Al-Mustansiriyah Journal of Science
ISSN: 1814-635X (print), ISSN: 2521-3520 (online)
Volume 28, Issue 3, 2017
DOI: http://doi.org/10.23851/mjs.v28i3.548

Research Article

Comparison Study on the Effect of Nano and Bulk Titanium Dioxide Particles on Seeds Germination, Growth and Chemical Composition of Wheat *Invitro* and *Invivo*

Raghad D. Abdul Jalil* , Alyaa M. Yousef
Department of Biology, College of Science, Mustansiriyah University, IRAQ
*Correspondent Author Email: raghadalshybany@yahoo.com

ArticleInfo

Received
20 Apr. 2017

Accepted
17 Oct. 2017

Abstract

The present study aimed to examine the effect of TiO2 nanoparticles (NPs) compared with bulk particles (BPs) on seed germination and growth of latefyha's cultivar wheat in vitro and in vivo and on chemical compositions with detecting the residuum of NPs in the plant. In the in vitro study, most concentrations of NPs and BPs have no effect on germination percentage, mean germination time, mean daily germination, promoter indicator, number of leaves, length and number of root and root tips viability but they reduced germination rate and germination value besides they induced shoot length and biomass. In the in vivo study, some parameters induced by most concentrations of NPs such as plant leaves area, leaf area index, length, of viability roots, height and total of plant length and biomass while no effect was seen on: mean daily germination, vigor index I and vigor index II, chlorophyll B, leaf area relative, in this regard, it reduced germination percentage, chlorophyll A, and carotene. There were some differences between the effect of NPs and those of BPs. There were increased in the total number of chemical compounds that identified in leaves of wheat plants treated with nanoparticles compared with control while the total numbers of compounds were decreased using bulk particles.

Keywords: TiO_2 nanoparticles, bulk particles, wheat, chemical composition, germination *in vitro* and *in vivo*.

الخلاصة

هدفت الدراسة الحالية لفحص تأثير جسيمات ثنائي أوكسيد التيتانيوم النانوية مقارنة مع الجسيمات الميكروية على انبات ونمو بذور الحنطة *T. aestivum* (صنف لطيفية) خارج الجسم الحي وداخل الجسم الحي وأيضا تأثير هذه الجسيمات على المركبات الكيميائية مع كشف بقايا الجسيمات النانوية في النبات. أظهرت نتائج هذه الدراسة في خارج الجسم الحي ان معظم تراكيز الجسيمات النانوية والجسيمات الميكروية لم تؤثر على نسبة الانبات, متوسط وقت الانبات متوسط الانبات اليومي، مؤشر تحفيز الانبات ، عدد الأوراق، طول وعدد الجذور وحيوية الجذور لكن قللت من سرعة الانبات وقيمة الانبات بالإضافة الى انها حفزت طول الجزء الهوائي والكتلة الحيوية للنبات .أظهرت نتائج الدراسة في داخل الجسم الحي حدوث تحفيز لمعلمات الانبات التالية بمعظم تراكيز الجسيمات النانوية: مساحة أوراق النبات, مؤشر مساحة الورقة، طول وحيوية الجذور، ارتفاع وطول النبات الكلي والكتلة الحيوية للنبات بينما لو تؤثر على متوسط الانبات اليومي ، مؤشر النشاط الأول، مؤشر النشاط الثاني ،كلوروفيل ب ، مساحة الورقة النسبي،بينما قللت الجسيمات النانوية نسبة الانبات ،كلوروفيل أ والكاروتين. كان هناك بعض الاختلافات في تأثير الجسيمات النانوية والجسيمات الميكروية. اظهرت النتائج زيادة في العدد الكلي للمركبات الكيمائية التي حددت في نباتات الحنطة المعاملة بالجسيمات النانوية مقارنة مع السيطرة بينما انخفض العدد الكلي للمركبات باستخدام الجسيمات الميكروية.

Introduction

Nanometer-sized materials have received considerable attention because of their unique chemical and physical properties, which differ greatly from those of bulk materials. These differences are frequently attributed to the effects of quantum confinement or of the particles 'finite size. TiO_2 nanoparticles less than 100 nm in diameter, have become a new generation of advanced materials due to their brilliant and interesting optical, dielectric, and photo catalytic characteristics from size quantization, [1]. They are widely used as an important kind of biomaterials due to their

large surface area, enhanced chemical reactivity and easy penetration into cells **[**2]. It is considered as nontoxic, an inert and safe material, photocatalysts and has been used in many applications such as: cosmetics, pharmaceuticals and biocompatible pigment products [3] [4] [5].

Nano-sized TiO_2 in various forms is used widely in everyday life in a variety of products, such as antifouling paints, household products, plastic goods, medications, cosmetics, pharmaceutical additives and food colorants, and many new applications are under development or already in pilot production, [5]. It can be used in, coatings, papers, inks, medicines, pharmaceuticals, food products, and toothpaste. It can even be used as a pigment to whiten skim milk, [6].

The increase in the production and use of manufactured nanoparticles (MNP) have initiated several scientific studies that investigate environmental risks and toxic effects on plants including wheat and rice, compared with bulk particles, [7] [8]. The study of Abdul Jalill and others in (2015) found in study on amber 33 variety of rice (*Oryza sativa*) *in vitro* that TiO_2 nanoparticles showed no toxic effects on shoots, roots, hairy roots length and total of plant's 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 is dose depending manner, [9].

Latyfia cultivar of *T. aestivum*, which chosen for this study, registered and certified in 10/9/1995, hybridization between an australian cultivar and arase cultivar, production 1000/kilograms/dunams, suitable for bread, The percentage of protein 12,5%, flour extraction rate 76%.

The TiO_2 NPs, considered having low solubility, remained in the soil for long periods and stuck to the plants' cell walls, which might create potential environmental risks for deeper soil layers. Thus, it is important to close research gaps of possible nano-risk on chemical composition of plant or residuum such material on tissues so that the chance of hazard and risks of using nanomaterials can be more accurately assess [10].

So present study aimed to exam the phytotoxicity of TiO_2 nanoparticles (NPs) compared with bulk TiO_2 particles on: germination parameters, vegetative traits, roots viability, biomass of seedling and photosynthetic pigments of latefyha variety of wheat (*T. aestivum*) *in vitro*. The same experiment would be carried out in *in vivo* with additional experiment: calculate the amount of TiO_2 in residue, chemical compositions and SEM analysis of plants.

Materials and Methodology

Preparation of nano, bulk particles and seeds

Dry titanium dioxide anatase nanoparticles (Sigma Aldrich, USA) were used. As in supplier's data its particle size was 50 nm, trace metal basis was 99.7% and surface area was 200–220 m^2/g. Bulk titanium dioxide particles (BPs) (Sigma Aldrich, China) was used to compare their effect with these of NPs. The size of nano and bulk examined by scanning electron microscope (SEM)/ Vega Tescan (USA) in Center of Nanotechnology and Advanced Materials/ University of Technology-Iraq. To prepare different concentrations of nano and bulk particles, sterilized distilled water was used.

The seeds of latyfia cultivar of *T. aestivum* were taken from Mabain Al-Nahrian Company for The Seeds Production in Baghdad- Iraq for culture season 2013-2014. They immerse in 1% sodium hypochlorite solution for 3 min and rinsed by sterilized distilled water three times. They soaked in bulk particles solutions or nanoparticles suspensions at various concentrations. All seeds incubated in an incubator at laboratory conditions (30±1 C°, 12 h. light: 12 h. dark) for four days. Sterilized distilled water was used for soaking other seeds as control. These seed used for *in vitro* and *in vivo* studies.

Experiments In vitro

A piece of filter paper (Whatman No. 42/ Zelpa, Belgium) putted in Petri dish (90 mm × 15 mm). For each concentration separately, one

hundred soaked seeds (that prepared above) planted in petri dishes. There were five seeds for each Petri dishes and four replications/ concentration including 100 seeds in each replicates. The distance between each seed was four centimeters. Five ml of sterilized distilled water added. Petri dishes were sealed with parafilm and placed in an incubator at: 30±1 Cº, 12 h. light, 12 h. dark for 10 days, [11]. The number of new germinated seeds recorded daily. A seed considered germinated when the radicle showed 2 mm in length at least. At the end of experiment, roots and shoots were separated and washed with distilled water. The following parameters counted at the end of experiment: germination percentage, [12] germination rate, [13] mean germination time, mean daily germination; germination value, [12] promoter Indicator, [14] number and lengths of: leaves, roots, hairy roots and the total length. Root tips viability, also, recorded using 2, 3, 5-triphenylte trazolium chloride (TTC) as a histopathologic stain for testing the viability of root tips, [15]. For biomass determination, the fresh and dry weight of roots and shoots was measured, [16].

Experiments In vivo

The soaked seeds of wheat latyfia cultivar were planted in pots (in diameter 18.5). The pots filled with soil. The experiment was randomized completely design with five replicates for each concentration. There were four concentrations (10, 1, 0.1 and 0.01 mg/ml) of each nanoparticles and bulk particles. Each treatment watered to field capacity and placed in a green house in mid-December for 45 days. The number of new germinated seeds recorded daily. At the end of experiment roots and shoots were separated and washed with distilled water.

The same parameters, described in in vitro study, recorded at the end of experiment. In addition, the fallowing parameter also, recorded: vigor index I, vigor index II, plant leaves area, leaf area relative, leaf area index, [13], pigments content of leaves (chlorophyll a, b and carotenoid (mg/g of fresh weight) content in the leave) according to the definition of Equation of Arnon in (1949) [17].

TiO_2 determination: TiO_2 amount of dry leaves determined using a flame atomic absorption spectrophotometer and expressed based on dry weight according [18]. TiO_2 concentration recorded according [19].

Gas Chromatography- Mass Spectrum analysis (GC-MS): The fresh aerial parts collected after the end of experiment. They washed with D.W and extracted with cooled acetone (100%) according to **[**20]. The extracts filtered by Whatman No.1 and 0.22 µ micro filters. GC-MS analyses were done using Shimadzu GC-2010 Plus coupled with Shimadzu GCMS-Q2010 Ultra. Capillary column was Inert Cap 1MS, 0.25µm, 30m, 0.25mm, Gl Sciences/ Japan.

Carrier gas was helium; constant flow rate was one ml per min.; Auto Injector was: AOC-20i, Shimadzu. Injection volume was 5 µl. Column oven Temperature: 100 °C. Oven temperature program: 100 °C for 3 min.; 240 °C for 9 min.; 280 °C for 5 min.; and 300 °C for 2min; rate was 15, [21].

Identification of all chemical components were direct comparison of the retention times and mass spectral data with computer matching of the standard components, the program was NIST mass spectral search program for the NIST/EPA/NIH mass spectral library version 2.0 f / 200).

Scanning Electron Microscope analysis (SEM):

The aerial parts examined by scanning electron microscope (SEM) in Center of Nanotechnology and Advanced Materials/ University of Technology/ Iraq. Analyzed samples dried at 40 Cº for 5 days by oven and prepared according to standard procedures of Center of Nanotechnology and Advanced Materials/ University of Technology/ Iraq.

Statistical analysis

All data analysis using analysis of variance (ANOVA) with the least significant difference (LSD) at levels ($P \leq 0.05$) P-values. These

calculations were carried out according to program SPSS, version 10.

Results and Discussion

In vitro.

Germination percentage: There was significant decreasing of germination percentage in concentration (0.01 mg/ml) of nanoparticles compared with same concentration of bulk particles and compared with control. All other treatments were not significant.

Germination rate: There were significant decreasing of germination rate by (0.01- 1 and 10) mg/ml of nanoparticles and (0.01- 0.1 mg/ml) of bulk particles compared with control. In this treatment, nanoparticles were more reducer than bulk particles Table 1.

Mean germination time: all treatments induced MGT, non-significantly, compared with control except the induction of 0.1 mg/ml of nanoparticles ($P \leq 0.05$). Nanoparticles were more inducer than bulk particles.

Mean daily germination: the changes were not significant at all treatments except (0.01 mg/ml) of nanoparticles which was reduced MDG significantly compared with each of control and bulk particles.

Germination Value: the reductions in GV were not important, (at $P \leq 0.05$) of all concentrations of nano and bulk particles compared with each of them and compared with control, except the reductions of (0.1-0.01) mg/ml, ($P \leq 0.05$).

Promoter indicator: the highest promoter indicator was observed in concentrations 1 mg/ml of bulk particles and 0.01 mg/ml of nanoparticles compared with control that gave lower promoter indicator (3.17). There were significant differences of promoter indicator at (1 and 0.01 mg/ml) between nano and bulk particles. All other treatments were not significant.

Leaves: There were inductions in length and number of leaves, ($P \leq 0.05$), at all treatments. In this treatment, nanoparticles were more inducer than bulk particles Table 2.

Root: The changing in length and number of roots per plant were not important, ($P \leq 0.05$), at all treatments. The same results found in root tips viability.

Hairy roots: different concentrations of nano and bulk particles induced length and number of hairy root per plant. These inductions were significant in bulk particles compared with control. Bulk particles were more inducer than nanoparticles, ($P \leq 0.05$).

Shoot length: They were significant increase in shoot length at concentration (1, 0.1 and 0.01) mg/ml of nanoparticles and at 0.01 mg/ml of bulk particles compared with control**.** Other concentrations of bulk particles were no significant effect on shoot length compared with control, nanoparticles were more inducer than bulk particles, ($P \leq 0.05$) Table 3.

Total length of plant and biomass: There were no significant effected in total length of plant, fresh and dry weight in plants treated with all concentrations of nano and bulk particles. The highest fresh weight (0.247 mg) was in concentrations 0.1 mg/ml of nanoparticles while the lowest fresh weight (0.188 mg) was in same concentration of bulk particles with no significant effect between them.Data shows Means; Con.: concentration (mg/ml); NPs.: Nanoparticles; BPs.: Bulk particles; Ctr: Control; GP: Germination percentage; GR: Germination rate; MGT: Mean germination time; MDG: mean daily germination; GV: Germination Value; PI: Promoter Indicator; horizontally, different litters are significant, ($P \leq 0.05$).Data shows Means; Con.: concentration (mg/ml); NPs.: Nanoparticles; BPs.: Bulk particles; Ctr: Control; L: length (cm); N.L.: number of leaves; N.R.: number of roots; L.Hr.: length of hairy roots (cm); N.Hr: number of hairy roots ; RTV: root tips viability.

Volume 28, Issue 3, 2017 DOI: http://doi.org/10.23851/mjs.v28i3.548

Table 1: The effect of nanoparticles compared with bulk particles on Germination percentage, Germination rate, Mean germination time, mean daily germination, germination value and promoter indicator of *T. aestivum in vitro*.

Con.	GP		GR		MGT		MDG		GV		PI	
	NPs	BPs	NPs	BPs	NPs	BPs	NPs	BPs	NPs	BPs	NPs	BPs
10	95.6	92.2	1.46	1.54	3.17	2.68	956	922	8022	7011	4.42	5.08
1	90.6	93.9	1.34[a]	1.44[b]	3.13	2.87	906	939	6622	6403	2.83a	7.33b
0.1	87.2	91.1	0.76[a]	1.38[b]	4.73	2.9	872	911	2283a	6644b	5.42	4.92
0.01	76.1	92.2	0.824a	1.33[b]	3.07	3.1	761	922	2650a	6744b	7.25a	2.67b
Ctr	93.9		1.7		2.67		939		8461		3.17	
LSD	12.66		0.3058		1.204		126.6		3039.9		2.286	

Table 2: The effect of nanoparticles compared with bulk particles on length and number of: leaves, roots, hairy roots and root tips viability of *T. aestivum in vitro*.

Con.	Leaves				Roots									
	L.		N.L.		L.		N.R.		L.Hr.		N.Hr.		RTV	
	NPs	BPs	NPs	BPs	NPs	BPs	NPs	BPs	NPs	BPs	NPs	BPs	NPs	BPs
10	12.17	13	2	2	19.33	25.33	5	5	4.13	7.27	23.3a	40.3b	100	100
1	15.5	13.5	2	2	23.5	22.67	4.67	4.67	4.53	5.67	26.3a	45b	100	100
0.1	14.17	12.83	2	1.7	21.5	18.5	4.67	5	6.4	6.83	20.3a	34.3b	100	100
0.01	16.5	14.67	2	2	18.17	19.67	5	5	6.47	8.67	43.7	41.3	100	100
Ctr	9.17		1.3		20.17		5		2.87		10.7		-	
LSD	4.86		0.2		5.77		0.79		3.813		13.94		-	

Table 3: The effect of nanoparticles compared with bulk particles on: Shoot length, total length of plant and biomass of *T. aestivum in vitro*.

Con.	/SL		T.L		F. W.		D.W.	
	NPs	BPs	NPs	BPs	NPs	BPs	NPs	BPs
10	12	13	31.5	38.33	0.261	0.224	0.022	0.0204
1	15.5	13.5	39	35.83	0.182	0.216	0.017	0.0188
0.1	14	12.9	35.5	31.33	0.247	0.189	0.019	0.0184
0.01	16.5	14.6	34.67	34.33	0.212	0.192	0.017	0.0165
Ctr	9		33		0.208		0.0185	
LSD	4.856		7.44		0.084		0.0069	

Data shows Means; Con.: concentration (mg/ml); NPs.: Nanoparticles; BPs.: Bulk particles Ctr: Control; SL: Shoot length (cm); T.L: total length of plant (cm); F.W: fresh weight (g); D.W: Dry weight (g).

- ***In vivo.***

Germination percentage: There were significant decreasing in germination percentage treated with (10, 0.1 and 0.01) mg/ml of bulk particles also significant decreasing with (1 and 0.1) mg/ml of nanoparticles compared with control. There were significant differences of germination percentage at all concentrations between nanoparticles and bulk particles.

Mean daily germination, vigor index II and I: The changing in them caused by different concentrations of nanoparticles were not important, ($P \leq 0.05$). The same effect had seen in these parameters at different concentrations of bulk particles except these of (0.1 and 10) mg in MDG and (1 mg) in SVI which were significant. There were differences between nano and bulk particles at (1-10) mg/ml concentrations in SVI and (0.01-1) mg/ml concentrations in SVII, (Table 4).

Plant leaves area and Leaf area index: There were significant increase in plant leaves area with concentrations (1-0.01) mg/ml of bulk particles and 1 mg/ml of nanoparticles

compared with control. There were significant increases in plant leaves area at (0.1 mg/ml) bulk particles compared with same concentrations of nanoparticles.

Leaf area relative: the differences of this test were not important, **(**$P \leq 0.05$) at all treatments except (0.01 mg/ml) bulk particles which induced LAR significantly. The lower concentrations (0.1, 0.01) mg/ml of bulk particles on LAR were difference's compared with same concentrations of nanoparticles, (Table 5).

Roots Length (cm): As shown in Table 6, all concentrations of bulk particles and nanoparticles were increase the Length of roots. This increasing was significance in (1-0.01) mg/ml of bulk particles and 0.1 mg/ml of nanoparticles compared with control. In addition, there were significance increasing at concentration (1 mg/ml) of bulk particles compared with same concentrations of nanoparticles, while the other treatments showed no significant different between them.

Roots number/plant: Nanoparticles and bulk particles increased number of roots at all concentrations compared with control.

Root tips viability: At 10 mg/ml of nanoparticles, there were decrease on root tips viability compared to control while all other treatments increased RTV significantly. Bulk particles (1- 0.01) mg/ml led to increase the root tips viability compared to these of NPs.

Shoot length (cm): There was significant increasing in shoot length at lower concentrations (0.01mg/ml) of nanoparticles and (0.1, 0.01 mg/ml) of bulk particles compared with control. There were no significant different between nanoparticles and bulk particles at all treatments.

Total of plant length: all treatments induced T.L; they were significant at (1-0.01) mg/ml of BPs and (0.1-0.01) mg/ml of NPs compared with control. Nanoparticles were more inducer than bulk particles.

Chlorophyll A: Nanoparticles and bulk particles did not effect in contain of chlorophyll A. There was significant decreasing in Chlorophyll A at lower concentrations (0.1, 0.01mg/ml) of nanoparticles compared with control.

Chlorophyll B: Nanoparticles and bulk particles did not effect in contain of chlorophyll B, ($P \leq 0.05$). **Carotene**: Carotenoid content significant decreased in plant treated with (0.1- 0.01) mg/ml of nanoparticle compared with control, While the other concentrations did not effect. There were no significant different between nanoparticles and bulk particles at all treatments in pigments.

Fresh and dry weight: There were significant increasing in these tests at (1, 0.01) mg/ml concentrations of NPs and compared with control. All other increasing in dry weight were not significant. There were significant effected in (0.01 mg/ml) between nanoparticles and bulk particles on fresh weight, (Table 7).

Data shows Means; Con.: concentration (mg/ml); NPs.: Nanoparticles; BPs.: Bulk particles Ctr: Control; GP: Germination percentage; MDG: mean daily germination; SVI: Vigor index I (cm); SVII: Vigor index II (g); horizontally, different litters are significant, ($P \leq 0.05$). Data shows Means; Con.: concentration (mg/ml); NPs.: Nanoparticles; BPs.: Bulk particles; Ctr: Control; N.L: number of leaves; L.A.: leaves area (cm^2); PLA: plant leaves area (cm^2); LAI: Leaf area index; LAR: Leaf area relative (cm^2/g); horizontally, different litters are significant, ($P \leq 0.05$).

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Table 4: The effect of nanoparticles compared with bulk particles on: Germination percentage, mean daily germination, vigor index I and vigor index II of *T. aestivum in vivo*.

Con.	GP		MDG		SVI		SVII	
	NPs	BPs	NPs	BPs	NPs	BPs	NPs	BPs
10	93.9[a]	77.6[b]	3756	3103	9737[a]	7610[b]	97.1	90
1	80[a]	100[b]	3199	4000	8308[a]	11850[b]	111.1[a]	157.9[b]
0.1	84[a]	69.6[b]	3359	2782	9584	8182	106.3[a]	79.3[b]
0.01	89.9[a]	80[b]	3596	3199	10053	10092	133.6[a]	54.3[b]
Ctr	100		4000		9300		113	
LSD	11.99		814.		2090.8		29.43	

Table 5: The effect of nanoparticles compared with bulk particles on plant leaves area, Leaf area relative and Leaf area index of *T. aestivum in vivo*.

Con.	PLA		LAI		LAR	
	NPs	BPs	NPs	BPs	NPs	BPs
10	74.2	56	2155	1626	797	458
1	84.5	78.3	2455	2275	601	464
0.1	52.8[a]	91.2[b]	1532[a]	2648[b]	461[a]	948[b]
0.01	74.4	90	2160	2615	484[a]	1271[b]
Ctr	52.3		1519		795	
LSD	23.67		687.4		449.3	

Table 6: The effect of nanoparticles compared with bulk particles on length, number and viability of roots, shoot length and total of plant length of *T. aestivum in vivo*.

Con.	RL.		N.R.		RTV		S.L		T.L	
	NPs	BPs	NPs	BPs	NPs	BPs	NPs	BPs	NPs	BPs
10	70.2	64	4.5[a]	8.75[b]	43	50	33.5	34.75	103.8	98.8
1	64.5[a]	81.2[b]	8.75	9	62.5	60	36.62	37.25	103.6	118.5
0.1	78.5	78	8.75	9.25	51	70	35.88	38.38	114.1	116.4
0.01	73.5	87.5	10	9.25	75	70	38.25	38.5	111.8	126
Ctr	59.5		7.25		50		33.5		93	
LSD	15.04		1.347		11		4.130		17.58	

Table 7: The effect of nanoparticles compared with bulk particles on concentrations of pigments and biomass of *T. aestivum in vivo*

Con.	Chlo. A		Chlo. B		Caro.		F. W.		D.W.	
	NPs	BPs	NPs	BPs	NPs	BPs	NPs	BPs	NPs	BPs
10	192.3	183.9	99.2	102.5	41	44.2	1.137	1.174	0.096	0.122
1	180.1	157.6	126.7	94.8	51.9	41	1.482	1.579	0.144	0.172
0.1	153.2	203.2	118.7	111.6	31.5[a]	57.9[b]	1.267	1.154	0.115	0.098
0.01	153.1	209.2	105.2	153.2	28.6	40.2	1.495[a]	0.676[b]	0.156[a]	0.072[b]
Ctr	219.3		125.1		48.2		1.13		0.0926	
LSD	61.82		55.07		14.22		0.3024		0.03031	

Data shows Means; Con.: concentration (mg/ml); NPs.: Nanoparticles; BPs.: Bulk particles; Ctr: Control; RL: Roots length (cm); N.R.: number of roots; RTV: root tips viability; S.L: Shoot length (cm); T.L: total of plant length (cm); horizontally, different litters are significant, ($P \leq 0.05$).

Data shows Means; Con.: concentration (mg/ml); NPs.: Nanoparticles; BPs.: Bulk particles; Ctr: Control; Chlo. A: Chlorophyl A (mg/gfw); Chlo. B: Chlorophyl B ((mg/gfw); Caro. Carotenoid (mg/gfw); F.W: fresh weight

(g); D.W: Dry weight (g); horizontally, different litters are significant, ($P \leq 0.05$).

TiO_2 determination:

The percentage of TiO_2 in residues of shoots treating with (0.01-10) mg/ml of nanoparticles or bulk particles were: (0.0310, 0.0263) % and (0.0490, 0.0573) % respectively. There was no significant effect of them compared with percentage of TiO_2 in control (0.0243) %.

GC-MS analyses of *T. aestivum*.

As show in (Table 8), there were increased in total number of compounds that identified in latyfia cultivar treated with higher and lower concentration of NPs (16 and 17 compounds) compared with 15 compounds in control. The total number of compounds was decreased in higher and lower concentrations of bulk particles (12, 9) compounds respectively.

There were increasing in percent of some compounds compared with control. l-(+)-Ascorbic acid 2,6-dihexadecanoate was increased in plant treated with all treatment of bulk and NPs except the lower concentration of NPs. This treatment was decreased the percent of compound. Slightly increasing in Phytol was observed in the plant treated with all concentration of NPs while this increase was very high in lower and higher concentrations of bulk. Another increased was found in Octadecanoic acid, 2,3-dihydroxypropyl ester and Tetradecanoic acid using higher concentrations of NPS. These compound was absent in other treatment.

In contrast, there were either decreasing or absent in percent of some compounds in treatments of plants. The percent of Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester was highly decreased in all treatments of NPs and bulk. Octadecanoic acid, 2-(2-hydroxyethoxy) ethyl ester were slightly decreased using higher concentrations of NPS. It was absent in other treatments compared with control. Chromatogram of latyfia cultivar treated different concentrations of: NPs, BPs and control were seeing in Figure 1.

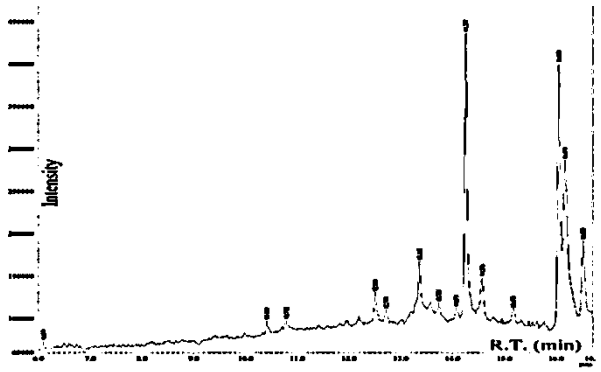
Table 8: effect of titanium dioxide (TiO2) nanoparticles and bulk titanium dioxide on chemical composition of *T. aestivum*.

N.	Nanoparticles				Bulk particles				Ctr.	
	(10 mg/ml)		(0.01 mg/ml)		(10 mg/ml)		(0.01 mg/ml)		(0 mg/ml)	
	Compound	%	Compound	%	Compound	%	Compound	%	Compound	%
1.	2,6-Dimethyl-6-nitro-2-hepten-4-one	0.22	2,6-Dimethyl-6-nitro-2-hepten-4-one	0.24	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	3.46	2,6-Dimethyl-6-nitro-2-hepten-4-one	1.51	Dodecanoic acid	0.91
2.	2-(Dimethylamino)methyl-1,2,3,4-tetrahydro-1-naphthol	0.30	Phenol,2,6 bis(1,1dimethyl ethyl)-4-methyl-, methylcarbamate	0.36	l-(+)Ascorbic acid 2,6dihexadecanoate	16.77	Phenol,2,6-bis(1,1dimethyl ethyl)-4-methyl-, methylcarbamate	1.21	Ketone,1cyclohexen-1-ylmethyl, semicarbazone	0.26
3.	Tridecanoic acid	0.23	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	2.31	1,6-Heptadiene, 2-methyl-6-phenyl-	2.79	2-Heptenedioic acid,4-cyclopropyl-, dimethyl ester, I-	2.89	Tetradecanoic acid	0.78
4.	Tetradecanoic acid	1.22	l-(+)-Ascorbic acid 2,6-dihexadecanoate	5.83	Phytol	25.96	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	7.32	Cyclopropanecarboxylic acid, 3-(3-methoxy-2-methyl-3-oxo-1-propenyl)-2,2-dimethyl	0.77

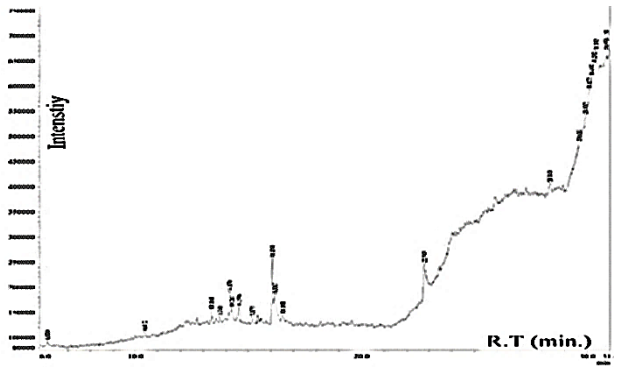
5.	Benzoic acid, 2-fluoro-5,6-dimethoxy	0.68	5-Methyl-1-phenylbicyclo[3.2.0]heptane	3.7	9,12,15-Octadecatrienoic acid, (Z,Z,Z)	21.38	l-(+)-Ascorbic acid 2,6-dihexadecanoate	19.51	l-(+)-Ascorbic acid 2,6-dihexadecanoate	7.01
6.	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	3.92	Phytol	10.72	Octadecanoic acid, 2-(2-hydroxyethoxy)ethyl ester	14.42	1,6-Heptadiene, 2-methyl-6-phenyl-	10.41	2-(2-Nitro-1-p-tolyl-ethyl)-cyclohexanone	5.42
7.	Cyclopentadecanone, 2-hydroxy	0.50	6-Octadecenoic acid, (Z)-	7.63	Hexadecanoic acid, 2-hydroxy-1(hydroxymethyl)ethyl ester	7.62	1-Cyclopenten-3-one, 1-(1-cyclohexen-1-yl)-2-[(carboxyethyl)(cyano)methyl]-	2.43	10-Oxatricyclo[6.4.0.09,12]dodecane-9-carboxylic acid, 11-oxo-12-phenyl-, methyl ester	1.18
8.	l-(+)-Ascorbic acid 2,6-dihexadecanoate	12.51	Octadecanoic acid, 2,3-bis[(1-oxotetradecyl)oxy]propyl ester	0.29	1-Octadecanol, methyl ether	6.00	Phytol	21.80	Phytol	10.18
9.	1,6-Heptadiene, 2-methyl-6-phenyl	2.89	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	7.13	1-(.beta.-d-Ribofuranosyl)-4-difluormethoxy-uracil	1.60	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	12.23	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	20.15
10.	Phytol	12.00	Squalene	3.60		100.00	2-Butene, 3-chloro-1-phenyl-, (Z)-	3.68	Octadecanoic acid, 2-(2-hydroxyethoxy) ethyl ester	3.09
11.	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	12.43	Cyclohexane, (1-butylhexadecyl)-	4.17			Octadecanoic acid	5.31	Phenol,2,2'methylenebis[6-(1,1-dimethylethyl)-4-methyl	1.47
12.	Octadecanoic acid, 2-(2-hydroxyethoxy)ethyl ester	3.38	Octadecane, 1,1'-[1,3-propanediylbis(oxy)]bis-	10.6			Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	11.70	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	31.7
13.	Hexadecanoic acid,2-hydroxy-1(hydroxymethyl)ethyl ester	23.15	1-Bromo-11-iodoundecane	8.99				100.00	Bis(2-ethylhexyl) phthalate	0.53
14.	Butyl 9,12,15-octadecatrienoate	5.06	E,E,Z-1,3,12-Nonadecatriene-5,14-diol	9.28					Octadecanoic acid, 2,3-dihydroxypropyl ester	15.28

15.	Octadecanoic acid, 2,3dihydroxypropyl ester	19.60	Acetic acid, chloro-, octadecyl ester	13.92					Octanoic acid, 3-oxo-4-(2-propenyl)-, methyl ester	1.27
16.	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)-	1.90	alpha.-L-Sorbofuranose, cyclic 2,3:4,6-bis(ethylboronate) 1-acetate	7.28						100.00
17.		100.00	i-Propyl 9-tetradecenoate	3.97						
				100						

N: number of peaks; NPs.: Nanoparticles; BPs.: Bulk particles; Ctr.: untreated control; Com.: Compounds.



(A)



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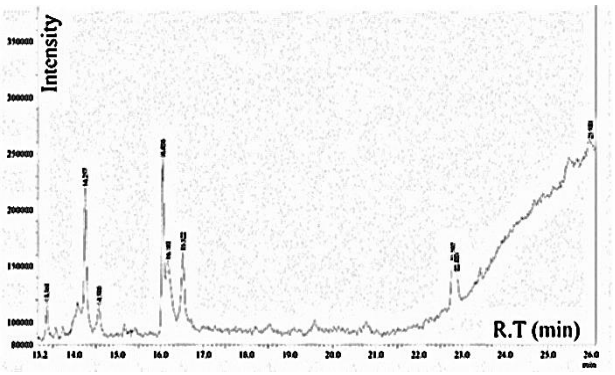

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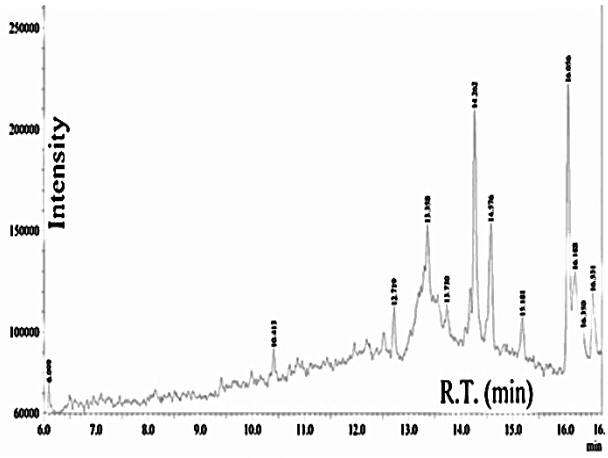

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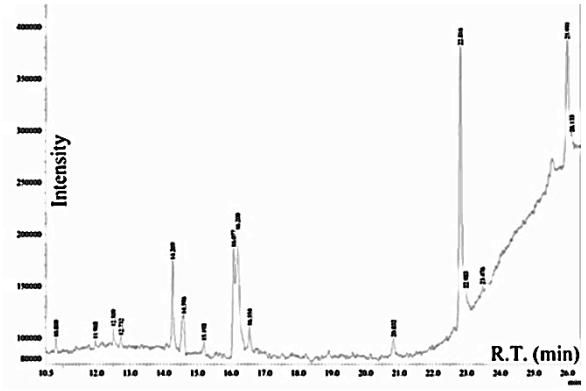


(E)

Figure 1: Chromatogram of T. aestivum treated with different concentrations of nano (NPs) and bulk (BPs) particles by GC-MS. (A): 10 mg/ml of (NPs), (B): 0.01 mg/ml of (NPs), (C): 10 mg/ml of (BPs), (D): 0.01 mg/ml of (BPs), (E): control.

SEM analysis

Figure 2 showed stander titanium dioxide nanoparticles. Their size was 50 nm. SEM of bulk titanium dioxide particles showed that their size arranges between (300-800 nm).

The aerial parts of *T. aestivum* were examined by SEM in order to detect the residuum of nanoparticles in plant treated with it and if there are any nano-size particles of titanium in plant treated with bulk particles. At a treatment of 0.01 mg/ ml concentration of NPs, the result found many particles. Their size arranges between (300-450 nm) (Figure 3, A). In plant treated with the higher concentration, (10 mg/ml), of nanoparticles, the result indicated that there were more than 30 particles, their size arranges between (450-850 nm), (Figure 3, B). Plants exposed to different concentration of bulk particles (10 and 0.01) mg/ml, showed very few particles (less than five) in SEM analysis. Their size was in micro- size (>1 µm), (Figure 4 A and B). Titanium dioxide (TiO_2) NPs are widely used as an important kind of biomaterials because they have large surface area which enhanced chemical reactivity and very easy penetration into cells **[**2]. Most of reports found positive effects of nanoparticles on growth of plants but others found negative effect [22]. This study focus on the effect of TiO_2 nanoparticles (NPs) compared with bulk particles (BPs) on seed germination and some growth parameters of latefyha's cultivar of wheat *in vitro* and *in vivo* and if there are any effect on chemical compositions of plant or if they remain in the plant's residue.

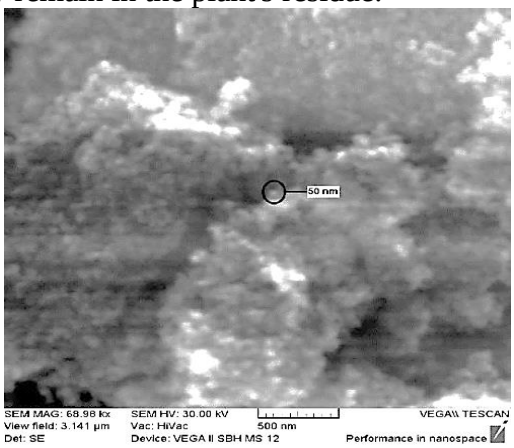
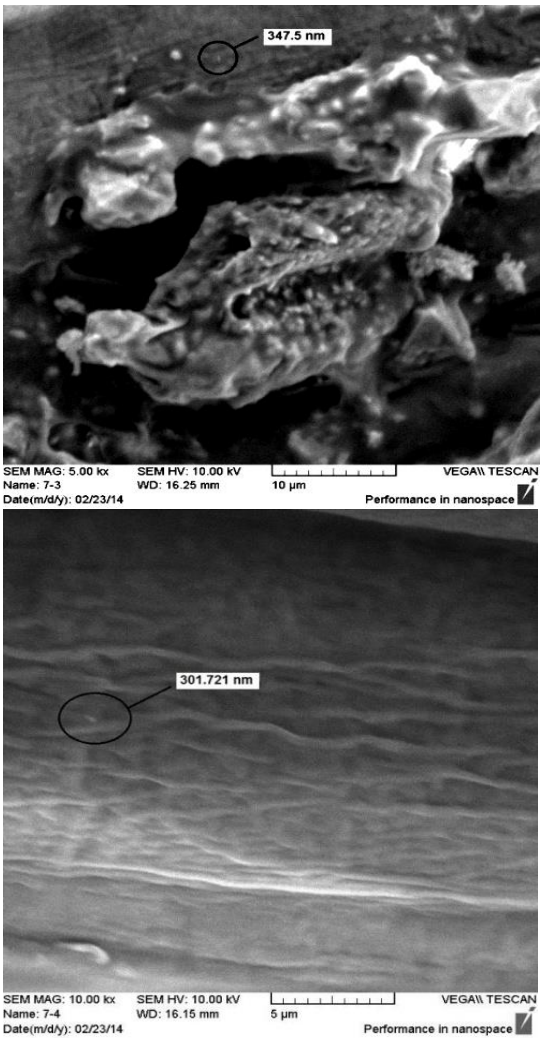
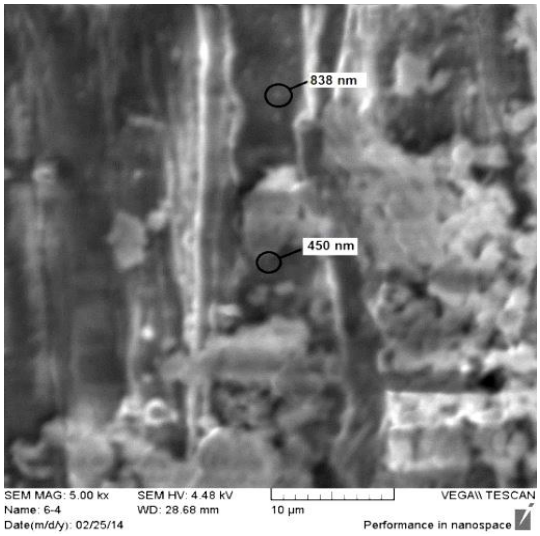


Figure 2: SEM image of standard TiO_2 nanoparticles. particle size 50 nm.

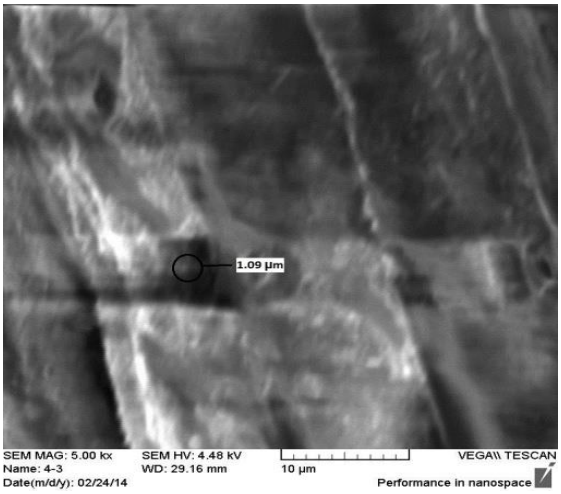


(A)

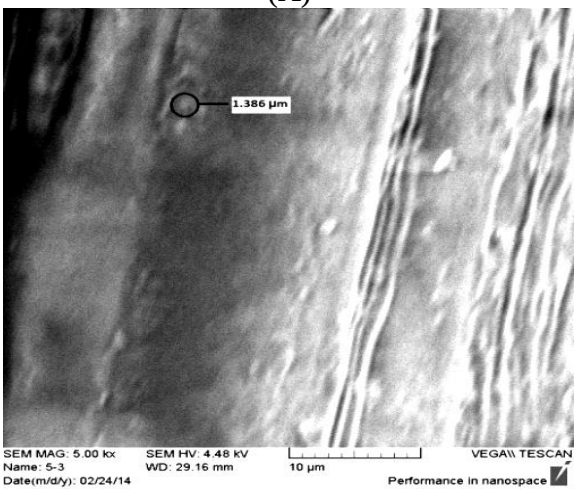


(B)

Figure 3: SEM image of plant tissues treating with nanoparticles: (A): 0.01 mg/ml, Particle size arrange between (300-450 nm). (B): 10 mg/ml, there were more than 30 particles, their size arranges between (450-850 nm).



(A)



(B)

Figure 4: SEM image of plant tissues treated with bulk particles. There were very few particles (<5), Particle size (>1 µm). (A): 0.01 mg/ml. (B): 10 mg/ml.

In *in vitro* of this study, most concentrations of NPs and BPs did not effect on: germination percentage, mean germination time, mean daily germination and promoter indicator, number of leaves, length and number of root, root tips viability but they reduced germination rate and germination value besides they induced shoot length and biomass with some differences between NPs and BPs. These, un effect, is probably because of selective permeability of seed coats that does not allow this material to pass through it [23]. Nanoparticles can explain their actions depending on both their chemical compound and on the size and/or shape of the particles. While in the case of induction it is most possible that nanoparticles could penetrate into the seed coat, depending in concentrations, and exert a beneficial effect on the process of seed germination, helped the water absorption by the seeds [24], increase nitrate reductase enzyme, increase seed abilities of absorbing and utilizing water and fertilizer, promote seed antioxidant system [25], reduced antioxidant stress by reducing H_2O_2, superoxide radicals and malonyldialdehyde content and increasing some enzymes such as superoxide dismutase, ascorbate peroxidase, guaiacol peroxidase, and catalase activities [26], but bulk particles, having a larger size, the physiological effects were related to the size of particles [24] thus they cannot easily enter, consequently may accumulate in the pores of a seed coat and prevent transition of water and oxygen [12].

Similar results of current inductions effect of germinations percentage and reduction in germination rat had seen in wheat, such as Feizi and others in (2012), indicated that the treatments of bulk and nanosized TiO_2 (2 ppm) had no effect on seed germination percentage [7]. Mahmoodzadeh and others (2013) approved that 1200 ppm of NPs of titanium could promote the seed germination and seedling growth of wheat in comparison to control plants and it could penetrate root only below a threshold of diameter, which was 36

nm [27]. The study of Azimi and others in (2013) showed that employment of TiO_2 nanoparticle with 5 ppm enhanced wheatgrass seed germination [28]. Exposure of wheatgrass seeds to high concentrations of nano TiO2 particles (80 ppm) led to diminished germination rate, [28]. TiO_2 nanoparticles can increased the germination of other plant like *Plantago psyllium* L., *S. officinalis (*5 mg L-1), [12] [29].

The present results of increasing shoot length not agree with the study of Feizi and others (2013), it was not affected by all concentrations of bulk particles and nanoparticles. It is probable that increasing the concentration of bulk-TiO_2 induced aggregation of particles and led to clog of root pores that interrupted water uptake by seeds [12].

Current results showed no significant effect of all concentration of nano and bulk particles on root length**.** Feizi and others (2012) showed that treatments on root length of wheat were not significant, but they had a significant effect on shoot and seedling lengths. Shoot and seedling lengths at 2 and10 ppm concentrations of nanosized TiO_2 (size 21 nm) were higher than those of the untreated control (8% and 7.3%, respectively) and bulk TiO_2 (10.2% and 7%, respectively) treatments. Increasing concentrations of nanosized TiO_2 after 10 ppm decreased shoot and seedling lengths [7].

The study of [30] found there were decrease in all plant's parameters at most concentrations of TiO2 biological synthetic (produced by *C. longa*) compare with industrial synthetic nanoparticles in Al-Rasheed variety of wheat, while there were inductions in some plant's parameters by biosynthetic nanoparticles compared with industrial synthetic in Tamuze-2 variety.

In *in vivo* study, the decreasing in germination percentage treated with most concentrations of bulk particles nanoparticles compared with control was not comfortable with [27]. The presence of NPs on the root surface could alter the surface chemistry of the root such that it affects how the roots interact with their environment. Plant development is negatively affected because NPs clog the root openings and both hydraulic and nutrient uptake in roots is inhibited [31]. X-ray fluorescence microspectroscopy showed that nano-TiO could attach to the *Vicia faba* root surface in 48 h, thus resulting in the inhibition of plant growth [32].

Such promotor effect of nanoscale SiO_2 and TiO_2 on germination was reported in soya bean [25] in which authors noticed increased nitrate reductase enzyme activity and enhanced antioxidant system. Many germination-related events (gene transcription and translation, respiration and energy metabolism, early reserve mobilization and DNA repair) could also take place during seed treatment [33], although often restricted due to reduced water supply compared to regular germination [34] [35]. It is possible that the seeds promote by nano TiO_2 and then cultivate in soil in field. In this condition, it is possible physico-chemical properties of soil modify adverse effects on plant growth and weights [12].

The increasing in shoot length, in present study, compatible with the reports on radish, rape, corn, lettuce and cucumber by Lin and Xing (2007) [36]. A results of (Mahmoodzadeh *et al*., 2013) revealed the promotor effect of nano scale TiO2 (20 nm) at optimum concentrations and inhibitory effect at high concentrations on root and shoot growth of wheat (*T.aestivum*), Nano scale TiO_2 at 100 mgL -1 decreased shoot and root length [37].

In current results, bulk particles did not effect in contain of all pigments, it seems that bulk TiO_2 could not penetrate inside the seeds of wheat in the same way which Zheng *et al*., 2005 reported, this is because their large size of crystals that might difficult entire cells or even chloroplast [24].

In the other hand, the decreasing in chlorophyll A and carotenoids at lower concentrations of nanoparticles in current results may be due to the fact that there is an oxidative [38]. This is depending on their small-sized TiO_2 NPs (around 20nm) which was able to penetrate the cell wall [39]. The size of seeds could be

render more sensitivity to NP exposure [40], this is because large seed species has a lower surface to volume ratio than a small seeded species and later might causes this decreasing in pigments. The toxicity of NPs in plants may base on plant-NP physical interactions, particle size and specific surface area, physio chemical properties of NPs, concentration of NPs, plant species, plant age or life cycle stage, growth media, NP stability, and diluting agents [41]. Ghosh and others in (2010) attributed such inhibition to DNA injury induced by TiO_2 NPs in *Nicotina tabacum* [42]. This evidence supports that some engineered NPs could exert physical or chemical toxicity on plants, depending on their chemical composition, size, surface energy and plant species [43].

Most studies found a positive effect of nanoparticles on chlorophyll content such as: wheat [27], spinach traits [44], *Zea mays* L. [45]. Study by (Samadi *et al*., 2014) on *Mentha piperita* which showed that the TiO_2 concentration in 200mg L-1 and 100 mg L-1 concentration of NP-TiO_2 had significantly increased the amount of chlorophyll a and chlorophyll b [46]. Other studies indicated that NP-TiO_2 and TiO_2 can raise the photosynthesis rate, chlorophyll formation and nitrogen metabolism at an optimum concentration. Samadi and others (2014) showed that TiO_2 and NP-TiO_2 concentrations in 200 mg L-1 and 100 mg L-1 had a significant stimulant effect on the amount of carotenoids of *Mentha Piperita* in comparison with the control group [47].

This is the first report to determine the effect of TiO_2 nanoparticles (NPs) and bulk titanium dioxide on qualitative and quantitative analysis of chemical components of the aerial parts of *T. aestivum* by GC-MS. The results found that there were increasing in total number of chemical compounds that identified in leaves of wheat plants treated with nanoparticles compared with control while the total numbers of compounds were decreased using bulk particles.

This increasing is may be a result of increasing growth of wheat (which a proved by current results) to increase the permeability of cell membrane lads to increase uptakes of minerals or important nutrition's by roots, or may be due to stimulate the activity of several enzymes and influence the uptake of nitrogen (Which is very important for growth and effect on chemical compositions later) this was proved and observed in spinach growth [8].

The level or concentration of TiO_2 NPs in the body system may depends on the rate (or kinetic) of absorption, distribution, metabolism, and excretion of TiO_2 NPs because 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 molecules [46]. So, properties of nanoparticles that might increase the chemical compositions: particle size, surface area and charge, shape/structure, solubility, and surface coatings. Small size of NPs give rise to a high surface area per unit mass, and this surface area is often correlated with higher biological reactivity [48]. The reductions in chemical compositions by bulk particles (in current study) is may due to their small surface area and large crystals than the nanoparticles, in addition, surface atom is more stable [49].

Conclusions

Despite TiO2 nanoparticles was found in wheat leaves residue, but it showed either no effects on growth or increased them with reduction in very few parameters in vitro and in vivo. In *in vitro* of this study, most concentrations of NPs and BPs did not effect on: germination percentage, mean germination time, mean daily germination and promoter indicator, number of leaves, length and number of root and root tips viability but they reduced germination rate and germination value besides they induced shoot length and biomass.

In *in vivo* study, the fallowing parameters induced by most concentrations of NPs: plant leaves area, leaf area index, length, of viability roots, plant number and height and total of plant length and biomass while it did not effect on: mean daily germination, vigor index I and vigor index II, chlorophyll B, leaf area relative, in this regard, it reduced germination percentage, chlorophyll A and carotene. There were some differences between the effect of

NPs and those of BPs. There were increased in total number of chemical compounds that identified in leaves of wheat plants treated with nanoparticles compared with control while the total number of compounds was decreased using bulk particles. Studying the genotoxic and environmental fate of nanoparticles on cereals may give more information about nano risk.

Acknowledgments

The authors wish to thank Dr. Raghad A. Ahmed and Asmaa A. Maryoosh, Department of Chemistry, College of Science/ Mustainsiriyah University for offering facilities to carry out this study.

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