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Research Article

The Genotoxic Effect of Heavy Metals on Plant Reproduction: A Case Study Of *Carthamus tinctorius* L

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Abstract
Present work investigates the impact of heavy metals, specifically Lead and Arsenic, on meiosis and related parameters in the dicotyledonous oil crop *Carthamus tinctorius* L. Plants were grown in culture tubes treated with heavy metals lead and arsenic, and various meiotic parameters analyzed. The results indicated significant meiotic irregularities including chromatin bridges, chromosome clumping and pollen sterility. The study highlights the genotoxic potential of lead and arsenic, emphasizing the need for further exploration of their effect on crop yield and genetic variability

Keywords: *Lead, Arsenic, meiotic anomalies.*

Introduction
In biological terms, sexual reproduction generates new gene combinations, both linked and unlinked, for future generations. This is achieved through two alternating processes: meiosis and fertilization. Meiosis is a reductional process that halves the chromosome number from 2n to n. This reduction from the sporophytic to the gametophytic level is crucial for sexual reproduction, allowing the sporophytic generation, which results from fertilization, to alternate with the gametophytic generation that produces gametes. Meiosis occurs once in a sporophytic cell and is sensitive to environmental changes such as atmospheric and terrestrial pollutants and xenobiotic compounds. Agricultural expansion and the use of agrochemicals may impact plants through groundwater contamination or spray drift, whereas industrial activities can release heavy metals into the environment (Fuchs *et al.*, 2018). Environmental changes that disrupt normal meiosis can lead to various meiotic anomalies, resulting in sterility. Heavy metals are particularly toxic due to their cumulative nature, causing adverse effects in different areas (Jarup, 2003, Sathwara *et al.*, 2004). Lead and arsenic are significant environmental pollutants that can negatively impact meiosis and related processes. Additionally, various physical and chemical agents, including water and air pollutants, can adversely affect meiosis or fertilization, potentially harming sexual reproductive potential by causing varying degrees of sterility and creating barriers to gametic union. These factors can limit the numerical increase of individuals within a species. In crops, abnormal meiosis can manifest as reduced seed and fruit yield and the rapid breakdown of agronomically superior genotypes. This analysis was conducted to assess and measure the effects of lead and arsenic on the parameters under study.

Materials and Methods
For the present experiment, a dicotyledonous oil crop *Carthamus tinctorius* was chosen. Plants were collected from the field and grown in culture tubes for a week, having lead (Pb) and arsenic (As). Control plants were raised in Hoagland's solution lacking lead (Pb) and arsenic (As).

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Young floral buds intended for meiotic analysis were fixed for 48 hours in a recently prepared solution of Carnoy's fluid II, composed of EtOH+$CHCl_3$+CH_3COOH in a 6:3:1 ratio, supplemented with a small amount of FeCl3. These specimens were subsequently preserved in 70% ethanol and kept in a refrigerator. Anthers of suitable size were smeared and squashed using 1.5% aceto-carmine. The studies were conducted on both temporary squashed and unsquashed preparations. Photomicrographs were also taken from these slides. Different parameters studied for evaluating the effect of lead and arsenic on male meiosis and fertility were:

(a) Chromosome configuration at diakinesis including chiasmata frequency,
(b) Meiotic anomalies types and frequencies,
(c) Spores per PMC,
(d) Pollen diameter (③m),
(e) Pollen fertility, and
(f) Number of pollens per anther.

Result

Lead and arsenic exposure in male meiosis was linked to notable meiotic irregularities. The information gathered from meiosis and 'meiosis related' parameters were as follows:

In the control sets, most of the bivalents were of ring type, while in the lead and arsenic treated sets, the frequency of ring bivalents and X-ta/PMC decreased significantly, with rare exceptions.

Analyses of metaphase I showed chromosome configurations varying from univalents to quadrivalents, observed exclusively in the sets treated with lead and arsenic. The quadrivalents were showing several types of orientation at metaphase I like twisted ring, open ring, chain of four and discordant type, consequently it lead to alternate as well as adjacent disjunction. The alternate disjunction resulted because of the presence of twisted ring quadrivalent at metaphase I. On the other hand, adjacent disjunction was the outcome of the presence of open ring, chain of four and discordant quadrivalent.

Both lead as well as arsenic could induce several types of meiotic anomalies listed below:

- Asynchrony in division within PMCs,
- Formation of chromatin bridge at ana-phase I,
- Formation of chromatin bridge at ana-phase II,
- Chromatin disintegration at metaphase I,
- Clumping of bivalents at metaphase I,
- Clumping of chromosomes at anaphase I,
- Clumping of chromosomes at metaphase II,
- Clumping of chromosomes at anaphase II,
- Grouping of bivalents in >1 groups at metaphase I,
- Grouping of chromosomes in >1 groups at metaphase II,
- Lagging of chromosomes at anaphase I,
- Lagging of chromosomes at anaphase II,
- Late movement of bivalents for metaphase I alignment,
- Formation of micronuclei at telophase I,
- Formation of micronuclei at telophase II,
- Precocious disjunction of bivalents at met-aphase I,
- Precocious disjunction of chromosomes at metaphase II,
- Restitution nucleus formation during first meiotic division, and
- Chromosomes are distributed unevenly during anaphase I.
- Unequal distribution of chromosomes at anaphase II.

Treatment with lead and arsenic induced changes in diameter of the pollen grains. Pollen sterility, pollen fusion and pollen disintegration were notified in both lead and arsenic treated sets.Both treated sets showed reduced pollen grains per anther.

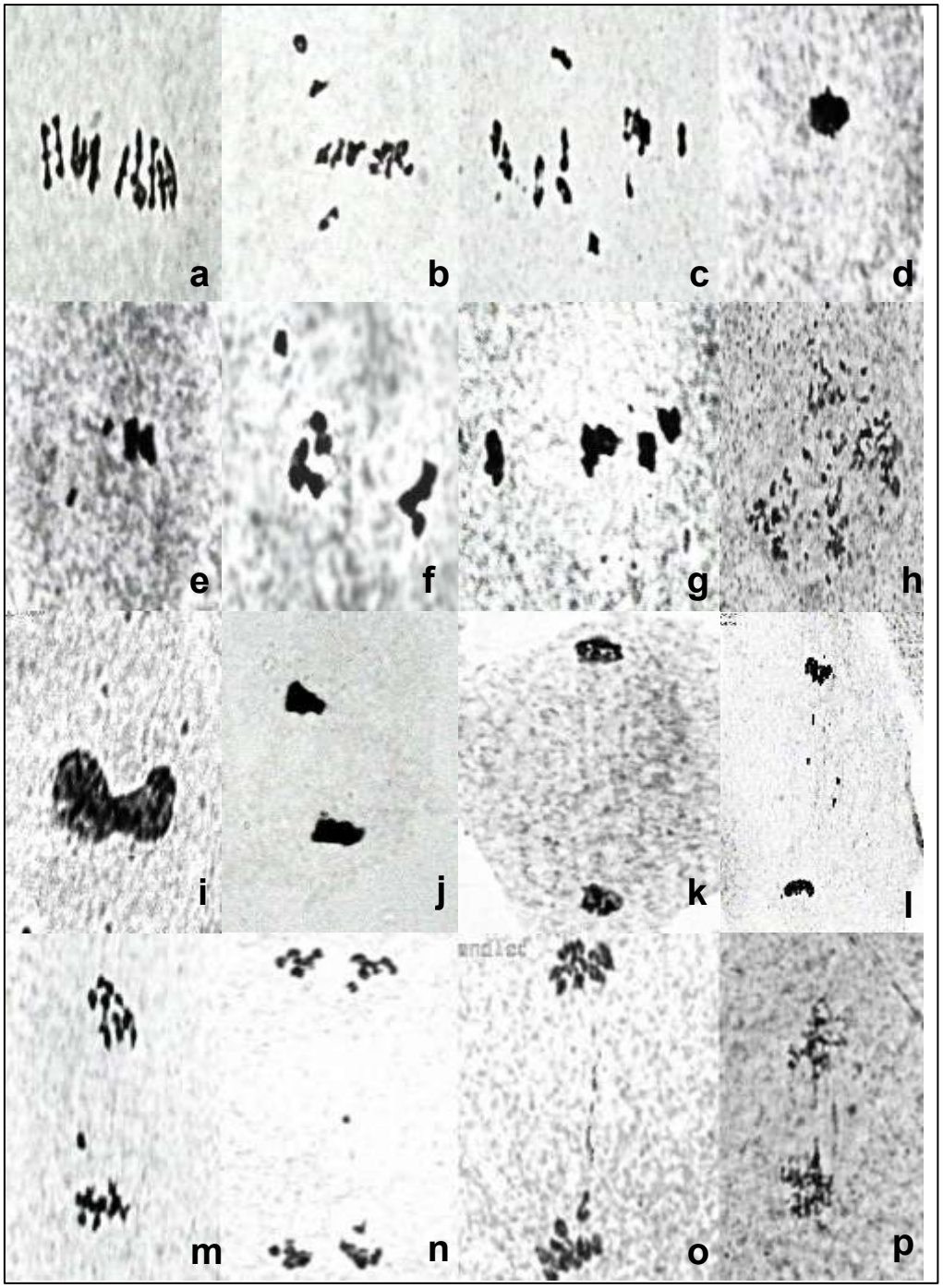


Figure 1. a: Bivalents at metaphase I, b: Late movements of bivalents for metaphase I, c: Precocious disjunction of bivalents at metaphase I, d,e: Clumping of bivalents at metaphase I, f,g: Grouping of bivalents in > 1 groups at metaphase I, h: chromatin disintegration at metaphse I, i: Restitution nucleus formation during first meiotic division, j,k: Clumping of chromosomes at anaphase I, l,m: Lagging of chromosomes at anaphase I, n: Grouping of chromosome at anphase I, o,p: Formation of chromatin bridge at anaphase I.

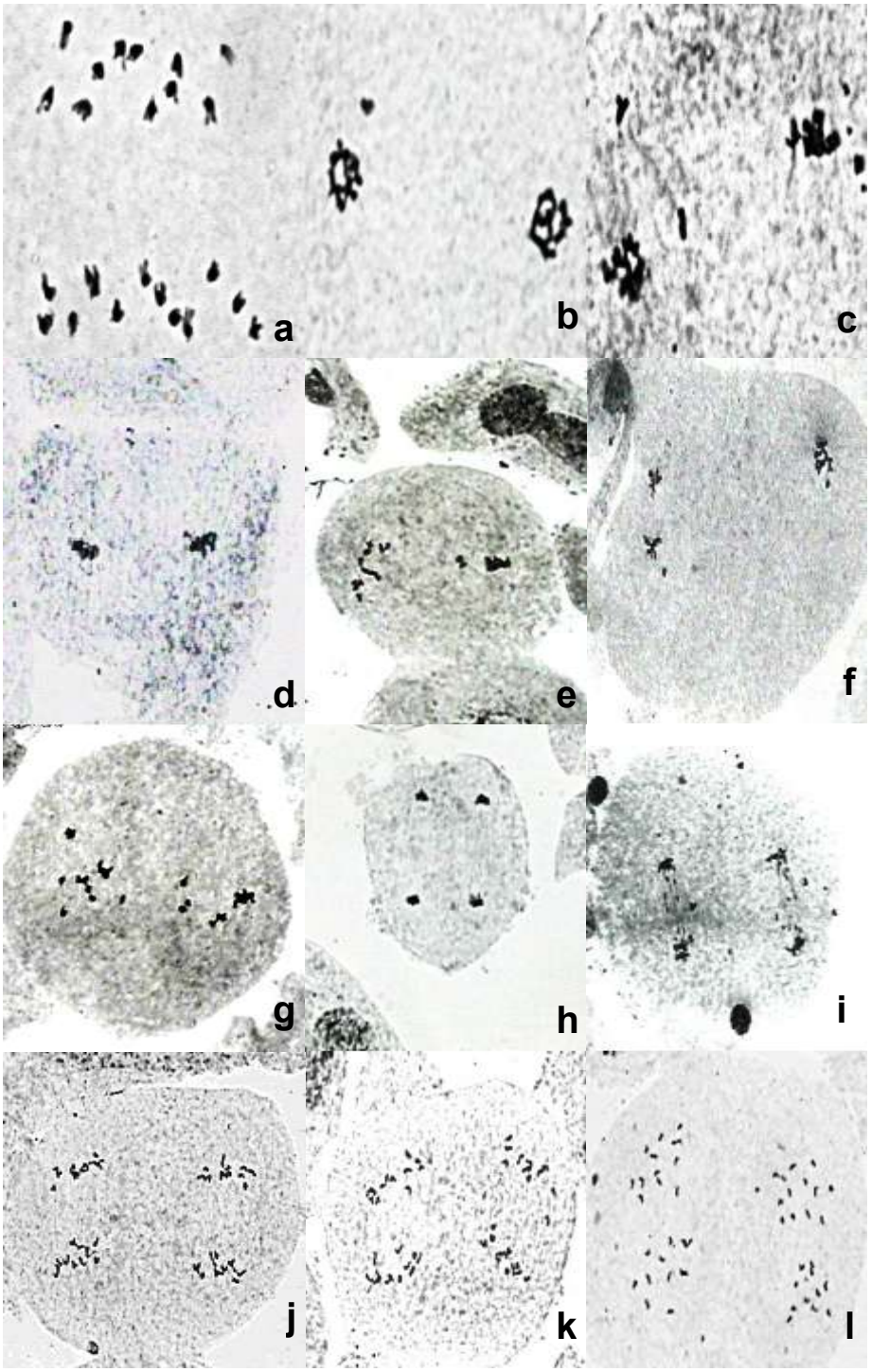


Figure 2. a: Unequal distridution of chromosomes at anaphase I, b: Formation of micronuclei at telophase I., c: Late movement of chromosomes at metaphase II, d: Clumping of chromosomes at metaphase II, e,f: Grouping of chromosomes in >1 groups at metaphase II, g: Precocious disjunction of chromosomes at metaphase II, h: Clumping of chromosomes at anaphase II, i: Formation of chromatin bridge at anaphase II, j: Grouping of chromosomes at anaphase II, k: Lagging of chromosomes at anaphase II, l: Unequal distribution of chromosomes at anaphase II

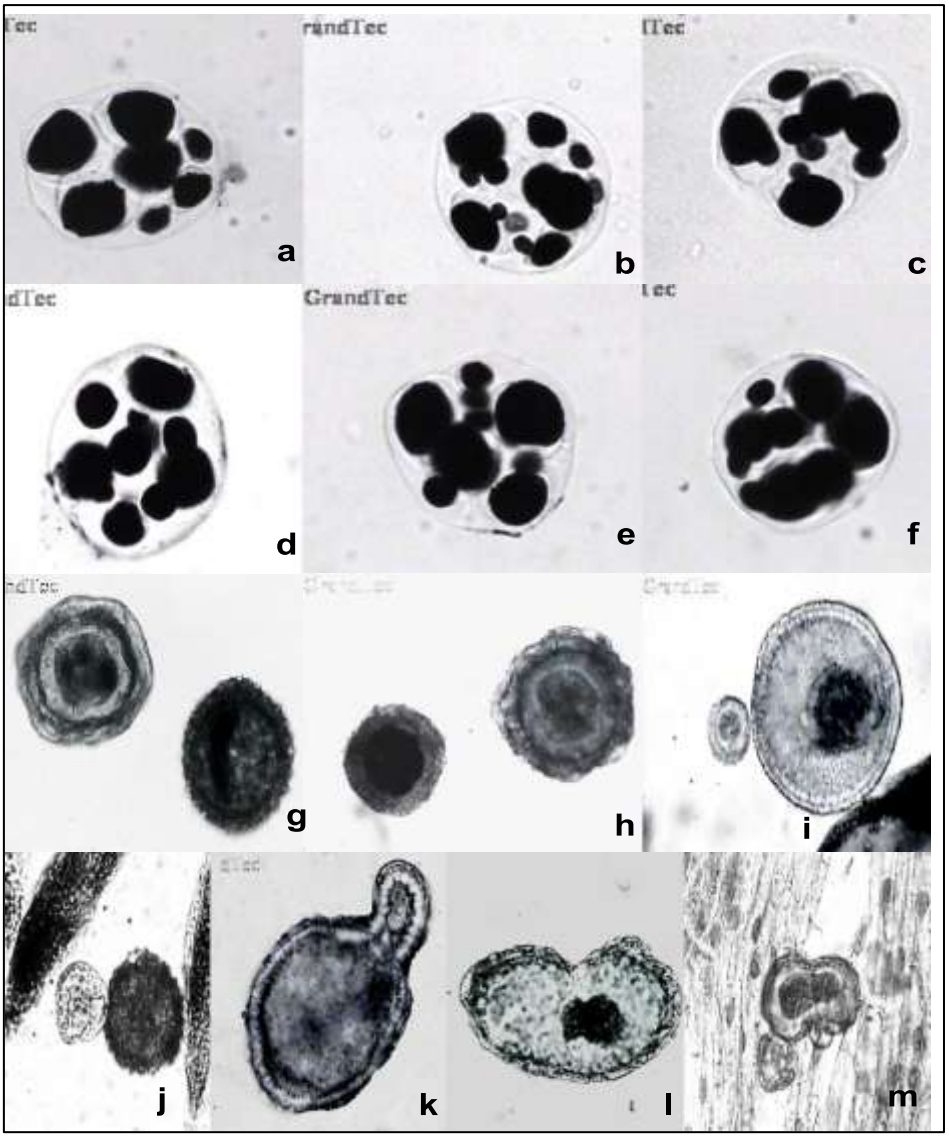


Figure 3. a-f: Polyads with variability in number of spores. g, h, j: Pollen wall differentiation, i: Variability in pollen size, k: Pollen fusion, l, m: Division of pollen grain.

Discussion

Meiosis is not only responsible for numerical reduction but is also responsible for random distribution of linked and unlinked genes to future generations. The recombination between unlinked genes is brought about by reshuffling the maternal and paternal chromosomes and randomly distributing one member of each pair to the initial cells of gametophytic generation. These initial cells, genetically programmed for developing into gametophytes, are called spores. However, meiosis and fertilization faithfully alternate with each other in all sexually reproducing eukaryotic individuals. These two processes together are also responsible for producing variations.

The treatments with lead and arsenic resulted in varying degrees of meiotic irregularities, potentially due to the different responses of genotypes to the same concentrations of these elements. The cytological effects of heavy metals and other water pollutants on plant systems have primarily been studied in root meristem cells of model test systems, with limited data available on their impact on meiosis and related parameters. (Ramel and Engstrom 1972) first studied how methyl mercury hydroxide affects *Tradescantia* microsporogenesis, observing that it inactivates spindle fibers. (Somashekhar 1987) examined induced meiotic abnormalities in *Chlorophytum amaniense* by directly treating

floral buds with water contaminated by dye industry effluent, observing several types of meiotic irregularities. The meiotic effects of various pollutants were assessed by (Wuu and Grant 1967, Amer and Ali 1968, 1974, Amer and Farah 1968, 1984, and Jabeen and Abraham 1998). Several researchers have thoroughly analyzed the effects of certain heavy metals and water pollutants on male meiosis and related parameters. (Jetly 1993 and Yadav 1993) studied the effects of Cr6+ and Cd++, respectively, on similar parameters in various crops. Treatments with sodium chromate solutions induced several types of meiotic irregularities (Jetly and Srivastava, 1994). (Bansikar 1993, Gautam 1996, Aggarwal 1998, Sharma 2001, and Tomar 2004) investigated the effects of different mercury compounds on various crops. These studies clearly demonstrated the genotoxic potential of water pollutants, including heavy metals, in causing meiotic anomalies. A review of the current data on the effects of lead and arsenic on male meiosis revealed the induction of spindle-dependent, chromatin agglutination-dependent, and chromosome pairing-dependent anomalies. Spindle-dependent meiotic anomalies may arise from either structural or functional defects in the spindle apparatus. Structural defects in spindle organization can be as severe as complete breakdown or may result in moderate effects like the presence of "longitudinal non-functional" zones. The former leads to the formation of restitution nuclei, while the latter causes chromosomes to cluster into multiple groups. Spindle malfunction results in differential chromosome movement during metaphase congregation, lagging of centric chromosomes, and micronuclei formation. Chromatin agglutination causes chromosome clumping at metaphase I and II, late disjunction of bivalents, and chromatin bridge formation, which can also lead to restitution nuclei formation. Chromosome pairing-dependent meiotic irregularities include precocious disjunction of bivalents and a reduction in the frequency of ring bivalents.

Lead and arsenic significantly decreased the occurrence of ring bivalents, thereby notably lowering X-ta/PMC, with only rare exceptions. If chiasmata represent the cytological evidence of genetic crossing over, then their reduction might suggest a decrease in recombination frequency, leading to a subsequent drop in genetic variability. To what extent this will affect the 'future' of a crop cultivar, needs critical exploration, since the preservation of agronomic qualities depend upon genetic segregation.

Movement of chromatin in organized form between PMCs was present in certain treated sets. The phenomenon was first described by (Gates 1911) as cytomixis and was later labeled nucleomixis by (Diamelidis 1951). Doubts about the existence of this phenomenon was raised by workers like, (Lewontin 1955 and Takala 1959) who argued that pressure applied during squashing may cause chromatin movement. Several workers provided evidence for its presence in different plant materials. (Singh and Srivastava 2014) found that applying pendimethalin and glyphosate to *Vigna mungo* foliage 21 days after sowing caused chromatin bridges, anaphase I laggards, spindle disruptions, and binucleate cells in 17% and 21% of pollen mother cells, respectively. During this work, unsquashed as well as squashed slides were studied for scrutinizing irregularities which may originate as an artifact. Moreover, fused chromatin material in the cytoplasmic channels, connecting the PMCs, cannot be produced due to pressure applied for squashing.

Currently, restitution nuclei are primarily observed because of chromatin clumping. These nuclei, formed due to chromatin agglutination, can be distinguished from those caused by spindle breakdown because they appear as a mass of clumped chromatin. The uneven movement of chromosomes during metaphase alignment is likely due to a disruption in the mechanism that pulls chromosomes to the spindle apparatus's equatorial plate. Another irregularity, the lagging of centric chromosomes, arises from spindle malfunction. However, since the former occurs before spindle formation and

the latter after, it is plausible that distinct biochemical processes are responsible for each. It is widely acknowledged that microtubules are crucial for both metaphase alignment and anaphase A movement of chromosomes. Thus, the presence of these irregularities indicates that lead and arsenic might have toxic effects on spindle components.

Chromatin agglutination led to the clustering of chromosomes, delayed separation of bivalents, and the formation of chromatin bridges. The delayed separation of bivalents might also result from the late resolution of X-ta, as early resolution of X-ta causes premature separation of bivalents. The chromatin bridges seen during anaphase I were not associated with fragments, indicating they were formed due to the stretching of chromosome segments between the poles. This was also the case for chromatin bridges observed during anaphase II. The arrangement of chromosomes into groups at metaphase I and II often resulted in the induction of "longitudinal splitting" of the spindle. Premature separation of bivalents and chromosomes, observed during the first and second meiotic divisions respectively, likely involved different sets of activities, as homologous chromosomes separate in the former, while sister chromatids separate in the latter. Furthermore, for bivalent separation, chiasma (-ta) must be resolved, and for chromatid separation, sister kinetochores must disjoin. Understanding how lead and arsenic induce premature separation may partially explain the mechanistic differences (or similarities) between the two at a biochemical level. The presence of univalents was noted in both treated sets, appearing alongside bivalents. These univalents may have arisen from pre-meiotic non-disjunction of chromatids, typically induced by lead and arsenic. Kuchey *et al.,* (2016) studied the impact of dichlorvos (an organophosphate) and endosulfan (an organochlorine) on *A. cepa*. Dichlorvos caused abnormalities in 11–27% of PMCs, versus 3% in controls. Endosulfan led to defects in 9–20% of PMCs, depending on concentration.

The most common abnormality observed was stickiness during metaphase I and II. A notable reduction in the number of pollen grains per anther was reported in all treated sets. This decline could be due to either a decrease in the number of PMCs per anther or the arrest of meiosis, resulting in fewer spores per PMC. Exposure to lead and arsenic significantly increased pollen sterility, fusion, and disintegration. This could be due to the induction of meiotic abnormalities and gene mutations affecting the development and maturation of pollen grains after meiosis.

Conclusion

Concludes that treatments with lead and arsenic result in various meiotic irregularities, which are likely due to the different responses of genotypes to these elements. The study highlights the genotoxic potential of heavy metals and water pollutants in causing meiotic anomalies, such as spindle-dependent, chromatin agglutination-dependent, and chromosome pairing-dependent irregularities.

Lead and arsenic significantly decrease the occurrence of ring bivalents, suggesting a reduction in recombination frequency and genetic variability. This could impact the future of crop cultivars, as genetic segregation is crucial for preserving agronomic qualities.

The study also observes chromatin movement between pollen mother cells (PMCs), known as cytomixis or nucleomixis, which is not an artifact of squashing during slide preparation. Restitution nuclei, primarily formed due to chromatin clumping, indicate spindle malfunction and uneven chromosome movement during metaphase alignment.

Chromatin agglutination leads to chromosome clustering, delayed separation of bivalents, and chromatin bridge formation. Premature separation of bivalents and chromosomes during meiotic divisions suggests distinct biochemical processes are responsible for these irregularities. The study also notes a significant increase in pollen sterility, fusion, and disintegration due to lead and arsenic exposure, which could be

attributed to meiotic abnormalities and gene mutations affecting pollen grain development.

Overall, the article emphasizes the need for further exploration of the genotoxic effects of heavy metals on plant reproduction and their potential impact on crop cultivars.

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