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A review on mitotic cell cycle phase and chromosemes in onion root tips using acetocarmine stain

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Abstract
The present work is designed so as to evaluate its cyto-toxic effect on onion root tip cells. Onion root tips were placed in container containing cypermethrin in a solution for 24 hrs root tips absorbed along with the distilled water which has residual pesticide. These tips were cultured with varied concentrations of streptomycin for three to five days in root tip cells like c- metaphase. In instances of growth in onion root tip cell mitotic inhibition leaf extract of marigold act as g0 biological activity at higher treatment duration. *Allium cepa* is used as an *in vitro* plant for evaluating the effects of chemical agents. Onion chromosomes have also been prepared for culturing purposes by employing the centred stain technique using the aceto -arsenic squashing procedures root tips are cut from each bulb and soaking is done for 1,3days in Aceto arsenic stain and then cytologically examined. The study aimed to unveil if there is any correlation between the division rate of onion root tip cells during the treatment and the apportionment of aceto orcin over the relationship amongst the cells of the roots. The study aimed to find out chromosomal aberration & its impact on the mitotic index of onion root. The tip of onion root cells was observed going through mitosis while actively undergoing mitotic division using the usual methods.

Keywords: Cell biology, chromosomes, cell cycle, Allium onion root, root tips, structural chromosomal abnormalities, mitotic index, piper nigrum, *Allium cepa*

Introduction
In this part of the project, we will discuss the application of onion root tip in biological research. Onion root tip is a plant system in biology that is popular for studies on growth and development. Onion root tips are derived from the roots of *Allium cepa* which are dense cells. Onion root tips are now commonplace in scientific research due to their, Rapid cell division, Easy to manipulate, Genetic stability, Low cost. Onion root tips have been used in various scientific applications like, cell biology, genetics, plant physiology, cytology. Examples include gardening techniques and insight on genetic processes or activities such as cell division and differentiation. Their enhanced cell division, easy of manipulation, genetic stability, and low of cost makes them an attractive choice for the scientist across various disciplines. Apart from this, in recent years, a number of studies have focused on medicinal herbs and their active components because they are effective for controlling and preventing life-threatening and chronic ailments such as malignancy and diabetes, stock and arthritis, as well as the management of psychiatric disorders. This plant process numerous pharmacological properties such as aet anti-hyperglycaemic, ant-inflammatory, Anti-diarrhoeal, anti-anaemic, hepatocyte, anti- hypertensive, Antibacterial, anti-oxidative properties, Hypoglycaemic, Anti-fungal. As well as exhibits many other pharmacological activities [1].

Morphology
The morphology, for the most part, includes:
1. External morphology
2. Internal morphology
3. Cellular morphology
4. Specialized structures, so let us delve deeper into them [2].

External morphology

1. **Form:** The onion root tip is elongated with an ovoid tip.
2. **Proportion:** Its length varies from one to five centimetres.
3. **Shade:** A whitish or light yellowish tint characterizes the root tip [3].

Internal Morphology

1. **Epidermis:** the outer most layer of the root tip consists of single later of cells.
2. **Cortex:** A layer of tissue beneath the epidermis composed of numerous cell layers.
3. **Endodermis:** This is a single layer of cells forming a sheath around the vascular bundles.
4. **Stele:** This is defined as the central part of the vascular system, which comprises xylem and phloem.
5. **Root cap:** A cluster of cells that are protective in function at the apex of the root [4].

Cellular Morphology

1. **Meristematic cells:** Cells that are active in cell division.
2. **Cell wall:** These are minute and pliable.
3. **Cytoplasm:** This is a thick and densely packed layer containing several cell organelles.
4. **Nucleus:** This is big and conspicuous [5].

Specialized Structures

1. **Root hair:** These are cells that are said to be specialized for absorption of nutrients and water for the plant.
2. **Lateral roots:** These are diminutive secondary roots borne along the length of the primary root [6].

Taxonomy

- **Kingdom:** Plantae.
- **Class:** Angiosperms, Monocots.
- **Order:** Asparagales.
- **Family:** Amaryllidaceae.
- **Genus:** Allium.
- **Species:** *Allium cepa*.
- **Tissue:** Root tip.
- **Cell type:** Meristematic [7].

Chemical constituents

Table 1: Chemical constituents found in onion root tips

S. No	Chemical Constituent	Percentage (%)
1	Flavonoids	0.5 - 1.5
2	Amino Acids	1 – 2
3	Organic Acids	1 – 2
4	Carbohydrates	10 – 15
5	Pectin	0.5 - 1.5
6	Phenolic Acids	0.5 - 1.5
7	Sulphur Compounds	0.5 - 1.5
8	Terpenes	0.1 - 0.5
9	Vitamins C and K	0.01 - 0.15
10	Potassium	0.5 - 1.5
11	Manganese	0.01 - 0.1
12	Allicin	0.1 - 0.5

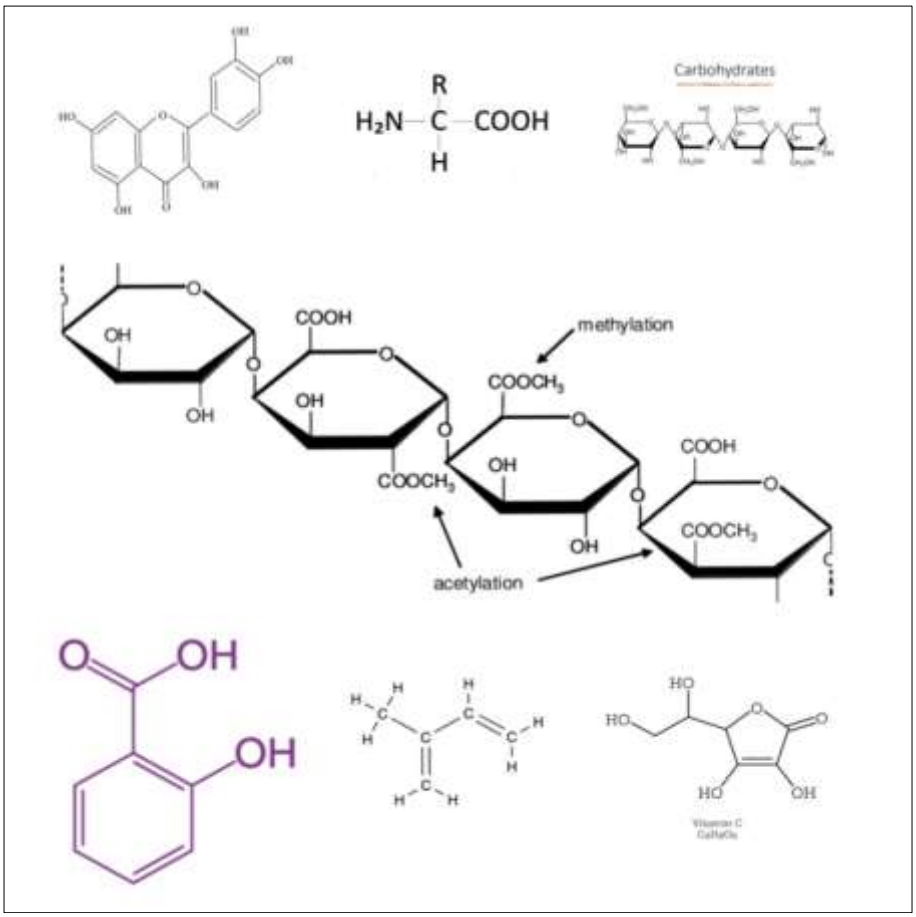

Fig 1: Microscopic view of mitotic stages in onion root tip cells stained with acetocarmine

Methodology

Equipment: Compound microscope, Water, acetocarmine dye, Brunner, HCL, Filter paper, cover slips, Aceto alcohol [glacial acetic acid & ethanol (1.3)], glass rod, peeled onion roots, blade, Petri dish, dropper, needle, and vial [8].

Procedures

1. Obtaining Onion Root Tips

The procedure itself is relatively simple, requiring only a healthy onion and some patience. Begin by taking an onion that has a firm structure and healthy outer layers. This guarantees the best possible root growth. You can use a cutting board and place the onion on one. The next step involves cutting off the dry and dead root with a sharp blade or scalpel in order to expose the fresh tissue underneath such growth can be submerged in clean water in a beaker with only the base of the onion being submerged in water. The cover does not need to be airtight; make sure the beaker is placed in a well-lit area, but not in direct sunshine. Wait four to five days allowing the onion to soak. Within that time, the base will begin to sprout roots, once the roots are around three cm long use a sharp blade to cut off a number of them and wash them before transferring them to a watch glass for further processing [9].

2. Fixation of Root Tips

Employ aceto-alcohol solution, a blend of glacial acetic acid and ethanol in a 1:3 ratio. This solution works to maintain cellular structures intact. Place the root tips into a vial with the fixating solution. Leave them for soaking for about 10 minutes. This step fixes the cells in what they are and prevents further division of the cells [10].

3. Staining of the Root Tips

Take a glass slide and add a drop or two of the N/10 HCl solution and then place the root tip into this glass slide. Using a dropper or forceps, gently remove one root tip and place it onto the clean glass slide Let's go back to Messy. Let's revert. In order to cut off the half of the rotated or split part of the root tip goes back. Add 1 drop of N/10 HCl solution to root. This allows the cells to be spread into a thin layer by softening the cell walls. Add 2-3 drops of aceto-carmine stain, which inhibit chromatin by staining, the chromosomes of the cells. It should be left for 2-3 minutes in order to enhance the effectiveness of the staining process. Use needle or tweezers to gently touch and remove dominantly less stained areas of the root tip. For clear examination, the more stained areas should be employed. In this solution two solutions are prepared; a solution of cypermethrin 2% (2 ml cypermethrin + 98 ml distilled water) and a solution of black pepper was prepared (4 g black pepper powder in 96 ml distilled water). The study employed a total of twelve onion bulbs where three were assigned to each solution with the remaining three utilized as a control. Once the roots had grown between one to two centimetres in length, the bulbs were submerged into each solution for a period of 72 hours while ensuring the control group remained untouched. Once the time elapsed, the roots were soaked in 80% alcohol, then stained using acetocarmine and treated for squash analysis in order to examine any abnormalities that existed in relation to the concentration of the solutions [11].

4. Preparing the Slide

Take a stained root and place a glass cover slip on it. With the help of a scalpel or a wider end of a tweezers, carefully push the cover slip over the tapered end of the root so that it forms a flat surface enabling the cells to be seen under a microscope. The raised edges of the cover slip can be lubricated with water to assist in visibility whilst also preventing it from drying out [12].

5. Observing Mitosis

Microscope Setup - Get the slide ready by putting it under a microscope. Begin the process by using the low-power objective lens(10x) to completely focus on cells located near the tip of the root and once identified switch to high-power lenses (40x or 100x) in order to closely observe cell division taking place.

You are required to identify stages of mitosis in onion root tip method by locating cells in different stages of cellular division. Prophase: The chromosomes become visible as they condense Metaphase: The chromosomes are lined up at the canter, the cell's equatorial plate Anaphase: The chromosomes separate to the two opposite poles of the cell. Telophase: The final stage of cell division, the condensation of chromosomes, and formation of two nucleuses Observations: The root tip is the region that contains a high concentration of dividing cells and meristematic cells [13].

Formula

$$\text{Mitotic Index} = \frac{\text{Number of cells in mitosis}}{\text{Total no. of cells}} \times 100$$ [14]

$$\text{S. ERROR} = \frac{A/b \times (100-\%)}{N}$$ [15]

Table 2: Frequency of cells observed in different phases of the cell cycle

Name of the cell division phase	No of stages	Total cell count
Inter phase	66	100
S phase	75	100
Pro phase	48	100
Meta phase	69	100
Ana phase	58	100
Telo phase	96	100
Cyto kinesis	67	100

Observation

Roots of onions are associated with cell divisions, and as such, it was expected that the
stained sections of roots tips would show different stages if the cells in the root tip on the microscope. And indeed, varying phases of the cell cycle was observed, starting with the interphase and ending with cytokinesis, passing through all the stages of mitosis that are in between. However, there is a no invariable count for each cell cycle point, which is the most frequently recorded, and uniformly distributed, is the number of cells at the stages of telophase. Furthermore, cypermethrin's effects on the meristem were noticed after 24 hours. In contrast, when coriander and cypermethrin were combined and observed for 72 hours, they had a much greater overall negative effect on root cells [16].

Results and Discussion

The bulbs of onion were soaked in a solution of cypermethrin and Piper nigrum (black pepper) in order to

test the harmful properties of the mist and the possible use of black pepper as therapy of the onion roots at the somewhat stages of mitotic cell division. The onion bulbs were sectioned into four specific categories and each category was treated separately. There was over a recording period of 18 hours investigation and recording of the mitotic activity in the roots. After 18 hours of fixing, the roots of the onion which were measuring up to 3 cm long were squashed and stained with a mixture of haematoxylin and eosin. The stained specimens were studied on a Metzger binocular equipped with a camera and the images were stored for records. After 18 hours of fixing, the roots of the onion which were measuring up to 3 cm long were squashed and stained with a mixture of haematoxylin and eosin. The stained specimens were studied on a Metzger binocular equipped with a camera and the images were stored for records [17].

Table 3: Effect of different treatment mixtures on root growth

Mixture	Root lengths in cm			
Cypermethrin	1.9	1.5	2.0	2.3
Concurrent	2.3	2.5	2.7	2.9
Black pepper	3.3	4.0	4.5	4.7
Control	2.8	3.0	2.9	3.1

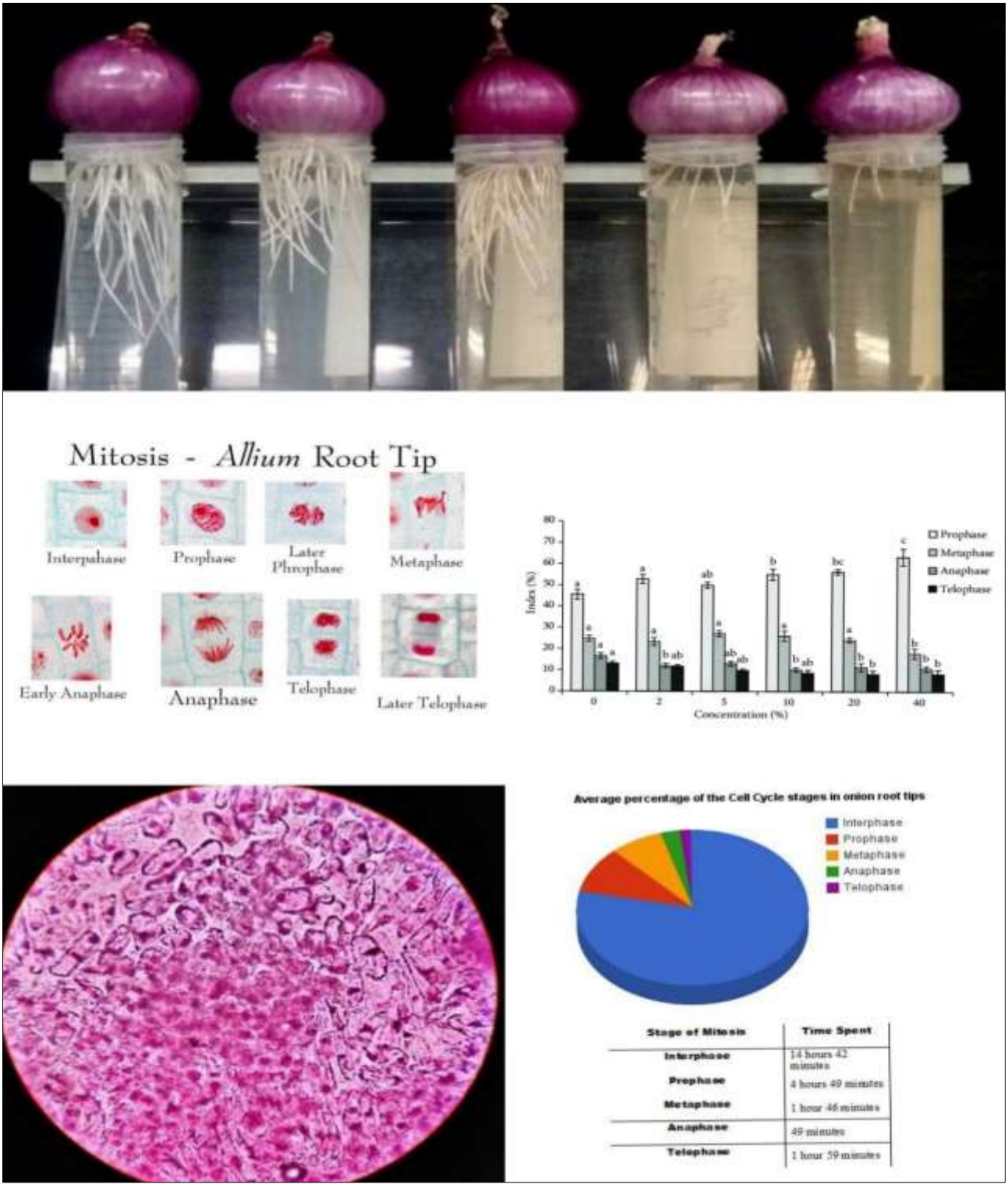

Fig 2: Comparative bar graph of average root lengths under different treatment conditions [1-5]

Conclusion

A slide with the dried onion root tip cells for the specimens is cleaved into place on the microscope stage, and the dissimilarities among the cells are studied. This cell cycle, along with the cell's phases, and stages are considered, as well as the number of cells in one phase. The graph provided depicts the proportion of cells that were dividing during the exposure to the different solutions. The research stem cells of onion root tip cells were examined and the effect of cypermethrin and black pepper was analysed and cypermethrin was found to have a negative effect. This pesticide causes damage by enhancing free radical generation which in turn results in increased ROS values in the root tip cells. Consequently, this damage leads to chromosome alterations and restrains growth.

Our experiments also show that cypermethrin apatite markedly decreases the mitotic index in a concentration-dependent manner, which indicates its genotoxic property. These cytotoxic effects emphasize the ecological threats that cypermethrin easily poses to agricultural environments.

Summary

From the analysis it is clear that cypermethrin is a chemically potent cytotoxic as it causes mushrooming of the palm cells as well as chromosomal alterations by stress oxygen species generation which stemmed from cypermethrin's normal use concentration. One of the consequences of the biotic damage is the reduction of the mitotic index which persists with increasing dose of the mitotic index's substance in agriculture's bio corridors.

Abbrevations

- **Cell Biology:** Cell biology is a branch of biology focusing on the study of cells as structural and functional basic building blocks of all living organisms.
- **Stain:** Particular red colour of a surface, substance or medium and which stands out from the colour of the object in question is known as stain.
- **ROS:** In biology and medicine, ROS is a term often used. This term can be simply defined as oxygen-containing reactive species.
- **Mitosis:** Mitosis is cell biological term that refers to a type of cell division which results in two identical daughter cells with the same chromosome number as the parent nucleus. Mitosis is usually followed by a process called cytokinesis where the cytoplasm divides and two daughter cells are formed.
- **Root tip:** The root tip is the terminal part of a root branch and automatically consists of root cap, meristematic area and at times differentiating, elongating and root hair forming areas.

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