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Comparative Effects of Colchicine, 8- Hydroxyquinoline and Paradichlorobenzene on the Lengths of Mitotic Chromosomes in *Allium cepa* L.

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Abstract: Studies were carried out to assess the comparative effects that 0.5% aqueous solution of colchicine, 0.004M 8-hydroxyquinoline and saturated solution of paradichlorobenzene may have on the lengths of mitotic chromosomes. The somatic chromosomes were studied in root meristems of *Allium cepa* L. Karyometric analysis of the onion root tips chromosomes revealed a chromosome number 2n = 16. The highest mean haploid chromosomes length of 8.443µm was observed for chromosomes treated with 0.5% colchicine, while 7.168µm and 7.188µm were recorded for chromosomes treated with 0.004M 8-hydroxyquinoline and paradichlorobenzene respectively. Analysis of Variance showed that there were significant differences among the treatments ($p<0.05$) in the lengths of chromosome 1, 2, 3 and 4 while there were no statistically significant differences ($p<0.05$) in the lengths of relatively shorter chromosomes (5 – 8) due to the three pre-treatments. Least Significant Difference (LSD) test for chromosomes 1-4 showed that these chromosomes pre-treated with paradichlorobenzene where significantly shorter ($p<0.05$) than those treated with colchicine, however there were no significant differences between these chromosomes treated with paradichlorobenzene and 8-hydroxyquinoline. Although advantageous, the differential contraction rates due to the three pretreatments, which may have been due to differences in the concentrations of the reagents and position of the sampled cells in the cell cycle, calls for a standardization of measurements of pre-treated chromosomes.

Key words: Chromosome lengths, mitosis, haploid complement, colchicine, 8-hydroxyquinoline, paradichlorobenzene

1. Introduction

Cytogenetic studies elucidate vital information on cell organisation under normal and artificial conditions. Examining the effects of exogenous substances on cell activities and structure remains one of the more

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promising approaches to mode of action studies. The lengths and thickness of chromosomes are important features that affect the visibility of somatic metaphase chromosomes (Osalou *et al*., 2013) and also find applications in cytotaxonomy. The relative size, the visual appearance of chromosomes during mitotic cell division and the degree of symmetry are key phenotypic markers for karyomorphological descriptions which are easily inferred from the karyotype (Lavania and Srivasta, 1992).

A critical look at the chromosomes of vegetative cells during mitosis reveals that at maximum condensation, chromosomes tend to resist spreading rendering chromosome number counts, size determination and structural studies strenuous at best and most often unattainable. Numerous methods have been developed for preparing mitotic chromosomes for easy viewing and in these methods, a number of substances have been used to enhance the spread of mitotic chromosomes during squashing. These substances include among others colchicine, 8-hydroxyquinoline, paradichlorobenzene, ice cold water and ȧ-bromonaphthalene (Osalou *et al*., 2013). Though these chemicals have been observed to produce desirable effects as it pertains to chromosome spread and visibility through the inhibition of spindle apparatus, and though the roles of these chemicals in spindle fibre inhibition have been elucidated by many authors (Burley, 1964; Amer, 1960) there is a dearth of reports on their effects on the lengths of mitotic chromosomes.

Allium cepa, the onion plant, is an outstanding test organism because of its sensitivity to xenobiotics (Leme and Marin-Morales, 2008) as well as its suitable chromosome features: the mitotic stages are distinct and spontaneous chromosomal aberrations are rare (Firbas and Amon, 2013). Indeed, almost all *Allium* species possess symmetrical median to submedian centromeric chromosomes with little deviation in size although a few telocentric chromosomes are present in a number of species (Hiroya, Kenji and Yukiko, 2002). A mean haploid idiogram is usually prepared from metrical data collected from the karyotype of a species. To do this, the chromosomes are arranged in pairs based on the data from the karyotype and in their descending lengths.
In his study on the karyotype of the sitka spruce, *Picea sitchensis*, Burley (1964) observed that at five hours of pretreatment, the total haploid complement of the chromosome had a 41.6% contraction due to the effect of 1% aqueous solution of colchicine but at twenty four hours in 0.002M 8-hydroxyquinoline, the total haploid compliment showed only a 37.2% contraction. Mergen and Burley (1963) however showed that the contraction of chromosomes by 0.002M 8–hydroxyquinoline was intermediate between those of 1% and 0.5% aqueous solution of colchicine. Ekong *et al*., (2014) investigating the comparative effects of colchicine, 8-hydroxyquinoline and paradichlorobenzene on the arm ratio of mitotic chromosome observed no significant differences among the three treatments although each treatment seem to have a differential effect that could have resulted from their interactions with the euchromatin.

It is clear that the effect of the three pretreatment chemicals on chromosome metrical characteristics have not been completely elucidated. It was therefore considered that the study of the comparative effects of colchicine, 8- hydroxyquinoline and paradichlorobenzene on the lengths of mitotic chromosomes could yield much insight into the use of the pretreatments during cytological studies. In this light, the present research sought to investigate the effects of colchicine, 8-hydroxyquinoline and paradichlorobenzene on the lengths of mitotic chromosomes in *Allium cepa* L.

2. Materials and Methods

For this study, healthy onion bulbs which had wintered and budded were obtained from the Uyo Main Market, Uyo, Akwa Ibom State, Nigeria. The dried external leaves and roots were removed before the onion bulbs were floated off in sterile distilled water with the stem disc just touching water, until the roots sprouted after 3-4 days.

A preliminary study showed a preponderance of the metaphase stage between 9am and 10 am at Uyo lying between Latitudes 5.02^0N – 6.10^0 N and Longitudes 7.92^0E – 9.48^0E (DGRPUU, 2009). Martens and Reisch (1988) reported that the time of sampling is a very crucial factor of note, especially when studying cell activity and mitosis. The root tips harvested at that time were immediately transferred to four glass flasks respectively containing:

(a) Distilled water
(b) 0.5% aqueous solution of colchicine
(c) 0.004M 8-hydroxyquinoline
(d) Saturated solution of paradichlorobenzene.

The flask containing the root tips were then stored in a refrigerator maintained at 15ºC for 4 hours to prevent chromosome fragmentation. At the end of the duration of pretreatments, samples were collected separately and fixed in acetic alcohol (1:3 v/v) for 24 hours at room temperature. Well-stoppered test-tubes containing 1N hydrochloric acid were partially immersed in a water bath maintained at 60°C for three (3) minutes to bring the temperature of the acid to 60°C. The root tips were thereafter hydrolyzed in the 1N HCl acid at 60ºC for 6 minutes. The hydrolyzed root tips were rinsed in three changes of sterile distilled water and excess fluid was removed with the help of Whatman filter paper. About 1.5mm of the root tip was cut off and placed in a drop of aceto-orcein stain on a clean microscope slide, macerated, covered with a clear coverslip and examined under the light microscope. The total lengths of the chromosomes were measured with the aid of an ocular micrometer and the mean chromosome lengths were calculated from six cells per treatment. The sixteen chromosomes in each cell were arranged in eight homologous pairs in descending order by the inspection of

individual arm lengths and total chromosome lengths. The means of the chromosome lengths due to the three chemicals were used to calculate a mean haploid compliment for each treatment. The means were then exposed to analysis of variance and significantly different means were separated using the Least Significant Difference (L.S.D) test. The relative lengths of the each pre-treated chromosomes were calculated as a percentage of the total haploid complement length. The ratio index was calculated as a ratio of the longest chromosome to the shortest.

Idiograms of the haploid compliments of *Allium cepa* pre-treated with the three chemicals were prepared. For each treatment, a mean haploid idiogram was built using metrical data from the karyotype. Chromosomes were then arranged in pairs according to descending lengths. The absolute lengths of the long and short arms of each chromosome pair were taken. The data were then pooled to produce a mean length of each chromosome pair for a treatment.

3. Results

The mean lengths of the *Allium cepa* chromosomes pre-treated with the three chemicals and the relative length of each chromosome expressed as a percentage of the total chromosome length due to that treatment are presented in Table 1. Overall, chromosomes pre-treated with colchicine varying between 0.734 and 1.388 recorded a total haploid complement length of 8.443, a mean length of 1.055±0.215 and a ratio index of 1.89. For those pre-treated with 8-hydroxyquinoline, individual chromosome length ranged from 0.671 to 1.085, recorded a total haploid compliment length of 7.168, a mean length of 0.896±0.144 and a ratio index of 1.62 as compared to the range of 0.654 to 1.167, a total haploid complement length of 7.188, a mean length of 0.899±0.171 and ratio index of 1.78 recorded for paradichlorobenzene. Subjecting the data to a One Way Analysis of Variance showed that there were significant differences among the treatments ($p<0.05$) in lengths of chromosome 1, 2, 3 and 4 while there were no statistically significant differences ($p>0.05$) in the lengths of relatively shorter chromosomes (5 – 8) due to the three pre-treatments. Least Significant Difference (LSD) test for chromosomes 1-4 showed that these chromosomes pre-treated with paradichlorobenzene where significantly shorter ($p<0.05$) than those treated with colchicine, there were no significance difference between these chromosomes treated with paradichlorobenzene and 8-hydroxyquinoline.

Table 1: Mean Chromosome lengths (μm ±s.e) and Relative lengths (%) for the pre-treatments

Chromosome number	COL		8HQ		PDCB	
	MCL ±SE	RL%	MCL ±SE	RL%	MCL ±SE	RL%
1	1.388 ±0.038[a]	16.439	1.085 ± 0.034[b]	15.137	1.167± 0.077[b]	16.235
2	1.250 ±0.029[a]	14.805	1.033 ± 0.025[b]	14.411	1.075± 0.073[b]	14.955
3	1.162 ±0.042[a]	13.762	0.979 ± 0.024[b]	13.658	0.988± 0.046[b]	13.745
4	1.092 ±0.046[a]	12.934	0.954± 0.027[b]	13.309	0.900± 0.062[b]	12.521
5	1.033 ±0.036[a]	12.235	0.883± 0.029[a]	12.319	0.854± 0.049[a]	11.881
6	0.933 ±0.059[a]	11.051	0.821± 0.038[a]	11.454	0.800± 0.043[a]	11.130
7	0.846 ±0.063[a]	10.020	0.742± 0.039[a]	10.351	0.750± 0.052[a]	10.434
8	0.734 ±0.057[a]	8.694	0.671± 0.033[a]	9.361	0.654± 0.056[a]	9.099
Total	**8.443**	**100.00**	**7.168**	**100.00**	**7.188**	**100.00**
Mean	**1.055**		**0.896**		**0.899**	
SD	**0.215**		**0.144**		**0.171**	
Ratio index	**1.89**		**1.62**		**1.78**	

Note: Figures in rows with the same alphabets are not significantly different ($p<0.05$).

COL = Colchicine; 8HQ = 8-Hydroxyquinoline; PDCB = Paradichlorobenzene; RL = Relative Length; MCL = Mean chromosome length; SE= Standard error of mean; SD = Standard deviation

Figure 1 represents a comparative plot of relative chromosome lengths for the 8 haploid complements due to the three pre-treatments. Although the decreasing trend is observed for the lengths of the eight chromosomes, the trend revealed that colchicine had a slightly higher contracting effect on shorter chromosomes than the longer chromosomes when compared with the other treatments. Furthermore, the result strongly suggests that each pretreatment chemical has a differential contracting effect on the chromosomes resulting in the observed differences among the chromosomes as shown by the differential ratio indices and relative chromosome lengths between chromosomes. This fact is more obvious when the mean haploid karyotypes due to three treatments are compared as shown in Figures 2 and 3. It is clear from these figures that the reported concentrations of paradichlorobenzene and 8 hydroxyquinoline contracted the chromosomes more at 3-4 hours than colchicine.

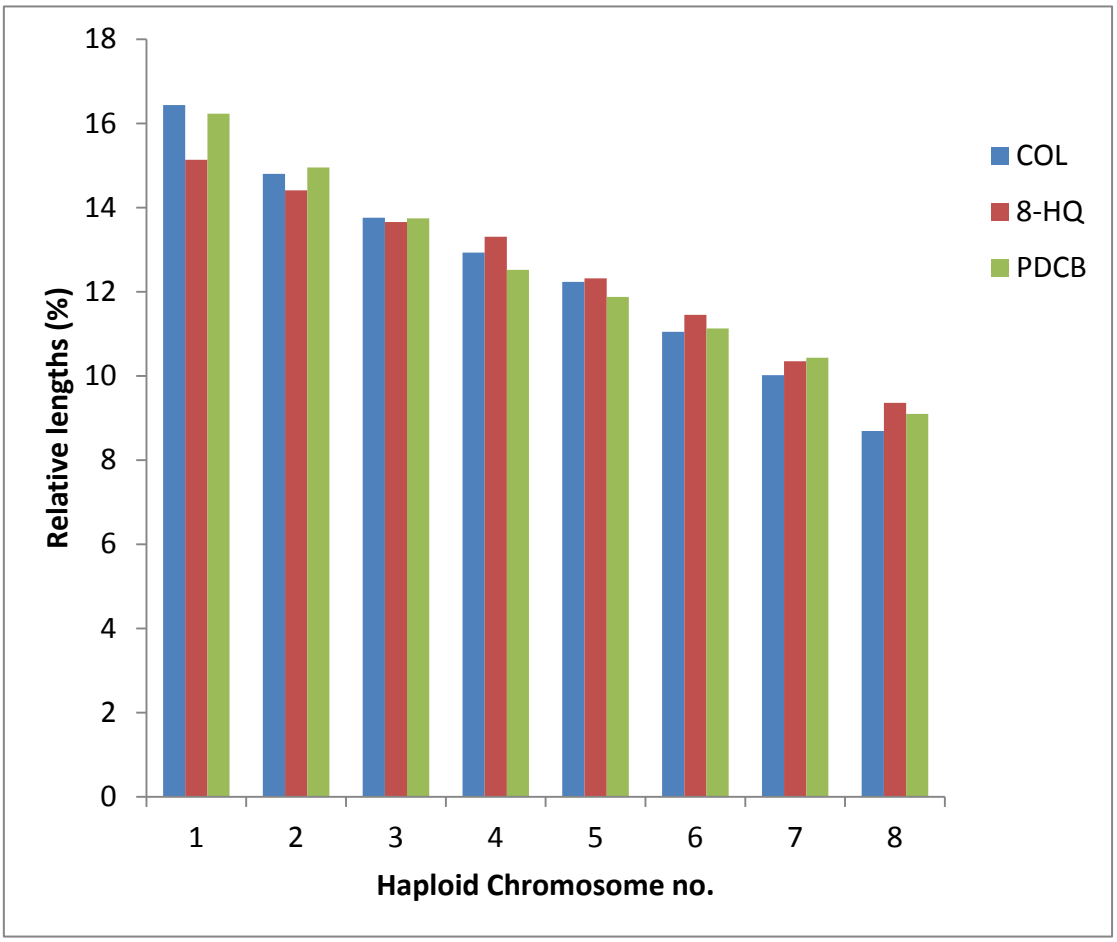

Figure 1: Comparative plot of relative chromosome lengths due to the three pre-treatments

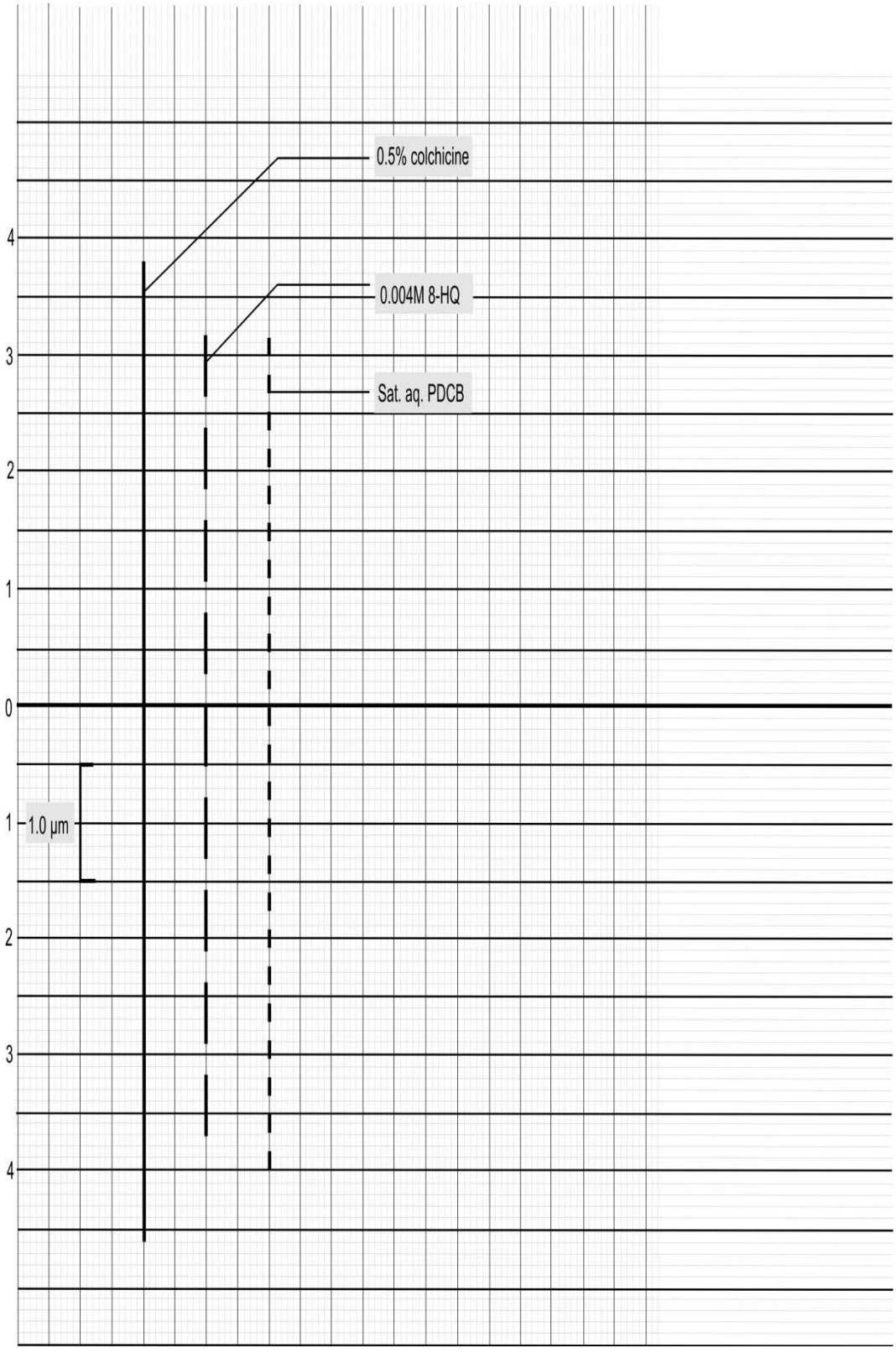

Figure 2: Mean haploid karyotypes due to three treatments based on six cells per treatment

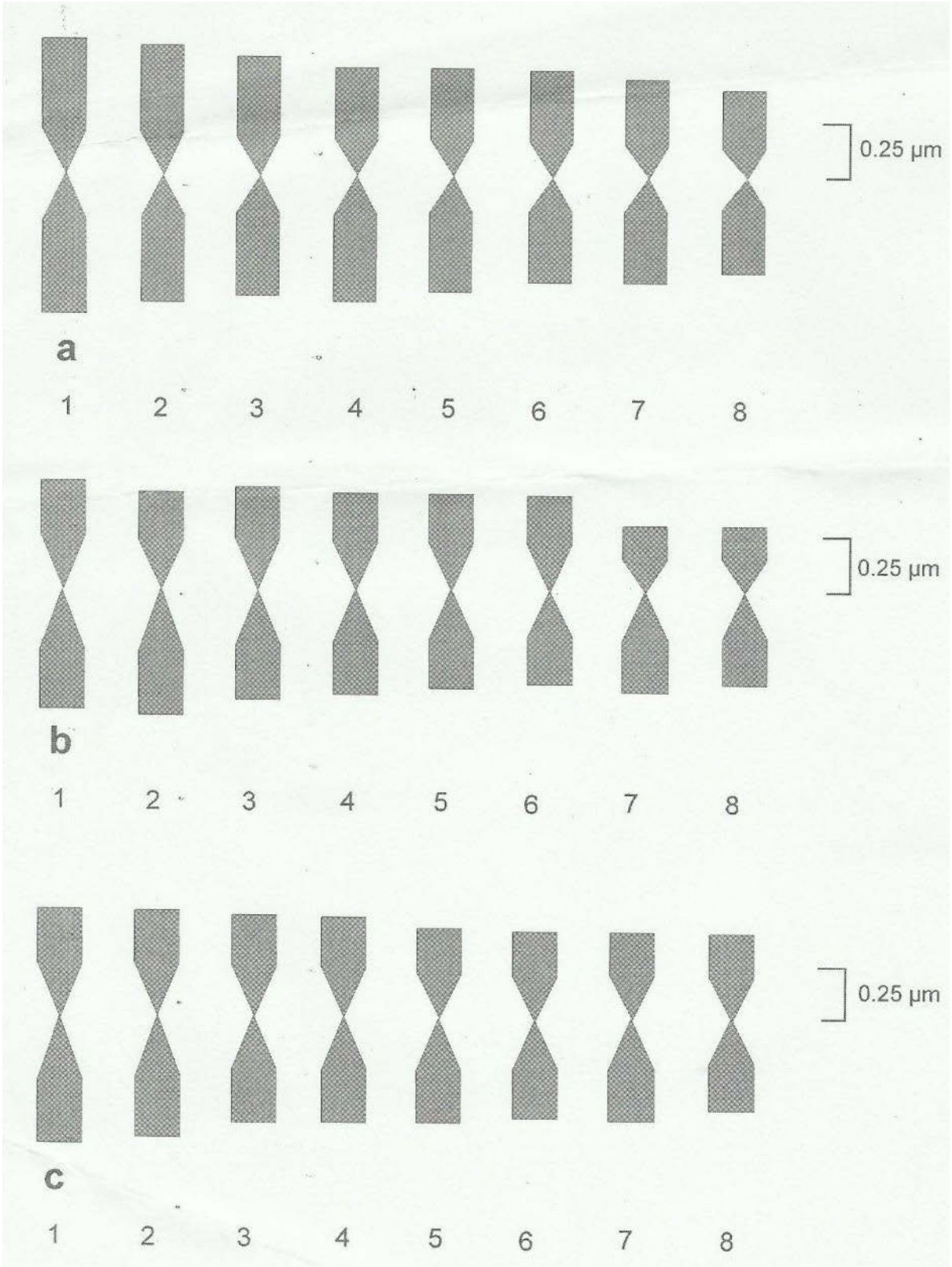

Figure 3: Idiograms of the haploid complements of A. cepa pretreated with (a) colchicines (b) 8-hydroxyquinoline and (c) paradichlorobenzene

The differential contraction in chromosome lengths due to the three treatments as seen in chromosome 1-4 of the haploid complement of *Allium cepa* in this study corresponds to the findings of Burley (1964) and Mergen and Burley (1963) that colchicine and 8-hydroxyquinoline contracted chromosomes differentially and that the contraction of the chromosomes by 8-Hydroxyquinoline was intermediate between those of 1% and 0.5% aqueous solutions of colchicine. The differential contraction could have also been due to the interactions of each pretreatments with euchromatin found in the different chromosomes arms as earlier reported by Ekong *et al*., (2014).

The result obtained from the present study also revealed that chromosomes 1-3 pre-treated with 8-hydroxyquinoline were shortest as compared to those of the same chromosomes pre-treated with colchicine which were the longest. This finding is in opposition to the findings of Burley (1964) that 1% aqueous solution of colchicine contracted the chromosomes slightly more than 0.002M 8-hydroxyquinoline did. The divergence of the findings of the present study from that of Burley (1964) can be reconciled by the fact that the concentrations used in the present study differed from those used by Burley (1964). This implies that contraction is a function of the concentration of the treatments and the time of exposure as espoused by Timari and Timari (1983).

The difference in mean length between the chemical treatments could have resulted from the difficulty of measuring relatively long and intertwining chromosomes 1-4 with the ocular micrometer as reported by Burley (1964). This finding agrees with the findings of Mergen and Burley (1963) that measurements of long chromosomes with the ocular micrometer were prone to errors.

The difference in length in chromosome 1-4 may also have been as a result of variability in chromosome lengths within the mitotic cycle. This finding corresponds with the findings of Burley (1963) that because mitosis is a continuous process, it was almost impossible to select cells in precisely identical stages of mitosis.

The absence of statistical difference in the contraction of the shorter chromosomes 5-8 chromosome was noted in this study. The three treatments showed very slight differential contraction in chromosomes 5-8. A similar result was obtained by Mergen and Burley (1964).

4. Conclusion

The findings of this study suggest that the three pre-treatment chemicals, colchicine, 8-Hydroxyquinoline and paradichlorobenzene are all effective in inhibiting the mitotic spindle and arresting the chromosomes at metaphase. However, each chemical elicits a differential contraction of the chromosomes. This differential chromosomes contraction by these three pre-treatments shows a concentration-dependence and may also have been impacted by the mensuration technique – the ocular micrometer - used to measure longer chromosomes.

The differential contraction rates due to the three pretreatment may also have be due to differences in the concentrations of the reagents and position of the sampled cells in the cell cycle. From this study it is obvious that though all the three treatments were useful in condensing chromosomes for cytological studies, colchicine had the best all round properties. However, in view of the cost implication and high carcinogenicity ascribed to colchicine, 8- hydroxyquinoline and paradichlorobenzene are both good substitutes for colchicine in contracting metaphase chromosomes for cytological studies although 8- hydroxyquinoline showed several undesirable visual effects. The differential rates of chromosome contraction therefore call for a standardization of chromosome lengths measurements.

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