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Research Article

Comparison the Phytotoxicity of TiO_2 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@ymail.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; TiO_2; 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 TiO_2 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 Cº, 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 Cº, 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 × GN / SN; GN is the total number of germinated seed; SN is the total number of seeds tested, [16].
2. Germination rate (GR) GR =Σ Gi /I; Gi is the number of seeds germinated on day I,[17].
3. Mean germination time (MGT), MGT=Σ Gi × i / Σ 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 at70◦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:

Chlo.A= (12.7 (D663)-(2.69(D645)) * V/ (1000*W)
Chlo.B= (22.9 (D645)-(4.68(D663)) * V/ (1000*W)
Carotenoid = ((4.695 *O.D 440) - (2.88 * O.D 663) + (O.D (645)) * (V/ (1000*W))

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 containing10 root tips, the temperature was kept at 35 ± 1C°. 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 arrange 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 were 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(10,1and 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 seen at(0.1 and0.01) mg/ml concentration of nanoparticles, ($P < 0.05$).The same features was seen at (1 and 10) mg/mlof bulk particles, ($P < 0.05$).Other concentrations were reduce the germination rate. There were significant different 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 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 different 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 increase: Vigor index I, Vigor index II, Germination Value and Promoter Indicator compared with control. Different result 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 different 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 were 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) shows 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 d_1) 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 germinationtime (3.99 d) but higher concentrations did not improve mean germinationtime. 40 ppm concentration of nanosized TiO2 treatment reduced mean germination time by 31.8%, whereas 40 ppm concentration of bulkTiO2 contributed to a reduction of mean germination time of about21% 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% and20% 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 Ibut 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±0.722bce	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±1.443bcef	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.

REFERENCES

1. Monica RC, Cremonini R; Nanoparticles and higher plants. Caryologia, 2009; 62:161-165.
2. Navarro E, Baun A, Behra R, Hartmann NB, Filser J, Miao AJ, Quigg A, Santschi PH, Sigg L; Environmental behavior and ecotoxicity of engineered nanoparticles to algae, plants and fungi, Ecotoxicology, 2008; 17 (5): 372–386.
3. Nowack B, Bucheli TD; Occurrence, behavior and effects of nanoparticles in the environment. Environ. Pollut, 2007; 150:5–22.
4. Larue C, Laurette J, Boime NH, Khodja H, Fayard B, Flank AM, Brisset F, Carriere M; Accumulation, translocation and impact of TiO2 nanoparticles in wheat (*Triticumaestivum* spp.): Influence of diameter and crystal phase. Science of the Total Environment, 2012; 431:197–208.
5. Salama HMH; Effects of silver nanoparticles in some crop plants, Common bean (*Phaseolus vulgaris* L.) and corn (*Zea mays* L.). International Research Journal of Biotechnology, 2012; 3:190-197.
6. Boonyanitipong P, Kositsup B, Kumar P, Baruah S, Dutta J; Toxicity of ZnO and TiO2 nanoparticles on germinating rice seed *Oryza sativa* L. International Journal of Bioscience, Biochemistry and Bioinformatics, 2011; 1:282-285.
7. Gelis C, Girard S, Mavon A, Delverdier M, Paillous N, Vicendo P; Assessment of the skin photoprotective capacities of an organo-mineral broad-spectrum sunblock on two ex vivo skin models. Photoimmunol Photomed, 2003;19:242–253.
8. Gurr JR, Wang AS, Chen CH, Jan KY; Ultrafine titanium dioxide particle in the absence of photoactivation can induce oxidative damage to human bronchial epithelial cells. Toxicology, 2005; 213: 66–73.
9. Huang S, Chueh PJ, Lin YW, Shih TS, Chuang SM;Disturbed mitotic progression and genome segregation are involved in cell transformation mediated by nano-TiO2 long-term exposure. Toxicol. Appl. Pharm, 2009; 241:182–194.
10. Chao SHL, Choi HS; Method for providing enhanced photosynthesis. Korea Research Institute of Chemical Technology.In: Owolade OF, Ogunleti DO. Effect of Titanium dioxide on the diseases, development and yield of Edible cowpea. J Plant Prot Res, 2005; 48: 330-335
11. Khot LR, Sankaran S, Maja JM, Ehsani R, Schuster EW; Applications of nanomaterials in agricultural production and crop protection: A review. Crop Protection, 2012; 35:64-70.
12. Wu SG, Huang L, Head J, Chen DR, Kong IC, Yinjie J, Tang YG;.Phytotoxicity of metal oxide nanoparticles is related to both dissolved metals ions and adsorption of particles on seed surfaces. J Pet Environ Biotechnol, 2012; 3:126.
13. Gui S, Sang X, Zheng L, Ze Y, Zhao X, Sheng L, Sun Q, Cheng Z, Cheng J, et al; Intragastric exposure to titanium dioxide nanoparticles induced nephrotoxicity in mice, assessed by physiological and gene expression modifications. Particle and Fibre Toxicology, 2013; 10(4).
14. Lu CM, Zhang CY, Wen JQ, Wu GR, Tao MX; Research of the effect of nanometer materials on germination and growth enhancement of *Glycinemax* and its mechanism. Soya Bean Science, 2002; 21: 168–172.(In chines).
15. Castiglione MR, Giorgetti L, Geri C, Cremonini R; The effects of nano-TiO2 on seed germination, development and mitosis of root tip cells of *Vicianarbonensis* L. and *Zea mays* L.Journal of Nanoparticle Research, 2011; 13:2443–2449.
16. Feizi H, Kamali M, Jafari L, Moghaddam PR; Phytotoxicity and stimulatory impacts of nanosized and bulk titanium dioxide on fennel *(Foeniculumvulgare* Mill). Chemosphere, 2013; 91:506–511
17. AL-Kaisi WA, Muhsen TAA, Hamed AS; Effect of mycorrhiza (*Glomus mosseae*) and superphosphate on physiological characters of *Hodeumvulgare*. Journal of the college of basic education, 2012; 18:765-784.
18. Vashisth A, Nagarajan S; Effect on germination and early growth characteristics in sunflower (*Helianthus annuus*) seeds exposed to static magnetic field. Journal Plant Physiology, 2010;167:149–156.
19. Bouslamo M, Schapangh WT; Stress tolerance in soybean. In: AL-Kais WA, AL-Hayani EA, AL-HadithyMA. Influence of Yeast (*Saccharomyces cereivisiae*) by foliar application, seed soaking and soil treatment of soil on growth of Petroselinum hortense.Magazine of Al-Kufa University for

Biology, 1984; 2:13-21.
20. Mahajan P, Dhoke SK, Khanna AS; Effect of nano-zno particle suspension on growth of mung (*Vignaradiata*) and gram (*Cicerarietinum*) seedlings using plant agar method. Journal of Nanotechnology, 2011; 7.
21. Arnon DI; Copper enzymes in isolated chloroplasts polyphenol oxidase in Beta vulgaris. Plant Physiol, 1949;24: 1-15.
22. Shaumurat T, Gu J, Xu C, Yang Z, Zhao Q, Liu Y, Liu Y; Phytotoxic and genotoxic effects of ZnO nanoparticles on garlic (*Allium sativum* L.): A morphological study. Nanotoxicology, 2011; 6: 241-8.
23. Ball P; Natural strategies for the molecular engineer. Nanotechnology, 2002; 13:15-28.
24. Buzea C, Pacheco BII, Robbie K; Nanomaterials and nanoparticles: Sources and toxicity. Biointerphases, 2007; 2:17-72.
25. Schwirn K, Tietjen L, Beer I; Why are nanomaterials different and how can they be appropriately regulated under REACH? Environmental Sciences Europe, 2004; 26: 9.
26. Aitken RJ, Creely KS, Tran CL; Nanoparticles: An occupational hygiene review. Express Polymer Letters, 2007; 1: 546–575.
27. Casals E, Campos SV, Bastús NG, Puntes V;Distribution and potential toxicityof engineered inorganic nanoparticles and carbon nanostructures in biological systems. Trends in Analytical Chemistr, 2008; 27: 672-683.
28. Ma X, Lee GJ, Deng Y, Kolmakov A; Interactions between engineered nanoparticles (ENPs) and plants: phytotoxicity, uptake and accumulation. Sci. Total Environ, 2010; 408: 3053–3061.