated by

researchers as a way of improving food supplies and nutrition [1]. African indigenous vegetables are also known for their medicinal uses apart from their high nutritional values [2].

Amaranthus, collectively known as amaranth consists of a genus of annual perennial plants, of 60-70 species [3]. Most of the species of Amaranthus are commonly referred to as pigweed [4]. *Amaranthus dubius* are cultivated mainly as leaf vegetables, but also it has some medicinal properties [5]. *A. dubius* leaves can also be used as fodder for animals [3]. Vegetable amaranths are the most widely eaten boiled greens throughout Africa's humid lowlands. They have exceptional protein quality making them useful supplements to cereals and root foods. The leaves are packed with vitamin A forming carotenoids, vitamin C, iron and calcium [6]. These plants are easy to produce and have a fast growth rate that they are ready for harvest two weeks after the onset of rainy seasons [7]. *A. dubius* also forms a crucial part in the rural economy as they are grown, harvested and sold close to the farmers' homes. The leaves make boiled vegetables with soft texture, mild flavor and no traces of bitterness [8]. *A. dubius* leaves are also boiled with other bitter vegetables such as *Cleome gynandra* to reduce the bitterness and improve the taste [7].

Bidens pilosa is an annual major weed in warm areas of the world and is also a serious weed in many farms. It is fast growing and very invasive. Invasive plant species may result into a number of ecological problems. Allelopathy is a process by which plants release toxic chemical compounds in their surroundings [9]. *Bidens pilosa* contains allelopathic substances which affect seed germination, plant growth and chlorophyll synthesis by plant leaves [10]. Its allelopathic effects are also useful in promoting its capacity in interspecific competition and its invasiveness [11].

According to Khanh, et al. [10] the bioassays and root exudates experiments it significantly suppressed the growth of test plants. Many secondary metabolites such as phenolics, polyacetylenes and triterpens which are involved in allelopathic action were found in *B. pilosa*. It exhibits allelopathic effect on numerous crops. *B. pilosa has* an inhibitory effect on other plants of between 70% to 90%. In Africa and many regions of the world *B. Pilosa* is used as a medicinal plant, cover crop and fodder [10]; [11].

Bidens pilosa and *Amaranthus dubius* grow together in the same environment as weeds. There have been other studies done on how allelochemicals found in *B. pilosa* affect the growth and germination of several plants such as maize, rice, sorghum etc. However, no study has yet been done on how *B. pilosa* affects growth of *A. dubius* since they occur in the same environment and they both equally have important economic and medicinal uses.

2. MATERIALS AND METHODS

2.1. Field Collection

Shoots of *Bidens pilosa* and seeds of *Amaranthus dubius* were collected from the Maseno University Botanic Garden and identified with reference to taxonomic keys [12]. The collected specimens were cleaned off before use.

2.2. Preparation of Extracts of *B. Pilosa*

One hundred and fifty grams of fresh shoots of *B. pilosa* were harvested at vegetative stage and cut into small pieces of about 4cm length. The small pieces were finely ground with pestle and mortar, and then soaked in 1 liter of tap water in a large beaker for 24hrs. The collected extracts were filtered through cheese cloth to remove debris and finally filtered using Whitman No. 1 filter paper to have 100% concentration. Aqueous extracts of 25%, 50%, 75% and 100% concentrations were made by diluting the original extract with distilled water according to procedure by Musyimi, et al. [9]

2.3. Germination Rate/Percentage

Germination tests were carried out in the Maseno University Microbiology Laboratory. Thirty uniform seeds were placed in dried Petri dishes lined with layers of Whatman No. 1 filter papers and moistened with 10ml of the respective aqueous extracts i.e. 25%, 50%, 75% and 100% and distilled water (control) according to procedure by

Musyimi, et al. [9]. The seeds and their respective treatments (aqueous extracts) were replicated four times. Data on seeds germinating each day were recorded and germination percentage calculated after two weeks of treatment.

2.4. Growth Experiment

Growth tests were carried out in the university greenhouse. Twenty four plastic pots were filled with 2kg of humus soils collected from Botanic garden. The soil were solarized (sun sterilized) for at least two days to prevent fungal growth. The pots were perforated at the bottom to avoid water logging. Twenty seeds of *A. dubius* were sown in each pot. Different amounts of fresh leaves of *B. pilosa* (20g, 40g, 60g, 80g, and 100g) were incorporated into the soil during sowing and each treatment was replicated in four pots. The control pots and the pots with *B. pilosa* leaves were all be supplied with 500ml tap water daily according to procedure by Bucki, et al. [13]

2.5. Determination of Shoots Length

Shoot length was measured from the soil level to the upper point of the terminal bud of the seedling using a meter rule, every week up to the end of the experiment according to procedure by Navidad [14].

2.6. Determination of Leaf Number

The number of mature leaves per plant was counted and recorded every week up to the end of the experiment according to procedure by Doku, et al. [15].

2.7. Determination of Root Length

Plants were uprooted and root length was measured from the soil level to the lower point of the longest root of the seedling using a meter rule at the end of the experiment according to procedure by Thomas [16].

2.8. Determination of Fresh and Dry Weight

At the end of the experiment, the plants were carefully uprooted from the soil, cleared off the attached soil, separated into root and shoot and then measured separately using an electronic weighing balance. Fresh plants (roots and shoots) were packaged separately in envelopes and dried to constant weight at 80^0c in an oven. Root and shoot dry weights were determined on an electronic weighing balance, and then mean weights calculated according to procedure by Riaz, et al. [17]

2.9. Determination of Leaf Area

Leaf area was measured on leaf number four from the top, on one plant from each pot, by placing a transparent ruler from the leaf apex to the leaf stalk, while the width was measured by placing the ruler at the center of the leaf blade and measurements read from one end of the leaf margin to the other margin. The leaf area was calculated using the following formula [18].

LA=0.5*L*W, Where: LA=Leaf area, L=Leaf length, W=Leaf breath

2.10. Determination of Chlorophyll Content

Measurement of chlorophyll content followed the procedure of Musyimi, et al. [9]. The fourth fully expanded leaf from shoot apex was sampled from all the treatments. 1g of these leaves were grounded in 20ml of 80 %(v/v) acetone using mortar and pestle. The resulting substrate was read at 664 and 647 nm using UV- Visible Spectrophotometer. Chlorophyll a, b and total chlorophyll concentration were calculated as follows:

Chlorophyll a= 13.19 A664-2.57 A647 (mg g^{-1} fresh weight).

Chlorophyll b= 22.1 A647- 5.26 A664 (mg g^{-1} fresh weight).

Total chlorophyll = 7.93 A664+ 19.53 A647 (mg g^{-1} fresh weight).

Where, A664 is the absorbance at 664nm A647 is the absorbance at 647 nm.

3. RESULTS

3.1. Germination Percentage

Table-1. Effect of *B. pilosa* extract on germination of *A. dubius* seeds

***B. pilosa* extract (%)**	**Germination percentage**
0	93.67a
25	63.17b
50	19.75c
75	5.25d
100	0.75e
LSD	2.91

Means with the same letters down the column are not significantly different at P≤0.05

Means with the same letters down the column are not significantly different at P≤0.05

There were significant differences (P≤0.05) in the germination percentage for all the treatments. The number of germinating seeds decreased with increase of extract concentration (plates, 2,3,4 and 5). Treatments with 100% extract had the lowest number of germinating seeds. The control had highest number of germinating seeds as presented in table (1) and plate 1. All treatments had an inhibitory effect on germination of *Amaranthus dubius* (plates,2,3,4 and 5).

Plate-1. Amaranthus seeds at day 10 treated with tap water (control)

Plate-2. Amaranthus seeds at day 10 treated with 25% *B. pilosa* extract

Plate-3. Amaranthus seeds at day 10 treated with 50% *B. pilosa* extract

Plate-4. Amaranthus seeds at day 10 treated with 75% *B. pilosa* extract

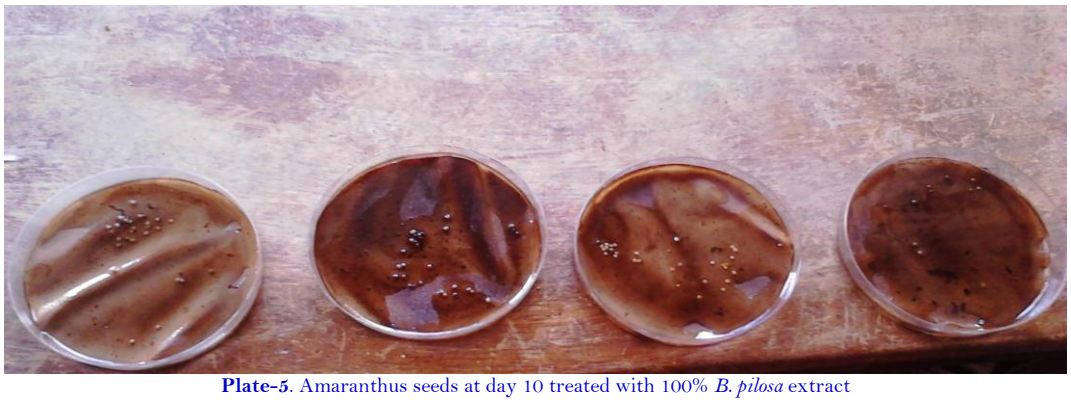

Plate-5. Amaranthus seeds at day 10 treated with 100% *B. pilosa* extract

3.2. Shoot Length

The results presented in table 4.2 show that there was significant difference (P≤ 0.05) between control and other treatments. Shoot height increased with increasing application of the various amounts of *B. pilosa* fresh leaves. Treatments with 100g of fresh leaf had the highest shoot height of 25.26cm while control had the lowest shoot height of 10.16cm. There was no significant difference (P≥0.05) between treatments with 20g and 40g, 60g, 80g and 100g.

Table-2. Effect of *B. pilosa* on shoot length, leaf number and leaf area

Trea□ments(g)	Shoot length	Leaf number	Leaf area
0	10.16d	3.96c	3.□7d
20	14.63□	4.83b	5.52cd
40	15.58c	5.13b	7.93bc
60	21.09b	5.21b	9□10b
80	22.83ab	6.46a	19.14a
100	25.26a	6.58a	19.16a
LSD	3.13	0.64	2.72

Means with the same letters down the column are not significantly different at P≤0.05

3.3. Leaf Number

Table 4.2 shows that leaf number increased with increase in amounts of fresh leaf of *B.pilosa*. There was significant difference (P≤ 0.05) between control and other treatments. There was no significant difference (P≥ 0.05) in treatments with 20g, 40g and 60g. The highest leaf number was recorded at 200g treatments and the lowest number at control. There was significant difference (P≥ 0.05) between treatments with 80g and 100g of fresh leaf material.

3.4. Leaf Area

There was significant difference (P≤ 0.05) between control and treatments with 40g, 60g, 80g and 100g. Leaf area increased with increase in fresh leaf material. Highest leaf area was in 100g (19.16) and lowest at control (3.8) as shown in table 4.2.There was no significant difference (P≥ 0.05) between treatments with 40g and 60g, 80g and 100g.

3.5. Shoot and Root Fresh Weight

There was significant difference (P≤ 0.05) between control and 100g treatment for both shoot and root fresh weights. Fresh weight increased with increase in amount of fresh leaf applied. There was no significant difference (P≥ 0.05) between control and 20g treatment. There was no significant difference (P≥0.05) between 20g, 40g and 60g treatments. Highest fresh weights were recorded in 80g treatment and lowest in control.

Table-3. Effect of *B. pilosa* on root length, shoot and root fresh weight and dry weight.

Treatments(g)	Shoot fresh weight	Root fresh weight	Shoot dry weight	Root dry weight	Root length
0	0.□1c	0.13c	0.09c	0.02b	2.65b
20	1.30bc	0.17bc	0.19bc	0.03b	2.93b
40	2.23b	0.29b	0.32bc	0.05b	4.08ab
60	1.97bc	0.25bc	0.43b	0.04b	3.53ab
80	4.50a	0.57a	1.13a	0.13a	4.88a
100	4.40a	0.53a	1.00a	0.13a	3.97ab
LSD	1.22	0.15	0.24	0.03	1.47

Means with the same letters down the column are not significantly different at P≤0.05

3.6. Shoot and Root Dry Weight

The results presented in table 4.3 indicate that there was significant difference (P≤ 0.05) between control and 100g treatment. Dry weight increased with increase in fresh leaf biomass. There was no significant difference (P≥0.05) between control, 20g, 40g and 60g treatments for both root and shoot. Highest dry weights were recorded at 80g for shoot and 80g and 100g for root (table 4.3). There was no significant difference (P≥ 0.05) between 80g and 100g treatments.

3.7. Root Length

There was significant difference (P≤ 0.05) between control and 80g treatment. Root length increased with increase in amount of fresh leaf material of *B.pilosa*. Longest root length was recorded at 80g (4.88cm) and shortest in control (2.65cm). There was no significant difference between control, 20g, 40g, 60g and 100g.

3.8. Chlorophyll Content

There was no significant difference (P≥0.05) in total chlorophyll in control and other treatments but the highest chlorophyll amount was recorded in control (49.09mg).

Table-4. Effect of *B. pilosa* on total chlorophyll.

Treatments(g)	Total chlorophyll(mg)
0	49.09a
20	47.82a
40	46.94a
60	44.98a
80	47.66a
100	47.26a
LSD	4.40

Means with the same letters down the column are not significantly different at P≤0.05

4. DISCUSSION

4.1. Germination Percentage

Seed germination is the most important stage in plant growth and development. During germination a lot of biochemical activities take place. Stress conditions can inhibit these biochemical activities.

The results in table 1 show that rate of germination was directly related to extract concentration, the highest concentration had high inhibitory effect. These results agree with those reported from various studies [10]; [19]; [20].The effects on germination may have been due to interference by allelochemicals on enzymatic activities Cruz-Silva, et al. [19] and Khanh, et al. [10].

4.2. Shoot Length

The application of fresh leaf biomass of *B. pilosa* positively influenced shoot growth. Shoot height increased with increase in amount of fresh leaf used. The decomposing leaves of *B .pilosa* became a source of green manure for the growing *A. dubius* seeds. Green manure provides a source of plant nutrients and organic matter; it also improves soil physical, biological and chemical conditions [21]. These results agree with those of Musyimi, et al. [18] and Mohammadi, et al. [22]. Increase in shoot height might be attributed to increase in cell division and enlargement as a result of high amounts of N from the decomposing leaves of *B. pilosa* [18]. Control plants had very poor growth compared to the others resulting in small shoot lengths.

4.3. Leaf Number And Leaf Area

Leaf number and area increased with increase in application of the various amounts of fresh leaf material. 100g treatments had the highest leaf number and area while control had the least number and area. This is because the amount of nutrients provided depends on the amount of leaf biomass used. Increase in N and other essential nutrients from the green manure promoted cell division and enlargement which led to increased leaf number and area [18]; [23]. Control plants did not receive any fresh leaf treatments thus may have lacked enough N and nutrients for foliage growth and development which resulted to inconsistent growth in leaf number and area [18].

4.4. Shoot And Root Fresh Weight

The data in table 4.3 shows that fresh weight for both shoot and root increased with increase in leaf biomass. Contrary to these results, studies done by Khanh, et al. [10] reported decrease in fresh weight up to 80%. The decomposing *B. pilosa* leaves released nutrients and other metabolites that promoted increased cell division and elongation which lead to increased fresh weight. High leaf number and area also contributed to photosynthesis and hence proper growth of the plants with fresh leaf treatments. Reduced leaf number and area in control plants may have lowered the rate of photosynthesis thus limited the plants growth [18].

4.5. Shoot And Root Dry Weight

Table 4.3 shows similarities in increase in dry weight to that of fresh weight. Highest dry weights were recorded in 80g and 100g. High amount of N from decomposing leaves of *B. pilosa* increased the rate of

photosynthesis which may have resulted to increased production of dry matter. Green manure also provided nutrients for root growth. In control plants, reduced leaf number and area resulted to reduced rate of photosynthesis and reduced plant growth consequently reducing the fresh and dry weights [18].

4.6. Root Length

The results in table 4.3 reveal that there was a slight difference in the root lengths; however these differences were not statistically significant. Longest root length was recorded at 80g treatment. Previous studies by Mohammadi, et al. [22] and Musyimi, et al. [18] showed that application at green manure improved soil structure and promoted root growth by providing nutrients and organic matter. This is contrary to the results presented in table 4.3 as there were no significant differences between the root lengths. This was probably due to lack of enough phosphorus in the soil.

4.7. Chlorophyll Content

Table 4.4 shows that there was no significant difference in the total chlorophyll in the leaves of the plants from all the treatments. This agrees with the study of Musyimi, et al. [18] that reported no significance differences in chlorophyll content in the treatments. According to studies by Mohammadi, et al. [22] green manure increased chlorophyll content as it provides elements such as nitrogen which are involved in chlorophyll structure. The lack of any significant differences could have been as a result of lack of sufficient magnesium or due to unfavorable greenhouse conditions such as overcrowding and shading from nearby trees.

5. CONCLUSION

This study has proved that *Bidens pilosa* aqueous extract has inhibitory effects on the germination of *Amaranthus dubius* seeds. The higher the concentration the higher the inhibitory effect. Therefore it can be concluded that the allelopathic effects of *B. pilosa* are concentration dependent.

Application of fresh leaf biomass of *B. pilosa* positively influenced the growth in shoot height, leaf number, leaf area, fresh weight and dry weight. This was possibly due to high amounts of nitrogen and other nutrients provided by the *B. pilosa* green manure. The green manure also improves the soil quality and structure. It also mulches the plants and prevents excessive loss of water. However, *B. pilosa* fresh leaf treatments did not significantly affect root length and chlorophyll content. The use of *B. pilosa* leaf biomass as green manure can greatly improve the growth and development of *Amaranthus dubius* seedlings.

6. RECOMMENDATIONS

There is need for farmers to be informed on the potential of *B. pilosa* as green manure. Instead of farmers cutting and discarding the *B. pilosa* as weeds they can reuse it as organic manure on their farms especially in production of amaranthus vegetables. Further studies should be carried out to determine which plants are inhibited by *B. pilosa* and which ones are not before using it as green manure for production of other crops. Also a study should be done to determine the proportions of *B. pilosa* leaves that are required for maximum yield without affecting the crops.

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