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# **Seed Preservatives and it's Cytotoxicity in plants**

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*Thesis Submitted to Midnapore City College  
for the Partial Fulfillment of the Degree of  
Master of Science (Botany)*

*Submitted by*

**Anuska Das, Koushiki Mondal, Piyali Ghosh,  
Raja Singha**

*Guided by*

Dr. Anulina Manna,  
Assistant Professor in Botany  
Department of Biological Sciences



Department of Biological Sciences  
**MIDNAPORE CITY COLLEGE**

Kuturiya, P.O. Bhadutala,  
Paschim Medinipur, Pin-721129  
West Bengal, India

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## Certificate



This is to certify that the project report entitled **Seed Preservatives and it's Cytotoxicity** in plants submitted by **Anuska Das Roll PG/VUWGP29/BOT- IVS No. 007; Koushiki Mondal ROLL PG/VUWGP29/BOT- IVS No. 028; Piyali Ghosh ROLL PG/VUWGP29/BOT- IVS No. 040; Raja Singha ROLL PG/VUWGP29/BOT- IVS No. 044** to the Midnapore City College, Midnapore, West Bengal, India during the year of 2023 in partial fulfillment for the award of the degree of M.Sc. in **Botany** is a bona fide record of project work carried out by him/her under my/our supervision. The contents of this report, in full or in parts, have not been submitted to any other Institution or University for the award of any degree.

(Dr. Anulina Manna)  
Assisntant Professor in Botany

(Dr. Kuntal Ghosh)  
Teacher- in charge  
MIDNAPORE CITY COLLEGE  
(Dr. Pradip Ghosh)  
Director  
MIDNAPORE CITY COLLEGE

Date:

Place: Midnapore City College, Paschim Medinipur

## Declaration

We do hereby declare that the present Master thesis entitled “*Seed Preservatives and it’s Cytotoxicity in plants*”embodies the original research work carried out by us in the Department of Biological Sciences, Midnapore City College, Paschim Medinipur, West Bengal, India under the supervision of Dr. Anulina Manna, Associate Professor, Department of Botany, Midnapore City College, Kuturiya, P.O.- Bhadutala, Pin- 721129, Paschim Medinipur, West Bengal, India. No part thereof has been submitted for any degree or diploma in any University.

Date:

Place: Midnapore City College, Paschim Medinipur

(Anuska Das)

(Koushiki Mondal)

(Piyali Ghosh)

(Raja Singha)

## Approval Sheet

This project report entitled “**Seed Preservatives and it’s Cytotoxicity in plants**” by Anuska Das, Koushiki Mondal, Piyali Ghosh, Raja Singha is approved for the degree of Master of Science, Botany.

Signature of Examiners

(Name.....)

.....  
(Signature of Guide)  
(Dr. Anulina Manna)

.....  
(Signature of T.I.C)  
(Dr. Kuntal Ghosh)

.....  
(Dr. Pradip Ghosh)  
Director

Date :

Place: Midnapore City College, Paschim Medinipur

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## Abstract

Ever since man learned to grow crops and store them, insects have been an associated problem. Most grains are harvested once per year so they must be stored throughout the year in order to have raw ingredients available for year round production of processed food. To fulfill the food demand of an increasing population remains a major global concern. More than one-third of food grains are lost or wasted in postharvest operations. Grain storage loss is a major contributor to post-harvest losses and is one of the main causes of food insecurity in developing countries. To prevent the grain storage loss some chemicals are used such as Celphos, Bavistin, Taqat, Ridomet 35, Pyriban Dust etc. In this study Grain Treat with the chemicals used for preservation and were evaluated for cytotoxicity and the induction of genotoxicity in the onion (*Allium cepa*) test. Onion seeds, chickpea seeds and lentils were germinated and exposed to chemicals for 24 hours to evaluate their germination percentages. For each concentration, three root tips were transferred to three microscope slides, stained with aceto-carmin, covered with cover slip, squashed and observed microscopically. The cytotoxicity and genotoxicity induced by each pesticide concentration was compared with the value for the concomitant negative control using t-test. Genotoxicity was determined by examining, 100 anaphase and telophase cells on each of three slides per concentration for chromosome aberration (CA). The induction of sticky chromosomes indicated that the chemicals caused abnormal DNA condensation, abnormal chromosome coiling and inactivated the spindles. Because abnormalities of the cell division process results from the genotoxic effects of environmental chemicals, the chemicals have the potential to cause aneuploidy in exposed organisms and adverse human health and environmental effects.

**Key words:** Chemical preservatives, post harvest preservation, cytotoxicity, chromosomal aberration.

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## **List of Abbreviations**

MI	Mitotic index
GI	Germination index
CF	Chromosomal aberration frequency
AP50%	Aluminium phosphide 50%

# **Chapter 1: Introduction**

## 1. Introduction

The preservation of gathered seeds for planting has been a challenge for man from the beginning of his nomadic life. All peoples continue to rely on seed supplies for survival, however temperate zone planters have fared better than those in the humid tropical regions of the earth. The storage needs for seeds for planting were discovered by primitive peoples to be distinct from the needs for seeds used for food.

Primitive man hung his unthreshed crops from roofs to dry and then they would have seed for the following crop. Then they stored the dried seed in pits, straw bundles, baskets, or pottery jars. Some of these techniques are still in use in some developing nations, but until the advent of plant science in the seventeenth century, it was unclear why seeds maintained increased viability under particular circumstances.

Systematic studies have revealed some aspects of seed lifespan; However, the issue of seed loss is yet to be resolved and longevity of seed of numerous species as well as seed storage for food and feed (Anderson and Alcock, 1954; James, 1963 and Owen, 1956). For this first, it's important to look at some assumptions about seed degradation.

India is the world's top producer of spices and the world's second-largest producer of fruits, vegetables, and grains after China (Dastagiri et al., 2013). In addition to being used to keep grains fresher for longer (Rao et al. 1993), preservatives can also generate cytotoxic and genotoxic effects. In order to reduce post-harvest grain losses caused by various insect pests, notably grain weevils, grain borers, grain beetles, and grain moths as well as other bio-agents, preservatives have been widely used to manage infestations (Jackai, 1998). Many chemicals are used for preservation, Aluminium Phosphide, Captan, Carbendazim, Chloropyrifos, Metalaxyl etc. Most of them has cytotoxic and genotoxic effects. The higher genotoxic effects of two preservatives, Aluminium Phosphide and Metalaxyl, were induced by these preservatives. These effects included chromosome breaks, ring chromosomes, chromatin bridges, and micronuclei. In vivo chromosomal aberrations included C-mitosis, despiralization, lagging chromosomes, and multipolar cells (Grover and Malhi 1988).

*L. monocytogenes* is one of the most significant psychrotrophic food pathogens associated with cooked meat products packaged anaerobically and shelf-life failures of preserved foods. This bacterium is the cause of listeriosis, a condition brought on by eating tainted food that can be deadly for those who are vulnerable to it (Cornu et al., 2006). Synthetic additives should

therefore be employed to protect against contamination during seed manufacturing, sale, and distribution as well as to increase the shelf life of raw and or processed seeds. However, there is considerable disagreement on the safety of these chemical preservatives because they are thought to be responsible for a number of teratogenic and carcinogenic characteristics as well as residual toxicity (Skandamis et al., 2001). Thus, natural chemicals from plants and herbs are receiving more attention as a new approach to stop the spread of microorganisms and shield food from oxidation.

Benzalkonium chloride(BAC), a bactericidal cationic tenside, is utilized as a preservative in a variety of medical preparations at concentrations ranging from 0.01% to 0.05%, as we can see in the case of preservation of some pharmaceutical concentration. The aliphatic alkyl chains in commercial preparations have lengths of 12, 14, and 16 carbon atoms (Gardner and Girard, 2000). At low concentrations, BAS forms positively loaded, boundary surface active ions with an amphipathic structure in aqueous solutions. When the amount of BAC in an aqueous solution exceeds the critical micellar concentration (CMC), these ions join together and form micelles. Micelles are globular approximately spherical aggregates with a hydrophobic inside and a hydrophilic outside. The physical and biological characteristics of the solution may suddenly alter as a result of micelle production. The permeability of the swine buccal mucosa for estradiol was significantly decreased by the ionic surfactant sodium dodecyl sulfate (SDS), probably as a result of micelle production (Nicolazzo et al., 2004).

Chemical preservation has several negative side effects, sulfites a popular preservative found in many fruits that can cause migraines, palpitations, allergies, and even cancer. Benzene hexachloride are additives that are used in seed products as agents. It is said to cause stomach cancer when ingested. Seeds are preserved with an antibacterial and antifungal preservative that has been linked to allergies, asthma, and skin rashes. As an antibacterial preservative, sorbates and sorbic acid are added to seeds. Sorbate reactions are generally uncommon; reports of urticarial and contact dermatitis have been made (Hatton, 1990) and antifungal preservative that has been linked to induced breathing problems like asthma, hyperactive behavior in children, weakened heart tissue, Increase the chances of obesity, and effects in platelet (Perkhofer et al., 2009). It's possible to discover whether or not an allergy is immediately triggered by eating a certain seeds like castor seeds, but people with seeds laced with preservatives experience allergy symptoms a day or two later, making it difficult to pinpoint the exact cause. Because people eat different types of seeds and they are preserved with different types of preservatives, identifying

the exact ingredient that triggers an symptoms can be challenging. These preservatives can have acute negative effects or cause cancer to form in the body over time. The long-term physical effects of these chemicals have recently been seriously studied by researchers (Pressinger, 1997). This project discusses different types of preservatives and how they can cause cytotoxicity and genotoxicity. Apart from their cytotoxic effects, synthetic pesticides such as Aluminium Phosphide and Metalaxyl also have several side effects that negatively impact the environment. These side effects include, environmental pollution, toxicity to non-target organisms, pesticide residues and non-biodegradable properties (Lee et al., 2004; Islam, 2006). Most genotoxic health effects are generated by genetic damage in both somatic and germ cells. Additionally, it has been proposed that abnormalities in the cell division process may account for any genotoxic effects of environmental contaminants (Parry et al., 1999). Therefore, exposure to chemical preservatives has the potential to cause aneuploidy in organisms and has a negative impact on both human health and the environment. This study looked at the numerous cytotoxic and genotoxic effects of various preservatives, which not only have negative health consequences for humans but also have negative effects on seeds.



## **Chapter 2: Literature Review**

## **2. Literature Review**

According to botany, a seed is an ovule that has reached maturity and is fertile. It consists of an embryonic plant which is usually surrounded by protective tissue and supplied with food storage tissue. However, the physiological organ for the reproduction of plant species is the seed. Being living things, seeds take in oxygen, give off carbon dioxide and water vapor and simultaneously generate heat. These events are very important for seed preservation. The genotype of the seed from which a seedling is produced determines the quality of the seed, which makes seed an important component in the production of high quality seedlings in nurseries. Therefore, to produce a high quality, one must sow high-quality seed and maintain the quality of that seed from harvest to germination (Feistritzer, 1975).

Threshing, drying to ideal moisture levels for storage, cleaning and grading, purity and germination testing, treatment for storage pests and seed-borne diseases, bagging, labeling and distribution are common steps involved in post-harvest seed processing. This is because the seed is practically never pure as it is mixed with other crops, weed seeds, trash, chaff, leaves, insects, small seeds etc. when it is picked from the field. Additionally, seeds are often harvested at moisture levels that are higher than recommended for storage. So the seed must be free from internal material, weed seeds, seeds of other varieties of the same crop, safe moisture, high germination and vigor and free from damage to a large extent. In addition, seeds need to be labelled, packaged and treated (Schmidt, 2000).

Although some people mistakenly believe that saving seeds is the same as putting them in storage, the actual biological, physiological and biochemical processes that go on inside the seeds and how they interact with their surroundings are most important. Seed storage begins essentially in the field, if we pay attention to how seeds work (Hartmann et al. 1997). It begins after the seed reaches physiological maturity because after that point, the mother plant no longer fully protects the seed. Instead, seeds at that physiological stage depend on the external environment in terms of moisture, temperature and even biomass. Therefore, the environment during seed development and threshing has a significant effect on seed viability and storability (Harrington and Kozlowski, 1972).

The crop is believed to have originated in the wild in Peru, Ecuador, and other tropical American countries (Rick and Butler, 1956). This crop is now grown all over the world because of its nutritional and economic relevance. Post-harvest, some post-harvest handling methods and

treatments will affect fruit, post-harvest quality and shelf life. After harvest, any fruit or vegetable can be used to maintain its quality, not improve it. Within hours of harvest, the quality and shelf life of fruits and vegetables can be significantly affected, the main issue being what happens to quality during storage characteristics of these products, especially physical characteristics including color size and shape (Jeffreys and Jaeger, 1990).

Fruits needed to be preserved properly to increase their shelf life, its important to manage the temperature and relative humidity during storage (Susan and Durward, 1995). Low temperature extends storage life by lowering respiration rate and the growth of bacteria that calls deterioration (R a et al., 2000; Watada et al., 1999). All other treatments can be ineffective against postharvest illness if temperature is not managed properly, seen as alternatives to refrigeration. Organic acids are one of the main cellular components experiencing modifications during ripening (Civello et al., 2006). When handling fruit and vegetables, its important to take into account the environments temperature and relative humidity for recently obtained fresh fruit any technique of raising the storage's relative humidity reducing the vapor pressure or the environment between the commodity and its deficit (VPD) slowing the rate of water loss to environmental factor additional metabolic process (Wu, 2010).

FAO (1983) promoted a cheap storage system founded on the idea that evaporative cooling for fruit and vegetable storage which are straightforward and generally efficient. In addition to using natural air, Redulla (1984) presented an evaporative cooler for the preservation of fruit and vegetables. A large portion of the germplasm is kept as seeds at a variety of location around the world, the U.S. National Sees Storage Laboratory (NSSL) at fort Collins in one such facility. The scientist who actually started a systematic seed research was Roberts, Ellis and their teams such as R.H. pioneering studies on seed longevity have been conducted (Roberts, 1973; Ellis and Roberts, 1980; Ellis et al., 1989; Ellis and Hong, 2007). They demonstrated that among abiotic parameters, oxygen, temperature, and humidity are the most crucial nevertheless, restorage and genetic factors also matters. More temperature reduction will increase seed lifetime and survival (Ellis and Roberts, 1980). Based on knowledge of the initial seed quality, Ellis and Roberts could determine the lifetime of any species. It is stated that with every one percent reduction in water content, and down to this equilibrium water content, seed longevity would be improved by proper drying, life expectancy could double (Harrington, 1973). Metallic nanoparticles may be created by plants, and this process is becoming recognized as a way to create cytotoxic chemicals that

can treat many types of cancer (Kuppurangan et al., 2016). Through non-specific cellular absorption as well as through cell processes like adhesion cytoskeleton organisation, migration proliferation, and apoptosis, nano particles can enter cells. The shape of the particles may have an impact on these activities (Huang et al., 2010). One of the most popular higher plant species for cytotoxicity and genotoxicity tests of different environmental contaminants is *Allium cepa* (Bonciu et al., 2018). *Allium cepa* is more sensitive than other test; this due to the sensitivity of onion roots, application is crucial in biomonitoring, to any hazardous substances. Plant bioassays are effective methods for detecting the genotoxicity of environmental contaminants. A common test for quickly and accurately identifying contaminants that pose environmental risks is the allium test. A number of authors have praised the use of *Allium cepa* for the bio-monitoring of genotoxicity (Datta et al., 2010). Since the 1940s, the *Allium cepa* has been employed as a test system to identify mutagens , it has also been used to evaluated a large range of chemical agents which contributes to its broad usage in environmental monitoring (Leme and Marin, 2009). *Allium cepa*'s root tip system has demonstrated a special sensitivity to the negative impacts of environmental risk (Bhat et al., 2015). In contrast the enhancement of abiotic stress tolerance has received more research than improving post-harvest preservation of fruit and vegetables. Entire work best on Musur (*Lens culinaris*), Chickpea (*Cicer arietinum*), Onion (*Allium cepa*). Humans have been growing pulses since prehistoric times. They have grown to be crucial to everyday nutrition. Most Indian families include at least one of these pulses- Chana (Chickpea), Musur, onion in their daily meanus. Pulses can enhance the protein consumption of meals that include cereal and root tubers along with pulses, according to (Kushwah et al.2002) eaten.

Many diseases can attack musur bean plnts. Fungicide-treated seed enhances seed health, plant stand, and crop, according to (Tanweer, 1982). Production and the prevention of seed-borne illnesses.

In asia, widespread farmer health issues have been caused by heavy pesticide use in food crops (Antle and pingali, 1994). The conclusion from their experiment on green beans is that careful control of spraying doses of dithiocarbamate fungicide is necessary.

The treatment of the fungicide reduced wax content and altered its morphology, resulting in ruptures and missing crystalloids that could render the plant more susceptible to disease. Herbivore and desiccation-prone (Lichston et al., 2006). Food production may be impacted by the indiscriminate use of agrochemicals on farms. An essential legume crop plant for agriculture and

nutrition is the chickpea. The availability of the chickpea transcriptome and draught genome sequences. Chickpea seed size is a significant end-user quality criterion. Chickpea with large seeds are highly desired since they sell for more money. Despite the fact that chickpea genotypes exhibit large variations in seed size, this phenotype heterogeneity was unable to due to limited understanding of the molecular mechanism underlying this crucial feature, enhance seed size in significant chickpe cultivars(Kujur et al., 2013; Verma et al., 2015; Singh et al., 2016). This work sheds light on the molecular processes behind seed formation and the variables affecting seed growth, weight/ size in chickpea.

Onion (*Allium cepa*) a member of the alliaceae family, is a significant spice and is frequently used as a condiment to flavor a variety of foods (Vazquez et al., 2016). In order to fulfill demand in the years to come, onion production must expand in response to rising demand.

The availability and usage of good quality seed with a high germination potential and in good health are the two most crucial factors in increasing onion yield, according to (Kameswara et al., 2017) The fundamental and indispensable component of all crop production is seed. High quality seed is a crucial input on which the effectiveness of all other inputs will depend (Thompson, 1979). If a seed is stored in less than ideal conditions, it may also suffer substantial deterioration. Condition causes the seed quality to decline and the subsequent loss of viability. Such containers make seeds vulnerable to infestation by storage fungus. The preservation of onion seeds is a significant issue in Bangladesh. The most crucial factors affecting the presence of fungi in seeds include seed moisture levels, storage temperature, and relative humidity. In order to determine how different storage methods, seed moisture levels, and storage conditions affect seed, an experiment was carried out. Occurrence and spread of fungus that live on onion seeds.

Preserving planting supplies from one season to the next is the goal of seed storage. In some circumstances (such as seed businesses), the goal of seed storage is to preserve seed quality for as long as feasible. Additionally, seed preservation allows for the long-term preservation of germplasm for a better plant breeding program. When the seed is ready to be collected, it should be cleaned, dried to a safe moisture content, cleaned again, stored under ideal circumstances, and guarded from damage and pests until planting. According to Babiker (2015), the type of seed crop, moisture content, storage conditions (temperature, relative humidity), and storage pests are the most crucial variables determining storability. The process of cleaning, purifying, and achieving high physiological quality (germinability) seeds that can be stored and handled with

ease during subsequent processes, such as pre-treatment, transport, and sowing, where applicability varies depending on seed type, the state of the seeds when they were collected, and any probable storage time. To keep seeds viable for a long time in storage, the right conditions are essential. The study's goal is to review the seed process and storage conditions in connection to both ecological elements and seed moisture (Desai, 2004).

Chemical preservatives are used to store seeds and grains. In this project we have used various chemical preservatives as test samples. We took these preservatives because we surveyed in the local market and found that these five preservatives are widely used in Paschim medinipur area to store the seeds and grains. These chemical preservatives are also available in the local market. We select *Allium cepa* because it's used as raw cooked food material add daily basis. Rest two Chickpea and Lentil of an consumed soaked condition and cooked by us. These preservatives save the seed grains from damaged but may cause many cytological or abnormalities or may affect their germination. The aim of this project find out chromosomal aberration like C-mitosis, de-spiralization, lagging chromosomes, multipolar cells etc, clastogenic effects like chromosome breaks, ring chromosome, chromatin bridges etc and clastogenic effects like chromosome breaks, ring chromosome, chromatin bridges etc. This experiment shows the increasing concentration of chemical preservatives shows a negative impact on plant germination and cytology. But it shows the right concentrations of preservatives to be used. And also shows which preservative is less harmful. Captan, Aluminum phosphide, Metalaxyl, Carbendazim and Gammexane at different concentrations in the seeds of three crops: Cicerarietinum, *Allium cepa* and *Lens curinalis*. Unfortunately, we observed negative effects of these preservatives on the seeds. However, it is important to note that many groups of seed preservatives are used worldwide and their potential negative effects remain unknown.

## **Chapter 3: Aims and Objective**

### **3. Aims and Objective**

#### **3.1. Aims:**

Chemical preservatives are used to store seeds and grains. The aim of this project to find out if the preservatives effects on the germination or cause any morphological changes to the plants and to find out the cytotoxic effects of these chemical preservatives. The optimum concentrations which are not hazardous to plant growth and suitability of chemical preservatives which are less harmful or show less abnormalities in plants will be disclosed. To fulfill this aim the following objectives are taken-

#### **3.2. Objectives:**

1. Comparative study of effect on germination and morphology in different concentrations.
2. Comparative study of chromosomal aberration like C-mitosis, despiralization, lagging chromosomes, multipolar cells etc.
3. Comparative study of clastogenic effects like chromosome breaks, ring chromosome, chromatin bridges etc.
4. To investigate the cytotoxic and genotoxic effects of some preservatives used in to control stored product insect pests using the *Allium cepa* anaphase-telophase chromosome aberration assay.



## **Chapter 4: Materials and Methods**

## 4. Materials and Methods:

### 4.1. Materials:

#### 4.1.1. List of test samples:

In this study five chemical preservatives are used as test sample for studying their effect on plants which are widely used in Paschim medinipur are to store the seed grains. All the chemicals used in this study are of analytical grade (AR).

**Table 1:** The Chemical preservatives used in the study:-

Sl.No.	Preservatives Name	Trade Name
1.	Aluminium phosphide 57%	Celphos
2.	Carbendazim 50% WP	Bavistin
3.	Captan 70% + Hexaconazole 5% WP	Taqat
4.	Metalaxyl 35% WS	Ridomet 35
5.	Chlorpyriphos 1.5%	Pyriban dust



Fig1: Chemical preservatives used in the study -A: Captan 70% + Hexaconazole 5% WP, B. Carbendazim 50% WP, C. Metalaxyl 35% WS, D: Aluminium phosphide 57%, E: Chlorpyriphos 1.5%

#### 4.1.2. List of chemicals used in different experiment:

**Table 2:** The chemicals used of different experiment:-

Sl. No.	Chemical name	Uses
1.	Absolute alcohol	Cytological study
2.	Acetic acid	
3.	Acetoorcein	

#### 4.1.3. Plant materials used:

In this study we have chosen 5 chemical preservatives as experimental samples that are used frequently to store food grains, seeds etc. Onion (*Allium cepa*) seeds and bulbs, Chickpea (*Cicer arietinum*) and Lentils (*Lens culinaris*) seeds as plant materials. Onion was chosen from the monocot plant caused of it's root, which is best for cytological study, as well as it is used as food as raw or cooked. So we can determine the effect of chemical preservatives on a specific level at which it can get harmful for human beings. In the case of chickpea and lentil, the production rate of these two grains are very high in west Bengal and are often consumed in soaked conditions by peoples.

#### 4.2. Methodology:

In this study we have studied the seeds treated with chemical preservatives form their germination to inner cytological changes. The methodology for them given below:-

##### 4.2.1. Experiments to select concentrations of preservatives to use:

The concentrations of each preservatives to be used in the real genotoxicity trials were established through preliminary experiments. The preservatives are serially diluted with distilled water in 0.2%, 0.4%, 0.6%, 0.8% and 1.0% in respect to their dosages used in store programmers. In separate Petri plates, seeds were dispersed on filter paper with varying doses of each pesticide or with water (negative control) for 72 hours at room temperature. The effective concentration (EC50) was the concentration that when 50% of the seeds are germinating or the effective concentration for preventing 50% growth inhibition for relative reduction of root length. The EC50s were too hazardous in trial studies, and it was impossible to see enough cells in the division phases, with the exception of Ridomet, which did suppress germination even at the limit

of solubility. The greatest concentration in each case during the genotoxicity trials was thus lower than the EC50 (Asita and Mokhobo, 2013).

#### **4.2.2. Chemical solution preparation:**

The stock solution of each chemical preservative were prepared as per their dosages used in grain preservation added in 100 ml of distilled water. From this different concentration (0.2%, 0.4%, 0.6%, 0.8% and 1.0%) of solutions are prepared keeping the conc. as highest. For controlled distilled water is added.

#### **4.2.3. Treatment of the plant samples with chemical preservatives:**

First labeled each container with the seed type (chickpea, lentil, or onion) and the concentration of each preservatives (normal, 0.2, 0.4, 0.6, 0.8, or 1.0). Then weighted the appropriate amount of samples for each concentration. Take 20 seeds of each type (chickpea, lentil, and onion) for each concentration. Ensure have a total of 120 seeds (20 seeds  $\times$  6 concentrations) for each seed type. Placed the seeds for each concentration in their respective containers.

For chickpea and lentil seeds were placed in chemical preservatives solution about 24 hours. Then, the seed were kept in chemical preservative solution soaked cotton pad.

For onion the basal portion is cut off. The bulbs were placed on a thermocol that is placed over plastic cup in that position where the basal portion is attached to the solution of chemical preservatives.

Placed all the containers in an incubator or a warm, well-lit area where they will receive consistent temperature and light. The containers checked regularly to ensure that the seeds remain moist. If the paper towels or filter paper inside the containers become dry, carefully added a small amount of water to rehydrate them, being careful not to disturb the seeds.

#### **4.2.4. Calculating the germination percentage:**

Observed the seeds daily and recorded the germination progress for each concentration. A seed is considered germinated when the root (radicle) emerges from the seed coat. After the germination period, carefully remove the seeds from the containers and transfer them onto a tray or plate lined with moist paper towels or filter paper. This will allow us to observe and compare the germinated seeds easily. Analyzed the germination results by calculating the germination percentage for each concentration. The germination percentage is determined by dividing the number of germinated

seeds by the total number of seeds and multiplying by 100. Recorded and compared the germination percentages for each concentration and seed type to evaluate the effects of chemical preservatives on seed germination.

#### 4.2.5. Genotoxicity assay:

The new emerged roots (10 – 15 mm in length) of *Allium cepa* treated with chemicals in different concentration of chemical preservatives were cut with sharp blade, fixed in Carnoy fixative (ethyl alcohol: glacial acetic acid) in 1:1, 1:2 and 1:3 ratio for 20 minutes in each. Then the root tips are transferred into 70% alcohol. The roots tips were stained 2% aceto carmine for 30 minutes and then heat fixed with a Bunsen burner. The microscopic preparations were performed by squash technique. For this purpose, the slide was placed and cover slip on a double layer of paper towel, then paper was folded over the cover slip and squash down on the cover slip with a strong vertical pressure, using the thumb. The pressure was applied to squash the root tip into a single cell layer. Five replicates were made for each concentration. The microscopic slides were examined at 40x magnification.

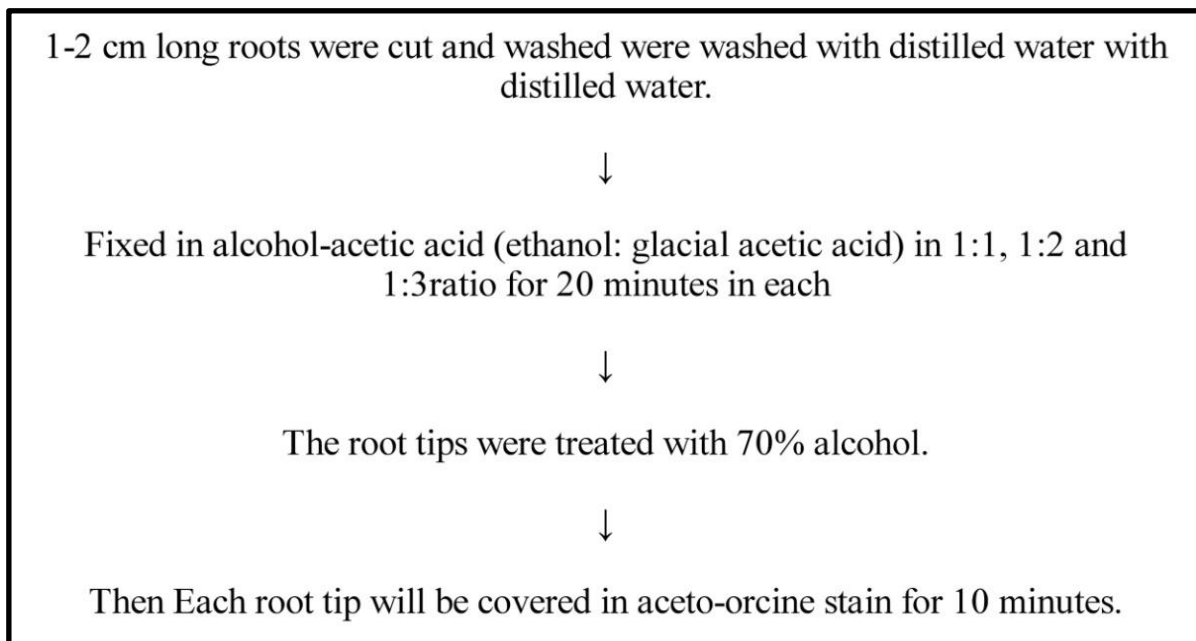


Fig 2: Diagrammatic representation for root tip analysis.

#### 4.2.5.1. Mitotic index:

Minimum of 1000 *Allium cepa* root meristematic cells were count from each prepared slide in a random manner to study interphase cells, cells in mitotic stage, and chromosomal aberrations in the dividing cells. The mitotic index (MI) was calculated for root tips of each onion bulb using

the following formula (the total number of dividing cells is the cells undergoing prophase, metaphase, anaphase, and telophase stages).

$$\text{Mitotic index(\%)} = \frac{\text{Number of dividing cells counted}}{\text{Total number of cells counted}} \times 100$$

#### **4.2.5.2. Abnormality index:**

The chromosomal abnormality are any abnormality occurs in structure, position or in number of chromosomes. Chromosome abnormalities mostly occur at metaphase and anaphase stages of mitotic cell division. Most types of chromosomal aberrations observed in high percentage were stickiness, disturbance, c-metaphase, chromosome bridges in anaphase, lagging chromosome etc. The % occurrence of each type of chromosomal abnormalities in root meristematic cells was calculated using the following equation.

$$\text{Chromosomal abnormalities(\%)} = \frac{\text{Total number of cells with abnormalities}}{\text{Total number of dividing cells}} \times 100$$

#### **4.2.6. Statistical Analysis:**

The average and standard deviations were calculated for each of the three experimental measures. Using the MS Excel 2007 programmed, the magnitude of the means, standard curve, standard errors, and standard deviations were computed. Divided among the 5 preservation samples are the findings and discussions.

## **Chapter 5: Results**

## 5. Results:

### 5.1. Germination index:

#### 5.1.1. *Cicer arietinum*:

The table no. 3 shows the germination index of *Cicer* seeds treated with five different chemical preservatives in different concentrations. The preservative names are- Captan 70% + Hexaconazole 5% WP, Carbendazim 50% WP, Metalaxyl 35% WS and Aluminium phosphide 57%. The concentrations used are 0.2%, 0.4%, 0.6%, 0.8%, 1.0% prepared as per their doses used per gram. For control we used only distilled water. After the germination of seed treated with chemical preservatives, it is clearly seen that the germination percentage decreased when the concentration of preservatives were increased. Seed germination percentage of distill water is 96%. In case of Captan 70% the germination percentage decreases from 85% to 75% as the concentrations are increased. In case of Carbendazim 50% the germination percentage decreases from 85% to 71% as the concentrations are increased. In case of Aluminium phosphide whis is commonly known as Celphos the germination percentage decreases from 85% to 70% as the concentrations are increased. Among these five preservatives the germination index is very low in case of Metalaxyl 35%. In 0.2% concentration only 40% seed are germinated. Then the germination percentage decreases dramatically to 3% and in 1.0% concentration the germination percentage become 0.

**Table 3:** Germination index of *Cicer arietinum* in different conc. of five different chemical preservatives:-

Preservatives	Concentrations	Total number of seeds	No. of seed germination			Germination index(%) [Mean±SD]
			R1	R2	R3	
Control		60	19	19	20	19.33±0.47
AP 57%	0.2%	60	16	18	17	17±0.81
	0.4%	60	17	17	17	17±0
	0.6%	60	15	16	15	15.33±0.47
	0.8%	60	12	16	14	14±1.63
	1.0%	60	15	14	15	14.66±0.47
Chlorpyrifos 1.5%	0.2%	60	18	19	19	18.66±0.47
	0.4%	60	19	18	19	18.66±0.47
	0.6%	60	17	20	19	18.66±1.24
	0.8%	60	20	19	18	19±0.81
	1.0%	60	19	20	8	15.66±1.43
Captan 70%	0.2%	60	16	19	16	17±1.41
	0.4%	60	18	15	17	16.66±1.24
	0.6%	60	16	17	14	15.66±1.24
	0.8%	60	18	19	8	15±2.96
	1.0%	60	16	16	13	15±1.41



Carbendazim 50%	0.2%	60	19	17	14	16.66±2.05
	0.4%	60	13	17	15	15±1.63
	0.6%	60	15	19	18	17.33±1.69
	0.8%	60	19	18	16	17.66±1.24
	1.0%	60	15	18	13	15.33±2.05
Metalaxyl 35%	0.2%	60	14	13	11	12.66±1.24
	0.4%	60	12	12	10	11.33±0.94
	0.6%	60	07	08	08	7.66±0.47
	0.8%	60	06	05	07	6±0.81
	1.0%	60	00	00	00	0±0

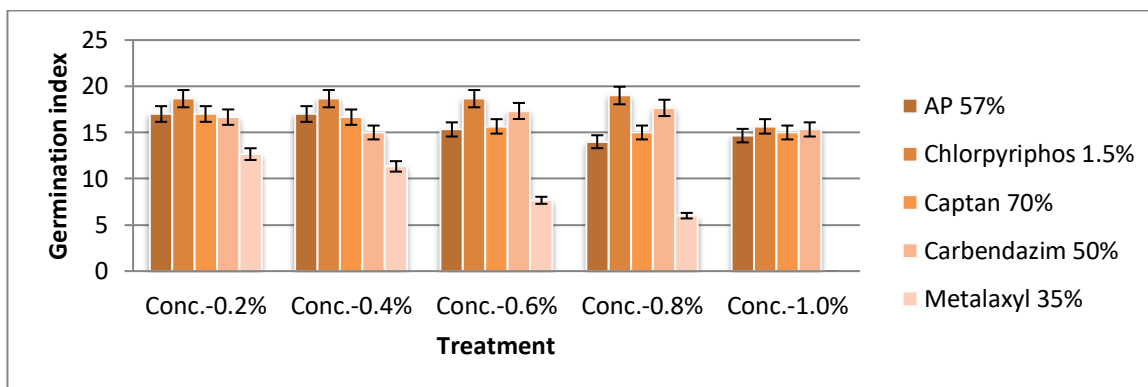


Fig.3: Graphical representation of germination of *Cicer arietinum* in different conc. of different preservatives.

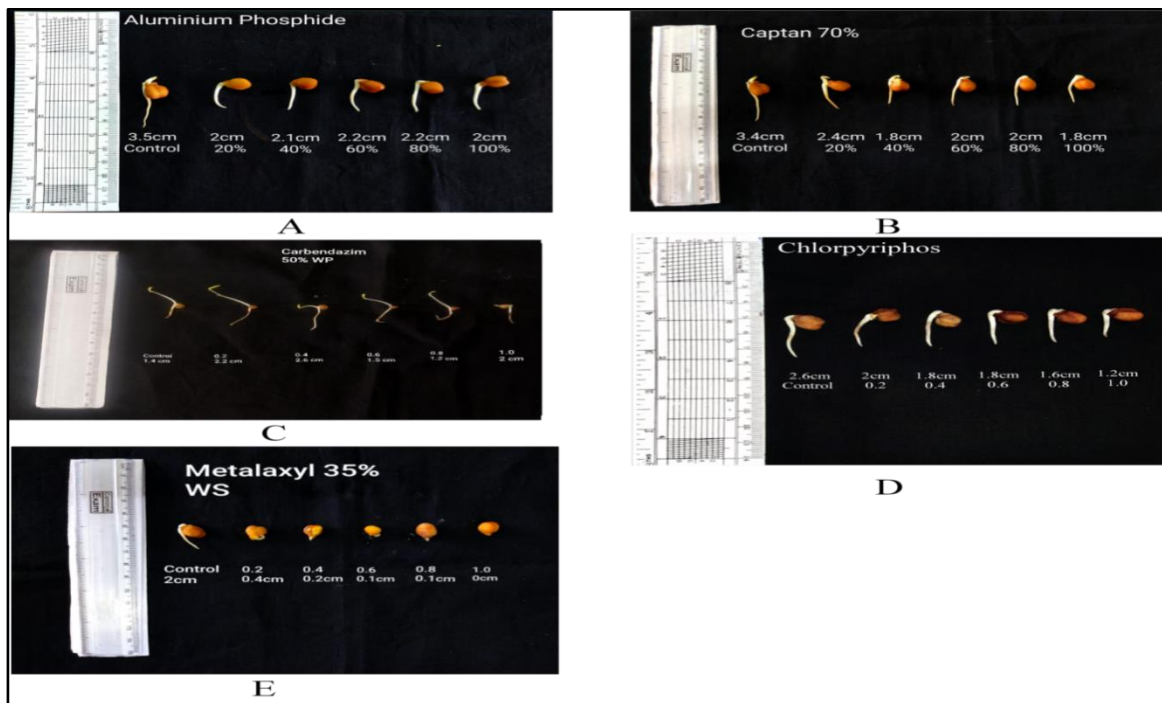


Fig 4: Photographs showing root germination of *Cicer arietinum* in different chemical preservatives- A. AP 57%; B. Captan 70%; C. Carbendazim 50% WP; D. Chlorpyrifos 1.5%; E. Metalaxyl 35%.

### 5.1.2 *Lens culinaris*:

The following table shows the gradual decrease of germination percentage when the concentration of chemical preservatives were increased. In cases of Captan 70%, Carbendazim 50% and metalaxyl 35% the germination index goes below 50% as the concentration of preservatives were increased where as in control the germination index scores 98%. Hence we can say over use of these preservatives can harm crop production.

**Table 4:** Germination index of *Lens culinaris* in different conc. of five different chemical preservatives:-

Preservative name	Concentrations	Total number of seeds	No. of seed germination			Germination index(%) [Mean± SD]
			R1	R2	R3	
Control		60	20	20	19	11.33±4.49
AP 57%	0.2%	60	16	15	15	12.66±0.47
	0.4%	60	13	14	13	10.33±0.47
	0.6%	60	12	12	11	12.33±0.94
	0.8%	60	13	15	14	11.66±1.24
	1.0%	60	14	16	15	10±1.63
Chlorpyriphos 1.5%	0.2%	60	8	12	16	9.66±9.66
	0.4%	60	10	16	13	9.33±1.69
	0.6%	60	6	17	11	12.66±0.47
	0.8%	60	13	13	12	10.33±3.24
	1.0%	60	10	11	10	10.33±2.62
Captan 70%	0.2%	60	13	11	13	11±0.81
	0.4%	60	12	10	13	12.33±1.69
	0.6%	60	10	8	12	14±1.41
	0.8%	60	12	11	6	11±0.81
	1.0%	60	11	10	7	10±1.41
Carbendazim 50%	0.2%	60	13	13	12	10.66±1.24
	0.4%	60	15	13	3	9.66±1.24
	0.6%	60	09	14	8	11.33±2.49
	0.8%	60	10	11	12	12.66±0.47
	1.0%	60	14	13	10	10.33±0.47
Metalaxyl 35%	0.2%	60	15	12	15	12.33±0.94
	0.4%	60	12	10	11	11.66±1.24
	0.6%	60	09	12	09	10±1.63
	0.8%	60	09	12	11	9.66±2.62
	1.0%	60	10	11	08	9.33±1.69

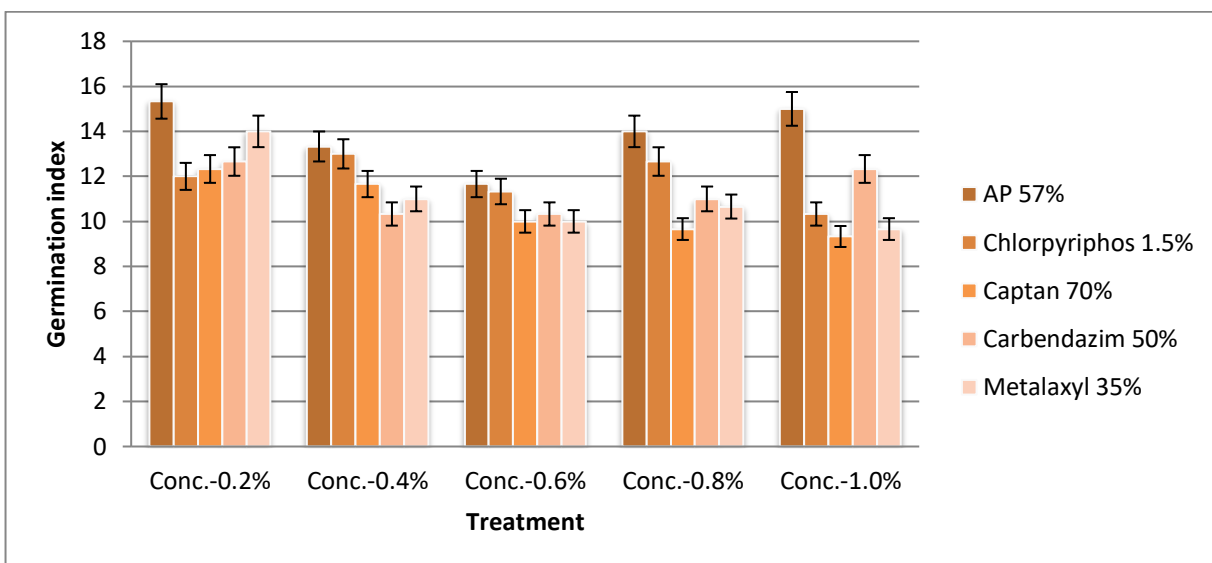


Fig.5: Graphical representation of germination of *Lens culinaris* in different conc. of different preservatives.

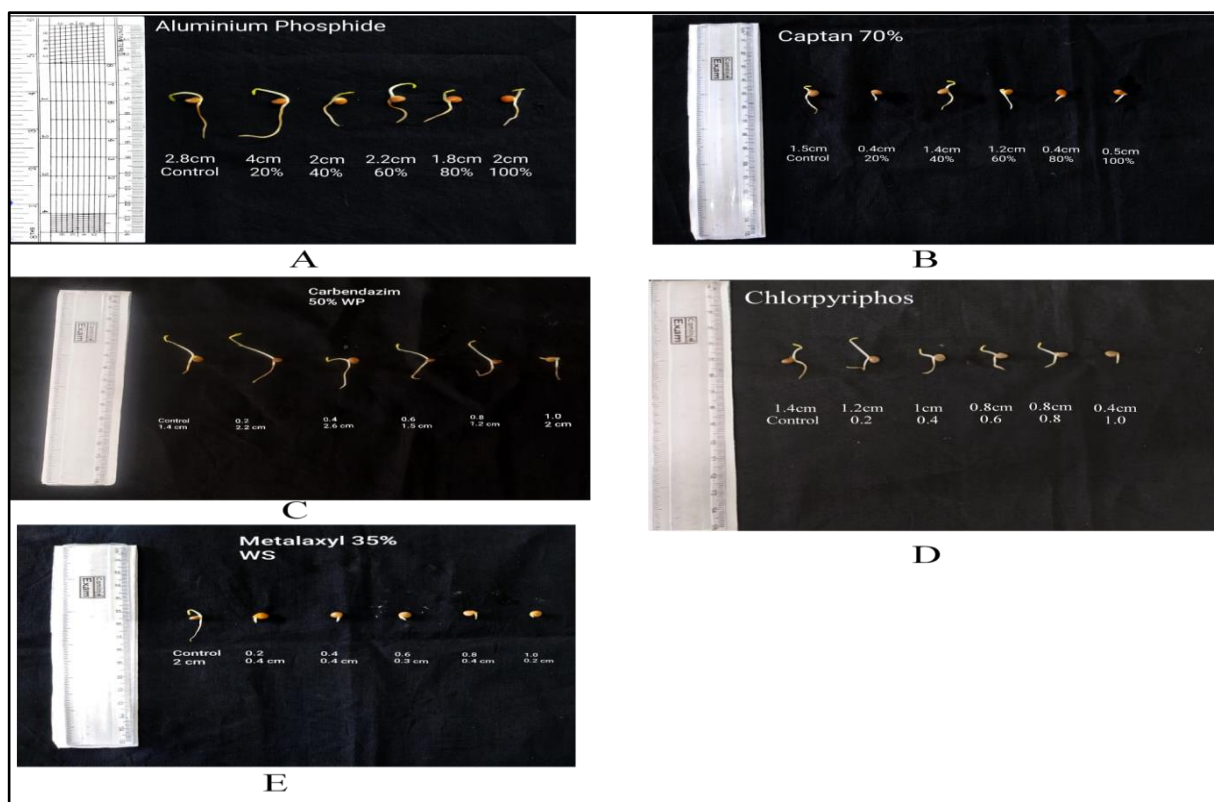


Fig 6: Photographs showing root germination of *Lens culinaris* in different chemical preservatives- A. AP 57%; B. Captan 70%; C. Carbendazim 50% WP; D Chlorpyrifos 1.5%; E. Metalaxyl 35%.

### 5.1.2. *Allium cepa*:

The following table shows the gradual decrease of germination percentage when the concentration of chemical preservatives were increased. In cases of Captan 70%, Carbendazim 50% and metalaxyl 35% the germination index goes below 50% as the concentration of preservatives were increases where as in control the germination index scores 98%. Hence we can say over use of these preservatives can harm crop production.

**Table 5:** Germination index of *Allium cepa* in different conc. of four different chemical preservatives:-

Preservative name	Concentrations	Total number of seeds	No. of seed germination			Germination index(%) [Mean± SD]
			R1	R2	R3	
Control		60	20	17	19	18.66±1.24
AP 57%	0.2%	60	14	12	13	13±0.81
	0.4%	60	9	10	9	9.33±0.47
	0.6%	60	7	8	8	7.66±0.47
	0.8%	60	9	12	11	10.66±1.24
	1.0%	60	9	6	9	8±1.41
Chlorpyrifos 1.5%	0.2%	60	16	14	18	16±1.63
	0.4%	60	16	17	12	15±2.16
	0.6%	60	13	9	8	10±2.16
	0.8%	60	7	11	6	8±2.16
	1.0%	60	9	9	4	7.33±2.35
Captan 70%	0.2%	60	17	16	16	16.33±0.47
	0.4%	60	14	17	11	14±0.44
	0.6%	60	16	13	9	12.66±2.86
	0.8%	60	11	14	12	12.33±1.24
	1.0%	60	10	8	13	10.33±2.05
Carbendazim 50%	0.2%	60	11	14	9	11.33±2.05
	0.4%	60	8	12	9	9.66±1.69
	0.6%	60	7	8	11	8.66±1.69
	0.8%	60	13	9	12	11.33±1.69
	1.0%	60	3	7	6	5.33±1.69
Metalaxyl 35%	0.2%	60	11	11	13	11.66±0.94
	0.4%	60	10	13	9	10.66±1.69
	0.6%	60	2	6	4	4±1.63
	0.8%	60	0	1	00	0.33±0.47
	1.0%	60	00	00	00	0±0

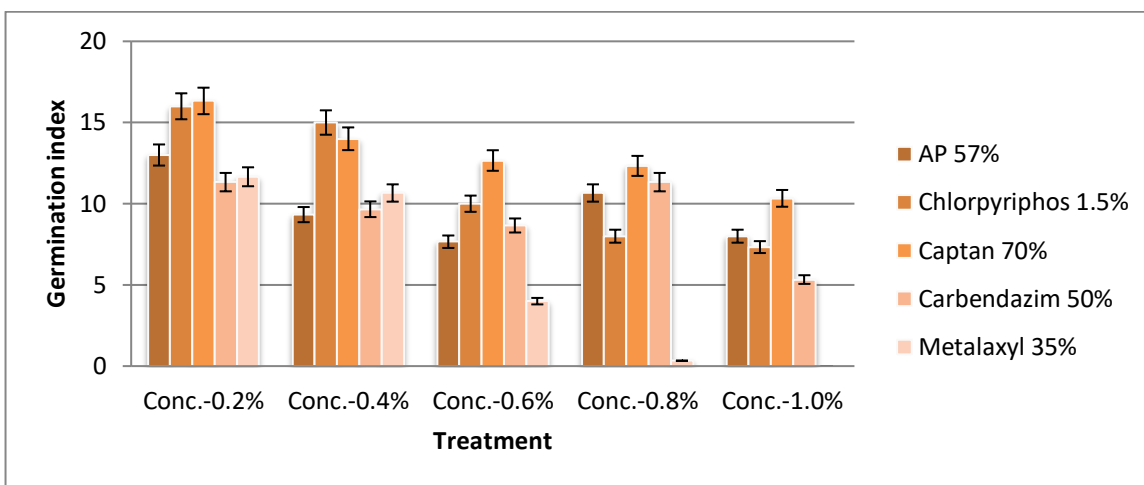


Fig.7: Graphical representation of germination of *Allium cepa* in different conc. of different preservatives

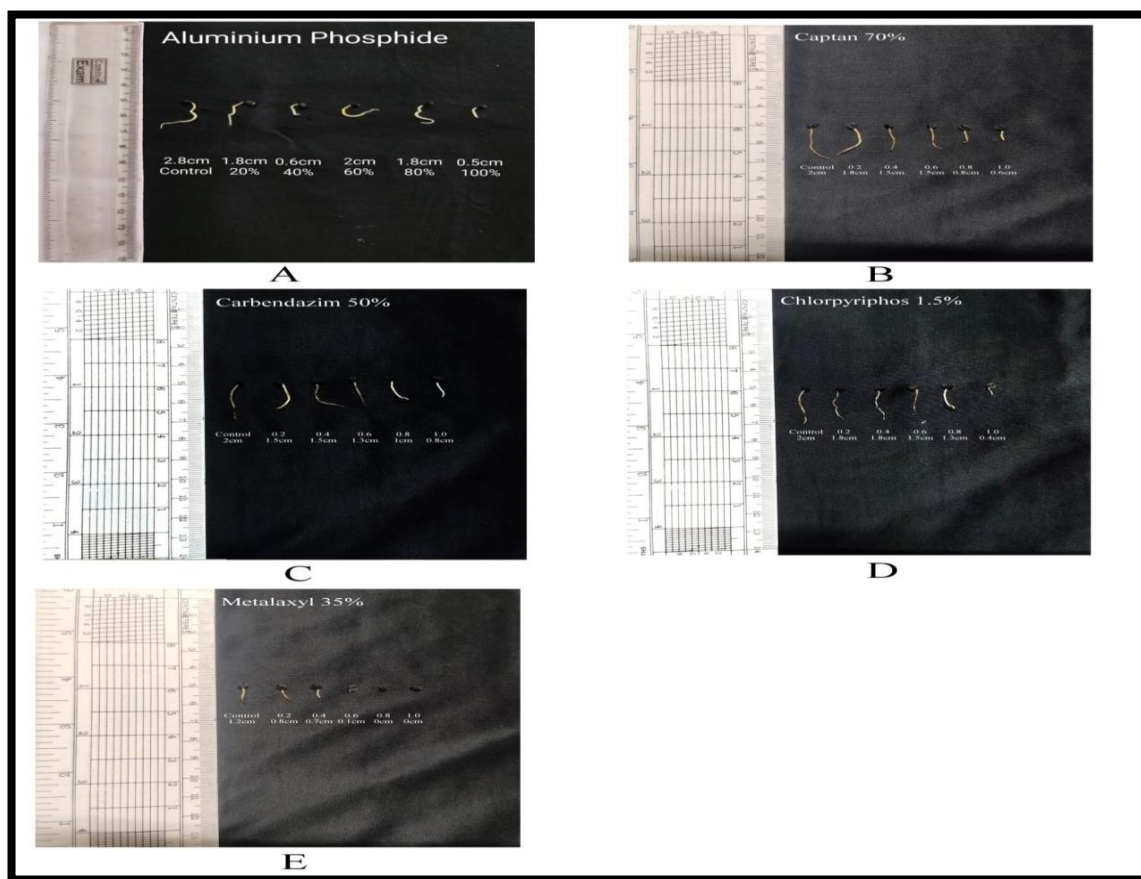


Fig 8: Photographs showing root germination *Allium cepa* in different chemical preservatives- A. AP 57%; B.Captan 70%; C. Carbendazim 50% WP; DChlorpyriphos 1.5%; E.Metalaxyl 35%

## 5.2. Morphological:

### 5.2.1. Effect on root length:

#### 5.2.1.1. *Cicer arietinum*:

Table no.5 shows the growth of cicer root in different preservatives in different concentrations. It shows a gradual decrease in root length as the concentrations are increased. In case Metalaxyl 35% no roots were found in 1.0% concentration. Where as in control root lengths are highest. So we can say chemical preservatives can effect in crop production.

**Table no. 6:** Average root lengths (cm) of *Cicer arietinum* treated with chemical preservatives at day 4:

Preservatives name	Concentrations	Root length in different concentrations			Mean± SD
		R1	R2	R3	
Control		3.7	3.2	3.2	3.36±0.23
AP 57%	Conc.-0.2%	2.7	2.6	2.4	2.56±1.12
	Conc.-0.4%	2.2	2	1.9	2.03±0.12
	Conc.-0.6%	2.2	2.2	2.1	2.16±0.04
	Conc.-0.8%	2	2.1	2	2.03±0.04
	Conc.-1.0%	1.8	2	1.9	1.9±0.1
Captan 70%	Conc.-0.2%	3.2	2.9	3	3.03±0.12
	Conc.-0.4%	2.2	1.8	2	2±0.160.04
	Conc.-0.6%	2	2.1	2	2.03±0.04
	Conc.-0.8%	2.1	2	2	2.03±0.12
	Conc.-1.0%	1.8	2.1	2	1.96±0.12
Carbendazim 50%	Conc.-0.2%	3.2	3	3.3	3.16±0.08
	Conc.-0.4%	3.2	3.1	3	3.1±0.12
	Conc.-0.6%	3.1	2.9	3.2	3.06±0.08
	Conc.-0.8%	2.8	2.6	2.7	2.7±0.08
	Conc.-1.0%	2.7	2.7	2.6	2.66±0.04
Chlorpyriphos 1.5%	Conc.-0.2%	2.6	2.4	2.5	2.5±0.08
	Conc.-0.4%	2	1.8	2	1.93±0.09
	Conc.-0.6%	1.8	2	2.1	1.96±0.12
	Conc.-0.8%	1.8	2.1	2	1.96±0.12
	Conc.-1.0%	1.6	1.9	1.7	1.73±0.12
Metalaxyl 35%	Conc.-0.2%	3.8	3.7	3.6	3.7±0.08
	Conc.-0.4%	0.7	0.6	0.5	0.6±0.04
	Conc.-0.6%	0.3	0.4	0.4	0.36±0.05
	Conc.-0.8%	0.1	0.1	0.2	0.13±00
	Conc.-1.0%	00	00	00	00±00

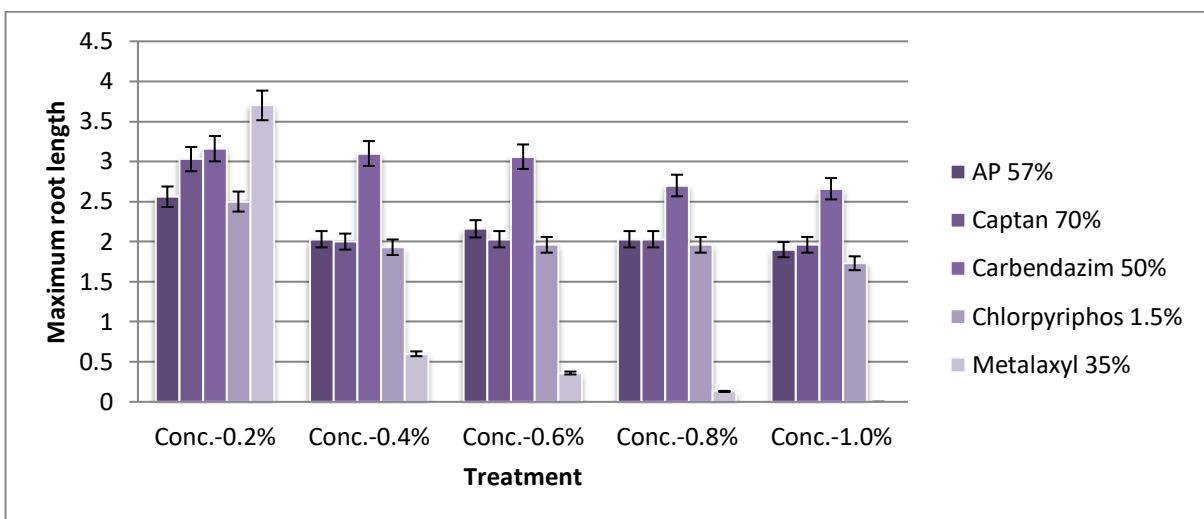


Fig. 9: Graphical representation of average root lengths (cm) of *Cicer arietinum* treated with chemical preservatives at day 4.

### 5.2.1.2. *Lens culinaris*

Table no.6 shows the growth of lentil root in different preservatives in different concentrations. It shows a gradual decrease in root length as the concentrations are increased. Where as in control root lengths are highest. So we can say chemical preservatives can effect in crop production.

**Table no. 7:** Average root lengths (cm) of *Lens culinaris* treated with chemical preservatives at day 4:

Preservatives name	Concentrations	Root length in different concentrations			Mean± SD
		R1	R2	R3	
Control		2.8	2.4	3.2	2.8±0.32
AP 57%	Conc.-0.2%	1.8	1.7	1.9	1.8±0.08
	Conc.-0.4%	0.8	0.6	0.7	0.7±0.08
	Conc.-0.6%	0.7	0.5	0.8	0.66±0.12
	Conc.-0.8%	0.7	0.6	0.7	0.66±0.04
	Conc.-1.0%	0.6	0.7	0.5	0.6±0.08
Captan 70%	Conc.-0.2%	1.2	1.1	1.3	1.2±0.08
	Conc.-0.4%	1.1	0.9	1	1±0.08
	Conc.-0.6%	0.8	0.7	0.7	0.7±0.04
	Conc.-0.8%	0.6	0.8	0.8	0.73±0.09
	Conc.-1.0%	0.5	0.6	0.4	0.5±0.08
Carbendazim 50%	Conc.-0.2%	3.2	3	3.1	3.1±0.08
	Conc.-0.4%	3.1	3.2	3	3.1±0.08
	Conc.-0.6%	2.8	2.6	3	2.8±0.16
	Conc.-0.8%	2.7	2.5	2.6	2.6±0.08
	Conc.-1.0%	2.6	2.7	2.5	2.6±0.08
Chlorpyriphos	Conc.-0.2%	1.2	1.1	1.1	1.13±0.04
	Conc.-0.4%	1	0.9	1	0.96±0.04

1.5%	Conc.-0.6%	0.8	0.9	1	0.9±0.08
	Conc.-0.8%	0.8	1	0.8	0.86±0.09
	Conc.-1.0%	0.4	0.5	0.4	0.43±0.04
Metalaxyl 35%	Conc.-0.2%	2	1.8	1.8	1.86±0.09
	Conc.-0.4%	0.4	0.3	0.3	0.33±0.04
	Conc.-0.6%	0.4	0.4	0.2	0.33±0.09
	Conc.-0.8%	0.3	0.3	0.2	0.26±0.04
	Conc.-1.0%	00	00	00	00±00

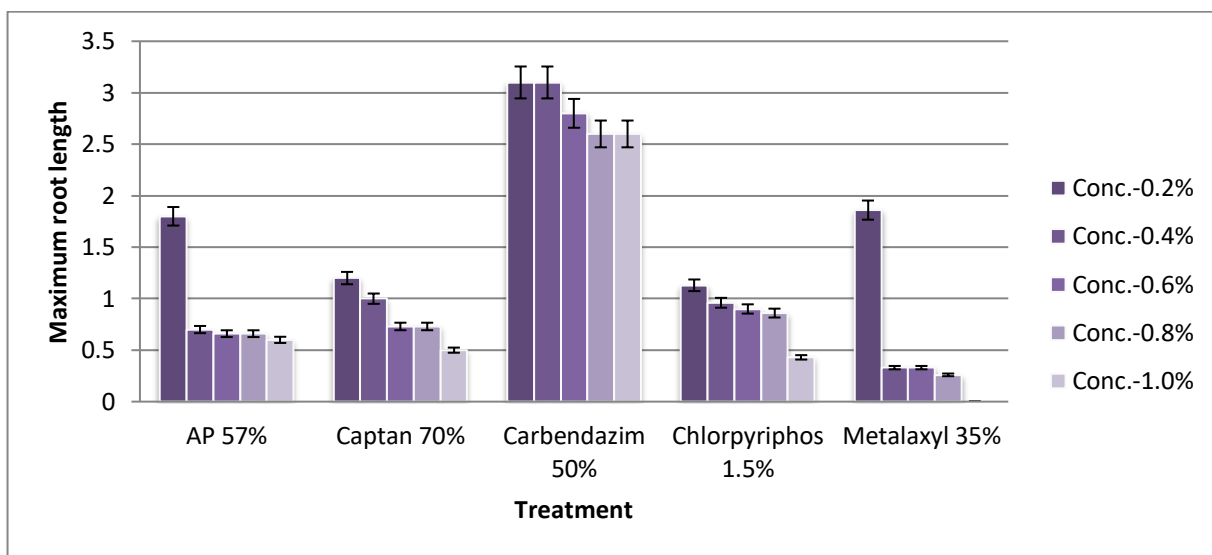


Fig.10: Graphical representation of average root lengths (cm) of *Lens culinaris* treated with chemical preservatives at day 4.

### 5.2.1.3. *Allium cepa*

Table no.7 shows the growth of lentil root in different preservatives in different concentrations. It shows a gradual decrease in root length as the concentrations are increased. Where as in control root lengths are highest. So we can say chemical preservatives can effect in crop production.

**Table no. 8:** Average root lengths (cm) of *Allium cepa* treated with chemical preservatives at day 4:

Preservatives name	Concentrations	Root length in different concentrations			Mean± SD
		R1	R2	R3	
Control		3	3.2	2.8	3±0.16
AP 57%	Conc.-0.2%	2.8	2.6	2.7	2.7±0.08
	Conc.-0.4%	1.7	1.6	1.6	1.63±0.04
	Conc.-0.6%	1.9	1.8	2	1.9±0.08
	Conc.-0.8%	1.8	1.6	1.7	1.7±0.08
	Conc.-1.0%	1.8	2	2	1.93±0.09
Captan 70%	Conc.-0.2%	2	1.9	1.7	1.86±0.15
	Conc.-0.4%	1.6	1.8	2	1.8±0.2
	Conc.-0.6%	1.6	1.7	1.7	1.66±0.04



	Conc.-0.8%	1.2	1	1.1	1.1±0.08
	Conc.-1.0%	0.8	1	1	0.93±0.09
Carbendazim 50%	Conc.-0.2%	2.2	2	1.9	2.03±0.12
	Conc.-0.4%	1.8	1.9	2	1.9±0.08
	Conc.-0.6%	1.4	1.6	1.8	1.6±0.16
	Conc.-0.8%	1.2	1.3	1	1.16±0.12
	Conc.-1.0%	1	0.9	0.8	0.9±0.08
Chlorpyriphos 1.5%	Conc.-0.2%	1.8	1.7	1.9	1.8±0.08
	Conc.-0.4%	1.6	1.5	1.4	1.5±0.08
	Conc.-0.6%	1.2	1	0.9	1.03±0.12
	Conc.-0.8%	1	0.8	1	0.93±0.09
	Conc.-1.0%	0.6	0.8	0.9	0.76±0.12
Metalaxyl 35%	Conc.-0.2%	1.5	1.4	1.4	1.43±0.04
	Conc.-0.4%	1.2	1.1	1.1	1.13±0.04
	Conc.-0.6%	1.2	1	1	1.06±0.09
	Conc.-0.8%	0.2	0.1	0.1	0.13±0.04
	Conc.-1.0%	00	00	00	00±00

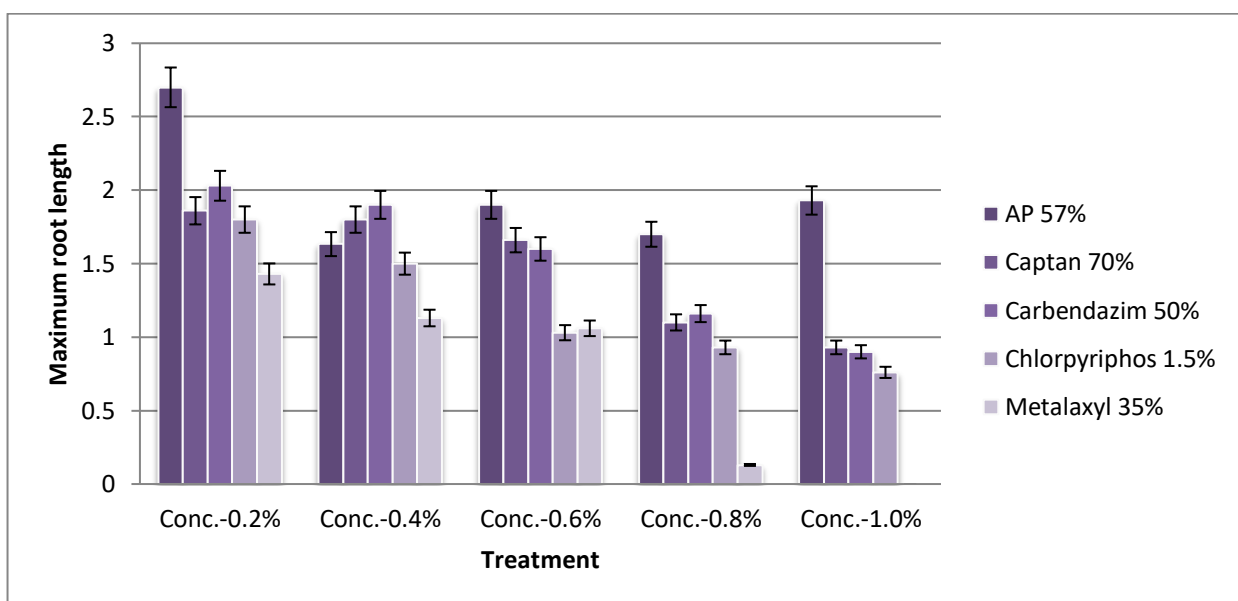


Fig.11: Graphical representation of average root lengths (cm) of *Allium cepa* treated with chemical preservatives at day 4.

### 5.3 Cytological study:

#### 5.3.1. *Allium cepa* :

Table no. 7 shows the microscopic observations of *Allium cepa*. The mitotic index of root tip cells treated with different concentrations of different chemical preservatives decreased comparatively of the control. Chromosomal aberration frequency estimation indicates that all the stages of mitotic cell division. Most types of chromosome aberrations observed in high percentage were sickness, disturbance c-metaphase, chromosome bridges in anaphase, lagging chromosome, micronuclei.

**Table no.9:** MI and Chromosomal aberration frequency index studied in *Allium cepa* root tips treated with different preservatives:-

Preservative name	Concentrations	Total number of cells analysed	No. of cells showing divisions			MI(%) [Mean± SD]	No. of cells showing aberration			CF(%) [Mean± SD]
			R1	R2	R3		R1	R2	R3	
Contro		600	48	52	36	45.33±1.79	0	0	0	0±0
AP 57%	0.2%	600	39	34	28	33.66±1.49	07	04	3	4.66±1.69
	0.4%	600	32	26	33	30.3±3.09	08	03	6	5.66±2.05
	0.6%	600	23	30	26	26.33±2.86	07	11	12	10±1.64
	0.8%	600	28	28	24	26.66±1.88	14	08	11	11±2.44
	1.0%	600	24	26	21	23.66±1.05	16	11	09	12±1.94
Captan 70%	0.2%	600	60	57	53	56.66±1.86	05	07	05	5.66±0.94
	0.4%	600	42	56	54	50.66±1.18	06	08	11	8.33±2.05
	0.6%	600	47	44	39	43.33±1.29	08	16	09	11±1.55
	0.8%	600	38	38	42	39.33±1.88	06	13	12	10.33±2.09
	1.0%	600	29	29	31	29.66±0.94	14	09	15	12.66±1.62
Carben dazim 50%	0.2%	600	30	39	20	29.66±0.77	09	06	03	6±2.44
	0.4%	600	30	22	43	31.66±2.65	10	09	08	9±0.81
	0.6%	600	11	13	09	11±1.63	11	10	09	10±0.81
	0.8%	600	13	10	07	10±2.44	11	09	13	11±1.63
	1.0%	600	20	40	20	26.6±62.42	10	12	14	12±1.63
Chlorp yripho s 1.5%	0.2%	600	63	57	52	57.33±1.42	08	02	05	5±2.44
	0.4%	600	65	51	54	56.66±1.49	06	07	08	7±0.81
	0.6%	600	38	44	39	40.33±1.01	09	16	06	10.33±1.18
	0.8%	600	29	24	22	25±2.62	14	15	08	12.33±1.09
	1.0%	600	33	44	31	36±2.94	06	11	13	10±1.94
Metala xyl 35%	0.2%	600	17	25	18	20±1.71	07	02	05	4.66±2.05
	0.4%	600	20	13	18	17±2.55	10	05	13	9.33±1.29
	0.6%	600	15	08	17	13.3±31.94	07	15	10	10.66±1.29
	0.8%	600	13	07	09	9.66±0.85	10	18	12	13.33±1.39
	1.0%	600	00	00	00	0±0	00	00	00	0±0

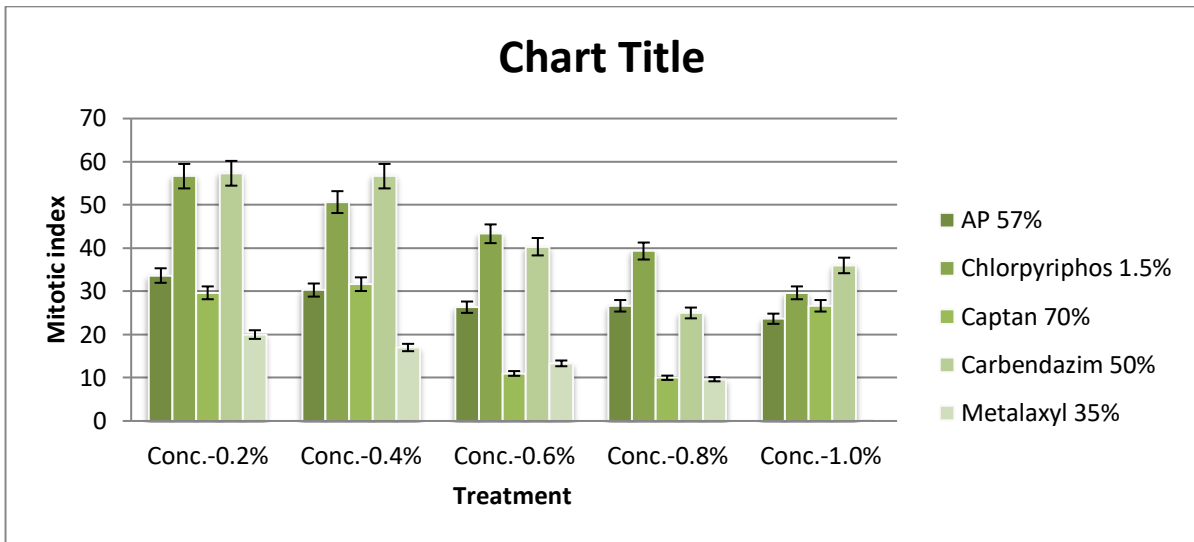


Fig 12: Graphical representation of MI in onion root tip cells treated with different preservatives

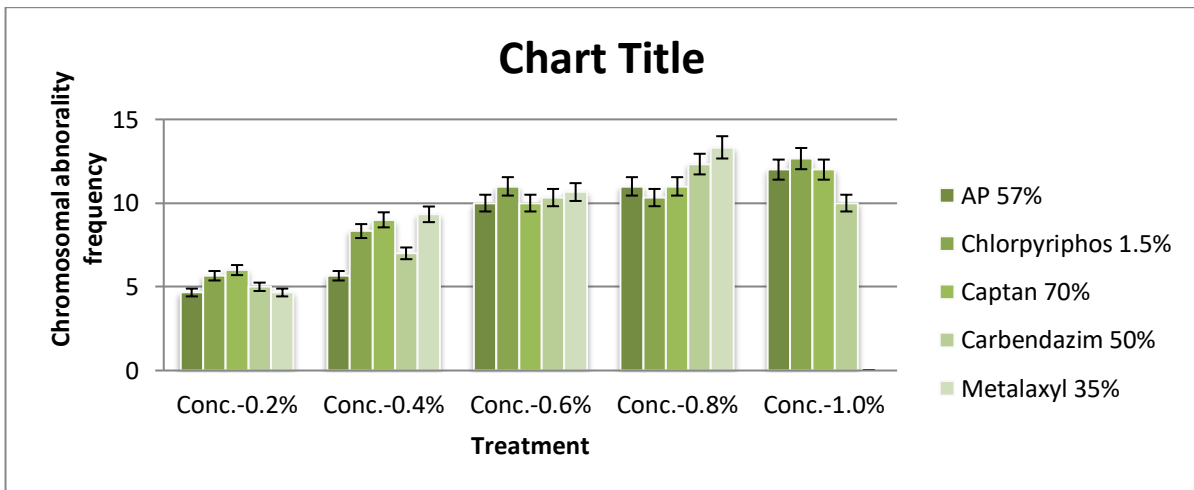


Fig . 13: Graphical representation of chromosomal abnormality frequency in onion root tip cells treated with different preservatives

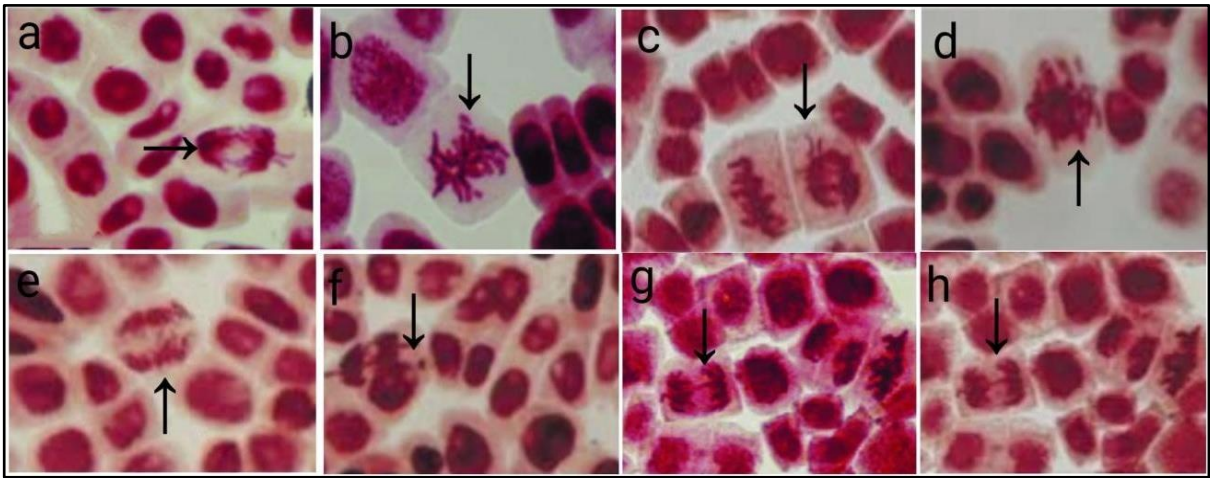


Fig. 14: Photographs showing chromosomal aberration in *Allium cepa* root tips -(a, e, f, g) double crossed bridge in anaphase; (b, d) multiple fragmentation of the chromosomes into aberrant metaphase with an implicit double nature of fragments; (c) configuration of single and double crossed bridge with a pair of long fragments in anaphase of a single cell; (h) bridge in the form of two linked chromatids like links in the chain;

### 5.3.2. *Cicer arietinum*:

Table no.8 shows the microscopic analysis of *Cicer arietinum* root tip treated with different concentrations of different preservatives. Chromosomal aberration frequency estimation indicates that all the tested conc. Of different preservatives (0.2%, 0.4%, 0.6%, 0.8%, 1.0%), induced chromosomal stages of mitotic cell division. The highest mitotic index observed in Captan 70% and lowest in Metalaxyl 35%. Highest chromosomal aberration frequency occurs in AP 57% and in Carbendazim 50%.

**Table no.10:** MI and Chromosomal aberration frequency index studied in *Cicer arietinum* root tips treated with different preservatives:-

Preservative Name	Concentration	Total number of cells analysed	No. of cells showing divisions			MI(%) [Mean±SD]	No. of cells showing aberration			CF(%) [Mean±SE]
			R1	R2	R3		R1	R2	R3	
Control		600	48	52	36	45.33±1.79	0	0	0	0±1.69
AP 57%	0.2%	600	39	34	28	33.66±1.49	07	04	3	4.66±2.05
	0.4%	600	32	26	33	30.33±2.09	08	03	6	5.66±2.16
	0.6%	600	23	30	26	26.33±2.86	07	11	12	10±2.44
	0.8%	600	28	28	24	26.66±1.88	14	08	11	11±1.94
	1.0%	600	24	26	21	23.66±1.05	16	11	09	12±0.94
Captan	0.2%	600	60	57	53	56.66±2.46	05	07	05	5.66±2.05

70%	0.4%	600	42	56	54	50.66±1.6	06	08	11	8.33±2.05
	0.6%	600	47	44	39	43.33±1.29	08	16	09	11±1.55
	0.8%	600	38	38	42	39.33±2.30	06	13	12	10.33±1.0
	1.0%	600	29	29	31	29.66±0.94	14	09	15	12.66±1.6
Carben dazim 50%	0.2%	600	30	39	20	29.66±0.77	09	06	03	6±2.44
	0.4%	600	30	22	43	31.66±1.6	10	09	08	9±0.81
	0.6%	600	11	13	09	11±2.44	11	10	09	10±0.81
	0.8%	600	13	10	07	10±1.42	11	09	13	11±1.63
	1.0%	600	20	40	20	26.66±1.42	10	12	14	12±1.63
Chlorp yriphos 1.5%	0.2%	600	63	57	52	57.33±1.49	08	02	05	5±1.44
	0.4%	600	65	51	54	56.66±2.01	06	07	08	7±0.81
	0.6%	600	38	44	39	40.33±2.62	09	16	06	10.33±0.4
	0.8%	600	29	24	22	25±1.94	14	15	08	12.33±1.0
	1.0%	600	33	44	31	36±1.71	06	11	13	10±1.94
Metala xyl 35%	0.2%	600	17	25	18	20±1.55	07	02	05	4.66±2.05
	0.4%	600	20	13	18	17±1.94	10	05	13	9.33±1.29
	0.6%	600	15	08	17	13.33±1.85	07	15	10	10.66±1.2
	0.8%	600	13	07	09	9.66±2.49	10	18	12	13.33±1.3
	1.0%	600	00	00	00	00±00	00	00	00	00±00

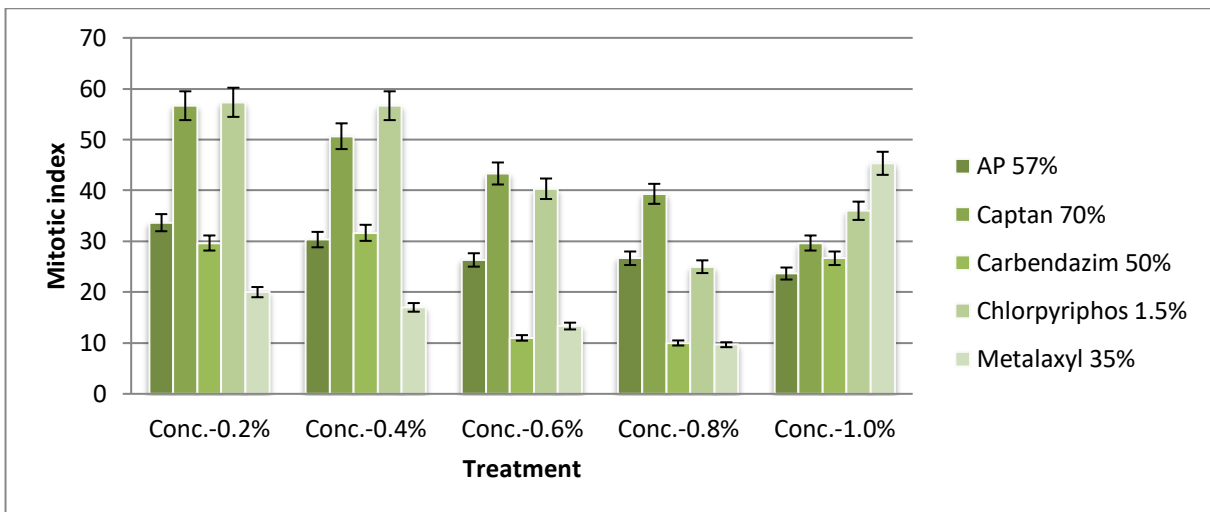


Fig 15: Graphical representation of MI in cicer root tip cells treated with different preservatives

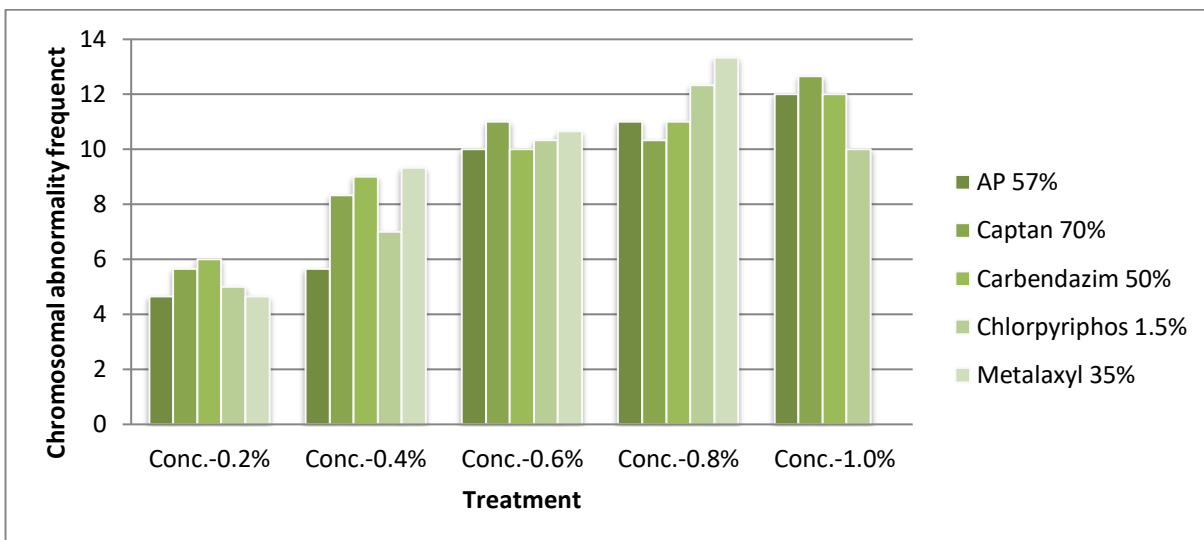


Fig . 16: Graphical representation of chromosomal abnormality frequency in cicer root tip cells treated with different preservatives

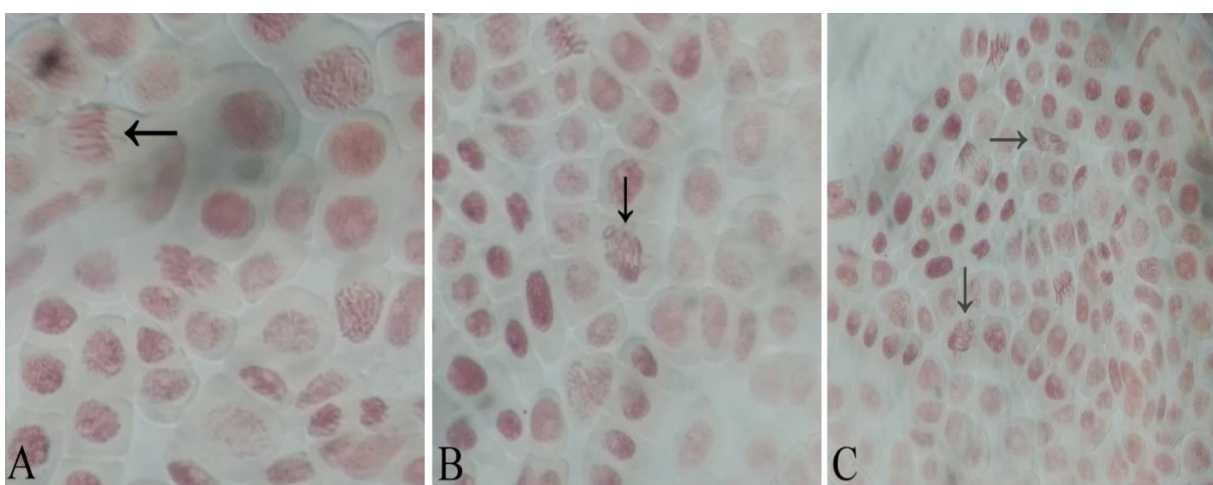


Fig:17: Photographs showing chromosomal aberration in *Cicer arietinum* root tips - (a) multiple fragmentation of the chromosomes into aberrant metaphase with an implicit double nature of fragments; (b, c) bridge in the form of two linked chromatids like links in the chain;

### 5.3.3. *Lens culinaris*:

Table no.8 shows the microscopic analysis of root tip of *Lens culinaris* treated with different concentrations of different preservatives. Chromosomal aberration frequency estimation indicates that all the tested conc. Of different preservatives (0.2%, 0.4%, 0.6%, 0.8%, 1.0%), induced chromosomal stages of mitotic cell division. The highest mitotic index observed in Captan 70%

and lowest in Metalaxyl 35%. Highest chromosomal aberration frequency occurs in AP 57% and in Carbendazim 50%.

**Table no.11:** MI and Chromosomal aberration frequency index studied in *Lens culinaris* root tips treated with different preservatives:-

Preservative name	Concentrations	Total number of cells analysed	No. of cells showing divisions			MI(%) [Mean±SD]	No. of cells showing abberation			CF(%) [Mean± SD]
			R1	R2	R3		R1	R2	R3	
Control		600	62	66	51	59.66±1.34	0	0	0	0±1.24
AP 57%	0.2%	600	36	38	32	35.33±2.49	05	08	06	6.33±1.35
	0.4%	600	27	26	34	29±1.55	05	05	10	6.66±2.29
	0.6%	600	26	28	22	25.33±2.49	07	12	04	7.66±1.85
	0.8%	600	33	26	28	29±2.94	15	08	06	9.66±1.41
	1.0%	600	21	18	19	19.33±1.68	12	09	12	3±1.69
Captan 70%	0.2%	600	54	52	58	54.66±1.86	05	02	06	4.33±2.16
	0.4%	600	53	44	49	48.66±0.34	03	08	07	6±2.05
	0.6%	600	46	43	39	42.66±0.94	09	11	06	8.5±1.39
	0.8%	600	37	41	26	34.66±1.49	05	13	11	9.66±1.86
	1.0%	600	28	36	19	27.66±1.76	12	16	09	12.33±1.241
Carben dazim 50%	0.2%	600	39	30	29	32.66±1.23	09	07	10	8.66±0.47
	0.4%	600	29	20	39	29.33±2.49	09	06	11	8.66±1.86
	0.6%	600	30	39	20	29.66±2.05	07	07	08	7.33±0.47
	0.8%	600	39	24	29	30.66±1.54	13	09	06	9.33±1.39
	1.0%	600	20	16	14	16.66±1.09	10	07	15	10.66±1.29
Chlorp yriphos 1.5%	0.2%	600	51	54	56	53.66±1.18	07	03	04	4.66±1.69
	0.4%	600	59	48	52	53±0.94	05	09	06	6.66±1.69
	0.6%	600	44	47	35	42±1.09	08	14	06	9.33±1.39
	0.8%	600	36	37	20	31±0.71	16	10	07	11±1.5
	1.0%	600	29	24	22	25±1.94	09	12	09	10±1.411.69
Metala xyl 35%	0.2%	600	30	27	18	25±2.09	06	07	10	7.66±1.69
	0.4%	600	27	13	31	23.66±1.71	06	15	08	9.66±1.85
	0.6%	600	13	20	15	16±0.94	18	08	19	15±0.96
	0.8%	600	16	14	09	13±1.94	20	14	21	18.33±2.09
	1.0%	600	00	00	00	00±00	00	00	00	00±00

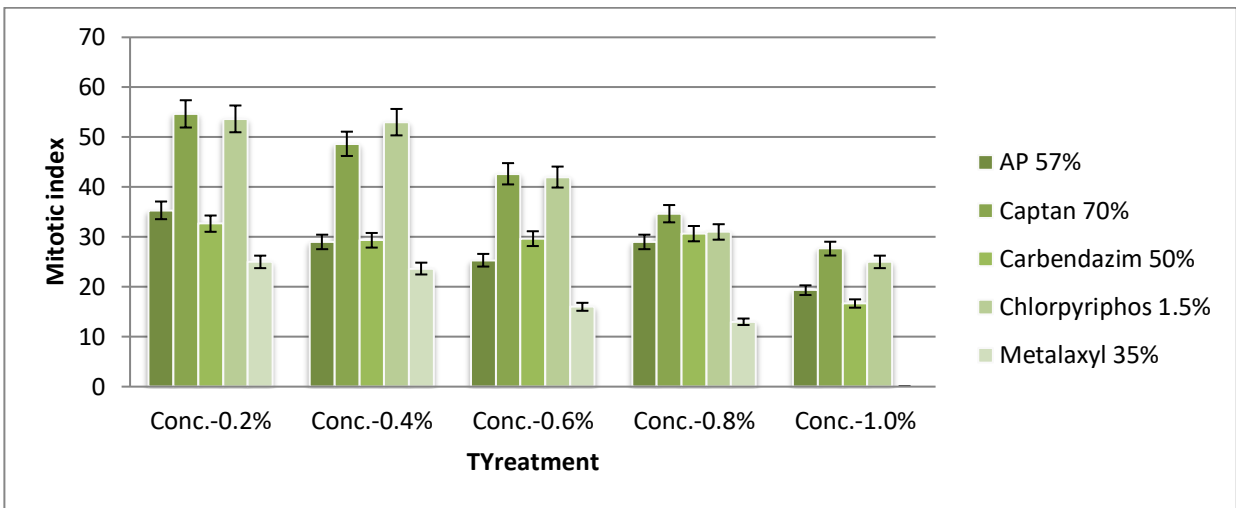


Fig 17: Graphical representation of MI in lentil root tip cells treated with different preservatives.

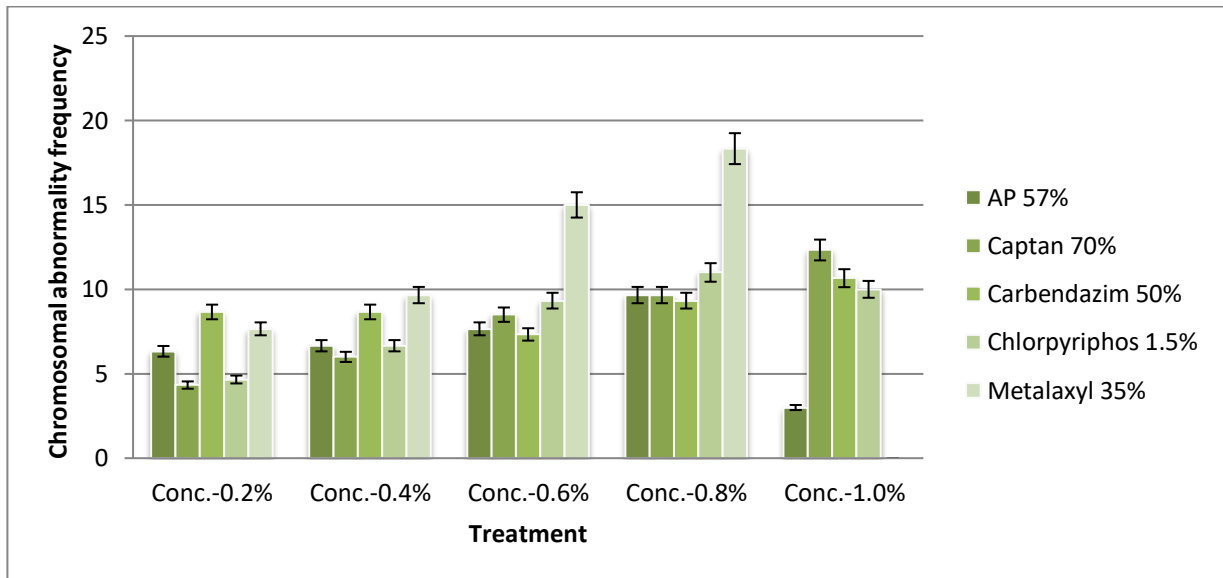


Fig . 18: Graphical representation of chromosomal abnormality frequency in lentil root tip cells treated with different preservatives.

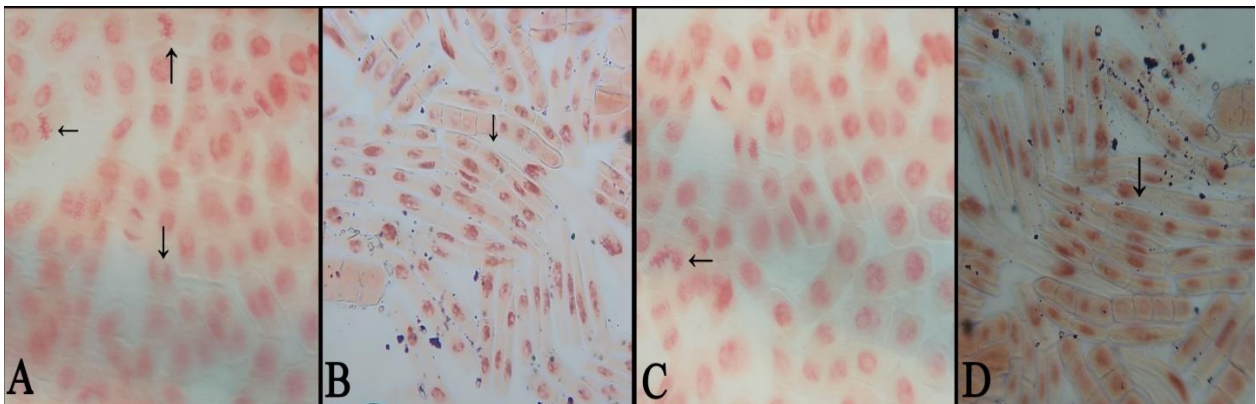


Fig. 19: Photographs showing chromosomal abnormality in *Lens culinaris* root tips -(A, B, C, D)



## **Chapter 6: Discussion**

## 6. Discussion

There are many causes to study and evaluate the effect of chemical preservatives on plants. We often consume seeds raw or in soaked condition that are treated with chemical preservatives to store them for long period and that is harmful for our health.

This experiment shows the increasing concentration of chemical preservatives shows a negative impact on plant germination and cytology. But it shows the right concentrations of preservatives to be used and also shows which preservative is less harmful. Different morphological features like germination index, root length were observed. GI is decreased when concentration of chemicals were increased. The average GI of *Cicer arietinum* in Chlorpyrifos 1.5% treatment (90.66) was highest and Captan 70% treatment was (16.33) shows the lowest. In case of *Lens culinaris* the GI was highest in AP 57% treatment (57.19) and in metalaxyl it was lowest (54.33). For *Allium cepa* the highest GI was shown in Captan 70% (65.8) and lowest in Metalaxyl 35% (33.4). The germination index decreases as the concentrations were increases in every chemical preservatives. In case of Metalaxyl 35% in the highest concentration GI was 0 for all the plant samples.

In case of root length observation the root lengths were decreases with increasing concentrations. In the lowest concentration average root length was 2.16cm and for the highest is was 1.34cm in *Cicer arietinum*. In case of *Lens culinaris* and *Allium cepa* same results were shown.

In this study the cytotoxic and genotoxic of five commonly used chemical preservatives were evaluated in *Allium cepa*, *Cicer arietinum* and *Lens culinaris*. In the present study the highest concentrations were cytotoxic i.e. significant reduction in MI in compare to control. Mitotic index is considered as a parameter helps to estimate the frequency of cellular division (Marcano et al., 2004) and the reduction of mitotic activities has been used frequently to trace substances that are cytotoxic (Linnainmaa et al., 1978; Smaka-Kincl et al., 1996). Here in his study the MI was greatly reduced with the increasing concentrations of the chemical preservatives. In case of *Allium cepa*, *Cicer arietinum* and *Lens culinaris* lowest MI found in Metalaxyl 35%. For In case of Carbendazim 50% and Metalaxyl 35% MI is below 20% and decreases gradually with increasing concentrations.

Also different chromosomal aberration like stickiness, chromosome laggards, double crossed bridge in anaphase c-metaphase were observed. In all cases most common chromosomal aberration induced by four pesticides is sticky chromosome. Stickiness of chromosomes can

cause abnormal DNA condensation (Österberg et al., 1984) and the entanglement of inter-chromosomal chromatin fibers (Patil & Bhat, 1992). It could therefore be concluded that the four test samples taken can cause abnormal DNA condensation, abnormal chromosome coiling and entanglement of inter chromosomal chromatin fibre. The mode of chromosomal aberration increases in higher concentration than lower concentration. For Metalaxyl the CF is 4.54 and for AP 57% it was 4.75. Whereas for AP 57% the CF is 4.75 in highest concentration in *Allium cepa*. In case of *Cicer arietinum* CF was highest in AP 57% and then Metalaxyl 35% comes in second.

Overall Metalaxyl 35% and Aluminium phosphide 57% is more toxic than the other two preservatives. According to Norppa (2004), most adverse effects on health, caused by genotoxins, result from genetic damage in somatic as well as germinal cells. It has also been suggested that any genotoxic effects of environmental chemicals, is likely to result from abnormalities of the cell division process (Parry et al., 1999). The five chemical preservatives therefore, have the potential to cause aneuploidy in exposed organisms and adverse human health and environmental effects.

This experiment shows the increasing concentration of chemical preservatives shows a negative impact on plant germination and cytology. But it shows the right concentrations of preservatives to be used and also shows which preservative is less harmful to plants.

## **Chapter 7: Conclusions**

## 7. Conclusions

From this study it can be concluded that the experiment demonstrates a negative impact on plant germination and cytology as the concentration of chemical preservatives increases. It also identifies the appropriate concentrations of preservatives to be used and determines which preservative is less harmful to plants. It is observed that the germination index (GI) decreases with increasing concentrations of chemical preservatives. The highest GI values were found in the treatment of Chlorpyrifos 1.5% for *Cicer areitinum*, AP 57% for *Lens culinaris*, and Captan 70% for *Allium cepa*, while the lowest GI values were observed in the treatment of Captan 70% for *Cicer areitinum*, metalaxyl 35% for *Lens culinaris*, and Metalaxyl 35% for *Allium cepa*. The root lengths were also found to decrease with increasing concentrations of preservatives. Furthermore, the study evaluated the cytotoxic and genotoxic effects of five commonly used chemical preservatives on *Allium cepa*, *Cicer arirtinum*, and *Lens culinaris*. The results showed that higher concentrations of the preservatives were cytotoxic, resulting in a significant reduction in the mitotic index (MI) compared to the control. Chromosomal aberrations such as stickiness, chromosome laggards, and double crossed bridges were observed, with sticky chromosomes being the most common aberration induced by the pesticides. The mode of chromosomal aberration increased with higher concentrations of the preservatives. Overall, it is concluded that Metalaxyl 35% and Aluminium phosphide 57% are more toxic than the other preservatives. The uses of these preservatives should be reduced or other substitutes can be used in post-harvest preservation for maintaining the seed. In the future, the abnormalities in seed germination and plants would be decreased. However, this study also provides insights into the appropriate concentrations of preservatives to be used and identifies the preservatives that are less harmful to post harvest preservation.

## **Chapter 8: Future Scope**

## **8. Future Scope:**

The current study evaluated the effects of five commonly used chemical preservatives. Future studies could expand this list to include a wider range of preservatives that are commonly used in seed preservation. This would provide a more comprehensive understanding of the potential negative effects of different preservatives on plant germination and cytology. It would be beneficial to compare the effects of different preservatives on seeds of the same crop. This would help identify which preservatives have the least harmful impact on germination and cytology. Such comparative studies can guide farmers and seed suppliers in selecting the most appropriate preservative for seed storage. Given the negative effects observed with chemical preservatives, exploring alternative methods for seed conservation is essential. Future research could focus on identifying and evaluating alternative conservation techniques such as natural or organic preservatives, biological agents, or physical treatments (e.g., temperature, humidity) that can effectively preserve seeds without compromising their germination and cytological properties. This study focused on the immediate impact of preservatives on seed germination and cytology. However, it would be valuable to investigate the long-term effects of preservative use on plant growth, development, and overall crop yield. Longitudinal studies that track the performance of plants grown from preserved seeds over multiple generations could provide valuable insights into the potential consequences of preservative use. In addition to evaluating the effects on plant health, future studies should also assess the environmental impact of chemical preservatives. This includes investigating their potential toxicity to non-target organisms, their persistence in soil and water systems, and their potential to contribute to pollution or other ecological disturbances. Such studies can help ensure that seed preservation practices are environmentally sustainable. While this study focused on the effects of preservatives on plants, it is important to consider potential implications for human health as well. Future research should investigate the transfer of preservatives or their breakdown products from preserved seeds to the human food chain. This would help assess any potential risks associated with consuming crops derived from preserved seeds and guide food safety regulations. To gain a deeper understanding of the mechanisms underlying the negative effects observed, future studies could employ genetic analysis techniques. These analyses could identify specific genes or pathways that are affected by preservatives, providing insights into the molecular mechanisms behind the observed cytological changes and potential genotoxic effects.

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