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EVALUATION OF CALCIUM CARBIDE'S CYTOTOXIC EFFECTS ON ONION (*Allium cepa*)

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ABSTRACT

The purpose of this investigation was to see if Calcium Carbide (CaC_2) had any harmful effects on onions (*Allium cepa* L.). *Allium cepa* root tips were grown in various concentrations of Calcium Carbide (CaC_2) (0.25g, 0.50g, 0.75g, and 1.00g)/ 250ml, with distilled water serving as a control. For cytological tests, the root tips of *Allium cepa* growing in the treatments and control were removed daily between 7:30am and 8:30am. Pretreatment, fixation, hydrolysis, squashing, and staining of cells for mitotic investigations were performed, and data on cytological parameters were collected using a light microscope at a magnification of X40. The mitotic index (MI) and karyotype analysis were used to assess the data collected on these parameters. The results of this study showed that root tips treated with 0.25g of Calcium Carbide (CaC_2) have a mitotic index of 45.83 and the rate of cell division decreases with an increase in the concentration of Calcium Carbide (CaC_2) as the cell divides the mitotic index dropped sharply. These finding indicated that Calcium Carbide is a strong mitotic inhibitor and could give rise to mitotic abnormalities with increase in concentration and also reduced cell division. We therefore recommended that there is need for further investigation using lower concentrations of Calcium Carbide as well as other mutagenic substances in order to ascertain their effect on the chromosomal behavior.

Keywords: Calcium Carbide; *Allium cepa*; Chromosome; Karyotype; Mutagen.

INTRODUCTION

Allium cepa is a member of the Plantae Kingdom, Magnoliophyta Division, Class Liliopsida, Order Asparagales, Family Liliaceae, Genus Allium, and Species *Allium cepa*. Carolus Linnaeus described it for the first time in his 1753 book Species Plantarum (Linnaeus, 1753). *Allium cepa* is one of the edible species in the Allium genus, which includes over 700 species (Burnie *et al*.,1999). In the warm-temperate hills of eastern Nepal, the onion (*Allium cepa* L.) is the most common edible Allium, followed by garlic (*Allium sativum*), and shallot (*Allium cepa* Aggregatum group) (Gautam, Neupane, Baral, Rood, & Pun, 1997).The most important properties of onions include antioxidant, anticancer, antimicrobial, asthma, and cardiovascular compounds such as sulphur, organo-sulphur, calcium, and riboflavin. Onions have a variety of health benefits including anti-carcinogenic, anti-platelet, anti-thrombotic, anti-diabetic, fibrinolytic, and hypocholesterolemic properties, as well as other biological actions such as antibi (Ashwini & Sathishkumar, 2014). Despite the abundance of information on onions and their curative effects on diabetes, cardiovascular disease, and respiratory disorders, it appears to be insufficient (Ashwini & Sathishkumar, 2014). Onion contains allins, which inhibit the formation of cancerous cells. In the mid-1990s, studies revealed that onions could help with cardiovascular issues. 3-mercapto-2-methylpentan-1-ol is an antioxidant that inhibits peroxynitrite-induced illnesses in onions (Shah & Gopal, 1988). The attribute of being hazardous to cells is known as cytotoxicity. An immune cell or some types of venom, such as from the puff adder (Bitis arietans) or the brown recluse spider, are examples of harmful substances (Loxosceles recluse). When cells are exposed to the cytotoxic chemical, they can develop a range of cell fates. The cells can either stop actively growing and dividing (a drop in cell viability) or start a genetic program of controlled cell death (a decrease in cell viability) (apoptosis).Necrosis causes cells to enlarge quickly, lose their membrane integrity, shut down metabolism, and spill their contents into the environment. Calcium carbide, or CaC_2, is a chemical compound with the formula CaC_2. It is primarily used in the manufacturing of acetylene and calcium cyanide in industry.

Calcium carbide is made in an electric arc furnace at a temperature of about 2200°C from a mixture of lime and coke. Since its creation in 1892, this approach has remained unchanged:

$$CaO + 3\,C \rightarrow CaC_2 + CO$$

Calcium carbide is sometimes employed as a source of acetylene gas, which is a ripening agent similar to ethylene, in the artificial ripening of fruit. However, in certain countries, this is prohibited since eating fruits that have been artificially ripened with calcium carbide can create major health concerns in individuals who consume them. Supplementing calcium during growth has been shown in numerous studies to improve the firmness of fruits and vegetables

(De Ell et al., 2001; Manganaris,et al., 2006; Tolvonen & Bowen 1999). Evidence of chromosomal aberration in the chromosome structure of *Allium cepa* are studied in this research work.

MATERIALS AND METHOD

Experimental Materials

Calcium Carbide (CaC_2) was obtained from a commercial chemical dealer in Ilorin, Kwara State, Nigeria, while *Allium cepa* bulbs were obtained from Ago Market in Ilorin, Kwara State, Nigeria.

Preparation of Test Materials
To make different concentrations of Calcium Carbide (CaC_2), 0.25g, 0.50g, 0.75g, and 1.0g of Calcium Carbide (CaC_2) were independently weighed (using a chemical weighing balance) into 250ml of distilled water within the fume cupboard. The study used distilled water as a control. The outer scale of *Allium cepa* bulbs approximately 1.5 to 2.2cm in diameter was gently removed after rinsing with running water. To avoid destroying the root primeval, old root remains were removed. By putting the shortened stems in touch with the containers, these healthy onion bulbs were able to establish roots. The onions were allowed to grow little roots before being moved to beakers with various calcium carbide concentrations (CaC_2). Onion roots were allowed to develop for 24 hours in each solution. Between 7:30am and 8:30am, root tips of onion samples measuring about 2cm in length were gathered into vials, and the investigation continued in stages according to the procedure stated by (Abu, Asomba, & Ubani, 2015).

Pretreatment
Each treatment's onion root tips were put into specimen vials containing 8-hydroxyquinoline for 3 hours of preparation.

Fixation
After removing the prepared root tips from the 8-hydroxyquinoline, they were rinsed in distilled water for five minutes to eliminate the 8-hydroxyquinoline, and then placed in fixative. In a 1:3 ratio, glacial acetic and absolute ethanol make up the fixation. The fixative aids in the killing of root cells while also keeping them in their natural state, preventing the contents of the cells from being leached out. The specimens were labeled properly.

Hydrolysis
After 3 hours in the fixative, the root tips were removed and rinsed in distilled water for 3 minutes before hydrolysis. The root tips were hydrolyzed using a 5N HCl solution;to soften the root tips, they were hydrolyzed for 12 minutes at room temperature.

Squashing and Staining
The hydrolyzed root tips were rinsed in distilled water for two minutes before being placed on a clean glass slide. On the root tips, two drops of 2% acetic orcein stain were applied and covered with cover slip. Squashing was carried out with the broader flat end of a cylindrical needle until a turbid suspension was visible. When necessary, the slides were gently flamed using a spirit lamp. For each treatment, five slides were produced and labeled in this manner.

Chromosome Observation
Under a light microscope, the slides were mounted and examined. The slides were viewed with the X4, X10, and X40 objectives. Using photomicrograph at X40 objectives, photographs of normal mitotic phases and aberrant cells were taken.

Data Analysis
The Mitotic index (MI) was computed using the method of Auti et al., (2010) for cells treated with varying doses of calcium carbide and controls

$$\text{INDEX OF MITOTIC INDEX} = \frac{\text{NUMBER OF DIVIDING CELLS IN TOTAL}}{\text{NUMBER OF CELLS EXAMINED IN TOTAL}} \times 100$$

Individual chromosomes were taken out, arranged in descending order of length, and matched on the basis of centromere position to provide a karyotype analysis. The chromosomes are characterized as metacentric (M), submetacentric (Sm), or sub telocentric (St) based on the centromere. Individual chromosomes were taken out, arranged in descending order of length, and matched on the basis of centromere position to provide a karyotype analysis.

$$\text{D.I} = \frac{\text{LONGEST CHROMOSOMES} - \text{SHORTEST CHROMOSOMES}}{\text{LONGEST CHROMOSOMES} + \text{SHORTEST CHROMOSOMES}} \times 100$$

RESULTS

Mitosis and karyotype were studied using cytological techniques. In mitotic division, chromosomes were investigated. The total number of cells, the number of dividing cells, the mitotic index, the length of the chromosome, and the karyotypic formular were all noted. As indicated in table 1, root tips treated with 0.25g of calcium carbide show fewer aberrations during cell division and have a mitotic index of 45.83. As the mitotic index reduces dramatically, the rate of cell division falls with increasing calcium carbide concentration.

Table I : Root tips treated with varying amounts of calcium carbide. Mitotic index of *Allium cepa* L.

S/N	CALCIUM CARBIDE CONCENTRATION	TOTAL NO. OF CELLS	TOTAL NO. OF DIVIDING CELLS	MITOTIC INDEX
1.	0.25g	24	11^{d}	45.83^{ab}
2.	0.50g	24	9^{c}	37.50^{ab}
3.	0.75g	24	8^{c}	33.33^{ab}
4.	1.00g	24	5^{b}	20.83^{b}
5.	CONTROL	24	14^{a}	58.33^{a}

Mean followed by different letter(s) along a column are significantly different at 0.05 probability level.

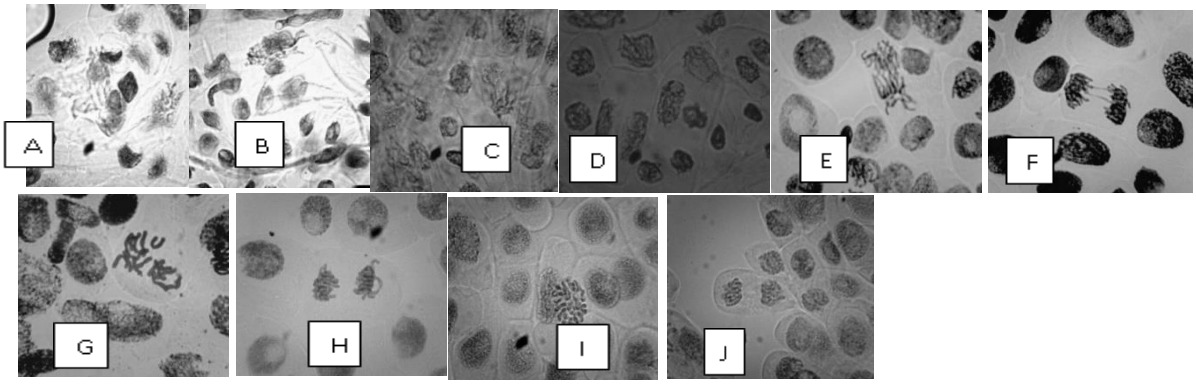

Figure I: Control experiment and photographs of *Allium cepa* roots revealing aberrant stages of cell division (plates A-D) (E-J). A: Chromosome fragmentation; B: Chromosome fragmentation; C: Chromosome fragment C: Adherent chromosome, B: Chromosome clumping D: Anaphase abnormality E stands for anaphase, F for anaphase bridge, G for metaphase, and H for normal anaphase. I stands for prophase, and J stands for late telophase (Magnification X40)

Karyotype Study

1M+6SM+1ST was the karyotypic formula. The longest chromosome is 2.1 meters long, while the shortest is 0.5 meters long. Using the formula above, the disparity index (D.I) can be determined as follows:

$$D.I = \frac{2.1 - 0.5}{2.1 + 0.5} X100$$

$$= 61.54\ \mu m$$

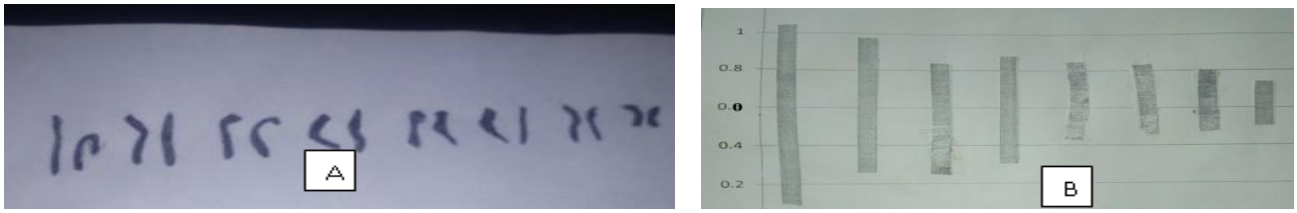

Figure II: Photograph of chromosomal pairs arranged in descending order (A) and Ideogram of chromosomes sorted in descending order of length and matching on the basis of centromere position (B).

DISCUSSION

Calcium carbide is a powerful mutagen, according to this study, and it can cause mitotic defects as concentration rises. Mitosis also decreases as concentration rises (i.e rate of cell division reduces). The accumulation of this mutagen (Calcium carbide) in onion cells stops the cells from growing. As the cells divide, root tips treated with 0.25g of calcium carbide exhibit reduced abnormalities and have a value of 45.83. The rate of cell division slows as the concentration of calcium carbide rises, and the mitotic index plummets. There are extremely few dividing cells at 1.00g concentration, and the mitotic index is at its lowest. It is clear from this that when the concentration of calcium carbide increases, the rate of cell division reduces. It causes nuclei to get disoriented, causing the chromosome to become deformed and unreadable. Calcium carbide (CaC_2) Induced chromosomal aberrations such as chromosome shrinkage, chromosome clumping, chromosome fragmentation, adherent chromosome, and abnormal anaphase (as shown in plates A-D) when compared to the control experiment where the chromosomes are very visible and stages such as Metaphase, Prophase, and Anaphase are present. Many writers noticed a similar type of outcome with the treatment with orange red, a food additive colour (Tripathy*et al.*, 2013).The detected chromosomal abnormalities were likewise similar to those described by Nwagburuka & Oyelana, 2011 in chloroquine and Yuzbasioglu,Unal,& Sancak, 2009 in illoxan. When compared to the control experiment, the anomalies discovered during cell division demonstrated that Calcium carbide has a negative effect (Plate E-J). The chromosomal abnormality seen in cells treated with calcium carbide, even at the lowest concentration (0.25g), may suggest that calcium carbide can be employed in cell mutagenesis investigations. The polyploidy level of the plant (*Allium cepa*) is indicated by the karyotype analysis, which shows that it is a diploid with 2n=16 chromosomes.

CONCLUSION AND RECOMMENDATION

Calcium carbide is a potent mitotic inhibitor, according to this study, and increased concentrations can cause mitotic abnormalities as well as slowed cell division. Their buildup in cells has the potential to stifle cell proliferation. Calcium carbide, which is employed as an artificial ripening agent in bananas, has been discovered to be useful in mutagenesis studies in plants such as onions because it interferes with DNA production. However, more research with lower concentrations of Calcium carbide, as well as other carcinogenic chemicals, is needed to determine their effect on chromosomal behavior. Furthermore, the current investigation's karyotype analysis will aid in understanding the quantity and form of chromosomes, which is useful in cyto-taxonomy and also advantageous for future cytogenetics research.

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