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Comparative Cytological Effects of Colchicine, 8-Hydroxyquinoline, and Paradichlorobenzene on Mitotic Activity in Allium sativum Root Meristem Cells

Geethanjali R.[1], Manjula A C.[2], Nalini T J.[3], Prathibha K Y.[4], Apoorva[5], Nandini[6]

[1,3]Associate professor, Department of Botany, Maharani Cluster University, Palace Road, Bengaluru, Karnataka, India 560001

[2]Professor, Department of Sericulture, Nrupathunga University, Banglore, Karnataka, India 560001

[4]Professor, Department of Botany, Maharani Cluster University, Bengaluru, Karnataka, India 560001

[5,6]B.sc Students, Maharani Cluster University, Bengaluru, Karnataka, India 560001

***Corresponding author:**

Prathibha K Y

Email ID: prathibha16ky@gmail.com

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ABSTRACT

Allium cepa, a widely used model organism in cytogenetic studies, is ideal for evaluating the effects of chemical agents on mitosis. *Allium sativum* (garlic), though known for its medicinal value, is less commonly used in such studies. This research focuses on the comparative effects of colchicine, 8-hydroxyquinoline, and paradichlorobenzene—three well-known mitotic inhibitors—on the root meristem cells of *Allium sativum*. Colchicine is a microtubule-disrupting alkaloid that causes metaphase arrest; 8-hydroxyquinoline acts as a chelating agent that interferes with spindle formation; paradichlorobenzene is an aromatic compound known to induce chromosomal abnormalities. Root tips of *Allium sativum* were treated with each chemical at standardized concentrations for specific durations, followed by fixation, staining, and microscopic analysis. The mitotic index, frequency of chromosomal aberrations, and phase-specific mitotic abnormalities were recorded and compared. Colchicine treatment led to a significant increase in metaphase frequency due to spindle inhibition, often resulting in c-metaphase configurations. 8-hydroxyquinoline exhibited a marked reduction in the mitotic index and induced metaphase arrest, along with chromosomal stickiness and clumping. Paradichlorobenzene exposure caused prominent chromosomal breaks and fragmentation, along with altered mitotic progression. The results demonstrate the differential impact of these chemicals on cell cycle progression, highlighting their effectiveness and mode of action as mitotic poisons. This study contributes to understanding their cytological effects and potential applications in plant cytogenetics and mutation breeding.

Keywords: *Allium sativum, mitotic index, colchicine, 8-hydroxyquinoline, paradichlorobenzene, cytogenetics, chromosomal aberrations, metaphase arrest, spindle inhibitors, cell division*

1. INTRODUCTION

Allium sativum L., commonly known as garlic, is a perennial bulbous plant belonging to the genus *Allium* in the family Liliaceae, though some taxonomists classify it under Alliaceae. Native to Central Asia, garlic is extensively cultivated worldwide for its culinary significance and remarkable medicinal properties. The plant produces a tall, unbranched flowering stem that may grow up to 1 meter in height and terminates in an umbel of small flowers or bulbils. Its underground bulb is made up of 10 to 20 cloves, each enclosed in a papery sheath. When crushed or cut, the cloves release sulfur-containing compounds responsible for garlic's characteristic pungent odor. Garlic is highly valued in both traditional and modern medicine for its wide-ranging therapeutic effects. Its bulbs are rich in biologically active sulfur compounds such as allicin, which exhibit strong antibacterial, antifungal, and antioxidant properties. *A. sativum* is known for its role in preventing cardiovascular diseases, modulating immune responses, and providing hepatoprotective and anti-inflammatory benefits. These attributes make it a vital component of numerous pharmacological applications. Cytogenetically, *Allium sativum var. sativum* possesses a diploid chromosome number of 2n = 16. Unlike *A. cepa*, garlic does not reproduce sexually and is propagated almost exclusively through vegetative means, resulting in genetically uniform clones. The species shows distinct chromosomal features, including structural asymmetries, particularly in chromosome 7, and variations in satellited chromosome pairs. Due to its large chromosome size and consistent genetic makeup, *A. sativum* serves as an excellent model

organism for studying cytological changes, especially in mutagenesis and polyploidy experiments induced by agents such as colchicine, paradichlorobenzene, and 8-hydroxyquinoline.

8-Hydroxyquinoline (8HQ), also known as oxine, is a quinoline derivative originating both from natural plant sources and synthetic processes. It was first synthesized by Hugo Weidel and Albert Cobenzl in 1880 by decarboxylating oxycinchoninic acid, with further developments by chemists like Zdenko Skraup and Otto Fischer. Among seven isomeric monohydroxyquinolines, 8HQ stands out for its potent ability to chelate metal ions, particularly divalent metals. This strong metal-coordinating property has led to its widespread use in analytical chemistry, agriculture (as a fungicide), and various industrial applications including textiles and wood preservation. In aqueous solutions, 8-hydroxyquinoline has a pKa of approximately 9.9 and forms stable 8-hydroxyquinolinato chelates upon deprotonation. It has also found applications in advanced materials such as organic light-emitting diodes (OLEDs), where substituted derivatives affect luminescence properties. Furthermore, it exhibits various biological activities, functioning as an antiseptic, disinfectant, and even showing potential as a transcription inhibitor and anti-cancer agent. Biologically, 8HQ plays a role in influencing metal homeostasis, which is critical for maintaining metabolic balance. Its bioactivity and therapeutic potential stem from its capacity to chelate metals, making it useful in managing conditions related to metal overload or deficiency. Notably, the compound is also released by the invasive plant *Centaurea diffusa*, demonstrating allelopathic effects on non-coevolved species. The relevance of 8-hydroxyquinoline in cytogenetics arises from its c-mitotic action—its ability to interfere with spindle fiber formation during mitosis. It disrupts normal chromosomal movements by arresting cells at metaphase, which is a key feature exploited in chromosome studies and polyploidy induction. However, in isolation, 8HQ may not always be effective in inducing polyploidy. Studies such as those by Prakhar and Swaminathan (1951) confirmed its c-mitotic properties, although no polyploid cells were observed in *Trigonella foenum-graecum* even after treatments up to 80 minutes. This limitation is likely due to insufficient exposure time. The mechanism behind 8HQ-induced polyploidy is believed to involve chemical imbalance at the cellular level, particularly affecting nucleic acid synthesis and spindle apparatus organization. The disruption causes the formation of multinucleate cells and irregular chromosomal separations, which, if followed by cell division, can yield polyploid progeny. Such somatic reduction and abnormal mitotic figures support the idea of 8HQ as an important tool in cytological research and plant breeding programs aimed at developing homozygous lines and improving genetic variability.

Paradichlorobenzene (1,4-dichlorobenzene, PDCB) is an aryl halide compound with the chemical formula $C_6H_4Cl_2$. It is a white crystalline solid with a distinctive odor and is primarily used as a fumigant insecticide, deodorizer, and disinfectant. Due to its high volatility and low water solubility, PDCB is commonly found in mothballs, urinal cakes, and air fresheners. It is also a precursor in the manufacture of polymers and other industrial chemicals. While PDCB is useful in pest control, prolonged exposure raises concerns due to its potential carcinogenicity and toxicological effects on humans and the environment. Recent studies have highlighted a novel use of PDCB in the field of plant biotechnology—its role in inducing polyploidy. Polyploidy refers to the condition of having more than two complete sets of chromosomes and is a key mechanism in plant breeding for enhancing desirable traits such as fruit size, disease resistance, and stress tolerance. Polyploidization can be achieved chemically by disrupting normal spindle fiber formation during cell division, preventing chromosome separation and resulting in chromosome doubling. Like traditional agents such as colchicine, PDCB has been found to interfere with mitotic processes, potentially by binding to microtubule proteins and inhibiting proper chromosome segregation. This disruption leads to the formation of polyploid cells. Chemically induced polyploidy can bring about significant morphological and physiological changes, including increased cell size, altered plant architecture, and enhanced biomass. In fruits and other horticultural crops, polyploidy can translate into commercially beneficial traits such as larger fruit size and improved shape—advantages that are difficult to consistently achieve through conventional cultural practices or hybridization at the diploid level.

Colchicine is an alkaloid that features a carbotricyclic structure, consisting of a 5,6,7,9-tetrahydrobenzo[a]heptalene core with four methoxy groups at positions 1, 2, 3, and 10, an oxo group at position 9, and an acetamido group at position 7. It is derived from plants of the *Colchicum* genus. Colchicine acts as a microtubule-destabilizing agent and is a plant metabolite. It is categorized as a carbotricyclic compound, an alkaloid, an aromatic ether, and an acetamide. The natural product, N-(1,2,3,10-Tetramethoxy-9-oxo-5,6,7,9-tetrahydrobenzo[a]heptalen-7-yl)acetamide, is found in *Colchicum crocifolium*, *Colchicum doerfleri*, and other organisms. Colchicine is present in individuals only if they have used or taken the drug. It is a major alkaloid from *Colchicum autumnale* L. and other *Colchicum* species. Primarily, colchicine is used to treat gout and has also been used for familial Mediterranean fever (periodic disease). Although the precise mechanism of action is not fully understood, in gout patients, colchicine seems to interrupt the cycle of monosodium urate crystal deposition in joint tissues and the subsequent inflammatory response, thereby preventing acute attacks. It reduces leukocyte chemotaxis and phagocytosis and inhibits the formation and release of a chemotactic glycoprotein during urate crystal phagocytosis. Colchicine also inhibits urate crystal deposition, which is enhanced by low pH in tissues, likely by inhibiting glucose oxidation and subsequent lactic acid production in leukocytes. Colchicine does not possess analgesic or antihyperuricemic properties. Colchicine interferes with microtubule assembly in various cells, including leukocytes, by binding to and disrupting the polymerization of the tubulin subunit. While some studies suggest this action does not significantly contribute to colchicine's antigout effects, recent in vitro research indicates it might play a partial role.

2. MATERIALS AND METHODS

The study was conducted at the Department of Botany, Maharani Cluster University, Bangalore. Root apical meristems of *Allium sativum* var sativum was used as plant models to determine cell cycle modulation and metaphase-arresting activities.

- Sample Collection and Preparation for treatment

Allium sativum var sativum was obtained from K R Market, Bengaluru. The dried external leaves and roots were removed before the bulbs were planted in soil until the roots sprouted. Rapidly growing root tips of *Allium sativum* (1.0-2.0 cm in length) were immersed in vials containing 0.004 N 8 hydroxyquinoline and 0.1% paradichlorobenzene separately and both of these combined together in a separate vial and both of these combined with colchicine solution in a separate vial. The immersion time for each treatment was 5 hours. The stem disc was positioned to just touch the solutions, and the samples were protected from direct sunlight. The effect of these treatments were tested for a duration of 5 hours. 0.004 N 8 hydroxyquinoline, 0.1% paradichlorobenzene and 0.1% colchicine were used to study their effects on mitosis inhibition.

- Chromosome Preparations

Root tips were harvested between 9 am and 12 pm and transferred into a beaker containing 1N hydrochloric acid, kept in a water bath for 6 minutes at 60°C. The cell walls were dissolved by acid hydrolysis. The hydrolyzed root tips were then transferred to a watch glass with 8-9 drops of acetoorcein per treatment and one drop of 1N HCl was added. The watch glass was warmed using a spirit lamp and left for 4-5 minutes.

- Root tips Preparation for Microscope

Approximately 1.5 mm of the root tip was cut off and placed in a drop of acetoorcein stain on a clean microscopic slide and gently tapped to create a squash. Additional acetoorcein stain was added and left for 2-3 minutes. Coverslips were placed over the squash, and excess stain was removed using blotting paper. The slides were then observed under a light microscope at different magnifications (10x, 40x, 100x) to observe various stages of mitosis, at 100x cedar wood oil was used.

3. RESULTS

The meristematic regions of *Allium sativum* roots without any treatment (control) showed a normal pattern of mitotic division. All four stages of the cell cycle—prophase, metaphase, anaphase, and telophase—were observed. Most cells were in prophase, while fewer cells were in metaphase, anaphase, and telophase. Metaphase chromosomes aligned along the equatorial plate, and during anaphase, chromosomes were evenly pulled toward opposite poles. No chromosomal abnormalities were recorded in the control group.

In treated samples, *Allium sativum* root meristem cells displayed several types of mitotic abnormalities upon exposure to paradichlorobenzene, and 8-hydroxyquinoline and combined treatments. Observed abnormalities included c-mitosis, vagrant chromosomes, laggards, chromatid breaks, stickiness, and polyploidy. Aberrations were more frequent in root tips treated with 8-hydroxyquinoline. PDCB mainly caused prophase arrest and polyploidy. Combined treatments of colchicine, PDCB, and 8-HQ produced more intense effects and higher frequencies of abnormalities. Prolonged exposure times further amplified mutation rates. In *Allium cepa* flower buds treated with 8-HQ, disturbed syncytial nuclei and severe chromosomal abnormalities were observed at diplotene and diakinesis stages, highlighting the mutagenic potential in reproductive tissues.

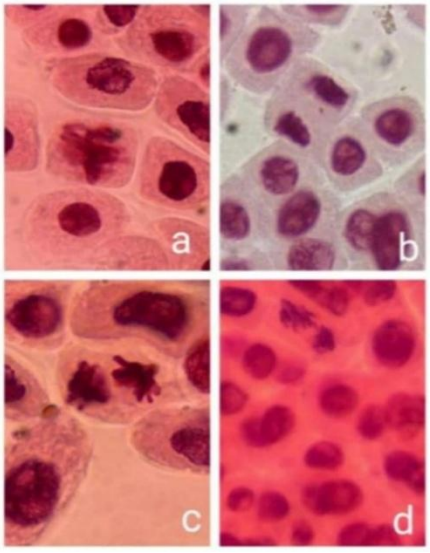

PLATE 1: Normal Root tips of Allium sativum, a-Normal Metaphase, b-Normal Prophase and Telophase, c-Normal Anaphase, d-Early Telophase

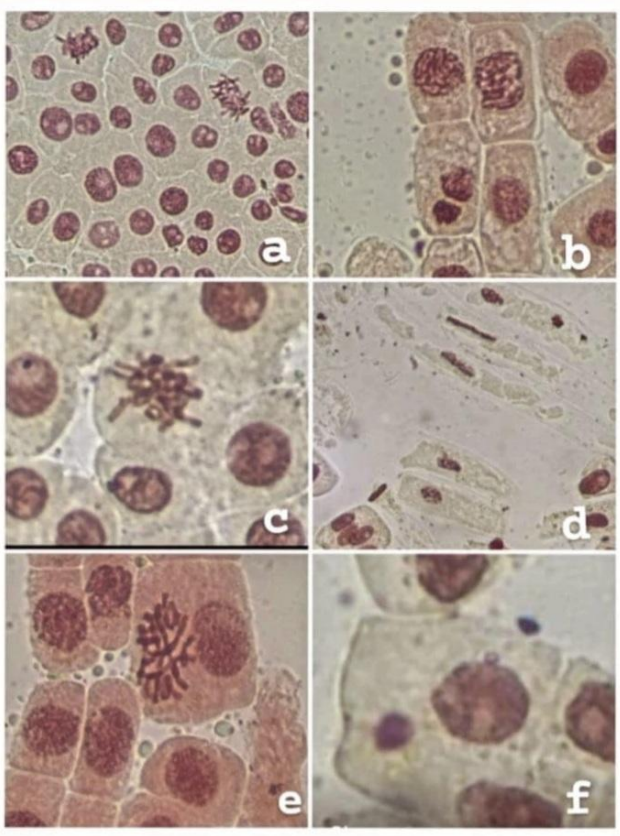

PLATE 2 : Root tips of Allium sativum treated with 0.004N 8 Hydroxy quinoline : a-Nuclear lesions and polyploid Metaphase, b-Polyploid prophase, c-Irregular sticky chromosome, d-Irregular cells with broken fragmented chromatin, e-C metaphase with polyploids, f-Micronuclei

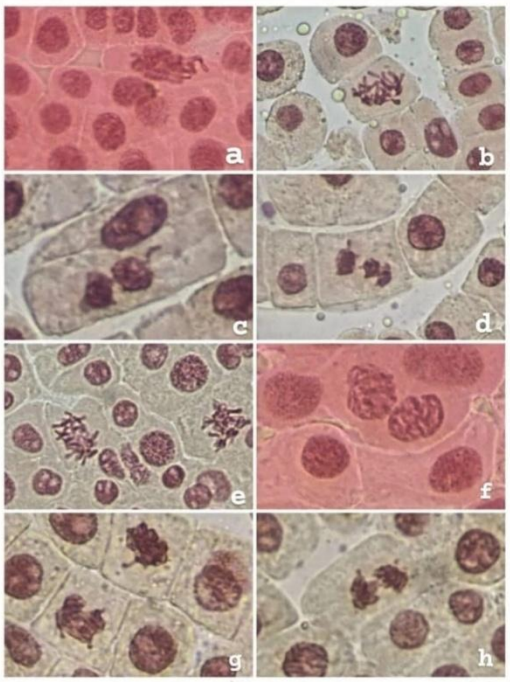

PLATE 3: Root tips of Allium sativum treated with 0.004N 8 Hydroxy quinoline : a-Irregularly arranged sticky chromosome with metaphase arrangement, b-Irregular anaphase, c-Irregular nuclei with vagrant and laggard, d-Multinucleate condition with vagrant, e-Sticky chromosome with C-metaphase, f- Polyploid anaphase, g-Vagrant and laggard, h- Vagrant and laggard

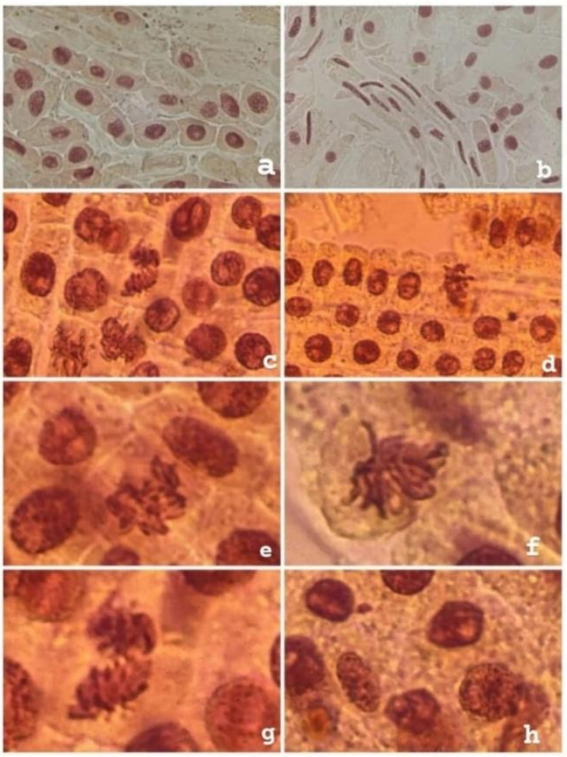

PLATE 4; Root tips of Allium sativum treated with 0.1 percent of Paradichlorobenzene: a-Nuclear lesion, b-Irregular cells with irregular nucleus, c-laggard and vagrant and C-metaphase, d-Sticky chromosome, e-Irregular anaphase, f-irregular metaphase, g-Irregular anaphase with laggard, h-micronuclei

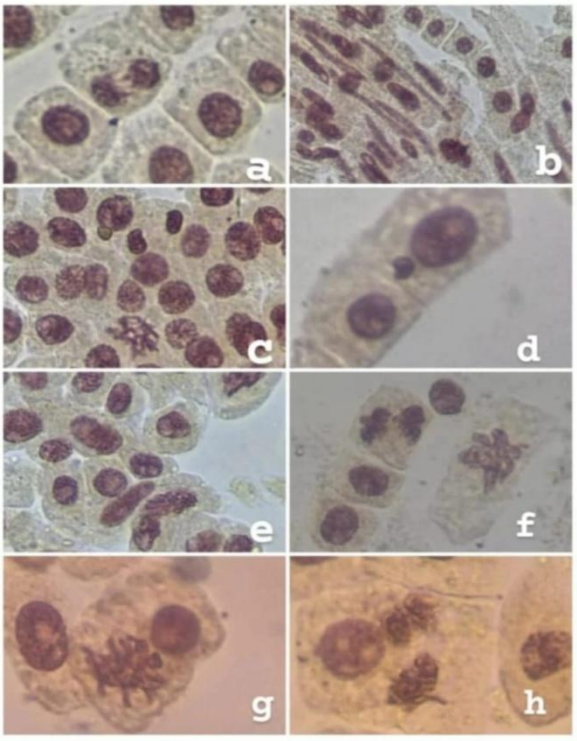

PLATE 5; Root tips of Allium sativum treated with 0.1 percent of Paradichlorobenzene + 0.004N 8 Hydroxy quinoline: a-Polyploid metaphase, b-Irregular anaphase with vagrant, c-Sticky chromosome, d-C metaphase, e-Laggard and vagrant, f-Irregular telophase and polyploid prophase

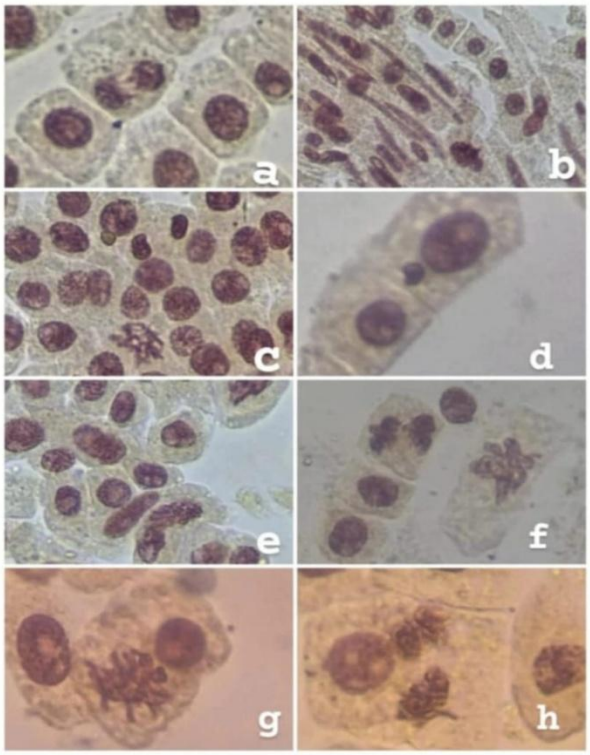

PLATE 6 ; Root tips of Allium sativum treated with 0.1 percent of Paradichlorobenzene + 0.004N 8 Hydroxy quinoline: a-Irregular anaphase with laggard, b-Irregular cells with nuclear lesions, c-C metaphase, d-Micronuclei, e-Polyploid prophase, f-Irregular anaphase and C metaphase, laggard, g-Sticky chromosome, h-Irregular anaphase and C metaphase

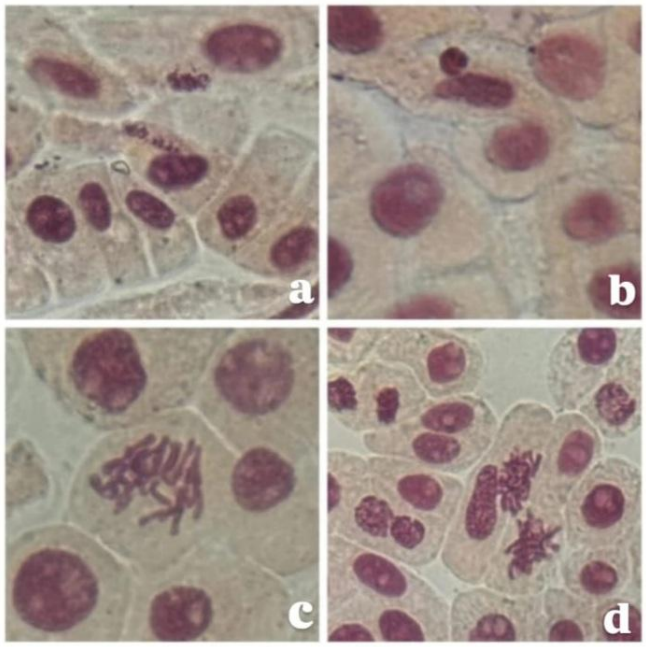

PLATE 7; Root tips of Allium sativum treated with 0.1 percent of Paradichlorobenzene + 0.004N 8 Hydroxy quinoline +0.1 percent colchicine: a-Fragmented chromosomes, b-Micronuclei, c-Sticky chromosome, d-C metaphase

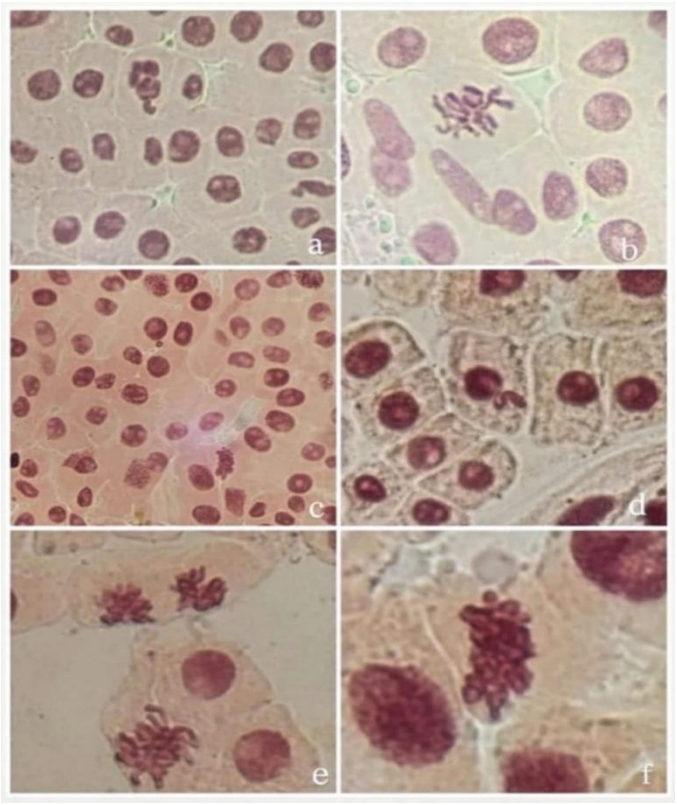

PLATE 8: Root tips of Allium sativum treated with 0.1 percent of Paradichlorobenzene + 0.004N 8 Hydroxy quinoline +0.1 percent colchicine : a-Multinucleate condition, b-Sticky chromosome, c-Micronuclei, d-Irregular nucleus, e-Sticky chromosome and Irregularly anaphase, f-Polyploid metaphase

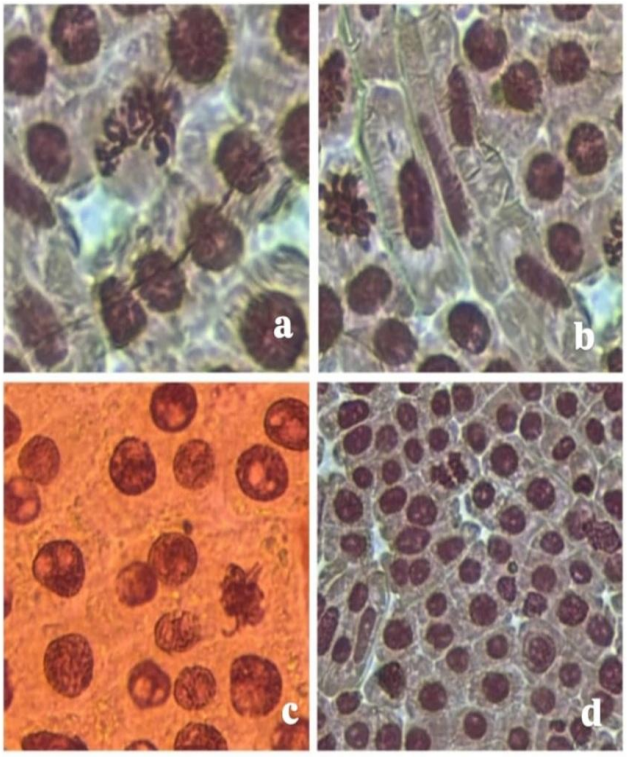

PLATE 9; Root tips of Allium sativum treated with 0.1 percent of Paradichlorobenzene + 0.004N 8 Hydroxy quinoline +0.1 percent colchicine ; a-Fragmented chromosomes, b-Irregular cells and C metaphase, c-Micronuclei and Sticky chromosome, d-Nuclear lesion with irregular anaphase

4. DISCUSSION

The current study comprehensively investigates the genotoxic and cytotoxic effects of colchicine, paradichlorobenzene (PDCB), and 8-hydroxyquinoline (8-HQ) on the root meristematic cells of *Allium sativum*. These species are widely recognized models for cytogenetic analysis due to their large chromosomes and high mitotic index (Fiskesjö, 1985; Bonciu et al., 2018). The untreated control cells of *A. sativum* displayed a normal mitotic sequence with proper chromosomal alignment and segregation, validating the suitability of the test system. In contrast, treated samples exhibited a range of chromosomal aberrations, indicating profound disruption of mitotic and meiotic processes. These abnormalities were both dose- and time-dependent, with increased frequency and severity correlating with prolonged exposure and combination treatments.

One of the most striking abnormalities observed in treated samples was c-metaphase, a form of metaphase arrest characterized by condensed chromosomes arranged randomly within the cell due to inhibition of spindle fiber formation. This phenomenon, first described by Levan (1938), is a well-established indicator of spindle poison activity. Colchicine, known for its microtubule-depolymerizing effects, prominently induced c-metaphase, particularly at higher concentrations and longer exposures. Similarly, 8-HQ exhibited a strong metaphase-arresting effect, confirming its role in disrupting the polymerization of tubulin, as documented by Fiskesjö (1985). The presence of c-metaphase in these treatments demonstrates interference with spindle dynamics and mitotic progression, halting cells in a metaphase-like state. Chromosomal stickiness was another frequently observed abnormality, especially in 8-HQ-treated *A. sativum* root cells. Chromosome stickiness reflects a loss of chromatin organization and may indicate DNA-protein interactions or inhibition of histone modifications (Yüzbaşioğlu et al., 2003). Stickiness often led to chromosomal bridges and breakages during anaphase, resulting in incomplete or faulty segregation. In this study, anaphase bridges were commonly recorded in 8-HQ and combined treatment groups. These bridges arise from dicentric chromosomes or fused chromatids and are indicative of clastogenic effects (Sudhakar et al., 2001). The presence of these abnormalities strongly supports the genotoxic potential of 8-HQ and its synergistic action when combined with other chemicals. The spindle fiber disruption caused by colchicine and 8-HQ also led to polar deviation and multipolar anaphases. These abnormalities suggest defective mitotic spindle formation, resulting in asymmetrical or tripolar segregation of chromosomes. Cells with polar deviation exhibited chromosomes deviating toward abnormal poles, contributing to unequal chromosomal distribution. Multipolarity was especially prominent in combination treatments, where more than two spindle poles formed, resulting in high rates of aneuploidy and genomic instability. Sharma and Sharma (1999) highlighted that such abnormalities compromise cell viability and can lead to severe developmental disruptions.

Abnormalities related to chromosome movement and alignment, such as vagrant and laggard chromosomes, were also prevalent. Vagrant chromosomes stray from the metaphase plate or move erratically during anaphase, while laggards fail to migrate to the poles. These anomalies result from disrupted spindle orientation and pose a risk of producing aneuploid or genetically unbalanced daughter cells. Their frequent appearance in 8-HQ and combined treatments confirms that these chemicals interfere with kinetochore-spindle attachment. The outcome of such missegregation is often the formation of micronuclei, which are small extranuclear bodies composed of acentric fragments or whole chromosomes excluded from the main nucleus. In this study, micronuclei were commonly observed following exposure to 8-HQ and combined chemicals, validating their use as biomarkers for genotoxic stress (Majewska et al., 2003). The occurrence of polyploidy, particularly in prophase and metaphase, was prominently associated with PDCB and colchicine treatments. Polyploid cells were characterized by enlarged nuclei and excess chromatin material, indicating chromosomal doubling or endoreduplication. PDCB, though not as extensively studied as colchicine, demonstrated a clear ability to arrest cells at the prophase stage and induce polyploidy. Chauhan and Sundararaman (1990) observed similar results with substituted ureas, suggesting that PDCB may act by a comparable mechanism. The appearance of giant cells, which were multinucleated and significantly larger than normal cells, further supports the conclusion that these chemicals can bypass cytokinetic checkpoints, leading to uninterrupted nuclear replication without cytoplasmic division.

Chromatid breaks, another critical indicator of genotoxicity, were recorded in colchicine- and 8-HQ-treated *A. sativum* cells. These breaks likely resulted from DNA strand fragmentation due to oxidative stress or inhibition of repair pathways. The production of acentric fragments poses a risk of lethal mutations or loss of essential genetic material. Distorted metaphase and anaphase figures, characterized by irregular chromosomal condensation and orientation, were frequent in PDCB- and colchicine-treated samples. These abnormalities are typically caused by incomplete spindle assembly or disturbed chromosome cohesion, leading to defective chromosomal segregation and increased mutational load. Abnormalities related to cell division completion, such as binucleated and multinucleated cells, were seen across all treatment groups, especially with prolonged exposure. These abnormalities arise from cytokinesis failure or mitotic slippage, leading to cells re-entering the cell cycle with abnormal chromosome content (Amer & Farah, 1974). The presence of nuclear lesions, observed as dark or irregular spots in interphase nuclei, indicates chromatin degradation or incomplete repair of DNA damage, which may be a precursor to apoptosis or senescence (Abdel-Salam et al., 2012).

5. CONCLUSION

The present study was conducted to assess the cytogenetic effects of chemical mutagens on *Allium cepa* root tips. The present investigation aimed to assess the cytological effects of colchicine, paradichlorobenzene (PDCB), and 8-hydroxyquinoline (8-HQ) on the root meristem cells of *Allium sativum*. Root tips were treated individually and in various combinations of the three chemicals for five hours. In untreated control root tips, a normal sequence of mitotic stages was observed, with clearly defined prophase, metaphase, anaphase, and telophase, and without any chromosomal abnormalities. However, the chemically treated samples displayed a wide spectrum of mitotic abnormalities that increased with treatment time and combination. C-metaphase, a classic marker of spindle inhibition, was prominent in 8-HQ treatments, especially when used in combination. Chromosomal stickiness, laggards, and vagrant chromosomes were frequently noted, especially in 8-HQ-treated root tips, suggesting interference with chromatin structure and spindle formation. Binucleated cells and giant polyploid cells were also observed, particularly in PDCB-treated root tips, indicating incomplete cytokinesis and chromosomal doubling. Nuclear lesions, distorted metaphase plates, and multipolar anaphases further confirmed the severity of genetic stress induced by these chemicals. The combined application of colchicine, PDCB, and 8-HQ had the most pronounced impact, leading to complex chromosomal aberrations and mitotic arrest. Prophase polyploidy and metaphase chromosomal misalignment were especially evident in PDCB treatments, indicating the chemical's strong metaphase-arresting capability.

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