Cytotoxicity testing of aqueous extract of bitter leaf (*Vernonia amygdalina Del*) and sniper 1000EC (2,3 dichlorovinyl dimethyl phosphate) using the Alium cepa test.

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Abstract:

Background: The unrefined nature of the herbal preparations from *Vernonia amygdalina* (VA) and toxicity potentials of Sniper may both have severe consequences on the biochemical and genetic systems.

Objectives: To assess the microscopic and macroscopic effects of these substances.

Methods: VA leafs and Sniper were prepared and dissolved in distilled water to give different concentrations. Series of baseline tests were carried out to establish concentration range for root growth. Series of twelve onion bulbs of three per series was prepared, with a series of three onion bulbs serving as control. Chromosomal aberrations were statistically analysed using chi-squared test. Root bundle mean length was obtained after 96 hours and EC_{50} values at 95% confidence interval was determined from a plot of root length against sample concentrations using Microsoft Excel software.

Results: Total cytotoxic effect was induced by 2% sniper and 70% VA. EC_{50} for VA and sniper were 33.07 and 0.346 respectively. The two substances induced chromosomal aberrations and the effect was concentration dependent.

Conclusion: There are risks of these widely used substances for therapeutic and environmental purposes.

Keywords: Chromosomal aberrations, Sniper 1000EC, *Vernonia amygdalina*, toxicity.

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Introduction

Africa is one of the richest phytodiversities in the World. The consumption of plant materials is believed to contribute immensely to the improvement of the health of man and animals.[1] It is estimated that 80% of the population of Africa depends on medicinal plants to satisfy their healthcare requirements.

Vernonia amygdalina (family of *asteraceae*) is a valuable medicinal plant that is widespread in East and West Africa[2]. It is known as bitter leaf and may be used as active anti-cancer[3], anti-bacteria, anti-malarial, and anti-parasitic agent[4]. This plant contains complex active components that are pharmacologically useful. The roots and the leaves are used in ethnomedicine to treat fever, hiccups, kidney problems, and stomach discomfort[5]. The stem and root divested of the bark are used as chew-sticks in many West Africa countries like Cameroon, Ghana, and Nigeria. *Vernonia amygdalina* (VA) leaves are one of the most widely leaf vegetables consumed by Cameroonians during special occasions such as marriages, baptisms, Christmas, and birthday. Pharmacological studies have also shown that the leaf extracts have both hypoglycaemic and hypolipidaemic properties in experimental animals and so could be used in managing diabetes mellitus[6]. Traditional medical practitioners, herbalists, and local healers in West Africa recommend aqueous VA for their patients. The beneficial use of VA in animal nutrition in Nigeria has been well documented[7,8],[9] reported that VA leaf extract enhanced the prophylactic and therapeutic efficacy of chloroquine against *Plasmodium berghei* malaria in mice. However, the unrefined nature of the herbal preparations, coupled with the apparent lack of specificity or precision in the application of the plant in traditional medicine could lead to over dosage of the herbal

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medicine, which can result in accumulation of essential and non-essential plant ingredients in the human system. The accumulation can reach a toxic level, especially in the systems of people who rely heavily on unrefined herbal products, with severe consequences on their biochemical and genetic systems.

Sniper 1000EC is an insecticide and acaricide that acts after coming in contact with plants and animals and is a stomach poison acting as fumigant and insecticide. The insecticide is used to control pests in households, stored product insects, mushroom flies, aphids, spider mites, caterpillars, thrips, whiteflies in grass house crops, outdoor fruits, vegetables, and weevils in stored grain. In Nigeria, due to increase in resistance to insecticides by disease vectors, there is an upsurge in the use of sniper 1000EC for indoor residual spray due to its quick knockdown effect. Hence the need to evaluate the genotoxic potentials of both widely used substances to which humans are exposed.

Allium test is a sensitive test that has often been used for the determination of cytotoxic and/or genotoxic effects of various substances[10,11]. The test has been shown to have a good correlation with tests in other living systems; hence, results obtained from Allium test are usually handled with care, because it could serve as an indicator of toxicity of the test materials[12].

Materials and methods

Preparation of *V. amygdalina aqueous* leaf extract

Leaves were collected from different stands of *V. amygdalina* growing in one location at Ijegun area in Lagos State Nigeria. The leaves were rinsed and air-dried at room temperature by spreading them on a laboratory table for 7 days. They were ground to a mesh size of 1mm. 400 g of powdered material was extracted with 1 litre of distilled water. The resultant formulation was filtered with whatman No. 1 filter paper and evaporated to dryness with the aid of a vacuum oven at 40°C. Ten (10) grams of the dried residue was dissolved in 500ml of distilled water to give a concentration of 0.02g/ml[13]. The following concentrations were then prepared from this stock solution: 5, 10, 20, 40 and 60 percent of aqueous solutions.

Preparation of Sniper 1000EC (2,3 dichlorovinyl dimethyl phosphate.)

The Sniper 1000EC was purchased from a shop in Lagos. The aerosol content of the 100ml container was poured into a tightly- fitted glass bottle. Aliquots were dissolved in appropriate volumes of distilled water to give concentrations of 0.06, 0.125, 0.25, 0.5 and 1 percent of aqueous solutions.

Preliminary tests

A series of baseline tests were carried out to establish a concentration range for root growth. Based on these results, a more elaborate experiment was carried out for the two test compounds.

Tests procedure

The modified Allium cepa test was used. A series of twelve onion bulbs of three per series was prepared. They were prepared by removal of the outer scales and brownish bottom plate. The onions were placed on top various concentration standards established for the two tests. A series of three onion bulbs was similarly prepared and maintained on distilled water as control. The ten onions that appeared to be developing the best in each series were selected for examination. The temperature range for this experiment was between 25^0C and 28^0C and was protected against sunlight.

The test and control samples were changed every 24 hours. After 48 hours, one root tip from each bulb was harvested, fixed and macerated in a solution of 9 parts 45% acetic acid and 1 part INHCL for 5 minutes for microscopic studies. Microscopic slides were prepared according to the standard procedure for orcein staining, followed by processing for cytological studies. The mitotic index (MI) was then determined by examination of 500 cells per slide and calculated as number of dividing cells per 500 observed cells. Characterization of mitosis and chromosome aberrations was scored in 100 cells per slide. All slides were coded and examined blind. After 96 hours, the root length of each bulb was measured and recorded as percentage of the control.

Data management

Chromosomal aberrations were statistically analyed using the chi- square test. The root length of each bulb was measured . The mean lengths of root bundles were obtained after 96 hours and EC_{50} values at 95% confidence interval were determined from a plot of root length against the sample concentrations using Microsoft Excel software.

Results and discussion

Macroscopic effects

It was observed that root growth inhibition is concentration dependent. Root growth inhibition increased as concentration of test substances increased. A total cytotoxic effect was induced by 2% concentration for Sniper and 70% concentration for VA. Effective concentration (EC_{50}) calculated were as follows: VA- EC_{50} =33.07 , and Sniper- EC_{50} = 0.346 (Figure 1 and 2).

Figure 1: Growth curve of Allium roots (in relation to control) after 96 hours cultivation exposure to different concentrations of *Vernonia amygdalina* extract.

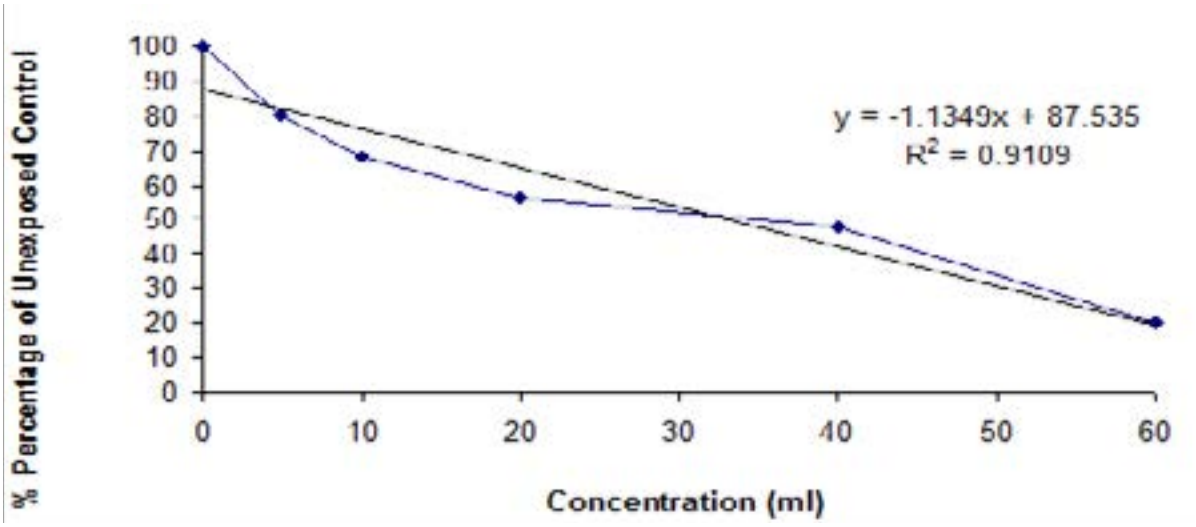
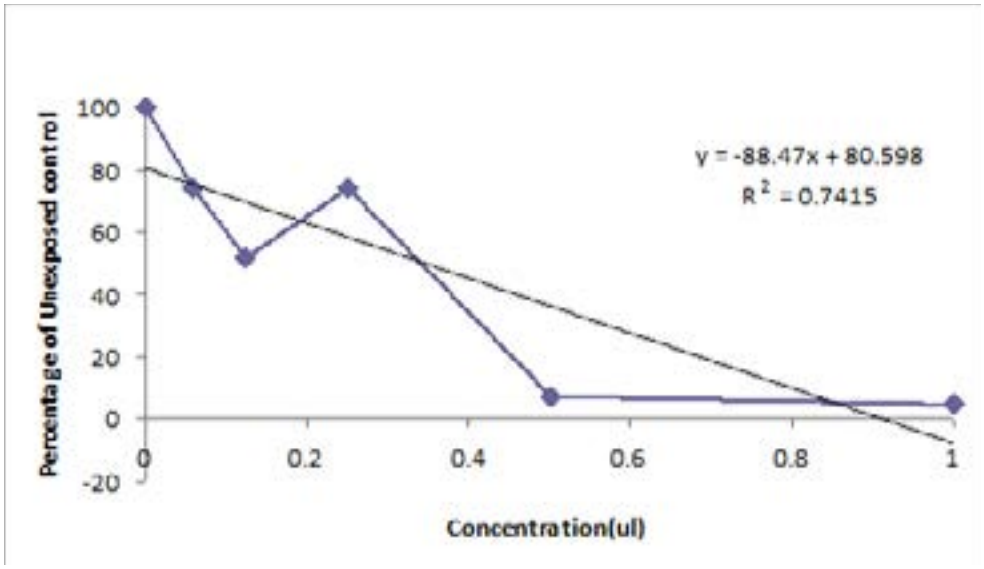


Figure 2: Growth curve of Allium roots (in relation to control) after 96 hours cultivation exposure to different concentrations of Sniper.



Microscopic effects

Table 1 summarises the results of the microscopic observations. The results showed decrease in mitotic index with increasing concentration of test substances. The decreases in mitotic index and number of mitosis scored are a reflection of the mitotic damage in the root tips. It was not possible to obtain as many as 500 mitosis for cytological screening in any of the test concentrations except in the control.

Table 1: Effect of treatment with *Vernonia amygdalina* and Sniper (2,3 dichlorovinyl dimethyl phosphate) in *Allium* test

Conc. (%)	Root length (% of control)	Mitotic Index	No of cells	Dividing cells	prophase	metaphase	Anaphase	Telophase	Chromosomal Aberrations							Total aberration(%)
									stickiness	c-mitosis	Bridges/ fragments	vagrant	Binucleus	Multipolar anaphase	translocation	
VA																
0	100	9.4	500	47	7	11	10	19	0	0	0	0	0	0	0	0
5	80	8.4	474	40	2	14	12	12	5	0	13	5	0	0	0	24.7
10	68	7.6	459	35	4	13	8	10	6	1	7	8	0	0	0	27.9
20	56	6.8	470	32	2	12	12	6	6	0	4	7	0	1	0	19.4
40	48	5.4	446	24	2	10	6	6	4	0	3	3	1	0	0	11.8
60	20	4.7	449	21	1	8	4	8	7	0	3	4	0	1	1	16.1
Sniper (2,3 dichlorovinyl dimethyl phosphate)																
0	100	9.4	500	47	7	11	10	19	0	0	0	0	0	0	0	0
0.06	74	8.1	454	37	1	15	10	11	5	0	7	11	0	0	0	27.4
0.125	52	6.9	452	31	2	9	7	13	8	1	5	5	1	0	0	23.8
0.25	74	5.5	434	24	2	7	9	6	7	0	5	6	0	0	1	22.6
0.5	7.4	6.4	437	28	5	9	6	6	5	0	3	6	0	0	0	16.7
1	5	2.3	394	9	1	3	3	2	2	1	3	2	0	0	0	9.5

The two substances induced chromosomal aberrations. The aberrations observed included stickiness, c- mitosis, vagrant chromosome, bridges/fragments, binucleus, multipolar anaphase and translocation (Figure 4 and 5). Sticky chromosomes are indications of a highly toxic usually irreversible effect, probably leading to cell death while in anaphase bridges and fragments are the results of chromosome and chromatid breaks. C- mitosis indicates weak, potentially reversible toxic effects.

The test substances (VA and Sniper) display to a great extent degree of toxicity which is clearly shown from the results presented in Table1, Figures 1,3 and 4.

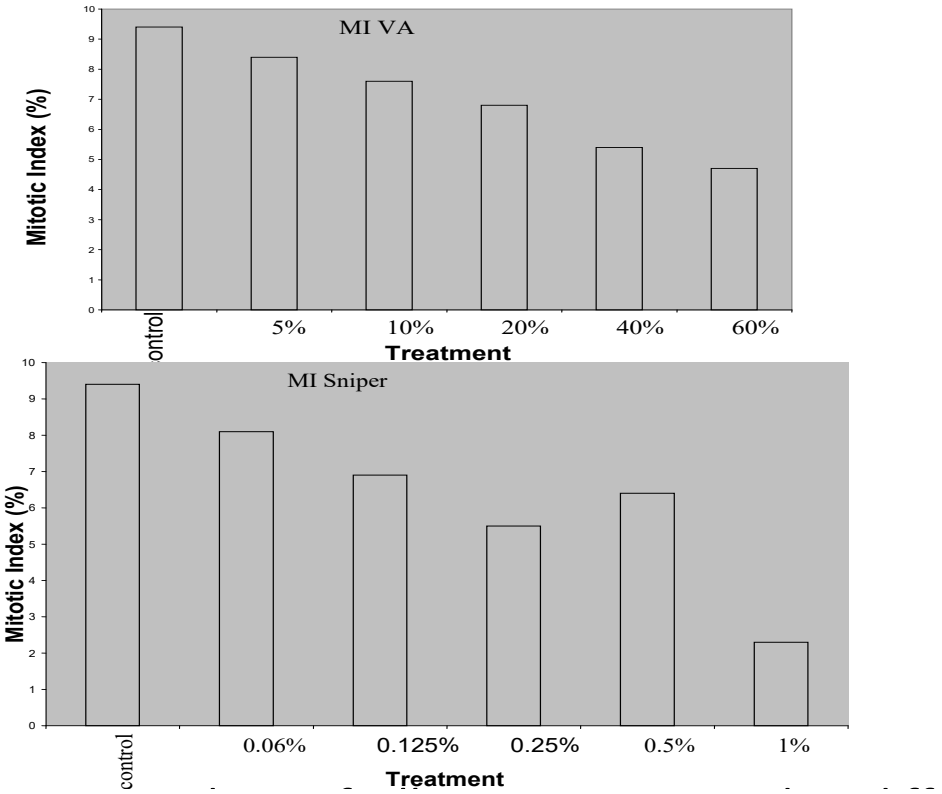
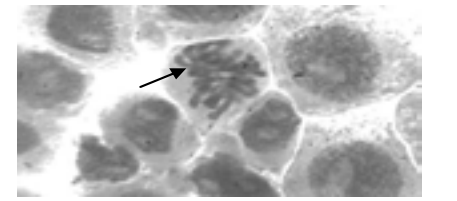
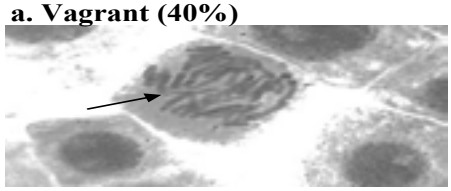


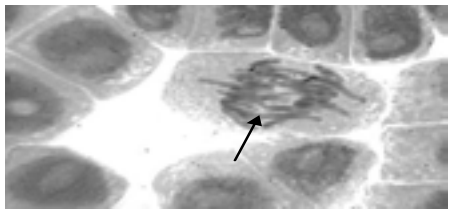
Figure 3: Mitotic indices of *Allium* roots exposed to different concentrations of VA and Sniper.



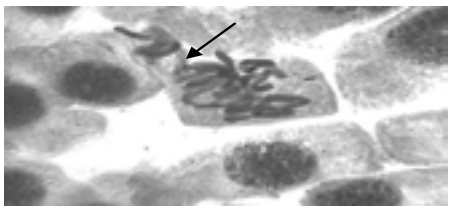
a. Vagrant (40%)



b. Multipolar (40%)



c. Bridge (60%)

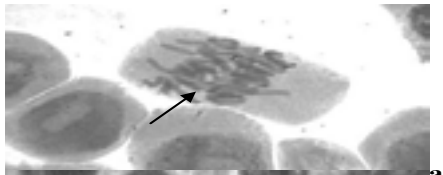


d. Translocated Chromosome (60%)

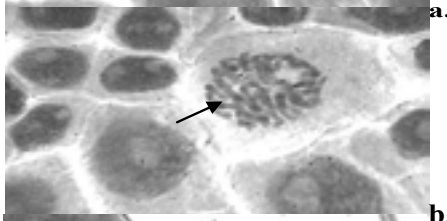
Figure 4 (a- d):Examples of microscopic effects on *Allium* roots exposed to different concentrations of *Vernonia amygadalina* (VA).

EC_{50} values gives information on general toxicity thus a possible comparison between the compounds. However, two aspects of toxicity were studied; general toxicity- estimated by percentage roots growth inhibition and genotoxicity- assessed by cytological studies of the chromosomes in roots tips. The growth curves for the two tests substances have a more or less sigmoid appearance, indicating a fundamental similarity in dose response. There was no root growth in onion bulb grown in 100% VA and Sniper samples. Root growth was achieved in concentration below 70% and 2% for VA and Sniper respectively.

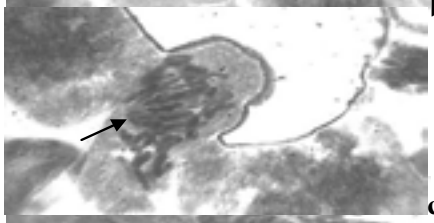
The two test substances studied induced a number of chromosome aberrations. Translocation was induced in 60% concentration of VA as well as in 0.25% of Sniper. Multipolar anaphase in 20% and 60% VA. Binucleus in 40% VA and in 0.125% Sniper. Also C-mitosis was induced by 10% VA and 0.125% and 1% Sniper. In addition to these, other chromosome aberrations; Stickiness, bridges/fragments, vagrant were mostly observed in both test substances. Highly toxic substances have been shown to induce such aberrations in the Allium[12,13]. These two substances were mitodepressive and the effect was concentration dependent.



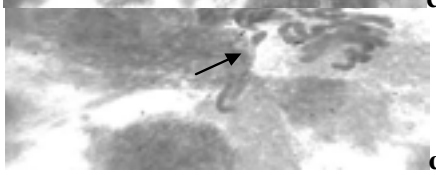
a. Bridge(0.5%)



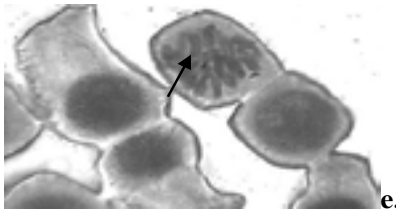
b. C- mitosis (0.06%)



c. Fragmented (0.125%)



d. Translocated chromosome (0.25%)



e. Vagrant (0.06%)

Figure 5 (a- e):Examples of microscopic effects on *Allium* roots exposed to different concentrations of 2,3 dichlorovinyl dimethyl phosphate (Sniper).

However, there was correlation in the frequency of chromosomal aberration and concentration of test substances. There was a decrease in the frequency of chromosomal aberrations at higher concentrations. Explanation for this could be that, with increasing concentration and consequently increasing toxicity, there was an inhibitory effect on cell division.

These results obtained are indicative of a probable risk of these test substances; which are widely used for therapeutic purposes (VA) or for environmental/ household use (Sniper); to the environment and human health.

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Conflict of interest

The authors however declare of no conflict of interest.

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