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Cytotoxic and genotoxic effects of textile effluent dilutions on *Zea mays* (maize plant)

Ahamefule Augustus Kelechi* and Ezeji Ethelbert Uchechukwu
Department of Biotechnology, Federal University of Technology, Owerri (FUTO), Nigeria
kcahams@gmail.com

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Abstract

This research investigated the cytotoxic and genotoxic effects of textile mill effluent on the maize plant (Zea mays). Seeds of maize (Zea mays) were grown in wood shavings (4/treatment) irrigated with different concentrations of textile effluent (0%, 25%, 50%, 75% and 100%) for 15 days. Most of the physicochemical parameters of the effluent, analyzed using specific instrument for each, were above permissible limits, examples are the COD (4208mg/L against 90mg/L), BOD (171mg/L against 50mg/L), Nitrate (71.2mg/L against 10mg/L), etc. There was complete loss of viability at concentration 100%, while germination reduced by 75%, 50% and 25% in 75%, 50% and 25% textile effluent concentrations respectively. Plant growth rate was inversely proportional to concentration increase; growth of the control significantly differed with other treatments at p<0.05. The cytotoxic effects were investigated using Automated Image Analyses Software and RAPD. RAPD analysis was performed on four pooled Genomic DNA extracted from shoots of the, 25%, 50%, 75% of the treatments and control (0%) plants after 15 days. Five decamer plant specific primers (OPB-11, OPT-11, OPH-08, OPK-11 and OPL-08) were utilized for screening of the Zea mays genome. Among them, 3 primers (OPB-11, OPT-11 and OPH-08) gave clear and stable bands. The RAPD profile obtained showed textile effluent had genotoxic effects on the plants. This was evident with the appearance and disappearance of bands in the treatments compared with the control. In all, 64 bands were scored, 31(48.4%) of these were polymorphic. Altogether, 13 new bands were formed while 15 were lost. A dendrogram of the four accessions using Weighted Neighbour-Joining (WNJ) procedure clustered the accessions into two major groups. The control (Maize-1) and treated 25% effluent (Maize-2) sample were clustered in one group with 67% bootstrap value. Group II, 50% effluent (Maize-3) and 75% effluent (Maize-4), were separated in another cluster, with 88% bootstrap value. The above results show that high concentrations of textile mill effluent have adverse genotoxic effects on the maize plant.

Keywords: Textile effluent, *Zea mays,* RAPD, cytology, DNA analysis, pollution.

Introduction

Extraordinary industrial and economic development is taking place. Examples of such developments are seen in the chemical industries, which led to the manufacture of large variety of products. The generality of usage of these products, mostly clothing of various designs, fertilizers, insecticides, etc, then followed. This, together with the almost simultaneous development of high-yield grains, led to unprecedented increase in local food production. These unfolded developments, coupled with improved medical technology, have helped to improve public health. While many were hailing the benefits of these advancements, just few became aware that these extraordinary developments came with costly implications.

Note that pollution is considered as one of the world's most dangerous threats[1]. We reside in a world exposed to different hazardous pollutants and chemicals, being generated from different sources per day. Industries, domestic and other human activities are among the anthropogenic factors that spread pollutants around our environment on a daily basis. Despite the fact that pollution had been known to exist from time immemorial, (at least, since people started making fire, mostly from woods, thousands of years ago), it reached explosive proportions only since the onset 19th century industrial revolution. Environmental pollution is a worldwide problem[2] known to cause a lot of distress not just to human and other animals, but to our sustainers (plants), driving many species to endangerment and possible extinction.

As one of the pioneer industries[3], the strength of textile industry flows from its strong production base of wide range of man-made fibres like polyester, nylon, acrylic, etc, from natural fibres like silk, cotton, jute, and wool. The act of local painting and designing of these fabrics have been taken by many Nigerians, especially those in the western hemisphere. These people, most of them are not aware of the actual contents of what they use, involve large quantity of water and discharge the resultant effluents in their surroundings, without proper treatment. With increasing demand for customized textile products, the activities of tie and dye producers, if left unchecked, may pose a big environmental and health hazards.

Textile effluents contributes significantly to environmental degradation and human illnesses[4]. Research showed that about

40% of usable colorants contain organically bound chlorine, a known carcinogen[3]. Some of these chemicals are volatile, and easily evaporate (sublime, for solids), into the air, and can as well be absorbed through the skin. The resultant effects could be allergies, which may cause prenatal defects. Also, as a result of these chemical pollutions, the normal functioning of cells could be disturbed. At this level, there may be alteration in the biochemical and physiological mechanisms of fauna and flora, which may hinder important cell functions like respiration, osmo-regulation, reproduction, and even mortality[5]. It thus mean that untreated or partially treated textile dye effluent can be harmful to living things by adversely affecting the natural ecosystem.

Materials and methods

Effluent Samples and Physicochemical Analysis: The effluent was collected from a textile industry located at Iganmu Industrial Estate, in Lagos State, Nigeria. Sample was taken using a clean Jerry-can, and transported to Biotechnology Research and Development Centre (Ebonyi State University, Abakaliki. Ebonyi State, Nigeria) laboratory for analysis. Physico-chemical parameters of the effluent were assessed using the appropriate instrument meant for each of the parameters that was considered, following the prescribed procedures by the manufacturers. Total Dissolved Solids (TDS), Turbidity, Electrical Conductivity (EC), pH, Biological Oxygen Demand (BOD), Total Chromium, Chemical Oxygen Demand (COD), etc, in the effluent were determined following standard method described by APHA[6]. Effluent pH was measured with digital pH meter (Metrohm, USA). EC was determined by conductivity meter (Thermo Orion, model-213H, USA). Total Chromium was analysed with Atomic Absorption Spectrometer. The Total Dissolved Solid (TDS), using Hanna instrument with model number-HI9811-5. Dissolved Oxygen (DO) was measured using Dissolved Oxygen meter by LT Luton (Model No. DO-5509).

Plant Source/Planting: Five (5) 100ml beakers were thoroughly washed with a detergent and 10% (v/v) sodium hypochlorite, rinsed with distilled water and allowed to air-dry. The beakers were filled to 1/3 volume with wood shavings (sawdust). There was serial dilution of the effluent, according to[7], to obtain 25% effluent (25ml effluent + 75ml of distilled water), 50% effluent (equal volume of effluent and distilled water), 75% effluent (75ml effluent + 25ml of distilled water), 100% effluent (no distilled water added). However, the 1st beaker served as the control (received only distilled water). The selected maize seeds, bought from a commercial market at Umuakah, in Imo State, were surface-sterilized with 75% (v/v) ethanol for 5 min, then 10% (v/v) sodium hypochlorite for 10 min, rinsed with distilled water and planted 4 seeds per beaker. The setting was replicated into six places, but the best four were analyzed.

Germination Rate/Growth: Seed germination rate was monitored by taking note of the day(s) individual/group(s) grain(s) germinated. The percentage germination amongst groups was calculated. Plants growth rate were measured by taking the highest shoot tip (in the early stage of germination) and the highest node of individual plants/treatments (after the leaves developed), using a centimeter calibrated scale. After 15 days, shoots were pooled from each of the plants group for DNA extraction.

Dna Extraction (CTAB Method): After 15 days of plant growth, 100mg of the shoots were ground with 100ul of extraction buffer in a sterile mortar and pestle. The mixture was poured into new sterile 1.5ml tube and briefly vortexed, incubated in water bath at 60^{o}C for 10min.

The mixture was brought to room temperature and 0.5ml of phenol, chloroform and iso-amyl alcohol at 25:24:1 ratio was added. The mixture was vortexed again and centrifuged at 12000rps for 10min, after which 450 microlitre of the supernatant was introduced into new and sterile 1.5ml tube. 400 microlitre of cold isopropanol was added, mixed and incubated for 45 minutes at -20^{o}C. Mixture was centrifuged at 12000rps for 10min to sediment the DNA, the supernatant was decanted gently, ensuring that DNA pellets were not disturbed. 500 microlitre of 70% ethanol was added to the pellets and centrifuge at 12000rps for 5min to wash it, the ethanol decanted while DNA was allowed to air dry at room temperature. DNA pellets were at this stage suspended in 200ul of TE buffer for further use.

RAPD Analysis: The five decamer plant specific primers used, with Sequences 5'→3' (from 1 to 10) were: OPB-11 (GTAGACCCGT), OPT-11 (TTCCCCGCGA), OPH-08 (GAAACACCCC), OPK-11 (AATGCCCCAG), and OPL-08 (AGCAGGTGGA). To each PCR tube, the following were added; Extracted DNA (50ng/µl), PCR Buffer (1X), $MgCl_2$ (5 Mm), dNTP mixture (10Mm), Primers (10pmole/µl), TaqDNA Polymerase (3unit).

Tube was tapped for two seconds to mix the contents thoroughly. Twenty five (25) microlitre of mineral oil was added in the tube to avoid evaporation of the contents, then the tube was place in the thermocycler block and the program set to get DNA amplified. DNA amplification was done, using a thermocycler for 40 cycles, in obedience to the following reaction conditions:

Onset denaturation of template DNA at 94-95°C for 10 minutes
↓
Denaturation into 2 strands at 94°C for 1 minute.
↓
Annealing of the 2 strands above at 37-45°C for 1 minute.
↓
New strands extension at 72°C for 1 minute.
↓
Final extension at72°C for 10 minutes
↓
Cooling at 4°C.

Band scoring and data analysis: 50ml of 0.8% agarose gel was prepared by adding 0.4g agarose to 50ml of 1X TAE buffer in a conical Flask. The mixture was heated with a microwave, constantly swirling the conical flask until the agarose desolved completely, allowed for five minutes to cool to 60^{o}C. 0.5 microlitre Ethidium bromide was added (this is a chemical that intercalates DNA and makes it visible under UV light), and well mixed. The electrophoretic trays and combs were prepared and balanced properly, the mixture was then turned into a casting tray with comb and allowed to solidify for 30 minutes at room temperature.

5µl of ready to use DNA ladder was loaded into the first well first, 2µl of 6X gel loading buffer was added to 10 µl of PCR product, and then loaded into the made wells.

The power cord was connected to the electrophoretic power supply according to its conventions, ie, red-anode and black-cathode, then the gel was run at constant voltage of 120 volts and 90 mA until band separation occurred.

The preparation (DNA bands) was placed under a UV trans-illuminator and visualized, resulted profiles were obtained and photographed with the help of a digital computer connected to the illuminator box.

Results and discussion

Physicochemical Analyses: Table-1 shows the results of the physico-chemical parameters analyzed in the textile effluent. The effluent had a slight harsh smell, alkaline in nature, with deep blue-black colour. Most of the parameters were above standard permissible limits, examples are the COD (4208 against 90), BOD (171 against 50), Nitrate (71.2 against 10), etc.

Effects of textile effluent on germination and growth of maize grains: The result of the germination days and growth response of individual groups, from day 1 to 15 is shown in Table-2. The control (0%) and 25% were the first to germinate on day 3 after planting. However, from the shoot measurement taken that day, the control measured more than the 25% treatment with about 1cm. 50% treatment germinated on day 5, with smaller shoot length (0.8000) compared to control (2.1000) and 25% treatment (1.0667). Treatment 75% germinated on day 7 with smaller shoot length (0.4667) compared to others, whereas treatment 100% did not germinate at all throughout the experiment.

Figure-1 shows the growth responses of *Zea mays* grains in glass beakers. Also, from the image below, one can calculate the percentage germination by taking note of the number(s) of seeds that germinated out of the four (4) planted per beaker.

Also, variation in growth rate was evident among the groups, it was observed that the control grew at much faster rate than others. Growth reduction was directly proportional to increase in effluent concentrations, in fact the differences were significant (at $p<0.05$) as shown in Table-2.

Table-1: The Physicochemical Parameters and Heavy Metals Contents of Textile Dye Effluent, and their National/ International Permissible Limits.

Parameters	Effluent	NESREA[a]	USEPA[b]
Colour	Blue-Black	-	-
pH	12.50	6.00-9.00	6.5-8.50
Turbidity	91.00	-	-
Salinity	18.10	-	-
BOD	171.00	50.00	-
COD	4208.00	90.00	-
DO	3.30	-	-
TDS	48160.00	-	-
TSS	11.60	-	-
Conductivity	749.00	-	-
Nitrate	71.20	10.00	10.00
Chloride	9104.50	250.00	250.00
Hardness	181.20	150.00	75.00
Alkalinity	2866.00	-	20.00
Manganese	0.05	-	-
Iron	0.09	0.30	0.30
Chromium	9.90	0.05	0.10

Note that all values are in mg/L, except; pH (no unit), Turbidity (FTU), Conductivity (mS/cm), and Salinity, in percentage (%). BOD = Biochemical Oxygen Demand, COD = Chemical Oxygen Demand, DO = Dissolved Oxygen, TDS = Total Dissolved Solids, TSS = Total Suspended Solids, NESREA = National Environmental Standards and Regulation Enforcement Agency, USEPA = United State Environmental Protection Agency, [a] = NESREA maximum permissible limits for wastewater[7]. [b] = USEPA maximum permissible limits for wastewater[8].

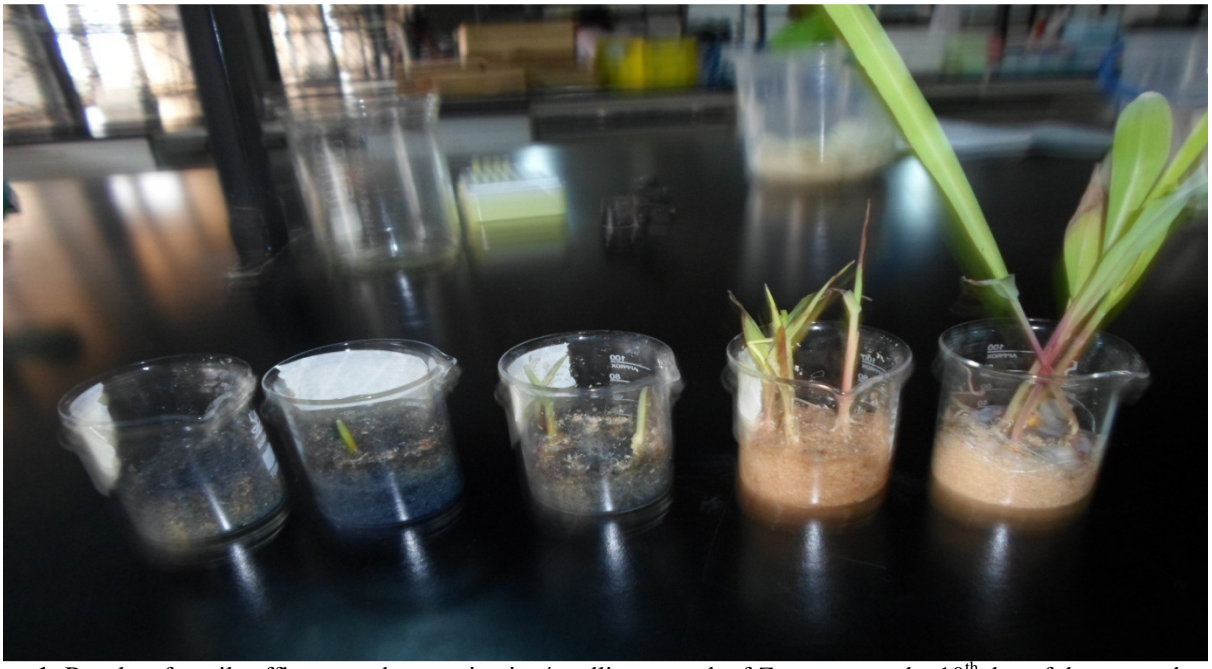

Figure-1: Results of textile effluent on the germination/seedling growth of *Zea mays* on the 10th day of the research work.

Table-2: Effects of Textile Effluent on Germination and Growth of Maize Seeds.

Days	Control	25%	50%	75%	100%
1	0.00 ± 0.00^{a}	$0 .00 \pm 0.00^{a}$	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
2	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
3	2.10 ± 0.10^{b}	1.07 ± 0.12^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
4	2.67 ± 0.15^{b}	1.50 ± 0.10^{c}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
5	3.47 ± 0.15^{c}	2.23 ± 0.15^{d}	0.80 ± 0.10^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
6	4.50 ± 0.26^{d}	2.97 ± 0.15^{e}	1.23 ± 0.12^{bc}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
7	5.67 ± 0.51^{e}	3.73 ± 0.12^{f}	1.83 ± 0.15^{c}	0.47 ± 0.05^{b}	0.00 ± 0.00^{a}
8	6.93 ± 0.57^{f}	4.57 ± 0.06^{g}	2.60 ± 0.17^{d}	0.77 ± 0.06^{c}	0.00 ± 0.00^{a}
9	8.20 ± 0.62^{g}	5.37 ± 0.06^{h}	3.40 ± 0.30^{e}	1.23 ± 0.15^{d}	0.00 ± 0.00^{a}
10	9.63 ± 0.55^{h}	6.33 ± 0.12^{i}	4.17 ± 0.40^{f}	1.80 ± 0.17^{e}	0.00 ± 0.00^{a}
11	11.10 ± 0.35^{i}	7.17 ± 0.06^{j}	4.90 ± 0.53^{g}	2.47 ± 0.12^{f}	0.00 ± 0.00^{a}
12	12.50 ± 0.44^{j}	8.10 ± 0.268^{k}	5.63 ± 0.57^{h}	3.10 ± 0.10^{g}	0.00 ± 0.00^{a}
13	13.20 ± 0.35^{k}	8.87 ± 0.29^{l}	6.23 ± 0.64^{hi}	3.63 ± 0.12^{h}	0.00 ± 0.00^{a}
14	13.77 ± 0.31^{kl}	9.60 ± 0.26^{m}	6.83 ± 0.72^{ij}	4.10 ± 0.17^{i}	0.00 ± 0.00^{a}
15	14.13 ± 0.25^{l}	$10.17 .30551^{n}$	7.27 ± 0.76^{j}	4.53 ± 0.21^{j}	0.00 ± 0.00^{a}

Values are mean ± standard deviations of triplicate determinations. Values in the same column bearing the same superscript letters are not significantly different at 5% confidence level ($p>0.05$).

RAPD Profile of the Control and Treated Plants: In this study, a total of five Random Amplified Polymorphic DNA (RAPD) primers were used to study DNA polymorphism among the accessions, to access the level of genetic diversity in the treatments. Out of the five primers used, only three produced distinct bands (Figure-2).

At the end, 64 bands were scored, 31 (48.4%) were polymorphic. Altogether, 13 new bands were formed while 15 were lost (Table-3). DNA damage/polymorphism was revealed in RAPD profiles through appearance and disappearance of bands in the treatments, compared to that of the control. Disappeared bands were likely due to changes in oligonucleotide priming sites, originated from rearrangements. They are less likely from point mutations and DNA damage in the primer binding sites[9]. DNA damage caused by textile effluent was evident in changes in RAPD profiles via changes in band intensity, appearance and disappearance of bands in the exposed plant. Note that bands disappearance may also be due to the formation of pyrimidine dimers, modified bases, single and double strand breaks, basic sites, bulky adducts, oxidized bases, DNA-protein cross-linked, point mutation and extended chromosomal rearrangement induced by genotoxins[10]. Note that any of these events can reduce or prevent the polymerization of DNA in PCR reactions. The bands appearance and disappearance were even observed at concentrations 25% of the effluent. This suggests that untreated textile effluent at this concentration can induce DNA damage that will result in band loss.

Note that appearance of new bands, as caused by mutation, can only be possible if they (the bands) occur at the same locus in a sufficient number of cells[11]. A minimum of 10% of mutation is likely required to get new PCR products to be visible in agarose gel. New bands appearance could be attributed to mutation, while bands disappearance were as a result of DNA damage[12]. Other studies obtained similar percentage of polymorphic bands, 48%[13] and 46%[14]. The appearance of new PCR products maybe a sign of change in some oligonucleotide priming sites due to mutations (new annealing event(s), homologous combination (juxtaposing two sequences that match the sequence of primer) and/or large deletions (bringing pre-existing annealing site closer)[13].

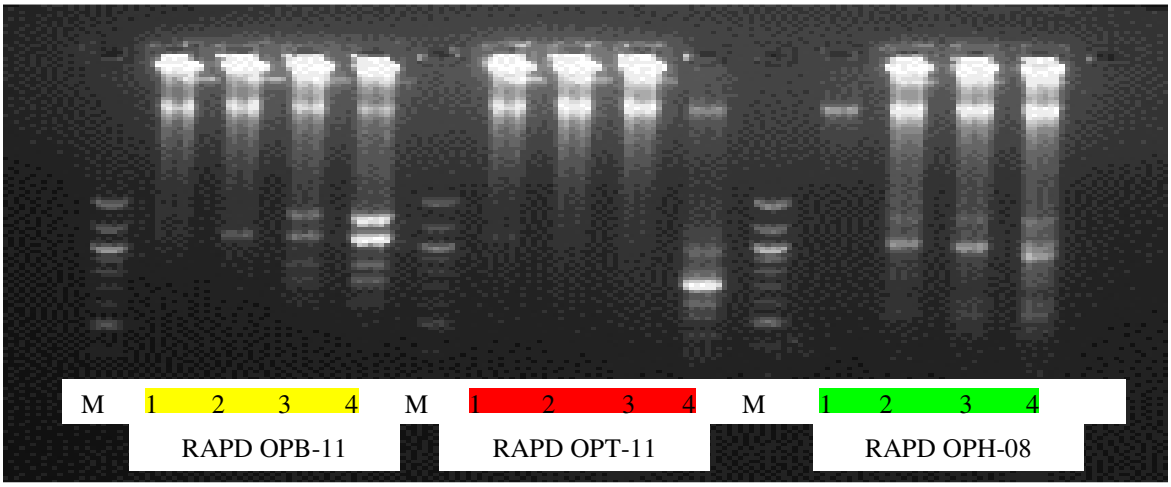

Figure-2: RAPD profiles generated by OPB-11, OPT-11 and OPH-08 from *Zea mays* plant irrigated with textile effluents. Lane M: 100 base-pairs (bp) step DNA ladder, 1: Control, 2: 25% effluent, 3: 50% effluent, 4: 75% effluent.

Table-3: Maize DNA bands following application of RAPD Primers.

RAPD Primer	Sequence	Total Bands	% Polymorphism	Band Gain	Band Loss
OPB-11	GTAGACCCGT	21	58	5	6
OPT-11	TTCCCCGCGA	19	42	3	4
OPH-08	GAAACACCCC	24	46	5	5
OPK-11	AATGCCCCAG	No Amplification	00	0	0
OPL-08	AGCAGGTGGA	No Amplification	00	0	0
		Total =	64	13	15

The cluster analysis, considered as one of the most effective numerical analysis methods with regards to RAPD finger printing and band scoring[14], because it can calculate the distances between every pair of entities and then summarize the community data sets, was done. It estimated the level of DNA polymorphism between the control plants and those irrigated with untreated textile effluent. The dendrogram result of the four accessions using Weighted Neighbour-Joining (WNJ) procedure clustered the accessions into two major groups (Figure-3 and 4). The control (Maize-1) and treated 25% effluent (Maize-2) sample were clustered in one group with 67% bootstrap value. Group II, 50% effluent (Maize-3) and 75% effluent (Maize-4), were separated in another cluster, with 88% bootstrap value. This result clearly showed that textile effluent above 25% contains much more genotoxic substances capable of deviations from normalcy, because the control and 25% treatment grouped in a cluster while the plants irrigated with above 25% textile effluent were grouped in another cluster joined at a larger distance.

Tree construction result of the four treatments: In applying the tree construction analysis, the average 'edge' distance between initial tree and bootstrapped trees was 0.224 with an edge length sum of 1.454. The dissimilarity maximum value was 1 while that of the minimum was 0.111 at 95% percentile value of 0.6667, as shown below.

The separation of the tree into two major branches was an indication of the concentrations at which textile effluent affected the treatments. Maize 1 and 2 (ie the control and 25% treatment respectively) separated on one side, while Maize 3 and 4 (ie the 50% and 75% treatments) appeared on a different major branch with a wider distance than the ones that exist among the sub-branches.

It entails that variations within the sub-branches were smaller compared to that of the major branch (following the separation distances) as shown in Figure-4.

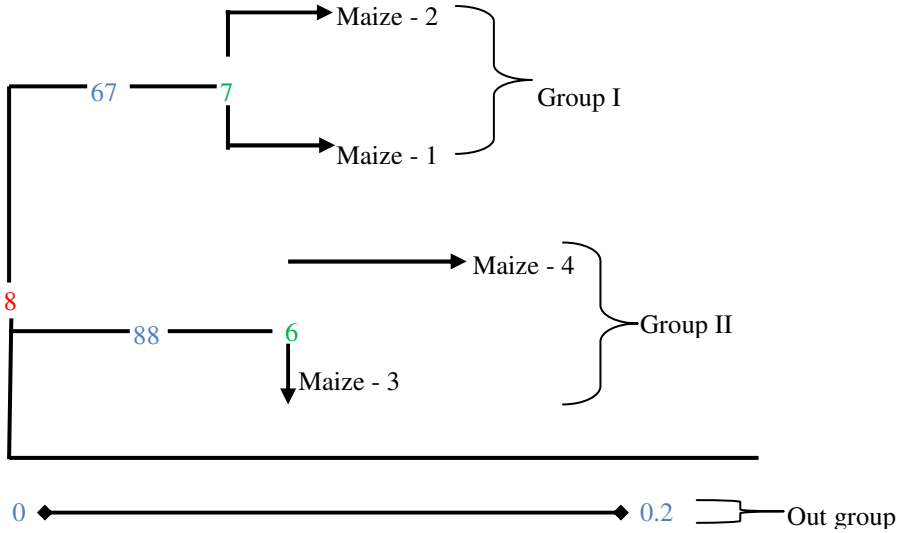

Figure-3: Dendrogram of four accessions of maize.

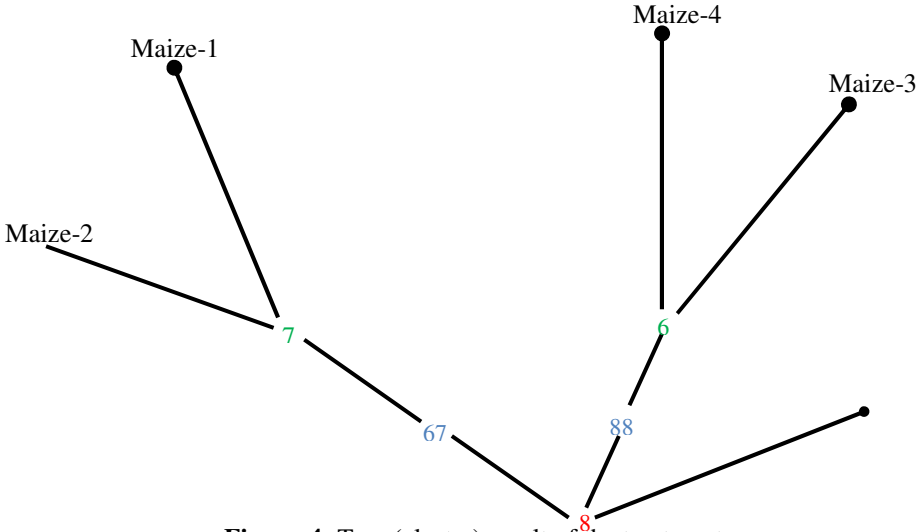

Figure-4: Tree (cluster) result of the treatments.

Conclusion

Following all the laid down facts, it means that textile effluent has been found to cause cytotoxic induction (chromosomal aberration) and genotoxic (DNA polymorphism/damage) effects in *Zea mays*. The induction of chromosomal aberrations observed in the shoot cytology of treated *Zea mays* compared to control showed the potential genotoxic effects of the textile effluent. Most of these aberrations were lethal and lead to cell death while others caused various degrees of genetic defects, which could even be expressed as congenital abnormalities in case of organisms (including humans), or be transferred from one generation to another if germ cells are affected[15]. It can also lead to loss of biodiversity by reducing the selective advantage or fitness of these plants to environmental selective pressures.

Besides the cytogenotoxic effects of the textile effluents, colour dyes in the effluents could obscure visibility in aquatic environments, affecting the aesthetic value of water bodies. It may be responsible for low water transparency (turbidity) recorded in the effluent and can lead to poor gaseous solubility in aquatic environment[16]. The presence of toxic metals as observed in the effluent can enhance the depletion of dissolved oxygen and destabilize the ability of the water to reduce microbial loads[17]. The analyzed metals in the effluent could be deposited as particulates in the aquatic environment, become bioaccumulated in aquatic forms and pose health risk to humans.

Data from this study revealed that untreated textile effluents contain toxic compounds deleterious to plants (using *Zea mays* as a case study). These bound elements, and/or compounds may pose a threat to (contaminate) surface water, rendering it unfit for irrigation and drinking. Therefore, indiscriminate discharge of textile wastewater into water bodies/farmlands should be prohibited and proper treatment of effluents before discharging into the environment should be enforced. Moreover, laws regulating pollution from all forms of anthropogenic activities should be enforced by appropriate authorities to mitigate its consequences on both plants and animals species. The continual discharge of untreated textile effluents into the environment will undoubtedly cause threat to the ecosystem and to human health. Environmental pollution from textile effluents have been the subject of much thought and research in recent times. It is expected that these studies will eventually lead to the promulgation and enforcement of regulations that will mitigate pollution of the natural environment by textile industries. All effluents (especially those of textile industries) should be treated (to levels below the standard permissive limit) before releasing them into the environment.

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