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Comparative Evaluation of the Allelopathic Effects of the Leaf Extracts of Three Asteraceae Species (*Ageratum conyzoides, Vernonia amygdalina, Artemisia annua*)

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Authors' contributions

This work was carried out in collaboration between both authors. Author CSB designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author EPO managed the analyses of the study and literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

Search for an alternative to chemical herbicides have prompted researchers to investigate the allelopathic potential of plants, which would serve as a remedy for contamination of soil, water bodies and crops products with toxic chemical residues derived from using synthetic herbicides. The allelopathic effects of the aqueous leaf extracts of *Ageratum* conyzoides, *Vernonia amygdalina* and *Artemisia annua* at 0.05, 0.1, 0.2 and 0.5 (part per volume) concentrations were evaluated on seed germination, root length and height of seedlings of *Lactuca sativa* after 28 days in vitro. Results obtained showed significant differences *P*=[0.5] among the different concentrations of aqueous leaf extracts used when compared with the control in all the three parameters studied. Significant reduction in seed germination, growth of root and height of seedlings were observed in all the treatments but at varying levels from moderate to severe effects for aqueous leaf extract of *A. conyzoides* and *A. annua* respectively, while on the contrary, aqueous extracts of *V. amygdalina*

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enhanced seed germination, root elongation and plant height. Cytological studies conducted to observe the mitotic behaviour of cells in root tips obtained from highest concentration of the leaf extracts indicated that aqueous leaf extracts of *A. conyzoides* and *A. annua* lowered the mitotic index of cells in root tips of *Lactuca sativa* while that of *V. amygdalina* greatly increased the mitotic index of cells in the root tips when compared with the control. For the number of dividing cells, T-test showed significance *P*=[0.001] only in aqueous leaf extract of *A. conyzoides*.

Keywords: Allelopathy; Ageratum conyzoides; Vernonia amygdalina; Artemisia annua; Lactuca sativa; aqueous and leaf extract.

1. INTRODUCTION

Recently, researchers have become interested in the allelopathic interactions of certain plants as a possible substitute for weed management. Current understanding in plant Physiology biochemistry, interaction and chemistry of natural products have shown that some plant residues and their allelochemicals may be useful for weed control thus overcoming the various problems linked with synthetic herbicides [1].

Allelopathic plants possess a store of poisons in their body parts [2]. They compete with other species through chemical warfare by releasing biochemicals known as ''allelochemicals'' which inhibit the growth of its competitors [3]. Allelochemicals in plants act like herbicides preventing the germination and growth of the seedlings of competing species [4]. Depending on the plant, allelochemicals can be released from a plant's flower, leaf debris and leaf mulch, stem, bark, root or soil surrounding the roots and can persist in soils affecting both neighbouring plants as well as those planted in succession [5].

Plants in the Asteraceae family, notably *chromolaena odorata*, *Helianthus annus* and *Tithonia diversifolia* (of which *Ageratum conyzoides, Vernonia amygdalina and Artemisia annua* belongs) have been reported to contain large amounts of allelochemicals, especially in their leaves, which inhibit the growth of many plants [6]. The basic approach used in allelopathic research for agricultural crops has been to screen both crop plants and natural vegetation for their capacities to suppress weeds.

To demonstrate allelopathy, plant origin, production and identification of allelochemicals as well as persistence in the environment over time in concentrations sufficient to affect plant species is first established [7]. This is carried out in the laboratory by screening plant extracts and leachates for their effects on seed germination with further isolation and identification of allelochemicals from green house tests [8]. An allelopathic crop can potentially be used for weed control by planting a variety, either as a smother crop in a rotational sequence or when left as a residue or mulch, especially in low-till systems to control subsequent weed growth [9].

Concerns about chemicals in the environment, prohibitive cost and other limitations have threatened the sustainability of chemical weed control measures [10]. Hence, the allelopathic suppression of weeds which is natural, environmental friendly and cost effective may prove to be an amazing alternative for weed control [11]. This study reveals the allelopathic influence of leaf extracts of some species of Asteraceae on growth parameters of *Lactuca sativa* (lettuce).

2. MATERIALS AND METHODS

Collection and Identification of Plant Samples: Leaves of *Ageratum conyzoides*, *Vernonia amygdalina* were locally sourced and identified by botanists at the University of calabar, Nigeria. Leaves of *Artemisia annua* were purchased from known and verified sellers in china. Seeds of *Lactuca sativa* were also identified and purchased from local vendors.

Preparation of Plant Extracts: Leaves from the three Asteraceae species were dried under shade at 28°C for 6 – 7 days to obtain a crisp-textured form. The crisp leaves were crushed into powdered forms using a manual blender. Thereafter, powdered leaves from individual plants were soaked separately in beakers containing distilled water in the ratios of 1:2, 1:5, 1:10 and 1:20 (parts per volume) for 48 hours (2 days). I part = 1 g powdered leaves weighed with electronic weighing balance, 1 volume = 10 ml of distilled water measured with the measuring cylinder. After 48 hours soaking period, the mixtures in beakers were filtered using a sieve,

poured into a centrifuge and spun for 10mins at 1000 rpm.

Inoculation of Seeds: Seeds of lettuce were placed in petri dishes containing a filter paper and 20ml of the plant extracts (each for the different concentrations). For the control, lettuce seeds were sown in distilled water. Petri dishes were arranged in a completely randomized design with 5 repetitions (each repetition corresponding to 40 seeds of lettuce placed in a petri dish). The percentage germination, root length, and height of seedlings were evaluated after 7 days, 14 days, 21 days and 28 days.

2.1 Statistical Analysis

The percentage germination of seeds was obtained by the mean of each of the five replicates for each treatment, while the average length of root of seedlings was obtained by summing only 10 from each repetition of the concentrations (taking the measurement of only two (2) from each petri dish. The data were subjected to one-way analysis of variance (ANOVA) and comparison between the means of treatment with the means of control was calculated.

2.2 Cytological Studies

Harvesting: For the control, roots from three day-old (72 hours) lettuce seedlings were harvested using coarse forceps to clip off the root portion from plants. Times for harvest were: 8.00am, 8.30am, 9.00am and 9.30am.

Fixation: For this study, the fixative used was Carnoy's solution (acetic alcohol), prepared in the ratio of 1 acetic acid: 3 absolute alcohol by volume. Using a fine forceps, harvested roots were transferred into specimen bottles (each with a tag indicating the time of harvest of roots) containing the fixative and left for 48 hours.

Hydrolysis: The fixed roots were rinsed three times in distilled water and transferred into tubes containing 1 normal Hcl. Thereafter, the test tubes were transferred to a digital water bath with water heated to a temperature of 60.7°C and left for 6mins. This loosens the cement substance between cells and allows the cells to spread out during squashing.

Staining and Squashing: Hydrolysed roots were washed in distilled water for three times. Thereafter, the roots were placed on a filter paper to drain off excess water and then onto a clean glass slide using a fine forceps. With the aid of a mounted needle, the milky portion of roots (root tips) was cut off and the other portion discarded. 1-2 drops of aceto orcein stain was placed on the specimen and squashed carefully using the back of a biro. A cover slip was then placed over the specimen and excess stain was removed by simply putting the slides between folds of an absorbent filter paper and applying thumb pressure. The preparation was gently tapped to further spread the cells. The periphery of the cover slip was smeared with a colourless nail vanish to prevent entry of air bubbles. Five slides were prepared for each extract treatment.

Examination of Slides: Slides were examined under a binocular microscope, starting with the lowest magnification (scanning x10, x40 and x100). Cells viewed under the microscope were counted using the tally counter.

Statistical Analysis: The total number of cells analysed and the total number of dividing cells was obtained by the mean of the five slides prepared for each extract treatment. Comparison between the mean of extract treatments with the mean of control (for total number of dividing cells) was calculated using the t-test P=[0.001]. The mitotic index and mitotic inhibition of cells for each extract treatment was calculated as well.

3. RESULTS

The following results were obtained to estimate the allelopathic effects of dried leaves extracts of three species of Asteraceae on the growth parameters of treated seeds of *Lactuca sativa.*

Allelopathic Effect on Seed Germination: Gradual reduction in seed germination percentage of *Lactuca sativa* was observed and reduction of germination was concentration dependent. *A. conyzoides* showed the highest allelopathic effects with decreased percentage seed germination in all the varying concentrations. *V. amygdalina* leaf extract enhanced seed germination in all the varying concentrations as shown in Fig. 1.

Allelopathic Effect on Average Root Length of Seedlings: Allelopathic effect of the leaf extracts of *A. conyzoides* and *A. annua* was evident on average root length of *Lactuca sativa* seedlings at all concentrations as shown on Fig. 2. It was observed that *A. annua* had the most significant allelopathic effect on root length. Seedlings

treated with *V. amygdalina* leaf extract further enhanced root elongation in all varying concentrations as compared to the control group. *A. annua* leaf extract had the highest allelopathic effect on root length reduction.

Allelopathic Effect on Average Height of Seedlings: Allelopathic effects on average height of seedlings of *Lactuca sativa* treated with the three Asteraceae leaf extracts was only visible in seeds treated with *A. conyzoides* and *A. annua* as shown in Fig. 3. *V. amygdalina* showed no allelopathic effect but rather enhanced seedlings height. It was observed that *A. annua* leaf extract inhibited the growth of *Lactuca sativa* the most in all concentrations when compared to seeds treated with leaf extracts of *A. conyzoides*.

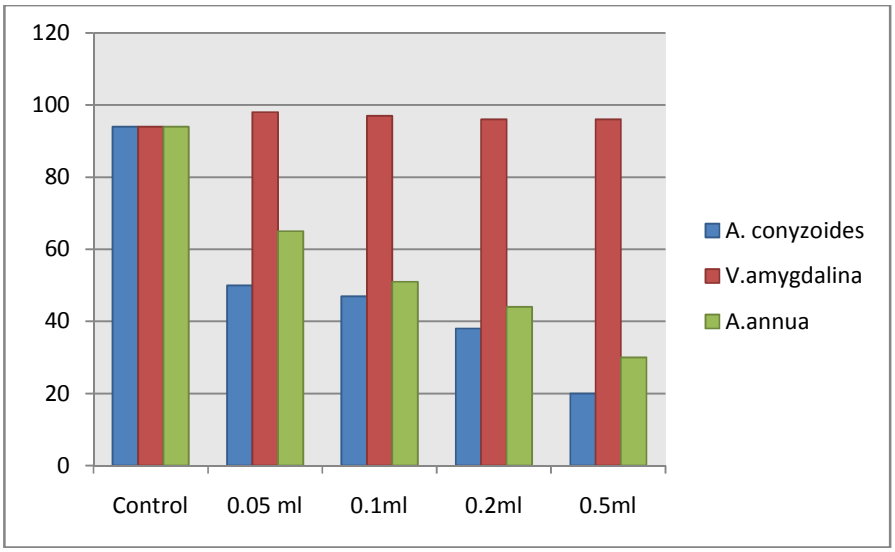

Fig. 1. The allelopathic effect of the three leaf extracts of *A. conyzoides, V. Amygdalina* and *A. annua* on seed germination percentage of *Lactuca sativa* after 28days of treatment

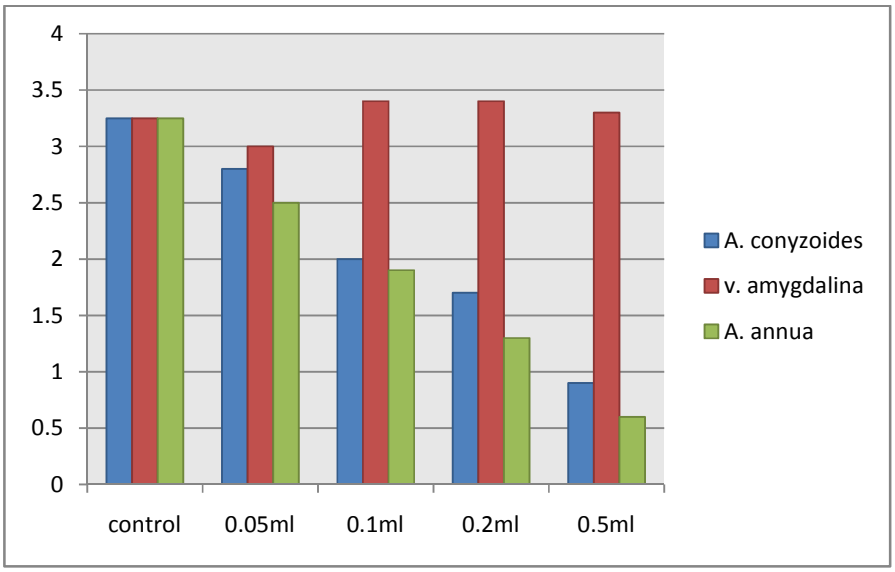

Fig. 2. The allelopathic effect of leaf extracts of *A. conyzoides, V. amygdalina* and *A. annua* on average root length of *Lactuca sativa* seedlings

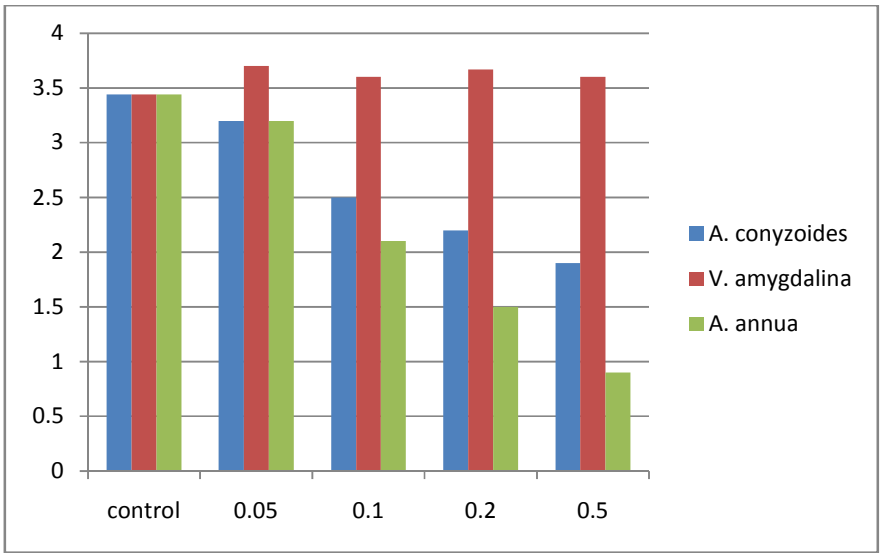

Fig. 3. The allelopathic effect of the three leaf extracts of *A. conyzoides*, *V. amygdalina* and *A. annua* on average height of seedlings of *Lactuca sativa*

Cytological Effects of the Three Asteraceae Leaf Extracts: To further estimate the allelopathic effect of the three Asteraceae species leaf extracts, cytological studies were carried out on treated root cells of *Lactuca sativa*. The cytological parameters under study were number of dividing cells, mitotic index and mitotic inhibition as shown in the Table 1. Leaf extracts of *A. conyzoides* and *A. annua* lowered the mitotic index of cells in root tips of *lactuca sativa* while *V. amygdalina* greatly enhanced the mitotic index of the cells as shown in Table 1. Significant mitotic inhibition was observed in only root cells treated with *A. conyzoides*.

4. DISCUSSION

Herbicides are frequently utilised to enhance crop yield through effective weed control, but excessive and non judicious use of herbicides has posed environmental and health problem [10] Interestingly, some allelopathic crops have proven to be efficient in weed control with little or no environmental and health risk [12]. In this study, a comparative evaluation of the allelopathic effects of leaf extracts of *A. conyzoides, V. amygdalina* and *A. annua* on seedlings of *Lactuca sativa* was carried out on the following parameters; percentage germination, average root length, average height, mitotic index and mitotic inhibition after 28 days. Allelopathic effects on percentage germination showed significant reduction in seedling germination in all concentrations of only two (*A. conyzoides* and *A. annua*) out of the three species leaf extracts thus depicting that the two Asteraceae leaf extracts had the potentials of being used to hinder the emergence and growth of other crops as reported by Deng et al. [13] and Huang et al. [14]. In his research, he observed the allelopathic effect of *A. conyzoides* on soybean seed germination. It was also observed that *A. conyzoides* had the most significant allelopathic effect in seed germination followed by extracts of *A. annua*. *V. amygdalina* on the contrary enhanced seed germination [15]. For average root length of treated *Lactuca sativa* seedlings after 28 days, results obtained showed significant root length reduction in seedling treated with *A. conyzoides* and *A. annua*. This shows that the two species could be used in the formulation of plant based herbicides that inhibit the growth and spread of roots in already emerged weeds in cultivated farmlands. Also the alleopathic effect of the two species (*A. conyzoides* and *A. annua*) on seedlings height has further confirmed their phytotoxic impact on the growth of other plants due to the presence of allelochemicals as documented by Salman et al. [16] and Dias et al. [17]. However, despite the non significance of the allelopathic effect of *V. amygdalina* leaf extract on all the parameters under study, its ability to enhance growth of treated seedlings shows that it can be utilised to develop ecofriendly, cheap and effective green growth promoter.

Table 1. Shows the cytological effect of the leaf extracts of *Ageratum conyzoides*, *Veronia amygdalina* and *Artemisia annua* at 0.5 (part per volume concentration on root cells of *Lactuca sativa*

Extract	Concentration (Part per volume)	Total number of dividing cells per 200 cells counted X±S.E	Mitotic index	Mitotic inhibition	CAL –T(4)	T-Test Tab – T4		
						5%	1%	0.1%
A	0.0	117 ± 2.3	58.5	-				
Ageratum conyzoides	0.5	56 ± 30	28.0	52.1	28.77***	2.78	4.60	8.61
B	0.0	117 ± 2.3	58.5	-				
Vernonia amygdalina	0.5	135 ± 4.2	67.5	-15.4	-4.16^{NS}	2.78	4.60	8.61
C	0.0	117 ± 2.3	58.5	-				
Artemisia annua	0.5	91 ± 3.1	45.5	22.2	6.5^{NS}	2.78	4.60	8.61

****Significant different at P<0.001*
NS = Not significant at P< 0.001

Cytological studies showed significant mitotic inhibition of root cells of *Lactuca sativa* which was achievable only by the allelopathic effects of *A. conyzoides*. A lowered cell division index suggests an inhibition effect often at the interphase stage [18]. This means that if used as a plant based herbicide, it will be most effective in inhibiting cellular division which is an essential factor in eradicating weeds as without cell division, there will be no plant growth hence plant death.

5. CONCLUSION

To further ascertain the efficacy of allelopathy in the three Asteraceae species under study, it is advisable to carry out molecular studies to determine the presence of allelopathic gene function. However, this species could be on the plant list for potential allelopathic properties until further molecular evidence are available as most data obtained in this study correlates with previous studies.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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