

Research Article

Comparison the Phytotoxicity of TiO₂ nanoparticles with bulk particles on Amber 33 variety of rice (*Oryza sativa*) *in vitro*.

Raghad DH Abdul Jalill*; Alyaa M. Yousef

College of Science, AL- Mustansiryia University, Baghdad, Iraq.

***Corresponding author**

Dr. Raghad DH Abdul Jalill

Email: raghadalshybany@gmail.com

Abstract: This study focus on the phytotoxicity of TiO₂ nanoparticles (NPs) compared with bulk TiO₂ particles B on: germination parameters, vegetative traits, roots viability, Biomass of seedling and photosynthetic pigments of Amber 33 variety of Rice (*Oryza Sativa*) *in vitro*. There were induction in seed germination percentage using different concentrations of B particles, while it decreased when the seeds exposed to NPs at concentrations (10, 1 and 0.01) mg/ml compared with control. There were differences between the effects of NPs compared with B particles on germination percentage. The highest germination rate had seen at (0.1 and 0.01) mg/ml concentration of NPs. The same features had seen at (1 and 10) mg/ml of B particles. Other concentrations were reduce the germination rate. All concentrations of NPs were reduced mean germination time. The same reduction has seen at (1-10) mg/ml of B particles. The lower concentrations (0.1-0.01) mg/ml of it were increased. The concentrations of B particles (10, 1 and 0.1) mg/ml showed increased in mean daily germination, whereas higher concentrations of NPs (10 and 1) mg/ml decreased it. However slight increase at 0.1 mg/ml and 0.01 mg/ml of it in MDG was observed. All concentrations of B particles were increase: vigor index I, vigor index II, germination value and promoter indicator compared with control. There were different changing in vigor index I in dose of NPs depending manner. There were differences between the effects of NPs (0.01, 1 and 10) mg/ml compared with B particles on vigor index I, vigor index II and promoter indicator. B particles and NPs were not effect on: shoots, roots, hairy roots length and total of plant lengths. While there were induction in number of hairy roots using 0.01 mg/ml concentration of NPs. NPs (10 and 1) mg/ml concentrations were reduced the number of hairy roots compared with control. The effect of these concentrations were different from the effect of the same concentrations of B particles. NPs and B particles were not effect on: biomass of seedling, chlorophyll A, chlorophyll B and root viability except the medium concentration of B particles, (0.01mg/ml), it reduce root viability.

Keywords: *Oryza sativa*; phytotoxicity; TiO₂; nanoparticles.

INTRODUCTION

Nanomaterials have been widely applied in the world in this last decade. Nanotechnology provides the tool and the technological platforms for the study and transformation of biological systems[1]. Some scientists believe that, with mass production of engineered nanoparticles, there is a realistic chance for these particles to interact with water, soil and air, and subsequently enter the environment[2-3]. Their ecotoxicological impact is still poorly documented, while their use in commercial goods is the increased constantly increasing[4].

Few studies have focused on the effects and mechanisms of nanomaterials on plants[1].The majority of the reported studies point to the positive impacts of nanoparticles on plant growth with a few isolated studies pertaining to negative effect[5]. A complete study on the toxic effects of these nanoparticles can help significantly in terms of use and safe disposal of

engineered nanoparticles for the reduction of adverse effects in both environmental and agricultural systems[6].

Titanium dioxide nanoparticles (TiO₂ NPs) have been used as nontoxic, chemical inert and biocompatible pigment products or photocatalysts in cosmetics, pharmaceuticals and paint industries[7-9]. Application of titanium dioxide (TiO₂) on food crops has been reported to promote plant growth, increase the photosynthetic rate, reduce disease severity and enhance yield by 30%[10].

Nowadays, various researchers have studied the effects of nanomaterials on plant germination and growth with the objective to promote its use for agricultural applications[11]. Some of them did not found any phytotoxicity of TiO₂ on seed germination and root elongation of lettuce, radish and cucumber seeds[12]. The potential human toxicity and

environmental impact of TiO₂ NPs have attracted considerable attention with their increased use in industrial applications[13].

Lu and other [14] studied the effect of mixtures of nano-SiO₂ and nano-TiO₂ on soybean seed. They found that the mixture of nanoparticles increases nitrate reductase in soy bean increasing its germination and growth. Nano-TiO₂ give rise to negative effect of *Vicianar bonensis* and *Zea mays* that can be evidenced as reduction and alteration in seed germination, development and mitosis of root tip cells[15].

A few studies have been done on the effects of nanoparticles on crops particularly on and rice[16], which are one of the most important crops cultivated in the Iraqi. This yields constitutes from the cultivated areas nearly 96% of the total cultivated land area in various types of cereals in the countryside.

The aims of the present study are study the phytotoxicity of TiO₂ nanoparticles (NPs) compared with bulk TiO₂ particles on: Germination parameters, vegetative traits, roots viability, Biomass of seedling and photosynthetic pigments of Amber 33 variety of Rice (*Oryza Sativa*) *in vitro*.

MATERIALS AND METHODS

Nanoparticles and Bulk particles

Dry titanium dioxide anatase nanoparticles powder was procured from Sigma Aldrich, USA. The supplier's data were: particle size 50 nm, 99.7% trace metal basis and surface area: 200–220 m²/g. White pigment powder of bulk titanium dioxide particles were procured from Sigma Aldrich, China. Molar mass was 79.87 g/mol and density was 4.2 g/cm³. The size of nanoparticles and bulk were examined by scanning electron microscope (SEM)/ Vega Tescan (USA) in Center of Nanotechnology and Advanced Materials/ University of Technology/ Iraq. Sterilized distilled water was used to prepare different concentrations of nanoparticles and bulk particles, (0.01, 0.1, 1, 10)mg/ml.

Seed Preparation:

Amber 33 variety of rice (*Oryza sativa* L.) seeds were taken from Mabain AL-Nahrian Company for the seeds production in Baghdad / Iraq for culture season 2012-2013. They were immerse in a 1% sodium hypochlorite solution for 1 min. Rinsed three times with sterilized distilled water. They were soaked in bulk particles solutions and nanoparticles suspensions at various concentrations (0.01, 0.1, 1 and 10)mg/ml. All seeds were incubate in an incubator at laboratory conditions (30±1 C°, 12 h. light: 12 h. dark) for four days. Sterilized distilled water was used in the soaking process for a control.

Experiments

A piece of filter paper (Whatman No. 42/ Zelpa, Belgium) was put into each Petri dish (90 mm × 15

mm). One hundred seeds of each concentrations were transferred onto petri dishes (five seeds for each Petri dishes and four replications including 100 seeds in each replicates). The distance between each seed was four cm. Five ml of sterilized distilled water was added. Petri dishes were sealed with parafilm and placed in an incubator. All seeds were incubate in an incubator at laboratory conditions (30±1 C°, 12 h. light: 12 h. dark) for 10 days. Sterilized distilled water was used in the soaking process for a control[6].

The number of germinated seeds was recorded daily. A seed was considered germinated when the radicle showed at least 2 mm in length. The following parameters were counted at the end of experiment:

Germination parameters

1. Germination percentage (GP, %), $GP = 100 \times GN / SN$; GN is the total number of germinated seed; SN is the total number of seeds tested, [16].
2. Germination rate (GR) $GR = \sum Gi / I$; Gi is the number of seeds germinated on day I, [17].
3. Mean germination time (MGT), $MGT = \sum Gi \times i / \sum G$; where i is the number of days since the day of sowing (day 0) and Gi is the number of seeds germinated on day i. Only seeds that germinated were included in the calculation[16].
4. MDG=Germination% (GP)/ total experiment day[16].
5. Vigor index I= Germination % × Seedling length (cm)[18].
6. Vigor index II = Germination % × Seedling weight (g), [18].
7. Germination Value: (GV) = PV × MDG, [16].
8. Promoter Indicator (PI) = (1* GP2 %) + (0. 75* GP4 %) + (0. 5* GP6 %) + (0. 25* GP8 %). GP2 %: Germination percentage in day two; GP4%: Germination percentage in day four; GP6 %: Germination percentage in day six; GP8 %: Germination percentage in day eight[19].

Vegetative traits: Roots and shoots were separated and washed with distilled water. Number and lengths of: leaves, roots, hairy roots and the total length of the plant were recorded.

Biomass: Roots and shoots were separated from seedlings for biomass determination. The fresh weight of roots and shoots was measured by sensitive balance, dry weights were recorded after dried on electric oven at 70°C for 24 h [20].

Pigments: The weight of leaves were recorded. The leaves were crushed with 80% of acetone/Medex (U.K) using ceramic mortar. The separation of the filtrate from the precipitate remaining using centrifuge / Hettich(Germany) on the speed of 4000 rpm for 5minutes. The absorbance has been read at wavelengths (663, 645,440) by spectrophotometer/Labomed, Inc(USA): [21] .The following formula were used to

calculate the amount of chlorophyll (A, B) and carotenoid:

$$\begin{aligned} \text{Chlo.A} &= (12.7 (D663) - (2.69(D645)) * V / (1000*W) \\ \text{Chlo.B} &= (22.9 (D645) - (4.68(D663)) * V / (1000*W) \\ \text{Carotenoid} &= ((4.695 * O.D 440) - (2.88 * O.D 663) + \\ & (O.D (645)) * (V / (1000*W)) \end{aligned}$$

D: the optical density. V: The final volume of the diluted concentration of acetone (80%). W: weight in grams of plant tissue that has been extracted.

TTC viability: 2, 3, 5-triphenylte trazolium chloride (TTC)/ BDH (England) was used as a histopathologic stain for testing the viability of root tips. The test was as follows: 5 mL of 0.5% solution of TTC was added to test tubes containing 10 root tips, the temperature was kept at $35 \pm 1^\circ\text{C}$. After 5 h in the dark, the TTC solution was removed with a syringe and root tips were thoroughly rinsed with distilled water and then examined. The red colored root tips were considered to be viable and others were non-viable or dead[22].

Statistical Analysis

Analysis of variance (ANOVA) and the least significant difference (LSD) were used for the statistical analysis of the results and P-values at levels ($P \leq 0.05$) were considered to be statistically significant. These calculations were carried out according to program SPSS, version 10.

RESULTS

The size of titanium dioxide nanoparticles were 50 nm, surface morphology was anatase. The size of bulk titanium dioxide was arranged between (300-800 nm).

The effect of nanoparticles compared with bulk particles on germination percentage, germination rate, mean germination time and mean daily germination.

Germination percentages: There was induction in seed germination percentage using different concentrations of bulk particles, while seed germination percentages decreased significantly when the seeds exposed to nanoparticles at concentrations (10, 1 and 0.01) mg/ml compared with control. There were differences between the effects of nanoparticles compared with bulk particles on germination percentage. The highest germination percentage (96.1%) was shown in 10 mg/ml of bulk particles concentrations, table (1).

Germination rate: The highest germination rate has been seen at (0.1 and 0.01) mg/ml concentration of nanoparticles, ($P < 0.05$). The same features were seen at (1 and 10) mg/ml of bulk particles, ($P < 0.05$). Other concentrations were reduced the germination rate. There were significant differences between the effects of nanoparticles compared with bulk particles.

Mean germination time: All concentrations of nanoparticles were reduced mean germination time. The same reduction has been seen at (1-10) mg/ml of bulk particles. The lower concentrations (0.1-0.01) mg/ml of it were increased, ($P < 0.05$). There were significant differences between the effects of nanoparticles compared with bulk particles.

Mean daily germination: The concentrations of bulk particles (10, 1 and 0.1) mg/ml showed significant increase in mean daily germination compared to the control, whereas higher concentrations of nanoparticles (10 and 1) mg/ml decreased mean daily germination ($P < 0.05$). However slight increase at 0.1 mg/ml and 0.01 mg/ml in MDG was observed.

The effect of nanoparticles compared with bulk particles on vigor index I, vigor index II, germination value and promoter indicator.

All concentrations of bulk particles were increased: Vigor index I, Vigor index II, Germination Value and Promoter Indicator compared with control. Different results were observed using different concentrations of nanoparticles. The increasing in above parameter were non-significant. The reduction of them were significant in most cases, table (2). There were significant differences between the effects of nanoparticles (0.01, 1 and 10) mg/ml compared with bulk particles on Vigor index I, Vigor index II and Promoter Indicator.

The effect of nanoparticles compared with bulk particles on length and number of: leaves, roots, hairy roots and total of plant length.

The results that appeared in table (3) showed that the length of: shoots, roots, hairy and number of roots, and total of plant lengths were not significantly affected by different concentrations of bulk particles and nanoparticles. While it had a significant effect on number of hairy roots. There was induction in number of hairy roots using 0.01 mg/ml concentration of nanoparticles. Nanoparticles (10 and 1) mg/ml concentrations were reduced the number of hairy roots compared with control. The effect of these concentrations were significant different from the effect of the same concentrations of bulk particles.

The effect of nanoparticles compared with bulk particles on biomass.

There were no significant effect of all concentration of nanoparticles and bulk particles on fresh and dry biomass compared to the control, Table (4).

The effect of nanoparticles compared with bulk particles on concentrations of pigments.

The results of table (5) show that the contents of chlo. A and chlo. B were not significantly affected by all concentrations of bulk particles and nanoparticles compared with control.

The effect of nanoparticles compared with bulk particles on root tips viability.

The results of table (6) shows root tips viability not affected by all concentrations of nanoparticles and bulk particles except the medium concentration of bulk particles, (0.01mg/ml), it reduce in root tips viability to (90%).

DISCUSSION

The widespread production and use of NPs, it is expected that they find their way into the environment, be taken up by living organisms (in particular plants) and consequently find their way into the food chain[3]. To confirm that nanoparticles played an important role in the observed phytotoxicity, this study focus on the phytotoxicity of TiO₂ nanoparticles (NPs) compared with bulk TiO₂ particles on: germination parameters, vegetative traits, roots viability, Biomass of seedling and photosynthetic pigments of amber 33 variety of Rice (*Oryza Sativa*) *in vitro*.

The results of nanoparticles in almost parameters of current study were different from result of bulk particles. Nanoparticles are particles between 1 and 100 nanometers in size [23]. In nanotechnology, a particle is defined as a small object that behaves as a whole unit with respect to its transport and properties[24]. Nanoparticles are of great scientific interest as they are, in effect, a bridge between bulk materials and atomic or molecular structures. A bulk material should have constant physical properties regardless of its size, but at the nano-scale size-dependent properties are often observed [22]. Thus, the properties of materials change as their size approaches the nanoscale and as the percentage of atoms at the surface of a material becomes significant[25]. For bulk materials larger than one micrometer (or micron), the percentage of atoms at the surface is insignificant in relation to the number of atoms in the bulk of the material.

Normally, nanoparticles are more reactive because of the high ratio of the surface area to the volume .The heterogeneous reaction occurs on the surface. Nanoparticles has a large surface area than the bulk one. It enhances the number of reaction site for the reaction to occur. In addition, surface atom is more unstable (and reactive). This instability related to their position on the lattice that force them to unbounded to their neighbor atoms or molecule[26] . For NPs case, as the surface/bulk atoms ratio increase, the instability (and reactivity) also increase. That's why surface chemistry and process is very important issue for handling NPs. The interesting and sometimes unexpected properties of nanoparticles are therefore largely due to the large surface area of the material, which dominates the contributions made by the small bulk of the material [16].

It has been declared that the biological activity and biokinetics of nanoparticles depends on parameters such

as size, shape, chemistry, crystallinity, surface properties (area, porosity, charge, surface modifications, coating), agglomeration state, bio persistence, and dose[27].

In this study, there were induction in seed germination using different concentrations of bulk particles, while it decreased when the seeds exposed to nanoparticles at concentrations (10, 1 and 0.01) mg/ml compared with control.

This result not agree with the result of Boonyanitipong and others in 2011, showing that nanoparticles of TiO₂, (50 nm), (10, 100, 500, 1000)mg/ml did not adversely effect on rice (*O. sativa* L.) seeds germination. The reason for this difference may due to the difference in variety of rice seeds. The results of NP phytotoxicity studies are highly dependent on the application method because apparent differences in the phytotoxicity of nanoparticles may arise from the properties of nanoparticles, plant species and ages, exposure time, and concentrations[28].

The result of current study, the highest germination rate of this study had seen at (0.1 and 0.01) mg/ml concentration of nanoparticles. The same features had seen at (1 and 10) mg/ml of bulk particles. Other concentrations were reduce the germination rate.

Feizi and others [16] observed that exposure of sage seeds (*Salvia officinalis* L.) to 60 mg L⁻¹ concentrations of bulk and nano TiO₂ particles led to enhanced germination rate .The maximum germination rate was found in 60 mg L⁻¹ bulk and nano-TiO₂ particles treatments (3.36 and 3.17 seed day⁻¹, respectively) and increasing concentration decreased the germination rate. The untreated group, 20 mg L⁻¹ bulk-TiO₂ and mg L⁻¹ nano TiO₂ treatments showed the lowest germination rate. Among the bulk-TiO₂ treatments only 60 and 80 mg L⁻¹ concentrations showed more values in germination rate in comparing to the control.

In similar study on fennel (*Foeniculum vulgare* Mill) Feizi and others in 2013 observed that fennel seeds exposure to low concentrations of nano TiO₂ particles led to enhanced germination rate. The highest germination rate was found in 5 ppm nano-TiO₂ particles(6.39 seed d₁) and increasing concentration decreased the germination rate. 60 ppm bulk-TiO₂ treatment showed the lowest germination rate. All of bulk TiO₂ particle treatments inhibited germination rates compared to the control[16].

This studies showed that all concentrations of nanoparticles were reduced mean germination time. The same reduction has seen at (1-10) mg/ml of bulk particles. The lower concentrations of it were increased. The concentrations of bulk particles (10, 1 and 0.1) mg/ml showed increased in mean daily germination,

whereas higher concentrations of nanoparticles decreased it.

Feizi and others [16] observed that exposure of sage seeds (*Salvia officinalis*L.) to 60 mg L⁻¹ bulk and nanosized TiO₂ obtained the lowest mean germination time (8.42 and 8.7 days, respectively) but higher concentrations did not improve mean germination time. Thus, 60 mg L⁻¹ concentration of bulk TiO₂ treatments reduced mean germination time by 20.4% whereas 60 mg L⁻¹ concentration of nano TiO₂ contributed to improve of mean germination time of about 17.5% in comparison with the control. It is proposed activation of respiration and rapid ATP production appears to be the primary metabolic events induced by early seed germination.

In the same way, fennel seeds exposed to 40 ppm nanosized TiO₂ reduced mean germination time (3.99 d) but higher concentrations did not improve mean germination time. 40 ppm concentration of nanosized TiO₂ treatment reduced mean germination time by 31.8%, whereas 40 ppm concentration of bulk TiO₂ contributed to a reduction of mean germination time of about 21% in comparison with the control [16]

In addition, Gurr JR and others [8] stated that the significant effect of nanosized TiO₂ on spinach germination in tests was maybe because of small particle size, which permitted nanoparticles to penetrate the seed during the treatment period, exerting its enhancing functions throughout growth.

In this study, all concentrations of bulk particles were increase: vigor index I, vigor index II, germination value and promoter indicator compared with control. There were different changing in vigor index I in dose of nanoparticles depending manner.

Feizi and others [16] observed that bulk-TiO₂ had a negative effect on vigor index I but the stimulating effect of nanoparticle treatments was seen on vigor index I of sage seeds. Exposure of seeds to 20 mg L⁻¹ bulk TiO₂ decreased vigor index I by 15% and 20% comparing to control and 20 mg L⁻¹ nano TiO₂. Additionally, the lowest vigor index II value was showed in bulk group treatments. Applying of 40 and 80 mg L⁻¹ bulk. In the same research, bulk TiO₂ particles decreased germination value of seeds except in 60 and 80 mg L⁻¹ concentrations while nanosized TiO₂ had a more positive effect than bulk TiO₂ treatments on germination value, TiO₂ showed 10 and 12% lesser value in vigor index II than control, respectively.

Furthermore, Feizi and others in 2013 observed that application of bulk-TiO₂ concentrations had a negative effect on vigor index I but the stimulating effect of nanoparticle treatments was seen on vigor index I and germination value of fennel seeds. Additionally, use of 5 ppm nanosized TiO₂ showed the greatest vigor index II value.

Finally, the results of current studies showed that bulk particles were either not effective or induction the plant growth. While even nanoparticles showed no toxic effects on shoots, roots, hairy roots length and total of plant lengths, biomass of seedling, chlorophyll A, chlorophyll B and root viability, but it decreased germination percentage, vigor index I, vigor index II, germination value and promoter indicator. In addition to vigor index I, number of hairy roots in dose depending manner. More studies of the effect of nanoparticles on the chemical composition of the plant, calculate the amount of TiO₂ in residue and in plant tissues will be beneficial in the safety uses of these materials.

Table-1: The effect of nanoparticles compared with bulk particles on Germination percentage, Germination rate, Mean germination time and mean daily germination.

Con. (mg/ml)	GP %		GR		MGT		MDG	
	Nano.	Bul.	Nano.	Bul.	Nano.	Bul.	Nano.	Bul.
10	52.2 ± 6.939c	96.1 ± 3.469a	0.605 ± 0.104cd	1.390 ± 0.341a	1.83 ± 0.404d	2.80 ± 0.872bcd	522. ± 69.389c	961. ± 34.694a
1	55.6 ± 3.849c	94.4 ± 5.092a	0.582 ± 0.096cd	1.138 ± 0.167ab	2.13 ± 0.493cd	3.10 ± 0.608bc	556. ± 38.490c	944. ± 50.918a
0.1	90.6 ± 8.221ab	94.4 ± 5.092a	1.287 ± 0.127a	0.760 ± 0.500bc	3.03 ± 0.751bc	4.67 ± 1.150a	906. ± 82.215ab	944. ± 50.918a
0.01	18.6 ± 2.493d	88.3 ± 4.410ab	1.265 ± 0.240a	0.329 ± 0.042d	2.90 ± 0.693bcd	5.33 ± 1.210a	894. ± 82.215ab	883. ± 44.096ab
Ctr	80.0 ± 13.333 ^b		0.776 ± 0.030 ^{bc}		3.53 ± 1.097 ^b		800. ± 133.333 ^b	
LSD	11.83		0.4127		1.083		129.7	

Data show Mean ± Standard Deviation; Con.: concentration; Nano.: Nanoparticles; Bul.: Bulk particles; Ctr: Control; GP: Germination percentage; GR: Germination rate; MGT: Mean germination time; MDG: mean daily germination; Similar letters are not significance at (P < 0.05).

Table-2: The effect of nanoparticles compared with bulk particles on Vigor index I, Vigor index II, Germination Value and Promoter Indicator.

Con. (mg/ml)	SVI		SVII		GV		PI	
	Nano.	Bul.	Nano.	Bul.	Nano.	Bul.	Nano.	Bul.
10	1242 ± 141.827de	2728 ± 701.191ab	10.99 ± 1.881d	26.43 ± 7.653a	1533 ± 416.333c	5497 ± 2378.380a	2.92 ± 1.876ef	8.58 ± 2.126ab
1	1196 ± 56.413 ^{de}	3016 ± 853.168 ^a	12.75 ± 2.340 ^{cd}	22.52 ± 4.603 ^{ab}	1289 ± 277.555 ^c	3606 ± 402.193 ^{abc}	2.67 ± 1.528 ^f	7.92 ± 1.588 ^{ab}
0.1	2084 ± 793.107 ^{bcd}	2878 ± 536.711 ^{ab}	18.52 ± 3.127 ^{bc}	18.17 ± 4.182 ^{bc}	5006 ± 1737.841ab	3672 ± 2115.573 ^{abc}	5.92 ± 0.722 ^{bce}	7.50 ± 3.307 ^{abc}
0.01	435 ± 80.866 ^e	2401 ± 430.239 ^{abc}	2.68 ± 0.684 ^e	17.46 ± 4.657 ^{bcd}	5378 ± 2973.463a	2267 ± 1059.874 ^{bc}	5.67 ± 1.443 ^{bcef}	9.08 ± 1.774 ^a
Ctr	1534 ± 349.327 cd		16.80 ± 1.785bcd		1652 ± 1502.316c		4.67 ± 0.520cef	
LSD	922.2		6.844		2925.6		3.061	

Data show Mean ± Standard Deviation; Con.: concentration; Nano.: Nanoparticles; Bul.: Bulk particles; Ctr: Control; SVI :Vigor index I; SVII: Vigor index II; GV: Germination Value ; PI: Promoter Indicator; Similar letters are not significance at (P < 0.05).

Table -3: The effect of nanoparticles compared with bulk particles on length and number of: leaves, roots and hairy roots.

Con. (mg/ml)	Leaves				Roots									
	L.		N.L.		L.		N.R.		L.Hr.		N.Hr.		T.L	
	Nano.	Bul.	Nano.	Bul.	Nano.	Bul.	Nano.	Bul.	Nano.	Bul.	Nano.	Bul.	Nano.	Bul.
10	13.33 ±1.528	11.40± 0.964	1	1	10.50± 0.500	17.17± 7.522	6.00± 1.732	8.33± 1.528	7.60± 2.227	5.03± 3.163	12.7± 6.658 ^{ab}	20.7± 19.502 ^{bc}	23.8± 1.041	28.6± 8.116
1	12.33± 1.041	11.27± 1.102	1	1	9.23± 2.040	20.83± 9.224	6.67± 2.082	6.33± 0.577	7.57± 1.762	4.57± 2.684	14.7± 7.506 ^{ab}	19.3± 14.572 ^c	21.6± 1.290	32.1± 9.996
0.1	11.00± 1.803	11.40± 0.656	1	1	11.63± 4.708	19.33± 6.526	6.33 ±0.577	7.00± 1.000	5.60± 1.600	6.67± 1.890	12.0± 10.583 ^{bc}	19.7± 14.154 ^{abc}	22.6± 6.385	30.7± 7.174
0.01	12.17± 0.611	11.67± 1.893	1	1	11.1± 1.353	15.67 ± 4.041	6.00 ±0.000	6.67± 0.577	9.07± 0.306	4.40± 1.637	20.7± 10.693 ^a	17.7± 12.583 ^c	23.3± 1.950	27.3± 5.752
Ctr	10.90±0.854		1		8.17±1.893		5.67±0.577		3.87±1.704		18.3±5.859 ^c		19.1±2.101	
LSD	1.886				8.945		2.080		2.898		20.53		9.96	

Data show Mean ± Standard Deviation; Con.: concentration; Nano.: Nanoparticles; Bul.: Bulk particles; Ctr: Control; L: length; N.L: number of leaves; N.R.: number of roots; Hr.: hairy roots; N.Hr: number of hairy roots ; T.L : total of plant length * : Similar letters are not significance at (P < 0.05).

Table -4: The effect of nanoparticles compared with bulk particles on biomass.

Con. (mg/ml)	F. W.		D.W.	
	Nano.	Bul.	Nano.	Bul.
10	0.2134±0.052	0.2756±0.084	0.01767±0.003	0.01577±0.004
1	0.2285±0.029	0.2375±0.038	0.01977±0.003	0.01503±0.001
0.1	0.2038±0.021	0.1942±0.054	0.01687±0.003	0.01117±0.003
0.01	0.1483±0.052	0.1968±0.048	0.01300±0.002	0.01253±0.004
Ctr	0.2116±0.017		0.01762±0.007	
LSD	0.08199		0.006474	

Data show Mean ± Standard Deviation; Con.: concentration; Nano.: Nanoparticles; Bul.: Bulk particles; Ctr: Control; F.W: fresh weight; D.W: Dry weight; * : (P < 0.05).

Table-5: The effect of nanoparticles compared with bulk particles on concentrations of pigments.

Con. (mg/ml)	Chlo. A		Chlo. B	
	Nano.	Bul.	Nano.	Bul.
10	63.6±52.861	60.9±4.682	62±16.029	62±43.745
1	49.7±27.363	48.6±9.130	126±109.876	125±79.350
0.1	57.9±14.240	30.6±15.357	60±8.984	61±24.370
0.01	48.2±28.644	47.0±27.637	39±36.363	46±23.911
Ctr	85.6±2.710		52±55.742	
LSD	43.56		93.3	

Data show Mean ± Standard Deviation; Con.: concentration; Nano.: Nanoparticles; Bul.: Bulk particles; Chlo. A: Chlorophyl A; Chlo. B: Chlorophyl B; Ctr: Control; * : (P < 0.05).

Table-6: The effect of nanoparticles compared with bulk particles on root tips viability.

Con. (mg/ml)	TTC %	
	Nano.	Bul.
10	100	100
1	100	100
0.1	100	100
0.01	100	90
Ctr	100	

Data show Mean; Con.: concentration; Nano.: Nanoparticles; Bul.: Bulk particles; Ctr : control.

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