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EFFECT OF LEAF CRUDE EXTRACTS OF *AZADIRACHATA INDICA* A. JUSS. ON THE GERMINATION AND GROWTH OF *AMARANTHUS HYBRIDUS* L. AND *AMARANTHUS SPINOSUS* L.

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ABSTRACT: *The effect of leaf crude extract of Azadirachta indica on the germination and growth of Amaranthus spinosus and Amaranthus hybridus was studied. The aim was to use crude water extract from the leaves of Azadirachta indica to germinate, nurture and compare the growth of A. hybridus and A. spinosus. The plants' seeds selected were planted in polyethylene bags filled with loamy soil, and different extract concentrations of 0ml, 5ml 10ml, and 15ml dilutions were used to water the plants. The plants were well watered with extracts of three different concentrations and allowed to grow under natural environmental conditions. Parameters measured include germination percentage, the height of plants, the number of leaves, and the leaf area of the plant. It was observed that there was delayed germination of Amaranthus hybrids and Amaranthus spinosus seeds as the concentration of A. indica increased. The study showed that the extract of A. indica had a negative effect on the growth of A. spinosus and A. hybridus. The effect was significantly higher at higher concentrations of the extracts. Azadirachta indica extract significantly reduced the leaf area, number of leaves, and stem height in A. spinosus and A. hybridus compared to the control. The study further showed that the sensitivity of A. spinosus and A. hybridus to the plant extract differed. From the study, A. indica had a more negative effect on A. spinosus, revealing more inhibitory activity on the growth of A. spinosusthan on A. hybridus.*

KEYWORDS: Extract, Germination, Growth, Neem, *Amaranthus*, *Hybridus*, *Spinosus*.

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INTRODUCTION

Neem (*Azadirachta indica*) is one of the very few trees that are prominent in the Indian subcontinent (Puri, 1999). This tree belongs to Meliceae family and grows vigorously in the tropic and semi-tropic climate. It is also observed that this tree can survive in very dry and arid conditions (Puri, 1999). The Neem Tree is a very unique plant that has been declared the tree of the 21st century by the United Nations (Puri, 1999). In some parts of Asia, it is known by various names such as 'Divine Tree', 'Life giving tree', 'Nature's Drugstore', 'Village Pharmacy' and 'Panacea for all diseases'. This plant is one of the major components in Ayurvedic medicine, which has been practiced in Asia for many centuries. Extracts from the Neem tree (*Azadirachta indica*) also called 'Dogonyaro' in Nigeria are most consistently recommended in ancient medical texts for gastrointestinal upsets, diarrhea and intestinal infections, skin ulcers and malaria (Dubey*et al*., 2009). All parts of Neem plant such as the leaves, bark, flower, fruit, seed and root have usefulness in medical treatments and industrial products. The plant leaves can be used as drug for diabetes, eczema and to reduce fever. Barks of Neem can be used to make toothbrush and the roots have the ability to heal diseases; they also work against insects (Akhar & Mahmood, 1995; Puri, 1999; Ascher, 2004). The seed of Neem tree has significantly high concentration of oil. Neem oil is widely used for insecticides, lubricants and drugs for variety of diseases such as diabetes and tuberculosis (Puri, 1999). The present study was undertaken to investigate the effects of crude extracts of *Azadirachta indica* on germination and growth of *Amaranthus hybridus* and *Amaranthus spinosus*.

MATERIALS AND METHOD

Study Area

The experiment was carried out from September to November 2015 at the Department of Applied Biology screen house in Ebonyi State University, Abakiliki, South East Nigeria. The study area lies within Ebonyi state; the area is geographically located between latitude 1°9’36°N and longitude 8°04'44°E and an altitude 92.0m above sea level, covering a total of area of 81km. The vegetation of Ebonyi state is a luxuriant vegetation of tropical rainforest; the vegetation is densely populated with grasses and trees of different sizes in the area. The climate of the study area is of humid tropical climatic region; it experiences one rainy season and one dry season (eight months of rainfall and four months of dryness).

Preparation of Crude Extracts

This was done by drying the collected leaves of *A. Indica* in an oven. The temperature was set at 65°C and left to dry for 48 hours. When the leaves had been properly dried, they were then pulverized into a fine powdered form. The powdered sample was boiled with water for 45 min for proper extraction. The concentration of the crude extracts was measured at 5 ml, 10 ml and 15 ml appropriately. Thus 5 ml, 10 ml and 15 ml of the crude extracts were diluted in 500 ml of distilled water and kept safe in a conical flask.

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Sowing of the Seeds

Amaranthus spp thrives well in all soil types especially loamy soil at normal temperature not more than 15°C. Loamy soil used in sowing of the seeds was gotten from a nearby farm land in Presco campus of Ebonyi State University, Abakaliki. 10 g each of the soil sample was collected and replicated 4 times inside a small dark polythene bag, ready for seed sowing.

Seed Viability Test

The seeds of *Amaranthus spinous* and *Amaranthus hybridus* L which were sourced from a farm within Mgbabor village in Abakaliki, Ebonyi State were subjected to thorough viability test. This involves soaking the seeds in water for a while and making observations. All healthy seeds that sank into the bottom of the water were presumed viable and worthy of use for the experiment while those that floated were presumed not viable and thus were discarded. However, after the viability test, the seeds were sown by broadcasting method in the polythene bags filled with loamy soil. They were left for three days before applying the crude extracts of *Azadirachta indica* in their different concentrations. After seven days of seed germination, four plants were selected from each of the replicates (including the control) for measurement. Measurement was made weekly. The following parameters were measured:

a. Percentage germination

b. Plant height

c. Leaf area (leaf length x leaf width)

d. Number of leaves

RESULTS

Table 1: Weekly height (cm) of *A. hybridus* grown with crude extract of *A. indica*

Concentrations	Percentage Germination	Week 2	Week 3	Week 4	Week 5
0ml	2.94±0.15	6.70±0.13	6.70±0.11	6 .65±0.06	6.68±0.9
5ml	1.89±0.08	5.28±0.09	4.10±0.14	4.25±0.06	4.20±0.09
10ml	0.94±0.11	3.83±0.09	3.70±0.13	3.55±0.17	3.40±0.22
15ml	-	2.50±0.13	2.30±0.13	2.28±0.13	2.25±0.10

Results are expressed as Mean±SEM.

Table 1 shows the percentage germination and weekly mean height of *A. hybridus* grown with extracts of *A. indica* at various concentrations. The table indicates that there was no germination at 15 ml concentration and the control had the highest percentage germination. The table also shows that comparatively, *A. hybridus* in the control (0 ml extract) had the highest height from the second week (6.70±0.13) to the fifth week (6.68±0.09). Samples treated with 5 ml concentration of the extract only had a mean height of 5.28±0.09 in the

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second week and 4.20±0.09 in the fifth week. The 10 ml concentration of extract only reported a mean height of 3.83±0.09 in the second week and 3.40±0.22 in the fifth week. Also, 15 ml concentration of the extract had a mean height of 2.55±0.10 in the second week and 2.25±0.10 in the fifth week.

Table 2: Weekly height (cm) of *A. spinosis* grown with crude extract of *A. indica*

Concentrations	Percentage Germination	Week 2	Week 3	Week 4	Week 5
0ml	1.94±0.12	6.50±0.13	6.30±0.13	6.38±0.11	6.38±0.13
5ml	0.83±0.08	4.50±0.13	4.25±0.10	4.20±0.08	4.15±0.06
10ml	-	3.30±0.13	3.38±0.07	3.28±0.05	3.33±0.05
15ml	-	2.28±0.09	2.15±0.12	2.03±0.19	2.08±0.17

Results are expressed as Mean±SEM.

Table 2 shows the percentage germination and weekly mean height of *A. spinosus* grown with extract of *A. indica* at various concentrations. The table indicates that there was no germination at 10 ml and 15 ml concentrations and the control had the highest percentage germination. The table also shows that *A. spinosus* in the control (0 ml extract) had the highest height from the second week (6.50±0.13) to the fifth week (6.38±0.13). Samples treated with 5 ml concentration of the extract only had a mean height of 4.50±0.13 in the second week and 4.15±0.06 in the fifth week. The 10 ml concentration of extract only reported a mean height of 3.30±0.13 in the second week and 3.33±0.05 in the fifth week. Also, 15 ml concentration of the extract had a mean height of 2.28±0.09 in the second week and 2.08±0.17 in the fifth week.

Table 3: Weekly leaf area (cm^2) of *A. hybridus* grown with crude extract of *A. indica*

Concentrations	Week 2	Week 3	Week 4	Week 5
0ml	137.04±22.10	158.40±17.64	155.35±13.72	153.03±14.97
5ml	16.49±4.06	12.17±2.07	13.60±1.72	12.61±1.69
10ml	7.27±2.20	6.22±1.62	3.02±0.86	2.47±0.56
15ml	3.17±1.04	2.82±0.67	1.45±0.53	1.21±0.60

Results are expressed as Mean±SEM; mean values in the same column with the same superscript are not significantly different at $P < 0.05$.

Table 3 shows the weekly leaf area of *A. hybrids* grown with crude water extract of *A. indica* at various concentrations. The table indicates that comparatively, *A. hybridus* in the Control had the highest leaf area of 158.04±17.64 in the second week to 153.03±14.97 in the fifth

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week. Samples treated with 5 ml concentration of the extract only had a mean leaf area of 16.94±24.06 in the second week and 12.61±71.69 in the fifth week. At 10 ml, the mean leaf area was 7.27±2.20 in the second week and 2.47+0.56 in the fifth week. Also at 15 ml, the mean leaf area of 3.17±1.04 in the second week and 1.21±0.60 in the fifth week was recorded.

Table 4: Weekly leaf area (cm^2) of *A. spinosis* grown with crude extract of *A. indica*

Concentrations	Week 2	Week 3	Week 4	Week 5
0ml	80.17±10.11	72.97±9.35	74.51±8.90	73.81±9.20
5ml	9.86±2.05	4.39±1.49	3.85±1.25	2.98±0.90
10ml	2.52±0.93	2.39±0.81	2.40±0.90	1.89±0.64
15ml	1.07±0.34	0.80±0.34	0.74±0.42	0.55±0.47

Results are expressed as Mean±SEM.

Table 4 shows the weekly mean leaf area of *A. spinosus* grown with crude water extract of *A. indica* at various concentrations. The table indicates that *A. spinous* in the control had the highest leaf area of 80.17±10.11 in the second week to 72.97±9.35 in the third week. Samples treated with 5 ml concentration of the extract had a mean leaf area of 9.86±2.05 in the second week and 2.98±0.90 in the fifth week. Similarly, samples treated with 10 ml of the extract had a leaf area of 2.52±0.93 in the second week and 1.89±0.64 in the fifth week. Samples treated with 15 ml of the extract had a leaf area of 1.07+0.34 in the second week and 0.55±0.47 in the fifth week.

Table 5: Weekly number of leaves of *A. hybridus* grown with crude extract of *A. indica*

Concentrations	Week 2	Week 3	Week 4	Week 5
0ml	15.23±3.12	18.97±4.35	24.51±6.10	30.01±9.11
5ml	6.86±1.05	4.32±1.00	3.88±1.00	2.98±0.92
10ml	3.62±0.73	2.49±0.91	2.40±0.80	1.79±0.64
15ml	1.08±0.44	0.82±0.32	0.70±0.22	0.55±0.27

Results are expressed as Mean±SEM.

Table 5 shows the weekly number of leaves of *A. hybridus* grown with crude water extract of *A. indica* at various concentrations. The table indicates that the control sample of *A. hybridus* had the highest number of leaves 15.23±3.12 in week two to 30.01±9.11 in the fifth week. Samples treated with 5 ml concentration of the extract had 6.86±1.05 number of leaves in the second week and 2.98±0.92 in the fifth week. At 10 ml the mean number of leaves was 3.62±0.73 in the second week and 1.79±0.64 in the fifth week. Also at 15 ml the mean number of leaves was 1.08±0.44 in the second week and 0.55±0.27 in the fifth week.

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Table 6: Weekly number of leaves of *A. spinosus* grown with crude extract of *A. indica*

Concentrations	Week 2	Week 3	Week 4	Week 5
0ml	12.42±2.06	16.17±4.07	22.60±5.72	26.61±5.89
5ml	7.37±2.00	6.00±1.82	3.03±0.89	2.27±0.66
10ml	3.17±1.04	2.62±0.67	1.55±0.53	1.31±0.60
15ml	1.15±0.24	0.77±0.12	0.60±0.12	0.44±0.06

Results are expressed as Mean±SEM.

Table 6 shows the weekly number of leaves of *A. spinosus* grown with crude water extract of *A. indica* at various concentrations. The table indicates that the control sample of *A. spinosus* had the highest number of leaves (12.42±2.06) in week two to 26.61±5.89 in the fifth week. Samples treated with 5 ml concentration of the extract had 7.37±2.00 number of leaves in the second week and 2.27±0.66 in the fifth week. At 10 ml the mean number of leaves was 3.17±1.04 in the second week and 1.31±0.60 in the fifth week. Also at 15 ml the mean number of leaves was 1.15±0.24 in the second week and 0.44±0.06 in the fifth week.

DISCUSSION AND CONCLUSION

The study showed that the extract of *A. indica* had significant effects which are detrimental to the germination and growth of *A. spinosus* and *A. hybridus*. The effect was successively higher at higher concentrations of the extract of *A. indica*and as the day went by. The result showed important decline in the leaf area, number of leaves and stem height of *A. spinosus* and *A. hybridus* when compared to the control. Additionally, the sprouting of *A. spinosus* and *A. hybridus*were delayed considerably at higher concentrations, and in some cases no germination was observed.

The results of this study were consistent with the findings of Okeke *et al*. (2015) who reported a general reduced crop performance in response to the application of leaf extracts of *M. oleifera*. Following on, the study of Singh *et al*. (2015) suggested that the negative effect of extracts from plants on the germination and growth of plants could be attributable to inhibitory substances in the plants. Elisante *et al*. (2013) reported that some extracts of plants such as *Eucalyptus* extract and *Morinda* extract have allelochemicals which could reduce and delay germination and crop yield.

This research further revealed that the sensitivity of *A. spinous* and *A. hybridus* to plant extracts differs. From the study, *A. indica* extract showed higher negative effect on *A. spinous* than on *A. hybridus*; thus, Kender *et al*. (2004) reported that the differences in sensitivity of *A. spinous* and *A. hybridus* to plant extracts could be attributed to differences in the selectiveness of inhibitory growth substances. According to Wang *et al*. (2010), the resistance to inhibitory substances in crops differs and therefore could account for the differences in sensitivity of *A. spinous* and *A. hybridus* to plant extracts.

Finally, this study revealed that the inhibitory activity of *A. indica* on the growth of *A. spinous* and *A. hybridus* was higher when compared to that of *M. oleifera*. Elisante *et al*. (2013) explained that most plants like *Eucalyptus* extract and *A. indica* have a higher concentration of allelochemicals in their leaves. This could account for the inhibitory activity of *A. indica* (Amadioha, 2000; Okeke *et al*., 2015). This study indicated that *Azadirachta*

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indica leaf extract was not suitable for the cultivation of *A. spinosus* and *A. hybrids* because it delayed germination and significantly reduced the leaf area, number of leaves and stem height of the two plants. This study therefore advocates for further research in the inhibitory substances in the leaf extracts of *Azadirachta indica*.

Conflict of Interest

Authors have declared no conflict of interest.

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