Original Research Article

Cytotoxicity of Different Ages of Exudates of *Ageratum conyzoides (*Billy Goat Weed*)* using *Allium cepa L.* Assay

Abstract

Considering the present inhibition of seed germination, reduction in agricultural yields and the unexpected death of plants in the field, an experiment was conducted to evaluate the cytotoxicity of exudates from soaked plants of *Ageratum conyzoides* (Billy goat weed) on plants using the *Allium cepa L*. assay. The onion bulbs were first placed in water for 48 hours to initiate root growth, then they were placed in exudates got from different soaking periods of all the plant parts of *A. conyzoides* (which served as the treatments) for 24 hours. Both macroscopic and microscopic parameters show the toxic effect of *A. conyzoides*. Root length and root number dropped in value with lower soaking period of *A. conyzoides.* The cells of the 36hrs treatment were charred; those of the 72hrs were not charred, but had their nuclei fallen out of the cells because the cell wall had broken open; while the cells of the 120hrs treatment had intact cells, but with no sign of division. Hence the mitotic index of the treatments could not be ascertained. It was observed that treatment effect on the cells decreased with increase in soaking period of the weed. This proves that *A. conyzoides* is allelopathic in nature, and time is required to get rid of its harmful allelochemicals for this weed debris to be useful in enriching the soil.
Keywords: Soaking periods, Cytotoxicity, allelochemicals, *Ageratum conyzoides*, *Allium cepa*.

Introduction

Ageratum conyzoides is an annual erect herb reported as an invasive, noxious weed in agricultural lands and as a colonizer of open fields and degraded areas, causing crop yield reductions and affecting biodiversity (Kohl*i et al*., 2006). It is also a host for pathogens and nematodes that affect crop species (BioNET-EAFRINET, 2016).

Ageratum conyzoides L. commonly known as Billy goat weed is an alien weed species native of Central America and Mexico. As a member of *Asteraceae* family, the plant is herbaceous in habit, found throughout the tropics and subtropical regions of the world including Nigeria. It is widespread across different agroecosystems and natural ecosystems owing to its wide ecological amplitude and adaptability (Oviedo-Prieto *et al*., 2012). Its production of extensive numbers of seeds and rapid spread to distant

places helps in its quick encroachment to wider areas. *A. conyzoides* forms dense stands which out-compete the native species in terms of space and resource utilizations, affecting the biomass of native species. The loss of biomass or productivity results in disruption of the local ecosystem in terms of structure and functioning (PIER, 2016)

Allelopathy is an interference mechanism in which plants release secondary metabolites into the environment that could have either inhibitory or stimulatory effects on the growth of nearby plants (Mallik, 2003). Secondary metabolites such as alkaloids, flavonoids, phenolics, chromenes and essential oils have been identified in A. *conyzoide*, some of which are considered as putative allelochemicals. Allelopathy is regarded as one of the causes of invasiveness in *A. conyzoides.* Enhanced phytotoxic effects are observed in *A. conyzoides* when conditions are extremely unfavourable for its growth. Water soluble phenolics as putative allelochemicals have been reported in significant amounts in *A. conyzoides* in a number of studies, deleteriously affecting the early growth of rice, wheat, chickpea and pea. The leaves were however used by Chinese farmers to increase the soil fertility for paddy fields. Weed debris is reported to enrich the soil nutrients, especially nitrogen (Field *et al*., 2006).

The *A. cepa* assay is an efficient test for *in situ* monitoring for toxicity of environmental contaminants. Its root meristem represents a normal proliferating plant cell population that is sensitive to changes in environmental conditions. The *A. cepa* assay provides a rapid procedure for screening chemicals which pose as environmental hazards.
In this study, the *Allium cepa* assay was used to evaluate the cytotoxicity of exudates of the weed *Ageratum conyzoides* soaked in water for increasing periods of time, using mitotic index parameters, root growth and root number.

Materials and methods

Cultivation of onion in water

Dry onion (*Allium cepa)* bulbs of about the same size, were locally obtained. Their dead roots and scales were carefully scraped away without destroying the primordia of the roots.
A total of twelve onion bulbs were prepared this way for the three treatment to be administered, and control, all in replicates of three. The twelve onion bulbs (between 90-100g) were then placed for 48

hours on plastic containers filled with distilled water such that only the base of the bulbs touched the distilled water for root induction, and to ascertain the viability of the onion bulbs. The number of roots found growing on each of the onion bulbs after 48 hours, were recorded and a few of these roots were measured and color - tagged for identification and subsequent measuring to ascertain growth.

Preparation of treatment extract

Fresh whole plants of *Ageratum conyzoides* were uprooted from a farm site, washed free of soil and air-dried. With a weighing scale, 500g each of the plants were weighed into three bowls. Each set was chopped into about 2cm pieces (stem, leaves and roots), and each soaked in 500ml of distilled water in a plastic bowl and covered. After 36 hours, the weeds in the first bowl were drained out and the water containing exudate was stored in a bottle and kept in a refrigerator. Likewise, after 72 hours, the weeds in the second bowl were drained out and the solution of exudate stored also in the fridge. The same procedure was carried out after 120 hours with the weeds in the third bowl. These three exudate concentrations were the treatments administered on the onion bulbs.

Treatment of Onion bulbs with weed exudate

After 48 hours in water, the number and lengths of roots growing were noted for each onion bulb, and the onions were transferred into containers containing the different treatments, each replicated three times. This however was after the exudates had been taken out of the refrigerator and left to warm to room temperature. The set up was kept in the dark at room temperature. After 24 hours in the different treatments, the number of roots per onion bulb were noted again, and the lengths of the color-tagged roots were measured for all the onion bulbs.

Harvesting and Fixing Onion Roots

After 24 hours of growing in the treatments, a few roots were harvested from the base of the onion bulb between 11am and 1pm as suggested by Ambrocio and Ian (2011). The harvested roots were fixed immediately in small McCartney bottles containing Carnoy's fluid (1 part of glacial acetic acid: 3 parts of ethanol) and placed in a refrigerator for 24 hours at 4°C. The roots were then rinsed and preserved in 70% ethanol in the refrigerator until used.

Slide Preparation

Onion roots were removed from the 70% ethanol, rinsed in distilled water and hydrolyzed in 1N hydrochloric acid in a water bath at 60°C for 5 minutes to soften cell walls and make the root tip malleable. The root tips were then rinsed and placed in a solution of 1% Ferric Chloride for an hour.

About 2mm of the root tip was cut unto a slide, a drop of acetocarmine stain placed on it and left for 10 minutes; then it was covered with a cover slip and squashed between paper towels.
The slides were then viewed under a compound light microscope. Photomicrographs were taken of dividing and non-dividing cells using a Samsung digital camera.

Results

Macroscopic parameters

After 24 hours of the onion bulbs being in the weed treatment, it was observed that there was a sharp reduction in the net root number (Table 1) and net root length (Table 2) compared to the control; and the highest reduction was observed in the treatment with the shortest soaking period of the weed in water.

Table 1: Onion root number before and after *A. conyzoides* exudate treatment

Treatment	Average root number after 48hrs in water.	Average root number after 24hrs in treatment.	Net root growth
Control (T_1)	27.33±6.2	32±8.9	4.67
120hrs (T_2)	24.33±5.4	27±6.4	2.67
72hrs (T_3)	12.66±3.5	13±4.8	0.34
36hrs (T_4)	31±6.8	30±6.5	-1.0

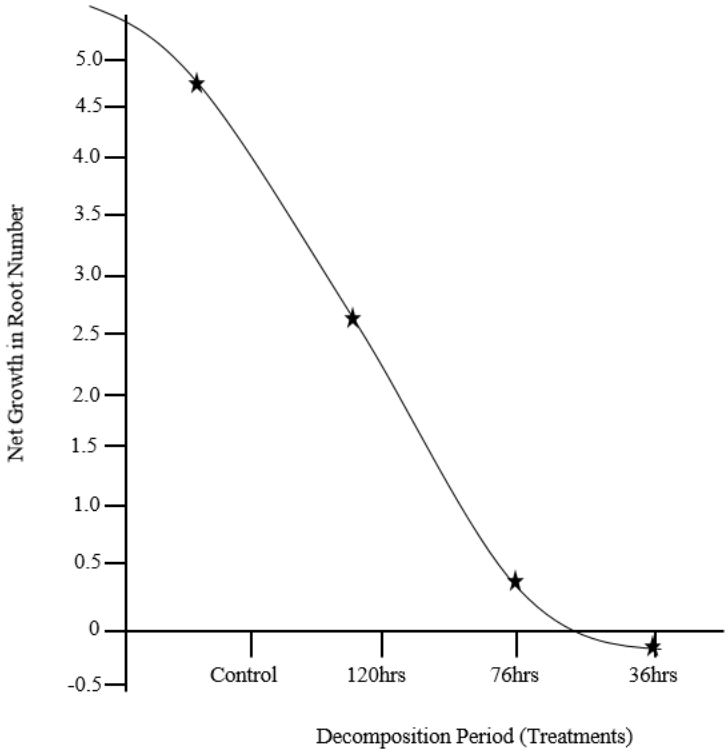

Figure 1: Effect of treatment concentrations on onion root number

Table 2: Onion root lengths before and after treatment with *A. conyzoides* exudate

Soaking period	Average root length after 48hrs in water (cm)	Average root length after 24hrs in treatment (cm)	Net increase in root length (cm)
Control (T_1)	0.97 ±0.14	1.72 ±0.25	0.75
120hrs (T_2)	0.63 ±0.40	0.60 ±0.12	-0.03
72hrs (T_3)	0.58 ±0.15	0.37 ±0.18	-0.21
36hrs (T_4)	0.94 ±0.41	0.82 ±0.36	-0.12

For both root number and root length, the drop in values from those of control was much. The treatment effect however reduced with increase in the soaking period of *A. conyzoides*. The 36 hour treatment had the worst effect on root number and root length (Table 1 & 2), and the roots appeared burnt up by the treatment effect.

Microscopic Parameters

It was observed that there were no dividing cells in the roots grown in the treatments, except in the control (Figure 2). In the treatment of 36hrs soaking period it was observed that the cells were burnt up (charred), as seen in Figure 2B. In the treatment with 76hrs soaking period it was observed that the chromosomes were fallen out of the cell because the cell walls had been disintegrated as a result of the treatment effects (Figure 2C). However, in the treatment with 120hrs soaking period, it was observed that the cells were intact but showed no sign of division as shown below (Figure 2D). Hence there was no need to calculate the mitotic index because dividing cells were only observed in the control, as the treatment effect damaged the cells (Fig. 2).

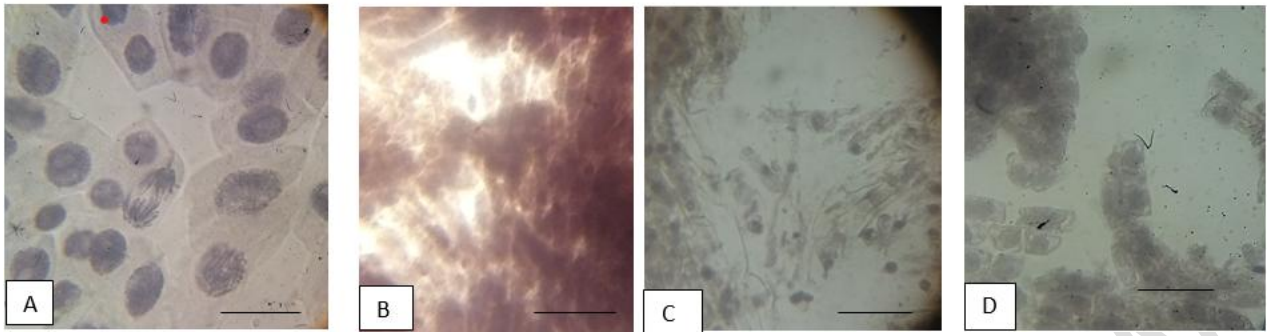

Figure 2: Treatment effects on onion cells: A is Control with dividing cells (bar= 10µ); B is 36hrs treatment with charred cells (bar=25µ); C is 72hrs treatment with broken up cells (bar= 25µ); D is 120hrs with intact but non-dividing cells (bar=25µ)

Discussion

Ageratum conyzoides (Billy goat weed) is one of the most common, invasive and harmful weeds in farmlands (GISD, 2006), and this is because it is known to be allelopathic. As a member of the Asteraceae family, A. conyzoides has aromatic polyacetylene compounds which are cytotoxic, amongst other activity (Konovalov, 2015). Xuan et al. (2004) on evaluating the effects of *A. conyzoides* on germination and growth of radish showed that all its plant parts inhibited radish germination. The phenolic acids detected in the leaves, stem, and root of *A. conyzoides* are putative allelochemicals which also show effects of inhibition on paddy weeds (Chung *et al.*, 2002).

This explains the damaging effect that the treatments had on the onion cells (Fig 2). However A. conyzoides is not only toxic to plants in a negative sense, for it has been proven that the secondary metabolites it possesses, such as alkaloids, flavonoids, phenols, and tannins could possibly be exploited for the development of natural herbicides and controlling pests for sustainable crop production (Batish *et al.*, 2009; Paul et al., 2021). It is also being used, amongst other purposes, to enrich soil nutrients as organic manure (Field et al, 2006; Anhar et al., 2018).

From this experiment, it has been further proven that if A. conyzoides is to be useful as manure, sufficient time should be allowed for the decomposition of its vegetation, oxidation and volatilization of its allelochemicals to prevent its inhibitory effect. The longer the soaking period, the less damaging was

the treatment, as was seen in both the macroscopic (Table 1 & 2) and microscopic parameters (Fig. 2). Moreover, natural polyacetylenes compounds have two or more triple bonds in their chemical structure (Christensen, 1998), and this makes them unstable, and therefore, chemically and biologically active. This brings about fast oxidation of these compounds in the weed, and their degradation when exposed to UV light (Konovalov, 2015). Hence the treatment effect was from charred cells in the 36 hrs treatment, to broken cell walls in 72hrs treatment, to intact but undividing cells in 120hrs treatment, improving with time. So the longer the period of soaking the vegetative parts of A. conyzoides, the less their damaging effect on the onion cells, and thus tomato seedling growth (Adeleke and Onyebuchi, 2022). Allelochemicals such as Phenolic compounds found in *A. conyzoides* can prevent plant growth by the endogenous regulation of auxin transport and enzymatic performance, resulting in the prevention of tumorigenesis. Hydroxycinnamic acids, coumarins, tannins, and ferulic acid have been some of the usual preventers of seed germination. It has been shown that phenolics can be active as germination preventers by preventing the transport of amino acids and the synthesis of proteins in seeds (Zhu and Yao, 2004).

Conclusion

In conclusion, in as much as the toxicity of *Ageratum conyzoides* is established, it is still a potential herbicide and manure, amongst other uses. Extraction of the unstable polyacetylene compounds it contains, to serve as an organic herbicide is a challenge. To serve as manure on the other hand, vegetation of *A. conyzoides* needs to be left for a considerable length of time in order not to inhibit the plants whose growth they are meant to improve. The abundance of this weed on farmlands makes it very much available, and its aggressive nature in taking over local ecosystems should make putting it to some good use on a large scale, a task to solving a problem.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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