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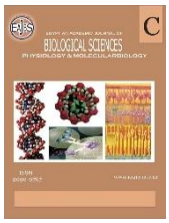

Cytogenotoxic Effects of Different Concentrations of Copper Sulfate Using the *Allium sativum* Assay

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ABSTRACT

An essential model for assessing the cyto-toxicity and geno-toxicity of heavy metals is garlic (*Allium sativum*) assay. Using *Allium sativum* model, the current study sought to determine the cytogenotoxicity of copper ($CuSO_4$). Garlic cells at the root tip cultured on distilled water added to with different $Cuso_4$ concentrations (0, 1, 5, and 10 ppm), were used to create slides with mitotic cells. In order to assess cytotoxicity and genotoxicity according to the evaluation of the mitotic index, mitotic inhibition, and length of the roots, the slides were examined under a light microscope. The findings demonstrated that the applied concentrations had a significant effect on the cell, resulting in negative effects on the chromosome structure, root growth, mitotic inhibition, and mitotic index. As the concentration of $CuSO_4$ increased, various chromosomal abnormalities were seen, including the formation of bridges, fragmentation fragments, lagging chromosomes, stickiness, bi-nucleate cells, and micronucleus formation. The cells were less affected by even the least concentration (1ppm) than by the higher concentrations, although they still exhibited significant cytotoxic and genotoxic effects ($p < 0.05$) in comparison to the control (0.0 ppm). The effects on clastogenic alterations increase with concentration. Even at low doses, copper sulfate is cytotoxic, and at larger quantities, it can have mito-depressive effects, as evidenced by the anomalies in both mitotic spread and root growth. The findings of this study can serve as a roadmap to ensure that individuals and organs are adequately protected when using $CuSO_4$ on a regular basis.

INTRODUCTION

A member of the Alliaceae family, garlic (*Allium sativum* L.) is one of the most important vegetables in the world. Central Asia is the center of origin, according to Vavilov (1926). Since ancient times, garlic's anticancer, antimicrobial, and antioxidant qualities have made it one of the most important plant species used in traditional medicine (Hirata 2016; Reiter *et al.*, 2017). The negative effects of heavy metals on living cells are referred to as cytotoxicity. If a heavy metal causes genetic abnormalities in a cell, it may also be genotoxic. A mutation is a change that may pose risks or potentially serious threats to different systems. The organism's germ or somatic cells may be affected by this alteration, which could have long-lasting and heritable effects on DNA that are passed down to subsequent generations (Kachout *et al.*, 2009). A preferred method of assessing mutagenicity is cytological observation of either mitotic or meiotic behavior (Feretti *et al.*, 2007).

Testing for cytotoxicity was crucial when carrying out biological evaluation of substances in cells. Tests on metals or medications can reveal the degree of cytotoxicity and harm to physiology and Structure of cells, including disturbance of the cell membrane,suppression of the production of proteins,as well as irreversible receptor binding. Rapid, precise, and economical cytotoxicity assays that assess toxicity to entire organisms by analyzing their effects *in vivo* or/*in vitro* are therefore desperately needed (Onciu *et al.*, 2018; Pistelli *et al.*, 2017 and Baktemur, 2023). Finding out how chemicals or biologics affect living tissues can be greatly aided by assays. According to Heldari and Sarani (2011), cytotoxicity assays seek to influence a number of cellular processes, including nucleotide uptake activity, ATP synthesis, enzyme activity, coenzyme production, and cell membrane permeability.The lethality of compounds and complex combinations *in vivo* can be experimentally determined using garlic (*Allium sativum*) as a model (Metwally, 2009). *A. sativum* assay is regarded as a simple and affordable screening technique that outperforms other short-term screening techniques that call for the inclusion of exogenous metabolic systems and the prior preparation of test samples. According to Yildiz *et al.* (2009), this assay can assess a substance's toxicity and genotoxicity. This involves evaluating mitotic abnormalities and chromosomal abnormalities (Nefic *et al.*, 2013). The *Allium sativum* test is frequently used to assess DNA damage brought on by mutagens. According to Amrita *et al.* (2008) and Reiter *et al.* (2017), this test has also proven successful in investigating the genotoxicity of environmental pollutants and the cytotoxicity of chemical agents. There is a strong correlation between the *Allium sativum* assay and testing methods used in mammals. Das *et al.* (2021) used an *Allium cepa* assay to examine the cytogenetic effects of the anticancer medications, Erlotinib and Doxorubicin. According to Onisan *et al.* (2025), the body needs between 1.4 and 2 milligrams of copper (Cu^{+2}) per kilogram of body weight for metabolic functions. In additionto being necessary to the living being, Cu^{+2} are employed as well extensively with the field of agricultural activities.

Copper sulphate has shown great efficacy as a fungicide against rot and other plant infections. The extensive usage of copper in water conduits, the electrical sector, and agriculture as a fertilizer or fungicide has brought attention to the necessity of promptly preventing dangerous concentrations of the metal. Despite being an important metal, consuming 10–20 mg of Cu^{+2} per day might result in poisoning symptoms, while consuming 60 mg or more per day can cause damage to the liver and, in certain situations, even demise. According to Hudcová *et al.* (2019), the legal limits for copper in each EU nation vary from 75 to 1750 mg/kg DM (≡1.180 mM–27.539 mM). Given the significant differences in heavy metal restrictions between nations, an early warning system is required. The use of bio-indicators, like the Allium test, can be a useful strategy for avoiding soil pollution. This method makes it possible to identify levels of heavy metals and other substances that might not yet be observable to plants but could jeopardize physiological functions including root system growth and nutrient absorption.

Despite the fact that $CuSO_4$ is poisonous and should not be handled carelessly, many firms and laboratories depend on it for everyday operations, and some people are exposed to it on a daily basis while working. Concern over copper compound pollution in labs and the environment is growing, and it has worldwide implications. $CuSO_4$ is extremely poisonous and corrosive, and it can lead to both acute and chronic poisoning (Elshkaki *et al.*, 2016). The kidneys and neurological system may suffer as a result of copper poisoning, which can happen both by ingestion and skin absorption into the bloodstream (Caglarırmak and Hepcimen, 2010 and Elshkaki *et al.*, 2016). According to Elshkaki *et al.* (2016), exposure to $CuSO_4$ can cause a number of

symptoms, such as skin rashes, visual impairment, memory loss, and mental issues. An inorganic chemical called copper sulfate is created when copper and sulfur are combined. Fungus, flora, molluscs, root systems, bacteria, and algae can all be killed by it. The amount of copper determines how dangerous copper sulfate is. Copper is a necessary mineral. It is present in food, water, and the environment. Assays for identifying genotoxic compounds involve assessing the degree of DNA damage in cells that have been exposed. This damage may result from point mutations, loss of excision repair, cross-linking, structural and numerical chromosomal aberrations, and chromosomal breakage, including single and double-strand breaks (Yadav *et al.*, 2009; Silveira *et al.*, 2017; Topal *et al.*, 2017 and Sarac *et al.*, 2019). In most cases, a variety of illnesses can arise from harmful changes in genetic material. To assess the genotoxicity of substances, a variety of techniques for performing toxicological testing in vitro and in vivo have been devised. Therefore, using the *Allium sativum* model in this study to evaluate the toxic effects of varying $CuSO_4$ concentrations on the root and mitotic characteristics to determine the presence of mitotic indices and evaluate chromosomal aberrations, nuclear abnormalities, and micronuclei at different stages of mitosis.

MATERIALS AND METHODS

1- Plant Material Preparation and Treatments:

Bulbs of *Allium sativum* (2n = 16) purchased from a local shop in Qalubia Governorate, Egypt. Healthy bulbs of almost equal sizes about 27 mm across, were selected for the experiment after being cleaned and allowed to dry in the sun. Concentrations of 0, 1, 5, and 10 ppm of copper sulfate ($CuSO_4.5H2O$) were used, which was obtained as a high purity product from Sigma-Aldrich (USA). Deionized water employed as negative control. The original roots' circle was left uninjured when the dry, scaly white covering leaves and ancient roots that were affixed to the basal stem were removed to prepare the garlic bulbs. The effects of the $CuSO_4$ dilute on development of roots and *in vivo* creation of *Allium sativum* L chromosomal abnormality were assessed. Ten distinct cloves served as repetition for each copper sulfate dilution other than control, as well as the basal stalk of the garlic heads was suspended in tiny 100 ml jars for each of the examined $CuSO_4$ concentrations. The sample was stored in a dark laboratory closet at 25 ± 1 °C. Garlic cloves were closely inspected every day to track root emergence, and the treatment solutions were switched every other day. After ten days, the cloves' root length was examined, which helped determine how $CuSO_4$ at different doses inhibited growth.

2- Root Harvest and Slide Preparation:

Sterilized scissors were used to cut off the tips of the *Allium sativum* roots after they reached a length of around 3 cm. After that, the tips are gathered and labelled with the treatments in specimen containers. After using forceps to remove the root tips from the solution, they were washed and then placed in newly made ethanol: glacial fixatives (3:1, v/v) for a whole day. The tips were then hydrolyzed for using 5 N HCl, and the HCl was disposed of. The tips were then placed to sterile microscope plates, where the remaining root portions were thrown away and 1-2 mm section of the rising tip was removed. The tip was then coated with 2% (w/v) aceto-carmine, allowed to sit for ten minutes, and then covered with a coverslip. In order to prepare the slide for microscopic examination, after being crushed in 45% acetic acid, the root tips then lightly struck to form a muddy solution, which was subsequently employed for staining. After that, a cover slide was placed above the slide.

3- Scoring of Slides and Data Analysis:

To see the impact of the CuSO4 concentrations at various phases of mitosis, slides were produced. A light microscope was used to view the slides, gradually increasing in objective from the lowest (× 10) to the highest (× 40). The dividing and nondividing cells were seen using a digital camera that was connected to the eyepiece of the microscope in order to compute the mitotic index. At each step of mitosis, chromosome shapes,

disruptions, or abnormalities were recorded. Three attempts of every remedy yielded about 800 cells, for a total of 2400–2500 cells per treatment. The mitotic index (MI), the frequency of cells exhibiting chromosomal aberrations (CAs), such as bridges, fragments, sticky chromosomes, polar deviation, pulverization, and others, and the frequency of micronucleus (MN) creation were evaluated.

4- Cytotoxicity Determination:

The response as well as shape of chromosomes in cells exposed to varying doses of CuSO4 or those not exposed (control) were compared. The CuSO4 dose was deemed harmful for cells exhibiting chromosomal destruction, cell death, lowered mitotic index, or mitotic abnormalities. Furthermore, the treatment groups' levels of micronuclei (MNs) were investigated. Eq (1) was used to calculate the mitotic index (MI) (Sehgal et al., 2006). Changes in the amount or shape of chromosomes after encountering chemical compounds are known as chromosomal aberrations (CAs). Fiskejo's (1993) approach, Eq. (2) (Mitotic inhibition= (Mitotic index in the control-mitotic index of test concentrations)/Mitotic index of the control)) X 100, was used to quantify mitotic inhibition. According to Pavlica et al. (2000), the frequency of MNs was calculated as ‰ MNs in interphase cells per 1000 cells. Frequent anomalies were visible in representative micrographs.

Eq. (1) Mitotic index = (Dividing cells in all phases / Total number of cells) × 100

Eq. (2) Mitotic inhibition = [(mitotic index in the control − mitotic index of test concentration)/Mitotic index of the control] × 100

5- Data Analysis:

Using SPSS version 20 (IBM Corp. Armonk, NY, USA), the data gathered on chromosomal aberrations, mitotic indices, and root parameters were subjected to the analysis of variance (ANOVA). Tukey's honest significant difference (HSD) test was used to evaluate the significant level between the treatment means at a p-value of less than 0.05. It was determined what proportion of cells had chromosomal abnormalities.

RESULTS

Data in Figure 1, shows the consequences of varying $CuSO_4$ concentrations on root development. All three dosages showed an inhibiting effect on garlic root growth comparing with control. Higher concentrations (5, 10 ppm) showed noticeably delayed root emergence, while garlic treated with with lesser amounts (1ppm) showed delayed rooting. The root growth was typical in the duplicates where the garlic was not treated with $CuSO_4$ (control). On the fifth day, the garlic began to sprout and grew to a length of more than 1 cm. As the concentration of $CuSO_4$ rose, so did the delay in root emergence. The untreated control and those treated with 1ppm $CuSO_4$ had average root lengths of 6.79, 4.06, 3.22, and 1.75 cm, respectively, even after 10 days. Higher dosages, however, markedly slowed the formation of roots. Samples treated with 10 ppm $CuSO_4$ showed the greatest harmful effect; at day 10, the roots' length was under two centimetres (Fig. 2). The number of dividing cells and the mitotic inhibition were directly correlated, according to an analysis of the cytological effects of various $CuSO_4$ concentrations (Table 1). The number of cells actively dividing increased at a lower concentration (1ppm) whereas the number of cells undergoing division reduced as the concentration increased.

Furthermore, the largest number of dividing cells in all phases was seen in the untreated cloves (control). Similar to this, concentration caused a corresponding decrease in the percentage of mitotic index (%MI), which shows the percentage of cells that are dividing rapidly relative to the overall quantity of cells seen. The MI was employed in this investigation as a quantitative indicator of cell division rates, which declined with increasing $CuSO_4$ concentration. On the other hand, a concentration-dependent pattern was seen in the percentage of mitotic inhibition. Greater mitotic inhibition was the outcome of higher $CuSO_4$ concentrations. Cellular division was thus more and more hindered as the concentration rose.

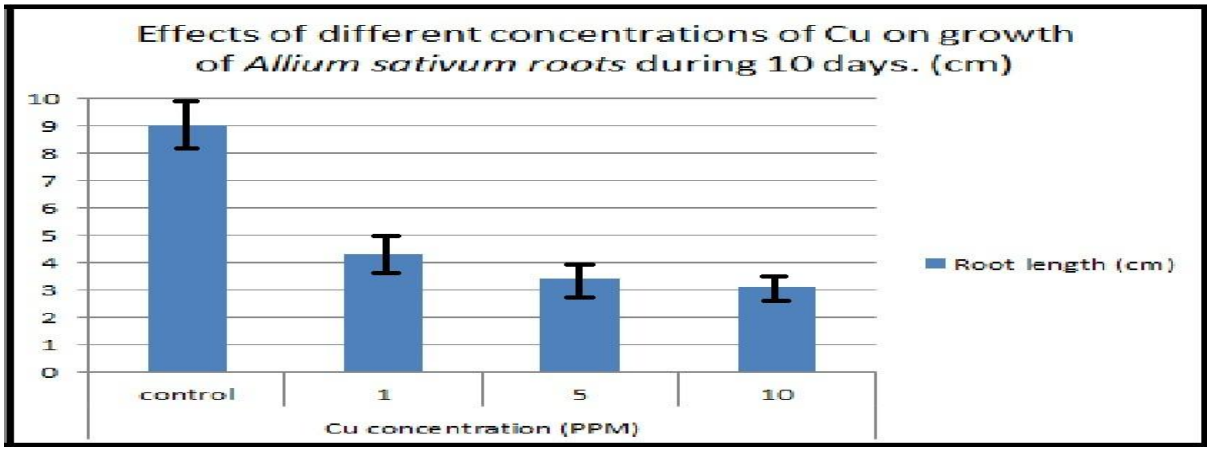

Fig. 1 Variations in root lengths of *Allium sativum* cloves were observed under different concentrations of $CuSO_4$. The mean value of three replicates ±SE is presented. The root number was determined by counting at least 0.5 cm long roots after ten days of treatment.

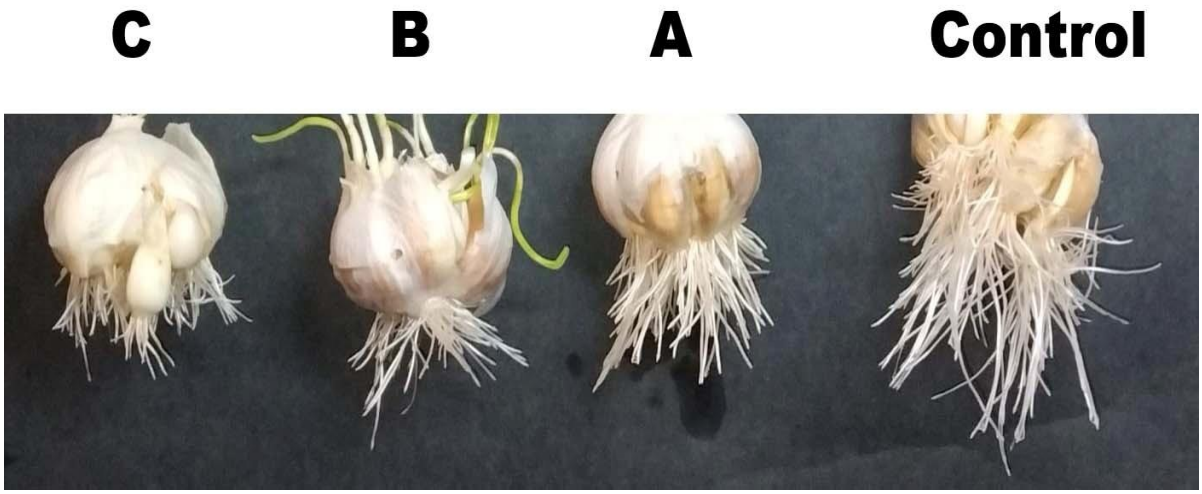

Fig. 2 *Allium sativum* root formation in response to exposure to varying concentrations of $CuSO_4$ in 10 days after (Control: Deionized water, A:-1 , B:-5, and C:-10 ppm).

The root meristem of *A. sativum* treated with different concentrations of $CuSO_4$, a surface sterilizing agent frequently employed in industry or laboratories, showed different types and frequencies of chromosomal aberrations, according to cytological examination.Figures 3 and 4, display the observed normal division, and chromosomal aberration data, and Table 2, displays the results. Among the different concentrations of $CuSO_4$, there were significant variations in the frequency of chromosomal abnormalities such as anaphase bridge, stickiness, C-mitosis, chromosome fragmentation, and micronucleus production. Onion root cells treated with greater concentrations (10 ppm) of $CuSO_4$ showed more anaphase bridges, which are defined by imperfect segregation of nuclear material to the poles due to persistent DNA tangling between sister chromatids. All of the other recognized abbreviations follow the same pattern. Chromosome abnormalities become more common when root cells are exposed to high amounts of $CuSO_4$. In cells treated with low doses (1ppm) of $CuSO_4$, chromosomal genotoxicity and damage were observed. However, these levels are harmful to cells, resulting in sticky masses of chromatin that can cause chromosome breakage and sterility (Table 2). Furthermore, a significant proportion of cells developed micronuclei at a

concentration of 5 ppm, and much more did so in cells exposed to 10 ppm. Onion root cells that were not treated showed a few chromosomal abnormalities, including irregular prophase, anaphase lagging, and pulverization.

However, their frequency rose with increasing $CuSO_4$ concentrations. The treated cloves showed higher frequencies of multipolar cells, binucleated cells, and nuclear abnormalities after being exposed to 10 ppm $CuSO_4$. In essence, the study found a significant proportion of chromosome aberrations, indicating cytogenotoxicity for doses of $CuSO_4$ between 1 and 10 ppm. High concentrations are linked to a higher risk of injury and genotoxicity, as seen by the order of aberrations caused by interventions, which was 0.0 < 1 ppm < 5 ppm < 10 ppm.

Table 1: Cytological effects of exposure to varying concentrations of $CuSO_4$ on *A. sativum* mitotic cells.

$CuSO_4$ concentrations (ppm)	**Total number of cells counted**	**Dividing cells**	**Mitotic index (%)**	**Mitotic inhibition (%)**
Control	2439,17±15.09	348.19±3.46	14.01±2.11	0.00±0.00
1	2023.37±36.46	39.73±5.53	2.89±0.09	79.37±7.01
5	1298.08±13.77	27.36±18.48	11.49±1.64	86.33±8.12
10	1302.67±11.95	23.66±6.69	9.77±1.05	90.54±5.90

Table 2: Chromosome aberrations induced in *A. sativum* root tip cells in response to exposure to varying concentrations of $CuSO_4$.

CuSO4 conc. Ppm	**Total no of detecting cells**	**Anaphase bridge**	**Stickness**	**Fragment**	**Micronucleus**	**Others**	**% Chrom aberration**
Control	$2439,17^{a}$±15.09	01.37^{c}	008.35^{e}	0.00^{c}	$08.93d^{e}$	05.90^{cd}	01.01
1	2023.37^{a}±36.46	23.42^{ab}	102.89^{cd}	79.37^{b}	$42.45b^{c}$	36.15^{b}	14.05
5	1298.08^{a}±13.77	29.43^{ab}	110.08^{cd}	82.09^{bc}	44.91^{b}	39.00^{a}	23.54
10	1302.67^{a}±11.95	32.12^{a}	118.34^{a}	89.03^{a}	47.12^{a}	43.56^{ab}	25.35

A-Three replicates were used to calculate the average total number of mitotic cells detected for each $CuSO_4$ treatment.

B- Nuclear anomalies, binucleated cells, irregular prophase, pulverization, anaphase lagging chromosome, multipolar cells, and polyploidy are further chromosomal abnormalities that have been found.

At a p-value of less than 0.05, the values indicated by various superscript letters in each column differ significantly from one another.

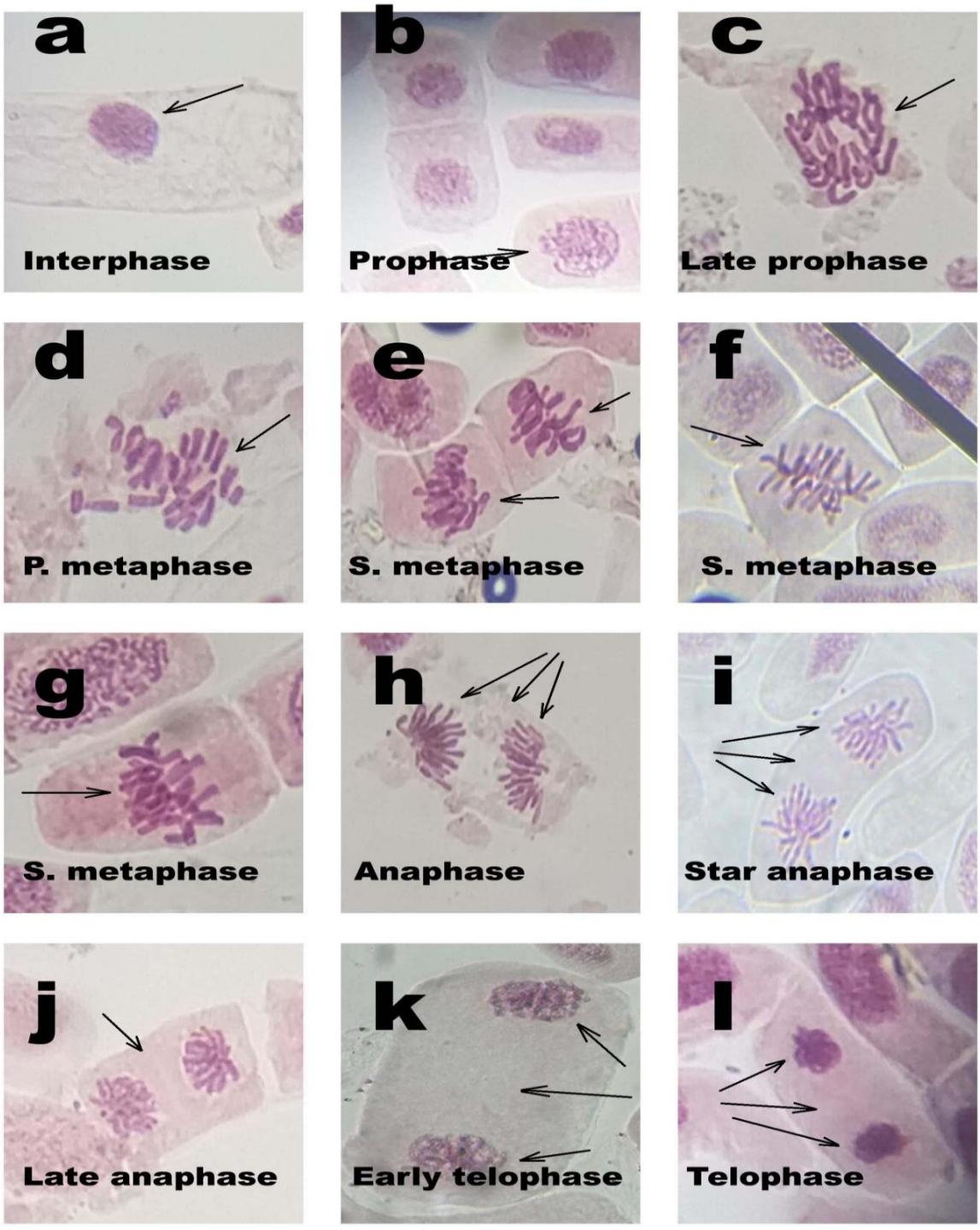

Fig. 3: Microscopic observation of normal interphase, prophase, metaphase, Anaphase and telophase cells in *Allium sativum* root tip meristematic region **a:** Interphase; **b:** prophase; **c:** Late prophase metaphase; **d:** P Metaphase; **e, f, g:** S metaphase; **h:** anaphase; **I:** Star anaphase; **j:** Late anaphase; **k:** Early telophase; and **L:** Telophase.

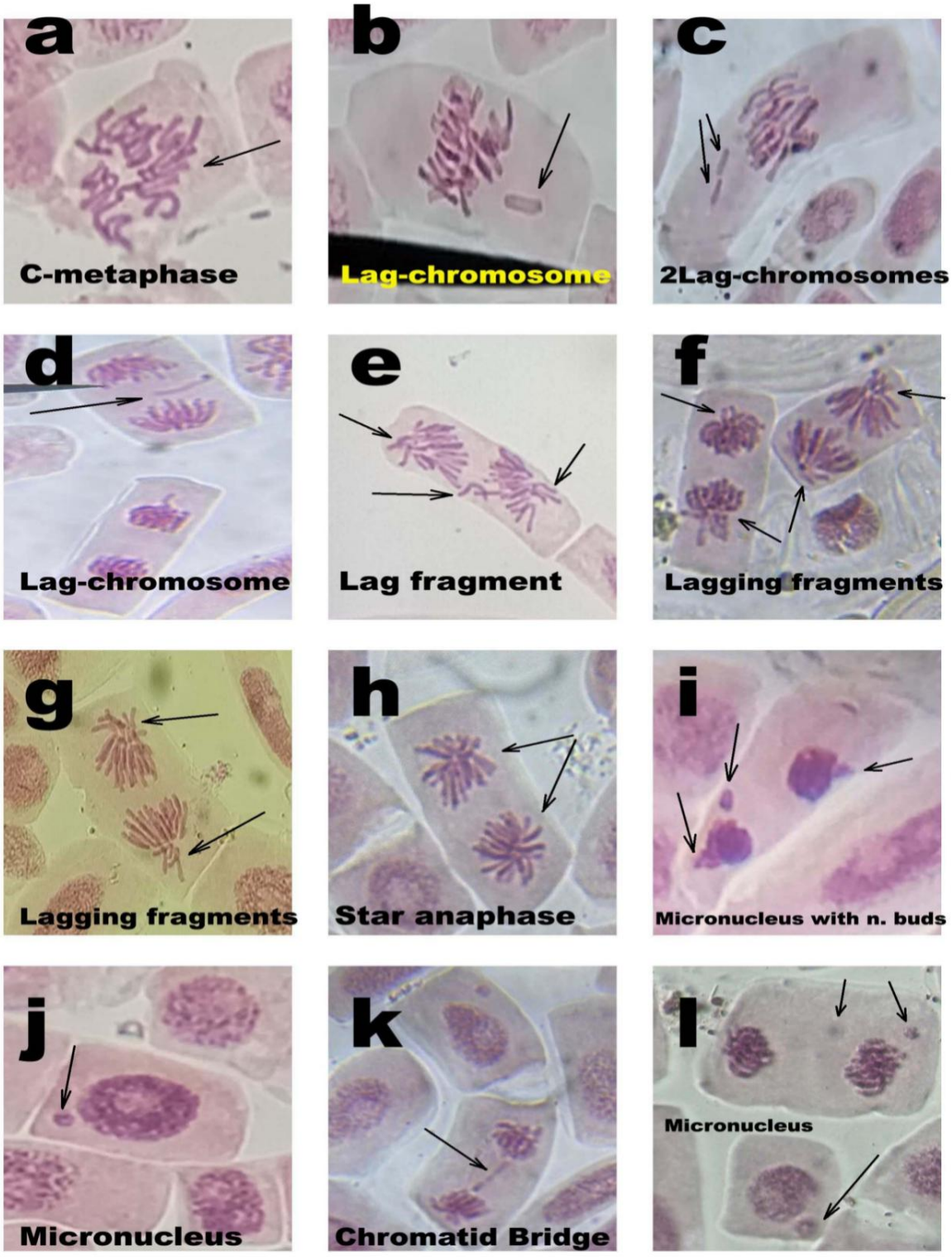

Fig. 4: revealed the nuclear abnormalities in the *Allium sativum* root tip cells exposed to 1 ppm concentration of $CuSO_4$. **a**: C- metaphase; **b, c**: lagging chromosomes in metaphase, **d, e, f, g:** Lagging fragments; **h:** star anaphase; **i:** Micronucleus with n. buds; **j:** Micronucleus **k:** chromatid bridge in telophase; **l:** Micronuclei in telophase.

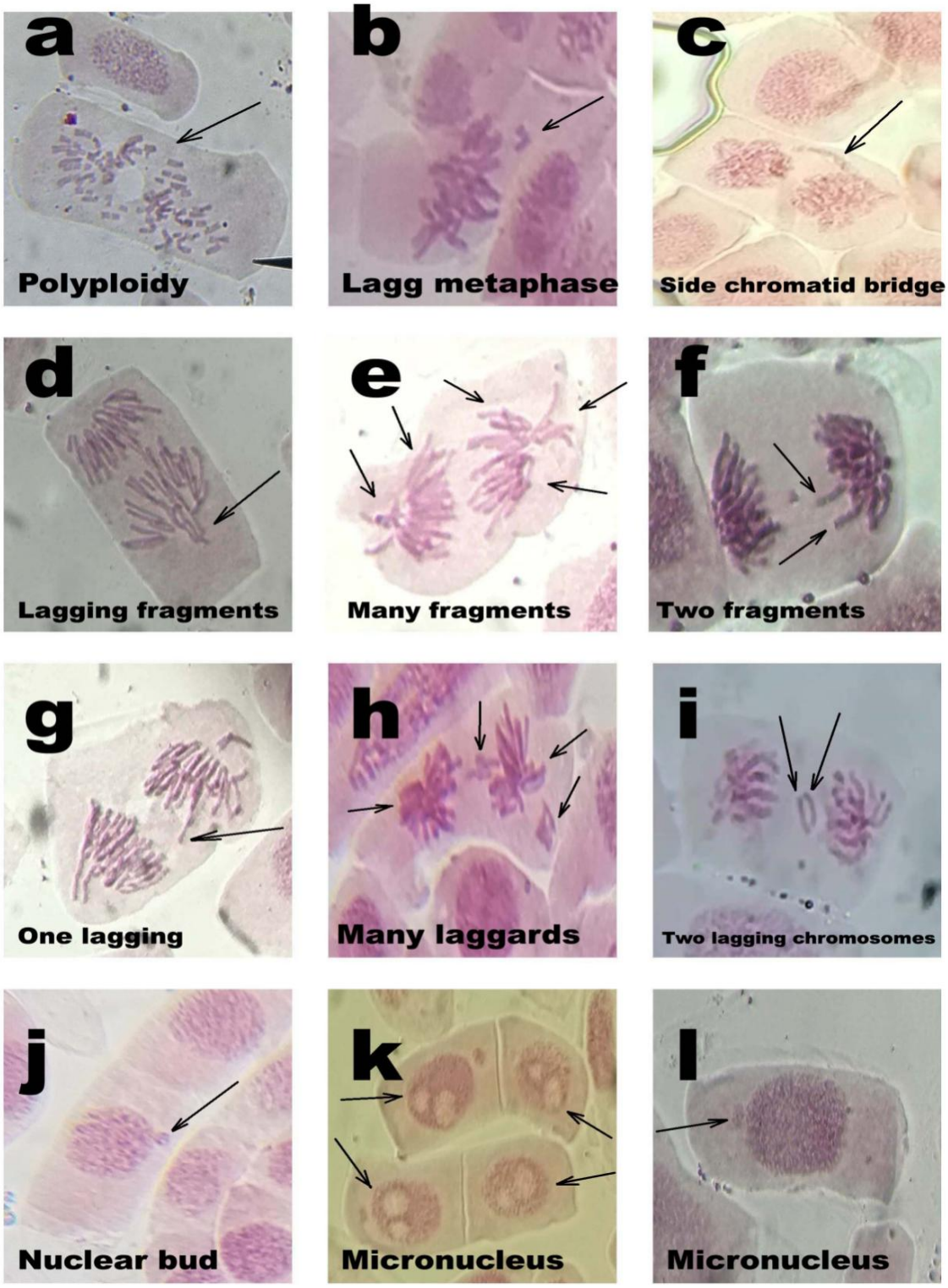

Fig. 5: revealed the nuclear abnormalities in the *Allium sativum* root tip cells exposed to 5 ppm concentration of $CuSO_4$ **a:** Polyploidy; **b:** Lagg metaphase; **c:** Side chromatid bridge; **d, e, f, g, h:** anaphase laggards; **i:** telophase with vagrant chromosome; **j:** nuclear buds; **k, l:** micronucleus.

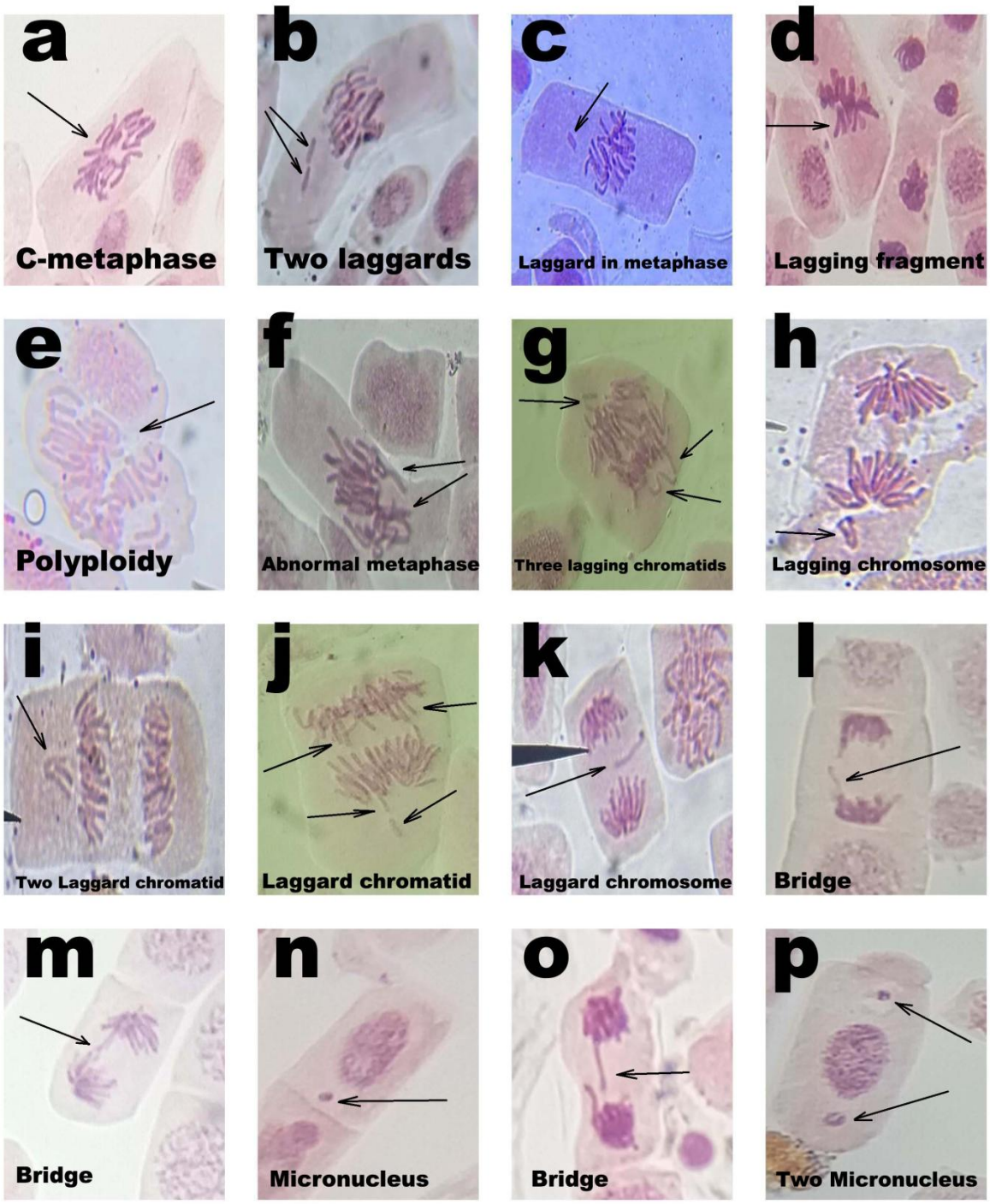

Fig. 6: Representative microscopic appearance of the nuclear abnormalities in the *Allium sativum* root tip cells exposed to 10 ppm concentration of $CuSO_4$ which is used in laboratories. **a**: C-metaphase; **b, c, d**: Lagging in metaphase; **e**: polyploidy; **f**: abnormal metaphase; **g, h, I, j, k:** lagging in anaphase; **l, m:** Bridges; **n:** micronucleus; **o:** Bridge; **p:** Micronucleus.

DISCUSSION

According to this study, chromosomal aberrations were seen in *Allium sativum* mitotic cells subjected to different concentrations of $CuSO_4$. The results also demonstrated how harmful the chemical was to the growth of *A. sativum* roots. According to the findings, garlic roots exposed to the chemical had a range of chromosomal aberrations at various stages of mitosis. This

implies that even if the chemical can be used alone or in conjunction with other chemicals as a catalyst or disinfectant, there may be dangers due to its mutagenic and genotoxic properties. Toxins or contaminants from the soil or other growing media usually come into contact with the plant at the tips of its roots. The evaluation of $CuSO_4$'s effects on *A. sativum* root production may therefore provide insight into the chemical's growth inhibition and impacts of different doses on plants, since it serves as a model of possible cytotoxicity in cells generally. Higher $CuSO_4$ concentrations were found to reduce root formation, which may have had a negative impact on root development by reducing nutrient conductivity and pressure-induced water flux. The levels evaluated in this study may have affected the enzymatic activity of the pathways and the physiological processes that enable root development and growth. The available data also clearly shows that even at low concentrations of 1 ppm $CuSO_4$, a variety of mitotic disturbances can occur, leading to widespread genetic mutation and modification. The likelihood of this happening increases with increasing concentrations. Copper's ability to react with tubulins' sulfhydryl groups is the main cause of its genotoxicity. Various chromosomal abnormalities are caused by this process, which also affects spindle function. This chemical can also cause polyploidy. Additionally, copper causes osmotic stress (Hossain *et al.*, 2021 and Khanna and Sharma 2013), which leads to apoptosis and the resulting DNA damage (Rajneet *et al.*, 2014 and Hossain *et al.*, 2021), hence inducing the generation of free radicals. $CuSO_4$'s capacity to interfere with the directed and sequential process of mitotic cell division and cycle advancement thus raises the possibility of cytotoxicity and genotoxicity. The plants' mitotic indices and the different clastogenic and aneugenic events seen in the roots exposed to $CuSO_4$ can be used to deduce these effects. Copper may impair cellular antioxidant systems, resulting in cell deterioration and necrosis. Because of its high affinity for proteins, $CuSO_4$ is the most toxic copper molecule. They discovered that cells exposed to any concentration of $CuSO_4$ had negative effects, suggesting that the chemical is harmful to living things. Significant chromosome damage was found in this investigation even at a low concentration of 1 ppm, suggesting that extended exposure to the material may be dangerous due to its capacity to penetrate live tissues and have negative effects on the genetic system. Nevertheless, despite the possible hazards, $CuSO_4$ is nevertheless used for a variety of purposes in farms as a fungicide, labs, and enterprises. Employees like farmers who might be exposed to the chemical's dangerous genotoxic effects are at risk because of this. The detrimental effects of $CuSO_4$ were observed by the authors to result in cell death and disturbance of the structure of the cell membrane. Numerous chromosomal problems, such as fragmentation, lagging, stickiness, bridge formation, polyploidy, binucleation, and nuclear budding, have been linked to $CuSO_4$ poisoning. These anomalies may result in mutations or illnesses, changes to the genetic system, and damage to DNA. According to the current study, elevated $CuSO_4$ inhibits cellular division, which lowers the mitotic index and causes a variety of noteworthy clastogenic alterations.

These results corroborate earlier observations (Khanna and Sharma, 2013) showing a high concentration of heavy metals in plant roots is associated with chromosomal abnormalities, including nuclear lesions, bridge forms, chromosome lagging, and a lower mitotic index. These abnormalities can cause problems and even cell death by interfering with cell division. The presence of chromosome lag, anaphase bridges, a reduced mitotic index, and other chromosomal abnormalities in this investigation depend on concentration. These results are in line with (Sarac *et al.*, 2019 and Sabeen *et al.*, 2020), which showed that the concentration also affects the genotoxicity caused by heavy metal accumulation. The primary cause of chromosomal bridging is chromosome stickiness, which causes these sticky chromosomes to separate slowly and fail to

detach.

Chromosome structures are bridged internally inside or between chromatids by a combination of homologous and nonhomologous chromosome exchanges, typically as a result of dicentric development or flawed replication processes. Disorders may arise from these occurrences (Rank 2003; Türkog˘lu, 2007 and Zhang *et al.*, 2015). Two processes can cause chromosomes to become adhesive: partial nucleoprotein breakdown, DNA depolymerization, and contraction or condensation. The high rate of stickiness found in this study points to $CuSO_4$'s detrimental effects on nuclear components, which could result in irreversible damage and cell death. Furthermore, the presence of misaligned chromosomes indicated that $CuSO_4$ had hampered the spindle apparatus's ability to organize and function.

The presence of toxic metals in the growing environment is suggested by the varying degrees of C-mitosis found in onion and eggplant root cells (Salt *et al.*, 1995; Yilmaz *et al.*, 2009 and Siddiqui *et al.*, 2014). Since the *Allium sativum* test may be used without raising ethical issues, it serves as a useful model for determining cytotoxicity and has shown a strong correlation with possible effects in animals (Sarac *et al.*, 2019). The potential chemical effects of chromosomal abnormalities and cytotoxicity have been assessed using the *Allium sativum* test (Fernandes *et al.*, 2007; Carita and Marin-Morales 2008 and Gedik *et al.*, 2013). When high concentrations of chemicals were used in these earlier investigations, the scientists noted significant cytotoxicity and chromosome deformation.

Because the data on the cytotoxicity of $CuSO_4$ gathered in this study could be a crucial reference for guaranteeing appropriate safety precautions for individuals regularly working with this compound, the findings of this study may be significant for tissue culturists, microbial laboratory technologists and industrial workers who are at risk of $CuSO_4$ toxicity because of their line of work. Considering the known adverse effects, such actions are essential.

Conclusion

The cytotoxic effects of varying $CuSO_4$ concentrations on garlic root tips were investigated in this work. The results demonstrated that the chemical can be harmful and toxic to cells at concentrations as low as 1 ppm because it prevents the development and formation of roots, interferes with the process of cell division, and results in chromosomal abnormalities such as lagging chromosomes, chromosome bridges at different stages of cell division, vagrants, sticky chromosomes, binucleated cells, chromosome fragmentation, and loculated nuclei. Concentration dependence was seen in most of the generated aberrations. Significant clastogenic alterations in garlic root tip cells were brought on by $CuSO_4$, indicating that it disrupts cell proliferation and the cell cycle, potentially influencing the genetic system and cell behavior. The $CuSO_4$ toxicity data collected in this study can be used as a guide to make sure that people who use $CuSO_4$ regularly take the right precautions to avoid its negative effects.

Declarations:

Ethical Approval: Not applicable.

Conflict of interests: The authors declare no conflicts of interest.

Authors Contributions: All authors contributed equally, and have read and agreed to the published version of the manuscript.

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ARABIC SUMMARY

تأثيرات السمية الخلوية الجينية للتركيزات المختلفة من كبريتات النحاس باستخدام نموذج الثوم

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يعتبر اختبار الثوم (*Allium sativum*) نموذجًا مهمًا لتقييم السمية الخلوية والسمية الجينية للمعادن الثقيلة. تهدف الدراسة الحالية إلى البحث في السمية الخلوية والجينية للنحاس، باستخدام اختبار *Allium sativum*. تم إختبار تأثير زيادة تركيز عنصر النحاس المتدرج من 0 ,وصولا إلي 10 جزء في المليون علي قدرة جذور الثوم علي النمو والإستطالة خلال 10 أيام. ودلت النتائج أن تركيز 1 جزء في المليون كان لة تأثير مثبط علي نمو الجذور. وأن هذا التأثير المثبط زاد بزيادة تركيز كبريتات النحاس إلي 5 ثم إلي 10 جزء في المليون. تم تحضير شرائح تحتوي على خلايا انقسامية من القمم النامية لجذور الثوم المنزرعة في الماء المقطر المضاف إليه تركيزات مختلفة من كبريتات النحاس $CuSo_4$ (0، 1، 5، 10 جزء في المليون) لمدة 48 ساعة. تمت ملاحظة الشرائح بواسطة المجهر الضوئي لتقييم السمية الخلوية والسمية الجينية اعتمادًا على تقييم معامل الانقسام، ومعامل تثبيط الانقسام وطول الجذر. أظهرت النتائج أن التركيزات المستخدمة لها تأثيرا معنويا على الخلية، مما أدى إلى تأثيرات ضارة على معامل الانقسام، ومعامل تثبيط الانقسام، ونمو الجذر، وتركيب الكروموسوم. لوحظت شذوذات كروموسومية مختلفة، مثل تكوين الجسور الكروموسومية، وشظايا التفتت، والكروموسومات المتأخرة، والخلايا ثنائية النواة، وتكوين النويات الصغيرة، وقد لوحظ أن نسبة هذة الشذوذات تتناسب طرديا مع زيادة تركيز كبريتات النحاس. حتى مع التركيز الأكثر إنخفاضا (1 جزء في المليون) كان له تأثيرات ولكنها أقل بصورة مءنوية على الخلايا. بالمقارنة بالتركيزات الأعلى والتي وجد لها تأثيرات سامة خلويا وجينيا أكبر بصورة معنوية عند درجة إحتمال ($p < 0.05$) مقارنة بمعاملة الكنترول (0.0 جزء في المليون). كلما زاد التركيز، زادت التأثيرات والتغيرات الكروموسومية. تشير التشوهات المنتشرة في خلايا الانقسام الميتوزي وأيضا تأثر نمو الجذور إلى أن كبريتات النحاس سامة خلويا وجينيا بدئا من التركيزات المنخفضة (1 جزء في المليون). علاوة علي ما سبق يمكن لهذا لعنصر النحاس أن يسبب تأثيرات مثبطة للانقسام الخلوي في التركيزات الأعلى. الهدف من هذة الدراسة هو توفير نتائج يمكن توظيفها كدليل لضمان احتياطات السلامة الكافية للأشخاص أثناء الاستخدام المنتظم لكبريتات النحاس.

الكلمات الدالة *Allium sativum* · اختبار السمية الخلوية · كبريتات النحاس · معامل الانقسام · معامل تثبيط الانقسام الخلوي · الانحرافات الكروموسومية