INVESTIGATIONS OF ACTIVITIES OF ALKALOID OF TRIFOLIATE YAM (*DIOSCOREA DUMETORUM,* (KUNTH) PAX.

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ABSTRACT

Alkaloid was extracted from the tubers of *Dioscorea dumetorum* by a procedure that consisted of acidification with dilute HCl, basification with dilute ammonia solution and extraction with chloroform. It was investigated for its cytotoxic, genotoxic and allelopathic activities on *Allium cepa* roots, tomato seeds and bean seedlings with a view to studying its safety or otherwise as famine food. The allelopathic, cytotoxic and genotoxic activities of the alkaloid were investigated using tomato seeds, bean seedlings and *A. cepa* assay techniques. The thin layer chromatography (tlc) of the alkaloid on pre-coated silica gel 60 F_{254} gave only one spot implying that alkaloid contained in trifoliate yam was chromatographically pure. The allelopathic studies revealed that the alkaloid did not have any significant effect on tomato seed germination, and root and shoot lengths of bean seedlings. The alkaloid caused slight reduction in the amount of chlorophyll a, chlorophyll b and total chlorophyll and elevated the levels of total proteins, soluble sugars and proline in bean seedlings. Mitotic Index decreased with increased alkaloid concentrations but no chromosomal aberration was observed. It is concluded from this study that alkaloid from *D. dumetorum* was cytotoxic but not genotoxic.

Key words: Cytotoxic, Genotoxic, Allelopathic, *Dioscorea dumetorum,* Chromosomal aberration, Mitotic index.

INTRODUCTION

Yams are staple tubers of West African origin. Botanically, they are flowering plants of the family Dioscoreaceae (genus *Dioscorea*). There are about 600 species of yams in the world, found mainly in the tropical and warm temperate regions of the world. Some are cultivated for their edible tubers; while the wild species are valuable famine food, and other species are sources of drugs both in traditional and western medicines (Eka 1998, IITA 2006, Stephens, 2009). Some commercially and nutritionally important varieties of yam tubers are *D. rotundata* (white yam), *D. alata* (water yam), *D. bulbifera* (aerial yam), *D. opposita* (Chinese yam), *D. cayenensis* (yellow yam) and *D. dumentorum* (trifoliate yam).Yams play a significant role in the diet of most Africans, the Caribbean and South Pacific where they have been reported to represent about 12% of the food consumed (FAO 2008; Alozie *et al.,* 2009).

D. dumetorum is one of the six species of yam cultivated in Nigeria. It is an important food security crop and is mostly consumed in West Africa. It originated in tropical Africa and occurs in both wild and cultivated forms. Its cultivation is mainly in West and Central Africa especially Nigeria and Cameroon (Sefa-Dedeh and Afoakwa, 2002). It is easily identifiable by its trifoliate compound leaves that twine clockwise unlike most other yams of economic importance. The local names in Nigeria are: *Kosanrogo* in Hausa, *Ona* in Ibo and *Esuru* in Yoruba. Other common names are three-leaved (trifoliate) yam, bitter yam, cluster yam and sweet yam in Cameroon. In Yoruba tribe of Nigeria, the wild type is called *Esuru-Igbo* or *Gudugudu*. It is the most nutritious of the commonly cultivated yam species. It is a good source of carbohydrate, protein, vitamins and minerals when compared with other common species of yam (Alozie *et al*., 2010). The amino acid profile of the yam has been reported to be quite balanced in essential amino acids with slight deficiency in sulphur containing amino acids and lysine as the most limiting (Alozie *et al*., 2009). The tubers of both varieties (edible and wild) are processed by boiling; but in the case of the wild variety, the tubers are detoxified by slicing and soaking and boiling, sometimes with addition of salt, or the sliced tuber is tied in a jute sack and left in a running water for 3 days to remove toxic and or bitter compounds that are believed to be injurious to health (Alozie *et al*., 2009). The wild varieties are not normally eaten except at times of food scarcity; they are commonly used for pharmaceutical or medicinal purposes by African

and Asian people (McAnuff *et al.*, 2003). The crude extracts of *D. dumetorum* tubers have been reported to have hypoglycaemic effects (Undie and Akubue, 1986) and the hypoglycaemic principle has been reported to be due to dioscoretine (Iwu *et al.*, 1990). The bitter principle has been identified as the alkaloid dihydrodioscorine, while that of the Malayan species, *D. hispida*, is dioscorine. These are water-soluble alkaloids, which on ingestion, produce severe and distressing symptoms. Severe cases of alkaloid intoxication may prove fatal (McAnuff *et al.*, 2003).

In this study attempts were made to extract and study the cytotoxic, genotoxic and allelopathic activities of the alkaloid of *D. Dumetorum* on *Allium cepa*, tomato seeds and bean seedlings. This was with a view to investigating the safety of *D. dumetorum* for consumption as famine food.

MATERIALS AND METHODS

Plant Materials: Collection and Identification. Fresh yam tubers were obtained from Kiire Village, Lagelu Local Government, Ibadan, Oyo State, Nigeria. The plant was identified at IFE Herbarium and authenticated by Dr. H.C. Illoh, Department of Botany, Obafemi Awolowo University, Ile-Ife, Nigeria. Fresh tomato fruits (*Solanum lycopersicum*), bean seeds (*Vigna unguiculata* (L) Walp) and Onion bulbs (*Allium cepa*) were obtained from Sekona Market, Ede North Local Government Council Area, Osun State, Nigeria. Fresh yam tubers were washed under running water, peeled and sliced into small chips and oven-dried at 50°C for 6 h as described by Alozie *et al.* (2010). The dried yam sample was ground into powder in a domestic warring blender and kept in an air-tight container.

Reagents and Chemicals.
All reagents used were of analytical grade obtained mainly from the following Chemical Manufacturing Companies: British Drug House (BDH) Poole, U.K., Sigma Chemical Company, St. Louis, U.S.A., Fluka Chemical Company and Pharmacia Fine Chemicals, Upsalla, Sweden. Aluminium -backed (Analytical) Silica gel 60 F_{254} pre-coated plates (20x20 cm) were obtained from Lab. Tech. Chemicals, U.S.A.

Preparation of Methanol Extract
The methanol extract was prepared as described by Djilani *et al.* (2006). Yam powder (600g) was first defatted in hexane (1L), suspended in 1.5 L of 70% (v/v) methanol for 4 h, stirred occasionally and followed by filtration through double layers of cheese-cloth. The extraction process was repeated four times until the filtrate became clear. The filtrates were pooled, allowed to settle at room temperature and the upper clear layer was carefully decanted into a clean beaker. The cloudy layer was filtered, pooled and concentrated *in vacuo* to dryness to obtain the crude methanol extract (CME).

Extraction and Fractionation of Alkaloid. The extraction of alkaloid mixture was carried out according to a procedure that was based on the methods earlier described (Iwu *et al.*, 1990; Djilani *et al.*, 2006) with slight modifications. The methanol extract (120 g) was dissolved in 3% (v/v) HCl (400 ml), extracted with chloroform (800 ml) and the acidic fraction was basified with dilute ammonia solution to pH 9. The basic aqueous solution was then extracted with chloroform four times until the aqueous layer tested negative for alkaloids. The chloroform fractions were pooled and washed with distilled water to neutral pH. The alkaloid was purified further by re-dissolving the chloroform fraction in 3% (v/v) HCl and re-extracted with chloroform. The chloroform fractions were pooled and concentrated to dryness *in vacuo* to yield 9.2 g of partially purified alkaloid; representing about 7.67% of the crude extract.

Assay of Allelopathic Activity.
The allelopathic potential of the alkaloid was investigated on tomato (*Solanum lycopersicum*) seeds and bean (*Vigna unguiculata* (L) Walp) seedlings as described by Vidysagar *et al.* (2009) and Pucklai and Kato-Noguchi (2011). The tomato seeds were used for pre-emergent studies and bean seedlings for post-emergent.

Pre-Emergent Allelopathic Studies
The tomato fruits were thoroughly washed under running water, cut open, the seeds were removed,

and washed with tap water. The non-viable seeds floated on the water and were removed by scooping while the viable seeds air dried. The dried seeds were kept in a plastic container in a cool dry place till further use. The effect of the alkaloids on tomato seeds germination was carried out in two different ways.

(a). Viable tomato seeds (n=150) were soaked in different concentrations of the alkaloid solution (0, 100, 200, 300 and 350 µg/ml) for 24 h.; distilled water was used as control. The seeds were removed after the 24 h., and air dried in the laboratory. The treated seeds (50) in triplicates were grown in Petri-dishes lined with cotton wool soaked with distilled water for 7 d in the dark under laboratory conditions. Then, data on seed germination were recorded at an interval of 24 h. for 7 d.

(b). Viable dried tomato seeds (n=50) in duplicates were grown in Petri-dishes as described earlier in different concentrations of alkaloid solutions (0, 100, 200, 300 and 350 µg/ml). The percentage germination for each alkaloid concentration and control was calculated using the equation:

$$\text{Germination (\%)} = \frac{\text{Germinated seeds}}{\text{Total seeds}} \times 100$$

Post-Emergent Allelopathic Studies

Viable bean seeds (n=4) were grown in triplicate on sand tray, irrigated with distilled water for 5 d. On the 6th day, for a period of 7 d, the seedlings were wetted with different concentrations of the alkaloid (0, 100, 200, 300 and 350µg/ml) in triplicates with distilled water as control. On the 8th day, the seedlings were uprooted; the root and shoot lengths for each group were measured. The plants were thoroughly washed, wrapped in foil paper, labeled according to group for easy identification and kept frozen for further investigations. The percentage growth inhibition was estimated for each group as described by Pucklai and Kato-Noguchi (2011) using the equation.

$$\text{Inhibition (\%)} = [1 - (\text{sample}/\text{control})] \times 100$$

Estimation of Total Soluble Protein, Proline and Total Soluble Sugar Concentrations

The total soluble protein was estimated according to the method Bradford (1976) as modified by Pandey and Budhathoki (2007) and Qin *et al.* (2010). Proline content was estimated according to the method described by Bates *et al.* (1973), while the total soluble sugar concentration was estimated using anthrone-sulphuric acid reaction method as earlier described by Farhad *et al.* (2011).

Estimation of Concentrations of Pigments

Chlorophyll a, chlorophyll b and total chlorophyll in the leaves of bean seedlings were determined according to the procedure of Gross (1991) as reported by Farhad *et al.* (2011) and Pirzad *et al.* (2011). The amount of chlorophyll a, chlorophyll b and total chlorophyll were determined using the expressions below:

$$\text{Chlorophyll a (g/l)} = (0.0127 \times OD_{663nm}) + (0.00269 \times OD_{645nm})$$

$$\text{Chlorophyll b (g/l)} = (0.0229 \times OD_{645nm}) + (0.00468 \times OD_{663nm})$$

$$\text{Total chlorophyll (g/l)} = (0.0202 \times OD_{645nm}) + (0.00820 \times OD_{663nm})$$

Root Growth Inhibition Assay: Using *Allium cepa*

The *Allium* test was carried out according to a procedure that was based on earlier methods (Fiskesjo 1985; Rank and Nielson 1993; Adegbite and Sanyaolu 2009). Typically the outer scales of the commercial onion bulbs (n=20) were carefully removed, and the dry bottom plates scraped without destroying the root primordial. The bulbs were first seated in distilled water in the dark to initiate rooting under laboratory condition for 24 h, after which the best (n=15) in terms of root growth were selected. One (in triplicate) was placed in medium sized beakers filled with different concentrations of the *D. dumentorum* alkaloid (0, 100, 200, 300, and 350 µg/ml) for another 72 h in the dark with distilled water as control. The test solutions and the water in the control were replaced daily throughout the duration of the experiment. The lengths of 5 longest roots from each bulb were measured and followed by the estimation of the mean root length of each treatment. The percentage root growth inhibition was calculated as earlier reported (Pucklai and Kato-Noguchi, 2011). The

total soluble protein and proline concentrations in *Allium cepa* roots were estimated as described earlier for bean seedling.

Assay of Genotoxicity Activity in *Allium cepa* Root Tips

Root tips (n=10) from each onion bulb were fixed in acetic acid/ethanol (1:3 v/v) for 24 h in well labeled bottles at room temperature and later stored at 4°C for genotoxicity analysis. The fixed root tips (n=5) from each onion bulb were hydrolyzed in 1N HCl at room temperature for 3-5 min., and then rinsed with distilled water. Slides were prepared using quashing and staining technique and stained with a drop of FLP-orcein for 3-5 min. as described by Adegbite and Olorode (2002). Then, replicates (5) for each alkaloid concentration and the control were prepared. The slides were viewed under the light microscope and data on total cells scored, total dividing cells, and cells carrying chromosomal aberrations were counted from 10 microscope fields.

Statistical Analysis

Data for biochemical assays were expressed as mean ± SEM. Statistical analysis was performed by one way ANOVA. Turkey-Kramer multiple comparison test was used to ascertain differences between treatment groups. All analyses were performed using Graph Pad Instant 3 (version 1.1, 2007).

RESULTS

Phytochemical screening of crude methanol extract confirmed the presence of alkaloid, flavonoid, saponin, tannins, terpenoids, and amino acids. Thin layer chromatographic analysis of the crude alkaloid on pre-coated silica gel 60 F_{254} plate using solvent mixture, MeOH : $CHCl_3$: NH_4OH (10 : 0.1 : 0.15 v/v/v) as mobile phase gave only one spot which implied that it might contain only one type of alkaloid (Plate 1). The alkaloid was adjudged chromatographically pure.

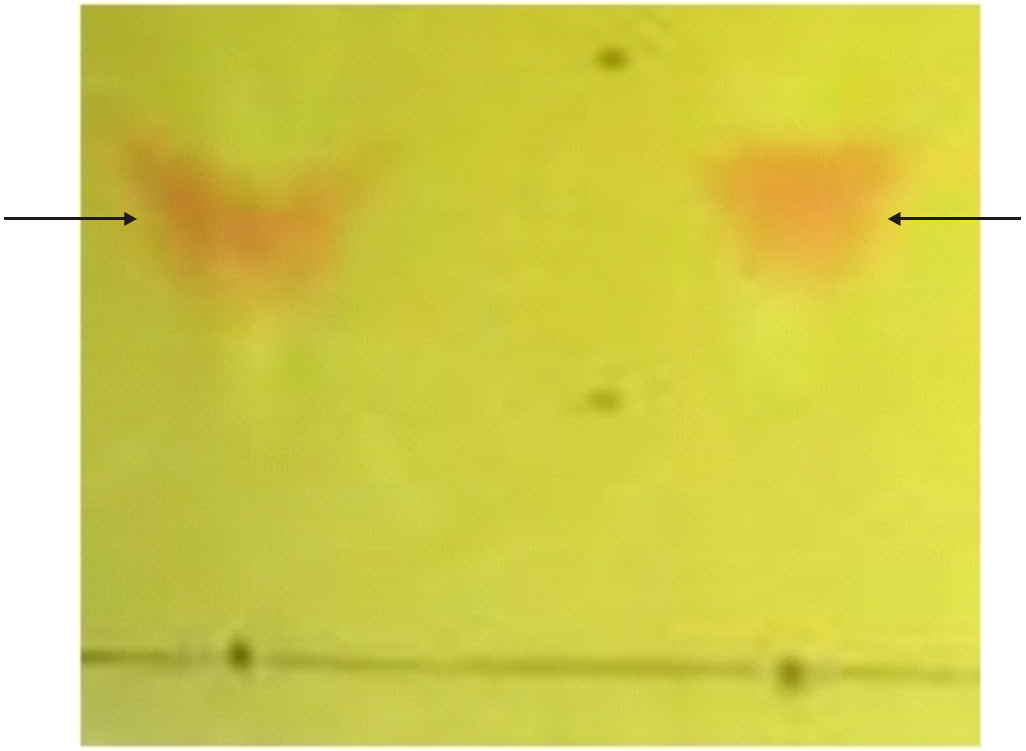


Plate 1: Chromatogram of Isolated Alkaloid from Tuber of *Discorea dumetorum* using (MeOH : $CHCl_3$: NH_4OH (10 : 0.1 : 0.15 v/v/v)) as solvent system. The chromatogram was sprayed with Dragendorff's reagent

→ = the alkaloid spots

Allelopathic Effects of *Dioscorea dumetorum* **Alkaloid**

The alkaloid did not have significant effect on the germination of tomato seeds (Table 1). Also it did not have any significant effect on the shoot and the root lengths of the bean seedlings (Table 2). In Table 3, the alkaloid caused significant increase in protein concentrations ($p < 0.05$) up to 286%, 257%, 368% and 379% respectively at 100, 200, 300 and 350 μg/ml alkaloid concentrations. Also, there was a slight increase in proline concentrations by 2%, 1%, 3% and 5% at 100, 200, 300 and 350 μg/ml alkaloid concentrations respectively; the increase was not statistically significant ($p<0.05$). The soluble sugar concentrations increased slightly by 9% and 12% respectively at 100 and 200 μg/ml concentrations of the alkaloid but at 300 and 350 μg/ml the increase was significant (54% and 56% respectively) ($p<0.05$). The pigments decreased slightly as the alkaloid concentrations increased; chlorophyll a by 5%, 12%, 20% and 21% respectively; chlorophyll b by 2%, 6%, 10% and 15% respectively and total chlorophyll by 3%, 6%, 13% and 16% respectively.

Table 1:Percentage Germination of Tomato Seeds

Conc. of Alkaloid (μg/ml)	No. of seeds	Pre-treated seeds		Seeds grown with alkaloid	
		No. of Germinated seeds	Percentage Germination	No. of Germinated seeds	Percentage germination
0	50	47	95	49	97
	50	48		48	
100	50	47	93	47	95
	50	46		48	
200	50	48	96	49	95
	50	48		46	
300	50	47	96	47	96
	50	49		49	
350	50	49	96	49	96
	50	48		47	

Table 2: Effect of the Alkaloid on Shoot and Root Lengths of Bean Seedlings.

Group	Conc. of alkaloid (μg/ml)	Shoot lengths (cm)	Root length (cm)
A	0 (Control)	10.75 ± 0.26	9.92 ± 2.05
B	100	9.27 ± 0.39	14.22 ± 1.13
C	200	10.17 ± 0.48	12.12 ± 1.5
D	300	9.90 ± 0.54	12.50 ± 1.79
E	350	10.72 ± 0.46	14.12 ± 1.07

Each value of the shoot and root lengths represents the mean of 4 readings ± SEM

Table 3: Effect of different Concentrations of *Dioscorea dumetorum* Alkaloid on the Concentrations of Chlorophylls, Protein, Soluble sugar and Proline of Bean Seedlings.

Conc. Of Alkaloid	Chlorophyll a (mg/g ft)	Chlorophyll b (mg/g ft)	Total chlorophyll (mg/g ft)	Protein (mg/g ft)	Total soluble sugar (µg/ml)	Proline (µmol/g ft)
0	4.08 ± 0.57	4.91 ± 0.55	5.52 ± 0.65	1.99 ± 0.08 3	.31 ± 0.37	0.127 ± 0.002
100	^{b}3.88 ± 0.48 (05%)	^{b}4.83 ± 0.58 (02%)	^{b}5.37 ± 0.65 (03%)	^{a}7.68 ± 0.04*** (286%)	^{a}3.61 ± 0.40 (09%)	^{a}0.129 ± 0.001 (02%)
200	^{b}3.61 ± 0.39 (12%)	^{b}4.60 ± 0.62 (06%)	^{b}5.06 ± 0.67 (06%)	^{a}7.11 ± 0.07*** (257%)	^{a}3.70 ± 0.51 (12%)	^{a}0.128 ± 0.002 (01%)
300	^{b}3.28 ± 0.34 (20%)	^{b}4.40 ± 0.63 (10%)	^{b}4.79 ± 0.66 (13%)	^{a}9.32 ± 0.03*** (368%)	^{a}5.11 ± 0.04* (54%)	^{a}0.131 ± 0.001 (03%)
350	^{b}3.24 ± 0.47 (21%)	^{b}4.18 ± 0.74 (15%)	^{b}4.64 ± 0.56 (16%)	^{a}9.53 ± 0.05*** (379%)	^{a}5.15 ± 0.02* (56%)	^{a}0.133 ± 0.003 (05%)

Each value represents the mean ± SEM of n = 4 readings
*Significant at $p<0.05$, *** Significant at $p<0.001$
g ft = weight in gram of fresh plant tissue
^{a} = Values greater than the control
^{B} = Values less than the control

Root Growth Inhibition Assay on *Allium cepa*

There were significant decreases in root lengths of *Allium cepa* as the concentration of the alkaloid increased. The percentage root growth inhibition also increased with increase in alkaloid concentrations (Table 4).

Effect of the Alkaloid on Protein and Proline Contents in *Allium cepa* Roots

At 100 µg/ml, there was a slight increase in protein concentration by 26%. At 200, 300 and 350 µg/ml concentrations of the alkaloid, the protein concentration increased by 108%, 67% and 44% respectively. The proline content also increased by 18%, 28%, 44% and 53% respectively as the alkaloid concentrations increased (Table 4).

Table 4: Effect of different Concentrations of *Dioscorea dumetorum* Alkaloid on Root lengths, Protein and Proline concentrations of *Allium cepa* roots

Conc. of Alkaloid µg/ml	Root length Mean ± sem	Percentage Growth inhibition	Protein (mg/g)	Proline (mg/g)
0	^{x}5.19 ± 0.19	0	^{y}30.63 ± 4.27	^{y}0.79 ± 0.246
100	^{x}3.94±0.08***	24.08	^{y}38.57 ± 3.17^{a}	^{y}0.93 ± 0.016^{a}
200	^{x}3.47±0.13***	33.14	^{y}63.61 ± 2.76^{a} ***	^{y}1.01 ± 0.045^{a}**
300	^{x}3.69±0.16***	28.90	^{y}51.13 ± 3.29^{a} **	^{y}1.14 ± 0.042^{a}***
350	^{x}3.29±0.12***	36.60	^{y}43.96 ± 3.54^{a} *	^{y}1.21 ± 0.047^{a}***

Percentage growth inhibition was calculated as 1- (sample/control) x 100
^{x}Value represents the mean ± SEM of 10 readings
^{y}Value represents the mean ± SEM of 3 readings
*Significant at $p<0.05$, ** Significant at $p<0.01$, *** Significant at $p<0.001$
mg/g = milligram per gram of fresh tissue
^{a} = Values greater than control
^{b} = Values less than control

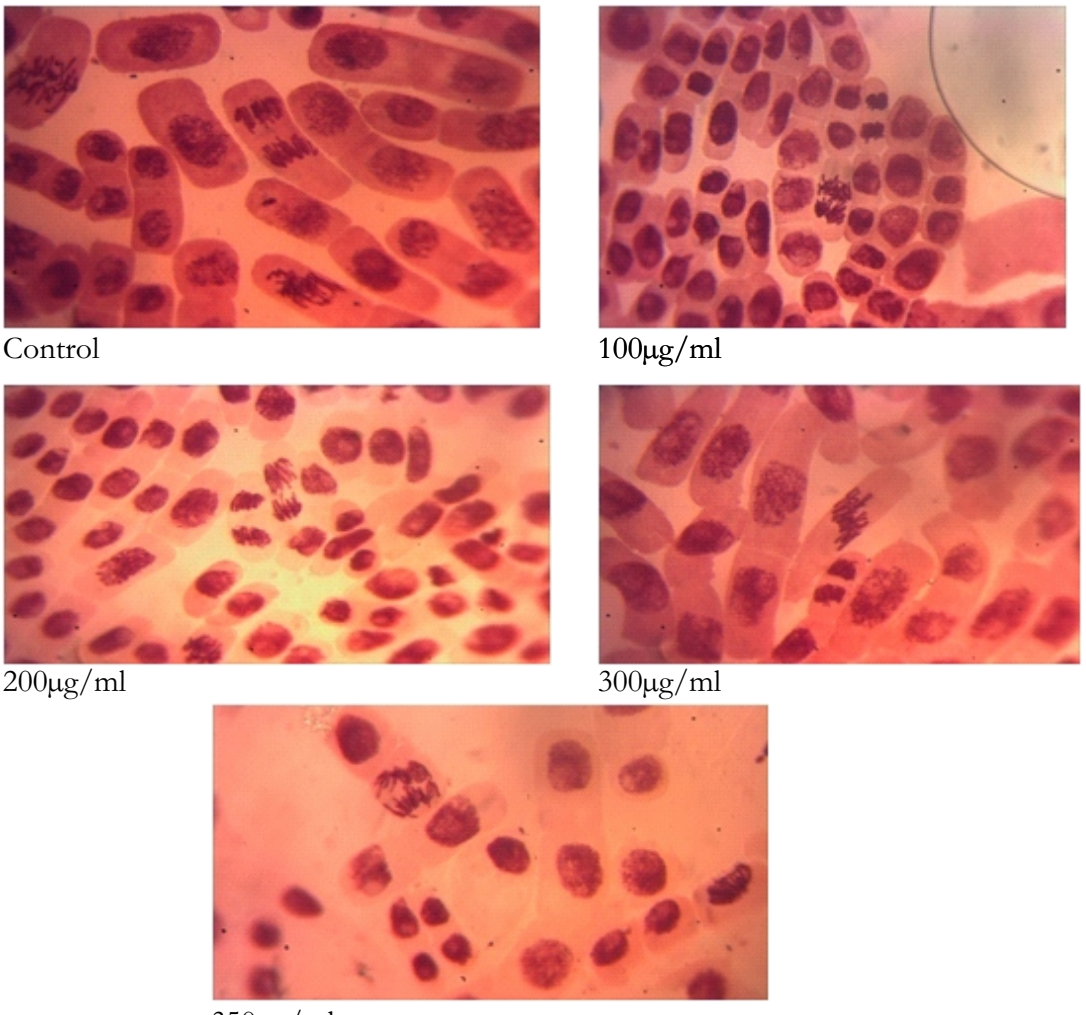
Genotoxic Effects of the Alkaloid on *Allium cepa* **Roots.**

No chromosomal aberration observed in any of the cells in each of the alkaloid concentrations (Plate 2), but there was a gradual decrease in the total dividing cells and mitotic index as the alkaloid concentration increased from 100 to 300µg/ml; but at 350µg/ml there was a slight increase in Mitotic Index (Table 5).

Table 5: Effect of Alkaloid Concentrations on Mitotic Index of *Allium cepa* Root.

Alkaloid Conc. (µg/ml)	Prophase	Metaphase	Anaphase	Telophase	Total Dividing cells	Total counted cells	Mitotic index
0	20	17	5	17	59	364	16.21
100	18	13	4	7	42	399	10.52
200	15	12	4	5	36	418	8.61
300	15	11	5	2	33	499	6.61
350	16	18	11	3	48	510	9.41

Data were obtained from 10 microscopic fields.
Mitotic index was calculated as: (number of dividing cells/total cells counted) x 100



Control 100µg/ml 200µg/ml 300µg/ml 350µg/ml

Plate 2: Photomicrgraph of mitotic divisions in *Allium cepa* root cells grown in water (control) and different concentrations of *Dioscorea dumetorum* alkaloid solutions; no chromosomal aberration was observed in any of the cells (control and the treated).

DISCUSSION

Thin layer chromatographic analysis of the chloroform fraction of the extract of *D. dumetorum* tuber on precoated silica gel plate gave only one spot; suggesting the presence of one type of alkaloid. This is in agreement with the observations of David and Michael (1985) who had earlier isolated a convulsion- causing alkaloid called dihydrodioscorine, a toxic alkaloid which triggered fatal paralysis of the nervous system (Bhandari and Kawabata, 2005).

Allelopathy refers to the beneficial or harmful effects of one plant on another plant around it by the release of biologically active compounds often referred to as allelopathics, allelochemicals or allele-compounds by leaching, root exudation, volatilization, residue decomposition and other processes in both natural and agricultural systems (Ferguson and Rathinasabapathi, 2009; Prasanta *et al*., 2003). There was no significant difference in percentage germination between the pre-treated tomato seeds with different concentrations of the *D. dumetorum* alkaloid and the control. Also, there was no significant difference in percentage germination between the tomato seeds grown with different concentrations of the *D. dumetorum* alkaloid and seeds grown with distilled water (control). Moreover, the *D. dumetorum* alkaloid did not exert significant effects on the root and shoot lengths of the bean seedlings (p 0.05). The effect of the alkaloid was more on the roots than the shoots. This observation agrees with the earlier reports of Krishnen and Kumori (2008) and Salam and Kato-Noguchi (2010) that extract of allelopathic plants and N-triacontanol had more inhibitory effect on root growth than hypocotyl growth because root was the first organ to absorb allelochemicals from the environments. Nishida *et al.* (2005) also reported that the permeability of allelochemicals to root tissue was greater than to shoot tissue.

There was a slight decrease (though not statistically significant, p 0.05) in chlorophyll levels in alkaloid- stressed plants. The slight decrease might not be unconnected with a number of factors such as decreased nitrogen availability (Parasher and Verma, 1993) and enhanced activity of the enzyme chlorophyllase (Ready and Vora, 1986). According to Pandolfini *et al.* (1992), the reduction in chlorophyll content could be due to inhibition of root and shoot growth, photosynthesis, nutrient uptake, leaf area and biomass.

The total soluble sugar content in the leaves increased significantly (p 0.05) as *D. dumetorum* alkaloid concentration increased. This is in consonance with the report of Farhad *et al*., (2011), that soluble sugar content in leaves significantly increased under environmental stress conditions. Mohammadkhani and Heidari (2008) also reported a significant increase in soluble sugars and significant decrease in starch in two maize (*Zea mays*) cultivars subjected to drought stress. This suggests that sugars may play an important role in osmotic adjustment in maize and probably also in bean seedling. The increase in sugar concentrations might be as a result of degradation of starch (Fischer and Holl, 1991). The accumulation of sugars in response to drought stress is well documented (Yancey *et al*., 1982; Kameli and Losel, 1993; Hakimi *et al*., 1995) and might function as a typical osmoprotectant, stabilizing cellular membranes and maintaining tugor pressure (Farhad *et al*., 2011). Other essential roles of soluble sugars in plant metabolism include substrates in biosynthetic processes, energy production and also in sugar sensing and signalling systems (Mohammadkhani and Heidari, 2008). It has also been reported that sugar flux might be a signal for metabolic regulation (Gibson, 2005).

According to Pirzad *et al.* (2011), under conditions of environmental stress (salinity, drought, high and low light), plants show ingenious adaptations at the physiological level, probably triggered by changes in various gene expressions. For example, biosynthesis of proline, a well-known osmo-protectant is accumulated in many plant species under various stress conditions (Delauney and Verma, 1993) which is a physiological response to osmotic stress (Szekely, 2004). Many solutes are involved in osmotic adjustment. Inorganic ions like Na^+, K^+ and Cl^- accounted for most of the osmotic potential in several species (Ford and Wilson, 1981), while sugars and amino acids especially proline are the major osmo-regulators

in vascular plants (Ruban *et al*., 1996; Adamska, 1997; Montane *et al*., 1997). This might probably be because of the convenience of osmolyte storage in large osmotically inactive molecules such as starch or protein, which serve several functions and from which they could be retrieved under conditions of stress (Pirzard *et al*., 2011). Proline acts as a hydrophobic protectant for enzymes and subcellular organelles (Lerudulier *et al*., 1994). In this study, the proline content of the bean seedling increased slightly with increase in *D. dumetorum* alkaloid concentration, while that of *Allium cepa* roots increased very significantly (p 0.001). These could be as a result of the stress produced by the alkaloid. Accumulation of proline in plants due to drought and temperature stress had been well documented (Gzik, 1996).

The protein concentrations increased significantly (p 0.001) in bean seedlings and also in *A. cepa* roots treated with *D. dumetorum* alkaloid the increase was directly proportional to alkaloid concentrations. This agreed with the observation of Azooz *et al*. (2011) that application of salicyclic acid stimulated soluble protein biosynthesis in broad bean seedlings grown under 25% sea water irrigation with a corresponding decrease in free amino acid concentrations. According to Hameed and Ashraf (2008) and Jaleel *et al*. (2008), soluble proteins may increase or decrease under salt stress. However, Batcheller and Romeo (1992) reported no change in soluble proteins under salt stress in *Spartina alternifolia*. Mommensen and Walsh (1992) reported that proteins are mainly involved in the architecture of plant cells, which were the chief source of nitrogenous metabolism and as well a source of energy during chronic period of stress. Singh *et al*. (1996) also reported that the quantity of protein depends on the rate of synthesis or its degradation. This might suggest that the alkaloid possibly stimulate protein biosynthesis in bean seedlings and also in *A. cepa*.

Mitotic index (MI) is a parameter that is often employed to estimate the frequency of cellular division (Marcano *et al*., 2004, Mustafa and Arnkon, 2008). The suppression of mitotic activity was often used in tracing and evaluating cytotoxic substances (Linnainmaa *et al*., 1978; Smaka-Kinel *et al*., 1996). In this study, decrease in mitotic index values observed coupled with the significant decrease in root lengths of the *A. cepa* when compared with the control, were indications of cytotoxicity of the *D. dumetorum* alkaloid. There was no chromosomal aberration in any of the onion meristematic cells (control and treated) and this could be an indication that the alkaloid was neither genotoxic nor mutagenic. This observation agreed with that of Adegbite and Sanyaolu (2009) that aqueous extract of *V. amygdalina* at a low concentration (100g/l) caused a reduction in mitotic index but did not induce any chromosomal aberration.

Conclusively, the observations by Oliver-Bever, (1989) and Okwu and Ndu, (2006) that *D. dumetorum* contains toxic alkaloid have been confirmed by the results of this study. The inhibition of roots growth coupled with decrease in Mitotic Index in *A. cepa* meristematic cells were proven evidences of cytotoxicity of the alkaloid. The alkaloid did not elicit any significant effect on germination of tomato seeds, thus the alkaloid may not be useful as a pre-emergent biological herbicide. The alkaloid also affected some metabolites in bean seedlings which are markers of environmental stress, further attesting to the cytotoxicity of the alkaloid.

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