domesticated. In Nigeria, three of the five domesticated species, namely, Capsicum annum L. (Tattasi group), Capsicum frutescence L. (Borkuno group) and Capsicum chinense Jacq. (Attarugu group) grow well in many communities. In the North Eastern sub region, peppers are grown by almost all local communities and it constitutes an important seasoning spice for most adult foods. Some of the communities from where seeds were collected claim to have cultivated this crop for time immemorial. The usually upright, herbaceous shoots of the Capsicum plant bears simple, estipulate leaves of entire margin with bisexual flowers whose corolla is white, milky white or milky green. The berry which ripens to red, yellow, orange or white bears shapes which are of sub specific categories.

Since the lunching of the Human Genome project in the United States, many researches are ongoing in a view to understanding the effect of chromosome abnormalities. Man's quest for food has landed him into what he naturally thinks is the best source of nutrition without minding if there is any side effect of over or under consumption of such food material. The use of Ash potash in cooking various foods particularly soup in North East region of Nigeria is a common phenomenon. However Cytogenetic information on the possible long-term side effect of consumption of Ash potash and its mutagenic effect are not readily available. Hence this study has provided information on safe consumption doses for Ash Potash.

MATERIALS AND METHODS

The materials predominantly used are the **Ash Potash** and ***Capsicun frutensence*** seeds. Ene-Obong & Usualla 1990 & Morris 2012, method was adopted in the research.

Procuring Ash Potash

Solidified Ash potash (concentrate) weighing about 1kg was purchased from market women at Fadama Rake village along Hong-Mubi road of Adamawa State. The potash was said to be obtained from the decantation and evaporation of dissolved Ash potash of corn stalks and corn chaffs.

Different measurements of 5g, log, 15g, 25g, of the solid potash was each dissolved in 100ml of distilled water to make up 5%, 10%, 15%, 20%,25%, of the solution respectively.

Planting

Dried seeds of Capsicum frutescence were soaked into the various concentrations of Ash potash for 48hours as treatment and in tap water and EMS, as control all in petri dishes and labelled accordingly. Each seed treatment was planted in germinating pots and labelled subsequently. Watering of the germinating pots with tap water and EMS in the morning and the evening was done. This was to avoid too much coloration of the root tip and possible over accumulation of potash which could be lethal to seedlings.

Harvesting

After 3-6 days of planting, when roots were expected to be 2-2.5cm long, about 2cm of the growing root tip was excised using forceps and scissor giving consideration to bulgy and creamy tips. This was done between the hours of 10-1 lam during which the metaphase spread of the cells of the growing roots are active (Oyewole, 1984),

Pre-Treatment

The excised good root tips were washed with running tap water to remove any dirt. It was then immersed in 0.05% Colchicine for 5-hours, this allowed for chromosome contraction and arresting of mitosis at metaphase and at the same time making the cytoplasm more transparent. It was however aerated at interval using electric bubble aerator so as to replenish lost oxygen (Ene-obong & Usualla, 1990).

Fixation

The modified method of Ene-obong, & Usually (1990) & Morris (2012), was used. The roots that were previously washed with running tap water were fixed V/V in glacial acetic acid for 12hrs. This was intended to kill the material rapidly in a way that the internal structures were preserved in a life like form. This was later washed in 30% ethanol transferred into absolute ethanol for hardening, then stored in 70% ethanol at 4c in a refrigerator until required for cytological analysis,

CYTOLOGICAL ANALYSIS

Hydrolysis

Stored root tips were washed thoroughly in changes of distilled water for 15minutes. (5 minutes interval) these was then rinsed in warn HCI in quick

succession and hydrolyzed in HCI for 8-10 minutes in a water maintained at 60^0 C (Morris, 2012).

Staining

Using sterilized pair of forceps, the root tips were picked, placed on a glass slide and the meristermatic tip measuring about 2mm to 2.5mm were cut and the remaining discarded.

Acidition of 2% acetic orcein was followed and covered with a cover slip. Each meristematic tip obtained was then squashed using the blunt end of a pen. The squashing was to ensure the formation of single layer of spread cells, Excess stain was removed using blotting paper which was placed carefully at the edge of the cover slip. (Akinboro *et al*., 2007; Fiskejo 1985)

Observation under microscope at XI0, X40 and XI00 oil immersion objective was done to see if there were any chromosomal abnormalities. The observations were micro graphed with a digital camera.

RESULTS AND DISCUSSION

The results of the *Capsicum frutescence* root test response to different concentrations of Ash potash extract are presented in Table 1 and Fig. 1. In comparison with the distilled water control, dose-dependent reduction in C. *frutescence* mean root length that was significant at 10- 25 g/L but not significant at 5 g/L treated concentrations of Ash potash for 48 hours was observed, suggesting cytotoxicity (Fig. 1). Significant reduction in Mitotic index (MI) of the C. *frutescence* meristematic cells with increasing concentration of Ash potash after 48 h of exposure was also observed after 48 h of exposure when compared to the control except at 5 g/L in which the MI disparity with the control was not significant. Unlike EMS, which elicited chromosomal aberrations in meristematic cells of C. *frutescence*, no chromosomal aberrations were seen in C. frutescence placed in the distilled water control. However chromosomal aberrations, suggestive of clastogenicity and impaired tubulin biogenesis were observed in 17-51 of the 300 C. *frutencences* cells examined at each of the different concentrations of Ash potash. Highest and lowest numbers of aberrant cells were recorded.

Chromosomal Aberrations Caused by Ash Potash

A total of 300 mother cells were scored for each treated group, Ash potash did not show any chromosomal aberration at 5 % concentration, the metaphase spread of this treated group appeared to be normal when compared to the control with tab water (plate 1). At 10 % concentration of ash potash, out of the 300 mother cells scored, 200 mother cells appeared to be normal, 36 mother cells showed chromosome dissolution (plate 2), 20 mother cells showed clumping of chromosomes (plate 3), 17 mother cells appeared with chromosome contraction (plate 4) while 27 mother cells showed fragments (plate 5). At 15 % concentration, 44 mother cells appeared with fragments (plate 5), 36 mother cells showed chromatic gap (plate 6), 22 mother cells showed lagging chromosomes (plate 7) and 28 mother cells appeared with chromosome bridges (plate 8). At 20 % concentration of Ash potash, 48 mother cells showed endoreduplication (plate 9), 33 mother cells appeared with laggard (plate 7), 51 mother cells showed clumping (plate 3) and 28 mother cells appeared with fragment (plate 5). However, at 25 % concentration of Ash potash, most of the treated seeds did not germinate, this could be due high concentration of Ash potash in this treated group which hinders the germination. Out of the few seedlings that germinated, 29 mother cells showed stickiness (plate 10), 40 mother cells showed agglutination (plate 1 1), 43 appeared with chromosome bridges (plate 8) and 35 mother cells showed contraction of chromosomes (Plate 4).

Table1: *Chromosome Aberrations due to Ash potash at Different Concentration on C. frutescence Root tips.*

Treatment group (g/l)	No. of mother cells scored	Dissolution	Stickiness	Fragment	Condensation	Laggards	Chromatid gap	Bridge	Agglutination	Endoreduplication	Clumping	No. of abertant mother cells	No. of normal mother cells	Frequency of abnormation (%)
5	300	-	-	-	-	-	-	-	-	-	-	-	300	0.00
10	300	17	-	27	-	-	-	36	-	-	20	100	200	14.3
15	300	-	-	44	-	22	36	28	-	-	-	130	170	21.3
20	300	-	-	28	-	33	-	-	-	48	51	160	140	26.3
25	300	35	29	-	36	-	9	43	40	-	-	192	108	38.1

Source – Authors Computation 2021

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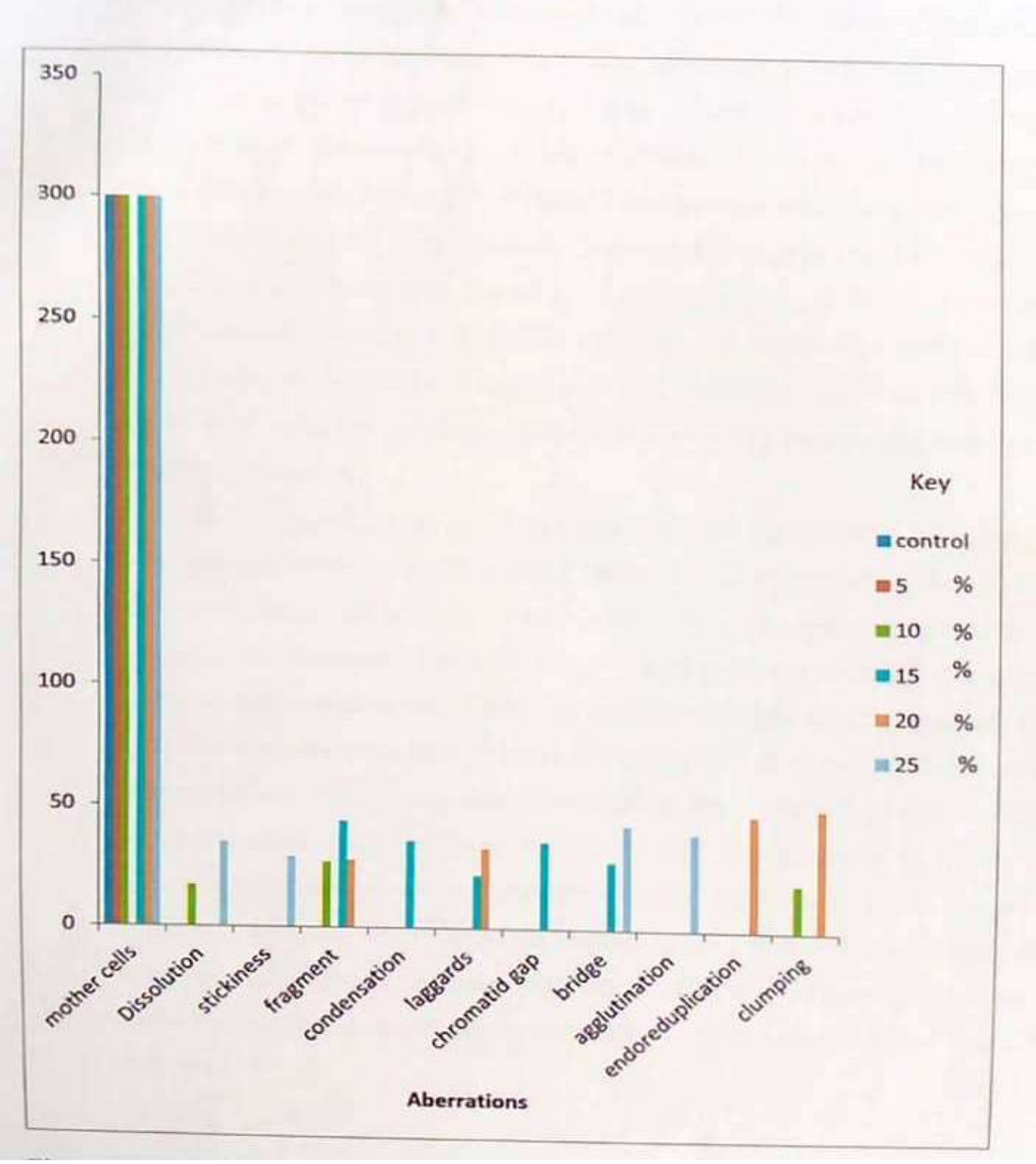


Source; Author's Computation 2021

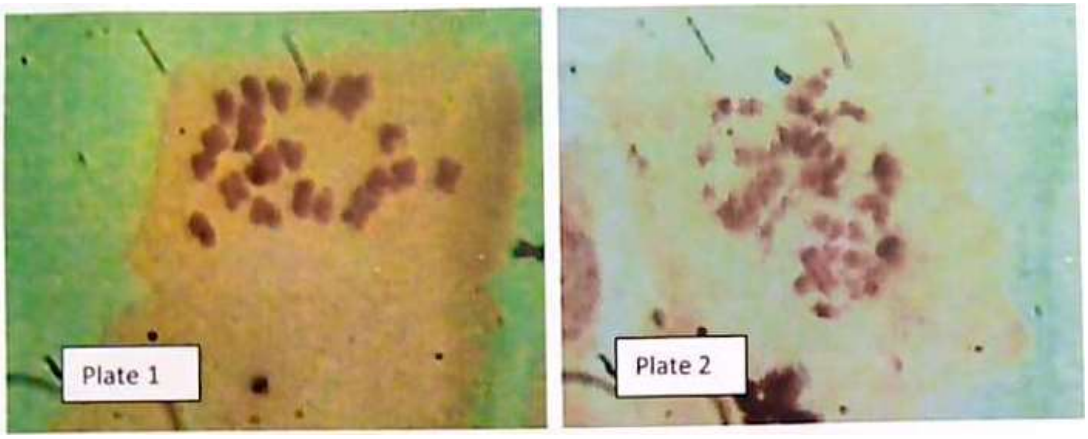
Fig. 1: *Types of Chromosomal Aberrations/Concentration of Ash Postash on C. frutescences Root Tips*

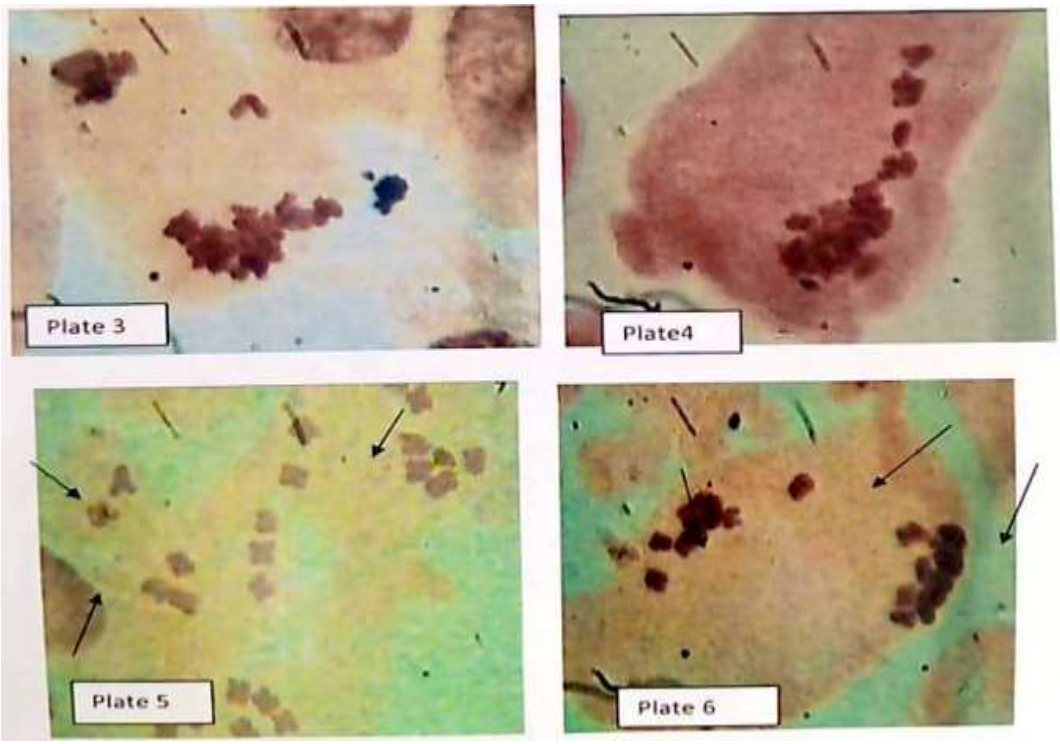
Comparative Analysis of the Effects of Ash Potash with the Control Groups.

Table I shows the comparative analysis of Ash potash with the negative and positive control. The negative control with tap water did not show any form of

aberration while the positive control showed high frequency of abberrations. At 5% concentration of EMS prepared with distilled water, the reagent revealed chromosomal stickiness, although not joined at their terminal ends. At 10% concentration, EMS revealed agglutinated and lagging chromosomes, the chromosomes were seen united as if joined by glue, at 15% concentration of EMS, the reagent showed dissolution, fragment and bridge chromosomes while at 20% concentration, more severe agglutination, bridge and ring chromosomes was observed which was an indication of induction of chromosome damage by this reagent and this can be referred to as chromosome poison.

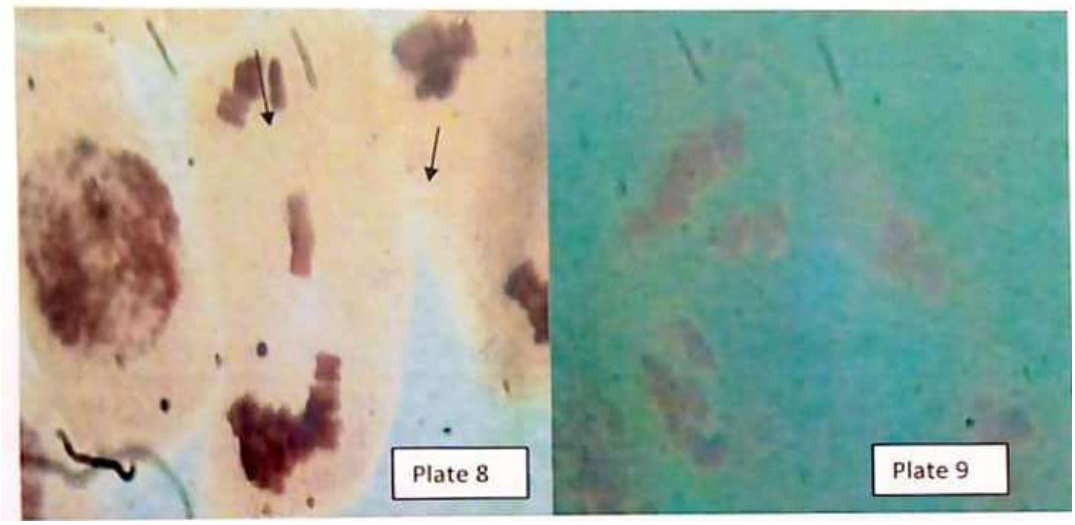
In the treated group with Ash potash, with the exception of 5 % concentration, all other tested concentrations (10-25), caused chromosomal aberrations. They aberrations observed were dose dependent and the most visible aberrations were chromosome contraction and condensation, fragments, laggards, bridges, stickiness, endoreduplication, ring chromosomes, clumping and agglutination. However, stickiness, laggards, ring chromosomes, bridges and fragments are indication of high level of toxicity in Ash potash, their appearance in this study characterize Ash potash as a potent clastogen at dose above 5 % concentration. Chromosome fragment results from multiple breaks of the chromosomes in which there is loss of chromosome integrity, endoreduplication is the duplication which occurs without nuclear division. Comparatively, some of the aberrations due to the effects of Ash potash are similar to those caused by Ethyl methane Sulphate (EMS), a well-known clastogen. Because of the similarities in effects of ash potash and EMS, it can be concluded that Ash potash behaves as a clastogen.

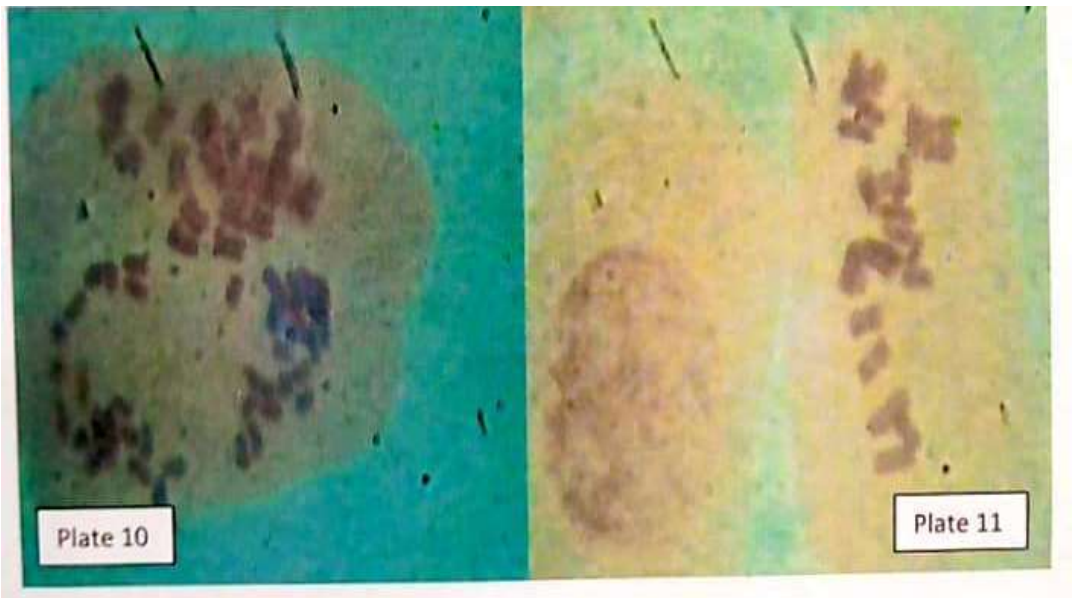




Source; Author's Computation 2021

Plates 1-6; Showing The Different Types of Abberrations
Plate 1: Normal metaphase spread, Plate 2: Dissolution, Plate 3: Clumping, Plate 4: Contration plate 5: Fragments, Plate6 Laggards and Chromatid gap,





Source; Author's Computation 2021

Pates8-11; Showing different types of abberrations
Plate8; Bridge, Plate 9; Endoreduplication Plate 10;
Stickness,
Platel l1; Agglutination.

CONCLUSION

The cytogenetic analysis of ash potash on root tips of *C. frutescence* indicates in vivo clastogenic, cytotoxic and tubergenic activity of Ash potash especially at higher concentration. These results from the plant assays provide strong evidence for Ash potash to be regarded as having a strong cytotoxic or mutagenic potential for man. Suggesting a need for safe dose administration for human consumption. The International Commission for Protection against Environmental Mutagens and Carcinogens had earlier written in 1985, that once plant data had been confirmed in a mammalian cell assay or from other eukaryotes, it may be prudent to designate the chemical in question as being potentially hazardous and that this may indicate that distribution and human exposure will be restricted. The Commission, however, suggested that it may be worthwhile to design further tests to either confirm this assessment or to determine the extent of the potential hazard if the chemical has huge positive potentials

RECOMMENDATIONS

1. The need for a safe dosage administration of Ash potash at long interval by human consumption is suggestive.
2. Ash potash can be used in low concentration at long or wide interval if it is to be used as a food additive in known dosage.
3. More research at the molecular level should be encouraged in this area so as to reveal the mutagenic potentials of ash potash used in evewday life for consumption purposes ensuring the revelation of the effects at the cellular level.
4. Government should assist in financing researches due to the capital intensive nature of cytogenetic research.

Further safety studies is recommended using any system, plant test system and chromosome aberration assays to determine the toxicity level of this Ash pot

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