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Effect of glyphosate toxin, nano silver and carbon nanotubes on the genetic material, germination and antioxidant enzymes of barley

Abstract

Globally, the application of nanoparticles has advanced significantly in recent years. Nonetheless, as nanoparticle usage escalates, there is a concomitant emission into the environment and detrimental effects on organisms. This study employed the Comet assay in vitro to determine the impact of agents containing silver nanoparticles, carbon nanotubes, and glyphosate at five different concentrations (control, 12.5, 25, 50, and 100 mg/L) on the DNA break index of barely (Hordeum vulgare L.). To accomplish this, the seeds were sterilized and cultivated using the sandwich method. Following a period of 72 hours, both the treated roots and the seeds were collected for the Comet assay. The results indicated that the 10-ppm treatment yielded the highest mean number of germinated seeds (25.3 value). However, as the concentration increased, this characteristic significantly decreased, and the 100-ppm treatment produced the lowest mean with 11.48 value. With respect to antioxidant activity, phosphonates produced the highest amounts of SOD, CAT, and APX at a concentration of 100 mg/L (10.3, 58.3, and 11.4 U.mg-1 protein, respectively). DNA fragmentation increased significantly in response to the experimental treatments. Additionally, it was observed that DNA damage exhibited a substantial increase as the concentrations rose. Additionally, among the three agents under investigation, glyphosate exhibited notably more detrimental effects compared to silver nanoparticles and carbon nanotubes.

Keywords: *DNA break; Nanoparticles; Plants; Toxicity*

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Introduction

Over the past decade, nanotechnology has been utilized to solve problems in numerous scientific and industrial fields, including agriculture. Throughout the entire process of agricultural production, processing, storage, packaging, and transportation, nanotechnology serves a multitude of purposes (malik et al., 2023). The application of engineered nanomaterials (ENMs) in environmental remediation, also known as nanoremediation, is an innovative and challenging strategy that ensures the efficient and timely elimination of pollutants from polluted sites (Corsi et al., 2018).

Nanotoxins and nanofertilizers are the most significant nanotechnology agents utilized during the production phase of agriculture. The implementation of nanofertilizers as opposed to conventional fertilizers facilitates the gradual and regulated provision of nutrients to plants. Nanotechnology increases the efficacy of fertilizers, reduces soil pollution, and mitigates the environmental hazards associated with chemical fertilizers (Chadwick et al., 2023). Presently, both the ability of nanoparticles to traverse cellular barriers, infiltrate cells, and engage with subcellular structures, as well as their propensity to induce oxidative stress as a principal mechanism of action, are firmly established. There is currently considerable interest in elucidating the effects of various physico-chemical attributes (particularly surface properties) of nanoparticles on mammalian cells and the environment, in addition to the critical considerations of their small size and surface area (Usman et al., 2020). The cytogenetic effects of chromium (III) oxide nanoparticles on onion root cells were assessed by Kumar et al. (2015). (Allium cepa). A notable reduction in mitotic index (MI) was detected after a duration of four hours of exposure to Cr2O3 NPs: from 35.56 percent (Control) to 35.26 percent (MI) at 0.1 µg/mL, 32.73 percent at 1 µg/mL, 29.6 percent at 10 µg/mL, and 20.92 percent at 100 µg/mL. At various exposure concentrations, optical, fluorescence, and confocal laser scanning analyses identified distinct chromosomal aberrations, including laggard and broken chromosomes, clumped chromosomes, multipolar phases, nuclear notches, and nuclear buds. In contrast to other cytogenetic indices, titanium nanoparticles had no effect on chromosome aberrations of barley or the induced aberration index in treated cells, as demonstrated by Takallu et al. (2013). (Takallo et al., 2013).

According to the fact that environmental effects of nanoparticles should be carefully assessed before widespread commercialization and that few studies have been conducted on cytotoxicity of nanoparticles on plants, this work was developed with the objective of effect of glyphosate, nano silver and carbon nanotubes on genetic material of barley using Comet assay.

Materials and Methods

Treatments and statistical design

In this study, agents containing silver nanoparticles, carbon nanotubes and glyphosate with five different concentrations (control, 12.5, 25, 50 and 100 mg/L) were used. The DNA break index was determined using the Comet assay in vitro. This research was done with 3×5 factorial experiment in a completely randomized design (CRD) using three replications.

Plant samples

In this research, the seeds of Valfajar barley cultivar were used, which were obtained from the Gene Bank of Agriculture and Plant Breeding Department of Agricultural and Natural Resources Campus.

Seed culture

For this purpose, at the first step, the seeds were sterilized and cultured by a sandwich method (Chilvers et al., 1986), a simple and easy approach for the preparation of suitable roots of the seed. After 72 hours, the seeds were harvested with suitable roots. Then, all the treated roots were harvested to perform the Comet assay.Over the past decade, nanotechnology has been utilized to solve problems in numerous scientific and industrial fields, including agriculture. Throughout the entire process of agricultural production, processing, storage, packaging, and transportation, nanotechnology serves a multitude of purposes (malik et al., 2023). The application of engineered nanomaterials (ENMs) in environmental remediation, also known as nanoremediation, is an innovative and challenging strategy that ensures the efficient and timely elimination of pollutants from polluted sites (Corsi et al., 2018).

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Antioxidant enzymes: in this research, the antioxidant enzymes were investigated such as superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX). The NPs engage with the cell walls first. Changes in the membranes, molecules, and cell organelles after entering cells may worsen, increasing the solubilization of dangerous NPs and the generation of ROS, The activity of superoxide dismutase enzyme was measured with the help of nitrobuterazolium (NBT) inhibition assay at 560 nm with a spectrophotometer (Shimadzu UV-1601PC model) according to Bradford's method (Giannopoliti and Ries., 1977). In order to measure the activity of catalase enzyme, it was done by using the method of Dhindsa and Motowe (1981) by calculating the reduction of $H2O2$ absorbance at 240 nm. Ascorbate peroxidase enzyme activity was reported in terms of enzyme units in the amount of total protein (mg) present in 50 microliters of the extract according to the Bradford method in 1976. Also, based on the reaction of hydrogen peroxide with potassium iodide (KI), the amount of hydrogen peroxide was determined.

Preparation of required solutions and suspensions

Silver nanoparticles were prepared by chemical reduction of nitrate salt and the carbon nanotube used in this test was provided by PlasmaChem Company; The size of nanoparticles is about 20 nanometers (Figure 1), in addition, glyphosate herbicide produced by Bahavarchimi Company was used.

Preparation of the fixative solution

The fixative solution was composed of 3: 1 ratio of ethanol to acetic acid. For this purpose, 15 ml of ethanol and 5 ml of acetic acid were used to prepare 20 ml of the fixative solution.

Comet assay

Comet assay [single cell gel electrophoresis (SCGE) assay] is a sensitive and rapid method for detection of DNA strand breaks in individual cells, which has been increasingly used in the past few years. In this investigation, the protocol of Vevers and Jha (2008) was used for comet assay (Vevers and Jha, 2008). Finally, the amount of DNA damage was measured and evaluated by related software such as CometScore ™ and Komet.

Statistical analysis

The data was analyzed by SAS 1.9 statistical software and Duncan test was applied for means comparison.

Results and Discussion

Germination parameters

Rooted Seed number

According to analysis of variances, concentration of genotoxic substrate had significant effects on rooted seed number at 1% statistically level (Table 1) Application of high concentration led to reduction in root seed number, so application of 12.5, 25, 50 and 100 ppm led to 10, 17, 26 and 36% reduction by compare to control (Table 2). At this order Helander et al. (2019) reported the seed germination and seed's growth of faba bean, oat and turnip rape, and sprouting of potato tubers was delayed in the greenhouse experiments in soils treated with glyphosate (Helander et al., 2019).

Radicle length

Treatments and them interactions had significant effect on radicle length at 1 % statistically level (Table 1). Between genotoxic substrate, the highest radicle length was observed by silver nanoparticles with 37.29 mm value and lowest mean was observed by glyphosate with 9.46 mm value (Table 2). In relation to concentration, highest and lowest means were obtained by 10 and 100 ppm with 42.39 mm and 9.74 mm value, respectively. On the other hand, Fayez et al. (2017) suggested that barley grain germination and seedling growth could benefit from the usage of silver nanoparticles (AgNPs) at low concentrations (0.1 mM). On the other hand, grain germination was inhibited and the root length was more strongly reduced at the higher concentrations of AgNPs (0.5 and 1 mM). The chlorosis of the leaves was confirmed by a decrease in photosynthetic pigments and a disarray of chloroplast grana thylakoids in plants treated with Ag+ and AgNPs (Fayez et al., 2017)..

3.1.3. Germinated's Seeds number

This trait was affected by simple effects of treatments and experimental treatment's interaction at 1% statistically level (Table 1), In relation to genotoxic substrate, silver nanoparticles, carbon nanotubes and glyphosate showed 28.9, 20.4 and 5 means (Table 2). The highest means were obtained by 10 ppm with 25.3 value and by the increase of concentration, this trait reduced significantly, so, 100 ppm treatment showed lowest mean with 11.48 value (Table 2). But Khodakovskaya et al. (2009) reported Carbon nanotubes were found to penetrate tomato seeds and affect their germination and growth rates. Gomes et al. (2017) resulted that the glyphosate herbicide reduced seed germination by unsettling the mitochondrial electron transport chain, leading to reduced energy (ATP) production

Plumule Length

Analysis of variance for plumule length showed significant effect of treatments at 1% statistically level and them interaction at 5% statistically level. Mean comparison showed that silver nanoparticles had highest mean with 17.4 mm value and lowest mean was related to glyphosate with 4.6 mm value (Table 2). Application of 12.5, 25, 50 and 100 ppm led to 51, 60, 67 and 81% reduction by compare to 10 ppm, respectively (Table 2). Khan et al (2020) reported that barley crops with glyphosate resulted in decreased levels of germination and biomass (Khan et al, 2020).

Antioxidant activity

According to analysis of variance, it was founded that treatments and them interactions had significant effects on antioxidant activity at 1% statistically level.

In relation to antioxidant activity, the highest activity of SOD, CAT and APX were obtained by Glyphosate at 100 mg/L with 10.3, 58.3 and 11.4 U.mg^{-1}protein values, respectively. The antioxidant activity was reduced by the reduction of concentration in all genotoxic substrate treatment. Chung et al (2019) reported that Reactive oxygen species (ROS), malondialdehyde (MDA), and hydrogen peroxide (H_2O_2) production were also enhanced in the Brassica rapa ssp. rapa seedlings exposed to CuO NPs, which could have caused DNA damage that was detected by a DNA laddering assay.

DNA properties

In relation to percentage of DNA in tail (%DNA) and according to analysis of variance (Table 5), genotoxic substrate and concentration had significant effects at 5 and 1% statistical

level on %DNA tail, and interaction of genotoxic substrate with concentration showed no significant effects on %DNA tail. According to mean comparisons, the %DNA tail increased through increase of concentration, and all concentration levels had significant differences with control (Table 6). The highest %DNA tail between genotoxic substrates was obtained by glyphosate.
(Table 7).

In this respect, Mattiello et al. (2015) in the evaluation of genotoxicity in barley exposed to CeO_2 and TiO_2 nanoparticles reported differences between treated and control plants at chromosomal level with a reduction of cell divisions (Mattiello et al., 2015).
Results on tail length showed that it was a trait affected by genotoxic substrate and concentration at 1% statistical level, also their interaction had significant effect at 5% statistical level. Control and 100-ppm treatments showed lowest (20.95) and highest (62.42) mean of tail length, respectively. Application of 12.5, 25, 50 and 100 ppm led to 1.82, 2.06, 2.40 and 2.97 folds increase of tail length compared to control. The lowest and highest means among genotoxic substrate treatments were obtained by nanosilver and glyphosate (Table 5).
Lee at al. (2013) studied the genotoxic effects of ZnO and CuO nanoparticles on early growth of buckwheat, and their results showed different DNA polymorphisms at 2,000 and 4,000 mg L^{-1} of ZnO and CuO NPs compared to controls (Lee et al., 2013). In a research by Moreno-Olivas *et al*. (2014) on *Cucurbita pepo*, RAPD profiles of TiO_2 NPs treated plants showed differences in band intensity, loss of bands, or appearance of new bands as compared to untreated plants (Moreno-Olivas et al., 2014).
In relation to tail moment, all simple and interaction treatments had significant effects at 1% statistical level and the tail moment increased by increasing concentrations, and all concentration levels had significant differences with control (Table 4). Application of 12.5, 25, 50 and 100-ppm concentrations led to 3.01, 4.49, 6.56, and 9.88-fold increase of tail moment as compared to control. The lowest and highest means among genotoxic substrate treatments were obtained by nanosilver and glyphosate (Table 5). Also, in relation to interaction between concentration and materials, it was founded that the highest value was obtained by 100ppm of Glyphosate (Table 6). Ma et al. (2023) reported changes in physiological and agronomical parameters of barley (*hordeum vulgare*) exposed to titanium dioxide nanoparticles (Marchiol et al., 2016). Nhung et al. (2018) evaluated biological effects of four iron-containing nanoremediation materials on the green alga *Chlamydomonas* sp, whose effects on *Chlamydomonas* sp. decreased in the order FerMEG12 > Carbo-Iron® > Fe-zeolite > Nano-Goethite (Nguyen et al., 2018).

Conclusion

A significant increase was observed in DNA fragmentation in experimental treatments, which was enhanced by increasing concentrations. Also, among the three studied agents, the damaging effect of toxic glyphosate on the genetic material was significantly higher than silver nanoparticles and carbon nanotubes.

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Table1: Analysis of variance for germination properties

		rooted Seed number	radicle length	Germinated Seed number	Plumule Length
Genotoxic substrate	2	21.71^{ns}	3065.4^{**}	2206.9^{**}	939.1^{**}
concentration	4	165.8^{**}	1386.7^{**}	220.2^{**}	384.1^{**}
Genotoxic*concentration	8	30.65^{ns}	284.2^{**}	178.5^{**}	51.2^{*}
error	30	15.07	63.11	33.72	21.36

ns, *and ** show no significant and significant at 5 and 1% statistically level

Table 2: Mean comparisons of germination properties in response to treatments interaction

Genotoxic substrate	Concentration mg/L	Rooted Seed number	Radicle length (mm)	Germinated Seed number	Plumule Length (mm)
Glyphosate	100	17h	2.28j	2e	1.5j
	50	19fgh	3.45ij	4e	1.5j
	25	22d-g	7.44hi	5e	2.8ij
	12.5	23cde	8.32gh	6e	4.7hi
	10	26bc	25.81d	9d	12.5e
Carbon nanotubes	100	18gh	5.95hij	13d	2.9ij
	50	22d-g	11.68fg	19c	5.9h
	25	24cd	15.67ef	20c	7.3gh
	12.5	26bc	16.55e	21c	9.2fg
	10	29ab	38.6b	27b	20.0b
Silver nanoparticles	100	20e-h	25.55d	22c	11.3ef
	50	23cde	31.28c	28b	14.3d
	25	26bc	35.27bc	29b	15.7cd
	12.5	28ab	36.15b	30b	17.6bc
	10	31a	58.2a	36a	28.4a

Table3: Analysis of variance for Antioxidant activity

	df	SOD	CAT	POD
Genotoxic substrate	2	43.33^{**}	15.8^{**}	17.1^{**}
concentration	4	50.8^{**}	11.2^{**}	19.1^{**}
Genotoxic*concentration	8	35.31^{**}	9.7^{**}	12.3^{**}
error	30	3.2	1.2	2.8

ns, *and ** show no significant and significant at 5 and 1% statistically level

Table 4: Mean comparisons of Antioxidant activity in response to treatments interaction

Genotoxic substrate	Concentration mg/L	SOD	CAT	APX
		U.mg^{-1}protein		
Glyphosate	100	10.3±a	58.3±a	11.4±a
	50	6.7±c	39.5±bc	10.1±ab
	25	5.4±d	22.6±de	8.3±bc
	12.5	4.1±def	12.6±efg	6.5±cde
	10	3.2±fg	10.5±fg	6±def
Carbon nanotubes	100	8.4±b	43.5±b	9.4±ab
	50	4.8±de	38.6±bc	7.4±cd
	25	3.9±df	20.1±e	4.4±efg
	12.5	3.3±fg	10.4±fg	4.3±fg
	10	2.5±ghi	7.8±fg	3±g
Silver nanoparticles	100	6.9±c	30.1±cd	7.4±cd
	50	3.8±ef	15.3±ef	5.4±ef
	25	3.5±f	7.3±fg	4.4±efg
	12.5	2.1±hi	5.5±g	3.2±g
	10	2±i	4.7±g	2.8±g

Table 5. Analysis of variance for studied traits (means of squares).

Source of variation	DNA Tail%	Tail Length	Tail Moment
Genotoxic substrate	196.84*	2257**	731.6**
concentration	1526**	2159**	998.3**
Genotoxic*concentration	73.07	262.8*	160.2**
Error	55.05	89.28	38.25

*and** show significant effects at 5 and 1% statistical levels

Table 6. Mean comparisons between treatment concentrations.

Concentration	DNA Tail%	Tail Length	Tail Moment
0 ppm	12.2144d	20.9556d	3.0971d
12.5 ppm	24.5000c	36.4722c	9.3367c
25 ppm	31.1500bc	43.2011bc	13.8886c
50 ppm	37.2678b	50.3856b	20.2829b
100 ppm	46.6878a	62.4167a	30.5475a

At each column, treatments with at least a similar alphabet show no significant differences.

Table 7. Mean comparisons between genotoxic substrate treatments.

Material	DNA Tail%	*Tail Length*	Tail Moment
Nanosilver	27.9613b	31.7207c	9.6179b
Carbon nanotubes	28.6000b	40.4020b	13.4955b
Glyphosate	34.5307a	55.9360a	23.1783a

At each column, treatments with at least a similar alphabet show no significant differences.

Table 8 Interaction between genotoxic substrate and concentration on tail length.

Concentration	Nanosilver	Carbon nanotubes	Glyphosate
0	21 hi	31 f	37e
12.5	47d	66b	17i
25	33ef	37e	35ef
50	36ef	25g	45d
100	56c	69b	85a

Treatments with at least a similar alphabet show no significant differences

Table 9 Interaction between genotoxic substrate and concentration on tail moment.

Concentration	Nanosilver	Carbon nanotubes	Glyphosate
0	3gh	7fg	10ef
12.5	17cd	31b	2h
25	10ef	12e	11ef
50	13de	4gh	11ef
100	20c	33b	48a

Treatments with at least a similar alphabet show no significant differences

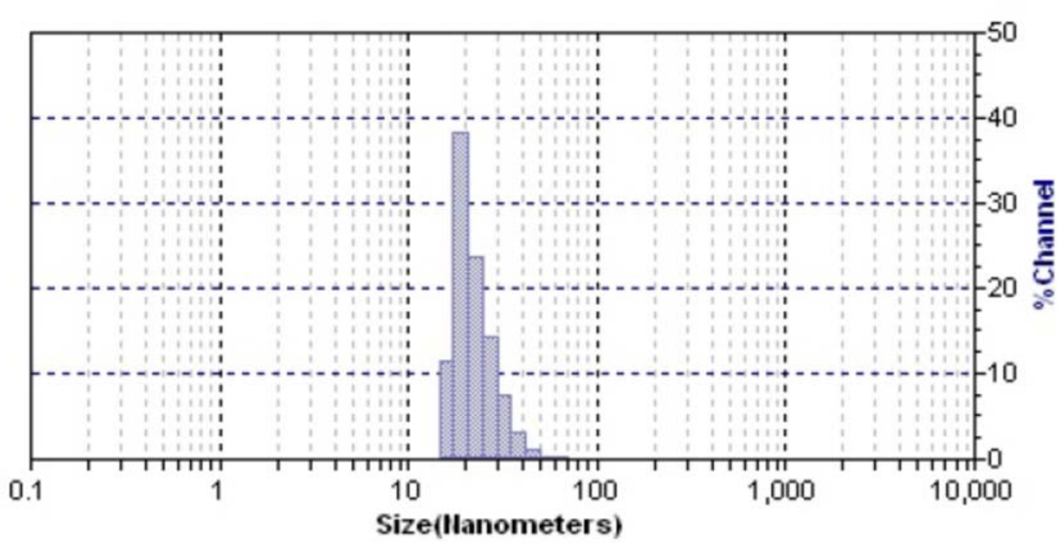

Figure 1. Distribution chart of silver nanoparticles: the size of nanoparticles is about 20 nanometers.