Pollution, 4(1): 83-92, Winter 2018
DOI: 10.22059/poll.2017.237168.293
Print ISSN: 2383-451X Online ISSN: 2383-4501
Web Page: https://jpoll.ut.ac.ir, Email: jpoll@ut.ac.ir

Physico-Chemical and Genotoxicity Assessments of Palm Oil Mill Effluent Generated by a Corporate Refinery In Nigeria

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Received: 05.06.2017 Accepted: 29.08.2017

ABSTRACT: The rising global demand for palm oil and its associated products has led to increased numbers of palm oil refineries with its attendant effluent discharge. Many researches have confirmed the ecological disruptive capability of Palm Oil Mill Effluent (POME), still further attention has to be directed at POME's potential genotoxicity. In this study, the physico-chemical and genotoxicity of POME obtained from a corporate refinery in Nigeria were evaluated using the American Public Health Association (APHA) procedures and Allium chromosome aberration assay respectively. *Allium cepa* roots were grown in graduated concentrations of POME and the roots were analyzed for chromosomal aberrations. Results suggest that POME caused growth inhibitions and chromosomal aberrations in *A. cepa* roots, with mitotic index of *A. cepa* roots dropping as POME concentrations were increased. The chromosomal aberrations induced in *A. cepa* were vagrant, sticky chromosomes, bi-nucleated cells, and C-mitosis. These results indicate that palm oil mill effluent is not only capable of causing ecological disruptions in the receiving environment, but is also potentially genotoxic to resident organisms. It is recommended that if effluents from palm oil mill refineries cannot be converted to other useful products and ought to be disposed of, it should first be properly treated and tested for genotoxicity.

Keywords: Chromosomes, Cytotoxicity, Mutagenicity, Environment.

INTRODUCTION

The oil palm fruit has multiple uses. It is sometimes eaten directly as fresh fruit, especially the fruits of species with small endocarp but thick and fleshy mesocarp. Palm oil, an extract from mesocarp, is one of the most consumed vegetable oil globally (Hartley, 1988; Igwe and Onyegbado, 2007). The oil extracted from the seed of palm fruit (kernel), together with palm oil, is used directly as edible oil in many food processes like manufacturing margarine, and non-nutritious processes like soap making. The cake, resulting from the extraction of oil from palm kernel, is used as livestock feed supplement. In Nigeria, palm oil is even used in informal traditional medicine as a therapy for food poisoning, cough, skin allergic reactions, and other minor ailments. The increasing demand for palm fruit products has continued to encourage more oil palm plantations and consequently, increasing palm oil processing mills.

Palm oil processing generates a number of waste by-products such as Empty Fruit Bunches (EFB), Oil Palm Shell (OPS), palm fibre, and Palm Oil Mill Effluent (POME)

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(Rupani et al., 2010; Otti et al., 2014). Among them, the latter (POME) is considered to be most environmentally-harmful when discharged untreated (TDIW, 1997; Rupani et al., 2010; Madaki and Seng, 2013). Palm oil mill effluent is a thick, brownish, colloidal slurry of water made up of oil, suspended solids, fatty acids, proteins, carbohydrates, and other plant materials (Okwute and Isu, 2007; Ohimain et al., 2012; Syirat et al., 2014). Untreated or under-treated POME can cause pollution-related problems, altering a number of environmental parameters like Dissolved Oxygen (DO), Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Carbon To Nitrogen (C/N) ratio, etc. (Okwute and Isu, 2007; Awotoye et al., 2011; Rupani et al., 2013).

As a one-time world's largest, and currently about the fifth largest, producer of palm oil (Parveen et al., 2010; Otti et al., 2014), Nigeria is home to a number of corporate and numerous traditional, small-scale, local palm oil refineries, which discharge their attendant effluent into the environment. Literature is awash with studies describing describing the environmental problems of POME, due to its observed toxic physico-chemical properties (Okwute and Isu, 2007; Igwe and Onyeagbado, 2007; Rupani et al., 2010; Ohiman et al., 2012; Syirat et al., 2014). However, research attention also needs to be focussed on the evaluation of genotoxic potentials of POME. The present study evaluates the physico-chemical and genotoxicity of POME, obtained from a corporate refinery in Nigeria, using the American Public Health Association (APHA) procedures and Allium chromosome aberration assay respectively; the latter being a fast, easy-to-handle genotoxicity test that gives reliable results (Rank and Nelson, 1994).

MATERIALS AND METHODS

The palm oil mill effluent, used for this study, was obtained from the Nigeria Institute for Oil Palm Research (NIFOR), Edo State, Nigeria. The effluent, which had been treated prior to disposal, was collected and transported to the laboratory in a 10-litre high, density plastic container, which was refrigerated at 4^{o}C throughout the study (Ademoroti, 2006).

The average-sized onion bulbs (*Allium cepa* L.), used for the study, were procured from Bariga market in Lagos, Nigeria. The onions were sun-dried for two weeks, after which the dry ones were selected for the test. The chemicals, beakers, test tubes, and other glass wares used for the study were collected from the laboratory store house of the Department of Cell Biology and Genetics, Faculty of Science, University of Lagos, Nigeria. All chemicals used were of analytical grade.

Palm oil mill effluent samples were analysed for physico-chemical properties (pH, DO, BOD_5, COD, suspended solids, total nitrogen oil, and grease), using the methods prescribed by the American Public Health Association (APHA, 1998) and Ademoroti (2006). A number of metals in the POME samples, namely lead (Pb), Zinc (Zn), Cadmium (Cd), Copper (Cu), and Chromium (Cr), were also analysed according to APHA (1998). Some of the results obtained from the physico-chemical analyses of POME were compared with the limits contained in the Environmental Management Guideline for the Palm Oil Industry published by Thai Department of Industrial Works (TDIW, 1997) as this was the only readily-available guideline, specifically released for palm oil industry.

To analyze the metals, 100 ml of the effluent sample was digested via heating with concentrated HNO_3 until the volume was reduced to 3 – 5 ml. This volume was made up to 10 ml with 0.1 N HNO_3. The concentration of the metal was estimated by using Atomic Spectrophotometer (Perkin Elmer Analyte A 200 model).

The Allium assay test was adapted from Fiskesjo (1997) and Olorunfemi et al.

(2011). The outer scales and brownish bottom plates of sun-dried bulbs were carefully removed, leaving the ring of primordial root intact. The peeled bulbs were placed in de-chlorinated tap water during the cleaning procedure to prevent the primordial from drying up.

The onion bulbs were grown in tap water at room temperature for 24 hours. When the roots were 1 – 2 cm long, they got transferred to the prepared POME concentrations of 10%, 20%, 30%, 100% (v/v, POME/water), and sole water (control). The test substrates were changed daily. Six onion bulbs were set up for each concentration and the control, out of which the best five were selected for evaluation.

To evaluate root growth inhibition, the roots of five onion bulbs with the best growth at each concentration were removed with a forceps. Their lengths were measured (in cm) with a meter rule at the end of the exposure period. The data were used to determine:

$$\text{Mean root length}(\text{cm}) = \frac{\text{Summation of root lengths}}{\text{Total number of root lengths counted}} \quad (1)$$

$$\text{Percentage root length} = \frac{\text{root length in test solution}}{\text{root length in control}} \times 100 \quad (2)$$

$$\text{Percentage root length inhibition} = \frac{\text{root lenth in control} - \text{root length in test solution}}{\text{root length in control}(\text{water})} \times 100 \quad (3)$$

The squash technique for onion root was used for chromosomal investigation, as described by Adegbite and Olorode (2002). Chromosome samples were prepared from the root tip meristem, containing actively-dividing cells. One root tip was squashed on each slide, stained with acetocarmine for 10 minutes. Cover slips were carefully lowered onto the slide to exclude air bubble. To prevent the possible drying out of the preparation by the heat of the microscope, the cover slips were sealed on the slides with clear fingernail polish. The slides were observed under the X40 objective of light microscope (Leica 2000 phase contrast microscope). Data on total cells, total dividing cells, and cells carrying chromosomal aberrations were taken from the slides, prepared for each of the different concentrations as well as the control.

Mitotic index was calculated by expressing the number of dividing cells as a percentage of total cells, counted for each of the treatments and the control.

$$\text{Mitotic Index} = \frac{\text{Number of dividing cells}}{\text{Total number of cells}} \times 100 \quad (4)$$

$$\text{Percentage Mitotic Inhibition} = \frac{\text{mitotic index in control} - \text{mitotic index in test solution}}{\text{mitotic index in control}} \times 100 \quad (5)$$

The frequency of chromosomal aberrations was calculated by expressing the number of aberrant cells as a percentage of total number of dividing cells for each treatment.

$$\text{Percentage aberration} = \frac{\text{Number of total chromosomal aberrations}}{\text{Total number of dividing cells}} \times 100 \quad (6)$$

The generated data was processed with Analysis of Variance (ANOVA) and the mean values were compared with Duncan Multiple Range Test (DMRT). Chi-square test was used to compare cell counts among POME concentration groups. All

analyses were done at 5% level of significance, using IBM SPSS version 22.

RESULTS AND DISCUSSION

Results from the analyses, carried out on POME, showed that total phosphate, potassium, and nitrogen levels in the POME were 195.00 mg/Lmg/L, 2049.00 mg/L, and 670.52 mg/L, respectively. The oil and grease level in POME was 893.11 mg/L while the pH was 5.6. The turbidity and alkalinity of POME were 500.0 mg/L and 498.4 mg/L, respectively. In addition, it was found that POME contained some trace/toxic metals. The levels of Pb, Cu, Cr, Zn, and Cd, found in the POME, were 0.02 mg/L, 0.75 mg/L, 0.19 mg/L, 2.96 mg/L, and 0.02 mg/L, respectively (Table 1).

Table 1. Physicochemical characteristics of POME

POME Characteristics	Concentrations	TDIW limit
Phosphate (mg/L)	195.00	-
Potassium (mg/L)	2,049.00	-
Total nitrogen (mg/L)	670.52	< 50
Moisture content (mg/L)	93.60	-
Oil and grease (mg/L)	893.11	< 25
pH (mg/L)	5.6	5 – 9
BOD (mg/L)	196.0	< 100
COD (mg/L)	1,841	< 1,000
Turbidity (NTU)	500.0	< 150
Chloride (mg/L)	528.0	-
Alkalinity (mg/L)	498.4	-
TDS (mg/L)	1,496.0	-
Conductivity (uS/cm)	2,992.0	-
DO (mg/L)	4.0	-
Lead (mg/L)	0.02	-
Copper (mg/L)	0.75	-
Chromium (mg/L)	0.19	-
Zinc (mg/L)	2.96	-
Cadmium (mg/L)	0.02	-

TDIW = Thai Department of Industrial Works (1997).

Some of these physico-chemical properties of POME, such as total nitrogen, oil, and grease, were far above the recommended limits set by Thai Department of Industrial Works (TDIW). In addition, the trace/toxic metals, including Cu, Zn, Cd, and Pb, found in the POME, were relatively high. Though these findings are in agreement with many other previous ones (Okwute and Isu, 2007; Igwe and Onyeagbado, 2007; Rupani et al., 2010; Ohiman et al., 2012; Syirat et al., 2014), the fact that the POME, used in this study, had undergone proper treatments prior to discharge, reinforces two facts: One, available POME treatment methods seem not effective enough to reduce the pollution load of POME to acceptable levels, and two, POME is a unique industrial effluent in this sense that it is such a strong pollutant that even in case of being subjected to full biological treatment and a highly-efficient one, the remaining effluent still had relatively high concentrations of pollutants, compared to general wastewater standards (TDIW, 1997).

The trace/toxic metals, contained in POME, could have come from a number of sources such as the soil, used for oil palm plantation, atmospheric deposition, and palm oil refining processes. Apart from the

fact that the poor physico-chemical qualities, observed in POME, pose a potential threat to ecological stability of the water bodies, into which it is discharged, the constituent metals in POME may indirectly find their way into human bodies via consumption of fish and other animals resident in these water bodies. Moreover, the presence of trace/toxic metals in POME suggests that the oil, extracted from the effluent, also contains these metals; therefore, it can be assumed that POME pollution has direct implications not only for ecological systems and the environment, but also human health.

Palm oil mill effluent inhibited root growth in *A. cepa* (Table 2), where the highest mean root length was recorded to be 1.91 ± 0.19 cm when exposed to water only (control) at 24 hours. Similarly, at 48 and 72 hours of exposure, the highest mean growth of 2.51±0.16 cm was recorded in the control. Onions exposed to 100% of POME concentration recorded higher mean root lengths than the ones, exposed to 10%, 20%, and 30% concentrations. Inhibition of the root growth by POME was generally higher at 24 hours exposure, with the highest inhibition of 55.5%, occurring in onions exposed to 20% of POME concentration. The least root growth inhibition of 5.98% was recorded in the onions, exposed to 10% of POME concentration at 48 and 72 hours of exposure.

As indicated in Table 3, POME had significantly high ($P < 0.01$) inhibitory effects on the mitotic activities of *A. cepa* in comparison to the control in this study. The number of dividing cells, mitotic index, and mitotic inhibition declined as POME concentrations increased. At 24 hours of exposure, POME concentrations of 10%, 20%, 30%, and 100% produced 16.76%, 24.31%, 35.20%, and 48.53% significantly high ($P < 0.01$) mitotic inhibitions in *A. cepa* roots, respectively, relative to the control. At 48 hours of exposure, the least (19.51%) and the highest (54.12%) mitotic inhibitions were produced by POME of 10% and 100% concentrations respectively. In the same vein, at 72 hours of exposure, the highest significant ($P < 0.01$) mitotic inhibition of 62.75% was produced by POME of 100% concentration. The differences in mitotic index among the *A. cepa* exposed to different POME concentrations were not significant ($P > 0.05$) at all evaluation intervals.

The chromosomal aberrations, observed in the roots of *A. cepa* that were exposed to different concentrations of POME, included multipolar, vagrant, C-mitosis, fragmented, bridged, sticky, bi-nucleated, and attached chromosomes (Table 4). There was no statistically-significant difference in chromosome aberrations of *A. cepa* roots, exposed to different concentrations of POME at 24 hours (χ^2 (3) = 1.845, $P > 0.05$), 48 hours (χ^2 (3) = 1.795, $P > 0.05$), and 72 hours (χ^2 (3) = 3.286, $P > 0.05$). Sticky chromosomes occurred most frequently throughout the observation phase, whereas multipolar and fragmented chromosomes were the least.

Table 2. Root growth and root inhibitions of *A. cepa* exposed to different concentrations of POME

POMEConcen-trations	Mean (cm) length			Percentage root length			Percentage root length inhibition		
	24hrs	48 hrs	72hrs	24hrs	48hrs	72hrs	24hrs	48hrs	72hrs
Control (water)	1.91±0.19	2.51±0.16	2.51±0.16	100.00	100.00	100.00	0.00	0.00	0.00
10%	0.90±0.13	2.36±0.16	2.36±0.16	47.12	94.02	94.02	52.88	5.98	5.98
20%	0.85±0.01	1.93±0.12	1.22±0.09	44.50	76.89	48.61	55.50	23.11	51.40
30%	0.98±0.71	1.36±0.09	1.36±0.13	51.31	54.18	54.18	48.69	45.82	45.82
100%	1.29±0.17	2.09±0.16	2.09±0.24	67.54	83.27	83.27	32.46	16.73	16.73

Table 3. The mitotic index and mitotic inhibition of *Allium cepa* exposed to the Effluent with different concentrations

POME Concentration	Number of dividing cells	Mitotic index (%)	Mitotic inhibition (%)
24 hours			
Control	51b	10.20	0.00a
10%	41b	8.49	16.76b
20%	37ab	7.72	24.31cb
30%	31ab	6.61	35.20cd
100%	24a	5.25	48.53d
χ^2 (P)	11.34 (< 0.05)*	1.74 (> 0.05)	52.25 (< 0.01)*
48 hours			
Control	51b	10.20	0.00a
10%	39ab	8.21	19.51b
20%	33ab	7.10	30.39c
30%	27a	5.96	41.57c
100%	21a	4.68	54.12c
χ^2 (P)	15.58 (< 0.01)*	2.06 (> 0.05)	56.44 (< 0.01)*
72 hours			
Control	51b	10.20	0.00a
10%	35ab	7.50	26.47b
20%	23a	4.97	51.28c
30%	20a	4.44	56.47c
100%	17a	3.8	62.75c
χ^2 (P)	26.74 (< 0.01)*	4.65 (> 0.05)	66.53 (< 0.01)*

Values with the same alphabet are not significantly different from one another (P > 0.05)
* = differences between groups are significantly different (P < 0.05).

Table 4. Chromosomal aberrations and percentage chromosomal aberrations in *Allium cepa* root cells, exposed to different POME concentrations.

POME Concen-tration	Chromosome aberration type									
	Stk	Cmit	Brg	Vag	Bnc	Mpl	Att	Frag	Total	% aberration
24 hours										
10%	7	0	4	5	0	0	2	0	20	48.78
20%	7	0	5	5	0	1	3	0	21	56.76
30%	6	0	4	4	0	0	2	0	16	51.61
100%	5	2	3	2	0	0	2	0	14	58.33
48 hours										
10%	8	0	6	4	0	0	3	0	22	56.41
20%	7	0	5	5	0	0	2	0	19	59.58
30%	6	0	4	3	0	0	3	1	18	59.26
100%	3	0	3	4	2	0	2	0	14	66.67
72 hours										
10%	6	1	5	2	0	0	4	0	16	51.43
20%	6	0	4	3	0	0	2	0	18	69.57
30%	5	0	3	3	0	0	2	0	13	65.00
100%	4	0	2	2	0	0	1	0	9	52.94
Total	70	3	46	42	2	1	28	1	182	-

Stk = sticky; Cmit = c-mitosis; Brg = bridged; Vag = vagrant; Bnc = bi-nucleated; Mpl= multipolar; Att: attached; Frag = Fragmented.

The lowest percentage chromosomal aberrations of 48.78% was observed in *A. cepa* roots exposed to 10% POME concentration, while the highest percentage chromosomal aberrations of 58.33% was recorded in the roots of *A. cepa* exposed to 100% POME concentration. Palm oil mill effluent of 20% concentration produced the highest total count of aberrations, which was 21, 19, and 18, at 24, 48, and 72 hours of exposure, respectively. Figures 1a-l illustrates photomicrographs of some representative pictures of normal mitotic cells, and cells containing aberrant chromosomes as observed in this study. Normal mitotic stages were observed in the root tip cells, exposed to water only (control) with proper divisions of the cell stages occurring in the control, too.

Inhibition of root growth and induction of chromosomal aberrations are indicative of cytotoxicity and genotocicity, occasioned by the contaminants, especially metals, in POME. Mitotic inhibition has been associated with cell cycle disruption and chromatin dysfunction, induced by metal-DNA interactions (Glinska et al., 2007). Some metals like Cd and Pb have been implicated in enzyme inhibition, mutation, and chromosomal irregularities (Zhang and Yang, 1994; Johnson, 1998).

Plant systems, like *A. cepa*, are among the bioassays, developed in order to detect mutagenicity, genotoxicity, and cytotoxicity, brought about by environmental pollutants. They are sensitive, cheap, and effective bioassays (Gopalan, 1999; Grant, 1999). Plant roots are generally used in bioassays since they are the first structures to be exposed to chemicals in both water and soil (Fiskesjo, 1997; Odeigah et al., 1997). *Allium* test is reported to have high correlations with other test systems (MIT-217 cell test with mice, rats, or humans *in vivo*) and could be used as an alternative to laboratory animals in toxicological research (Fiskesjo and Levan, 1993). As a result, the harmful effects observed in plant genetic materials could also be similarly observed in man and other animals. Hence, the chromosomal aberrations, induced by POME in this study, suggest that POME may be potentially genotoxic to both man and other animals. In view of this undesirable reality, there should be specific attention paid to finding alternative uses of POME rather than its mere disposal. It has been recorded that POME is potentially capable of being converted to a number of value-added products such as (1) citric acid, (2) carotenoid, which can be further utilized for the production of vitamin A and E (Alam et al., 2008), (3) fertilizer (Basiron and Weng, 2004), and (4) biodiesel (Gutiérrez et al., 2009). The increased mean root lengths observed in onions exposed to 100% of POME concentration, compared to the ones exposed to lower POME concentrations in this study, could be due to maximal availability of organic nitrogen and organic sulphur in 100% POME (Nwoko et al., 2012), which gives credence to POME's potential usage as an organic fertilizer.

CONCLUSION

The results of this study indicate that palm oil mill effluent is not only capable of causing ecological disruptions in the environment receiving it, but is also potentially genotoxic to resident organisms. Therefore, sustained research efforts are needed to transform POME to other value-added products, instead of merely disposing it. In the meantime, if POME would not be converted to other useful products and needs to be disposed, it should first be subjected to adequate and effective treatments to reduce its pollutant constituents to safe limits.

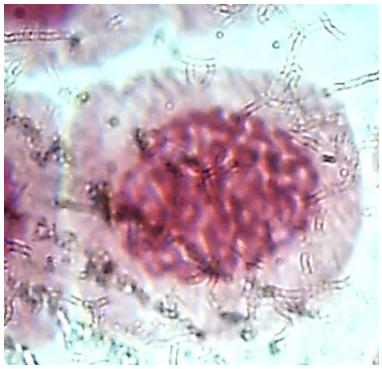

Fig. 1a. Normal prophase

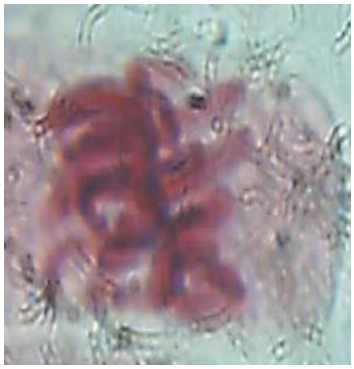

Fig. 1b. Normal metaphase

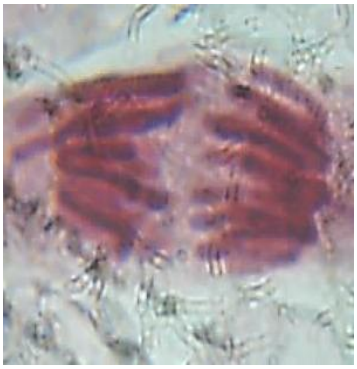

Fig. 1c. Normal anaphase

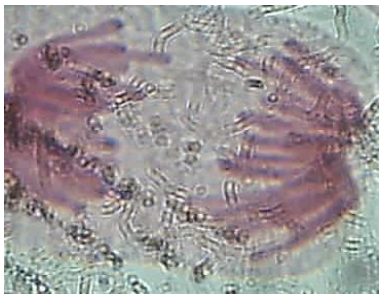

Fig. 1d. Normal telophase

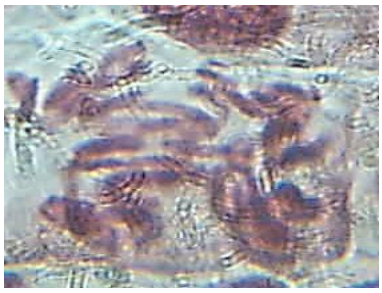

Fig. 1e. Multipolar chromosomes

Fig. 1f. Vagrant chromosome

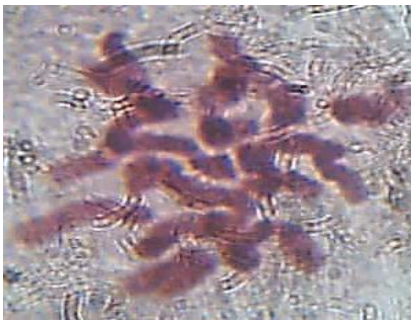

Fig. 1g. C- Mitosis

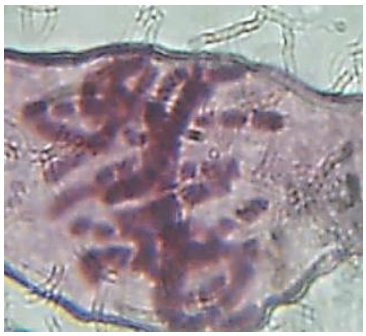

Fig. 1h. Fragmented chromosomes

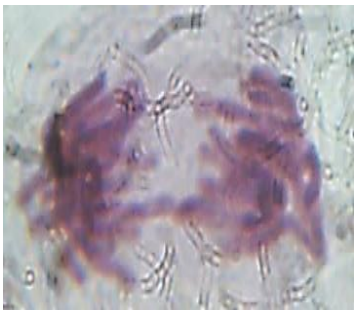

Fig. 1i. Bridged chromosomes

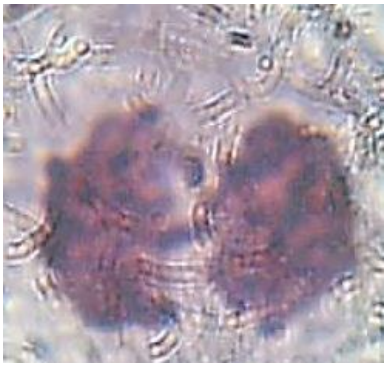

Fig. 1j. Sticky chromosome

Fig. 1k. Bi-nucleated chromosome

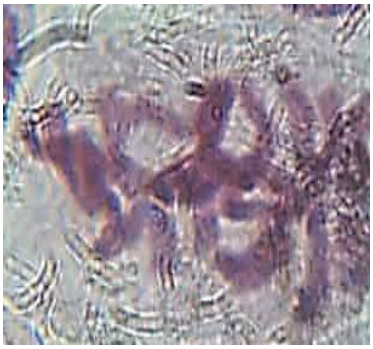

Fig. 1l. Attached chromosomes

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