Faculty of Women for, Arts, Science, and Education

Scientific Publishing Unit

Journal of Scientific Research in Science

Biological Sciences

Volume 41 , Issue 2 , 2024

ISSN 2356-8372 (Online) \ ISSN 2356-8364 (print)

Journal of Scientific Research in Science 2024, 41, (2),95:121

Contents lists available at EKB

Journal of Scientific Research in Science

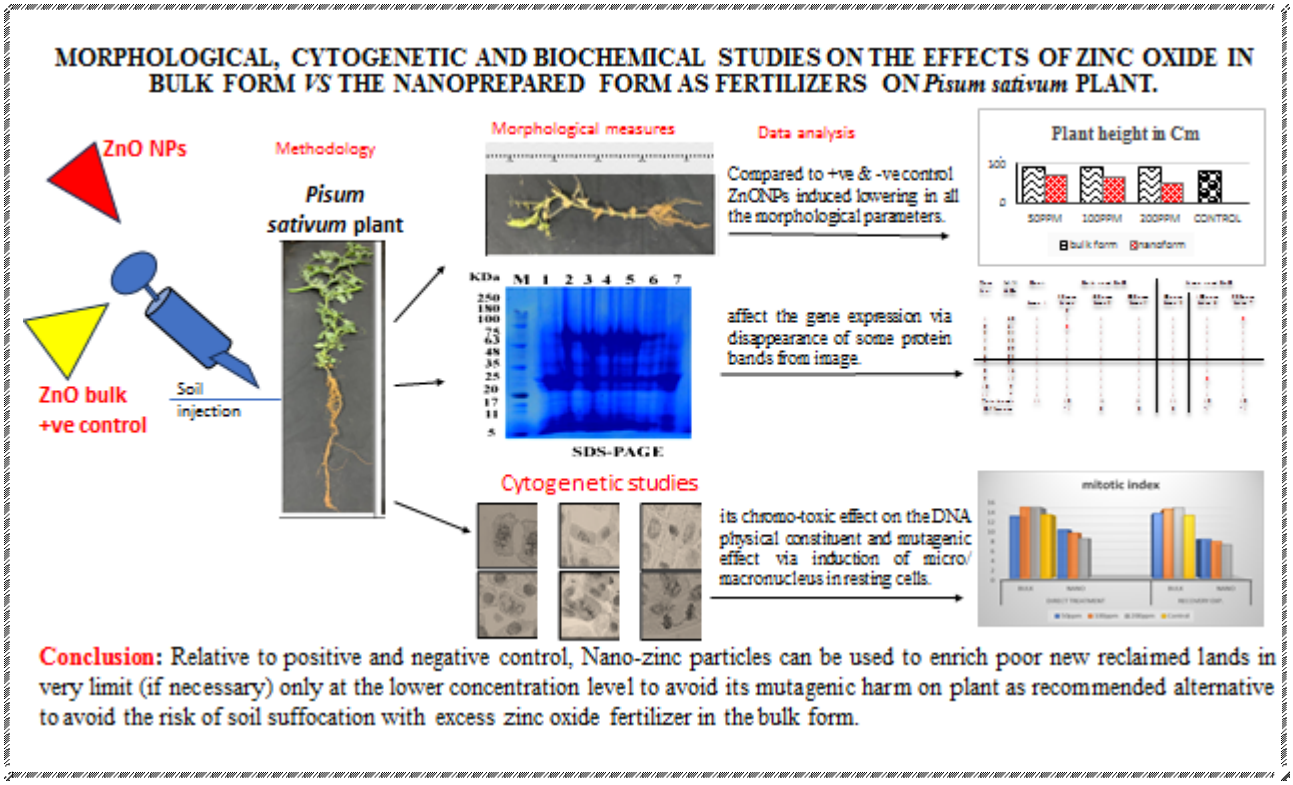
Journal homepage: https://jsrs.journals.ekb.eg/

Morphological, cytogenetic and biochemical studies on the effects of zinc oxide in bulk form *vs* the nanoprepared form as fertilizers on *Pisum sativum* L. plant.

Safaa, M. Saleh[1]*, Nahla, O.M.Ehsan[1], Rania, T. Ali [2], Emad, A. Abdeldaym[3], Abd-Elwahab, M. Mahmoud [4] and Thoria, R. Mohamed[1].

[1]Botany Department, Faculty of Women for Arts, Science and Education Ain Shams University, Cairo, Egypt.
[2]Genetics and Cytology Department, National Research Center, Dokki, Egypt.
[3]Vegetable Crops Department, Faculty of Agriculture, Cairo University, Giza, Egypt.
[4]Physiology Department, Faculty of Agriculture, Cairo University, Giza, Egypt.

Graphical abstract



***Corresponding author**: **Safaa, M. Saleh ,**Botany Department, Faculty of Women for Arts, Science and Education Ain Shams University, Cairo, Egypt.
Email: safaaismael28@gmail.com
(Received 19 Nov 2024, revised 14 Dec 2024, accepted 18 Dec 2024)
https://doi.org/10.21608/jsrs.2024.337476.1140

Abstract

Zinc element is very essential in planting process; supplying plants with zinc element improve plant growth, development and performances in norm and under stresses. The new introduced nano zinc preparations should be evaluated and revised for their safety usage in agriculture. **Methods**: in this work the zinc oxide fertilizer in naturally bulk form is used as positive control to visualize effect of zinc oxide in nano form in different concentrations (50, 100 & 200ppm) on *Pisum sativum* plant. The study concerned some morphological traits and the total soluble seedling's protein profile after the soil around root was injected with used materials, in addition to cytogenetic studies on the mitosis system after the direct treatment for 3hr and after the 24hr of recovery experiment. **Results:** the study confirms the great risk of the nanoforms usage on all the studied parameters on the level of the two higher concentrations if compared to -ve and +ve control. It induced lowering in all the morphological parameters. On the level of cytogenetic parameters, it empathized its chromo-toxic effect on the DNA physical constituent and mutagenic effect via induction of micro/ macronucleus in resting cells. It also affected the gene expression via disappearance of some protein bands from image. **Conclusion:** lower concentration of nano zinc oxide particles can be used to enrich new reclaimed lands in limited conditions (if necessary) to avoid its mutagenic harm on plant as recommended alternative and to evade the risk of soil suffocation with excess ZnO fertilizer in bulk form.

Keywords: *Pisum sativum* L., ZnO-NPs, plant growth, mitosis apparatus, protein profile

I. Introduction

Nanotechnology is one of the most-trendy approaches in all areas of science and technology. It creates and utilizes nano-sized particles ranges between 1 and 100 nm in at least one dimension **[1].** Nanoparticles display completely new physical, chemical and biological properties compared to larger particles of bulk material and these novel properties have led to a wide range of applications in agriculture, crop improvement and medical fields **[2].** In recent years, nanotechnology has a great application in the field of agriculture; especially in pest and disease management. It can be used in the preparation of new formulations like insecticides, pesticides, pheromones, insect repellents and fertilizers **[3, 4].**

The speedy expansion of using nanotechnology has increased attention over the impact of nanoparticles (NPs) on the environment, elevating concerns for environmental hazards and adverse health effects **[5, 6].** Nanoparticles can arrive to the environment due to different human activities, such as chemical manufacturing; or during environmental remediation efforts, domestic wastewater and crop improvement **[7].** Release of nanoparticles in the environment may have harmful effects on plants, thus affecting the whole food chain. Nanoparticles may binds to some vital cellular

constituents leading to production of reactive oxygen species (ROS) causing oxidative stress, cytotoxicity and genotoxicity **[8, 9, 10].**

Zinc oxide is one of the nanoparticles that have attracted research attention in the recent years. ZnO-NPs have several uses, involving sunscreens, personal care, and paints **[11].** Also, they have been used as delivery mediator for drug treatment and applied for tumor cell **[12].** ZnO-NPs could reach to plants either directly or indirectly. Direct exposure includes their application as fertilizers and as fungicides **[13, 14].** While indirect way could occur through the release of ZnO-NPs into the soil, water, and air because of excess production, use, and disposal. Thus, the ecological risk of using ZnO-NPs must be validated for its' benefit /harm issue and it represents essential research point.

Lately, phytotoxicity of ZnO-NPs on plant growth has been documented in cabbage **[15],** rice **[16],** black mustard **[17],** corn **[18],** Arabidopsis **[19]** and Pea **[20].**

Additionally, **Wang et al., [21]** reported a significant decline in the dry weights of shoots and roots of maize after ZnO-NPs treatment. ZnO-NPs caused genotoxicity in broad bean **[22].** Also, **Wang et al., [23]** declared that, 50 and 100 mg/L of ZnO-NPs had an inhibitory effect on plant growth on *Ginkgo biloba*.

A limited number of studies have been focused on the genotoxic impacts of NPs on plant systems. Studies on the assessment of toxicity of zinc NPs on plants are insufficient. Since plants provide efficient systems for screening the cytotoxic and genotoxic potentials by several types of chemicals **[24, 25].** Several plant bioassays have been suggested to reveal the toxic effects of nanoparticles (NPs). Pea plant is an excellent model plant to evaluate genotoxic effect of environmental contaminants due to its sensitive mitotic dynamics and large size of chromosomes.

The pea (*Pisum sativum* L.) is one of the cultivated legume crops that belong to the Fabaceae family, a diploid crop with the number of chromosomes (2n = 14) **[26].** The pea is an important legume cultivated and widely consumed throughout the world. As a rich source of protein, essential amino acids (lysine and leucine) carbohydrates, vitamins and minerals like potassium, phosphorus, calcium, copper, iron, and Zn, peas are important in human nutrition. Consumed mainly as green peas, total production worldwide is around 21.22 million tons **[27].**

Assessment of the mitotic index, phase index, chromosomal aberrations, and micronuclei induction would be important to give a confirmation and explanation to the cytogenicity of NPs in plants. Previous studies pointed to a decrease in MI values in the root tips of *Allium cepa* and *Vicia faba* with increasing concentration of ZnO-NPs **[28, 29].**

Protein content represents the final basic product of gene expression. Therefore, any noticeable alteration in protein-banding pattern brought by any mutagen is considered a reflection for genetic variations **[30].** Based on that, any alteration in protein profile could be beneficial for revealing the mutagenic effects of pollutants or chemicals such as nanoparticles.

The aim of this study was to evaluate the toxic hazard effects of ZnO-NPs on morphology, cytogenicity, and biochemistry levels in *Pisum sativum* L. plant.

II. Methodology

The field work of the present study was carried out in green house of Cairo University, faculty of agriculture, Giza, Egypt.

The cytology and cytogenetic works were carried out in the Department of Genetics and Cytology, Biotechnology research institute, National Research Centre, Dokki, Cairo Egypt.

- **Plant materials:**

The Pea (*Pisum sativum* L.) seeds (Var. Master B) were used for all the conducted experiments and were obtained from the vegetable crops research department, Horticulture Research Institute, Agricultural Research Centre, Giza, Egypt.

- **Chemicals:**

- Zinc element symbol (Zn) with atomic weight 65.38 in bulk size (EDTA Zn) and Nano zinc oxide were kindly afforded and prepared by Dr. Mohamed Abd-Elwahab in physiology department, faculty of agriculture, Cairo University, Giza, Egypt.

- Cytogenetics study: The chemicals for cytogenetic studies were prepared according to **[31]** with some modifications.

- Biochemical studies: the chemicals for protein fingerprint were prepared after **[32].**

- **Methods:**

1- Preparation of Zinc nanoparticles:

All the used reagents were of analytical grade and the nanoparticles were prepared from their precursors. Zinc in the form of zinc chloride ($ZnCl_2$) was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Zinc nanoparticles were obtained by the top to bottom molecular chemical method [33]. Nano zinc was prepared from an aqueous solution of zinc chloride. Sodium hydroxide solution was added slowly in a molar ratio of 1:2 under vigorous stirring for 8 h. The obtained precipitate was

filtered and washed thoroughly with deionized water in a mixed water/toluene system using a high-speed stirrer, and then washed again with ionized water alone for 3 h. The precipitate was dried in an oven at 100 °C, (2.75 g nanoparticles powder) then exposed to 1.5 psi of pressure for 3 days discontinuously (7 h per day) **[34].**

2- Field work:

Seeds of pea were planted at the beginning of November under normal field conditions in 40 cm diameter pots filled with soil mixture (sand & clay) were arranged into three separate groups in three rows, a row for each examined concentration (50, 100 and 200ppm) apart from each other, as described:

1- Group (1) for non-treated soil as negative control.

2- Group (2) for treatment with zinc oxide in bulk size as a positive control.

3- Group (3) for treatment with zinc oxide in nanoform.

Seeds were sown in each pot, and injected twice after 40 days of sown, with time interval 20 days between two injections.

The plants in each pot were tested for the variation in metric traits (plants height, fresh & dry weights of shoots, fresh & dry weights of roots, number of leaves, number & weight of pods/plant and weight of 100 fresh seeds).

3- Cytogenetic studies:

Nearly equal sized pea seeds were germinated on filter paper rolls in large beakers with 3cm height tap water at the bottom, after germination 2-3 cm long root were directly treated for three hours with negative & positive control and the tested material in the three used concentrations (50,100 &200 ppm), then after that, the roots were fixed in carnoy's fixative for 24 hours at 4°C followed by rinsed several times in tap water stored in 70% ethyl alcohol in refrigerator till slides preparation. At the time of preparation of the slide, the roots were rinsed in a descending series of ethanol concentration from 70% to 30% to distilled water for 30 min. in each.

Hydrolysis followed in 1N HCl at 60^{o}C for 10 min. after cooling, the roots were rinsed with distilled water and the root tip stained with a drop of aceto-carmine stain immediately and covered with cover slip, then squashed between blotting paper folds.

The prepared slides were examined under light microscope, 1000 cells were scored from each root of 9 plants (3plants/ replicate) the frequencies of the different stages of mitosis, as well as the frequencies of different mitotic abnormalities were determined.

4- Biochemical analysis:

Total proteins were extracted from the treated plant's leaves to perform sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). 1g of leaves was grinded and mixed with 1 ml of extraction buffer in Eppendorf tube and left in refrigerator overnight, then vortexed for 15 seconds and centrifuged at 12,000 rpm at 4˚C for 20 min. The supernatants containing total proteins were transferred to new Eppendorf tubes and kept at deep-freeze until use for electrophoretic analysis. The marker of the used protein is BLUltra Pre stained Protein Ladder (GeneDirex, Cat No. PM001- 0500). In this method, 10% protein separating gel were used. Protein fractionations were performed on vertical slab gel (19.8 cm x 26.8 cm x 0.2 cm) using the electrophoresis apparatus manufactured by Cleaver, UK. The images were captured by digital camera (Sony, made in Japan) and transferred directly to the computer and then the protein bands were analyzed by Total Lab program to find out the molecular weight of each band and that to find the effect of each treatment on gene expression of different genes responsible for the formation of proteins in pea plant.

5- Statistical analysis:

The mean values of 3 replicates in obtained data were tested via analysis of variance (one-way ANOVA) using Statistica 7 software to evaluate the significant differences between treatments and Tukey's tests at $p \leq 0.05$ were applied to compare means.

III. Results & discussion

The difference in the morphological measurements of the pea's plant is the interaction of plant metabolism and environmental effects. Plant height, number of leaves, and shoot weight are important and simply noticeable forms of growth variations in the plant which eventually lead to the upsurge in total biomass of the plant and which has been noted with the addition of different types and concentrations of zinc. Compared with negative control (water) & positive control (bulk form), soil application of ZnO in nano form at different concentrations (50, 100 and 200 ppm) showed lower

values of vegetative growth parameters, including plant height, shoot fresh weight, root fresh weight, shoot dry weight, root dry weight, and number of leaves **Table (1)** and **Figure(1).**

A similar trend was observed in total yield and its components. Whereas, the minimum values of the number of pods per plant, weight of pods per plant and weight of 100 fresh seeds were observed in plants treated with nano ZnO at different concentrations. The maximum values were noted in plants supplied with bulk ZnO at different concentrations. It can be observed from **Table (1)** that the pea's plants treated with ZnO-NPs at 200 ppm showed the lowest values of vegetative growth measurements and total yield per plant compared with all of the other treatments.

On the same track, **[35]** pointed to the inhibitory effect of ZnO-NPs on the root growth of *Allium cepa* plant and referred to this inhibition as the cytotoxic effect of released Zn^{+2} dissociated from ZnO-NPs. **Yang et al., [19]** pointed to the effects of different concentrations of ZnO nanoparticles (ZnO-NPs) on the growth performance of *Arabidopsis thaliana* seedlings illustrating that ZnO-NPs caused strong inhibitory effects on several plant growth indices such as root length. Also, **Sorahinobar et al., [36]** referred to the suppression in the growth parameters after treatment of the mung bean plant with ZnO-NPs to the induced oxidative stress (ROS).

Several studies related to our findings such as **[37, 38, 39, 40]** reported the negative effect of higher levels of ZnO-NPs on plant growth indices. **Srivastav et al., [41]** referred to the ZnO-NPs toxicity (at a concentration of 50 to 200 ppm) to the perturbed homeostasis of Zn and its impacts on other elements' homeostasis.

The relative suppression in the studied parameters was previously obtained by **[42]** when testing the Ag-NPs on the *Vicia faba* plant; and referred to this suppression as the effect of nano-silver on the cellular energy level *via* affecting the ATP production. The study conducted by **[43]** showed a decrease in the fresh and dry weights of the seedlings of *Portulaca oleracea* L. in the treated groups compared to the control group and they declared that loss may be due to the disruption of the biosynthesis and transfer of growth regulators, such as gibberellic acid and auxin, in the plants treated with ZnO-NPs. This outcome is consistent with the studies on the *Brassica napus* plant conducted by **[44].** Also, study of **[45]** on the toxic effects of 200, 500, 1000 and 1500 mg L^{-1} of ZnO NPs on the growth and metabolism of *Brassica juncea* displayed a decline in the fresh and dry weights of plants in a dose-dependent manner. Similarly, a high concentration (2000 mg/mL) of ZnO-NPs displayed poor growth, crop yield and pod development in *Arachis hypogaea* **[46].**

The obtained data were in conflict with **[47]** who observed ZnO-NPs increased root elongation whereas, the bulk treatments showed both root and stem elongation, this conflict may have resulted from the different experimental conditions and used concentrations.

The outcome is consistent with **[48]** who recorded that, yield parameters (the number of mature pods per plant, number of seeds per pod and weight of 100 seeds) for the second generation of pea plant treated with silver nanoparticles were reduced.

Table(1): Influence of different treatments of ZnO in bulk and Nano form on growth indices of pea plant (plant height, shoot fresh weight of shoot & root, dry weight of shoot& root, number of leaves, number & weight of pods/plant and weight of 100 fresh seeds).

Treatment		Plant height	Shoot fresh wt.	Root fresh wt.	Shoot dry wt.	Root dry wt.	No. of leaves	No. of pods /plant	Wt of pods /plant	Wt of 100 fresh seeds
	Control	84.67 b	9.50 b	5.60 b	1.77 c	3.50 c	11.07 b	2.33 b	12.33 c	36.80 c
B	**50ppm**	92.67 a	11.60 a	8.60 a	2.80 b	5.90 a	13.00 a	4.33 a	19.83 a	39.09 b
	100ppm	91.67 a	10.70 a	7.80 a	2.70 b	5.10 b	13.33 a	3.33 ab	22.93 a	47.33 a
	200ppm	92.33 a	10.10 ab	8.80 a	3.90 a	6.10 a	13.67 a	3.00 ab	16.13 b	33.30 d
N	**50ppm**	69.33 c	9.80 b	1.50 c	1.50 cd	0.90 d	10.67 b	1.67 c	9.20 d	25.10 e
	100ppm	63.67 c	8.60 bc	1.90 c	1.40 d	1.30 d	8.67 c	1.33 c	7.10 d	20.90 f
	200ppm	52.33 d	7.10 c	2.50 c	0.80 d	0.40 e	7.33 c	0.00 b	0.00 e	0.00 g

Means followed by different letters point to significant differences between the treatments according to Tukey HSD test ($p \leq 0.05$). B: bulk - N: Nano.

Figure 1(a-g): Snapped photograph from field experiment show the detected changes in the whole pea plant after soil injection for two times with zinc oxide in bulk and prepared nanoform.

Cytogenetic studies on the *Pisum sativum* root tip meristems were carried on to reveal the direct effect of zinc oxide fertilizer in nanoform relative to -ve control (water) and to +ve control (bulk sized) on the mitosis apparatus -as a sign to plant healthy growth- to confirm its advantage or disadvantage if applied as fertilizers.

The obtained results revealed that zinc oxide in bulk size enhanced the mitotic index over control (13.78%) to reach (15.36%) after 200ppm of zinc oxide.

On the other hand, the nano form zinc oxide induced a concentration dependent decrease in the mitotic index to reach its highest value 8.62% after 200ppm **(Figure 2& Table 2).** This reduction in the mitotic index was previously obtained by **[49]** when applying the Ag-NPs (1%, 3% and 5%) on the *Allium cepa* plant; and referred to this decline as the ability of nano-silver to inhibit or block the formation of several metabolites essential for a normal sequence of mitosis. The mitotic normal currency is affected by many factors such as the rate of the DNA synthesis **[50],** the easygoing cell cycle **[51],** protein synthesis and metabolites' availability **[52].**

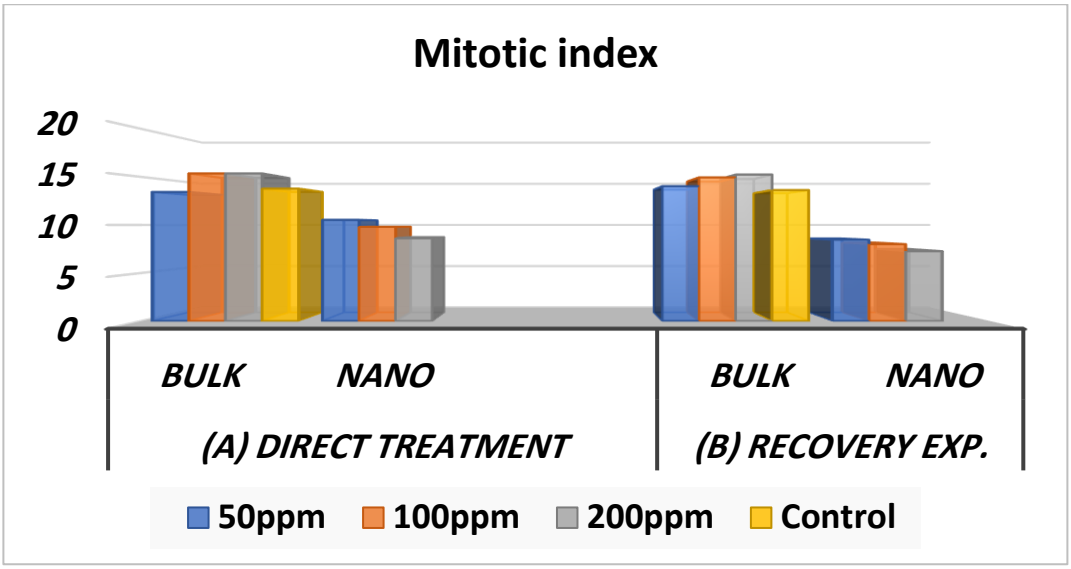


Figure (2): The mean performance of mitotic index in *Pisum sativum* root-tip meristems after 3hours of direct treatment with bulk & Nano zinc (A) and after the 24 hr of recovery after treatment with bulk & Nano zinc (B).

Table (2): The mean performance of mitotic index, percentage of abnormalities and phase index in *Pisum sativum* root-tip meristems after 3hours of direct treatment with bulk & Nano zinc (a) and after the 24 hr of recovery after treatment with bulk & Nano zinc (b).

Treatment	Mitotic index		% abnormalities		Phase index					
					Prophase		Metaphase		Ana-telophase	
	Bulk	Nano	Bulk	Nano	Bulk	Nano	Bulk	Nano	Bulk	Nano
(a) 3h Direct treatment										
50ppm	13.42 b	10.52 c	14.49 b	31.78 a	47.61 a	32.95 c	23.10 b	35.20 a	29.29 b	31.85 ab
100ppm	15.36 a	9.80 cd	14.83 b	35.71 a	37.77 b	41.25 ab	25.24 b	25.35 b	36.99 a	33.40 ab
200ppm	15.36 a	8.62 d	15.12 b	34.28 a	44.76 a	36.90 bc	24.82 b	28.81 b	30.42 ab	34.29 a
Control	13.78 b		4.76 c		48.19 a		23.06 b		28.75 b	
(b) 24h recovery after treatment										
50ppm	14.02 b	8.46 c	14.10 c	27.60 b	45.07 a	40.34 b	24.08 b	30.43 a	30.85 ab	29.22 ab
100ppm	14.94 a	8.00 cd	12.79 c	32.78 a	40.13 b	42.20 ab	24.78 b	31.75 a	35.10 a	26.05 b
200ppm	15.23 a	7.27 d	15.31 c	33.18 a	43.49 a	37.30 c	23.46 b	33.62 a	33.05 ab	29.08 ab
Control	13.62 b		5.87 d		46.08 a		23.73 b		30.19 ab	

Means followed by different letters point to significant differences between the treatments according to Tukey HSD test ($p \leq 0.05$).

The decrease in the growth parameters as result of mitotic index after nano zinc oxide was previously obtained by **[35]** after treatment the *Allium cepa* plant with 5& 50µg/ml of ZnO-NPs for 12, 24 and 36 h.

The mitosis decline observed in our experiment came in contradiction with **[53],** who highlighted mito-accelerating effect of the nano zinc oxide on *Vicia faba* root tip meristems. Thus, it may be due to the used concentrations, experimental conditions and the nano-particles preparation method.

It is worth mentioning that, the recovery experiment for 24 hr after treatment didn't fix the mitotic index after nano zinc oxide treatment which reflects the irreversible damage on the mitotic apparatus **(Figure 2).**

Regarding that the mitotic index sheds its effect on plant growth; the collected data about the increasing in plant growth indices after treatment with zinc oxide in bulk form; and about the decline in same studied parameters after treatment with zinc oxide in nanoform are very reasonable.

The progressive concentration dependent reduction in the mitotic indexes after the nano treatment was accompanied by lowering the prophases on response to the expanding the metaphases and ana-telophases after all used concentrations. The changes in phase's duration pointed to acceleration in the cell cycle which is often followed by genome instability, allowing cell survival on count of mutation induction **[54].** It is worth mentioning that, direct treatment with zinc in bulk size did not affect the phase indexes except after 100ppm concentration which expand the ana-telophases on response to prophase shortening **(Table 2).**

Recovery after treatment with bulk form of zinc oxide showed little shrinkage in prophase on response to ana-telophase expansion after 100ppm concentration only. The same concentration (100ppm) of nano zinc oxide expanded metaphases on response to the ana-telophase shrinkage, 200ppm expand the metaphases over prophases **(Table 2).**

The mitodepressive effect of ZnO-NPs was combined with abnormal mitosis; this came in agree with **[55]** when carry on a comparative study on the ZnO-NPs (0.5, 1 and 3%) as soil fertilizer with two bio-fertilizers Moringa and yeast extracts on *Vicia faba* plant and referred this depression to the direct effect of nano zinc oxide on the cellular mitochondria structure and integrity which is expected to handicap the mitochondria to carry normal function.

The treatment with nano- zinc oxide raised the risk of abnormal mitosis relative to-ve and + ve controls. Worth to mention that, the induced percentage of anomalies in mitosis after nano zinc

oxide exceeds over twice the induced anomalies after bulk sized particles. This finding came in agree with **[56, 53]** may be explained by the massive penetration of the very tiny particles of nano-zinc into the root cells to reach the chromosome modules.

In contradiction with **[57]** who pointed to the concentration dependent increase in the anomalies and the cytotoxic effect in *Pisum sativum* L. and *Hordeum vulgare* L. plants after exposure to nano- ZnO at 1, 10, and 30 times of maximal permissible concentration (MPC). Our data revealed that, the induced damage after treatment with nano zinc oxide was highly sever and in concentration independent manner as the 100ppm of nano zinc oxide induced the highest significant percentage of abnormal mitosis to reach 35.71% **(Table 2).** This contradiction may be related to the used concentrations or to the experiment conditions. Also, **Daphedar et al., [58]** referred to the decline in mitotic index which was associated with a significant increase in abnormality index after treat the *Cicer arietinum* plant with the higher concentration of ZnO-NPs to the remarkable damage of DNA and to arrest of the cell division at various phases of cell cycle and induce the formation of chromosomal abnormalities.

Despite that zinc oxide in bulk form enhanced the plant growth through mitotic high performance; it induced a considerable percentage of abnormalities classified into three classes of disorder in the following arrangement (disturbances, DNA liquification and structural aberrations) according to their occurrence **(Table 3&Plate 1, 2, 3).**

Our data revealed that treatment with zinc oxide in bulk size for three hours induced the percentage of DNA liquification in concentration dependent decrease from 49.71% after 50ppm to 38.76% after 200ppm. Considering the second class of aberration types, bulk ZnO induced rising percentages of disturbances to reach 54.07% after 200ppm ~ double the percentage in control (27.12%). The third class also, exceeded over control in a fluctuated way to reach 7.18% after 200ppm **(Table 3).** 50& 100ppm induced highest percentage of liquification while the 200ppm of ZnO induced the highest percentage of disturbances and structural aberrations which means that it affects the chromosome mobility and structure more than it affects the DNA physical constituent.

The 24hr. of recovery after treatment lowered the percentage of liquified DNA relative to control with the exception after 100ppm of concentration which induce nearly the same percentage as control. The increase in the stickiness after this concentration came on response the second class of aberration (disturbance) shrinkage to reach 28.49% **(Table 4).**

Treatment with nano zinc oxide affect the DNA constituent as it induced a concentration fluctuated increase in the percentage of stickiness (sticky & sticky bridges) to reach the highest value after 100ppm (48.89%). Chromosomes liquification was attributed to partial dissociation and alteration of the nucleoproteins as chromosome content **[59, 35],** or to the alteration in DNA depolymerization and folding proteins **[60].** Nano zinc oxide recorded the highest significant percentage of disturbances to reach after 50ppm (65.12%). Alteration in chromosomes mobility was observed earlier with **[53]** when tested the ZnO-NPS coated with PVP on the *Vicia faba* plant root meristem cells and referred this to the indirect effect on: microtubules which is protein in nature and constitute the spindle fibers, on the centrioles and/ or the associated protein in cell division. Also, it increased the percentage of aberration of the third class (structural in types such as: fragments, ring and micro/ macronucleus) over control and among treatment to reach 10.90% after 200ppm **(Table 3& Plate1, 2, 3).**

Table (3): Percentage of different types of abnormality relative to the abnormal mitosis and its distribution among phases in means in *Pisum sativum* root tip meristems after the 3h of direct treatment with zinc oxide in bulk and nano form.

Treatment		% of different types of abnormality														
		Chromatin liquification					Disturbances& Spindle disorder					Structural aberration				
		Pro.	Meta.	Ana-telo	Sum	% of stick.	Pro.	Meta.	Ana-telo.	Sum	% of dist.	Pro.	Meta.	Ana-telo.	Sum	% of stru.
		3h. Direct treatment														
	Control	0.22 a	2.11 b	2.44 d	4.78 c	72.88 a	0.11 b	1.44 d	0.22 c	1.78 c	27.12 c	0.00 a	0.00 d	0.00 b	0.00 c	0.00 c
B	50ppm	0.11 a	3.44 b	6.11 bc	9.67 b	49.71 b	2.11 a	6.33 c	0.67 b	9.11 b	46.86 ab	0.00 a	0.56 c	0.11 a	0.67 b	3.43 b
	100ppm	0.00 b	3.78 ab	7.22 b	11.00 b	48.29 b	2.33 a	5.89 c	2.89 a	11.11 b	48.78 a	0.00 a	0.44 c	0.22 a	0.67 b	2.93 b
	200ppm	0.11 a	3.11 b	5.78 c	9.00 b	38.76 c	2.56 a	9.22 b	0.78 b	12.56 b	54.07 a	0.00 a	1.33 ab	0.33 a	1.67 a	7.18 a
N	50ppm	0.00 b	3.78 ab	6.89 b	10.67 b	31.89 c	2.78 a	15.33 a	3.67 a	21.78 a	65.12 a	0.00 a	1.00 b	0.00 b	1.00 ab	2.99 b
	100ppm	0.33 a	5.89 a	10.89 a	17.11 a	48.89 b	3.78 a	10.33 b	1.56 a	15.67 ab	44.76 b	0.00 a	1.89 ab	0.33 a	2.22 a	6.35 a
	200ppm	0.11 a	2.78 b	11.22 a	14.11 a	47.74 b	2.78 a	8.11 b	1.33 a	12.22 b	41.35 b	0.00 a	3.00 a	0.22 a	3.22 a	10.90 a

Means followed by different letters point to significant differences between the treatments according to Tukey HSD test ($p \leq 0.05$).
B: bulk - N: Nano – Pro: prophase – Meta: metaphase – Ana-telo: anaphase-telohase – Stick: stickness – Dist: disturbance – Stru: structural.

Table (4): Percentage of different types of abnormality relative to the abnormal mitosis and its distribution among phases in means in *Pisum sativum* root tip meristems after the 24h of recovery after treatment with zinc oxide in bulk and Nano form.

Treatment		% of different types of abnormality														
		Chromatin liquification					Disturbances& Spindle disorder					Structural aberration				
		Pro	Meta	Ana-telo	Sum	% of stick	Pro	Meta	Ana-telo	Sum	% of dist.	Pro	Meta	Ana-telo	Sum	% of struc.
		24h. recovery after treatment														
	Control	0.00 a	2.33 b	3.33 d	5.67 c	70.83 a	0.22 b	1.56 d	0.00 d	1.78 e	22.22 e	0.00 a	0.56 d	0.00 b	0.56 c	6.94 c
B	50ppm	0.00 a	2.78 b	6.56 c	9.33 b	47.19 b	2.67 a	4.89 c	0.44 c	8.00 c	40.45 c	0.00 a	1.78 c	0.67 a	2.44 b	12.36 ab
	100ppm	0.00 a	4.44 a	9.00 a	13.44 a	70.35 a	1.89 a	3.56 cd	0.00 d	5.44 d	28.49 d	0.00 a	0.22 d	0.00 b	0.22 d	1.16 d
	200ppm	0.22 b	2.67 b	7.44 b	10.33 b	44.29 b	1.78 a	8.33 b	0.33 c	10.44 b	44.76 b	0.00 a	2.22 b	0.33 a	2.56 b	10.95 b
N	50ppm	0.00 a	1.33 b	5.00 cd	6.33 c	27.14 c	2.00 a	10.11 ab	2.33 a	14.44 ab	61.90 a	0.00 a	2.22 b	0.33 a	2.56 b	10.95 b
	100ppm	0.00 a	1.00 b	4.22 d	5.22 c	19.92 c	2.44 a	12.44 a	2.56 a	17.44 a	66.53 a	0.00 a	3.11 ab	0.44 a	3.56 ab	13.56 ab
	200ppm	0.00 a	1.56 b	4.89 cd	6.44 c	26.73 c	2.00 a	9.89 ab	0.89 b	12.78 b	52.99 ab	0.00 a	4.22 a	0.67 a	4.89 a	20.28 a

Means followed by different letters point to significant differences between the treatments according to Tukey HSD test ($p \leq 0.05$).
B: bulk - N: Nano – Pro: prophase – Meta: metaphase – Ana-telo: anaphase-telohase – Stick: stickness – Dist: disturbance – Stru: structural.

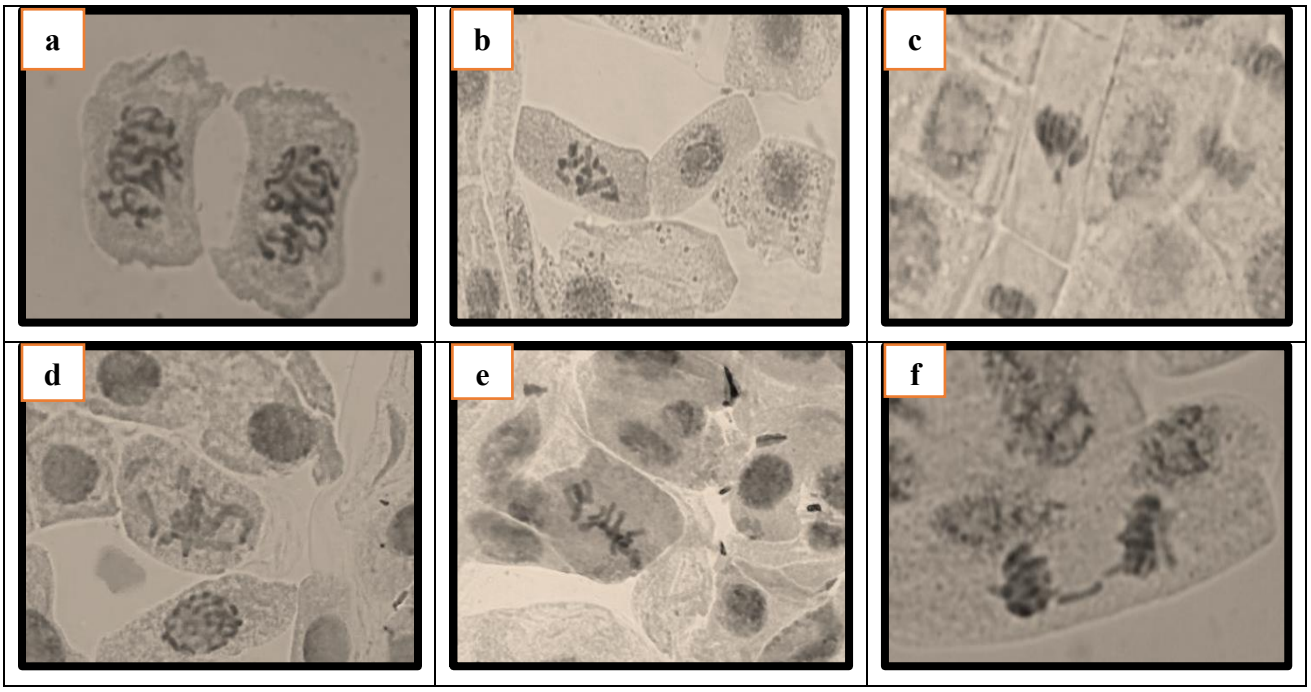


Plate 1(a-f): Show the induced defect on spindle mechanism and chromosome movement (turbogenecity) after treatment with zinc oxide: irregular prophases after 50ppm in bulk form (a), prometaphase after 50ppm in nanoform (b), clumped metaphase after 100ppm in bulk form (c), partial sticky metaphases with laggared chromosomes after recovery exp. from100ppm in nano(d)& bulk forms(e) and sticky anaphase with laggard chromosomes after100ppm in bulk form(f).

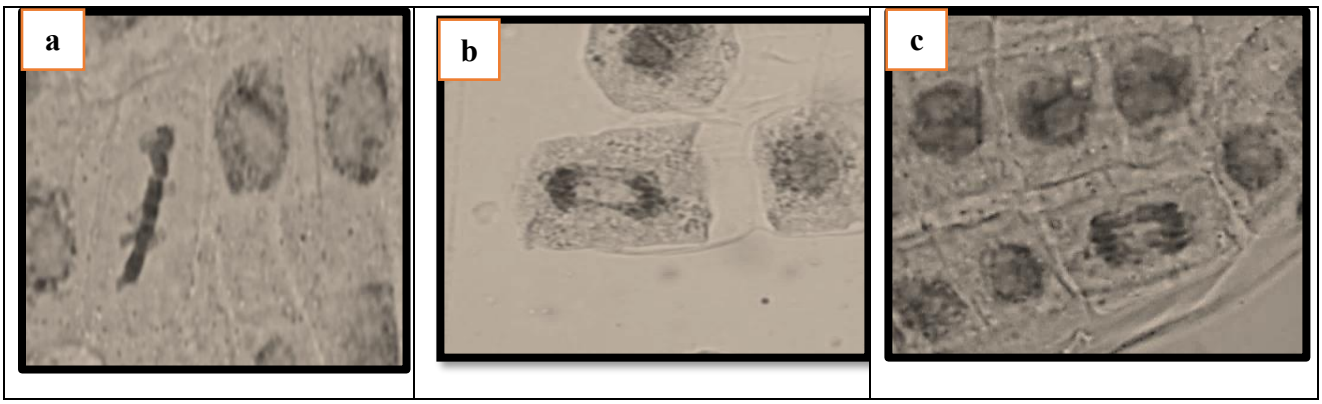


Plate 2(a-c): show the induced alteration in physical nature of DNA (chromotoxicity) after treatment with ZnO: sticky metaphase after 50ppm in bulk form, and sticky anaphases with multi-bridges after 100ppm of bulk (b)&nanoform(c).

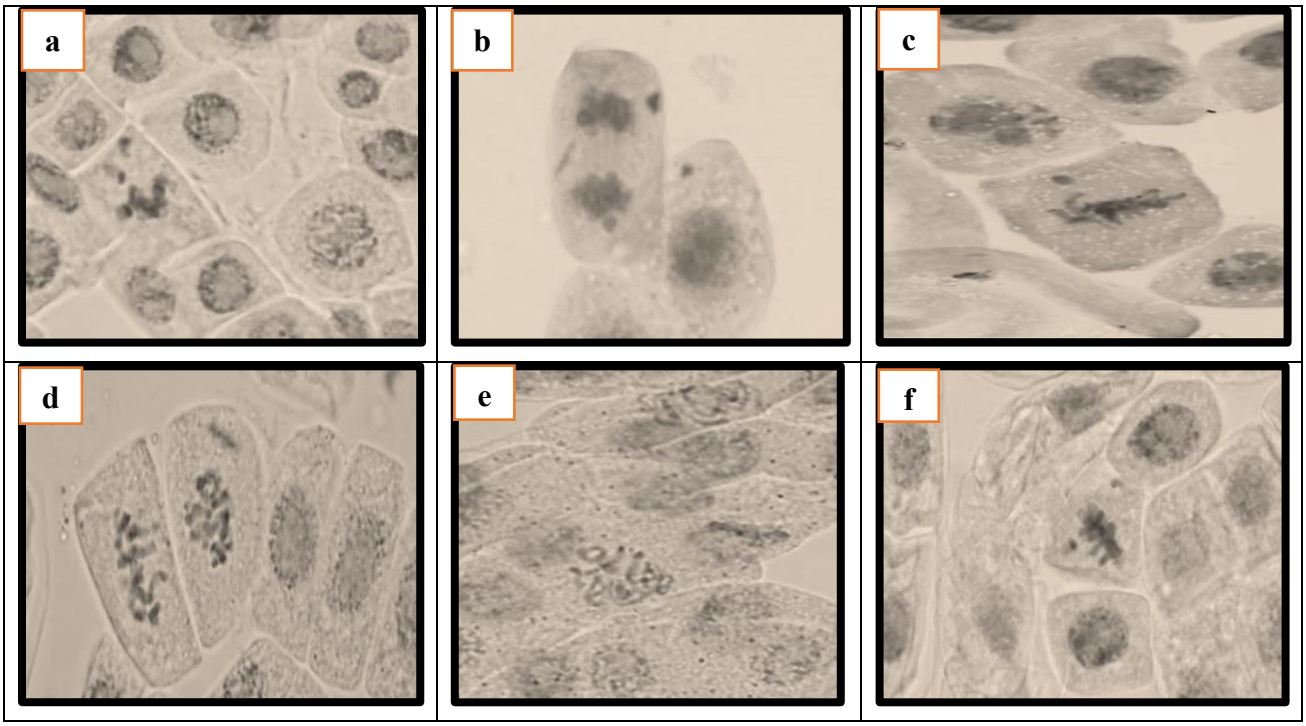


Plate 3(a-f): Show the induced alteration in chromosomes integrity and structure (Clastogenecity) after treatment with ZnO: sticky metaphase with fragment and laggard after 100ppm in nano form(a), sticky anaphase with fragment& micronucleus in resting after 200ppm in nanoform (b), split prophase & fragment in metaphase after the recovery exp. From 100ppm in bulk form (c) and ring chromosomes in pro-metaphase after100ppm in bulk form (d) and disturbed metaphase with ring chromosome after recovery exp. from 100ppm in bulk form (e), sticky metaphase with fragment after 50ppm in nanoform.(f).

Recovery experiment emphasizes the irreversible damage on chromosome structure to reach 20.28% after treatment with 200ppm. One the other hand the second class of aberration includes spindle disorder such as: pro-metaphase, clumped metaphases, disturbance and laggard chromosomes came on the front of other two classes induced higher percentages to reach 66.53% after the 100ppm **(Table 4).**

The micro/macro nucleus induction refers to the defect in the cell mend system and to the structural damaged DNA. The absence of the micro/ macro nucleus in resting cells investigated after the treatment with lower concentration 50&100ppm and very slight percentage after 200ppm of zinc oxide in bulk form declared its safety on the genome constituents, yet the surprisingly scored micro/ macro nucleus after the recovery experiment argue this assumption.

It is clear that, nano zinc oxide is more mutagenic factor as it induced a remarkable number of micro/macro nucleus to reach 0.23 & 0.15% after direct treatment with 100 &200ppm respectively.

Follow the micro/macro nucleus after the recovery experiment declares irreversible damage after treatment **(Table 5 & Plate 4).**

Table (5): The mean percentage of interphase cells with micro/macro nuclei in *Pisum sativum* root-tip meristems after 3hours of direct treatment with bulk & Nano zinc (a) and after the 24 hr of recovery after treatment with bulk & Nano zinc (b).

Treatment	Number of scored interphase cells		% of micro/ macro. nuclei	
	Bulk	Nano	Bulk	Nano
(a) 3h Direct treatment				
50ppm	865.78 c	894.78 b	0.00 c	0.02 c
100ppm	846.44 d	902.00 ab	0.00 c	0.23 a
200ppm	846.44 d	913.78 a	0.01 c	0.15 b
Control	862.22 c		0.00 c	
(b) 24h recovery after treatment				
50ppm	859.78 c	915.44 b	0.04 d	0.12 c
100ppm	850.56 d	920.00 ab	0.03 d	0.24 a
200ppm	847.67 d	927.33 a	0.05 d	0.18 b
Control	863.78 c		0.00 e	

Means followed by different letters point to significant differences between the treatments according to Tukey HSD test ($p \leq 0.05$).

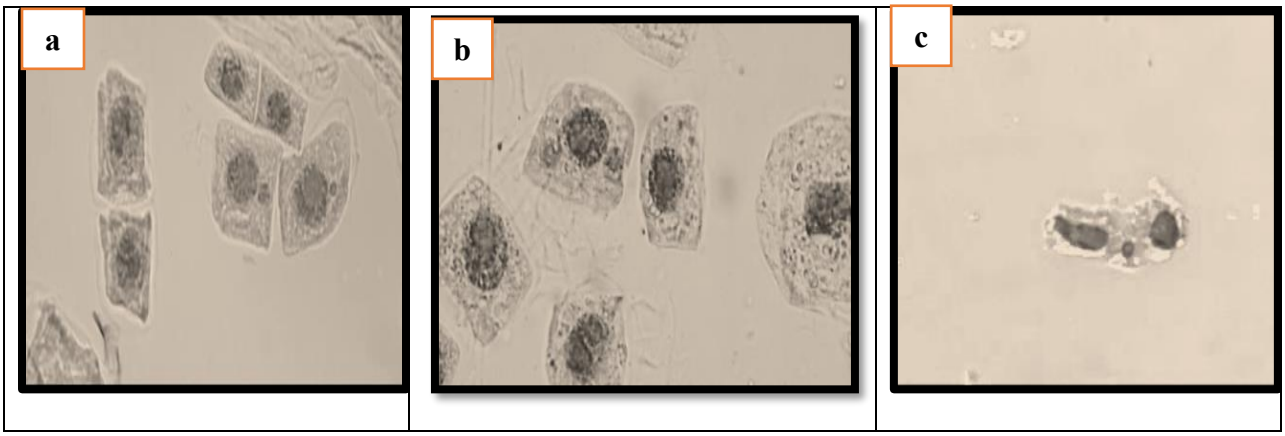


Plate 4(a-c): Show the scored micro/ macro nucleus in resting cells after treatment with ZnO after 200ppm in bulk form (a), 100ppm in nanoform (b) and after the recovery exp. from 200ppm in nanoform(c).

Data analysis of the SDS-PAGE image was summarized in 0/1 **(Table 6).** Eleven protein bands were recorded in the control plants (non-treated seedlings) ranged from 68 to 7 KD. The soil-injection with the examined materials induced some variation in the Protein PAGE extracted from seedlings leaves by induction of 8 monomorphic bands recorded at molecular wt. 54, 35, 29, 23, 21, 16, 9 and 7 KD in addition to three missing bands considered as negative markers at molecular wt. 68,61, and 14KD.

Soil injection around root with ZnO in bulk form affected the protein profile of the *Pissum sativum* only after the lowest used concentration 50ppm; the same concentration which scored the highest percentage of DNA stickiness. After the 50ppm concentration of bulk zinc oxide the total scored protein bands were 10 bands with one missing band at molecular weight 61 KD, while the higher used concentrations did not affect the protein profile **(Table 6 and Figure 3).**

Treatment with the ZnO in nanoform seemed to be more serious as the two higher used concentrations 100 &200 ppm scored 10 bands for each with recorded missing bands at molecular weight 14 & 68 KD respectively **(Table 6 and Figure 3).** It is worth mentioning that; these two concentrations induced higher percentages of chromosomal aberration at DNA liquification class in addition to the induction of micro/ macro nucleus at interphase (resting cells). Missing bands from protein profile after treatment with ZnO-NPs was observed and recorded earlier by **[55]** and referred this result to the aroused DNA stickiness.

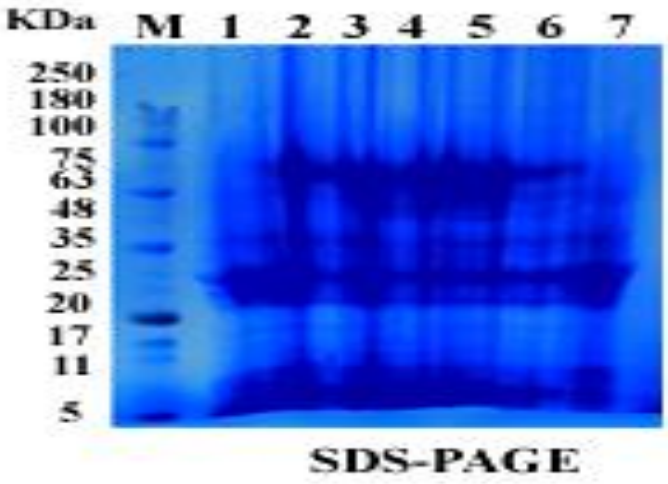
Sever stickiness of DNA may prevent some genes expressing themselves into protein *via* the transcription -translation process; thus, may explain the missing of the protein band related to the genes. This deduction came in accordance with **[61].**

In agree with **Salama et al., [62]** who referred the missing of the bands to the complete blocking of the correlated gene as kind of response to the treatment stress.

Induction of micro /macro nucleus is considered as remarkable sign for the mutagenic effect of the treatment on the plant. The mutagenic effect also, shed its light on the protein profile and gene expression. **[63, 64]** pointed to the effect of nanoparticles on the plant cells and referred it to the easily penetration, absorption, translocation, and accumulation of very small sized nanoparticles in plant through cell wall to reach deep inside, and to modulate gene expression **[65, 66].**

Table (6): The effect of treatment with zinc oxide in bulk and nano-sized forms on the *Pisum sativum* seedling's- soluble protein electrophoresis in 0/1 analysis.

Row No.	M.W KDa	Cont.	Bulk sized No			Nano sized No			Band description
			50ppm	100ppm	200ppm	50ppm	100ppm	200ppm	
		Lane 1	Lane 2	Lane 3	Lane 4	Lane 5	Lane 6	Lane 7	
1	68	1	1	1	1	1	1	**0**	-ve marker
2	61	1	**0**	1	1	1	1	1	-ve marker
3	54	1	1	1	1	1	1	1	monomorphic
4	35	1	1	1	1	1	1	1	monomorphic
5	29	1	1	1	1	1	1	1	monomorphic
6	23	1	1	1	1	1	1	1	monomorphic
7	21	1	1	1	1	1	1	1	monomorphic
8	16	1	1	1	1	1	1	1	monomorphic
9	14	1	1	1	1	1	**0**	1	-ve marker
10	9	1	1	1	1	1	1	1	monomorphic
11	7	1	1	1	1	1	1	1	monomorphic
Total bands		11	10	11	11	11	10	10	
Difference			-1	0	0	0	-1	-1	



Lane 1: Control.
Lane 2-4: Zinc oxide in bulk form (50,100&200ppm).
Lane 5-7: Zinc oxide in nanoform (50,100&200ppm).
M: Molecular weight protein marker.

Figure (3): SDS-PAGE total proteins of *Pisum sativum* seedlings grown under stress of around soil injected with different concentration of ZnO in bulk form and nanoform fertilizers.

IV. Conclusion:

Application of nano zinc oxide fertilizers *via* soil injection around roots of pea plant showed concentration dependent reduction in all vegetative growth parameters, also the two higher concentrations showed a direct effect on mitotic apparatus, induced chromosome misleading, alteration in DNA physical nature, in addition to slight clastogenic effect which shed its light on the protein profile. Based on that, the nano-zinc particles can be used to enrich poor new reclaimed lands in very limited conditions (if necessary) only at the lower concentration level to avoid its mutagenic harm on plant as recommended alternative and to evade the risk of soil suffocation with excess zinc oxide fertilizer in the bulk form.

V. Conflict of interest:

The authors declare that they have no conflict of interest.

References:

[1] Nikalje, A.P. (2015). Nanotechnology and its applications in medicine. Medicinal Chemistry, 5: 81-89.

[2] Lu, P.J.; Shou, C.H.; Pen, C.L.; Ching, C.D. and Chih S.Y. (2015). Analysis of titanium dioxide and zinc oxide nanoparticles in cosmetics. Journal of Food and Drug Analysis, 23(3): 587-594.

[3] Parisi, C.; Vigani, M. and Rodríguez-Cerezo, E. (2015). Agricultural nanotechnologies: What are the current possibilities? Nano Today, 10(2): 124-127.

[4] Grillo, R.; Abhilash, P.C. and Fraceto, L.F. (2016). Nanotechnology applied to bio-encapsulation of pesticides. Journal of Nanoscience and Nanotechnology, 16(1): 1231-1234.

[5] Colvin, V.L. (2003).The potential environmental impact of engineered nanomaterials. Nature Biotechnology, 21(10): 1166-1170.

[6] Ahmed, B.; Dwivedi, S.; Abdin, M.Z.; Azam, A.; Al-Shaeri, M.; Khan, M.S.; Saquib, Q.; Al-Khedhairy, A.A. and Musarrat, J. (2017). Mitochondrial and chromosomal damage induced by oxidative stress in Zn^{2+} ions, ZnO-Bulk and ZnO-NPs treated *Allium cepa* roots. Scientific Reports, 7: 40685.

[7] Shweta, J.h.a. and Ramesh N.P. (2016). Molecular mechanism of plant nanoparticle interactions. In: "Molecular Mechanism of Plant –Nanoparticle Interactions. (Kole C, Kumar DS, Khodakovskaya MV. Eds)". Chapter 7. Springer International Publishing, 155-181.

[8] Wang, L.; Mao, J.; Zhang, G.H. and Tu, M.J. (2007). Nanocerium- element-doped titanium dioxide induces apoptosis of Bel 7402 human hepatoma cells in the presence of visible light. World Journal of Gastroenterology, 13(29): 4011-4014.

[9] Dietz, K.J. and Herth S. (2011). Plant nanotoxicology. Trends Plant Science, 16(11): 582-589.

[10] Pakrashi, S.; Jain N.; Dalai, S.; Jayakumar, J.; Chandrasekaran, P.T.; Raichur, A.M.; Chandrasekaran, N. and Mukherjee, A. (2014). In vivo genotoxicity assessment of titanium dioxide nanoparticles by *Allium cepa* root tip assay at high exposure concentrations. PLoS One, 9(2): e87789. http://doi:10.1371/journal.pone.0087789.

[11] Osmond, M.J. and McCall, M.J. (2010). Zinc oxide nanoparticles in modern sunscreens: an analysis of potential exposure and hazard. Nanotoxicology, 4(1):15-41.

[12] Rasmussen, J.W.; Martinez, E.; Louka, P. and Wingett, D.G. (2011). Zinc oxide nanoparticles for selective destruction of tumor cells and potential for drug delivery applications. NIH Public Access, 7(9):1063-1077.

[13] Liu, R. and Lal, R. (2015). Potentials of engineered nanoparticles as fertilizers for increasing agronomic productions. Science of the Total Environment, 514:131-139.

[14] He, L.; Liu, Y.; Mustapha, A. and Lin, M. (2011). Antifungal activity of zinc oxide nanoparticles against *Botrytis cinerea* and *Penicillium expansum*. Microbiological Research, 166:207-215.

[15] Pokhrel, L.R. and Dubey, B. (2013). Evaluation of developmental responses of two crop plants exposed to silver and zinc oxide nanoparticles. Science of the Total Environment, 452: 321-332. http://doi:10.1016/j.scitotenv.02.059.

[16] Chen, J.; Liu, X.; Wang, C.; Yin, S.S.; Li, X.L.; Hu, W.J.; Simon, M.; Shen, Z.J.; Xiao, Q.; Chu, C.C.; Peng, X.X. and Zheng, H.L. (2015). Nitric oxide ameliorates zinc oxide nanoparticles-induced phytotoxicity in rice seedlings. Journal of Hazardous Materials, 297:173-182. https://doi.org/10.1016/j.jhazmat.

[17] Zafar, H.; Ali, A.; Ali, J.S.; Haq, I.U. and Zia, M. (2016). Effect of ZnO nanoparticles on *Brassica nigra* seedlings and stem explants: growth dynamics and antioxidative response. Frontiers in Plant Science, 7: 535. https://doi.org/10.3389/fpls.2016.00535.

[18] López-Moreno, M.L.; RosaG, de la.; Cruz-Jiménez, G.; Castellano, L.; Peralta- Videa J.R. and Gardea-Torresdey, J.L. (2017). Effect of ZnO nanoparticles on corn seedlings at different temperatures; X-ray absorption spectroscopy and ICP/OES studies. Microchemical Journal, 134:54-61. https://doi.org/10.1016/j.microc.2017.05.007.

[19] Yang, S.; Yin, R., Wang, C.; Yang, Y.; and Wang, J. (2023). Phytotoxicity of zinc oxide nanoparticles and multi-walled carbon nanotubes, alone or in combination, on *Arabidopsis thaliana* and their mutual effects on oxidative homeostasis. PLoS ONE, 18(2): e0281756. https://doi.org/10.1371/journal.pone.0281756.

[20] Stałanowska, K.; Szablińska-Piernik, J.; Okorski, A.; Lahuta, L.B. (2023). Zinc Oxide Nanoparticles Affect Early Seedlings' Growth and Polar Metabolite Profiles of Pea (*Pisum sativum* L.) and Wheat (*Triticum aestivum* L.). International Journal of Molecular Sciences, 24(19): 14992. https://doi.org/10.3390/ijms241914992.

[21] Wang, F.; Jing, X.; Adams, C.A.; Shi, Z. and Sun, Y. (2018). Decreased ZnO nanoparticle phytotoxicity to maize by arbuscular mycorrhizal fungus and organic phosphorus. Environmental Science & Pollution Research, 25:23736–23747. https://doi.org/10.1007/s11356-018-2452-x.

[22] Manzo, S.; Rocco, A.; Carotenuto, R.; Picione, F.D.L.; Miglietta, M.L.; Rametta, G. and Francia, G. (2011). Investigation of ZnO nanoparticles 'ecotoxicological effects towards different soil organisms. Environmental Science & Pollution Research, 18:756-763. http://doi:10.1007/s11356-010-0421-0.

[23] Wang, Q.; Xu, S.; Zhong, L.; Zhao, X. and Wang, L. (2023). Effects of Zinc Oxide Nanoparticles on Growth Development, and Flavonoid Synthesis in *Ginkgo biloba*. International Journal of Molecular Sciences, 24(21): 15775. https://doi.org/10.3390/ijms242115775.

[24] Grant, W.F. (1999). Higher plant assays for the detection of chromosomal aberrations and gene mutations-a brief historical background on their use for screening and monitoring environmental chemicals. Mutation Research, 426:107-112. https://doi:10.1016/s0027-510700050-0.

[25] Haq, I.; Kumari, V.; Kumar, S.; Raj, A.; Lohani, M. and Bhargava, R.N. (2016). Evaluation of the phytotoxic and genotoxic potential of pulp and paper mill effluent using *Vigna radiata* and *Allium cepa*. Advances in Biology, 1-10. https://doi:10.1155/2016/8065736.

[26] Sato, S.; Isobe, S. and Tabata, S. (2010). Structural analyses of the genomes in legumes. Current opinion in plant biology, 13(2): 146-152.

[27] Food and Agriculture Organization of United Nations (FAO) (2018). FAOSTAT Database. Crop Statistics Rome, Italy. http://www.fao.org/faostat/en/#data/QC.

[28] Taranath, T.C.; Patil, B.N.; Santosh, T.U. and Sharath, B.S. (2015). Cytotoxicity of zinc nanoparticles fabricated by *Justicia adhatoda* L. on root tips of *Allium cepa* L. a model approach. Environmental Science and Pollution Research, 22(11): 8611-8617.

[29] Ghosh, M.; Jana, A.; Sinha, S.; Jothiramajayam, M; Nag, A.; Chakraborty, A.; Mukherjee, A. and Mukherjee, A. (2016). Effects of ZnO nanoparticles in plants: Cytotoxicity, genotoxicity, deregulation of antioxidant defenses, and cell-cycle arrest. Mutation Research: Genetic Toxicology & Environmental Mutagenesis, 1(807):25-32. https://doi:10.1016/j.mrgentox.07.006.

[30] Hamoud, M.A.; El-Shanshoury, A.d.; Al-Sodany, Y.M. and Gad El-Karim, M.S. (2005). Genetic diversity among *Ipomoea carnea Jacq*. populations from different habitat types in Nile-Delta region of Egypt. Egyptian Journal of Experimental Biology (Botany), 1: 1-10.

[31] Sharma, A.K. and Sharma A. (1980). Chromosome techniques theory and practice. 3rd. ed. Buttertworths, London, 145-150.

[32] Laemmli, U.K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature, 227:680-685.

[33] Seifolazadeh, A. and Mohammadi, S. (2012). Synthesis and characterization of nano-boron powders prepared with mechano-chemical reaction between B_2O_3 and Mg powders. Bulletin of Materials Science, 39: 479-486.

[34] Daneshvar, N.; Aber, S.; Sayed, D.; Khataee, M.S.; Rasoulifard, A.R. (2007). Preparation and investigation of photocatalytic properties of ZnO nanocrystals: Effect of operational parameters and kinetic study. International Journal of Nuclear & Quantum Engineering, 1: 24-29.

[35] Sun, Z.; Xiong, T.; Zhang, T.; Wang, N.; Chen, D. and Li, S. (2019). Influences of zinc oxide nanoparticles on *Allium cepa* root cells and the primary cause of phytotoxicity. Ecotoxicology, 28: 175–188. https://doi.org/10.1007/s10646-018-2010-9.

[36] Sorahinobar, M.; Deldari, T.; Nazem Bokaeei, Z. and Mehdinia, A. (2023). Effect of zinc nanoparticles on the growth and biofortification capability of mungbean (*Vigna radiata*) seedlings. Biologia, 78: 951-960. https://doi.org/10.1007/s11756-022-01269-3.

[37] Zhang, R.; Zhang H.; Tu, C.; Hu, X.; Li, L.; Luo, Y. and Christie, P. (2015). Phytotoxicity of ZnO nanoparticles and the released Zn(II) ion to corn (*Zea mays* L.) and cucumber (*Cucumis sativus* L.) during germination. Environmental Science and Pollution Research, 22:11109-11117. https://doi.org/10.1007/s11356-015-4325-x.

[38] Javed, R.; Usman, M.; Yücesan, B.; Zia, M. and Gürel, E. (2017). Effect of zinc oxide (ZnO) nanoparticles on physiology and steviol glycosides production in micropropagated shoots of *Stevia rebaudiana* Bertoni. Plant Physiology & Biochemistry, 110:94–99. https://doi.org/10.1016/j.plaphy.2016.05.032.

[39] Tymoszuk, A. and Wojnarowicz, J. (2020). Zinc oxide and zinc oxide nanoparticles impact on in vitro germination and seedling growth in *Allium cepa* L. Materials, 13(12):2784. https://doi.org/10.3390/ma13122784.

[40] Rani, N.; Kumari, K.; Sangwan, P.; Barala, P.; Yadav, J. and Hooda, V. (2022). Nano-iron and nano-zinc induced growth and metabolic changes in *Vigna radiate.* Sustainability, 14(14):8251. https://doi.org/10.3390/su14148251.

[41] Srivastav, A.; Ganjewala, D.; Singhal, R.K.; Rajput, V.D.; Minkina, T.; Voloshina, M. and Shrivastava, M. (2021). Effect of ZnO nanoparticles on growth and biochemical responses of wheat and maize. Plants, 10(12): 2556. https://doi.org/10.3390/plants10122556.

[42] Galal, O.A. and Thabet, A.F. (2018). Cytological and molecular effects of silver nanoparticles (AgNPs) on *Vicia faba* M1 plants. Journal of Agricultural Chemistry and Biotechnology, 9: 269–27.

[43] Iziy, E.; Majd, A.; Vaezi-Kakhki, M.R.; Nejadsattari, T. and Kazemi Noureini, S. (2019). Effects of zinc oxide nanoparticles on enzymatic and nonenzymatic antioxidant content, germination, and biochemical and ultrastructural cell characteristics of *Portulaca oleracea* L. Acta Societatis Botanicorum Poloniae, 88(4):3639. https://doi.org/10.5586/asbp.3639.

[44] Kouhi, S.M.M.; Lahouti, M.; Ganjeali, A. and Entezari, M.H. (2015). Comparative effects of ZnO nanoparticles, ZnO bulk particles, and Zn^{2+} on *Brassica napus* after long-term exposure: changes in growth, biochemical compounds, antioxidant enzyme activities, and Zn bioaccumulation. Water Air Soil Pollution, 226:364.https://doi.org/10.1007/s11270-015-2628-7.

[45] Rao, S. and Shekhawat G. (2014). Toxicity of ZnO engineered nanoparticles and evaluation of their effect on growth, metabolism and tissue specific accumulation in *Brassica juncea*. Journal of Environmental Chemical Engineering, 2:105-114. https://doi.org/10.1016/j.jece.2013.11.029.

[46] Prasad, T.; Sudhakar, P.; Sreenivasulu, Y.; Latha, P.; Munaswamy, V. and Reddy K.R. (2012). Effect of nanoscale zinc oxide particles on the germination, growth and yield of peanut. Journal of Plant Nutrition, 35(6): 905-927.

[47] Mukherjee, A.; Peralta-Videa, J.R.; Bandyopadhyay, S.; Rico, C.M.; Zhao, L.; Gardea-Torresdey, J.L. (2014). Physiological effects of nanoparticulate ZnO in green peas (*Pisum sativum* L.) cultivated in soil. Metallomics, 6(1):132-8. http://doi:10.1039/c3mt00064h.

[48] Labeeb, M.; Haroun, S.; Badr, A.; Matter, M. and El-kholy, A. (2023). Impact of ecofriendly synthesized silver nanoparticles on yield parameters and molecular traits of pea (*Pisum sativum* L.). Catrina: The International Journal of Environmental Sciences. 27: 1-11. https://doi.org/10.21608/cat.2023.288519.

[49] Sobieh, S.S.; Kheiralla, Z.M.H.; Rushdy, A.A. and Yakob, N.A.N. (2016). *In vitro* and *in vivo* genotoxicity and molecular response of silver nanoparticles on different biological modelsystems. *Caryologia*, *69*(2):147-161. https://doi.org/10.1080/00087114.2016.1139416.

[50] Harrison, J.C. and Haber, J.E. (2006). Surviving the breakup: the DNA damage checkpoint. Annual Review of Genetics, 40: 209-235.

[51] Hartwell, L.H. and Weinert, T.A. (1989). Checkpoints: control that ensure the order of cell cycle events. Science, 246: 629-634. https://bit.ly/3nGMZus.

[52] Kim, J.C. and Bendixen, E.L. (1987). Effect of haloxyfop and CGA -82725 on cell cycle and cell division of oat (*Avene sativa*) root-tips. Weed Science, 35: 769-774. https://doi.org/10.1017/S0043174500079315.

[53] Salah, M.; Yehia, S. and Ali, R.T. (2022). Cytogenetic effect of some nanostructure polymers prepared *via* gamma irradiation on *Vicia faba* plant. Chemical and Biological Technologies in Agriculture, 9(1): 6. https://doi.org/10.1186/s40538-021-00279-8.

[54] Hartig, K. and Beck, E. (2006). Crosstalk between auxins, cytokinins and sugars in the plant cell cycle. Plant Biology, 8: 1-8.

[55] Ali R.T.; Mohammed S. and Mohammed F.I. (2022). Comparative study on the cytogenetic and biochemical effects of nanozinc oxide *vs* other two recommended natural fertilizers on *Vicia faba* plant. International Journal of Advances in Science Engineering and Technology, 10:122-134.

[56] Masarovičová E and Kráľová K. (2017). Essential elements and toxic metals in some crops, medicinal plants, and trees. In: Phytoremediation, 183–255.

[57] Kumari, A.; Chokheli, V.A.; Lysenko, V.S.; Mandzhieva, S.; Minkina, T.; Mazarji, M.; Rajput, V.; Shuvaeva, V.; Sushkova, S. and Barakhov, A. (2022). Genotoxic and morpho-physiological responses of ZnO macro- and nano-forms in plants. Environmental Geochemistry and Health, 1-13. https://doi.org/10.1007/s10653-022-01428-0.

[58] Daphedar, A.B.; Kakkalameli, S.B.; Melappa, G.; Taranath, T.C.; Srinivasa, C.; Shivamallu, C.; Syed, A.; Marraiki, N.; Elgorban, A.; Veerapur, R.; Patil, S. and Kollur, S.P. (2021). Genotoxic assay of silver and zinc oxide nanoparticles synthesized by leaf extract of *Garcinia livingstonei* T. Anderson: A comparative study. Pharmacognosy Magazine, 17: S114-21. https://doi.org/10.4103/pm.pm_536_20.

[59] Kumar, G.; Kesarwani, S. and Sharma, V. (2003). Clastogenic effect of individual and combined treatment of gamma rays and EMS in *Lens culinary*. Journal of Cytology and Genetics, 4: 149-154.

[60] Asita, A.O. and Mokhobo, M.M. (2013). Clastogenic and cytotoxic effects of four pesticides used to control insect pests of stored products on root meristems of *Allium cepa*. L Environment and Natural Resources Research, 3(2): 133-145.

[61] Adam, Z.M.; Mikhael, E.; El- Ashry, Z.M.; Ehsan, N.O. and Ali, R.T. (2017). Potential mutagenic effect of dusting *Vicia faba* seeds with Malathion 1% powder or fumigating by Phosphine for storage. African Journal of Biological Sciences, 13(1):23-44. https://doi.org/10.21608/ajbs.2017.68628.

[62] Salama, D.M.; Abd El-Aziz, M.E.; Osman, S.A.; Abd Elwahed, M.S.A. and Shaaban, E.A. (2022). Foliar spraying of MnO_2-NPs and its effect on vegetative growth, production, genomic stability, and chemical quality of the common dry bean, Arab Journal of Basic and Applied Sciences, 29:(1): 26-39. https://doi.org/10.1080/25765299.2032921.

[63] Hossain, Z.; Mustafa, G. and Komatsu, S. (2015). Plant responses to nanoparticle stress. International Journal of Molecular Sciences, 16(11): 26644-26653. https://doi:10.3390/ijms161125980.

[64] Nair, P.G.M. and Chung, I.M. (2014). Impact of copper oxide nanoparticles exposure on *Arabidopsis thaliana* growth, root system development, root lignificaion, and molecular level changes. Environmental Science & Pollution Research, 21: 12709-12722. http://dx.doi.org/10.1007/s11356-014-3210-3.

[65] Khiew, P.; Chiu, W.; Tan, T.; Radiman, S.; Shukor, R.A. and Chia, C.H. (2011). Capping effect of palm-oil based organometallic ligand towards the production of highly monodispersed nanostructured material. In Palm oil: Nutrition, uses and impacts, 189–219.

[66] Salama, D.M.; Osman, S.A.; Abd El-Aziz, M.E.; Abd Elwahed, M.S. and Shaaban E.A. (2019). Effect of zinc oxide nanoparticles on the growth, genomic DNA, production and the quality of common dry bean (*Phaseolus vulgaris*). Biocatalysis & Agricultural Biotechnology, 18:101083. https://doi.org/10.1016/j.bcab.2019.101083.

الملخص العربي

الدراسات المورفولوجية والخلوية والكيميائية الحيوية على تأثير أكسيد الزنك في الصورة السائبة مقابل الصورة المحضرة نانوياً كأسمدة على نبات البسلة

صفاء محمد صالح[١،*], نهله عثمان احسان[١], رانية توفيق علي[٢], عماد عبد الحميد عبد الدايم[٣], محمد عبد الوهاب محمود[٤], ثريا رشاد محمد[١].

[١] قسم النبات - كلية البنات للأداب والعلوم والتربية - جامعه عين شمس- القاهرة - مصر.
[٢] قسم الوراثة والخلية - المركز القومي للبحوث- الدقي - مصر.
[٣] قسم الخضر - كلية الزراعة - جامعه القاهرة- الجيزة - مصر.
[٤] قسم الفسيولوجي - كلية الزراعة - جامعه القاهرة- الجيزة - مصر.

الملخص العربي:

عنصر الزنك ضرورياً جداً في عملية الزراعة؛حيث يعمل إمداد النباتات بعنصر الزنك على تحسين نمو النبات وتطوره وأدائه بشكل طبيعي وتحت الضغوط. يجب تقييم ومراجعة مستحضرات الزنك النانوية الجديدة المقدمة لاستخدامها بأمان في الزراعة. **الطرق**: في هذا العمل تم استخدام سماد أكسيد الزنك في صورته السائبة الطبيعية كعينة قياسية ايجابية لتصور الدراسة على أكسيد الزنك في الصورة المحضرة بتقنية النانو وبتركيزات مختلفة (٥٠، ١٠٠، ٢٠٠ جزء في المليون) على نبات البسلة. تناولت الدراسة بعض الصفات المظهرية (السمات المورفولوجية) والمحتوى البروتيني الكلي الذائب للشتلة بعد حقن التربة حول الجذر، بالإضافة إلى الدراسات الوراثية الخلوية على نظام الانقسام الميتوزي بعد المعاملة المباشرة لمدة ٣ ساعات وبعد تجربة الاستشفاء لمدة ٢٤ ساعة. **النتائج:** أكدت الدراسة الخطورة الكبيرة لاستخدام الأشكال النانوية على جميع العوامل المدروسة على مستوى التركيزين الأعلى بالمقارنة العينة القياسية السلبية والايجابية حيث أدي ذلك إلى انخفاض في جميع المعايير المورفولوجية وعلى مستوى المعايير الوراثية الخلوية، فقد اظهرت تأثيرها السام على المكون الطبيعي للحمض النووي وتأثيرها المسبب للطفرات عن طريق تكوين النواة الصغيرة / الكبيرة في الخلايا الساكنة (المهيئة لدخول الانقسام). كما اثرت ايضا على التعبير الجيني من خلال اختفاء بعض حزم البروتين من الصورة. **الاستنتاج:** يمكن استخدام تركيز اقل من جزيئات اكسيد الزنك النانونية لتخصيب الأراضي المستصلحة الجديدة الفقيرة في ظروف محدودة جداً (إذا لزم الأمر) لتجنب التأثير المطفر على النبات كبديل موصى به ولتجنب مخاطر اختناق التربة بسماد أكسيد الزنك الزائد في الشكل السائب.

الكلمات الدالة: نبات البسلة - جزيئات اكسيد الزنك النانونية - نموالنبات - الانقسام الميتوزي - ملف البروتين