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Genotoxic studies of cooked and uncooked processed spices using *Allium cepa* Test

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Abstract

Spices are commonly used in modern culture for varieties of delicacies; some spices are eaten raw, while others are cooked with the meal. However, as important as spices are in our everyday life, the methods through which some of them are being produced are questionable and as such may pose a significant health risk to the society. This study was therefore designed to investigate the genotoxicity of four well known spices (thyme, curry, suya and pepper soup) using *Allium cepa*assay, as well as to evaluate their nutrient value, microbial load and heavy metal contents. The spice caused a reduction in the mean root length in onion bulbs exposed to the treatment of both the cooked and uncooked forms of the spices. Analysis of variance showed that there was no significant difference ($p>0.05$) in the mean root length and mitotic indices of *A. cepa* exposed to different concentrations (1%, 5%, 25%, 50% and 100%) of the spices. The mitotic index was inversely proportional to the mitotic inhibition. The spices induced chromosomal aberrations such as vagrant, stick anaphase and telophase, bridges etc in exposed *Allium cepa*compared to control. Microbial evaluation of the spices showed that the suya and pepper soup spice had the most significant microbial load above the standardized permissible level. Proximate analysis of the spices showed that suya spice had the highest fat (14.85%) and protein (37.33%) content, while thyme had the highest moisture (12.45%) and crude fibre (18.70%) content. Heavy metal analysis showed that lead (Pb) was absent from of all the spices evaluated, while all the spice had cadmium contents above the permissible limit. However only suya spice was found to contain nickel (Ni); content above the permissible limit. The findings of this study therefore showed that the uncooked/ unprocessed spices are less safe than the cooked/ processed spices.

Keywords: Genotoxicity, *Allium cepa*, chromosomal aberrations, spice, heavy metals

Introduction

A spice is a seed, fruit, root, bark, berry, bud or vegetable substance primarily used for flavouring, colouring or preserving food (Czarra, 2009). Spices are distinguished from herbs, which are parts of leafy green plants used for flavouring or as a garnish. Many spices have antimicrobial properties, which may explain why spices are more commonly used in warmer climates, which have more infectious disease, and why the use of spices is prominent in meat, which is particularly susceptible to spoiling (Czarra, 2009). A spice may have other uses, including medicinal, religious ritual, cosmetics or perfume production, or as a vegetable (Turner, 2004).

Spices can be grouped into two classes based on their mode of production, which include: processed spices and unprocessed spices (Czarra, 2009). The processed spices are produced in industrial quantities and under

standardised conditions. Examples include curry powder and thyme. On the other hand unprocessed spices are not necessarily produced under standardised conditions. These types of spices are more commonly used in African cultural food preparation. For example in Nigeria, "yaji" (Suya spice) is an unprocessed spice that is traditionally used to prepare a meat delicacy called suya by the hausa ethnic group (Okonkwo, 1987). Another example is the pepper soup spice which is used to prepare pepper soup; a delicacy usually taken among the southern ethnic group of the country especially the Yorubas.

Genotoxicity is a property possessed by some substances that makes them harmful to the genetic information, contained in organisms (Mishra*et al.*, 2013). While there are many different factors that can affect DNA, RNA and other genetic materials, the property of genotoxicity only applies to those substances that actually cause harm to the genetic information (Mishra*et al.*, 2013). A substance that has the property of genotoxicity is known as a geno-toxin. Micronuclei are cytoplasmic chromatin-containing bodies that appears in the cell like a small satellite nucleus around the cell nucleus, due to chromosome fragments or entire chromosomes that are not incorporated in the main nucleus after cell division (Mishra*et al.*, 2013). The presence of micronuclei (MN) in cells is considered as a biomarker of damage to the DNA (Mishra*et al.*, 2013). The micronucleus test is an *in vivo* and *in vitro* short-time screening cytogenetic test is a widely used method for assessing genotoxicity of chemicals in organisms (Mishra*et al.*, 2013).

The *Allium cepa*test has been used by many researchers mainly to evaluate the genotoxic potential of various substances, because this test uses a model that is adequately sensitive to detect in numerous substances that cause chromosomal alterations (Leme and Marin-Morales, 2009). This test is important since it is an excellent model *in vivo*, where the roots grow in direct contact with the substance of interest (i.e. effluent or complex medicinal mix being tested) enabling possible damage to the DNA of eukaryotes to be predicted. Therefore, the data can be extrapolated for all animal and plant biodiversity (Leme and Marin-Morales, 2009). The analysis of chromosomal alterations can be equal to the test of mutagenicity mainly for the detection of structural alterations; however, it is possible to observe numerical chromosomal alterations, as well (Leme and Marin-Morales, 2009). This study was aimed to investigate the genotoxicity of four well known spices (thyme, curry, suya and pepper soup) using *Allium cepa*assay, as well as to evaluate their nutrient value, microbial load and heavy metal contents.

Materials and Methods

Allium cepa assay

Sources of materials

Common purple onion (*Allium cepa*) bulbs of average size (6cm) used for his investigation were obtained from the popular mile 12 market, Lagos, Nigeria. The processed spices used in carrying out this study were purchased from Otta market, Ogun State, Nigeria.

Preparation of spices concentration

Stock was prepared from "market-sold" curry and thyme spice from which serial dilutions was done by measuring 10 g of curry spice powder and diluted in 100 ml of distilled water. This was absolute concentration (of 100%). The stock was diluted into 100%, 50%, 25%, 5% and 1%.

Viability tests of *Allium cepa*

The viability assay was carried out as described by Olorunfemi*et al*., (2011). The outer scales of the dried onion bulb were carefully removed and dry brownish roots at the bottom were scraped away without damaging the ring of root primordial. The onion bulb was introduced to distilled water for 48 h to determine the viable ones. The water was changed daily and those onions that sprouted properly were used to carry out the experiment while those with poor or no growth were discarded.

Morphological studies

The root structural form was studied by observing the nature of the root for the presence of straight and turgid, bent and flaccid, straight and turgid, withered and flaccidand the change in root colour was also observed and recorded (Ozmen and Summer 2004)

Root length and root length inhibition assay

The onion bulb exposed to distilled water only was used as the control. The onion bulbs that were transferred into the various spices solutions was studied for root growth inhibition assay that was performed at 24 h, 48 h, 72 h and 96 h based on the exposure tests (Samuel *et al*., 2010). For each

treatment there were five replicates. The root lengths of the onion were measured at 24h, 48h, 72h and 96 h. The root length was measured using a ruler and was expressed in centimetre (cm).

$$\text{Mean root length} = \frac{\text{Addition of root length}}{\text{Total number of root counted}}$$

Percentage root length inhibition (%)

$$= \frac{\text{root length in control} - \text{root length in spices concentration}}{\text{Root length in control (water)}} \times 100$$

Cytological and chromosomal aberration studies

Harvesting and fixing of root tips

The control (in water) roots were harvested after 48 h of growths while the treated roots were harvested after 24 h, 48 h, 72 h and 96 h of exposure in the spices concentrations, and were introduced into the fixative immediately (aceto-alcohol 1:3) slide preparation was carried out in order to arrest mitosis (Fiskesjo, 1987).

Slide preparation

Root tips exposed to the spices concentrations were gently removed using forceps and cut into 5 mm. This was treated with a drop of 1 normal HCl for 5 min. Excess 1N HCl was blotted out neatly using a filter paper (Sharma and Dphil, 1980).

Maceration was done using a dissecting needle to enhance stain uptake and to ensure the spreading of the cells in a monolayer for easy microscopic examination. A drop of lactic- acetic orcein stain was placed on the tissues and was left for 20 min to allow the stain penetrate the cells thoroughly (Fiskesjo, 1993).

After staining, the slide was gently covered with a cover slip allowing the stain to spread evenly over the square parts of the cover slip and the glass slide was then placed in between two folds of filter paperand pressure was applied around the square area of the cover slip for evenly squashing of the specimen. Finally, the square edges of the cover slip were sealed with the clean fingernail polish. This was to prevent drying out of the preparation by the heat of microscope (Sharma, 1983).

Cytological studies

Each prepared slide was placed on the stage of the microscope and viewed under the ×40 objective for its mitotic stages. The total number of cells and total number of dividing cells was recorded. Scoring of chromosomal aberrations was taken from 5 microscopic fields for each of the different tests solution and having control having a total count about 500 cells per spices concentration (Fiskesjo, 1985). Photomicrographs of normal and aberrant dividing cells were taken with National microscope (TSViewCxImage Application)

Mitotic index

Mitotic index was determined by the examination of 500 cells per point source (100 cells per slide) (Samuel *et al*., 2010).

$$\text{Mitotic index} = \frac{\text{Number of dividing cells}}{\text{Total number of cells counted}} \times 100$$

Mitotic inhibition

The percentage mitotic inhibition was calculated (Borooah, 2011).

Mitotic inhibition =

$$\frac{\text{mitotic index in control} - \text{mitotic index in test}}{\text{Mitotic index in control}} \times 100$$

Phase index

The phase index was derived from the cytological study (Fiskesjo (1997).

Phase index =

$$\frac{\text{number of cells on each mitotic phase}}{\text{Number of dividing cells}} \times 100$$

Scoring for chromosomal aberrations

Various aberrations were characterized such as bridges, vagrant, sticky chromosomes, binucleated cells, c-mitosis and attached chromosomes. The frequency of aberrant cells was calculated based on the number of the aberrant cells per total cells scored at each spices concentration (El-Shahaby*et al*., (2003).

Percentage (%) chromosomal aberration

To determine the percentage chromosomal aberration, the number of total aberration was divided by the total dividing cells and multiplied by 100 (Okoli and Russom 1986). It was calculated as:

Percentage aberration =

$$\frac{\text{Number of total chromosomal aberration}}{\text{Total number of dividing cells}} \times 100$$

Microbial analysis and identification of isolates from suya and pepper soup spices

Sterilization of materials

All the glass wares used for the experiment were washed, air dried and sterilized in an oven set 160 ºC for 3 h. The inoculating loop (nichrome wired) was sterilized using bunsen flame. Culture media were sterilized using autoclave (Buchanian*et al.*, 1994).

Media preparation

All the nutrient media (Oxoid) used for this study were prepared according to the manufacturers' instruction.

Isolation of micro-organism from spice samples

About 10 g of each spice sample was thoroughly mixed with 90 ml of sterile distilled water to form a suspension. Ten-fold serial dilution was carried out using 1 ml of the prepared suspension. Appropriately 1 ml of the diluents was inoculated into sterile petri dish, overlaid with melted agar (45 ºC) and mixed thoroughly. This was allowed to solidified and incubated at optimum growth condition (Ayanda*et al.*, 2013). After incubation, micro-organisms that emerged from the plates were sub-cultured to obtain pure cultures.

Identification of bacteria from spice samples

Pure cultures of bacterial isolates from the spices were identified based on their biochemical, morphological and colonial characteristics.

Colonial morphology on agar plates

Colony morphology include the type pigment (if present), size colony, texture (opaque, translucent or transparent), adherence to agar and shape (undulating, round, serrated edges).

Gram staining

Pure isolate from the spices were gram stained and examined under oil immersion objective to determine the shapes, arrangements and gram reaction of the bacterial isolates.

Biochemical tests

Biochemical tests were performed to identify the bacterial isolates. Young cultures of bacteria were used to inoculate the test media. Biochemical tests performed include, spore staining, motility test, catalase production, oxidase test, indole production, citrate utilizationn, nitrate reduction, coagulase test, urease activity, methyl-red vogesproskauer (mr-vp) test, gelatin hydrolysis, starch hydrolysis, carbohydrate utilization (sugar fermentation), casein hydrolysis (Ayanda*et al.*, 2013).

Fungi identification from spice samples

The morphological and cultural characteristics with special reference to the sporulation of the fungi isolates were used for identification. The two methods that were used are direct observation of agar plates and riddle's slide culture technique (Ayanda*et al.*, 2013).

Observation of fungal plates

The plates were observed daily growth rate of each of the isolates. The colour and morphology of the colonies were noted. The base of the plates (reverse), rate of growth, colony texture, colour (spores and mycelium) was noted.

Slide culture technique

A wet mount of each fungus was prepared by suspending a loopful of fungal culture in a few drops of lacto-phenol cotton blue solution on a microscope slide and then cover with a slip then view under ×40 objective.

Proximate analysis

Moisture content

The sample was analysed using the Moisture Analyzer (MS-70 by A and D Company limited), the samples were first scanned from 100 ºC to 200 ºC to know the optimum temperature suitable for the sample to be dried. The sample's optimal temperature was 140 ºC. The moisture content was then determined and the percentage shown automatically on the LED of the analyser (A.O.A.C., 2010).

Ash content

About 1g of the sample was weighed in an already dried and weighed crucible. The samples were then charred on a hot plate to be decarbonized. After complete de-carbonization, they were placed in the furnace for 3 h at 560 ºC to obtain the ash content (A.O.A.C., 2010).

$$\frac{W_2\text{-}W_1}{W} \times 100$$

Where, W_2 is the weight of crucible + ash
W_1 is the weight of dried crucible
W is the weight of sample taken

Protein content

Using the kjehdahl apparatus, about 0.5 g of the sample was weighed into a digestion tube, 1 tablet of kjehdahl catalyst and 10 ml of concentrated sulphuric acid were added and placed in the digestion block to digest to 430 ºC for 2 h. The resulting digestate was placed in the distiller; 70-80 ml of 40 % sodium hydroxide was dispensed into it and distilled. The resulting nitrogen was trapped inside 50 ml boric acid indicator (boric acid indicator is a mixture of 40 g boric acid, 0.03g methyl red, 0.06g bromocresol green in 2 L of ethanol water) which changes to green indicating the presence of protein in the sample (A.O.A.C., 2010). The trapped Nitrogen in the boric acid is then titrated against 0.1N HCl to give a red coloration at the end point (A.O.A.C., 2010).

$$\frac{\text{Titre value} \times \text{normality of acid} \times 1.4007 \times 6.25}{\text{Weight of sample taken}}\%$$

Where: 1.4007 is factor of nitrogen and 6.25 is the general conversion factor for most samples from nitrogen to protein.

Fat content

Using the Soxhlet extraction method, about 1-2 g of sample was weighed on a filter paper, inserted in a thimble and put in the soxhlet set up. 150 ml of 40-60º Pet ether was added in an already dried and weighed round bottom flask placed on a hot plate and set to about 80ºC. This was allowed to extract for about 3 h. The resulting extract was then dried in the oven and weighed (A.O.A.C., 2010).

Crude fibre

About 0.5g of swlite is weighed into crucible and heated in a fyrnace foe 20 mins. The conditoned selites was then mixed with 1g of seitw in the rucible. The mixture was then analysed by The fibretec machine (A.O.A.C., 2010).

Heavy metal analysis

The spice was burnt to ashes in a furnaces, the cooled ash was then washed into a beaker. 30 ml of concentrated trioxonitrate (V) acid (HNO_3) were added to dissolve the white ash. The solution was evaporated to dryness and the residue was further heated for 30 minutes.The residue was then digested in 40 ml of Hydrochloric acid (HCl) for 2 hours with heating and continuous stirring with a magnetic stirrer. About 1 ml of diluted hydrochloric acid solution was added to the sample and boiled for an hour. The solution was filtered while hot using Whatman No 4 filter paper, and washed with HCl solution. The volume was then made up to 100 ml with distilled water. The minerals were then determined using the spectrometry method of Atomic Absorption Spectrometer (AAS) with a hollow cathode lamp and afuel-rich flame (A.O.A.C, 2010).

Statistical Analysis

Data obtained from the assay were analysed using IBM SPSS v23 (2015). Analysis of Variance (ANOVA) was used to test for significant differences between all treatments, with the P value set at P=0.05. Independent T-test was used to test for significant differences between the cooked and uncooked spices. Duncan's Multiple Range Test (DMRT) was used to separate significant mean difference.

Results

Allium cepa assay

The control (0%) and 1% concentration of thyme spice showed increased root growth of *A. cepa* as shown in figure 1. The root growth in the control was higher than the 1% thyme concentration throughout the period of the experiment; maximum growth was observed at 72 h. Other concentrations showed a decrease in root growth throughout the experiment, with 100% thyme concentration having the highest inhibitory effect. There was no significant growth ($P > 0.05$)between 72-96 hours of the control.

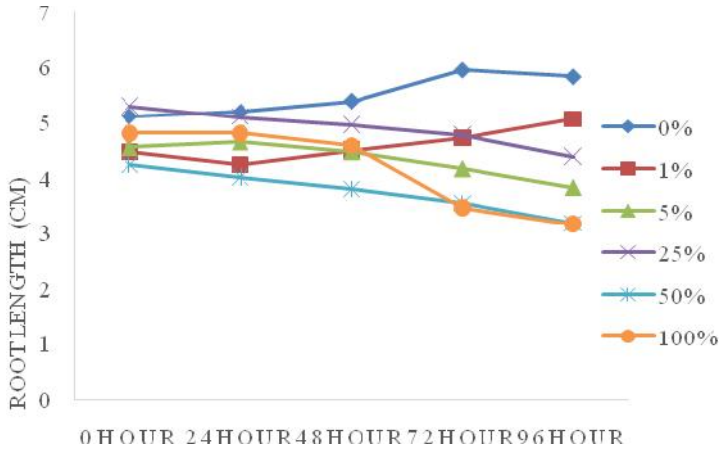


Figure 1: Root length of *A. cepa*treated with thyme spice.

The control (0%) and 1% concentration of curry spice in figure 2 showed increased root growth of *A. cepa*. The growth in the control was higher than the 1% during the 24 and 48 h while during 72 h the growth of 1% concentration was higher than the control, maximum growth was observed at 72 h. Other concentrations showed a gradual declined root growth as the treatment time increased. There was no significant growth ($P > 0.05$) between 72-96 h of the control.

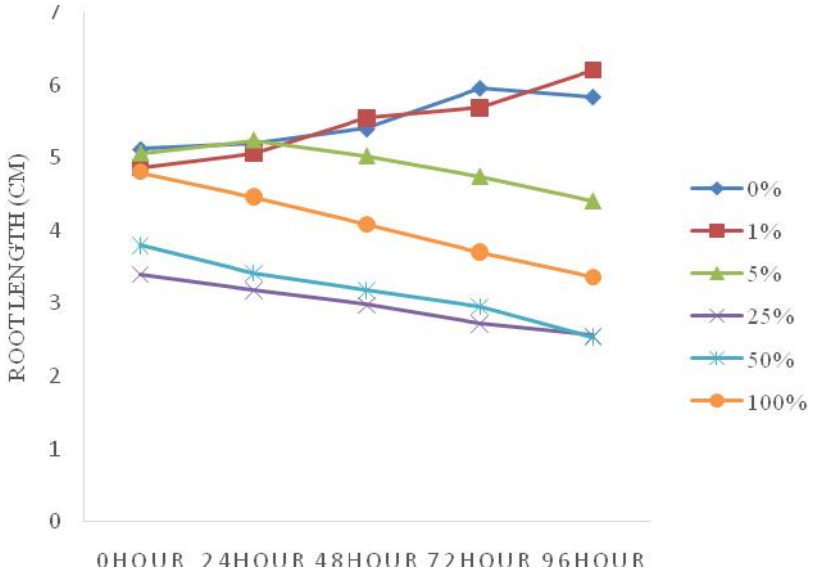


Figure 2: Root length of *A. cepa*treated with curry spice

The control (0%) and 1% concentration of pepper soup spice in figure 3 indicated increased root growth; maximum growth was noticed at 72 h. Growth in the control was higher than the 1% throughout the period of the experiment. Other concentrations showed a gradual decrease in root growth as the treatment time increased. There was no significant growth ($P > 0.05$) between 72-96 h of the control.

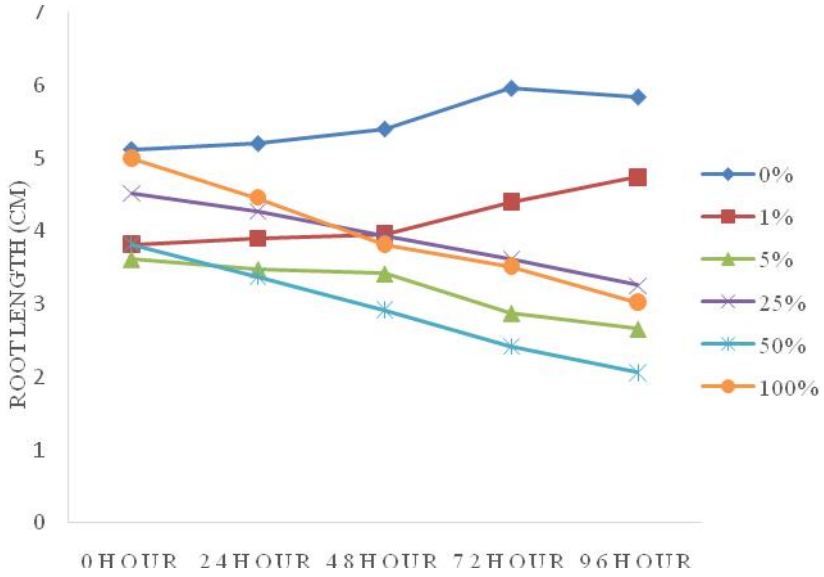


Figure 3: Root length of *A. cepa* treated with pepper soup spice

The control (0%) and 1% concentration of suya spice showed increased root growth of *A. cepa*; maximum growth was observed at 72 h. The growth in the control was higher than the 1% throughout the period of the experiment. Other concentrations showed a gradual decrease in root growth as the treatment time increased with 100% concentration showing the highest inhibitory effect. There was no significant growth ($P > 0.05$) between 72-96 h of the control.

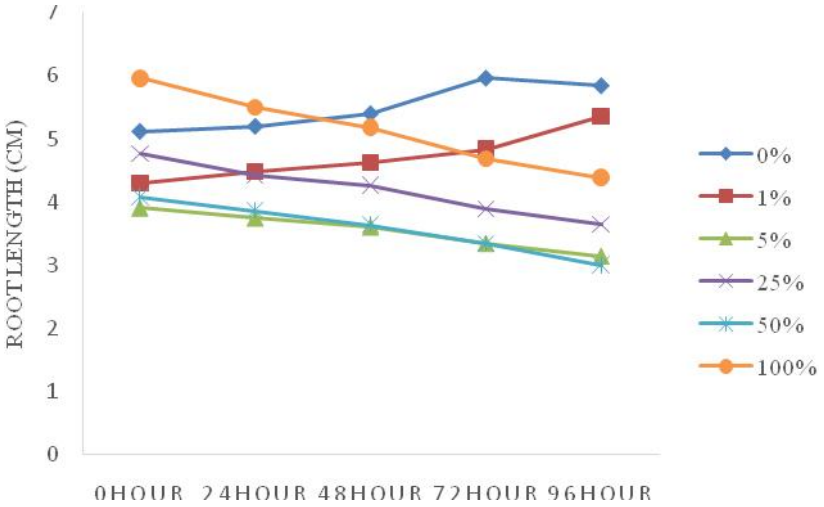


Figure 4: Root length of *A. cepa* treated with suya spice

The control (0%), 1% and 5% concentration of boiled thyme spice in figure 5 showed increased root growth of *A. cepa;* maximum growth was observed at 72 h. The control had the highest growth followed by 1% and 5% concentration throughout the treatment period. Other concentrations showed a gradual decreased in root growth as the treatment time increase. There was no significant growth ($P > 0.05$) between 72-96 h of the control.

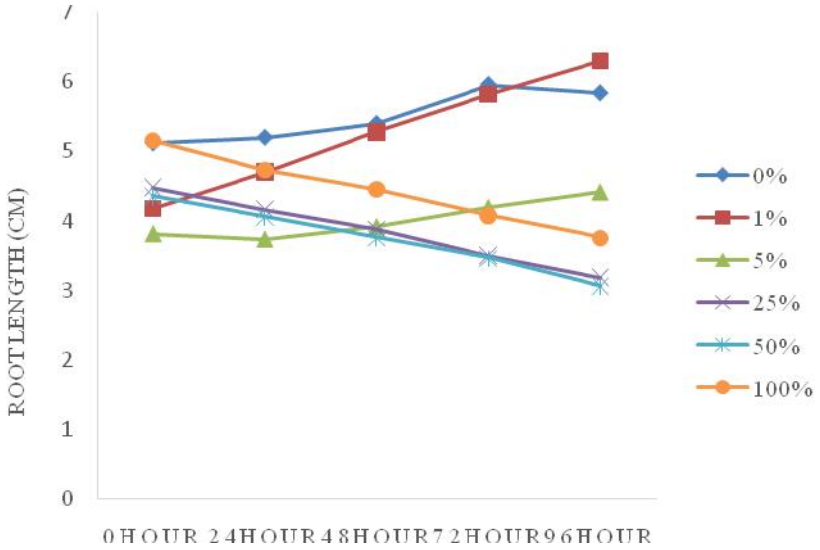


Figure 5: Root length of *A. cepa* treated with boiled thyme spice

The control (0%) and 1% concentration of boiled curry spice in figure 6 showed increased root growth of *A. cepa;* maximum growth was observed at 72 h. The growth in the control was higher than the 1% throughout the treatment time. Other concentrations showed a gradual decrease in root growth as the treatment time increase. There was no significant growth (P > 0.05) between 72-96 h of the control.

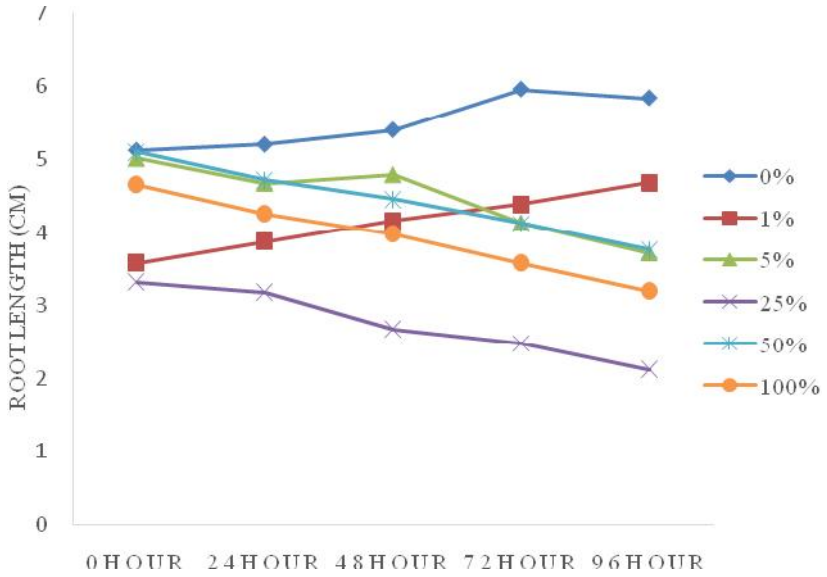


Figure 6: Root length of *A. cepa*treated with boiled curry spice

The control (0%) and 1% concentration of boiled pepper soup spice in figure 7 showed increased root growth of *A. cepa;* maximum growth was observed at 72 h. Growth in the control was higher than 1% concentration throughout the treatment time. Other concentrations showed a gradual decrease in root growth as the treatment time increase. There was no significant growth (P > 0.05) between 72-96 h of the control.

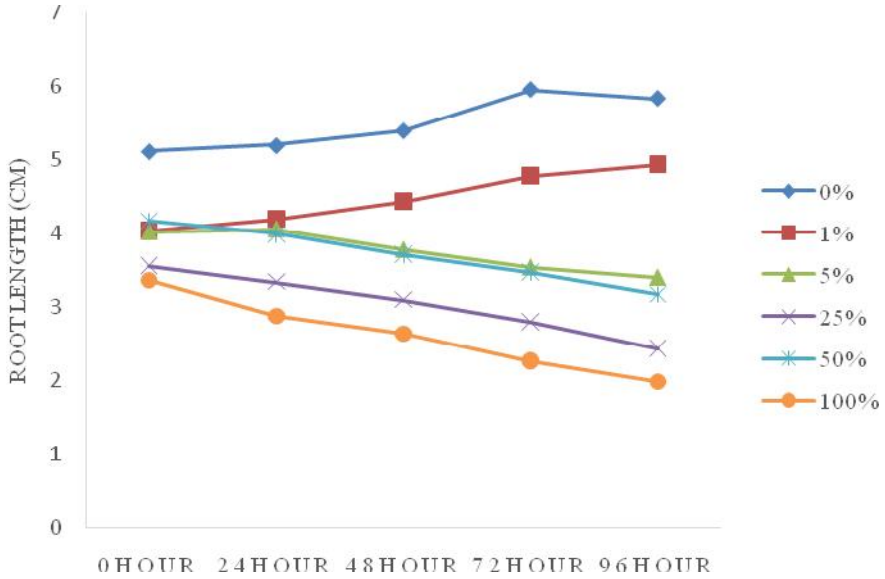
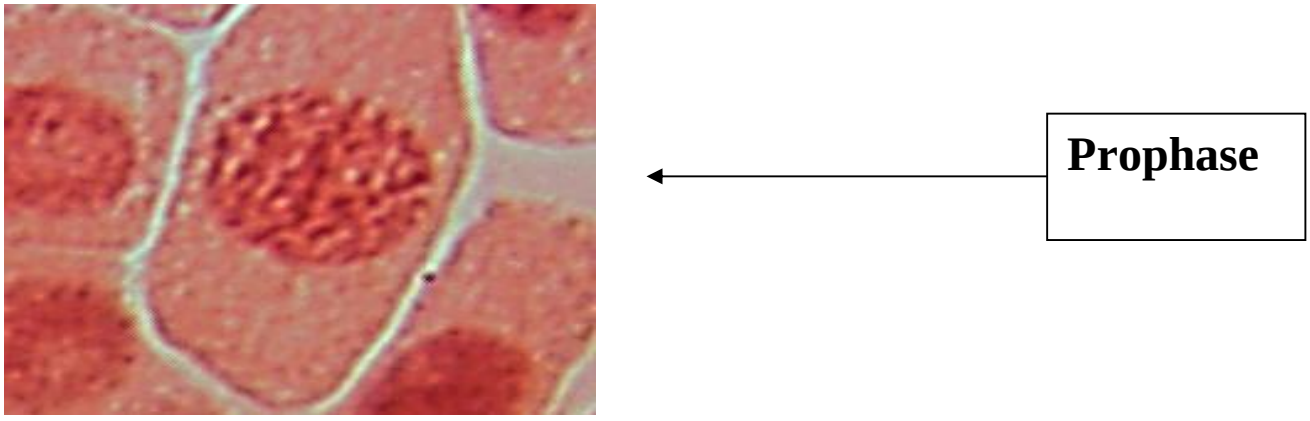


Figure 7: Root length of *A. cepa* treated with boiled pepper soup spice

Chromosomal aberrations

Photomicrographs of the most representative pictures of normal mitotic cells and cells containing the different types of chromosome aberration observed are shown in plates 3-5. Plate 3 showed normal mitotic stages observed in the root tip cells of the control; proper division of cells was observed throughout in the control. The following aberrations were observed and scored as endpoints for determination of cytogenetic effects of the spices: sticky chromosome, bridged anaphase, vagrant, bi-nucleated, sticky anaphase and C-mitosis (Plate 4-5).



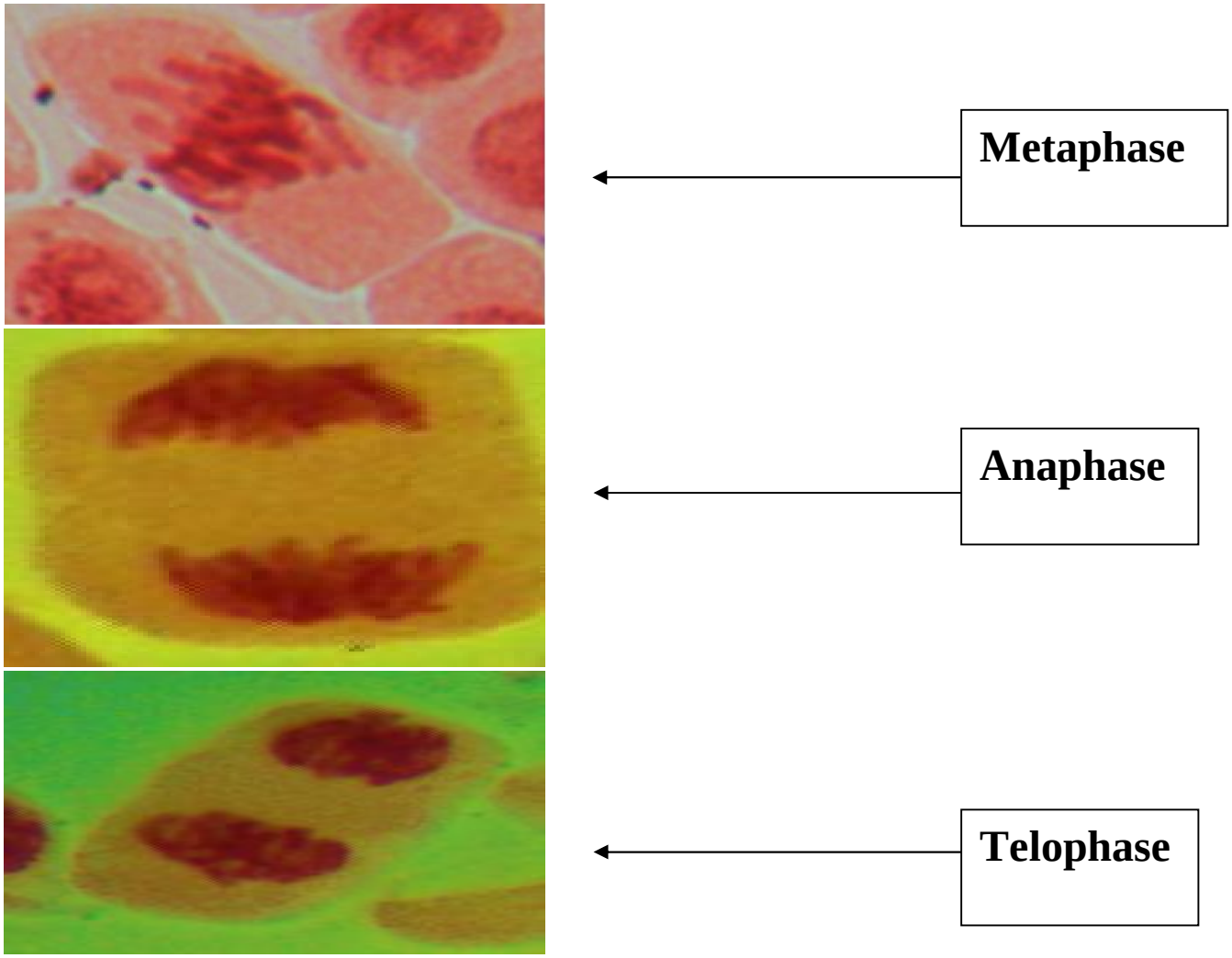
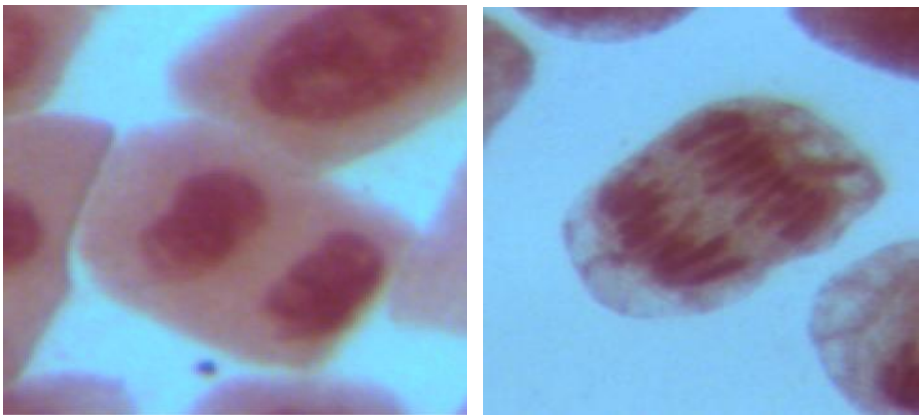


PLATE 3: *A. cepa* cells in normal division



Vagrant telophase **Vagrant anaphase**

Table 1a: Mitotic index of the analyzed spices

Uncooked Spice	Conc. (%)	Mitotic Index				Cooked Spice	Conc. (%)	Mitotic Index			
		24h	48h	72h	96h			24h	48h	72h	96h
Thyme	1	4.72	4.42	4.31	3.98	Boiled thyme	1	2.70	2.73	2.69	2.24
Thyme	5	4.55	3.93	3.66	2.87	Boiled thyme	5	2.93	2.35	2.07	1.49
Thyme	25	2.88	2.34	2.13	1.60	Boiled thyme	25	2.66	2.28	1.49	1.28
Thyme	50	2.50	2.12	1.91	1.26	Boiled thyme	50	2.78	2.00	1.63	1.31
Thyme	100	1.56	1.61	1.35	0.98	Boiled thyme	100	2.30	2.03	1.19	0.69
Curry	1	2.64	2.73	2.60	2.26	Boiled curry	1	2.76	2.31	1.91	1.82
Curry	5	2.96	2.59	2.29	1.98	Boiled curry	5	2.75	2.47	1.62	1.19
Curry	25	2.44	2.32	2.14	2.10	Boiled curry	25	2.54	1.85	1.47	1.02
Curry	50	2.18	1.40	1.82	1.21	Boiled curry	50	2.36	1.65	1.37	1.05
Curry	100	2.25	1.95	4.25	0.82	Boiled curry	100	2.16	1.58	1.00	0.53
Pepper Soup	1	3.45	3.21	2.69	2.19	Boiled pepper soup	1	4.37	3.92	3.37	2.85
Pepper Soup	5	3.23	2.83	2.44	2.13	Boiled pepper soup	5	4.15	2.26	3.31	3.15
Pepper Soup	25	3.08	2.68	2.28	1.72	Boiled pepper soup	25	3.93	3.29	2.75	2.03
Pepper Soup	50	3.09	2.67	2.22	1.64	Boiled pepper soup	50	3.55	2.60	2.20	1.58
Pepper Soup	100	2.69	2.74	2.23	1.53	Boiled pepper soup	100	3.10	2.35	1.57	1.46
Suya	1	4.58	3.92	3.68	3.41						
Suya	5	3.96	3.70	3.36	2.87						
Suya	25	3.81	3.49	3.12	1.42						
Suya	50	3.21	3.29	2.80	2.12						
Suya	100	3.44	3.19	2.03	2.10						

Table 1b: Aberration percentage of the analyzed spices

Uncooked spice	Percentage Aberration (%)				Cooked Spice	Percentage Aberration (%)			
	24h	48h	72h	96h		24h	48h	72h	96h
Thyme	2.66	1.47	0.63	0.40	Boiled thyme	0.34	0.23	0.27	0.14
Thyme	2.21	1.39	0.78	0.41	Boiled thyme	0.35	0.35	0.12	0.25
Thyme	1.79	1.04	0.28	0.00	Boiled thyme	0.36	0.24	0.12	0.13
Thyme	1.76	0.56	0.29	0.00	Boiled thyme	0.42	0.25	0.13	0.00
Thyme	1.87	1.02	0.60	0.16	Boiled thyme	0.29	0.25	0.26	0.14
Curry	1.32	1.02	0.52	0.40	Boiled curry	0.74	0.44	0.45	0.23
Curry	2.01	1.29	0.60	0.49	Boiled curry	0.36	0.34	0.12	0.24
Curry	1.16	0.61	0.13	0.00	Boiled curry	0.38	0.12	0.00	0.00
Curry	1.09	0.38	0.13	0.13	Boiled curry	0.52	0.12	0.12	0.00
Curry	1.19	0.91	0.53	0.00	Boiled curry	0.58	0.24	0.13	0.00
Pepper Soup	1.94	1.11	0.45	0.23	Boiled pepper soup	0.85	0.58	0.46	0.47
Pepper Soup	1.85	1.25	0.35	0.24	Boiled pepper soup	0.59	0.30	0.30	0.00
Pepper Soup	1.42	1.17	0.84	0.49	Boiled pepper soup	0.63	0.16	0.00	0.00
Pepper Soup	2.10	0.73	0.25	0.13	Boiled pepper soup	1.86	0.49	0.00	0.00
Pepper Soup	1.92	1.73	0.74	0.31	Boiled pepper soup	0.69	0.50	0.00	0.00
Suya	0.42	0.21	0.22	0.11					
Suya	0.32	0.22	0.11	0.00					
Suya	0.67	0.45	0.12	0.12					
Suya	0.41	0.23	0.12	0.12					
Suya	1.42	0.37	0.13	0.13					

Table 2 shows that all the sources and interaction of sources of variation showed no significant effect on mitotic index and also show significant effect on percentage aberration evaluated from the *A. cepa* assay.

Table 2: Variation effect on mitotic index and percentage aberration

Source of variation	Mitotic Index	Percentage Aberration
Spice	9.692ns	4.232*
Concentration	13.963ns	0.436*
Time	19.744ns	12.050*
Spice x Concentration	1.202ns	0.241*
Spice x Time	0.409ns	0.794*
Time x Concentration	0.187ns	0.089*
Spice x Concentration x Time	0.241ns	0.071*
Error	0.000	2.299e-30

***Effects significant at 5% level of significance; ns: effects not significant at 5% level of significance; x: interaction**

Microbial analysis and identification of isolates from suya and pepper soup spices

Microbial evaluation of the four spices shows that there was a significant difference ($P<0.05$) observed in total bacteria counts with pepper soup having the highest bacteria count. All spices evaluated except curry and thyme had bacteria counts well above standard limits. The spices did not show microbial counts above standard limits for mold, *E. coli* and Coliform counts. Thyme and Curry spices (processed spices) showed no observation of any microbial growth in all the media prepared. The microbial evaluation of the raw spices is shown in table 3. No microbial growth on plates containing curry and thyme.

Table 3: Microbial evaluation of the analysed spices

Spice	TBC	TMC	E. *coli*	SSA	Identified Organisms
Curry	Nil	Nil	Nil	Nil	Nil
Thyme	Nil	Nil	Nil	Nil	Nil
Suya	8.2×10^6a	7.5×10^3	7.0×10^1	Nil	See table 3.1
Pepper soup	1.37×10^7b	5.5×10^3	Nil	1.0×10^1	See table 3.1
P<0.05	0.00*	0.11	-	-	
SL	10^4-10^5	10^3-10^4	50-10^2	10^2-10^3	

Mean of value (n=3) in cfu/gm; Cfu/gm: colony forming unit per gram evaluated; TBC: total bacteria counts; TMC: total mold counts; EMB: SSA: salmonella shigella agar; SL: Standard Limits; *significant at 5% level of significance. Values with the same alphabet are not significantly different at 5% level of significance.

Table 3.1: Identified microbes in pepper soup and suya spices

Spice	Probable Bacteria present	Probable Mould present	Spice	Probable Bacteria present	Probable Mould present
PepperSoup Spice	*Acinetobacter ivotoffi* *Alcaligenes* eutrophs* *Bacillus brevis* *Bacillus cereus** *Bacillus polymyxa* *Bacillus luterosporus* *Bacillus subtilin* *Citrobacter freundii* *Corynebacterium* Kutscheri* *Corynebacterium* pilosum* *Corynebacterium striatum* *Escherichia coli* *Euterobacter agglomerans* *Euterobacter amnigemus* *Flavobacterium rigense* *Proteusvulgaris* *Salmonella* arizonae* *Salmonella* bongori* *Staphylococcus albus* *Staphylococus curreas*	*Aspergillus niger* *Aspergillus chevalieri* *Nugrospora oryzae* *Taloromyces thermophilue*	Suya spice	*Alcaligenes* eutrophs* *Baccillus sphaericus* *Bacillus cereus** *Bacillus coagulans* *Bacillus megaterium* *Corynebacterium* Kutscheri* *Corynebacterium* pilosium* *Euterobacter aerogenes* *Flavobacterium aquantile* *Klebsiella aerogenes* *Micrococcus sedentarius* *Micrococcus luteus* *Micrococcus roseus* *Pseudomonas aeruginosa* *Pseudomonas mallei* *Salmonella* arizonae* *Salmonella* bongori*	*Absiclia spinosa* *Aspergillus amstelodami* *Fusarium oxysporium* *Mucor plumbeus*

* Bacteria common to both suya and pepper soup spice.

Bacteria isolates from the spices were identified based on their colonial morphology, cellular morphology and biochemical characteristics according to the taxonomic scheme of Buchanan and Gibbons (1994).

The colonial morphology of the mould isolates was observed based on the size, colour, and aerial mycelia growth as stated above and microscopy morphology was determined using staining methods called wet mount with lacto-phenol cotton blue stain. (Cowan *et al.*,1990).

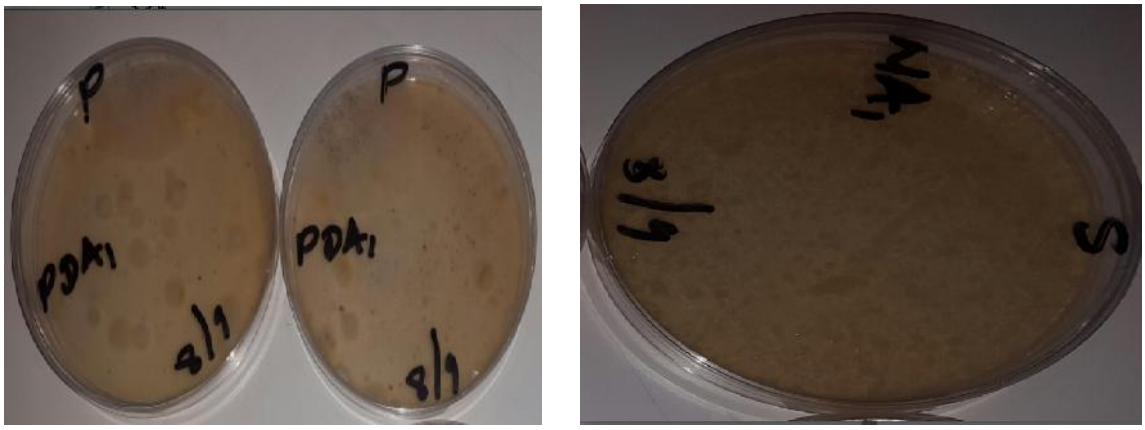


Plate 6: Cultured plate showing growth of micro-organism

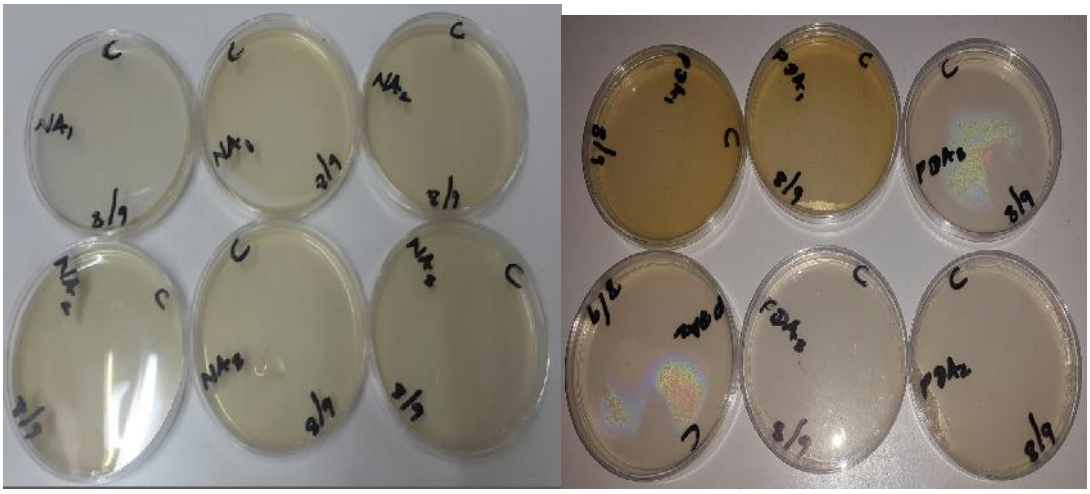


Plate 7: Cultured plate showing absence of growth of micro-organism

Proximate analysis

Proximate analysis of spices evaluated showed significant difference in all the parameters measured between the spices evaluated. Suya contained the highest fat (14.85) and protein content (37.33) while it was least in thyme spice (2.48, 7.21) respectively; thyme contained the highest moisture (12.17), crude fiber (13.45) and carbohydrate (44.25) content while it was least in suya spice (7.50, 10.39, 18.26) respectively; curry contained the highest ash content (13.82) while it was least in thyme spice (4.71).

Table 4: Proximate analysis result of cooking spices evaluated

Spices	Fat (%)	Moisture (%)	Ash (%)	Protein (%)	Crude fiber (%)	CHO (%)
Curry	4.31b	12.17c	13.82d	12.00b	13.45c	44.25c
Thyme	2.48a	12.45d	4.71a	7.21a	18.70d	54.46d
Suya	14.85d	7.50a	11.68b	37.33d	10.39a	18.26a
Pepper soup	9.86c	8.77b	12.51c	13.76c	11.70b	43.41b
P<0.05	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*

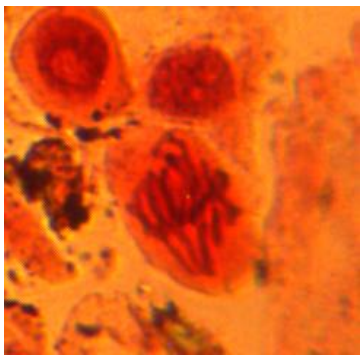
CHO: Carbohydrate; *significant at 5% level of significance; values with the same alphabet are not significantly different at 5% level of significance.

Heavy metal analysis of the spices show that all the spices evaluated did not contain lead metal. Although, there was no significant difference between the cadmium (P=0.222) contained in all the spices evaluated, all the spices however contained cadmium (0.426) more than the standard permissible limit (SL=0.02). Chromium was not detected in all the spices evaluated except pepper soup spice. There were no significant differences in nickel (P=0.199) contained in all the spices, only suya spice (3.258) was above the standard permissible limit of nickel (SL=0.163).

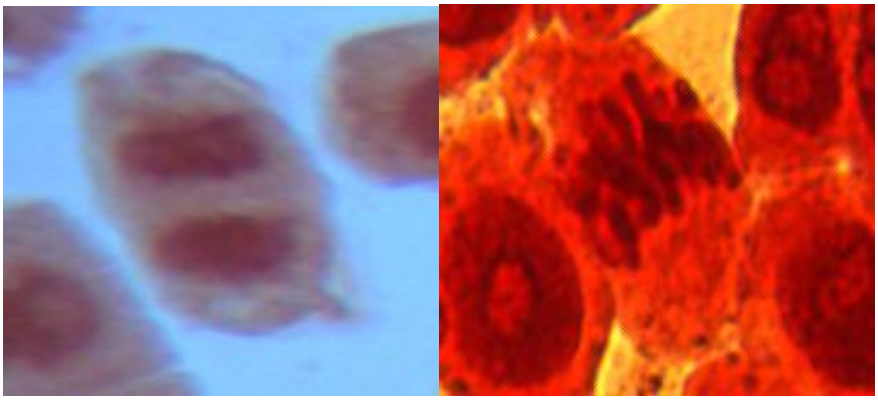
Table 5: Heavy metal analysis of cooking spices

Spice	Pb	Cd	Cr	Ni
Curry	ND	0.657	ND	1.085
Thyme	ND	0.314	ND	1.142
Suya	ND	0.256	ND	3.258
Pepper soup	ND	0.478	0.188	1.164
P<0.05	-	0.222	-	0.199
SL	5.0	0.20		1.63

Pb: Lead; Cd: Cadmium; Cr: Chromium; Ni: Nickel; SL: Standard limits; values are measure in mg/kg

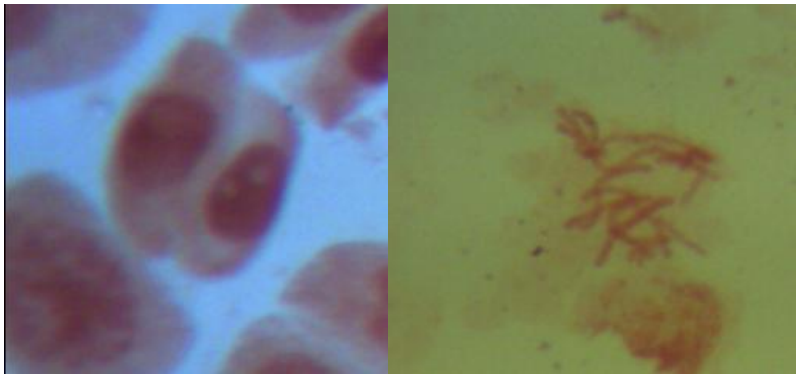


Vagrant metaphase

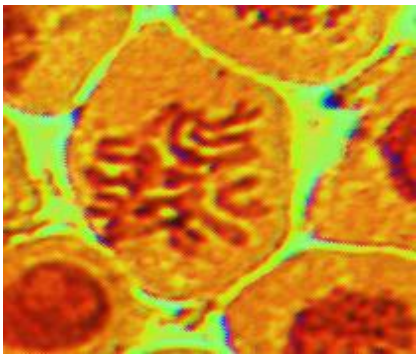


Sticky telophase **Sticky metaphase**

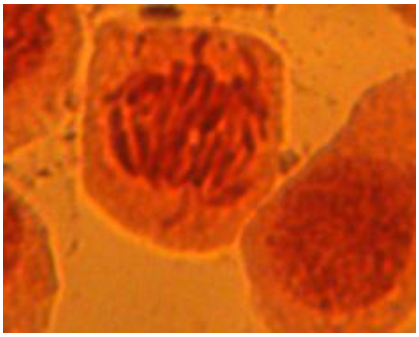
PLATE 4: ***A. cepa*** **cell showing the aberration due to the action of spices**



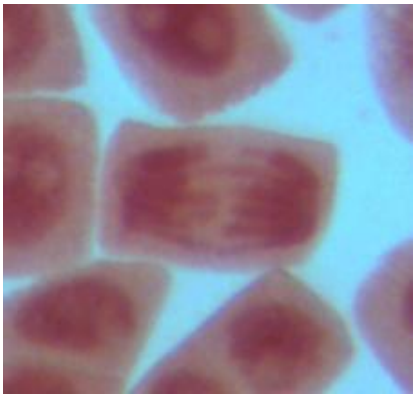
Binucleated chromosome **Distorted chromosome**



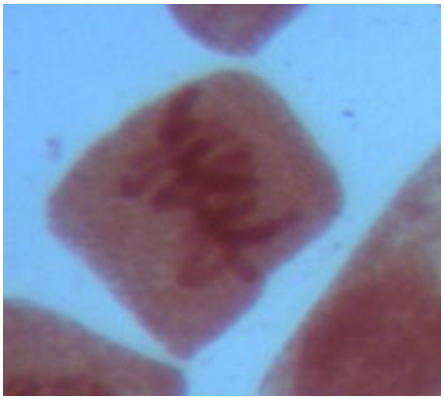
C-mitosis



Multiple bridge anaphase



Bridge anaphase



Bridge metaphase

Plate 5: **Aberration of *A. cepa*cell due to spices treatment**

Discussion

Spices contains compounds such as sulphites, nitrates, nitrites and antibiotics, that are harmful to human health and have many side effects including nausea, wide spread weakness, mental retardation, seizures, cancer and anorexia (Turkoglu, 2009). The A*llium cepa* test has many advantages as genotoxicity screening assay. In the *Allium cepa* test, inhibition of rooting and appearance of stunted root indicated retardation of growth and cytotoxicity, while root wilting explains toxicity (Ukaegbu and Odiegah, 2009).

Parameters such as frequency of mitosis and abnormal cell division can be used to estimate cytotoxicity, genotoxicity and mutagenicity of spices. The effects of spices were based on microscopic and macroscopic evaluations which include abnormalities like stickiness, vagrant type, sticky chromosome, laggard binucleate etc. The present study provides evidence that the uncooked spice (curry, thyme, suya and pepper soup spice) and the cooked spice (curry, thyme and pepper soup spice) inhibits root growth and caused retardation which is in agreement to the findings of Odeigah *et al.* (1997).

Mitotic index is used as an indicator of cell proliferation biomarkers which measures the formation of cells in the mitotic phase of the cell cycle. The spices used in this study caused a reduction in the mitotic index in the onions treated with the spices. The mitotic indices in the treated onion root cells with the spices were significantly lower than the mitotic index of the control. In this study, different types of chromosomal aberrations were recorded including: sticky chromosome, bridged anaphase, vagrant, bi-nucleated, sticky anaphase and C-mitosis. Analysis of the various aberrations showed that the cooked spices induced lesser aberration than the uncooked spices. The aberrations could be due to the effect of spices on the spindle formation, thus resulting in cell division disturbances as suggested by Abdel Migid and Abdel-rahman (2013). The most frequent type of aberrations that occurred was vagrant and sticky aberrations.

Chromosomal stickiness is caused probably through immediate reactions with DNA-DNA or DNA- protein cross linking (Amin, 2002). According to El-Ghamery *et al*. (2003) the induction of vagrant chromosome leads to the production of daughter cells with unequal number of chromosome in their nuclei, which as a consequence are unequal in size, or are shaped irregularly at anaphase.

In this study, the cells of the *A. cepa* root tip after treatment with the spices showed decrease in mitotic index values and increase in the incidence of chromosomal aberrations with increasing concentration of spices. This conforms to the finding of Bakare *et al.* (2003), who reported reduction in mitotic indices obtained in the treated root *A. cepa* with increase in concentration of neem extract. With increasing concentration and increasing toxicity, there was an inhibitory effect on cell division (Odeigah *et al.*, 1997).

The presence of bi-nucleated cells reveals the inhibition of cell wall formation between cells (Fiskejo, 1985). Fragmentation observed in this study after may be attributed to dissolution of chromatin (Salam *et al.*, 1993). The observation of laggard has been suggested by Yildiz and Arikan (2008) to be an indicator that a laggard chromosome indicates that a test compound acts as a strong spindle inhibitor. Badr *et al.* (1992) attributed the induction of anaphase/telophase bridges to chromosome breaks, stickiness and breakage and reunion of the broken ends of chromosomes. Therefore the observed anaphase/ telophase bridge suggested a clastogenic effect of the spices.

Heavy metal analysis indicated that all the spices contain cadmium and nickel, while only pepper soup spices contains chromium. This has critical health implications for the general population because these spices are consumed in large quantities regularly. Cadmium found in all of the spices analysed, is classified as human carcinogenic. Therefore, increase in this content in food is always harmful. The Cadmium content in all the spice samples were above the maximum permissible limit (0.2 ppm) as recommended by FAO/WHO (1984). This high level of cadmium might be due to the use of cadmium containing fertilizers or from the practice of growing this spices crops on the soil amended with sewage sludge. Nickel also found in all of the spices analysed has been reported to have low toxicity, due to its low absorption in the body (Oehme, 1989). All spices contained nickel concentration below the permissible limit set by FAO/WHO (1984) in edible plants except for suya spice that has a higher concentration (3.223ppm).

Proximate analysis of spices evaluated showed significant difference in all the parameters measured between the spices evaluated (Ihekoronye and Ngoddy, 2013). Suya contained the highest fat and protein content while it was least in thyme spice. This high fat content of the suya promotes micro-organism growth. Thyme contained the highest moisture, crude oil and carbohydrate content while it was least in suya spice. High moisture content of spices should normally aid the growth of micro-organisms, but the result of the study showed thyme had no microbial growth; this can be attributed to the preservative it has been subjected to, during its process of production (Ihekoronye and Ngoddy, 2013).

Conclusion

This study showed that the spices analysed had negative effect on the mitotic index, also they were found to inhibit mitosis in the roots of the onion bulbs. Comparison of the aberration induced by the spices showed that the cooked spices had lesser genotoxic potentials than the uncooked spices. Furthermore, the unprocessed spices (pepper soup and suya spice) showed heavy microbial load, while the processed spice had negligible microbial load. The same distribution was observed in the result of the heavy metal analysis. Therefore, the study concluded that the cooked/ processed spices are less toxic than the uncooked/ unprocessed spice.

References

Amin, A. (2002). Cytotoxicity testing of sewage water treatment using allium cepa. *Pakistan Journal of Biological Sciences*, **5**: 184-188.

Association of official Analytical Chemists (A.O.A.C). (2010). *Official Methods of Analysis*, 19th edition. Washington, D. C. 45-78 Pp.

Ayanda, O.I., Ajayi A. A., Olasehinde, G. I. and Dare, O. T. (2013) Isolation and characterization of extracellular enzyme detection of microbial isolates from deteroriated apple (*Malus domestica*) fruits. *Int. J. Biol. Chem. Sci.* 7(2):641-648.

Badr, A., Ghareeb, A., and El-Din, H. M. (1992). Cytotoxicity of some spesticides in mitotic cell of *Vicia fabaroots. EgyptianJournal of Applied Sciences*, **7:** 457 - 468.

Bakare, A. A., Monsura, A. A. and Osibanjo, O. (2003). Effects of simulated leachates on chromosomes and mitosis of allium cepa. *Journal of Environmental Biology*. **21**: 263-271.

Borooah D. D. (2011). Genotoxicity assessment of water extract of *ocium gratissium* using *Allium cepa* assay. *Journal of Plant, Animal and Environmental Science*. **1**(2)**:** 185-188

Buchanan, R. E. and Gibbons, N. E. (1994). *Bergy's manual of determinative bacteriology. 19th edition.* The Williams and Wilkins Company. Baltimore. 23-67 Pp.

Czarra, F. (2009). *Spices: A Global History*. Reaktion Books (2nd Edition). ISBN 978-1-86189-426-7. Pp. 128- 142.

El-Ghamery, A. El-kholy, M. A. and Abou- El-Yousser, M. A. (2003). Evaluation of cytological effect of $zinc^{+2}$in relation to germination and root growth of *Nigella Sativa L Mutation Research,* **53**(7): 29-41.

EL-Shahaby, A. O., Abdel-Migid H. M., Soliman, M. I. and Mashaly, I. A. (2003). Genotoxicity Screening of industrial waste-water using the *Allium cepa* chromosome aberration assay. *Pakistan Journal of Biological Sciences*. **6**(1): 23-28

Fiskesjo, G. (1985). Allium test on river water from bran and sexan before and after closure of a chemical factory. *Ambiologia*. **14**: 99- 103.

Fiskesjo, G. (1987). The allium test, an alternative in environmental studies; the relative toxicity of metal ions, *Mutation Research*.**197**: 243-280.

Fiskesjo, G. (1993): A 2-3 day plant test for toxicity assessment by measuring the mean root growth of onions (*Allium cepa L.*). *Environmental Quality and water control*. **24**:612-618

Fiskesjo, G. (1997). Allium test for screening chemicals: Evaluation of cytological parameters: In Plant for environmental studies *Environmental and risk assessment Toxicology*. **8:** 291-298.

Fiskesjö, G. (1985). The *Allium*-test as a standard in environmental monitoring. *Hereditas*, **102**: 99-112.

Ihekoronye, A. I. and Ngoddy, P. O. (2013). *Integrated Food Science and Technology For the Tropics*. Macmillan Publishers, London. 55 Pp.

Leme, D. M. and Marin-Morales, M. A. (2009). *Allium cepa* test in environmental monitoring: A review on its application. *Mutation Research*, **682**: 71–81.

Mishra, A., Gupta, V., Nagar H. andShrivastav V. (2013). Protective effect of *Murraya koenigii* (curry leaf) leaves extract against genotoxicity induced by cyclophosphamide in mouse bone marrow cells. *Global veterinaria*, **10**(2): 128-133.

Odeigah, P. G. C., Nurudeen, O. and Amund, O.O. (1997). Genotoxity of oil field waste water in Nigeria. *Heriditas,***126**:161-167

Oehme,F. M.1989.Toxicity of heavy metals in the environment. Marcel Dekker, NewYork,Part pp 76-79

Okoli, B. E. and Russom, Z. (1986). Effects of an aqueous extract of *Cassia alata* L. on mitosis of *Allium cepa* roots. *Biologia Africana*. **1**(2):31-37.

Okonkwo, T. (1987). About Suya and Yaji. *Journal of Food and Agriculture,***1**(1): 51 - 52.

Olorunfemi, D. L., Ogieseri, U. M. and Akinboro, A. (2011). Genotoxicty screening of industrial effluent using onion bulbs (*Allium cepa L.*).*Journal of Applied Science and Environmental Management*. **15**(1):211-216.

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