REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 25, No.1 (2024)
http://www.veterinaria.org
Article Received: Revised: Published:

Colchicine-Induced Chromosomal Aberrations and Mitotic Inhibition in *Allium sativum* var sativum Root Meristems

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Abstract
Allium sativum, commonly known as garlic, is a perennial flowering plant that develops from a bulb. Colchicine acts as a microtubule-destabilizing agent and is a plant metabolite. This study explores the effects of colchicine on mitosis and chromosomal behavior in *Allium sativum* var sativum (garlic) root apical meristem cells. Garlic cloves were treated with varying concentrations of colchicine (0.5%, 1.0%, and 1.5%) to observe the impact on mitotic inhibition and metaphase arrest. Root tips were processed and examined microscopically, revealing a range of chromosomal abnormalities. These included c-metaphase, anaphase bridges, vagrant and laggard chromosomes, chromosomal stickiness, and multinucleated cells. The frequency and severity of these abnormalities increased with higher colchicine concentrations. Observations also indicated the presence of polyploid cells, nuclear lesions, and distorted cell division stages. The study highlights the dose-dependent effects of colchicine in disrupting normal cell division processes in *Allium sativum* var sativum contributing to a deeper understanding of its role in cytogenetic research.

Keywords: *Allium sativum* var sativum, colchicine, chromosomal aberrations, mitosis inhibition, metaphase arrest, c-metaphase, anaphase bridges, polyploidy, cytogenetics

Introduction

Allium sativum, commonly known as garlic, is a perennial flowering plant that develops from a bulb. It features a tall, erect flowering stem that can reach up to 1 meter (3 feet) in height. The leaves are flat, linear, and solid, measuring about 1.25 to 2.5 cm (0.5 to 1 inch) in width, ending in a pointed tip. Garlic plants bloom with pink to purple flowers from July to September in the Northern Hemisphere. The bulb, known for its strong odor, typically contains 10 to 20 cloves. The central cloves are symmetrical, while those on the outer parts are often asymmetrical. Each clove is wrapped in an inner sheathing leaf, which is further encased in layers of outer sheathing leaves. Garlic can be cultivated in northern regions, including as far north as Alaska, provided it is planted at the right time and depth. The plant produces hermaphroditic flowers that are pollinated by various insects, such as bees, butterflies, and moths. The sulfur compounds in garlic are responsible for the green or blue color changes observed during pickling and cooking. In acidic or heated conditions, the sulfur-containing compound alliin reacts with common amino acids to form pyrroles, which are clusters of carbon-nitrogen rings. These pyrrole molecules, depending on their structure, absorb different wavelengths of light and thus appear colored. For instance, two-pyrrole molecules appear red, three-pyrrole molecules appear blue, and four-pyrrole molecules, similar to chlorophyll, appear green. These pyrrole pigments are safe to consume. When fresh or crushed, garlic produces several sulfur-containing compounds such as allicin, ajoene, diallyl polysulfides, vinyldithiins, and S-allylcysteine. It also yields enzymes, saponins, flavonoids, and Maillard reaction products when cooked, which do not contain sulfur. The sharp flavor of garlic comes from phytochemicals that are released when the plant's cells are damaged. Chopping, chewing, or crushing garlic cells triggers enzymes in cell vacuoles to break down sulfur-containing compounds in the cell fluids. These compounds are responsible for garlic's strong taste and smell, and some continue to react and change over time. Garlic has the highest concentrations of these reaction products among alliums, making it much more potent than onions, shallots, or leeks. These compounds likely evolved as a defense mechanism to deter animals like birds, insects, and worms from consuming the plant. Allicin is the main compound responsible for the "hot" sensation of raw garlic. It activates thermo-transient receptor potential channels, which create a burning sensation. Cooking garlic removes allicin, reducing its spiciness. Allicin and its breakdown products, such as diallyl disulfide and diallyl trisulfide, contribute significantly to garlic's characteristic odor, along with other compounds like vinyldithiins and ajoene. Garlic is sometimes referred to as the "stinking rose" due to its strong smell. When consumed in large quantities, garlic's sulfur compounds can be detected

in a person's sweat and breath the following day. This is because the body metabolizes these compounds into allyl methyl sulfide (AMS), which cannot be digested and is instead excreted through the lungs and skin. This process takes several hours, causing the odor to linger. Eating fresh parsley is believed to alleviate garlic breath, which is why it is often included in garlic recipes such as pistou, persillade, and garlic butter spread used in garlic bread. Despite the belief that consuming garlic can repel mosquitoes because of sulfur compounds in the blood, there is no scientific evidence to support this claim.[1,2,3,4,5]

Garlic (*Allium sativum*) has 16 chromosomes, or 2n = 2x = 16. One study found that each strain of garlic can be considered a clone because garlic does not produce seeds. The study also found that the shorter chromosome 7 is an m chromosome that is more asymmetric than the longer m chromosomes. The two satellited pairs have a similar gross organization, with a short arm that is divided into a small proximal segment and a large satellite.[6]

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]

Materials and Methods

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

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➢ Sample Collection and Preparation for treatment

Allium sativum var sativum was obtained from K R Market, Bengaluru. *Allium sativum* cloves took a longer time to form roots and were grown in soil with water. 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 sativum* (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.

Results and Observations

The meristematic regions of *Allium sativum* roots without colchicine treatment (control) displayed a normal mitotic distribution. All four stages of cell division—prophase, metaphase, anaphase, and telophase were observed in *Allium sativam* [plate1,figs.a,b,c,d]. 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 sativum, 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 sativum* roots treated with 0.5% colchicine (Plates 6 and 7) showed various chromosomal abnormalities, including polyploidy in anaphase and metaphase, unequal anaphase, anaphase bridges, C-metaphase, fragmented chromosomes, distorted metaphase, and sticky chromosomes, as well as an aphasic bridges and vagrants.

The *Allium sativum* roots treated with 1% colchicine (Plate 8 [a-f]) exhibited severe chromosomal damage, including fragmented chromosomes, depolarized anaphase with vagrants, sticky chromosomes, deformed cells with tapering nuclei, laggard and vagrant chromosomes, and anaphase bridges, indicating a high level of disruption to normal cell division processes.

Allium sativum roots treated with 1.5% colchicine (Plates 9 and 10) showed extreme chromosomal abnormalities, including dumbled-shaped telophase, micronuclei, multinucleate cells, irregular cells, C-metaphase, vagrants, and disrupted cell division processes.

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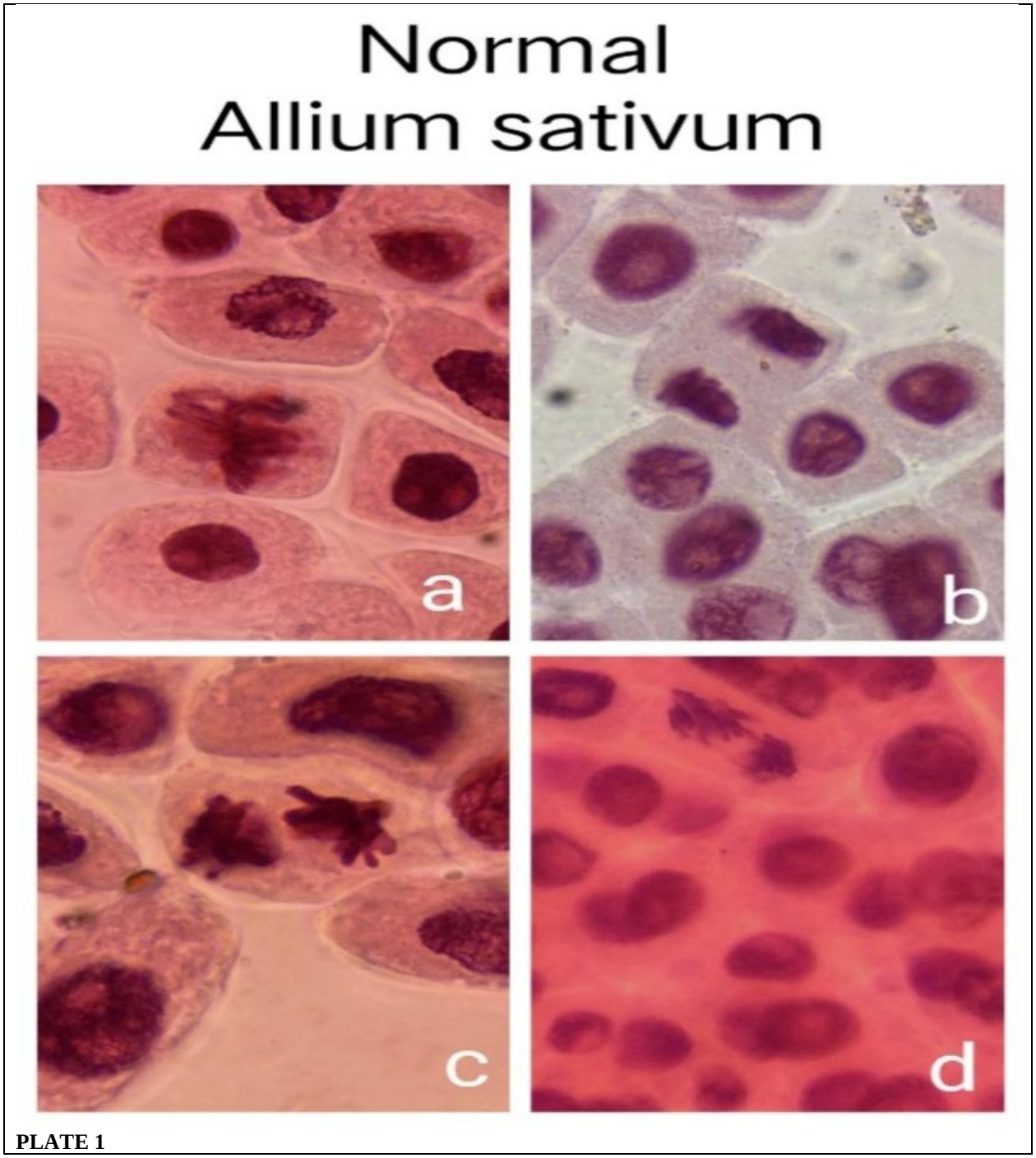

Fig 1: *Allium sativum* Plate 1 [a-d] Normal mitosis: a. Normal metaphase, b. Normal prophase, c. Normal anaphase, d. Early telophase

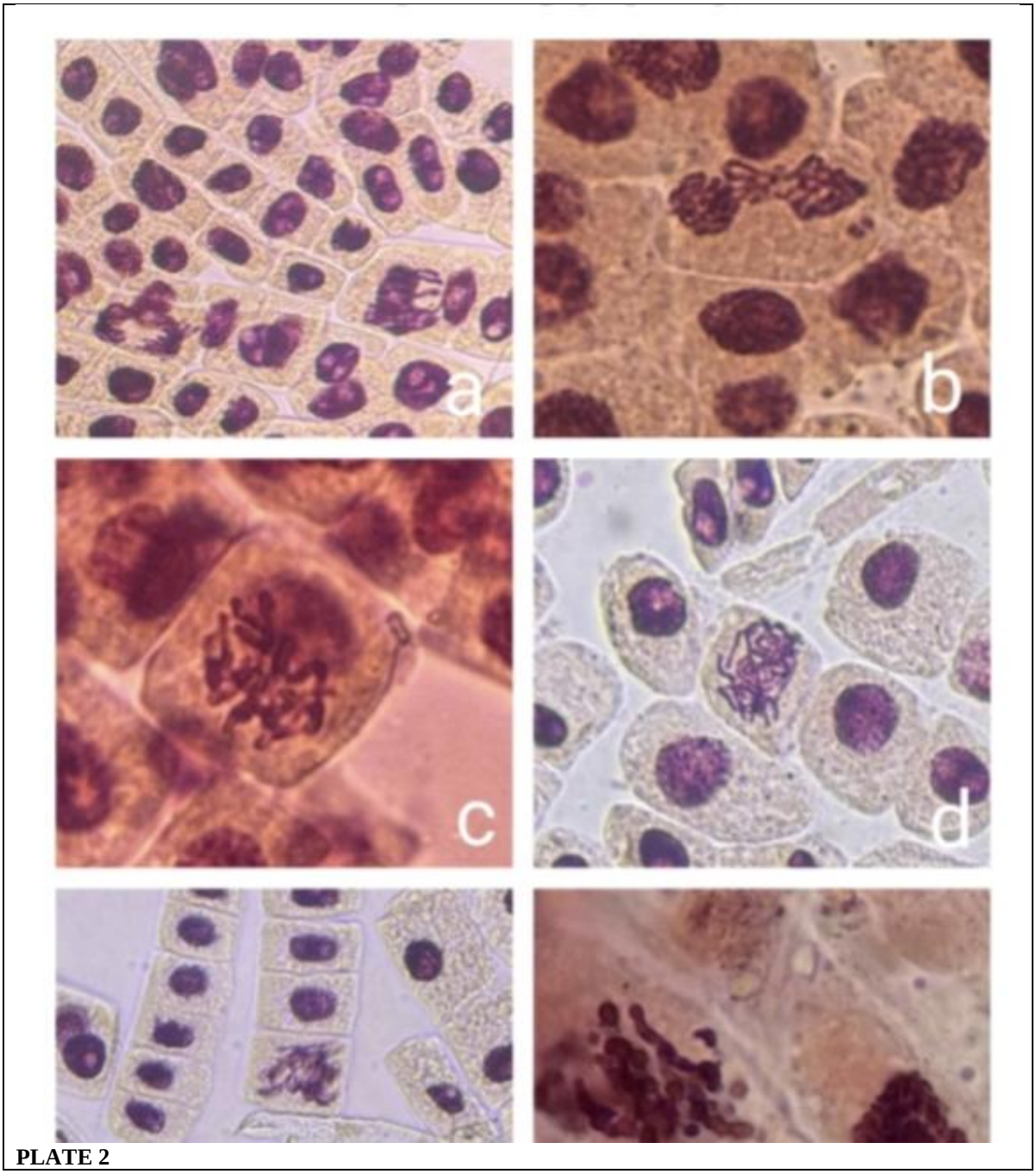

PLATE 2

Fig 2: *Allium sativum* plate 2 [a-f] 0.5 percent colchicine treated roots: a. Polyploid anaphase/unequal anaphase, b. Anaphase bridge, c. C-metaphase, d. Polyploid metaphase, e. C-metaphase, f. Fragmented chromosome

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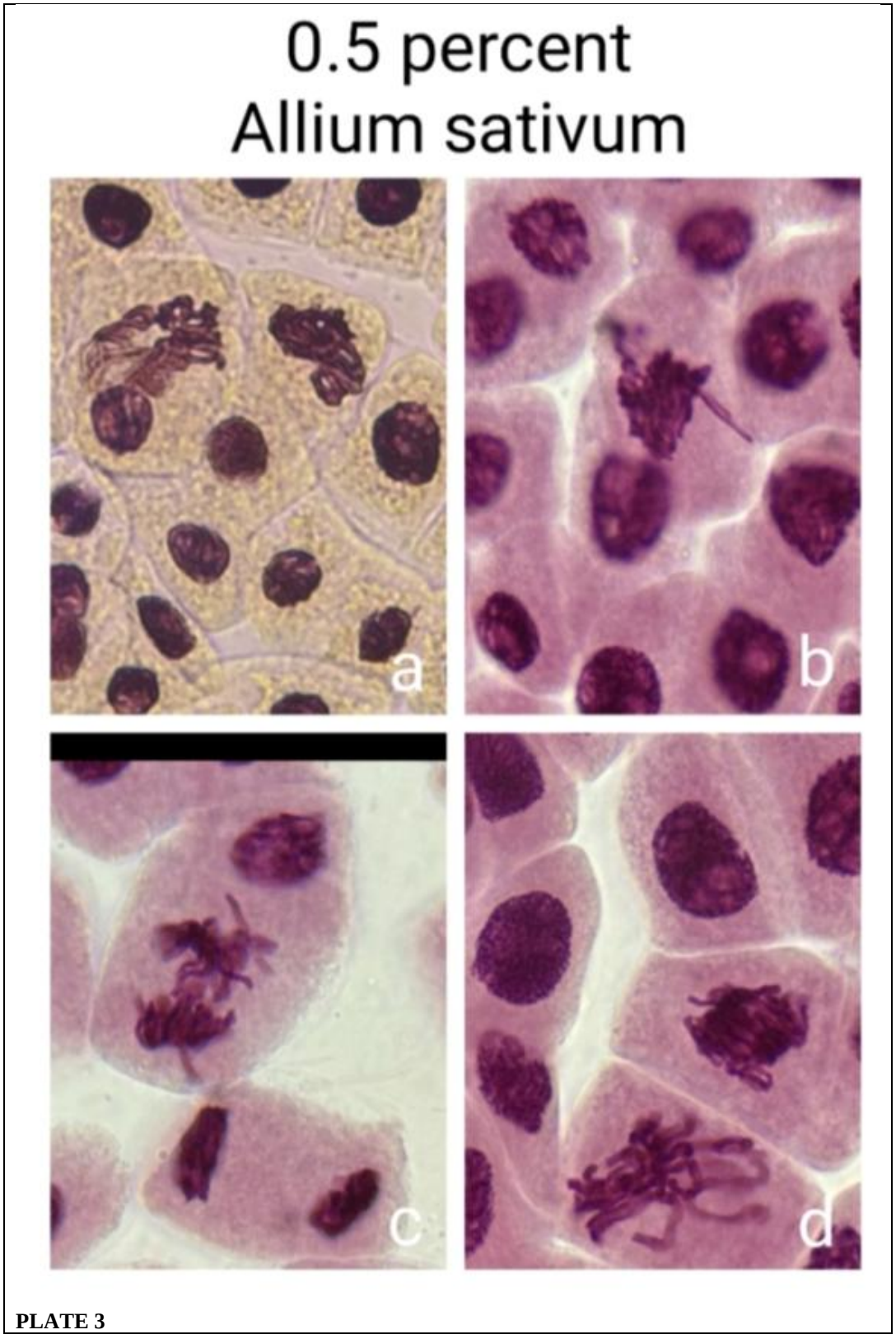

Fig 3: ***Allium sativum*** **plate 3 [a-d] 0.5 percent colchicine treated roots: a. Distorted metaphase, b. Distorted metaphase, c. Anaphasic bridge and vagrant, d. Sticky chromosome**

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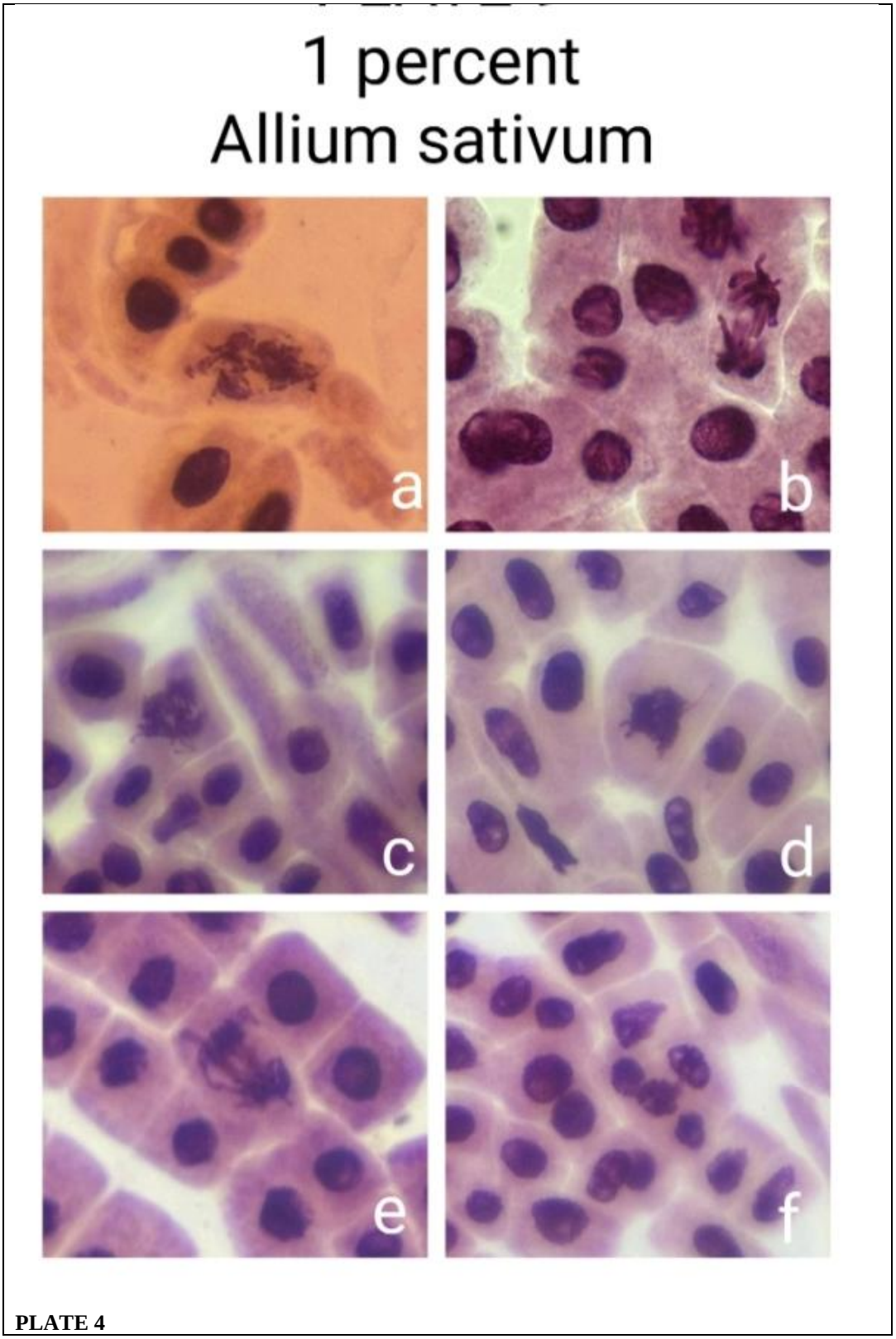

PLATE 4

Fig 4: *Allium sativum* plate 4[a-f] 1 percent colchicine treated roots: a. Fragmented chromosome, b. Depolarized anaphase with vagrant, c. Sticky chromosome, d. Deformed cells with tapering nucleus, e. Laggard and vagrant, f. Anaphase bridge

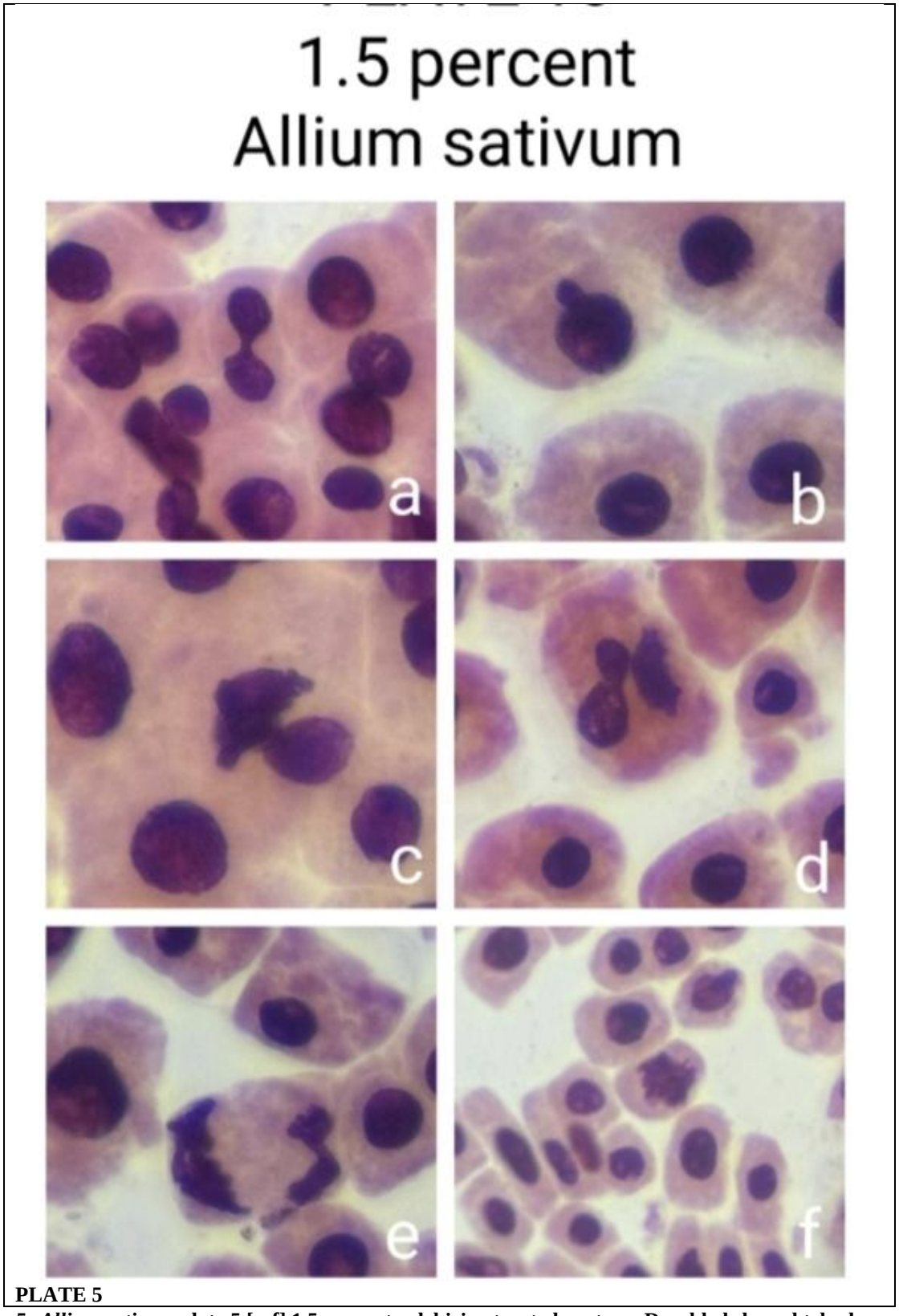

Fig 5: *Allium sativum* plate 5 [a-f] 1.5 percent colchicine treated roots: a. Dumbled shaped telophase, b. Micronucleii, c. Multinucleate, d. Irregular cells, e. Irregular anaphase, f. Irregular cells

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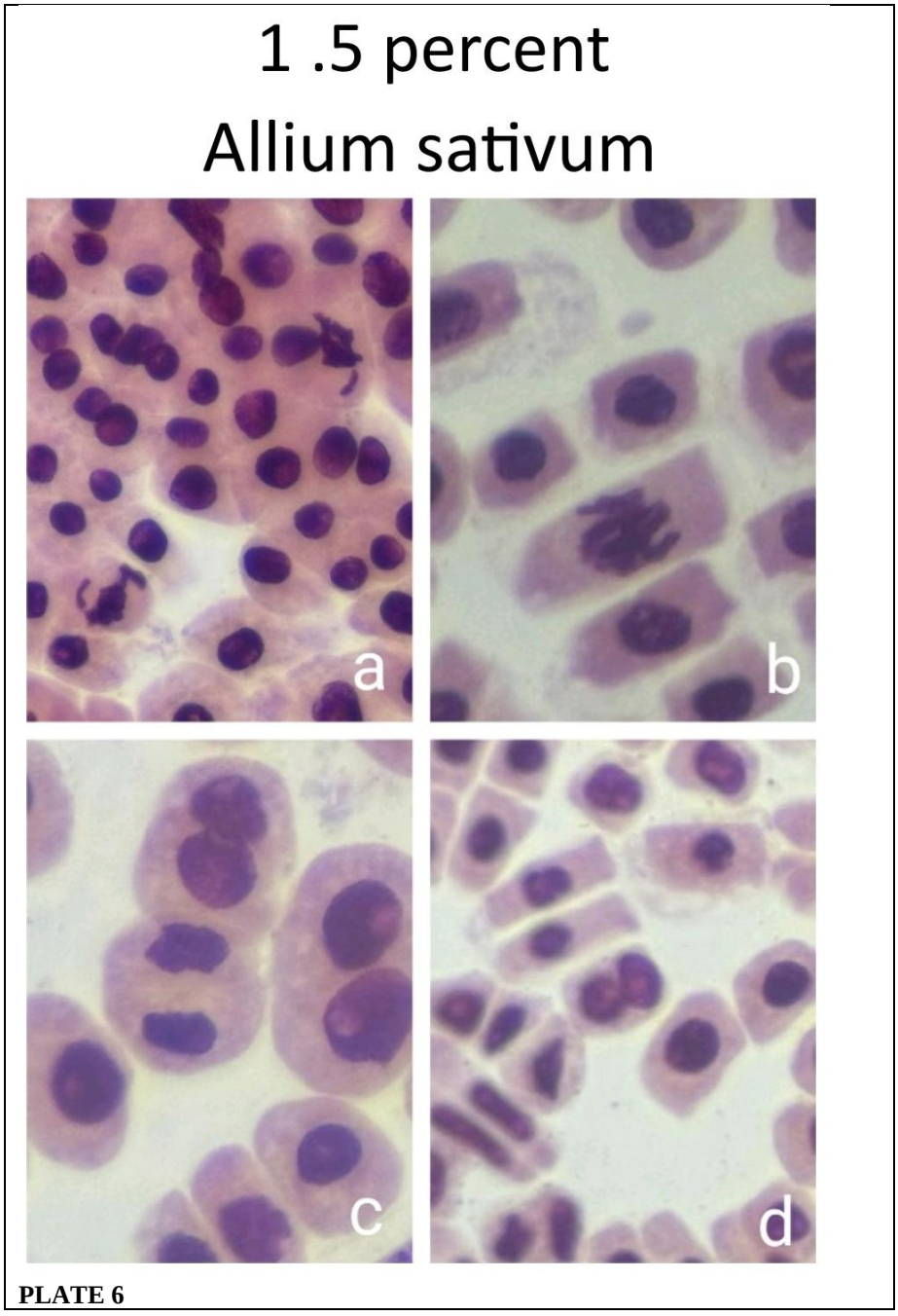

Fig 6: *Allium sativum* plate 6 [a-d] 1.5 percent colchicine treated roots: a. Vagrant, b. Sticky chromosome, c. Multinucleate, d. Irregular cells

Discussion

Following are some of the abnormalities observed in the present work

Mitotic Abnormalities

The mitotic abnormalities induced by colchicine treatment in *Allium sativum* root tip cells included c-metaphase, vagrant chromosomes, laggard chromosomes, chromosome stickiness, anaphase bridges, micronuclei, and polyploidy were induced by colchicine treated root tips cells of *Allium sativum.*

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,figs.c,e],

[plate5,fig.f]. 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 [plate1, figd], [plate2, figs.a, b]. The colchicine treatment induced an increase in the anaphase bridge in *Allium sativum* root apical meristem cells. In the case of treated samples 12-15h showed more anaphase bridge in 1% colchicine treated root cells [plate3,fig.c], [plate4,figs.b,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. The colchicine treatment to garlic root tip cells induced the highest frequency of chromosomal stickiness and was maximum in 1% colchicine treated root cells [plate1,fig.b].

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], [plate3, fig.a].
Polar deviation increases in root apical meristem cells treated with colchicine (1%) and also in (1.5%) [plate4, fig.e], [plate5, fig.6], [plate6, fig.a].

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.a], [plate3, figs. b, c]. The increased frequencies of vagrant and laggard chromosomes were observed in the all the concentrations (0.5, 1, 1.5%) [plate4, figs .b, e, f].

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 [plate2,fig.b], [plate3,fig.c], [plate4,figs.a,b,e,f].

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. The colchicine treated root cells showed an increase in micronuclei frequency [plate5, fig.b].

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.d], [plate3, 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,figs.d,f], [plate3,figs.a,d], [plate4,figs.a,c,d].

10. Chromatid Break:
Chromatid aberrations have two typical appearances at metaphase some appear to be simple breaks while others are evidently reciprocal chromatid exchanges. The breaks may be in single chromatids or they may be in both chromatids at the same site. Chromatid break is a discontinuity of a single chromatid in which there is a clear misalignment of one of the chromatid, originating acentric fragments and consequently generating a misalignment of chromosome that occurs. Colchicine treated roots showed chromatid break [plate2, fig.f], [plate3, fig.b], [plate5, fig.e].

11. 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, figs. a, b], [plate3, fig.a], [plate5, fig.e].

12. 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 [plate4,fig.f], [plate5,figs.a,c,d,f], [plate6,figs.a,d].

13. Distorted Anaphase:
Irregular anaphase was recorded in present work [plate5, fig.e].

14. Nuclear Lesions:
Nuclear lesions are abnormalities that can be observed in the interphase cells when treated with certain substances. Nuclear lesions were the most common feature when mitotic cells were treated with colchicine [plate2, fig .a].
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. Consequently, colchicine's toxic effects are most pronounced in tissues with high mitotic rates, such as hair follicles, bone marrow, and gastrointestinal epithelium. Recently, histopathologic features of colchicine toxicity have been described in gastrointestinal tract biopsies, showing epithelial pseudostratification, loss of cellular polarity, and increased ring mitotis. Our observation of polyploidy in *Allium sativum* var sativum roots treated with 0.5% colchicine is consistent with the findings of Othman et al. (2017), who reported colchicine-induced polyploidy in *Allium* species by inhibiting spindle formation during mitosis (Othman et al., 2017). Other studies, such as those by Kumar et al. (2020), also corroborate that colchicine can effectively induce polyploidy in various plant species, including *Allium sativum* (Kumar et al., 2020). The occurrence of unequal anaphase in our study at 0.5% colchicine treatment mirrors the results of Sharma et al. (2021), who found similar abnormalities in *Allium sativum* due to colchicine's impact on chromosome segregation and spindle function (Sharma et al., 2021). This suggests that colchicine consistently disrupts the normal distribution of chromosomes during anaphase. Our detection of anaphase bridges at both 0.5% and 1% colchicine treatments aligns with the findings of Saeed et al. (2017), who observed that colchicine induces anaphase bridges in *Allium* species by causing unresolved chromosomal entanglements (Saeed et al., 2017). This phenomenon is indicative of severe chromosomal damage and mitotic disruption. The formation of C-metaphase in *Allium sativum* roots at all colchicine concentrations is in agreement with the results of Zhang et al. (2018), who reported that colchicine leads to C-metaphase by affecting chromosome alignment and spindle apparatus (Zhang et al., 2018). This is a common outcome of colchicine treatment, reflecting its impact on mitosis. The presence of fragmented chromosomes in our study at both 0.5% and 1% colchicine treatments is consistent with the findings of Fathy et al. (2018), who noted similar chromosomal fragmentation in *Allium sativum* under colchicine stress (Fathy et al., 2018). Fragmented chromosomes indicate severe genetic damage induced by colchicine.

Our observation of sticky chromosomes at all colchicine concentrations supports the findings of Lee et al. (2020), who documented that colchicine causes chromosome stickiness due to its effects on spindle apparatus and chromosome alignment in *Allium sativum* (Lee et al., 2020). Sticky chromosomes are a hallmark of colchicine-induced chromosomal abnormalities. The formation of micronuclei at 1.5% colchicine treatment in our study correlates with the observations of Morsy et al. (2015), who found micronuclei as a consequence of colchicine treatment in *Allium* species, indicating chromosomal fragmentation and mis-segregation (Morsy et al., 2015). This confirms colchicine's role in inducing genetic instability. The occurrence of multinucleate cells at 1.5% colchicine treatment in our study is consistent with the findings of Mavi et al. (2020), who reported that colchicine-induced mitotic disruptions lead to the formation of multinucleate cells in *Allium sativum* (Mavi et al., 2020). This reflects the severe impact of colchicine on normal cell division. The observation of laggard chromosomes at 1% colchicine treatment in our study supports the results of Fathy et al. (2018), who noted that colchicine caused lagging chromosomes due to impaired spindle apparatus function in *Allium* species (Fathy et al., 2018). Laggard chromosomes are indicative of disrupted mitotic processes. The extreme chromosomal abnormalities, including dumbled-shaped telophase, observed at 1.5% colchicine treatment in our study, align with the findings of Saeed et al. (2017), who documented similar abnormalities at high colchicine concentrations in *Allium sativum*, indicating severe mitotic disturbances (Saeed et al., 2017). Similar abnormalities were seen in the previous work done on *Allium cepa.*[42]

Conclusion

The present study investigated the effects of colchicine on mitotic abnormality induction in *Allium sativum* root apical meristems. The results confirmed that colchicine causes cell cycle delay, pro-metaphase arrest, and mitotic abnormality induction in both species. 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. Similar abnormalities were seen in the previous work done on *Allium cepa.*[42]

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