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Original Research Article

Effects of 0.004 N 8-Hydroxyquinoline and 0.1% Paradichlorobenzene on Meiosis and Chromosomal Behaviour in Allium cepa Flower Buds

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Name of Author:
Prathibha K Y.[1], Geethanjali R,[2] Nalini T J,[3] Tanushree R.,[4] Shantha.,[5] Sridevi M Y[6] and Marhoob Banu[7*]

Affiliation: *[1]Professor, Department of Botany, Maharani Cluster University, Palace Road, Bangalore, Karnataka, India 560001*
[2,3]Associate Professor, Department of Botany, Maharani Cluster University, Palace Road, Bangalore, Karnataka, India 560001
[4]M.sc student, Department of Botany, Bengaluru City University, Bangalore, Karnataka, India 560001
[5,6]M.sc student, Department of Biotechnology, Nrupathunga University, Bangalore, Karnataka, India 560001
[7]M.sc student, Department of Botany, Bangalore University, Bangalore, Karnataka, India 560056

Corresponding Author:
Prathibha K Y

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Abstract:
The present study aimed to investigate the effect of pre-treatment with 0.004 N 8-hydroxyquinoline and 0.1% paradichlorobenzene on meiosis in Allium cepa flower buds. Flower buds of Allium cepa var. aggregatum were collected from Thanushree Farm, Bengaluru, and treated with 0.004 N 8-hydroxyquinoline and 0.1% paradichlorobenzene for five hours. Post-treatment, anthers were stained with acetocarmine, squashed, and observed under a microscope to assess chromosomal behaviour. Results indicated that meiotic abnormalities were higher in flower buds treated with 0.004 N 8-hydroxyquinoline compared to 0.1% paradichlorobenzene, including irregular pairing, hyperploid pollen mother cells, and transfer of chromatin material. Irregular telophase stages, including triad, dyad, and polyploid conditions, were also observed. These findings demonstrate that pre-treatment with 0.004 N 8-hydroxyquinoline and 0.1% paradichlorobenzene distinctly influence chromosomal behaviour and meiosis in Allium cepa flower buds.

Keywords: Allium cepa, 0.004 N 8-hydroxyquinoline, 0.1% paradichlorobenzene, meiosis, chromosomal aberrations, pollen mother cells, polyploidy.

INTRODUCTION

Allium cepa, commonly known as onion, is a widely cultivated and economically important plant species belonging to the family Liliaceae, which comprises more than 250 genera and approximately 3700 species. Due to its characteristic pungent odour and distinct flavour, *Allium cepa* is extensively used in fresh form, as powdered seasoning, and as essential oils to enhance the taste of various foods. Historically, *Allium cepa* was regarded as a medicinal plant by ancient Egyptians due to its antimicrobial, anti-inflammatory, and therapeutic properties. Traditionally, onion extracts and onion tea have been used in the treatment of fever, headaches, cholera, dysentery, the common cold, and arthritis. *Allium cepa* contains a wide array of pharmacologically active components, including flavonoids such as quercetin, phenolic compounds, and various sulphur-containing organic molecules like

dialkyl polysulfides. These biologically active constituents contribute to its antibacterial, antioxidant, antihypertensive, antifungal, anticancer, anti-inflammatory, antispasmodic, antimicrobial, antimutagenic, and anti-diabetic effects. Furthermore, Allium cepa and its bioactive compounds have shown protective effects against chemical- and toxin-induced pathologies, and have been reported to enhance growth performance and lipid profiles in animals under stress conditions.[1] *Allium cepa* is also an excellent model organism for cytological studies because of the large size and clear visibility of its chromosomes. The actively dividing meristematic cells in onion root tips are commonly used to study mitosis and evaluate the cytotoxic and genotoxic effects of various chemicals and pollutants. Along with mitotic studies, the flowers of *Allium cepa* provide ideal material for studying meiosis, as their floral buds contain actively dividing cells undergoing reduction division. The ease of availability, simple handling, low cost, high sensitivity, reproducibility, and rapid experimental setup make *Allium cepa* one of the most widely preferred plants for cytogenetic and toxicity assessments.[2]

Meiosis is a specialized type of cell division that occurs in diploid germ-line cells located in the ovaries and testes, ensuring the production of haploid gametes—sperm and egg—that contain only a single set of chromosomes. The discovery of meiosis began with Theodor Boveri's finding in 1888 that fertilized eggs of roundworms contain twice the number of chromosomes found in their gametes, proving that gametes are haploid while the other cells of the body are diploid. Meiosis begins with a single round of DNA replication similar to mitosis, but instead of one cell division, it involves two successive cell divisions—meiosis I and meiosis II—without any additional DNA replication in between. During meiosis I, each duplicated paternal chromosome pairs with its corresponding duplicated maternal chromosome in a process called pairing, forming structures called bivalents that contain four sister chromatids. This pairing is essential for proper segregation, and the homologous chromosomes undergo crossing-over, where non-sister chromatids exchange segments of DNA. Crossing-over is supported by the synaptonemal complex and creates chiasmata that physically link homologs, helping ensure that each gamete receives one copy of each chromosome. At anaphase I, cohesin proteins holding chromosome arms together are removed, allowing maternal and paternal homologs to separate into different daughter cells, while sister chromatids remain paired. Meiosis II resembles mitosis: the remaining cohesins at the centromere are degraded, sister chromatids separate, and four haploid cells are ultimately produced. Meiosis generates extensive genetic variation in two main ways. First, the random orientation of bivalents during metaphase I causes a random assortment of maternal and paternal chromosomes into gametes. Second, crossing-over shuffles alleles along each chromosome, creating new combinations of genetic information. These two randomizing processes ensure that no two gametes, and therefore no two offspring, are genetically identical except in rare cases such as identical twins. Despite its precision, meiosis is not flawless. Errors can occur when homologous chromosomes fail to segregate properly during meiosis I, a condition known as nondisjunction, producing gametes with too many or too few chromosomes. Such mistakes often result in embryonic death, but some individuals survive with abnormalities such as those seen in Down syndrome, caused by an extra copy of chromosome 21. Aneuploidy occurs particularly frequently in human oocytes, where roughly 10% of meiotic events show chromosome mis segregation. Thus, meiosis not only ensures the correct halving of chromosome number during sexual reproduction but also plays a central role in generating the genetic diversity that is fundamental to life.[3]

Oxyquinoline (OQ) belongs to the important quinoline group of compounds used in chromosome structure studies. Tjio and Levan (1950) introduced the use of 8-hydroxyquinoline in chromosome analysis, where it demonstrated a c-mitotic property by causing mitotic arrest. It also shows additional characteristics that colchicine does not. OQ inactivates the spindle, allowing chromosomes to spread easily during squashing, and it causes equal contraction of the chromosome arms. Unlike colchicine, it preserves the relative arrangement of metaphase chromosomes at the equatorial plane. It is especially useful for plants having long chromosomes, and its best effects are achieved at low temperatures.[4] p-Dichlorobenzene ($C_6H_4Cl_2$) is considered the most suitable benzene derivative for chromosome studies. It has limited solubility in water and works by inhibiting spindle formation, which enhances the visibility of chromosome constrictions through contraction and differential hydration of chromosome segments. This compound has wide applicability, and it is effective for plants with both long and short chromosomes, although the treatment duration may need to be adjusted. However, its use is limited by the relatively long exposure time of about three hours and the requirement for a specific temperature range of 10–16°C to obtain the best results.[4] The present Study aimed to study the effect of pre-treatment with 8 hydroxy quinoline and Paradichlorobenzene on meiosis in *Allium cepa* flower buds.

MATERIALS AND METHODS

The study was conducted at the Department of Botany, Maharani Cluster University, Bangalore. Flower buds of *Allium cepa* var aggregatum (onion) were used as plant models to determine cell cycle modulation due to pre-treatment.

Sample Collection and pre-treatment

Flower buds of *Allium cepa* were collected from Thanushree farm, Bengaluru. Allium cepa flower buds were treated with 0.1% paradichlorobenzene and 0.004 N 8 hydroxyquinoline for 5hours to study its effects on meiosis inhibition.

Flower bud Preparation for Microscope

The bud was placed in a watch glass, and 1–2 drops of acetocarmine stain were added to it. The stain was gently heated for a few seconds to enhance staining. Using a needle, the anthers were carefully removed from the flower bud and transferred onto a clean microscope slide. A drop of stain was added if necessary, and a cover slip was placed over the anthers. The cover slip was then gently pressed using the blunt end of a pencil or a thumb wrapped in tissue paper to squash the material and spread the cells into a thin layer. Finally, the slide was observed under the microscope, starting with low power and then switching to high power for a clearer view of the cells.

RESULTS

Effect of 0.1% Paradichlorobenzene on Meiosis; Flower buds of Allium cepa treated with 0.1% paradichlorobenzene exhibited high susceptibility to chromosomal mutations. Disturbed syncytium and multiple chromosomal aberrations were observed, particularly during diplotene and diakinesis stages.

Effect of 0.004 N 8-Hydroxyquinoline on Meiosis; Flower buds treated with 0.004 N 8-hydroxyquinoline displayed more pronounced chromosomal abnormalities compared to paradichlorobenzene. Irregular pairing of chromosomes and hyperploid pollen mother cells were observed. Transfer of chromatin material between pollen mother cells was noted. Irregular telophase stages, including triad and dyad formations as well as polyploid conditions, were observed. Abnormal pollen grain development was also seen.

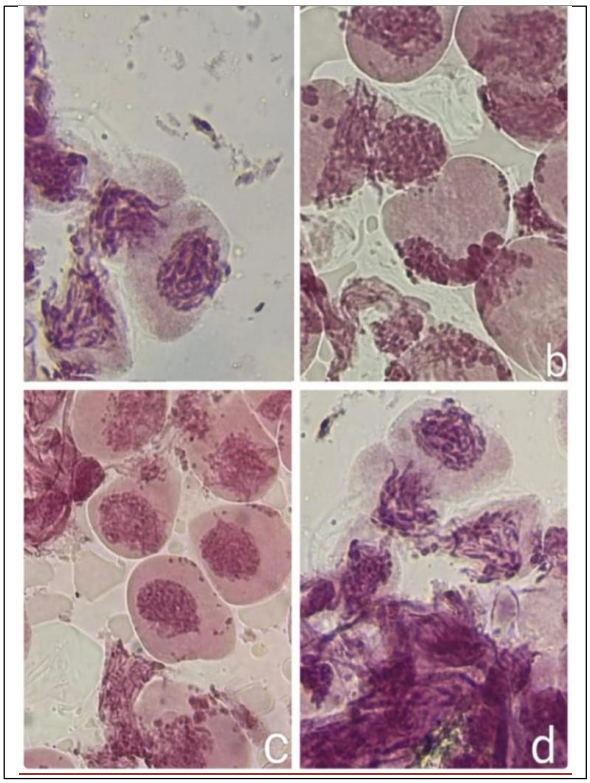

Fig 1; Plate 1 (a-f) *Allium cepa* - 0.1 percent Paradichlorobenzene treated *Allium cepa*; a. Meiotic cell showing polyploidy and transfer of chromatin, b. Group of PMCs involved in transfer of chromatin material at prophase, c. Meiotic polyploidy with micronuclei, d. Hyperploidy PMCs and transfer of chromatin material

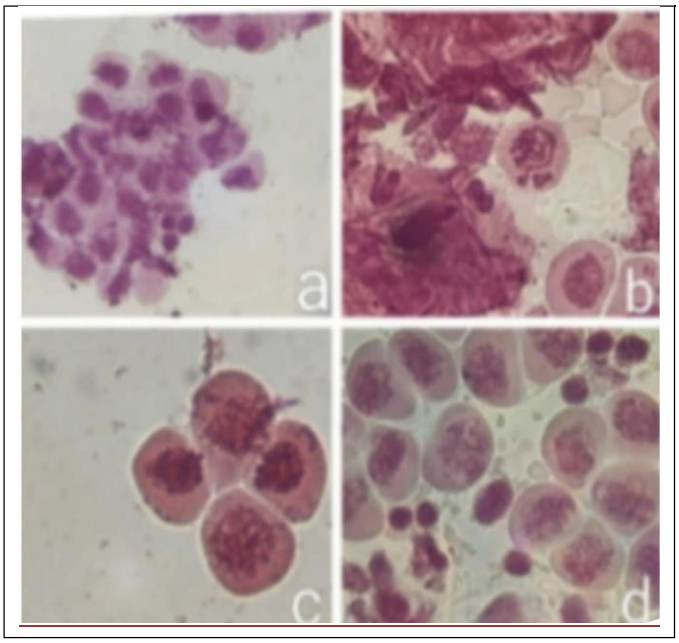

Fig 2; Plate 2 (a-d) *Allium cepa* - 0.1 percent Paradichlorobenzene treated flower buds a. Irregular prophase 1, b. Irregular pairing scene at diplotene, c. Transfer of chromatin between two PMC, d. Hyperploid PMCs

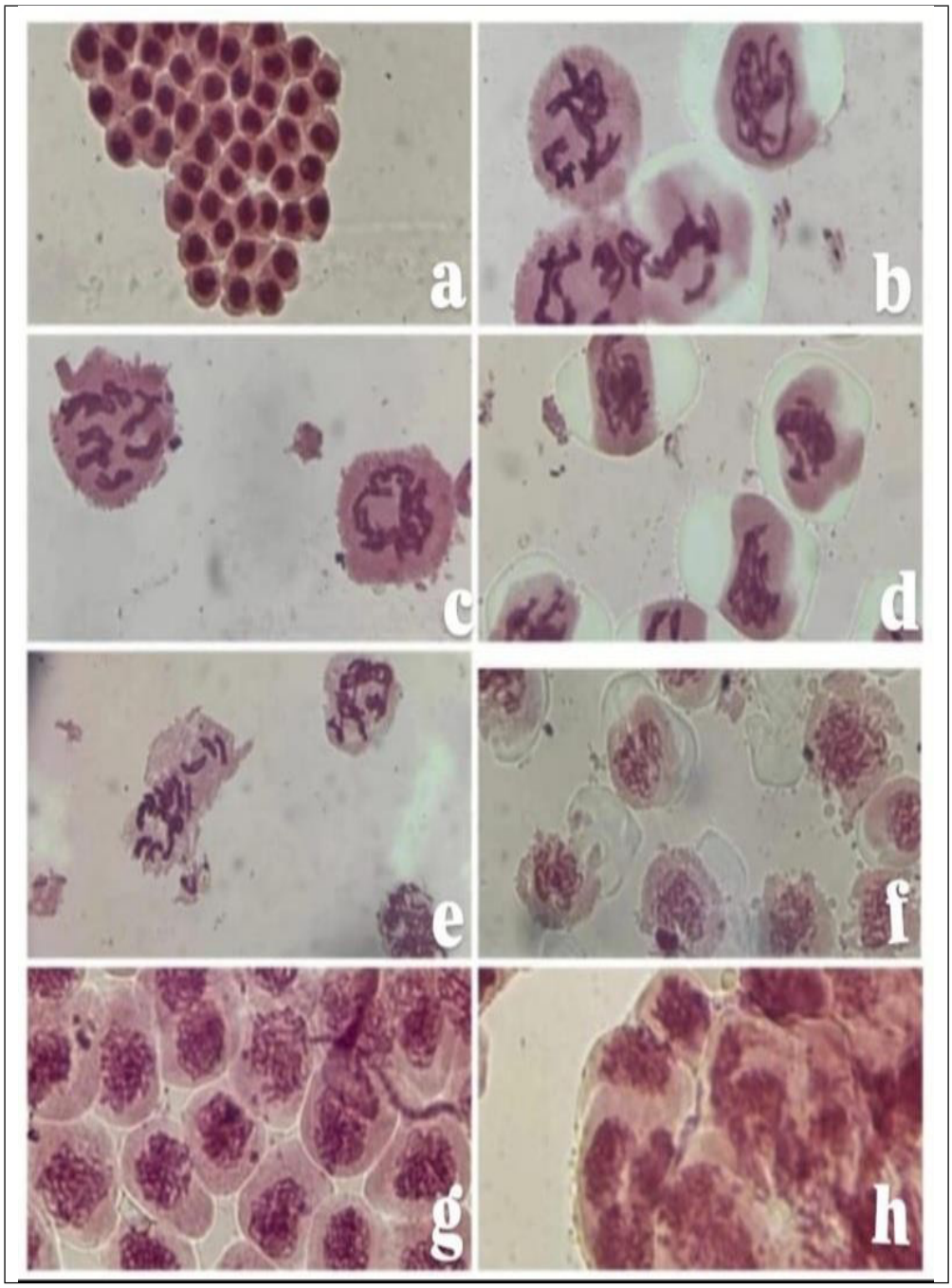

Fig 3; Plate 3 (a-h) *Allium cepa* - 0.004N 8 Hyroxy quinoline treated flower buds; a-Hyperploid pachytene stage, b-Irregular pairing with laggard, c-Irregular pairing with broken chaismata, d-Hyperploid pollen mother cell, e-Distorted metaphase, f-pollen mother cells showing micronuclei, g-Irregular diplotene stage with two pollen mother cells showing transfer of chromatin material, h-Cells showing syncytium Hyperploid pollen mother cells showing transfer of chromatin material

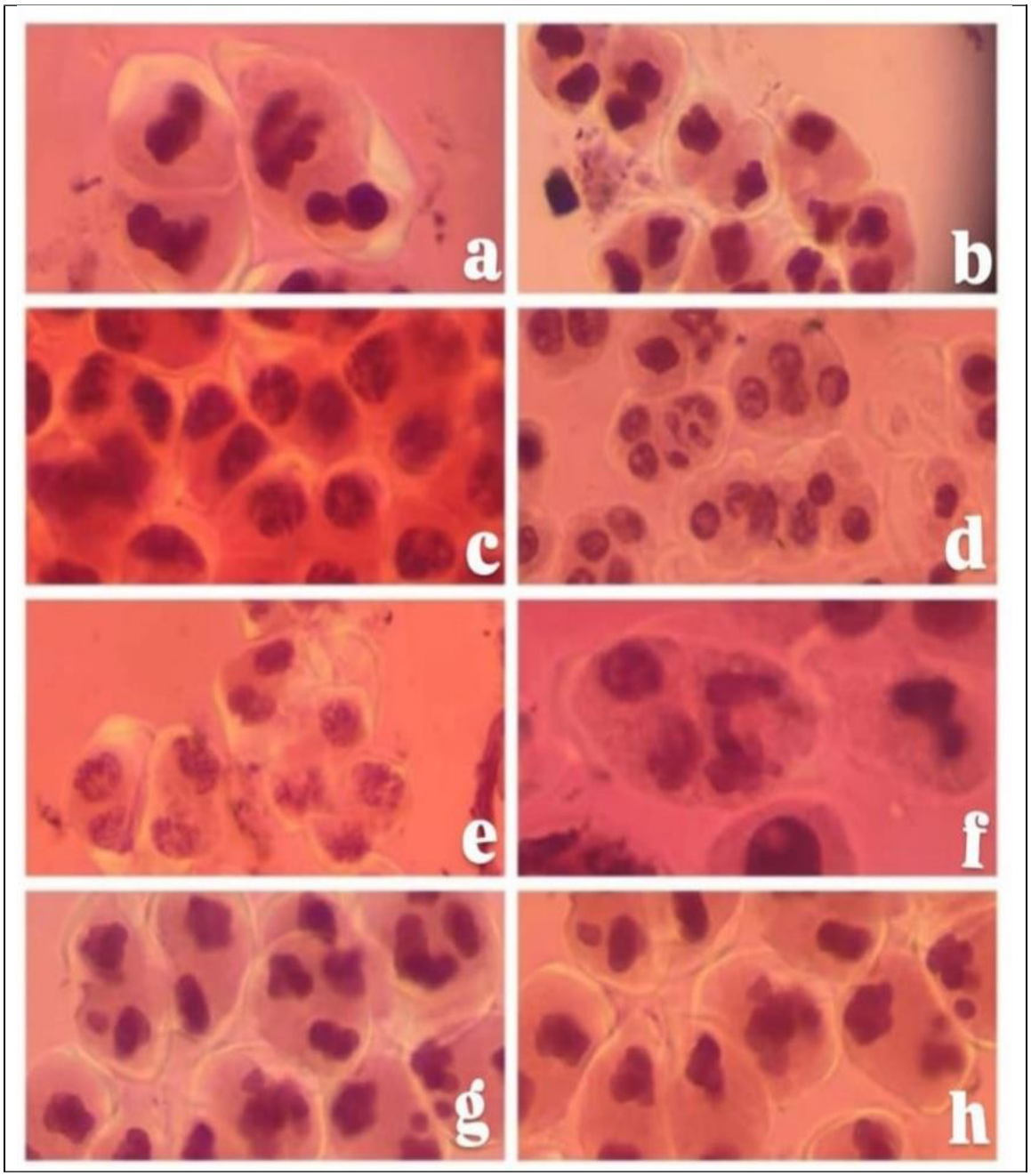

Fig 4; Plate 4 (a-h) *Allium cepa* - 0.004N 8 Hyroxy quinoline treated flower buds; a-Microspores with Multinucleate condition,b- Microspores with Multinucleate condition, c-Irregular pachytene, d-Pollen mother cells with micronuclei, e-Pollen mother cells with laggard, f-Pollen mother cells with bridge, g-Microspores showing transfer of chromatin, h-Microspores with multinucleate condition

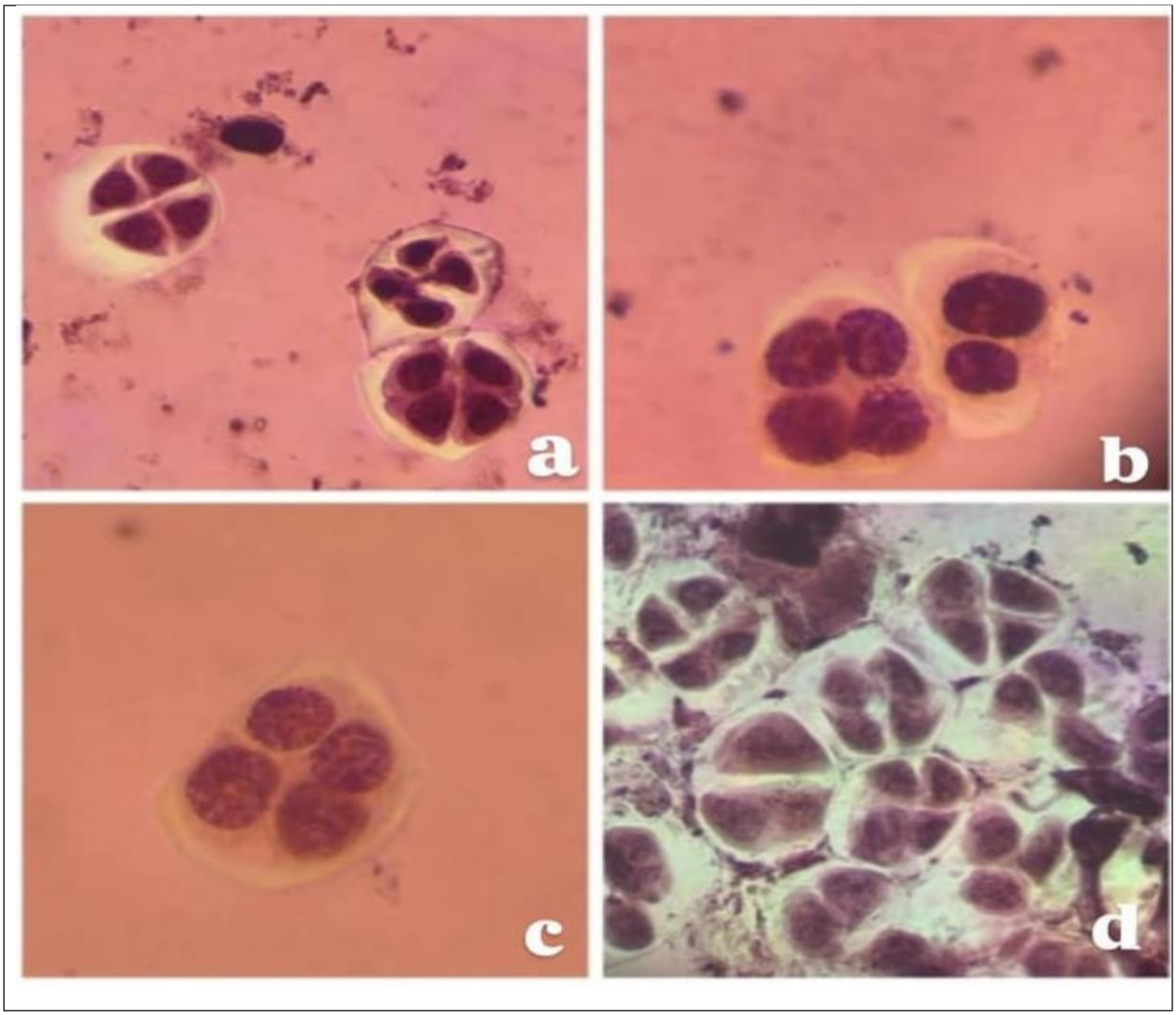

Fig 5; Plate 5 (a-h) *Allium cepa* - 0.004N 8 Hyroxy quinoline treated flower buds; a-Normal telophase, b-Normal and Diad, c-Telophase showing polyploid condition, d-Triad and Diad

DISCUSSION

Flower buds of *Allium cepa* treated with 0.1% paradichlorobenzene (PDB) showed widespread meiotic disturbance, with disturbed syncytium and multiple chromosomal aberrations concentrated at diplotene and diakinesis. Such abnormalities are consistent with the known clastogenic and spindle-disrupting properties of PDB and related benzene-derivatives, which have been reported to reduce mitotic activity and cause a variety of mitotic and chromosomal anomalies when applied to plant tissues. These effects commonly include delayed or disrupted chromosome condensation, stickiness, laggards, bridges and fragmentation, which explain the high susceptibility to chromosomal mutations seen in the present material.2

Treatment with 0.004 N 8-hydroxyquinoline (8-HQ) produced more pronounced abnormalities than PDB in the observed material, manifesting as irregular chromosome pairing, hyperploid pollen mother cells (PMCs), chromatin transfer between adjacent PMCs, abnormal telophase configurations (triads, dyads) and abnormal pollen grain development. These observations align with the documented action of 8-HQ as an antimitotic agent that chelates divalent cations and interferes with spindle function and nucleic acid metabolism; such interference tends to produce metaphase arrest, chromosome stickiness/clumping, aneuploidy and sometimes polyploid cells. The stronger disturbance with 8-HQ at the applied concentration supports the conclusion that 8-HQ caused both spindle-related (aneugenic) and structural (clastogenic-like appearance) meiotic effects in this experiment.5

The observed transfer of chromatin between pollen mother cells (cytomixis) is a recognized phenomenon in plant meiosis and is frequently reported following stress or chemical treatment. Cytomixis can produce PMCs with altered chromosome numbers (hyperploid or hypoploid), syncytial or fused PMCs, and irregular

gametes; this mechanism plausibly explains the hyperploid PMCs, triad/dyad telophase stages and ultimately abnormal pollen grains seen after 8-HQ treatment. Cytomixis is not uniquely diagnostic of one chemical mechanism but is commonly induced by physical or chemical stressors that compromise cell-wall or cell-plate formation or induce cytoplasmic channels between adjacent PMCs. Reporting cytomixis together with the other aberrations supports interpretation that treatments caused both direct chromosomal disruption and altered cell connectivity during microsporogenesis.6

The present results show quantitative and qualitative differences: PDB primarily produced chromosomal aberrations visible at diplotene/diakinesis and disturbed syncytium, while 8-HQ produced a broader spectrum (irregular pairing, metaphase-type disturbances, chromatin transfer, abnormal telophases and defective pollen) and greater overall disruption. This pattern is consistent with comparative cytogenetic studies in Allium and other plant models showing that although both agents act as antimitotic pretreatments useful for chromosome studies, they differ in potency and in the balance of spindle inhibition versus effects on chromatin condensation and cell connectivity.7

The presence of hyperploid PMCs, dyads/triads and abnormal pollen grains implies a likely reduction in viable, balanced gametes after treatment; this consequence is a predictable outcome of uneven chromosome segregation and cytomixis. Where the goal is to use pretreatment to obtain well-spread metaphases for karyotyping, lower concentrations or shorter exposures may reduce the frequency of severe meiotic disruptions while still providing metaphase arrest. Where the goal is to assay genotoxicity, the observed mix of clastogenic-appearing and aneugenic outcomes indicates that both chromosome breakage/fragmentation and spindle/segregation failure contributed to the aberrations, so scoring methods should record both structural and numerical abnormalities.8

Microscopy-based descriptions do not directly distinguish primary DNA breakage from secondary segregation defects; cytomixis can be influenced by technical factors (fixation, sectioning, pressure) as well as treatment; and concentration, exposure time and developmental stage of buds strongly influence the phenotype. Future work to support mechanism would include dose–response series, time-course sampling, assays for micronuclei and aneuploidy quantification, and, if available, immunostaining for spindle microtubules.9

CONCLUSION

The present study conducted at Department of Botany, Maharani Cluster University, Bangalore aimed to investigate the effect of pre-treatment with 0.004 N 8-hydroxyquinoline and 0.1% paradichlorobenzene on meiosis in Allium cepa flower buds. Flower buds of Allium cepa var. aggregatum were collected from Thanushree Farm, Bengaluru, and treated with 0.004 N 8-hydroxyquinoline and 0.1% paradichlorobenzene for five hours. Post-treatment, anthers were stained with acetocarmine, squashed, and observed under a microscope to assess chromosomal behaviour. Results indicated that meiotic abnormalities were higher in flower buds treated with 0.004 N 8-hydroxyquinoline compared to 0.1% paradichlorobenzene, including irregular pairing, hyperploid pollen mother cells, and transfer of chromatin material. Irregular telophase stages, including triad, dyad, and polyploid conditions, were also observed. These findings demonstrate that pre-treatment with 0.004 N 8-hydroxyquinoline and 0.1% paradichlorobenzene distinctly influence chromosomal behaviour and meiosis in Allium cepa flower buds.

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