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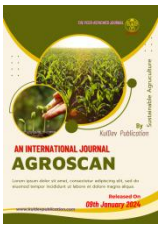

Research Article

Synthetic Silver Nanoparticles' Cytotoxic and Genotoxic Effects on *Allium* Species Cells

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ABSTRACT

Over the last ten years, nanotechnology has grown at a fast rate. However, widespread usage of nanotechnology may have negative effects on the environment and major health issues. Due to its extensive use in many fields, particularly the agricultural sector, silver nanoparticles are of particular significance among the other nanoparticles. The purpose of the current research is to assess the cytotoxic and genotoxic effects of artificial silver nanoparticles on Allium species root tip cells. For three distinct periods of time, 10 nm synthetic silver nanoparticles in varying concentrations were applied to the root tips of both species. Chromosome aberration, damaged cell count, and mitotic index all increased when the findings were compared to the control sample. Cell wall abnormalities such as blebs, rupture, and elongated cells are examples of damaged cells, whereas chromosomal aberrations include ring, bridge, laggard, and stickiness. It was discovered that a 10 nm particle may readily pass through a cell wall.

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Introduction

Smarter particles with sizes ranging from 1 to 100 nm are called nanoparticles. These days, a variety of engineered nanomaterials are used in daily tasks ranging from home to research. Of these, silver nanoparticles make up around 25% [40]. AgNPs are used in the agricultural sector as a fungicide [1], a plant growth stimulant [35], and a fruit ripening agent [34, 42]. Additionally, they have antimicrobial qualities, which is why they are used in a variety of consumer goods, including face cream, deodorants, athletic socks, food packaging, and cleaning solutions [4]. As silver nanoparticles are added to more items, there is a greater likelihood that they will be exposed to humans and the environment, which will lead to bioaccumulation and biomagnification. Waste water from sewage treatment was analysed to detect their presence in the water [15]. Larger AgNPs are filtered out of the root cell's cell wall whereas smaller AgNPs are allowed to pass through the porous network of the cell wall [38]. Large size nanoparticles may also enter via the cell wall because tiny size silver nanoparticles have the potential to enlarge pores as they enter [9].

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Silver nanoparticles have the ability to pass through 50–60 nm-diameter plasmodesmata pores [29], [14], and [26]. AgNPs have been shown by Geisler et al. to accumulate in Arabidopsis plasmodesmata and cell walls [13]. AgNPs were reported to be absorbed by Arabidopsis stomata in addition to being taken up by the roots and plasmodesmata [12]. According to Li et al.'s research, foliar areas accumulate 17–200 times more silver nanoparticles than root areas [24]. AgNPs may penetrate vascular tissue in crops and then travel over great distances to reach leaves and other organs [6, [29], [12]. Therefore, it's likely that AgNPs may translocate and infect edible plant parts like fruit and seeds. Following AgNP treatment, significant morphological alterations in plants were observed. The phytotoxicity of AgNPs was evaluated in plants using a variety of factors, including biomass, leaf surface area, growth potential, and seed germination in Arabidopsis. AgNP toxicity was documented in a number of plant species, including Arabidopsis [33], Phaseolous radiates and Sorghum bicolor [23], rice [8], wheat [43], and others.

It also affected seed germination, biomass accumulation, root and shoot development, and more. Research revealed that AgNPs may modify cell division and structure, leading to cell aberration [44]. For this reason, Allium cepa has been shown to exhibit a variety of aberrations, including aberrant metaphase, stickiness, chromatin bridge, and cell disintegration, as well as a significant drop in the mitotic index and impaired cell division [21]. AgNPs treatment reduced the mitotic index and increased the development of moicronuclei and chromosomal abnormalities in the root tips of Vicia faba L. [32]. Tests for cytotoxicity and genotoxicity may be performed on plant cells to detect the toxicity resulting from a mutagen. An established bioassay is the chromosomal aberration test for Allium root tip cells. It has been approved by the United Nations Environment Programme (UNEP) and the International Programme on Chemical Safety (IPCS, WHO) for systematic calibration in chemical screening and laboratory monitoring of genotoxicity resulting from environmental agents [16]. Of all the A. cepa material, A. faba, A. fistulosum, and proliferum have shown to be preferred materials [17].

RESOURCES AND TECHNIQUES

Nanoparticles

The suspension of silver nanoparticles was obtained from Sigma Aldrich; the created particles had a size of 10 nm, a purity of 99.7%, and a concentration of 0.02µg/ml. Three distinct concentrations—20 ppm, 10 ppm, and 5 ppm—were produced for the experiment.

Treatment and testing system

From the stock solution, silver nanoparticles with varying concentrations—20 ppm, 10 ppm, and 5 ppm—were made. Healthy seeds of Allium fistulosum and Allium cepa were obtained for the experiment from IARI, New Delhi, and ICAR Plandu, Ranchi, respectively. For nine to ten hours, the seeds were steeped in double-distilled water. Both species' soaked seeds were stored in separate petri dishes for germination on damp filter paper. moved as soon as germination to the various silver nanoparticle concentrations during the various time intervals listed in the tables.

Microscopic analysis

After being cut with a sterile blade to the appropriate size of 1-2 cm, the root tips were preserved for 24 hours in Carnoy's fixative (1:3 acetoalcohol) before being moved to 70% alcohol. Slides were produced for cytological studies using the squash method with 1.5% acetocarmine stain. Under a Magnus s/n: C197050239 microscope, slides were examined, and 40X and 100X photos were obtained.

END RESULTS AND TALK

The effects of varying concentrations of 10 nm silver nanoparticles on A. cepa L. and A. fistulosum L. chromosomal behaviour over time are reported in (Table 1-2). There were five duplicates used throughout the

whole experiment. Using the following formulas, the mitotic index (MI) and total abnormality percentage (TAB) were determined:

$$\text{Mitotic index (MI)} = \frac{\text{Total number of dividing cell}}{\text{Total cell count}}$$

$$\text{Total abnormality (\%)} = \frac{\text{Total number of abnormal cell}}{\text{Total cell count}}$$

Table 1 Effect of different concentration of 10 nm AgNPs on mitotic abnormalities in root tip of cells of *Allium cepa* L.

AgNPs		TCC	NCD	M.I%±SD	TAC	BB	BD	CP	LG	MN	NPB	PC	SK	SM	Abn%±SD
	Control	3000	521	17.36±1.23	-	-	-	-	-	-	-	-	-	-	-
20ppm	1hr	3000	361	12.03±1.46	53	6	10	2	12	3	1	2	3	15	1.75±0.57
	3hr	3000	348	11.60±0.83	58	4	12	3	8	5	2	1	5	18	1.92±0.52
	5hr	3000	321	10.70±2.57	67	7	16	2	11	9	1	5	2	14	2.23±0.45
10ppm	1hr	3000	447	14.90±1.11	37	3	9	1	7	2	2	1	2	10	1.26±0.42
	3hr	3000	417	13.90±1.87	40	2	5	2	8	6	3	3	4	7	1.35±0.44
	5hr	3000	406	13.53±2.54	42	2	8	2	10	4	1	1	3	11	1.42±0.45
5ppm	1hr	3000	461	15.36±1.77	20	2	3	1	4	3	0	1	1	5	0.66±0.32
	3hr	3000	461	15.36±1.25	24	1	3	1	7	1	1	1	2	7	0.75±0.43
	5hr	3000	457	15.23±1.18	26	1	5	1	3	2	2	2	4	6	0.81±0.98

Table 2 Effect of different concentration of 10 nm AgNPs on mitotic abnormalities in root tip of cells of *Allium fistulosum* L.

AgNPs		TCC	NCD	M.I%±SD	TAC	BB	BD	CP	LG	MN	NPB	PC	SK	SM	Abn%±SD
	Control	3000	561	18.7±0.57	0										
20ppm	1hr	3000	402	13.4±2.03	63	3	13	4	12	2	2	3	6	18	2.07±0.61
	3hr	3000	381	12.7±1.48	70	5	11	5	19	4	1	6	4	15	2.24±0.59
	5hr	3000	351	11.7±1.43	72	8	8	6	18	2	1	1	9	19	2.38±0.53
10ppm	1hr	3000	460	15.3±1.73	44	4	8	1	11	5	2	1	3	9	1.62±0.49
	3hr	3000	442	14.7±1.36	56	6	13	7	10	3	1	3	2	11	1.85±0.51
	5hr	3000	423	14.1±1.27	61	1	19	9	16	1	2	1	6	16	2.01±0.42
5ppm	1hr	3000	511	17.03±1.76		2	3	1	5	1	0	0	1	7	0.67±0.33
	3hr	3000	494	16.46±1.82		4	6	4	5	2	0	1	3	3	0.92±0.96
	5hr	3000	487	16.23±1.79		3	8	3	7	0	2	1	1	6	1.03±0.98

TCC- Total cell count, NCD- Number of cell division, TAC- Total abnormal cell,BB- Bleb, BD- Bridge, CP- concave plasmolysis, LG- Laggard, MN- Micronuclei, NPB- Nucleoplasmic bridge, PC- pulverized cell, SK- Stathmokinesis, SM- Sticky metaphase, Abn- Abnormality, ± - Standard Deviation

The experiment's findings demonstrated that, in contrast to the control, which exhibited no aberration, silver nanoparticles might produce clastogenic, aneugenic, and non-clastogenic (physiological) aberration. The link between dosage and duration and M.I. is inverse, meaning that as dose and duration rise, M.I. lowers, whereas the relationship between dose and duration and chromosomal aberrations is linear, meaning that as dose and duration increase, chromosomal aberration increases. The different abnormalities demonstrated that AgNPs may function as a clastogen, causing chromosomal disruption and breaking that can result in structural alterations as well as anagenetic, or numerical changes, in contrast to non-clastogens, which result in physiological changes. As the cells advanced through mitosis, several chromosomal abnormalities were seen in them; during interphase or very early prophase, cells displayed hypo- and hyperchromatization, karyopycnosis, followed by karyorrehxis, the first stages of death. Higher concentrations of bleb and plasmolysis were observed.

Metaphase investigations

Excessive chromosomal clumping was seen in the sticky chromosome at the equatorial plate during metaphase (Fig 5). Ring development and breakage of metaphasic chromosomes were also seen. A set of misaligned chromosomes was seen during metaphase (Fig. 6).

Anaphase research

Chromatid bridge and lagging anomalies were the most frequently seen abnormalities at this period (Fig 9). Anaphase bridges came in a variety of forms, including single, multiple, and ring bridges (Figs. 8–10). The distinctive aspect of this experiment is the occurrence of many bridge kinds. Early chromosomal mobility and fragmented anaphase chromosome (Fig. 11) were similarly brought about in three and five hours by 10 nm synthetic silver nanoparticles at a concentration of 20 ppm.

Studies on telophase

The most frequent abnormalities during telophase are multiple laggards, bridge (Fig. 12), and premature chromosomal migration. At all doses, chromosomal disorientation during telophase was seen.

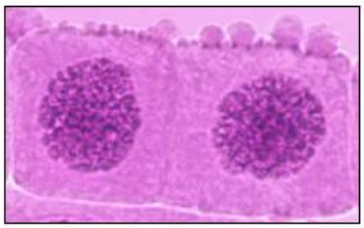

Fig 1 Blebs

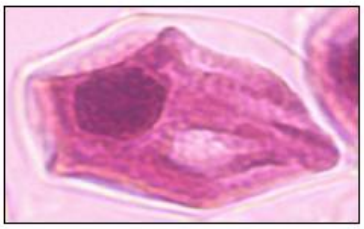

Fig 2 Concave plasmolysis

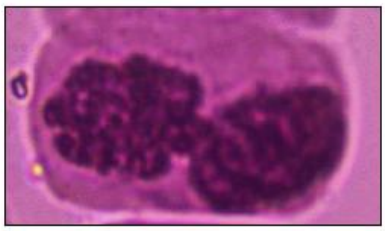

Fig 3 Nucleoplasmic bridge

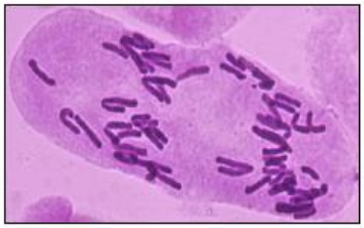

Fig 4 Stathmokinesis

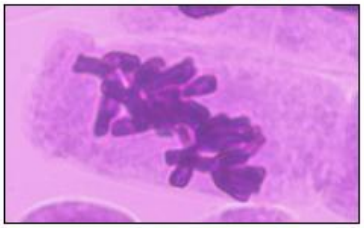

Fig 5 Sticky metaphase

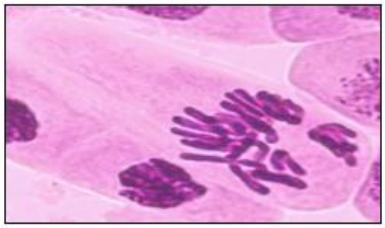

Fig 6 Abnormal metaphase with a group of unaligned chromosomes

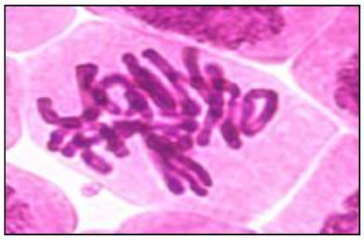

Fig 7 Ring at metaphase

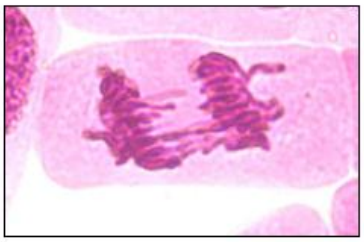

Fig 8 Thick anaphase bridge

Fig 9 Anaphase with multiple laggard

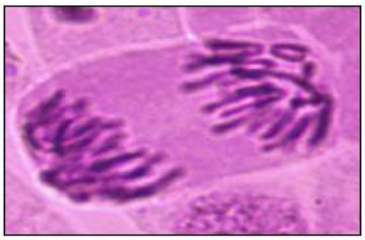

Fig 10 Ring at anaphase

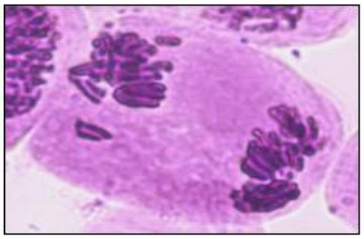

Fig 11 Abnormal anaphase with broken chromosome

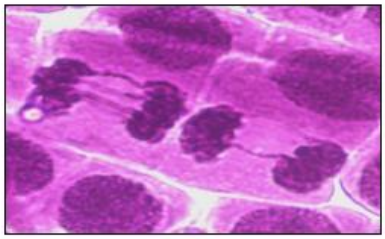

Fig 12 Single and multiple bridge at telophase

Chromosome clumping that results in sticky metaphase, anaphase bridge, and stathmokinesis (Fig. 4) may be caused by AgNPs disrupting the nucleoprotein's protein moiety. Venema [41] demonstrated in 1959 that these anomalies result from disrupted RNA synthesis, which stops protein metabolism. In the current investigation, pulverized chromosomes were detected at concentrations of 10 ppm and 20 ppm of AgNPs, which are the most prevalent characteristics. The bulge in the cell's plasma membrane known as a "bleb" (Fig. 1) is the result of intracellular pressure in the cytoplasm damaging the cytoskeleton. The majority of chromosomal stickiness (Fig. 5) is seen during metaphase, with a smaller amount occurring during anaphase. This phenomenon may be caused by AgNPs' strong nucleic acid polymerization action [18]. During anaphase

segregation, anaphase bridges (Fig. 8) are DNA threads that connect two DNA clumps. It results from a non-protein source of cohesion between sister chromatids, which during the replication process creates sister chromatid intertwines, or SCIs. Persistent unresolved SCIs may create an anaphase bridge during mitosis, however SCIs are resolved in S phase [3]. Stress causes a rise in the number of anaphase bridges, which may be the cause of the production of distinctive anaphase bridges in my experiment since stress increases with AgNP concentration, which enhances the internal environment of the cell. The anaphase bridge creation model known as BFB (Breaking-Fusion-Bridge) differs from the UFB (Ultra-fine bridges) formation model, which involves cleavage.

Immediately after the start of anaphase and is not reliant on cytokinesis [3], [7]. The delayed spindle fiber terminalization is the cause of laggards' presence during metaphase, anaphase, and telophase. Chromosome breakage and deletion caused by spindle fibers dragging the chromosomes toward the poles is the cause of the fragment that developed in between bridges [19]. Ring formation was seen during anaphase (Fig. 10) and metaphase (Fig. 7). This could be caused by an incorrectly attached kinetochore to the spindle, which would join the ends [20], or it could be the result of two terminal breaks in each chromosomal arm, which would fuse the proximal broken ends together or by fusing the dysfunctional telomeres of the same chromosomes, as the shortening of telomeric DNA repeats causes protective proteins to separate from the chromosomal ends. Apart from bridge and laggard, the most prevalent characteristic in most cases was stathmokinesis, a condition where chromosomes are dispersed throughout the cell and may be caused by a disruption or delay in the nucleation of spindle fibers. In all three doses, plasmolyzed cells were less common; however, at 20 ppm, a distinctive concave plasmolysis was seen (Fig. 2), in which the plasma membrane separated from the cell wall by forming concave pockets, which may have resulted from a shift in the cytoplasm's viscosity. The plasmolysis process is influenced by heavy metals and other stressors, hence testing cellular viability may also be done using the plasmolysis test [11], [27], [36].

Neutralizing chemicals, according to Fernandes et al., encourage the total inactivation of the mitotic cycle, which might result in alterations such polyploidy, multinucleated, and micronucleated cells [10]. Kuriyama and Sakai demonstrated in 1974 that the interaction between AgNPs and the tubulin-SH group impairs the mitotic cycle [22]. AgNPs' clastogenic capacity is shown by chromosome breakage (Fig. 11), which may result in the loss of genetic material [9, 5]. When plant cells are treated with AgNPs, an excess of reactive oxygen species (ROS) is produced. This may lead to lipid peroxidation, damage to cell membrane permeability and structure, direct damage to protein and DNA, and ultimately, cell death [2], [45]. Research indicates that exposure to reactive oxygen species (ROS) produced by superoxide, hydrogen peroxide, and activated neutrophils resulted in a large dose-related increase in nucleoplasmic bridge (Fig. 3) [39]. AgNP toxicity may be the reason for the incidence of NPB as AgNPs enhance ROS. Allium cepa and Allium fistulosum chromosomes had almost identical effects across all dosage and time ranges. According to what I've learned from reading publications, among the many clastogenic and non-clastogenic abnormalities brought on by AgNPs, the nucleoplasmic bridge, bleb development, concave plasmolysis, ring at metaphase, anaphase, and thick anaphase bridge were the first to be noticed.

FINAL VERDICT

Ten nm-sized silver nanoparticles obstruct the various M-phase phases. Chromosome aberrations and M.I. variance were noted in relation to the control. These effects depend on both concentration and duration. In our investigations, 10 nm-sized silver nanoparticles demonstrated possible aneugenic and clastogenic effects at all concentrations (5 ppm, 10 ppm, and 20 ppm), with a maximum at 20 ppm. As the concentration increased from 5 ppm to 20 ppm, the M.I. fell compared to the control. Significant chromosomal abnormalities, including as various bridge types, metaphase, and anaphase rings, were seen during anaphase. As AgNP concentrations rise from lower to higher, the frequency of NPB in the root tips of Allium species progressively rises. My observations and data contribute to the understanding of the possible toxicity of artificial silver nanoparticles. According to my research, the use of AgNPs in agricultural activities should be done with extreme caution since there is always a chance of mutagenesis.

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