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Comparative Study On The Effects Of Colchicine On Mitosis In *Allium Cepa* Var Aggregatum And *Allium Sativum* Var Sativum Root Meristem Cells

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Abstract:
Colchicine acts as a microtubule-destabilizing agent and is a plant metabolite. *Allium cepa* (onion) and *Allium sativum* (garlic) are members of the *Allium* genus and belong to the Liliaceae family. Both *Allium cepa* var aggregatum and *Allium sativum* var sativum are excellent model organisms for studying the cytogenetic effects of colchicine treatment due to their large, easily observable chromosomes. This study investigates the effects of colchicine on mitosis in *Allium cepa* var aggregatum and *Allium sativum* var sativum root meristem cells, with a focus on colchicine-induced chromosomal abnormalities and metaphase arrest. Roots of both plants were treated with different concentrations of colchicine (0.5%, 1%, and 1.5%) for varying durations. Mitotic abnormalities such as c-metaphase, chromosomal stickiness, laggard chromosomes, anaphase bridges, and polyploidy were observed in both species. The results showed a higher incidence of c-metaphase in *Allium cepa* var aggregatum, whereas *Allium sativum* var sativum exhibited more severe chromosomal fragmentation and dumbbell shaped nucleus was observed for the first time. The frequency and severity of abnormalities increased with colchicine concentration and exposure duration, highlighting differential sensitivities of *Allium cepa* var aggregatum and *Allium sativum* var sativum to colchicine-induced mitotic arrest.

Keywords: Colchicine, Mitosis, Chromosomal Abnormalities, *Allium cepa* var aggregatum, *Allium sativum* var sativum, Metaphase Arrest, Cytogenetics

Introduction:
Allium cepa is characterized by its underground bulb, which serves as a storage organ. It produces flower stalks that can reach a height of 75–180 cm and is widely cultivated for its culinary and medicinal properties. Onions contain about 90% water and are rich in dietary fiber, vitamins (B1, B2, C), selenium, and potassium. They have been linked to various health benefits, including cancer prevention and antidiabetic properties. Onion peels, for instance, have therapeutic uses, such as preventing hypertrophic scars.
Allium sativum, on the other hand, features a tall flowering stem that can grow up to 1 meter in height. Its bulb typically consists of 10 to 20 cloves wrapped in sheathing leaves, which release sulfur compounds when damaged, giving garlic its characteristic pungent odor. Garlic's health benefits, much like onion's, are extensive, including its role in combating cardiovascular diseases, immune disorders, and even exhibiting hepatoprotective and anti-inflammatory properties. Garlic is also known for its potent sulfur-containing compounds, such as allicin, which have antibacterial and antifungal effects. Both plants' medicinal benefits make them vital in folk and modern medicine.
Both *Allium cepa* (onion) and *Allium sativum* (garlic) are members of the *Allium* genus and belong to the Liliaceae family, though some sources suggest Alliaceae. They are perennial plants that grow from bulbs and serve as essential food crops worldwide. Despite these similarities, they display unique biological and cytogenetic characteristics, making them valuable model organisms for studies like colchicine-induced chromosomal abnormalities.
Cytogenetically, both *Allium cepa* var aggregatum and *Allium sativum* var sativum have 16 chromosomes (2n = 16) in a normal diploid cell. However, the organization and morphology of these chromosomes slightly differ between the two species. *Allium cepa* exhibits a karyotype formula of 12m + 2sm + 2st, consisting of six pairs of metacentric chromosomes, one pair of submetacentric chromosomes, and one pair of subtelocentric chromosomes. These chromosomes are relatively large, making onion an ideal organism for chromosomal studies, as its cells allow for clear observation of mitosis and meiosis stages under a microscope.
Similarly, *Allium sativum* var sativum has 16 chromosomes, but the plant primarily reproduces vegetatively as it does not produce seeds. This asexual reproduction leads to little genetic diversity, and each strain of garlic can be considered a clone. Cytogenetic studies have identified asymmetries in garlic chromosomes, particularly in chromosome 7, which has

been observed to be more asymmetric compared to the longer chromosomes. The satellited pairs in garlic share similar gross organization but display variations in chromosomal structure.

Both *Allium cepa* var aggregatum and *Allium sativum* var sativum are excellent model organisms for studying the cytogenetic effects of colchicine treatment due to their large, easily observable chromosomes. Their rapid growth rates and ability to produce multiple generations in a short period make them highly suitable for research on the effects of colchicine on cell division. Moreover, these species' tolerance for genetic manipulation allows for detailed investigation into polyploidy and mutagenesis induced by colchicine. [1-10]

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. [11-15]

Materials and Methods:

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

➢ **Sample Collection and Preparation for treatment**

Allium cepa var aggregatum and *Allium sativum* var sativum were 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. *Allium sativum* var sativum cloves took a longer time to form roots and were also 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 cepa* and *Allium sativum* var 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:

The meristematic regions of *Allium cepa* and *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 both species of *Allium cepa* [plate 1, figs. a,b,c,d,e,f] and *Allium sativam* [plate6,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 cepa and *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 cepa* 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* 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* roots treated 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.

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.

➢ ***Comparative analysis of different concentrations of colchicine treatment on Allium cepa var aggregatum and Allium sativum var sativum***

- **0.5% Colchicine Concentration**

Allium cepa var aggregatum:
Chromosomal abnormalities included micronuclei, elongated cells with abnormal nuclei, polyploidy in prophase, sticky chromosomes, depolarized anaphase vagrants, and C-metaphase conversion.
Allium sativum var sativum:
Similar abnormalities were observed, including polyploidy in anaphase and metaphase, unequal anaphase, anaphase bridges, C-metaphase, fragmented chromosomes, distorted metaphase, and sticky chromosomes. Anaphasic bridges and vagrant chromosomes were also noted.
At 0.5%, both species exhibited similar chromosomal abnormalities, but *Allium cepa* var aggregatum showed a higher frequency of depolarized anaphase vagrants, while *Allium sativum* var sativum had more fragmented chromosomes.

- **1% Colchicine Concentration**

Allium cepa var aggregatum:
Abnormalities included multinucleate cells, polyploidy in metaphase, sticky chromosomes, unequal anaphase, C-metaphase, and the conversion of cells to C-metaphase.
Allium sativum var sativum:
Severe chromosomal damage occurred, including fragmented chromosomes, depolarized anaphase with vagrants, sticky chromosomes, deformed cells with tapering nuclei, laggard and vagrant chromosomes, and anaphase bridges.
At 1%, *Allium sativum* var sativum showed a higher level of disruption, with more frequent fragmented chromosomes and deformed cells, while *Allium cepa* var aggregatum had a greater prevalence of C-metaphase.

- **1.5% Colchicine Concentration**

Allium cepa var aggregatum:
Abnormalities included anaphase bridges, sticky chromosomes, C-metaphase, polyploidy in prophase, and laggard and vagrant chromosomes. The results indicated significant disruption to the normal cell division process.
Allium sativum var sativum:
Extreme chromosomal abnormalities were observed, such as dumbled-shaped telophase, micronuclei, multinucleate cells, irregular cells, C-metaphase, vagrants, and severely disrupted cell division.
At 1.5%, both species exhibited extreme abnormalities, but *Allium sativum* var sativum showed more pronounced disruptions, such as dumbled-shaped telophase and irregular cells, while *Allium cepa* var aggregatum demonstrated more consistent presence of laggard and vagrant chromosomes.

Table 1: Comparison of results between *Allium cepa* var aggregatum and *Allium sativum* var sativum

Concentration	*Allium cepa* var aggregatum	*Allium sativum* var sativum
0.5% Colchicine	Micronuclei, elongated cells, polyploidy in prophase, sticky chromosomes, depolarized anaphase vagrants, C-metaphase	Polyploidy in anaphase and metaphase, unequal anaphase, anaphase bridges, fragmented chromosomes, distorted metaphase, sticky chromosomes, vagrant chromosomes
1% Colchicine	Multinucleate cells, polyploidy in metaphase, sticky chromosomes, unequal anaphase, C-metaphase, C-metaphase conversion	Fragmented chromosomes, depolarized anaphase with vagrants, sticky chromosomes, tapering nuclei, laggard chromosomes, anaphase bridges
1.5% Colchicine	Anaphase bridges, sticky chromosomes, C-metaphase, polyploidy in prophase, laggard and vagrant chromosomes	Dumbled-shaped telophase, micronuclei, multinucleate cells, irregular cells, C-metaphase, vagrant chromosomes

Both *Allium cepa* var aggregatum and *Allium sativum* var sativum exhibited similar patterns of mitotic abnormalities upon colchicine treatment, with the severity and type of chromosomal disruptions increasing with higher concentrations.

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However, *Allium sativum* var sativum appeared to exhibit more severe damage at higher colchicine concentrations, particularly at 1% and 1.5%, compared to *Allium cepa* var aggregatum, which showed more frequent occurrences of specific abnormalities like C-metaphase and laggard chromosomes.

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

Table 2: 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

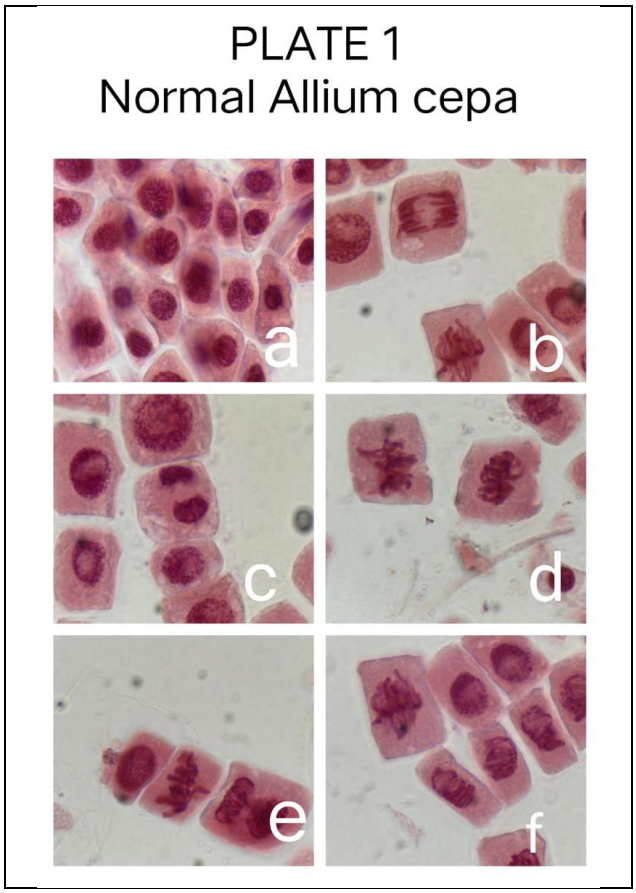

Fig 1*: Allium cepa* plate 1 [a-f] Normal mitosis: a. Normal prophase, b,c,d. Normal metaphase, e. Normal anaphase, f. Normal telophase

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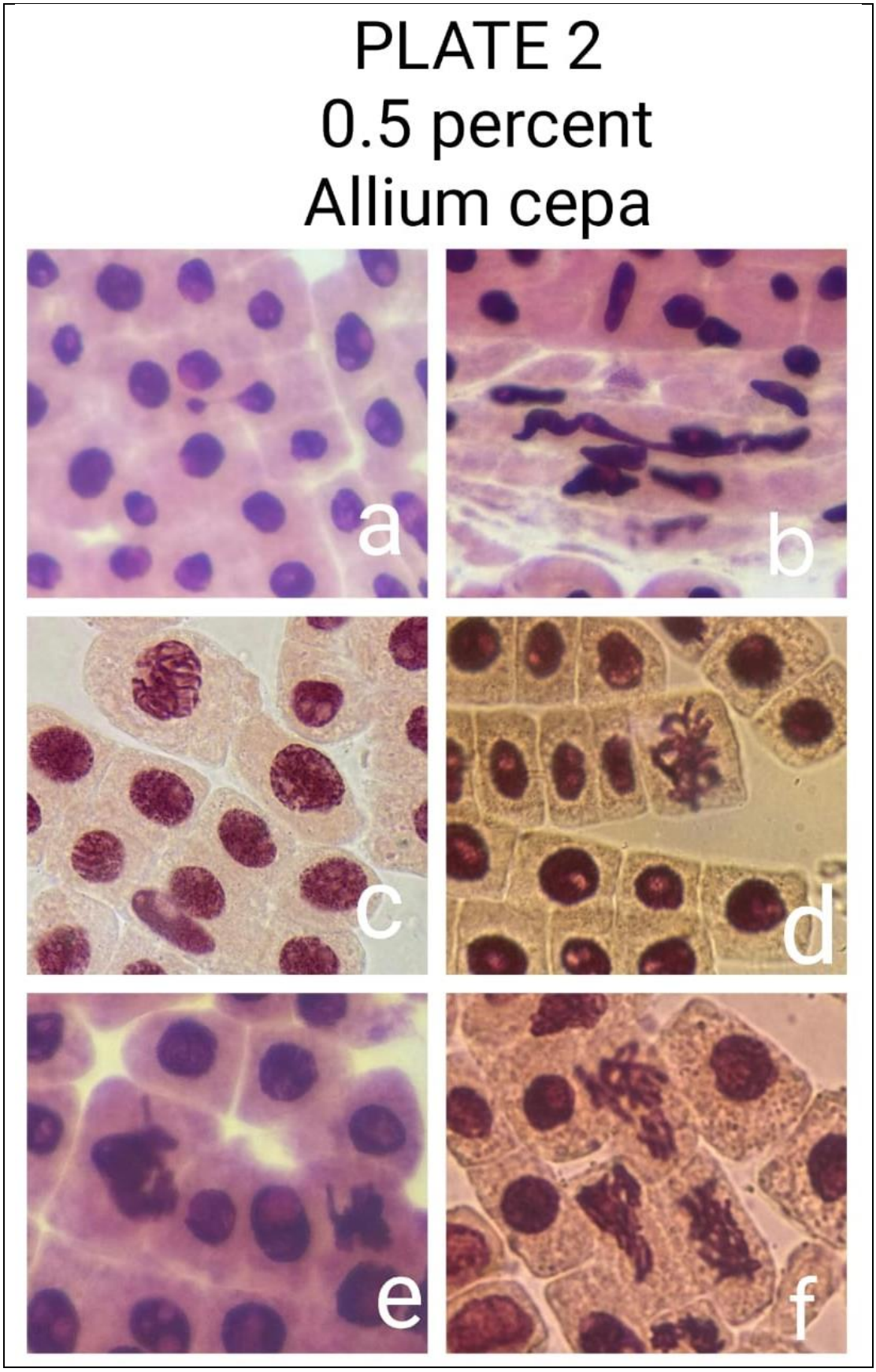

Fig 2: *Allium cepa* plate 2 [a-f] 0.5 percent colchicine treated roots: a. Micronucleii, b. Elongated cells with abnormal nucleus, c. polyploid prophase, d. Sticky chromosome, e. Depolarized anaphase showing vagrant, f. C-metaphase and depolarized anaphase

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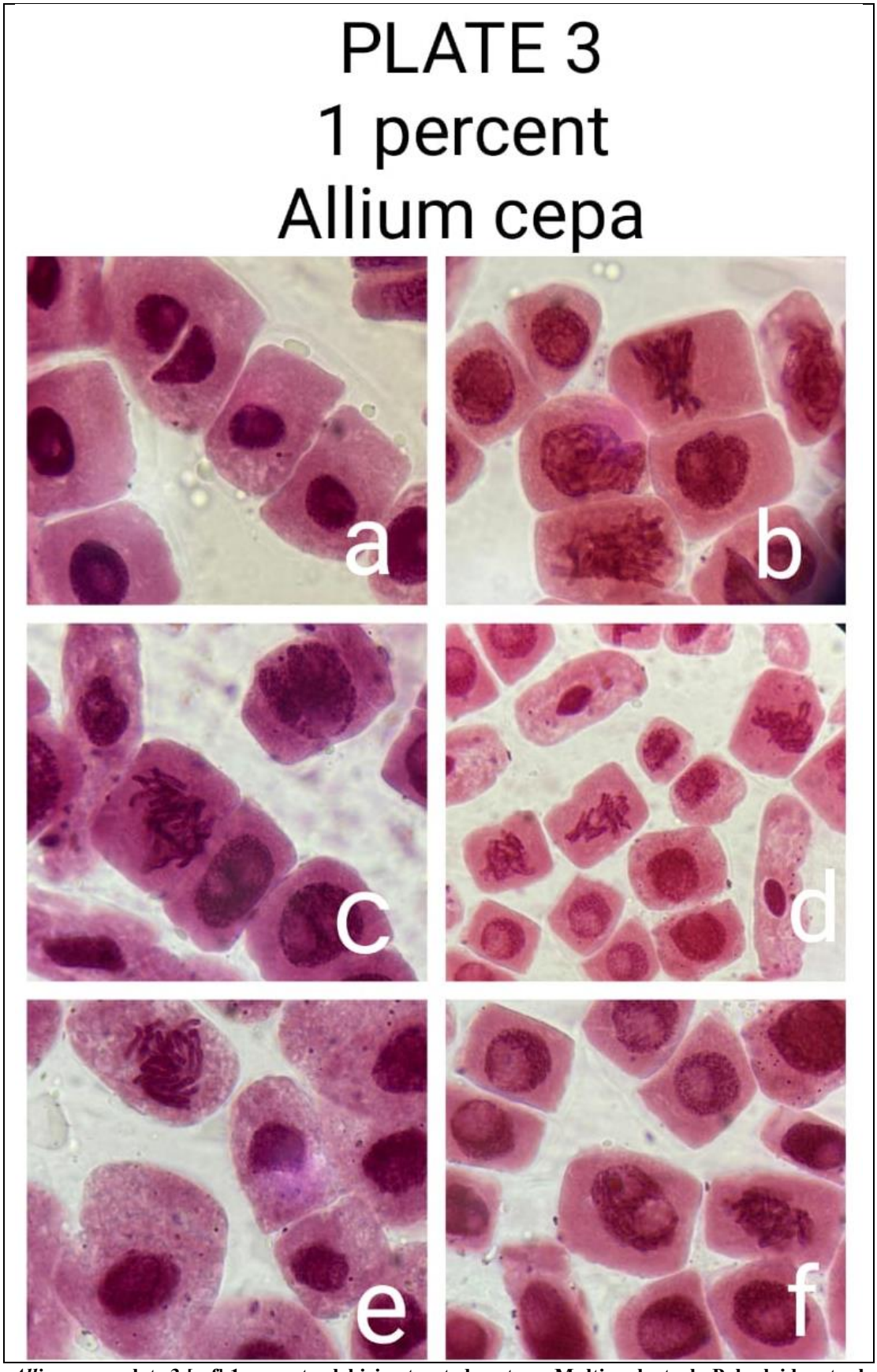

Fig 3: *Allium cepa* plate 3 [a-f] 1percent colchicine treated roots: a. Multinucleate, b. Polyploid metaphase, c. Sticky chromosome, d.Unequal anaphase, e. C-metaphase, f. Nuclear lesions

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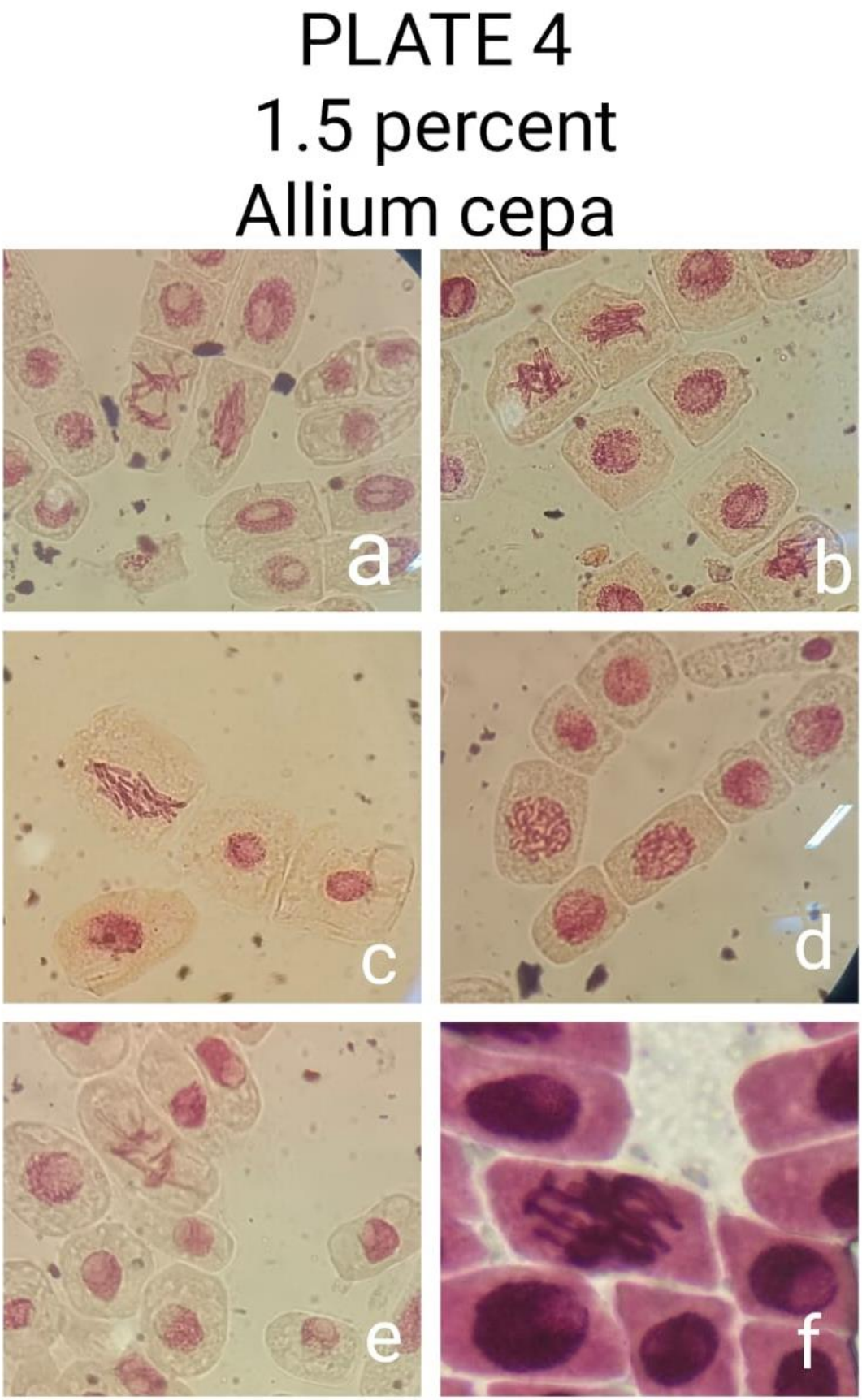

Fig 4: ***Allium cepa*** **plate 4 [a-f] 1.5 percent colchicine treated roots: a. Anaphase bridge and vagrant, b. Sticky chromosome, c. C-metaphase, d. Polyploid prophase, e. Laggard and vagrant f. Sticky chromosome**

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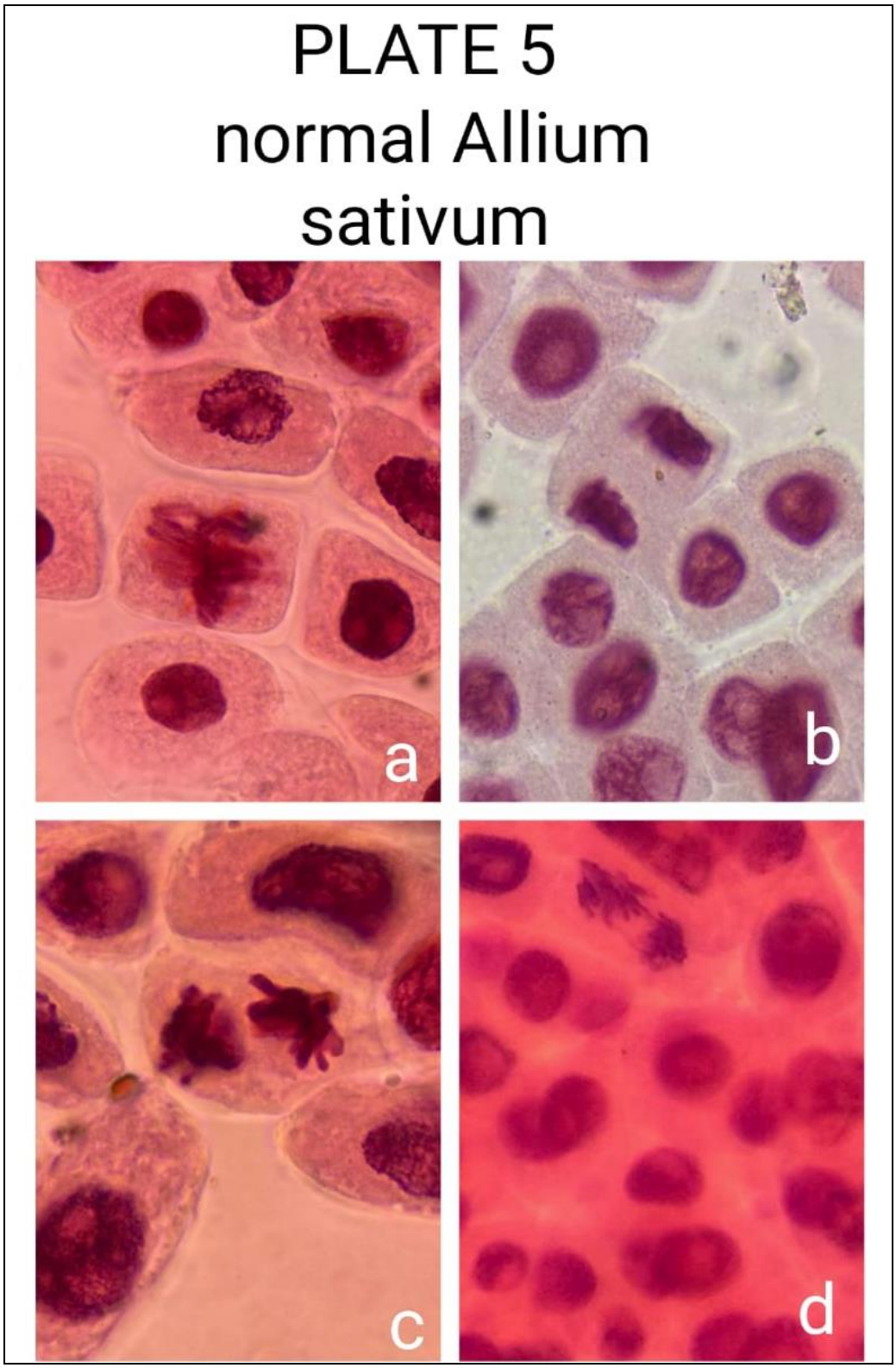

Fig 5: *Allium cepa* plate 5 [a-b] 0.5percent, [c-d] 1percent, [e-f] 1.5percent: a. sticky chromosome, b. Irregular cells and C-metaphase, c. polyploidy metaphase, d. Nuclear lesions, e. C-metaphase, f. Nuclear lesions

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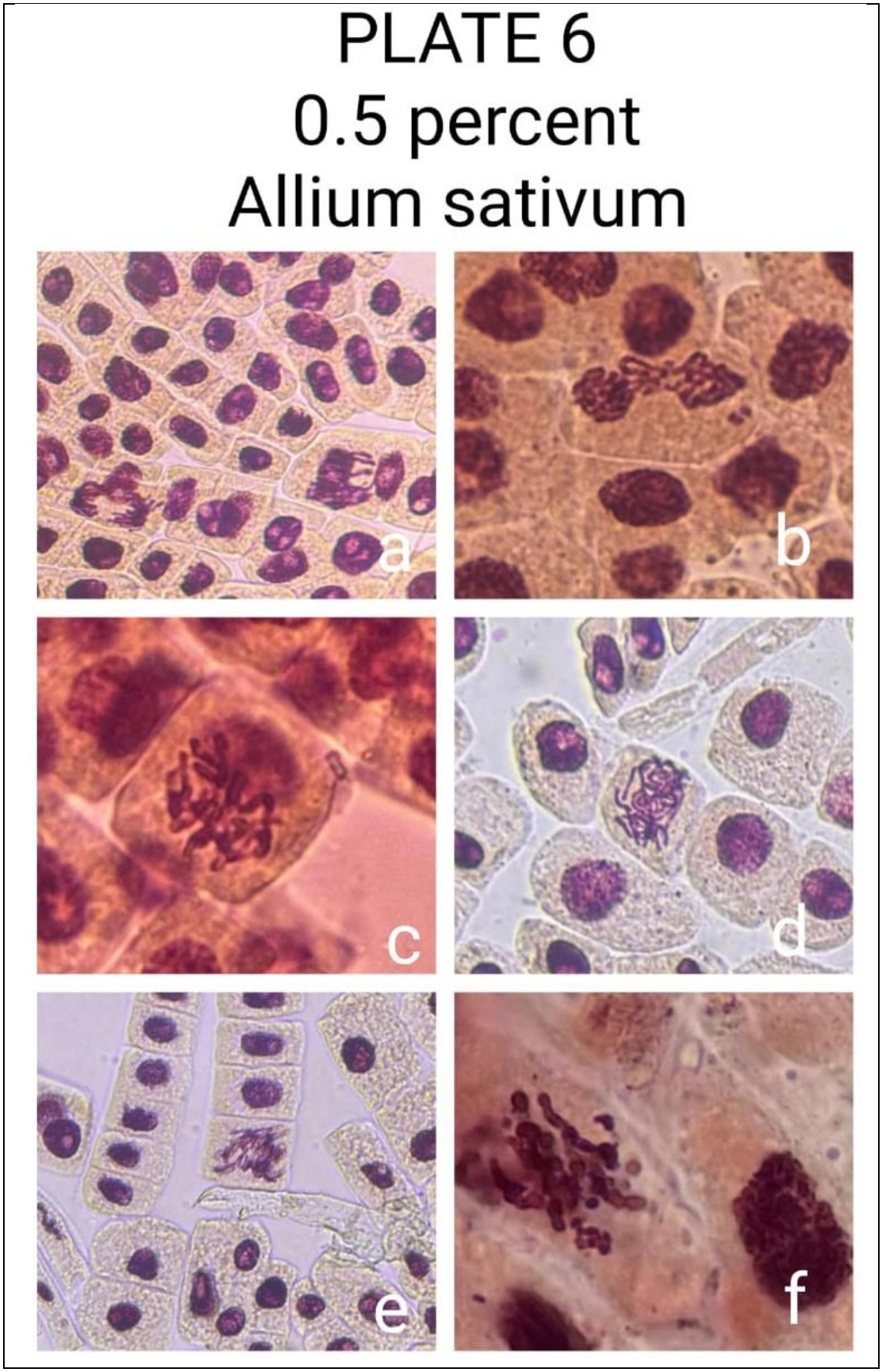

Fig 6: *Allium sativum* Plate 6 [a-d] Normal mitosis: a. Normal metaphase, b. Normal prophase, c. Normal anaphase, d. Early telophase

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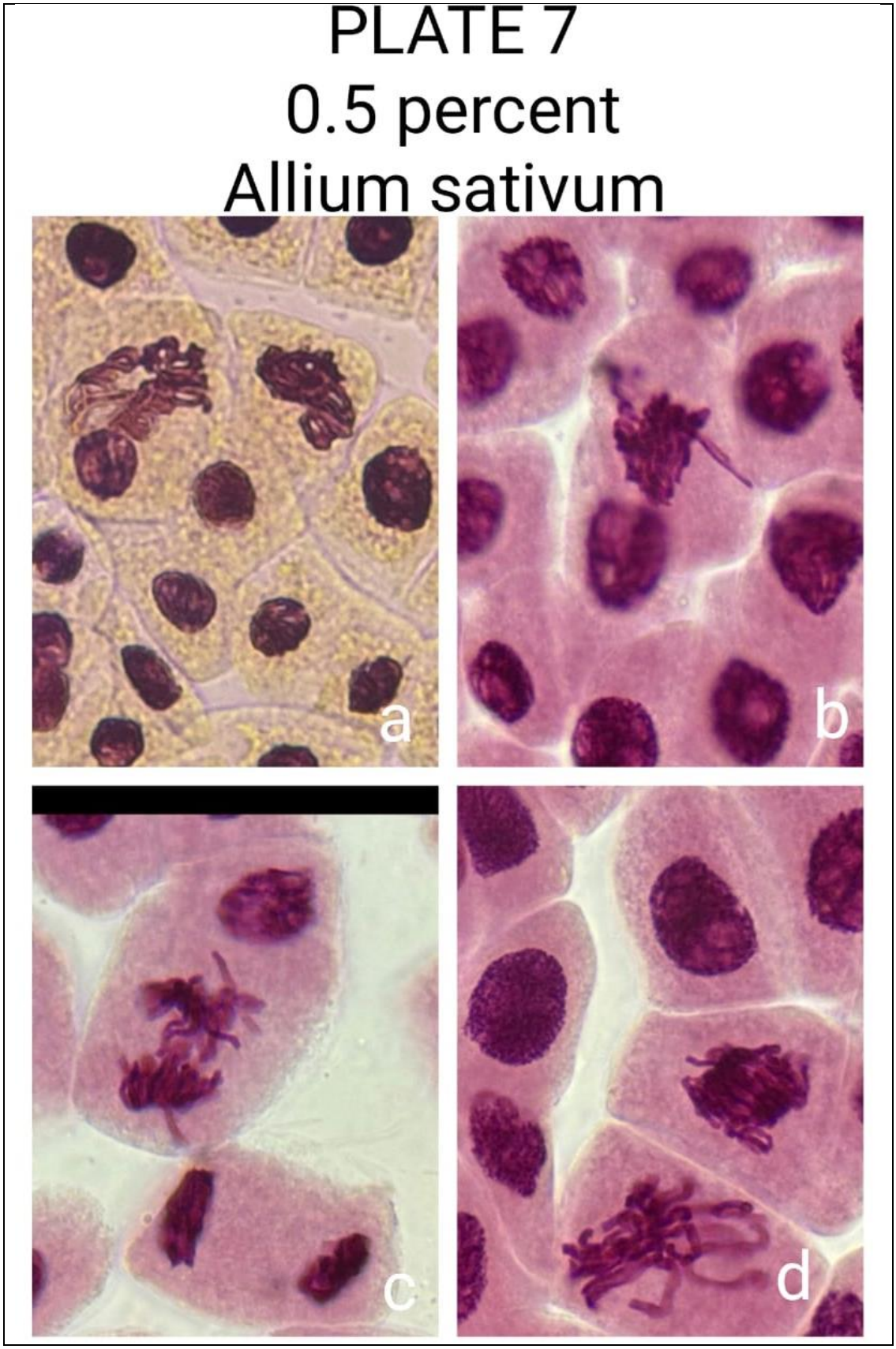

Fig 7: *Allium sativum* plate 7 [a-f] 0.5 percent colchicine treated roots: a. Polyploid anaphase/unequal anaphase, b. Anaphase bridge, c. C-metaphase, d. Polyploid metaphase, e. C-metaphase, f. Fragmented chromosome

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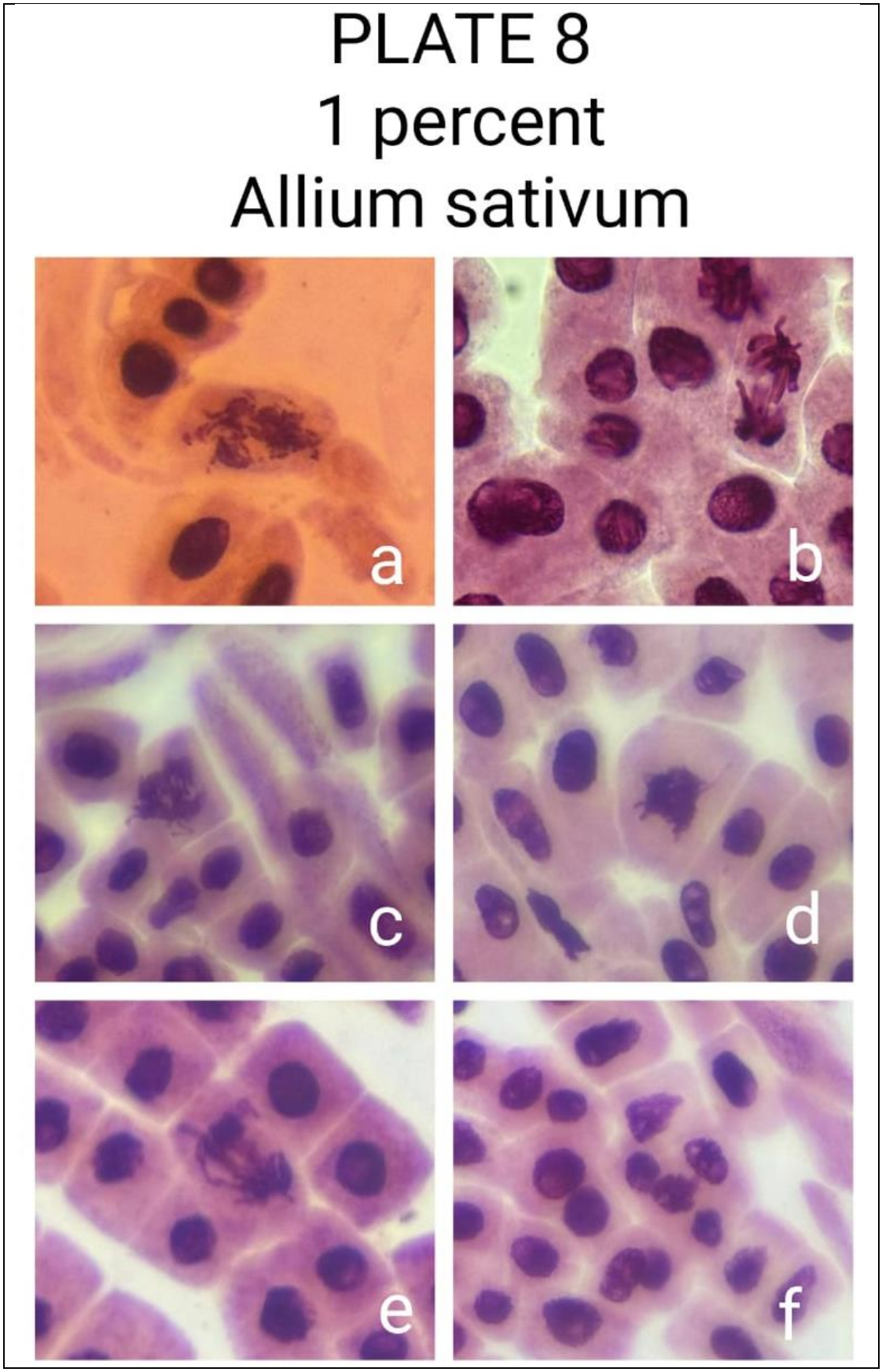

Fig 8: ***Allium sativum*** **plate 8 [a-d] 0.5 percent colchicine treated roots: a. Distorted metaphase, b. Distorted metaphase, c. Anaphasic bridge and vagrant, d. Sticky chromosome**

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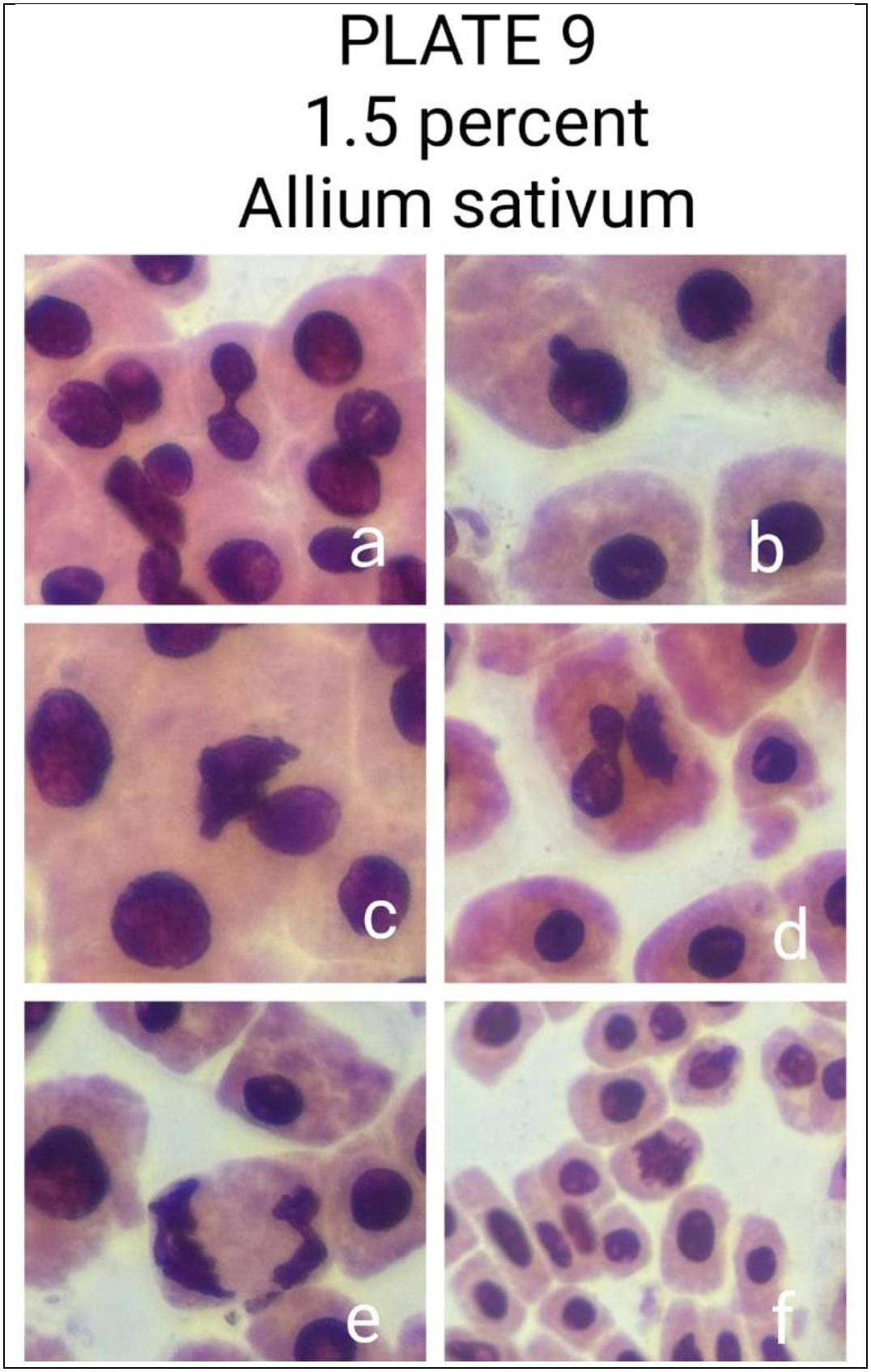

Fig 9: *Allium sativum* plate 9 [a-f] 1 percent colchicine treated roots: a. Fragmented chromosome, b. Depolarized anaphase with vagrant, c. Sticky chromosome, d. Deformed cells with tapering nucleus, e. Laggard and vagrant, f. Anaphase bridge

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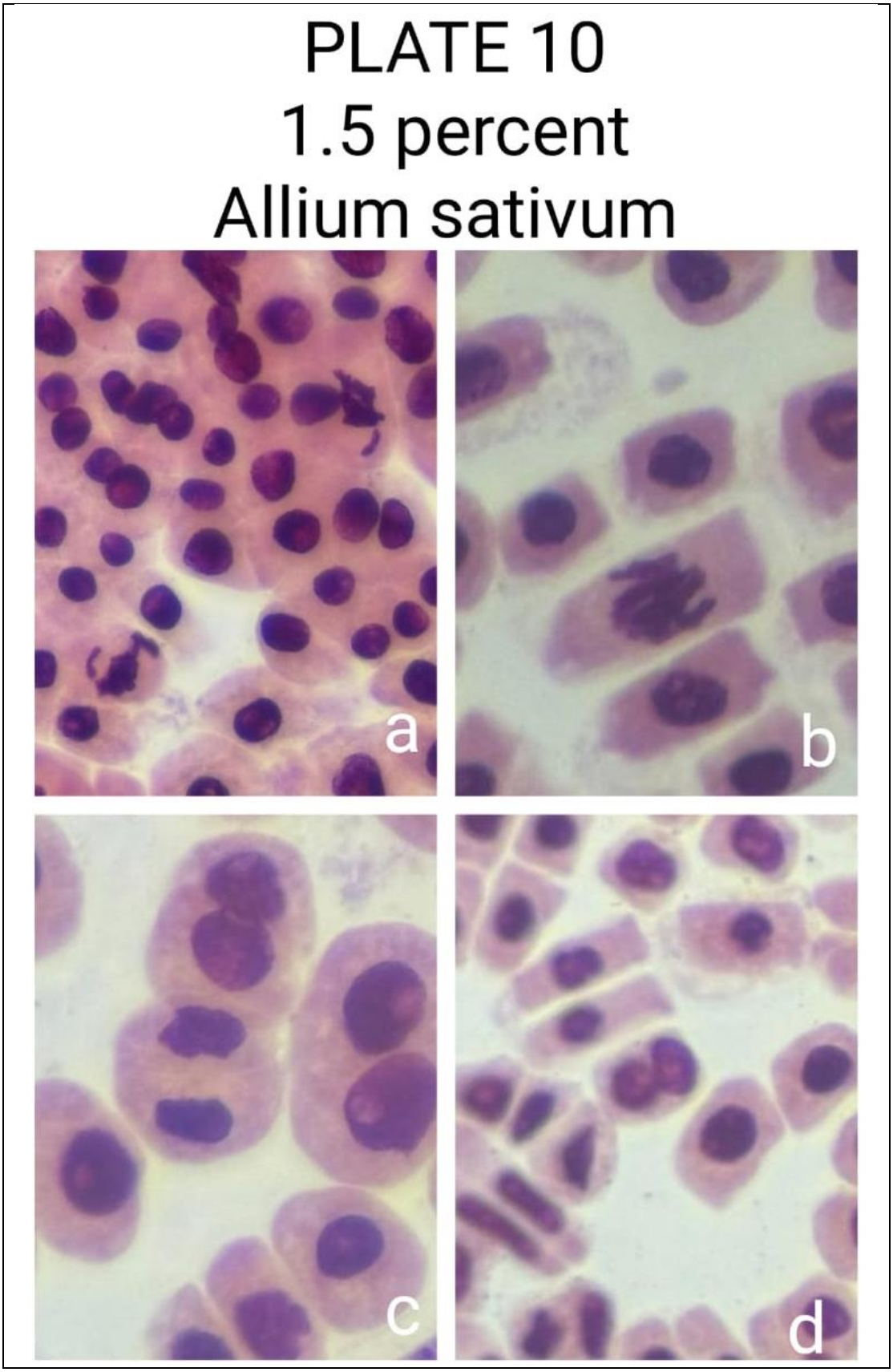

Fig 10: ***Allium sativum*** **plate 10 [a-f] 1.5 percent colchicine treated roots: a. Dumbled shaped telophase, b. Micronucleii, c. Multinucleate, d. Irregular cells, e. Irregular anaphase, f. Irregular cells**

REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
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http://www.veterinaria.org
Article Received: Revised: Accepted:

Plate 11

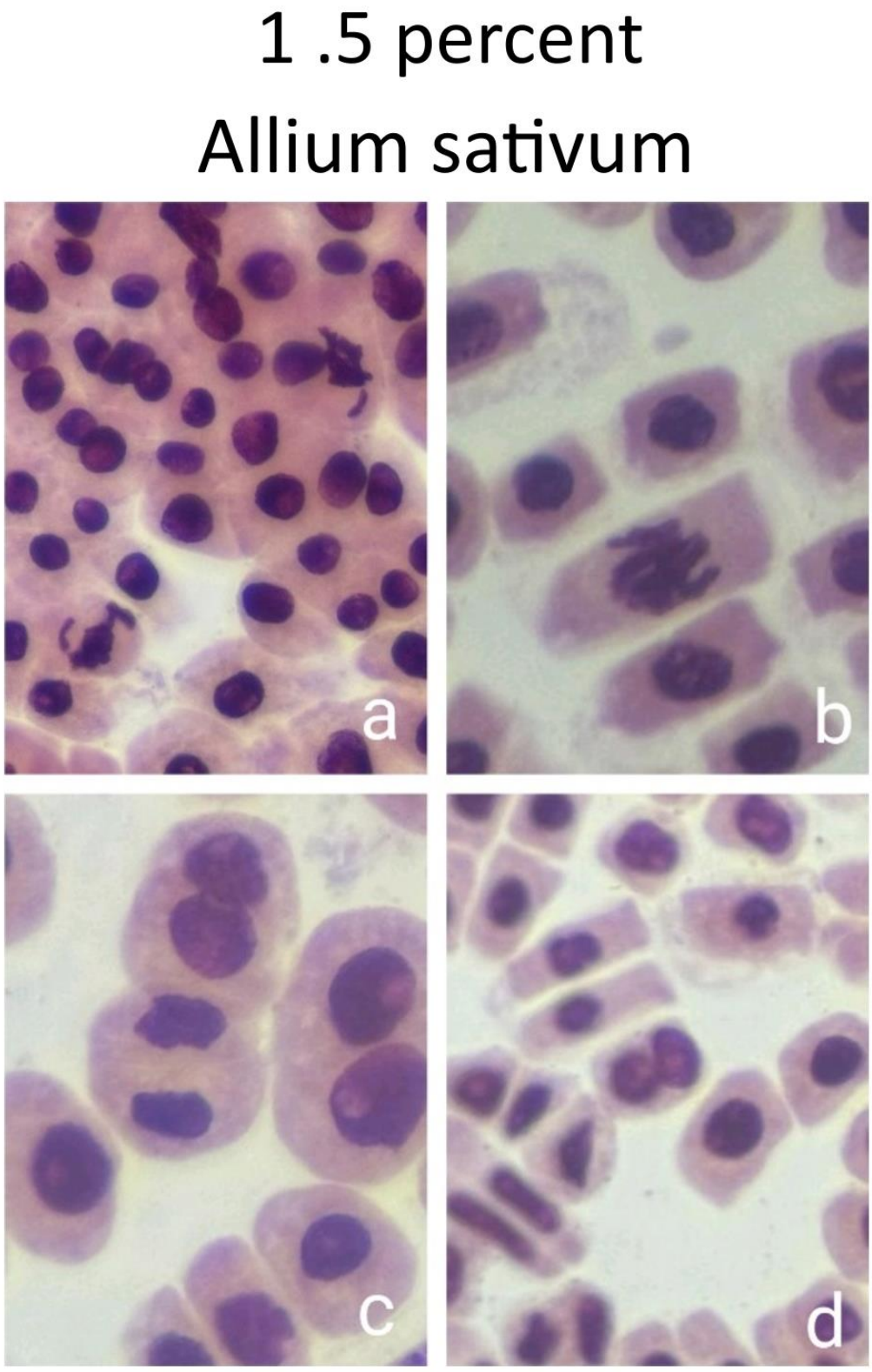

Fig 11: *Allium sativum* plate 11 [a-d] 1.5 percent colchicine treated roots: a. Vagrant, b. Sticky chromosome, c. Multinucleate, d. Irregular cells

Discussion:
The results from this study indicate a dose-dependent increase in chromosomal aberrations, consistent with previous studies that suggest colchicine induces a wide range of mitotic anomalies in plant cells (Singh & Roy, 2020; Roy & Banerjee, 2018).

The abnormalities observed in the present work:
I Aberrant Cells
An increase in colchicine concentration led to the observation of irregularly shaped elongated cells with large nuclei [plate2, fig. b], [plate11, and fig. d]. Giant cells, arise due to endomitosis or endoreplication [plate2, fig. f], [plate3, fig. b]. Damaged cells [plate2, fig. b], [plate5, fig. b] and cells with tapering nuclei were also recorded [plate7, fig. c], [plate8, fig. d], [plate9, fig. d].

II Mitotic Abnormalities
The mitotic abnormalities induced by colchicine treatment in Allium cepa and 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 cepa and 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, fig. f], [plate3, fig. e], [plate4, figs. A,b,c], [plate7, figs. C,e], [plate10, 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. Increase in concentration of colchicine results in a significant increase in the frequency of C-metaphase in Allium cepa compared to Allium sativum root apical meristem cells [plate5, figs. D, e, and f].

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], [plate6, figd], [plate7, figs.a, b]. The colchicine treatment induced an increase in the anaphase bridge in both the species of Allium cepa and Allium sativum root apical meristem cells. In the case of treated samples 12-15h showed more anaphase bridge in 1% colchicine treated root cells [plate8, fig. c], [plate9, 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. Such chromosomal sickness was observed in present study also [plate2, fig.d], [plate3, fig.c], [plate4, figs. B, f].
Similar to anaphase bridge frequency, an increased frequency of chromosomal stickiness was also observed in the colchicine treated onion root tip cells. The colchicine treatment to onion root tip cells induced the highest frequency of chromosomal stickiness and was maximum in 1% colchicine treated root cells[plate8,fig.d], [plate9,fig.c], [plate11,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], [plate7, fig. e], [plate8, fig. a].
Polar deviation increases in root apical meristem cells treated with colchicine (1%) and also in (1.5%) [plate9, fig. e], [plate10, fig. 6], [plate11, 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.e], [plate4, figs .a, e], [plate7, fig.a], [plate8, figs. B, c]. The increased frequencies of vagrant and laggard chromosomes were observed in the all the concentrations (0.5, 1, 1.5%) [plate9, 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 [plate4, figs. A,e], [plate5, figs. E,f], [plate7, fig. b], [plate8, fig. c], [plate9, 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. Based on the analysis carried out in the root tip cells from allium cepa, it was observed that colchicine treated several cells showed micronuclei. The colchicine treated root cells showed an increase in micronuclei frequency [plate2, fig. a], [plate10, 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.c], [plate3, fig.b], [plate4, fig. d], [plate5, figs. C, e, f], [plate7, fig. d], [plate8, 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, figs. B,c,e], [plate4, fig. f], [plate5, fig. c], [plate7,figs. D,f], [plate8, figs. A,d], [plate9, 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 [plate7, fig. f], [plate8, fig. b], [plate10, 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, fig. f], [plate7, figs. A,b], [plate8, fig. a], [plate10, 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[plate2,fig.a], [plate3,fig.a], [plate9, fig. f], [plate10, figs. A,c,d,f], [plate11, figs. A,d].

13. Distorted Anaphase:
Irregular anaphase was recorded in present work [plate10, fig. e].

14. Distorted Metaphase:
At metaphase chromosomes showed different orientation and condensation [plate5, figs. D,e, and f].

15. Nuclear Lesions:
Nuclear lesions are abnormalities that can be observed in the interphase cells of onions (Allium cepa) 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], [plate5, fig. d], [plate7, fig. a].
The comparative analysis of the effects of different colchicine concentrations on *Allium cepa* var. aggregatum and *Allium sativum* var. sativum revealed significant chromosomal abnormalities, with variations in the type and severity of disruptions between the two species. While both displayed similar mitotic irregularities at all concentrations, the severity of these abnormalities increased with higher colchicine doses. However, the frequency and nature of these aberrations varied between *Allium cepa* and *Allium sativum*, indicating species-specific responses to colchicine-induced mitotic inhibition.

Comparative Analysis of Chromosomal Abnormalities
At 0.5% colchicine, both species exhibited polyploidy, sticky chromosomes, anaphase bridges, and fragmented chromosomes. However, *Allium cepa* var. aggregatum showed a higher frequency of depolarized anaphase vagrants, whereas *Allium sativum* var. sativum had more fragmented chromosomes and distorted metaphases. These differences may arise from variations in chromosome structure or spindle dynamics in these species, which influence their responses to mitotic spindle inhibition.
At 1% colchicine, *Allium sativum* var. sativum exhibited more severe chromosomal damage, including deformed cells with tapering nuclei and anaphase bridges. The greater frequency of fragmented chromosomes in *Allium sativum* suggests a heightened sensitivity to colchicine-induced mitotic arrest compared to *Allium cepa* var. aggregatum. This could be due to structural differences in chromatin condensation or differences in microtubule organization, leading to increased susceptibility to spindle disruption in *Allium sativum*.
At 1.5% colchicine, both species displayed extreme chromosomal abnormalities, but *Allium sativum* var. sativum demonstrated a higher degree of mitotic disruption, including the presence of dumbled-shaped telophase and multinucleate cells. In contrast, *Allium cepa* var. aggregatum showed a more consistent presence of laggard and vagrant chromosomes, which suggests that while both species experience severe mitotic disturbances, *Allium cepa* may exhibit slightly more controlled chromosomal missegregation, while *Allium sativum* is prone to more drastic mitotic failures.
Mechanisms Underlying Chromosomal Abnormalities
The observed abnormalities can be attributed to the mechanism of colchicine, which disrupts microtubule polymerization, thereby inhibiting spindle fiber formation and leading to mitotic arrest at metaphase (Eren & Koca, 2014). The induction of polyploidy in both species results from the inability of cells to complete anaphase due to the lack of spindle function, leading to the retention of chromosomes within the same nuclear envelope (Sikora et al., 2017). Sticky chromosomes arise from improper chromatin condensation, possibly due to altered histone modifications or defects in cohesin proteins, which regulate chromatid separation (Badr et al., 2012).
The presence of laggard and vagrant chromosomes indicates partial spindle disruptions, leading to improper chromosomal alignment and segregation errors (Liu et al., 2020). The higher frequency of fragmented chromosomes in *Allium sativum* var. sativum suggests greater chromosomal fragility, which could result from DNA damage induced by prolonged mitotic arrest (Hodžic et al., 2018). The formation of multinucleate cells and dumbled-shaped telophase in *Allium sativum* further implies an extreme response to colchicine, possibly due to differences in nuclear envelope stability or mitotic checkpoint regulation compared to *Allium cepa* (Guerra, 2012).

Conclusion:
Colchicine treatment results in a broad spectrum of mitotic abnormalities in both *Allium cepa* var. aggregatum and *Allium sativum* var. sativum, with the severity of these abnormalities increasing with colchicine concentration. Dumbbell shaped nucleus was observed for the first time. Although both species are affected by colchicine in similar ways, *Allium cepa* appears to be more sensitive to its effects, exhibiting higher frequencies of chromosomal stickiness, anaphase bridges, and laggard chromosomes. These results are consistent with previous studies that have documented colchicine's effects on plant mitosis and highlight the utility of both *Allium cepa* and *Allium sativum* as models for studying chromosomal aberrations and polyploidy induction (Fiskesjö, 1985; Souza et al., 2016).

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