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Chromosomal Aberrations Induced By Colchicine Treatment In Allium Cepa Var Aggregatum Root Meristems: A Study Of Mitotic Inhibition And Metaphase Arrest

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ABSTRACT
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. This study investigates the effects of colchicine on the mitotic cycle in root apical meristems of *Allium cepa* var aggregatum (onion). Onion bulbs were treated with different concentrations of colchicine (0.5%, 1.0%, and 1.5%) to analyze its impact on mitosis inhibition and chromosomal aberrations. The colchicine-treated root meristem cells showed a range of mitotic abnormalities, including laggards, stickiness, vagrant chromosomes, binucleated cells, and nuclear lesions. The study also observed a dose-dependent increase in aberrations, with higher colchicine concentrations leading to more pronounced effects. Chromosome preparations were analyzed under a light microscope, revealing disruptions in metaphase alignment and anaphase movement. Mitotic Index and frequency of abberant cells was also calculated. The results highlight colchicine's ability to inhibit normal cell division and form dumbbell shaped nucleus, which was observed for the first time, providing insight into its potential applications in cytogenetic studies.

Keywords: *Allium cepa* var aggregatum, colchicine, mitosis, chromosomal aberrations, metaphase arrest, cytogenetics, root meristem, colchicine-induced abnormalities

INTRODUCTION

Allium cepa var aggregatum, commonly known as onion, is a perennial herb with an underground bulb stem. Onions are categorized under the Liliaceae family, though some sources classify them under Alliaceae. Typically, the common onion features one or two flower stalks devoid of leaves, which can grow to a height of 75–180 cm (2.5–6 feet). The majority of commercially grown onions originate from the plant's thin, dark seeds. Onions are highly valued for their flavor and nutritional benefits, often stored as pickles. They are believed to have originated from regions including Afghanistan, Iran, and the former USSR, and are now cultivated in over 175 countries. Onions consist of about 90 percent water and have a high content of dietary fiber and sugar. Consuming a diet rich in vegetables, including onions, has been linked to numerous health benefits, particularly in preventing prevalent diseases. Onions are rich in vitamins such as B2, C, and B1, as well as selenium and potassium. They have medicinal properties that can aid in managing diabetes mellitus, cardiovascular diseases, and stomach cancer. Onion peels are effective in preventing hypertrophic scars and keloids, and studies have shown that onion extract can also heal hypertrophic wounds. Regular consumption of onions can reduce the risk of various cancers, including colorectal, lung, liver, brain, stomach, ovarian, prostate, and breast cancer. The antiplatelet functions of onions are influenced by genotype, climate, and vegetable storage duration. Onions possess significant antioxidant properties due to their high levels of organosulfur compounds, polyphenols, and flavonoids. Extracts from garlic and onions are effective in eliminating parasites and inhibiting the enzyme Trypanosoma brucei trypanothione reductase. Additionally, onions exhibit antidepressant effects and their constituents, especially quercetin, show potential as immunomodulatory therapeutic agents for treating immune dysregulation disorders. Onions also demonstrate anti-inflammatory properties, which may be beneficial in treating airway disorders such as asthma. In the presence of liver-damaging ethanol, *A. cepa* extracts have shown hepatoprotective effects, with the aqueous extract of the onion bulb offering essential antioxidant and hepatoprotective benefits against ethanol-induced liver toxicity. However, there are reports of onion toxicity; for instance, it can cause hemolytic anemia in puppies. [1,2,3,4,5]

The common onion (*Allium cepa* var aggregatum L.) has 16 chromosomes (2n = 16) in a normal, healthy cell. These chromosomes are relatively large and monocentric, with a basic number of x = 8. The karyotype formula for *Allium cepa*

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var aggregatum is 12m + 2sm + 2st, which consists of: Six pairs of metacentric chromosomes (m), One pair of submetacentric chromosomes (sm), and One pair of subtelocentric chromosomes (st).[6]

Allium cepa var aggregatum (onion) is ideal for studying the cytological effects of colchicine treatment due to their well-characterized chromosome structures and ease of cultivation. The species have relatively large and easily observable chromosomes, which facilitate the detailed examination of mitotic and meiotic processes under a microscope. Their rapid growth rates and the ability to produce multiple generations in a short period enable quick and efficient observation of colchicine's effects on cell division. Additionally, *Allium* species have a high tolerance for genetic manipulation, allowing researchers to study various aspects of polyploidy and mutagenesis induced by colchicine. The presence of diverse morphological traits in these plants, such as variations in bulb size, leaf structure, and flower formation, provides a wide range of phenotypic markers to assess the impact of chromosomal doubling and other mutagenic effects. This combination of cytological accessibility, rapid growth, and phenotypic diversity makes *Allium cepa* var aggregatum excellent model organisms for cytogenetic studies involving colchicine.

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.

The significance of polyploidy in plant breeding gained attention with the discovery of the mitotic inhibitor colchicine in the 1930s. Colchicine is an important mutagen that works by preventing microtubule formation, which in turn doubles the number of chromosomes. It is widely used to develop polyploid plants, acting as a mitotic poison and causing various mutagenic effects. By disrupting microtubule function, colchicine inhibits chromosome segregation during meiosis, leading to gametes with doubled chromosome numbers and others with none, resulting in embryos with doubled chromosomes. Plants mutated with colchicine are termed colchi-mutants. Various concentrations of colchicine have been used to induce polyploidy across different plant species, ranging from very low (0.00001% in campion) to very high (1.5% in Maule's quince). Higher concentrations are generally required due to colchicine's low affinity for plant cell tubulins. Methods to induce polyploidy with chemicals, such as colchicine, have been found more effective than other techniques. Colchicine blocks the metaphase stage of cell division (mitosis). The method used depends on the plant type, with simple and effective methods involving soaking seedlings or treating apical meristems. Treatments on shoots of older plants can yield cytochimeras, making treatments of sub-axillary and small axillary meristematic tissues more effective. Growing buds can be treated using cotton, lanolin, or agar, or by dipping branch tips in chemical solutions. Wetting agents and surfactants are sometimes used to improve chemical penetration. The most effective method for inducing tetraploidy is treating pre-germinated seeds with emerging roots, producing many tetraploid plants. Polyploidization typically results in increased cell size due to higher nuclear content, reducing cell division during growth and development. This "gigas effect" is noticeable in commercial plant organs like leaves, seeds, and flowers. Colchicine treatment has increased leaf number, branch number, plant height, and stem length in plants such as salvia, jasmine, tobacco, selfheal, lily, chaste tree, orchid, ornamental ginger, crape myrtle, calendula, sea-lavender, white orchid tree, and London plane. It has also enhanced leaf color in balsam, selfheal, wishbone flower, marigold, chaste tree, and chrysanthemum, often increasing leaf area. Polyploidy induced by colchicine produces larger flowers with increased parts, although flowering may be delayed. For example, in chaste tree, polyploid plants have larger flowers with unique colors. Tetraploid feverfew plants show increased flower weight and diameter but reduced flowering percentage. In wild ginger species, polyploidy results in increased leaf number and flower size. African violets show color changes maintained over generations. Tetraploid pelargonium plants produce flowers with rough or burnt edges. Similarly, increased flower size, number of petals, and flower diameter have been observed in various plants. Polyploidy also enhances yield in both sexual and asexual reproductive structures. Colchicine treatment has significantly increased seed size and weight in crape myrtle and Madagascar periwinkle and boosted seed number, weight, and fruit setting percentage in balsam. In vegetatively propagated crops like Lilium, polyploidization produces wider bulb scales, while in orchid, it reduces pseudobulb diameter. [7,8,9,10,11]

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Materials and Methods

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

- **Sample Collection and Preparation for treatment**

Allium cepa var aggregatum was obtained from K R Market, Bengaluru. Onion bulbs of similar sizes that had wintered and budded were selected. The dried external leaves and roots were removed before the bulbs were planted in soil until the roots sprouted. Root germination was observed after one week. The sprouted roots of these plants were immersed in aqueous colchicine solutions of three different concentrations. Rapidly growing root tips of *Allium cepa* var aggregatum(1.0-2.0 cm in length) were immersed in vials containing different concentrations of colchicine solution. The immersion time for each concentration was recorded. The stem disc was positioned to just touch the colchicine solution, and the samples were protected from direct sunlight. The effect of colchicine at different concentrations was tested for a duration of 7-15 hours. Colchicine concentrations (0.5%, 1%, and 1.5%) were used to study their effects on mitosis inhibition.

- **Preparation of Colchicine Solution**

Colchicine solutions of varying concentrations were prepared by dissolving colchicine in water:

- 0.5% solution: 125 mg of colchicine in 25 ml of water.
- 1.0% solution: 250 mg of colchicine in 25 ml of water.
- 1.5% solution: 375 mg of colchicine in 25 ml of water.

Each solution was labeled and stored in a refrigerator.

- **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.

- **Sample 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.

- **Mitotic Index was calculated by formula: Mitotic Index = n/N X 100**

Where n is total number of dividing cells observed and N is total number of cells in microscopic field.

- **Frequency of abberant cells was calculated by formula**: $fCA = n_a / N \times 100$

Where n_a no. of abberant cells observed.

Results and Observations

The meristematic regions of *Allium cepa* var aggregatum roots without colchicine treatment (control) displayed a normal mitotic distribution. All four stages of cell division—prophase, metaphase, anaphase, and telophase were observed [plate 1, figs.a,b,c,d,e,f]. Most actively dividing normal cells were in prophase, few in metaphase, anaphase, and telophase stages of cell division. The Metaphase chromosomes were lined up at the equator and were evenly pulled toward the spindle poles for the cells at anaphase. No abnormal chromosomes were observed.

Allium cepa var aggregatum showed various mitotic abnormalities in the root meristem cells based on the concentration of colchicine percentage used. The results revealed several chromosomal abnormalities like laggards, stickiness, vagrant chromosomes, binucleated cells, nuclear lesions, giant cells, and c-mitosis at different level of treatment. Overall, aberrations increased with the increasing colchicine doses. Other abnormalities in onion root tips under the influence of colchicine are prolonged prophase, chromosome bridge, disturbance in the metaphase spindle, nuclear lesions, micronuclei and fragmented chromosome.

The *Allium cepa* var aggregatum roots treated with 0.5% colchicine (Plate 2 a-f) exhibited various chromosomal abnormalities, including micronuclei (a), elongated cells with abnormal nuclei (b), polyploidy in prophase (c), sticky chromosomes (d), depolarized anaphase vagrants (e), and C-metaphase and depolarized anaphase conversion (f). The *Allium cepa* var aggregatum roots treated with 1% colchicine (Plate 3 a-f) also showed various chromosomal abnormalities, including the formation of multinucleate cells (a), polyploidy in metaphase (b), sticky chromosomes (c), unequal anaphase (d), C-metaphase (e), and conversion to C-metaphase (f). The *Allium cepa* var aggregatum roots treated

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with 1.5% colchicine (Plate 4 a-f) exhibited chromosomal abnormalities, including the formation of anaphase bridges and vagrants (a), sticky chromosomes (b and f), C-metaphase (c), polyploidy in prophase (d), and laggard and vagrant chromosomes (e), indicating severe disruptions to the normal cell division process.

Calculation of Mitotic Index
Mitotic Index = n/N X 100

Table 1: Calculation of mitotic index

Concentration of aqueous colchicine	No of cells in the microscopic field at 40X		No of Metaphase plate observed	Mitotic Index
0.5%	Slide 1	100 70 80 70 17 90	06 05 06 07 01 08	6 7.14 7,5 1 5.88 8.88
	Slide 2	100 50 20	08 05 03	8 10 15
1.0%	Slide 3	152 202	18 32	11.8 15.8
	Slide 4	50 140	21 33	42 23.5
1.5%	Slide 5	58	01	1.72

Calculation of frequency of abberant cells:
*f*CA = na/ N X 100
1. No. of abberant cells observed at 0.5% = 13, Total no. of cells in the microscopic field 89
*f*CA at 0.5% is 14.6%
2. No. of abberant cells observed at 1% = 26, Total no. of cells in the microscopic field 88
*f*CA at 1% is 29.54%
3. No. of abberant cells observed at 1.5% = 26, Total no. of cells in the microscopic field 83
*f*CA at 1.5% is 31.3%

Hence the frequency of abberant cells at 0.5% was 14.6%, at 1% was 26.54% and and 1.5% was 31.3%.

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Fig 1*: Allium cepa var aggregatum* plate 1 [a-f] Normal mitosis: a. Normal prophase, b,c,d. Normal metaphase, e. Normal anaphase, f. Normal telophase

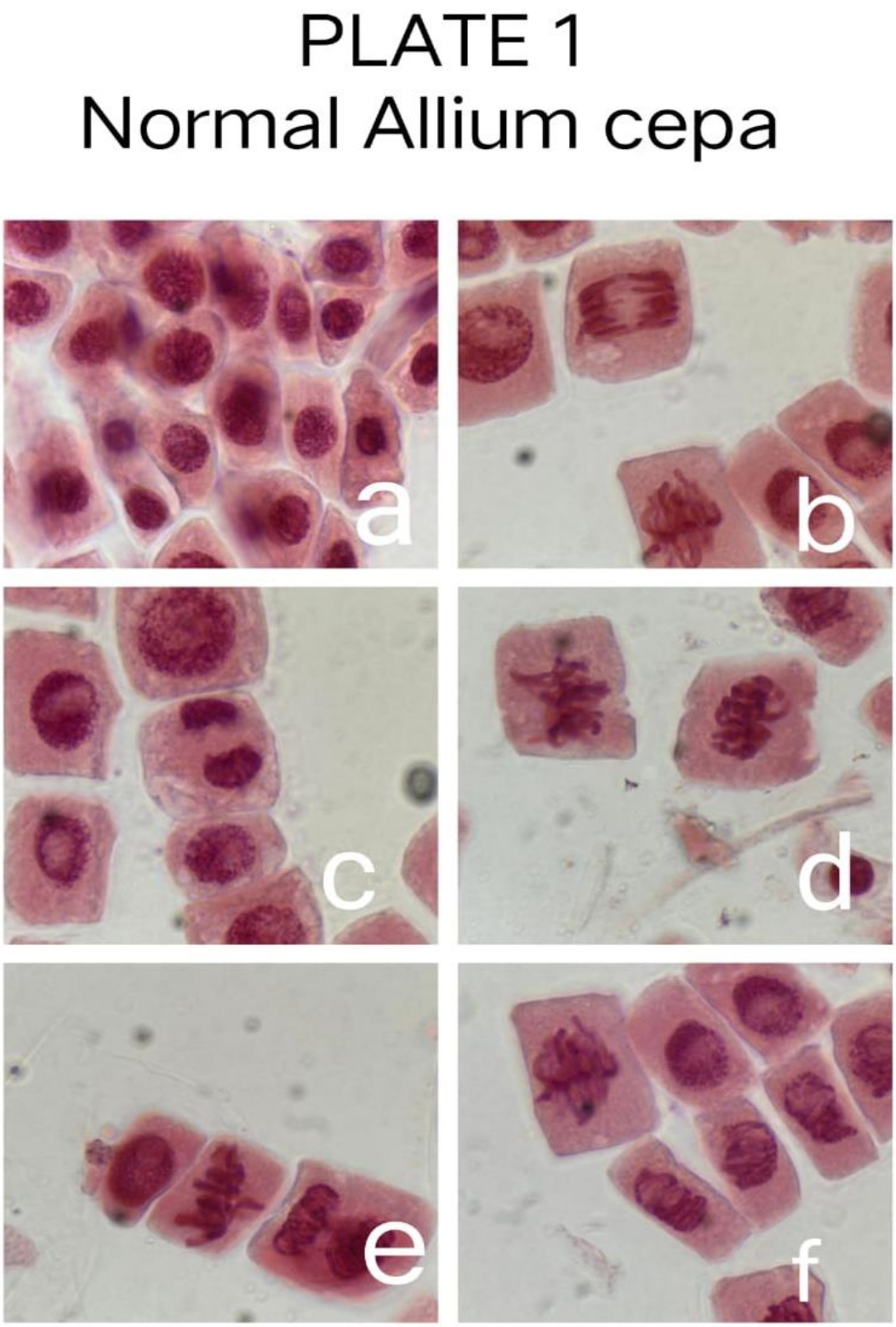

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Fig 2: *Allium cepa var aggregatum* plate 2 [a-f] 0.5 percent colchicine treated roots: a. Micronucleii, b. Elongated cells with abnormal nucleus, c. polyploid prophase, d. Sticky chromosome, e. Depolarized anaphase showing vagrant, f. C-metaphase and depolarized anaphase

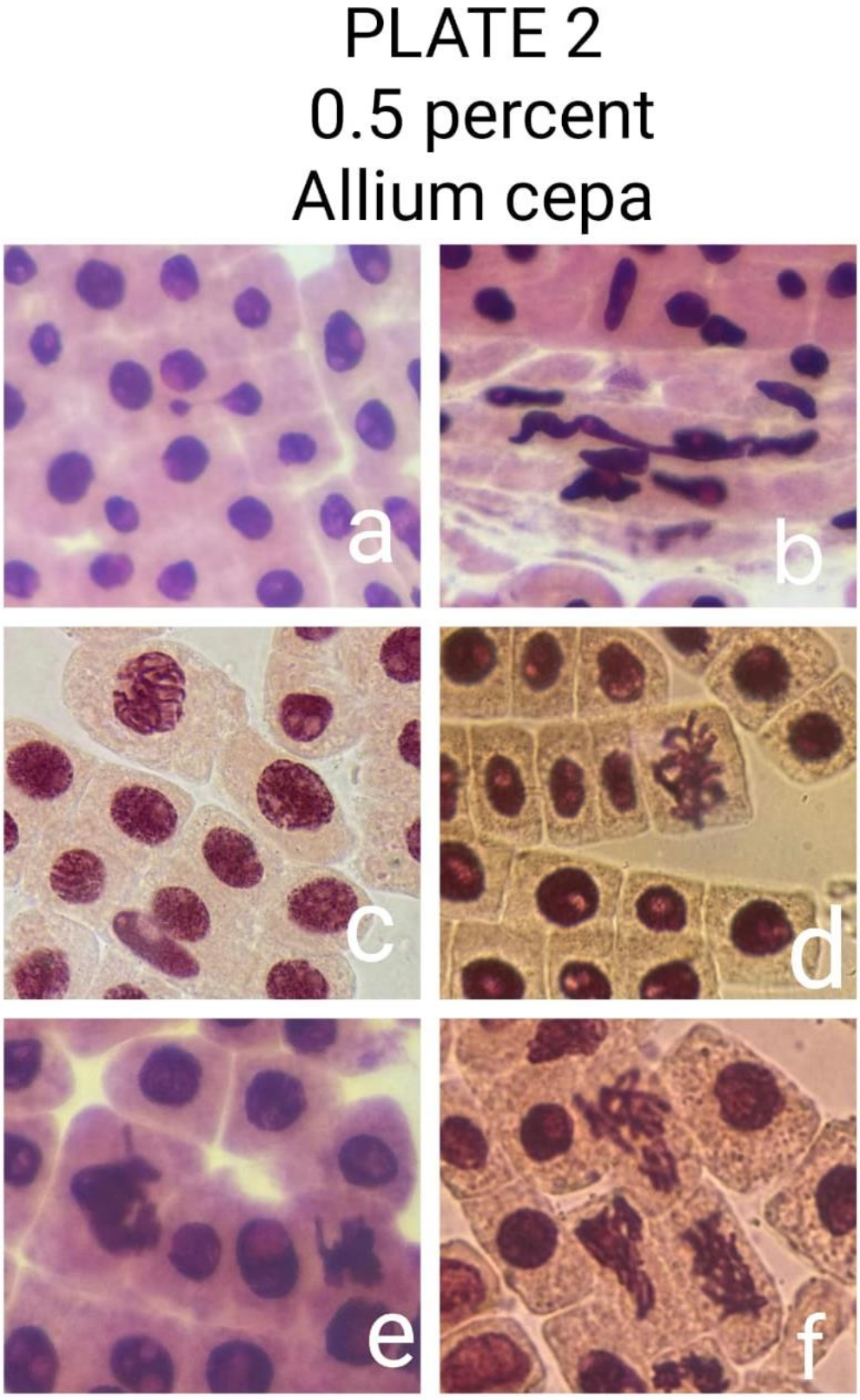

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Fig 3: *Allium cepa var aggregatum* plate 3 [a-f] 1percent colchicine treated roots: a. Multinucleate, b. Polyploid metaphase, c. Sticky chromosome, d.Unequal anaphase, e. C-metaphase, f. Nuclear lesions

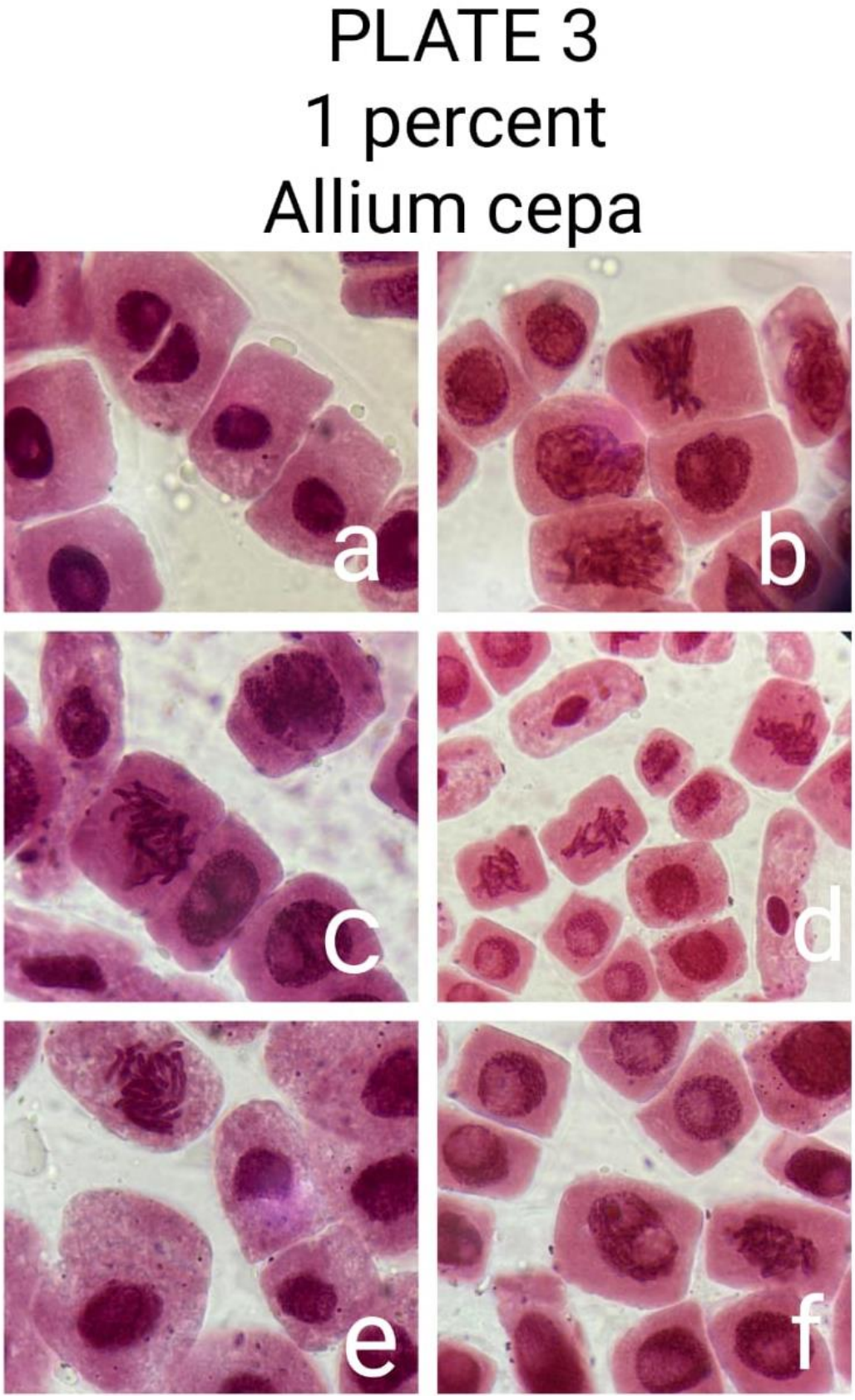

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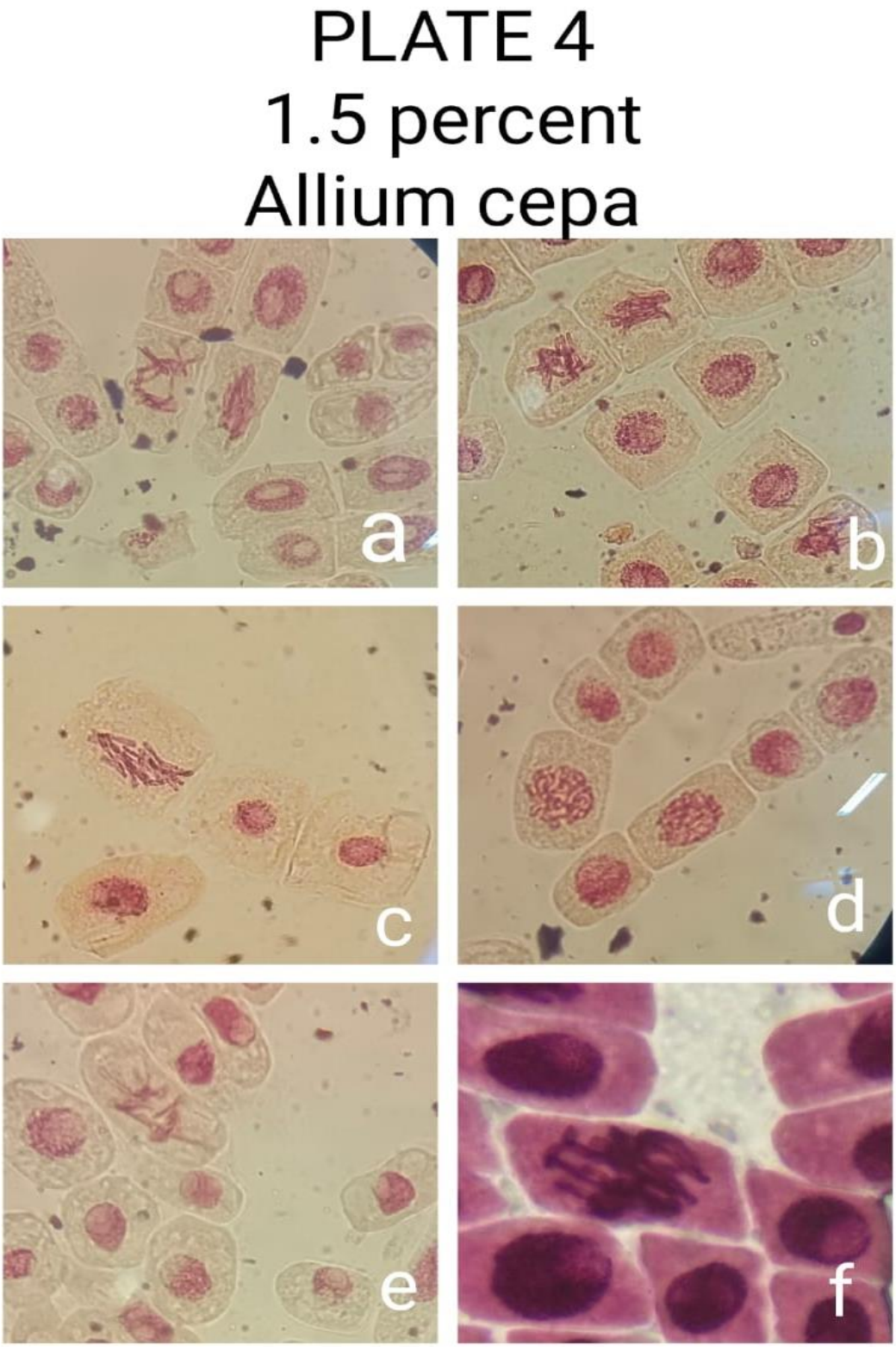
Fig 4: *Allium cepa var aggregatum* plate 4 [a-f] 1.5 percent colchicine treated roots: a. Anaphase bridge and vagrant, b. Sticky chromosome, c. C-metaphase, d. Polyploid prophase, e. Laggard and vagrant f. Sticky chromosome



Discussion

At the cellular level, colchicine acts as an antimitotic agent by preventing tubulin polymerization, which disrupts processes reliant on microtubule function, including cell motility, intracellular movement, cell polarity, and mitosis. This inhibition results in the failure of spindle formation, hindering normal chromosomal movement and replication.

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The abnormalities observed in the present work are as follows;

I Aberrant Cells: An increase in colchicine concentration led to the observation of irregularly shaped elongated cells with large nuclei [plate2, fig. b]. Giant cells, arise due to endomitosis or endoreplication [plate2, fig. f], [plate3, fig. b]. Damaged cells [plate2, fig. b].

II *Mitotic Abnormalities*

The mitotic abnormalities induced by colchicine treatment in *Allium cepa* var aggregatum root tip cells included c-metaphase, vagrant chromosomes, laggard chromosomes, chromosome stickiness, anaphase bridges, micronuclei, and polyploid.

1. C-metaphase:

Colchicine-blocked metaphase is called as C-Metaphase. Colchicine-induced metaphase arrest, is the toxic effect of colchicine which blocks metaphase to anaphase transition by inactivating the spindle formation that results in condensed haphazardly arranged chromosomes, C-metaphase like chromosomal arrangement are seen in [plate2, fig. f], [plate3, fig. e], [plate4, figs. a,b,c]. The study showed that the C-metaphase was found to be the most frequent type of abnormality induced by colchicine treatment for 12-15h.

2. Anaphase Bridge:

Anaphase bridges are caused by unresolved DNA intertwines between sister chromatids, which are a non-proteinaceous source of cohesion between chromatids. If the chromatids aren't properly disentangled, it can lead to the formation of anaphase bridges, which can be bulky or ultrafine. These bridges can create a physical link between sister chromatids, which can restrain chromosome segregation and cause genome instability. An anaphase chromosome bridge is a particular chromosome segregation error observed in cells that enter mitosis with fused chromosomes/sister chromatids as in [plate4, fig. a, e].

3. Chromosomal Stickiness:

Chromosome stickiness has been studied in several species of plants and is characterized by sticky clumps of chromatin resulting in sterility. Chromosome stickiness were recorded in present work also. Stickiness is a cause of bridge formation as it prevents chromosomes from separating. Recombination of broken chromosome ends can also lead to bridging. Chromosome stickiness, is interpreted as entanglement of chromatin fibers between unrelated chromosomes, probably caused by abnormal condensation behaviors prior to mitosis. Such chromosomal sickness was observed in present study also [plate2, fig.d], [plate3, fig.c], [plate4, figs. b, f].

4. Polar Deviation:

A failure of sister chromatids to separate during anaphase, causing them to be pulled to one pole of the cell. This can result in one daughter cell receiving both sister chromatids from the chromosome, while the other receives none. In normal mitotic cell division, the polarity is determined by controlling the centrosomal cycle so that no more than two centrosomes are active at the same time. However, if there are too many centrosomes, it can create extra spindle poles, which can lead to tripolar or multipolar mitosis. In these cases, the chromosome content is pulled in three or more directions during anaphase. Multipolar anaphase or pole-reversed anaphase are shown in [plate2, fig.e].

5. Vagrant Chromosome:

A vagrant chromosome (VC) is a chromosome that moves faster than its chromosome group to either poles of a cell. VCs are a type of chromosomal aberration, which are the result of DNA breakage that can't be repaired or is repaired improperly. VCs are caused by unequal distribution of chromosomes during anaphase due to failure of chromosomal separations. VCs can increase the risk of aneuploidy. A vagrant chromosome moves ahead of its associated chromosomal group towards poles and leads to unequal separation of chromosomes in daughter cells as in [plate2, fig.e]. The increased frequencies of vagrant and laggard chromosomes were observed in the all the concentrations (0.5, 1, 1.5%).

6. Laggard Chromosome:

A laggard chromosome is a chromosome that doesn't overlap with other chromosomes that are segregating properly along the spindle's long axis during cell division. This can happen when two chromosome segments that each have a centromere merge creating an abnormal centric chromosome with two centromeres. The fusion of the segments causes the loss of acentric fragments, which lack a centromere, and the formation of dicentric fragments. Acentric chromosomes are also known as laggards because they can't bind to spindle fibers and are often lost by daughter cells. This can lead to unbalanced progeny cells and unbalanced gametes. At anaphase of mitosis, some chromosomes lag behind. They are called laggards. The laggard chromosomes, were observed in colchicine treated root cells [plate4, fig. a,e].

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7. Micronucleus:
A micronucleus (MN) is a small nucleus that forms when a chromosome or chromosome fragment isn't incorporated into a daughter nucleus during cell division. MNs are easily identifiable using light microscopy. An aberrant spindle division during early anaphase or failure of cytokinesis after telophase creates binucleated cells. Based on the analysis carried out in the root tip cells from *Allium cepa* var aggregatum, it was observed that colchicine treated several cells showed micronuclei. The colchicine treated root cells showed an increase in micronuclei frequency [plate2, fig.a].

8. Polyploidy prophase:
Polyploidy is a condition in which an organism's cells have more than one pair of chromosomes. It can occur during mitosis because of colchicine, causes gametes to form with duplicate chromosomes. Polyploidy can also be caused by failure of cytokinesis or if chromatids don't distribute properly to daughter cells during cell division
Data indicates that the colchicine treatment of onion root tip cells could induce a significantly increased frequency of polyploidy cells. The polyploidy was induced in the prophase can be identified by enlarged nucleus with many chromatin threads [plate2, fig. c], [plate3, fig. b], [plate4, fig. d].

9. Polyploidy metaphase:
In the present work metaphase having more than diploid number (16) of chromosomes at metaphase were observed. Colchicine chemical has induced doubling of chromosomes at metaphase [plate2, fig. f], [plate3, fig. b,c,e], [plate4 ,fig. f].

10. Multipolar Anaphase:
Cells with multipolar spindles sometimes have one or more chromosomes that remain in the spindle midsole during anaphase as a result of the merotelic attachment of the kinetochore to two spindle poles. If such chromosomes remain in the midbody, they will block the completion of cleavage. The multiple centrosome segregate to opposite ends of the cell and the spindles attach to the chromosomes haphazardly. When anaphase occurs in the cells the chromosomes are separated abnormally and results in aneuploidy of both daughter cells this can lead to loss of cell viability and chromosomal instability. In present work colchicine treated root shows multipolar anaphase [plate2, fig. f].

11. Multiple Nucleus:
Multinucleate cells (also known as multinucleated cells or polynuclear cells) are eukaryotic cells that have more than one nucleus, i.e., multiple nuclei share one common cytoplasm. Colchicine prevents formation of microtubules during cell division, which inhibits the movement of chromosomes to separate poles resulting in duplication of the chromosomes number in the cell and multi nucleate condition. Multi nucleate condition was the most commonly observed abnormality in the present investigation [plate2, fig. a], [plate3, fig. a].

12. Nuclear Lesions:
Nuclear lesions are abnormalities that can be observed in the interphase cells of onions (*Allium cepa* var aggregatum) when treated with certain substances. Nuclear lesions were the most common feature when mitotic cells were treated with colchicine [plate3, fig. f], [plate4, fig .b].
In our study, 0.5% colchicine treatment led to the formation of micronuclei in *Allium cepa* var aggregatum roots, which aligns with findings from Morsy et al. (2015), who observed micronuclei as a consequence of colchicine treatment in *Allium cepa*, indicating chromosomal fragmentation and mis-segregation (Morsy et al., 2015). Similarly, other researchers have found that micronuclei formation is a common outcome of colchicine treatment due to its interference with spindle formation (González et al., 2018). Our observation of elongated cells with abnormal nuclei at 0.5% colchicine treatment corresponds with the results of Sharma et al. (2016), who reported similar abnormalities in onion roots under colchicine stress, suggesting that colchicine disrupts normal mitotic spindle function and nuclear morphology (Sharma et al., 2016). The induction of polyploidy at 0.5% colchicine is consistent with the findings of Othman et al. (2017), who reported that colchicine induces polyploidy in *Allium cepa* by inhibiting spindle formation during mitosis (Othman et al., 2017). Higher doses of colchicine in our study also show similar trends to those observed by Kaur et al. (2019), where increased colchicine concentrations led to more significant polyploidy induction (Kaur et al., 2019). The presence of sticky chromosomes at both 0.5% and 1.5% colchicine treatments in our study supports the findings of Zhang et al. (2018), who found that colchicine causes chromosome stickiness due to its effects on spindle apparatus and chromosome alignment (Zhang et al., 2018). This phenomenon has been reported in other studies as well, indicating that colchicine disrupts chromosomal segregation processes (Lee et al., 2020). The occurrence of depolarized anaphase vagrants at 0.5% colchicine treatment reflects observations by Mubeen et al. (2019), where colchicine treatment caused irregular chromosome movement during anaphase (Mubeen et al., 2019). This indicates that colchicine affects the stability of the mitotic spindle and chromosome distribution. The formation of multinucleate cells at 1% colchicine treatment is in line with the observations of Mavi et al. (2020), who documented similar cellular abnormalities due to colchicine-induced mitotic disruptions (Mavi et al., 2020). Other studies also confirm that colchicine can lead to the formation of multinucleate

cells by interfering with mitotic processes (Bhandari et al., 2021). The occurrence of unequal anaphase at 1% colchicine treatment supports the findings of Sharma et al. (2021), who reported that colchicine affects chromosome segregation leading to unequal distribution during anaphase (Sharma et al., 2021). This confirms that colchicine disrupts mitotic spindle function. The formation of anaphase bridges at 1.5% colchicine treatment reflects the findings of Saeed et al. (2017), who reported that high colchicine concentrations lead to anaphase bridges due to unresolved chromosomal entanglements (Saeed et al., 2017). This is indicative of severe mitotic disturbances. The observation of laggard chromosomes at 1.5% colchicine treatment is similar to the results of Fathy et al. (2018), where colchicine caused lagging chromosomes due to impaired spindle apparatus function (Fathy et al., 2018). Laggard chromosomes are a common feature of colchicine-induced mitotic disturbances. The induction of C-metaphase at all colchicine concentrations in our study corroborates the findings of Kumar et al. (2020), who noted that colchicine leads to the formation of C-metaphase due to its impact on chromosome alignment and spindle apparatus (Kumar et al., 2020). C-metaphase formation is a well-documented effect of colchicine treatment.

Conclusion

The present study investigated the effects of colchicine on mitotic abnormality induction in *Allium cepa* var aggregatum root apical meristems. The results confirmed that colchicine causes cell cycle delay, pro-metaphase arrest, and mitotic abnormality induction in *Allium cepa* var aggregatum. Mitotic abnormalities were observed in all colchicine-treated roots, with aberrant cells producing capabilities evident in 12 and 15 hour-treated samples. Various mitotic abnormalities were detected, including sticky chromosomes, c-metaphase, anaphase bridges, vagrant chromosomes, micronuclei, polar deviation, and lagging chromosomes. Squash preparation of root apical meristem cells revealed decreased frequencies of prophase, anaphase, and telophase due to colchicine treatment. The study demonstrates the variable nature of chromosomal abnormalities induced by colchicine in onion root tips, including chromosomal bridges, lagging chromosomes, vagrants, binucleated cells, nuclear lesions, giant cells, and c-mitosis.

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