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Original Article

Phytotoxicity of Chitosan and SiO_2 Nanoparticles to Seed Germination of Wheat (*Triticum aestivum L.*) and Barley (*Hordeum vulgare* L.) Plants

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Abstract

Plants such as wheat and barley that are strategically important crops need to be considered to develop a comprehensive toxicity profile for nanoparticles (NPs). The present study was aimed to investigate the effects of chitosan and SiO_2 NPs on wheat and barley plants. Two factorial experiments (seeds priming and direct exposure) were performed based on a completely randomized design in four replications. Results showed that the seeds priming with the NPs had not significant effect on germination parameters such as Germination Percentage (GP), Germination Rate (GR), Germination Value (GV), Mean Germination Time (MGT), Pick Value (PV) and Mean Daily Germination (MDG). In contrast, exposure of the seeds to the NPs had significant effects on these parameters. In both experiments, treatments had significant effects on shoot, seedling, root length, fresh and dry weight, as well as vigor indexes as compared to the control. In most traits, the best concentration of NPs was 30 ppm, whereas applications of the NPs with 90 ppm displayed adverse effects on majority of the studied traits. According to these results, selectivity in applications of NPs with suitable concentration and method is essential for different plant species.

Keywords: chitosan, germination rate, seedling, toxicity, vigor index

Introduction

Nowadays, various researchers have studied the effects of nanoparticles (NPs) on plant germination and growth with the objective to promote its use for agricultural applications (Khot *et al.*, 2012). NPs have a high surface to volume ratio that increases their reactivity and possible biochemical activity (Dubchak *et al.*, 2010). The interaction mechanisms at the molecular level between NPs and biological systems are largely unknown (Barrena *et al.*, 2009). Therefore, it is important to understand the course of plant germination and growth in relation to NPs. The reported data from various studies suggested that effect of NPs on seed germination was concentration dependent (Siddiqui et al., 2015). For example, application of 15 mg kg^{-1} of Fe/SiO_2 NPs increased the shoot length of barley and maize; however, the concentration of 25 mg kg-1 had a negative effect on shoot length in barley (Najafi Disfani *et al.*, 2016). Also ZnO NPs was reported to be one of the most toxic NPs that could terminate root growth of plants (cucumber, ryegrass, radish, corn, lettuce, and rape) (Lin and Xing, 2007).

On the other hand, SiO_2 NPs are frequently used nanomaterials in a variety of technological applications such as packaging, industrial manufacturing, composite and ceramics materials, adsorption, drug delivery, bio sensing and catalytic applications (Martinez *et al.*, 2010; Ghorbani *et al.*, 2015). The great interest shown to SiO_2 NPs is attributed to their low toxicity, high chemical and physical stability, and straightforward surface chemistry, which allows them to be combined or functionalized with a variety of functional species or molecules (Ghorbani *et al.*, 2015). Silicon (Si) is the second most abundant element in soil, which accounts for approximately 32% of the total weight of soil (Abdel Latef and Tran, 2016). It plays an important role as a physic mechanical barrier, and is deposited on the walls of epidermis and vascular tissues of the stem, leaf sheath and hull in most plants especially monocots (Ma and Yamaji, 2006; Parven and Ashraf, 2010).

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Study on the influence of metal NPs (Si, Pd, Au, Cu) on germination of lettuce seeds indicated that NPs (Pd, Au at low concentrations; Si, Cu at higher concentrations, and combination of Au and Cu) had a positive influence on seed germination, shoot to root ratio and growth of seedling (Shah and Belozerova, 2009). Lu *et al*. (2002) reported that application of SiO_2 and TiO_2 NPs mixture on soybean (Glycine max) could increase nitrate reductase, enhance water and fertilizer absorbing capacity, stimulate antioxidant defense system and also hasten seed germination and seedling growth. Also, Bao-shan *et al*. (2004) immersed the roots of changbai larch (Larix olgensis) seedlings in SiO_2 NPs for 6 hours. Their results clearly showed positive effects of SiO_2 NPs on growth and quality of the seedlings.

Chitosan is a linear β-(1, 4)-glucosamine polymer produced by de-acetylation of chitin (Radman *et al*., 2003). It has great potentials for being used in a wide range of industries such as pharmacology, medicine and agriculture (Bautista-Banos *et al*., 2004). Chitosan is known for its unique properties like biodegradability, antimicrobial activity and nontoxicity (Saharan et al., 2015). Such unique properties of the chitosan biopolymer can be further enhanced by using it in the form of NPs, as in this form it can instill different biological activities with altered physicochemical properties like surface area, size, cationic nature etc. Chitosan has promoted growth of various plants such as soybean sprouts (Lee *et al*., 2005) and sweet basil (Kim *et al*., 2005). It was also reported to increase wheat seeds resistance to certain diseases and improve their quality and ability to germinate (Reddy *et al*., 1999). Anusuya and Banu (2016) stated that Ag-Chitosan NPs significantly promoted growth and biochemical variation capabilities of chickpea (*Cicer arietinum* L.). Similarly, rice seed coated with chitosan could accelerate their germination and improve their tolerance to stress conditions (Ruan and Xue, 2002).

Therefore, attention was given to appropriate experimental design to study the effects of chitosan and SiO_2 NPs with two consumption methods on germination of wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.).

Materials and Methods

Two factorial experiments were carried out to assess the effects of SiO_2 and chitosan NPs on wheat and barley germinations in a completely randomized design with four replications. The experimental factors included the NPs with concentrations 0 (control, no NPs), 30, 60 and 90 ppm as well as plant species (wheat and barley). The experiment was performed in a germinator with an average temperature of 21 ± 1 °C for 16/8 hours (day/night) in laboratory of the College of Agriculture, Tarbiat Modares University, Tehran, Iran (May, 2016).

Description of materials

NPs

Two types of NPs were used in the experiment. Average primary particles sizes were > 80 nm. The size of SiO_2 and chitosan NPs (Fig. 1) were determined through Field Emission-Scanning Electron Microscope (FE-SEM).

Plant materials

Seeds of wheat (*Triticum aestivum* cv. 'Pishtaz') and barley (*Hordeum vulgare* cv. 'Reyhan') were used as plant materials for the present investigation. The seeds were purchased from the Plant, Breeding and Seed Institute of Karaj.

SiO_2 NPs suspension

The SiO2 NPs (5 g) were suspended directly in distilled water (1,000 mL) and dispersed by ultrasonic vibration (100 W, 40 KHz) for 30 min. Small magnetic bars were placed in the suspensions for stirring before use, to avoid aggregation of the particles (Adhikari *et al*., 2013). The suspension of SiO_2 NPs was diluted with deionized water for preparing different doses of SiO2 NPs suspensions (0, 30, 60 and 90 ppm).

Chitosan NPs suspension

The chitosan NPs (5 g) was solubilized in deionized water (1,000 mL) with acetic acid (10 mL) under constant stirring (80-90 °C) for 2 h until complete dissolution of the chitosan NPs. Then, the suspension was alkalized to pH 6.5

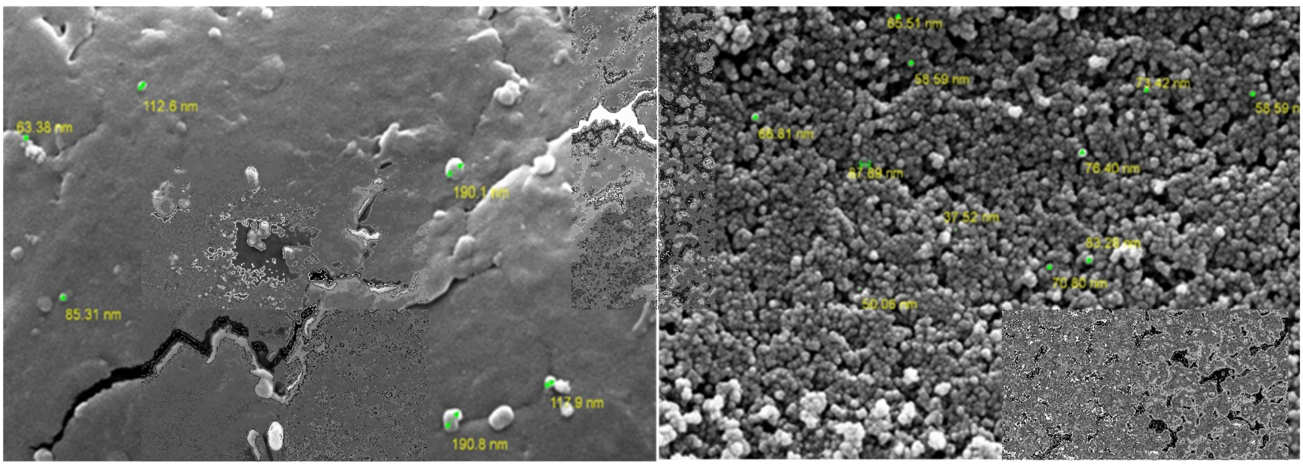

Fig. 1. Images of SiO_2 NPs (right) and chitosan NPs (left) by Field Emission-Scanning Electron Microscope (FE-SEM)

with 1 N NaOH (Li *et al*., 2008). Further, the suspension of chitosan NPs was diluted with deionized water for preparing different doses of chitosan NPs suspensions (0, 30, 60 and 90 ppm).

Seeds treatment

Plants culture system and test procedures for two experiments have been adapted from U.S. EPA and ISTA guidelines (USEPA, 1996; ISTA, 2009). In the first experiment, the seeds of wheat and barley were immersed in a 5% sodium hypochlorite solution for 10 min to ensure surface sterility. Then they were soaked in distilled water, SiO_2 and chitosan NPs suspension for about 12 hours at 25 °C, after being rinsed three times with distilled water. Treated wheat and barley seeds were shade-dried for 1 hour. Then the 25 seeds were placed in a Petri dish (100 mm × 15 mm) with one piece of sterilized filter paper (Whatman No. 1) and 5 mL of distilled water was added. Watering was done for all Petri dish.

For the second experiment, the seeds of wheat and barley were immersed in a 5% sodium hypochlorite solution for 10 min to ensure surface sterility. Then seeds after being rinsed three times with distilled water were shade-dried for 1 hour. The 25 seeds were placed in a Petri dish (100 mm x 15 mm) with one piece of sterilized filter paper (Whatman No. 1) and 5 mL of distilled water, SiO_2 and chitosan NPs suspension were added to them. Finally, petri dishes for two experiments were covered and placed in a germinator at 21 ± 1 °C for 16/8 hours (day/night) for fourteen days.

Measurements

Number of germinated seeds was noted daily for 7 days. Seeds were considered germinated when the radicle showed at least 2 mm in length (ISTA, 2009). On the 14th day, from each Petri dish, 10 seedlings were randomly sampled and morphological parameters including root, shoot and seedling length were measured. Subsequently, they were oven-dried at 70 °C overnight to estimate their dry weight with a sensitive scale (Gubbins *et al*., 2011). Also, the germination parameters were used according to the following formula:

MGT (mean germination time) was calculated based on equation 1 (Matthews and Khajeh-Hosseini, 2007):

$$MGT = (\Sigma(F \times X))/\Sigma F$$

Eq. 1

Where F is the number of seeds newly germinated at the time of X, and X is the number of days from sowing.

GR was determined based on equation 2 (Maguire, 1982):

GR = (a/1) + (b-a/2) +(c-b/3) +.....+ (n-n-1/N)

Eq. 2

Where a, b, c… n are numbers of germinated seeds after 1, 2, 3… N days from the start of imbibition.

Seedling vigor index were computed based on equations 3 and 4 (Vashisth and Nagarajan, 2010):

Vigor index I = Germination% × Seedling length (cm)

Eq. 3

Vigor index II = Germination % × Seedling weight (g)

Eq. 4

Evaluations of MDG, PV and GV were calculated by the following equation 5 (Hartmann *et al*., 1990):

MDG = Germination % / total experiment days

Eq. 5

PV = Maximum germinated seed number at one day / day number

GV= PV × MDG

GP was calculated by the following equation (Hosseini *et al*., 2013):

$$GP=\frac{\Sigma n}{N}\times 100$$

Eq. 6

Where Σn is the number of seeds germinated until the last day of experiments, and N is the total number of seeds.

Statistical analysis

Data were statistically analyzed using two way analysis of variance (SAS Institute, 9.1.3). The significance of differences among treatment means were compared by Duncan's multiple range tests at $p < 0.05$.

Results

Characterization of chitosan and SiO_2 NPs suspension

Specific surface area of SiO_2 NPs was > 80 m2 g-1, average primary particle size was > 80 nm and purity was > 99.5%. The pH of SiO_2 NPs suspension was 6.8. Also, specific surface area of chitosan NPs was > 80 m2 g-1, average primary particle size was > 80 nm and purity was >99%. The pH of chitosan NPs suspension was 6.8.

First experiment

Germination parameters

Analysis of variance showed that wheat and barley seeds priming with chitosan and SiO_2 NPs had not significant effect on GP, GR, GV, PV, MDG and MGT of the seeds (Table 1). Different concentration of SiO_2 or chitosan NPs led to 97-100% seeds germination of wheat and barley. Also, analysis of variance showed that main effects and two-way interaction between plant species and NPs had significant effect on vigor index I (Table 1). Pre-treatment of the barley seeds with 60 ppm chitosan NPs demonstrated the lowest value of vigor index I (1,134.24 cm), while the highest this value (1,642.33 cm) was encountered in 30 ppm chitosan NPs for wheat seeds (Table 2). Wheat seeds priming with 90 ppm of both NPs adversely affected vigor index I as

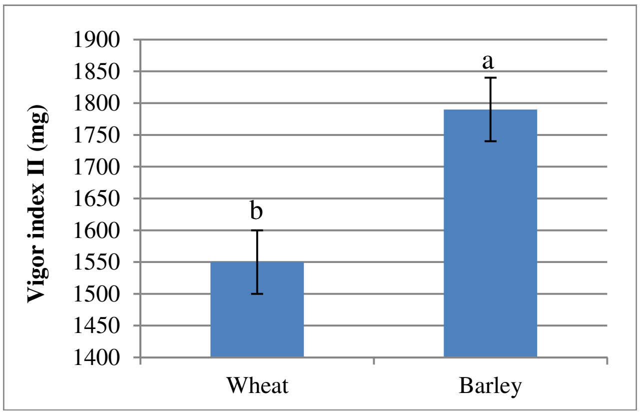

Fig. 2. Effect of plant species on vigor index II. Means by the uncommon letter are significantly different according to Duncan tests ($p < 0.05$)

Table 1. Analysis of variance for some related germination parameters of barley and wheat

		Mean square							
Source	df	GP	GR	GV	PV	MDG	MGT	VI (I)	VI (II)
NPs (A)	6	7.85 ^{ns}	0.000018 ^{ns}	96.73 ^{ns}	0.1344 ^{ns}	0.49 ^{ns}	0.007237 ^{ns}	78,418.68 **	80,921.62 ^{ns}
Plant species (B)	1	6.62 ^{ns}	0.000002 ^{ns}	3.02 ^{ns}	0.0003 ^{ns}	0.41 ^{ns}	0.000002 ^{ns}	99,884.86 **	81,5760.48 **
A×B	6	2.91 ^{ns}	0.000016 ^{ns}	95.14 ^{ns}	0.1443 ^{ns}	0.18 ^{ns}	0.006935 ^{ns}	84,482.80 **	77,206.57 ^{ns}
Error	26	9.02	0.00001	88.81	0.1179	0.53	0.007878	3,569.99	41,268.95
CV (%)	-	3.04	5.82	11.58	10.40	4.86	8.07	4.29	12.35

VI show vigor index. ns Non- significant, ** and * significant at the 1% , 5% probability levels, respectively

Table 2. Effects of SiO_2 and chitosan NPs on wheat and barley growth parameters

Traits / Treatments	VI (I) (cm)	L (cm)			F.W. (mg)			D.W. (mg)
		Seedling	Shoot	Root	Seedling	Shoot	Root	Shoot
Control/Wheat	1,544.33 ^{ab}	15.44 ^{bc}	8.26 ^{d}	7.18 ^{b}	141.79 ^{bcd}	57.50 ^{c}	84.28 ^{a}	8.56 ^{ef}
Control/Barley	1,277.91 ^{de}	12.90 ^{ef}	7.89 ^{de}	5.01 ^{e}	159.52 ^{abc}	94.64 ^{a}	64.88 ^{abcd}	11.68 ^{ab}
30 SiO_2 NPs/Wheat	1,452.27 ^{bc}	14.86 ^{d}	8.78 ^{c}	6.07 ^{d}	123.33 ^{de}	57.14 ^{c}	66.19 ^{abc}	8.64 ^{ef}
60 SiO_2 NPs/Wheat	1,322.24 ^{d}	13.52 ^{de}	7.35 ^{gf}	6.16 ^{d}	127.14 ^{cde}	57.38 ^{c}	69.76 ^{ab}	8.71 ^{ef}
90 SiO_2 NPs/Wheat	1,196.27 ^{ef}	12.52 ^{fg}	7.34 ^{fg}	5.18 ^{e}	97.14 ^{e}	47.38 ^{c}	49.76 ^{bcd}	6.42 ^{g}
30 SiO_2 NPs/Barley	1,540.60 ^{ab}	15.75 ^{b}	9.13 ^{bc}	6.61 ^{c}	176.19 ^{a}	100.71 ^{a}	75.47 ^{a}	11.35 ^{ab}
60 SiO_2 NPs/Barley	1,357.80 ^{cd}	13.87 ^{d}	7.96 ^{de}	5.91 ^{d}	132.62 ^{bcd}	89.28 ^{a}	43.33 ^{d}	12.52 ^{a}
90 SiO_2 NPs/Barley	1,302.67 ^{de}	13.02 ^{ef}	7.73 ^{ef}	5.29 ^{e}	142.38 ^{bcd}	74.52 ^{b}	67.85 ^{abc}	10.78 ^{bc}
30 chitosan NPs/Wheat	1,642.33 ^{a}	16.42 ^{a}	8.22 ^{d}	8.20 ^{a}	132.38 ^{bcd}	59.76 ^{bc}	78.57 ^{a}	8.92 ^{def}
60 chitosan NPs/Wheat	1,605.29 ^{a}	16.41 ^{a}	9.45 ^{ab}	6.95 ^{bc}	122.86 ^{de}	53.80 ^{c}	63.09 ^{abcd}	8.11 ^{f}
90 chitosan NPs/Wheat	1,302.89 ^{de}	13.32 ^{de}	6.52 ^{h}	6.79 ^{bc}	96.19 ^{e}	45.47 ^{c}	50.71 ^{bcd}	9.59 ^{de}
30 chitosan NPs/Barley	1,561.33 ^{ab}	15.61 ^{b}	9.77 ^{a}	5.83 ^{d}	184.05 ^{a}	102.14 ^{a}	81.90 ^{a}	11.95 ^{ab}
60 chitosan NPs/Barley	1,134.24 ^{f}	11.60 ^{h}	6.24 ^{h}	5.36 ^{e}	161.43 ^{ab}	96.19 ^{a}	65.23 ^{abcd}	10.83 ^{bc}
90 chitosan NPs/Barley	1,208.33 ^{ef}	12.08 ^{gh}	7.00 ^{g}	5.08 ^{e}	119.29 ^{de}	73.33 ^{b}	45.95 ^{cd}	9.90 ^{cd}

VI, L, F.W, D.W show vigor index, length, fresh weight, dry weight, respectively. Means by the uncommon letter in each row and column are significantly different according to Duncan tests ($p<0.05$)

compared to the control (NPs = 0 ppm), but had not significant effect on barley seeds. Using of 30 ppm of both NPs in barley seeds significantly increased vigor index I as compared to the control. Also, analysis of variance showed that plant species significantly affected the seedling vigor index II (Table 1), among which barley showed the maximum value (Fig. 2).

Seedling, shoot and root lengths

Results in Table 3 showed that shoot, root and seedling elongation of wheat and barley were significantly affected by main effects, and tow-way interaction. Wheat seeds priming with 30 ppm chitosan NPs led to relatively highe increase in seedling and root length (Table 2). In contrast, wheat seeds priming with SiO_2 NPs significantly decreased seedling and root length. The highest shoot length (9.77 cm) was observed at barley seeds priming with 30 ppm chitosan NPs. The lowest shoot and seedling length was obtained in 60 ppm chitosan NPs in barley seeds. Wheat seeds priming with 30 ppm chitosan NPs was led to the highest root length (8.20 cm).

Seedling, shoot and root weight

Seedling, shoot and root fresh weight, as well as shoot dry weight, were significantly affected by two-way interaction between plant species and NPs (Table 3). Also, plant species had a significant effect on seedling dry weight.

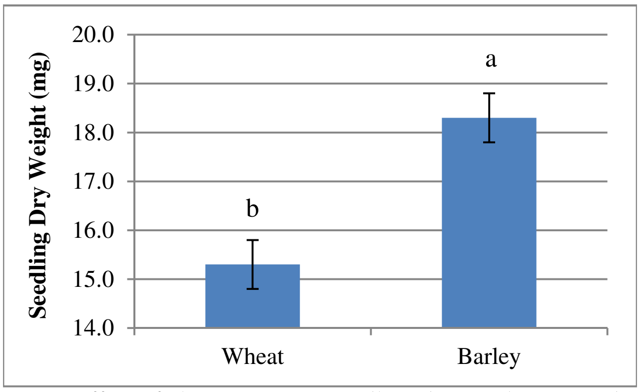

Fig. 3. Effect of plant species on seedling dry weight. Means by the uncommon letter are significantly different according to Duncan tests ($p < 0.05$)

Table 3. Analysis of variance for some growth parameters of barley and wheat

		Mean square								
Source	df	L			F.W.			D.W.		
		Seedling	Shoot	Root	Seedling	Shoot	Root	Seedling	Shoot	Root
NPs (A)	6	6.76 **	2.806 **	1.42 **	716.76 ^{ns}	213.07 *	285.78 ^{ns}	7.90 ^{ns}	3.21 **	1.90 ^{ns}
Plant species (B)	1	12.50 **	0.008 ^{ns}	11.87 **	11,797.69 **	13,649.25 **	67.40 ^{ns}	75.68 **	94.94 **	1.08 ^{ns}
A×B	6	8.38 **	4.448 **	2.29 **	1,815.61 **	298.46 **	830.69 **	8.38 ^{ns}	4.38 **	3.15 ^{ns}
Error	26	0.12	0.055	0.06	294.98	73.08	140.39	3.47	0.42	2.29
CV (%)	-	2.48	5.96	4.19	12.39	11.08	17.94	11.18	6.66	19.68

L, F.W, D.W show vigor index, length, fresh weight, dry weight, respectively. ns Non- significant, ** and * significant at the 1% , 5% probability levels, respectively

Treatments had not significant effect on root dry weight of barley and wheat plants. Use of both NPs in 90 ppm greatly decreased shoot, root and seedling fresh weight of both plants as compared to the control (Table 2). Whereas, use of both NPs in low concentration had not significant effect on fresh weight and shoot dry weight as compared to the control. The lowest shoot dry weight was observed in wheat seeds priming with 90 ppm SiO_2 NPs. Also, seedling dry weight in barley was more than wheat (Fig. 3).

Second experiment

Germination parameters

Analysis of variance showed that the main effects and two-way interaction between plant species, and NPs significantly affected all measured traits (Table 4). Results in Table 5 showed that usage 90 ppm SiO_2 NPs in barley seeds significantly decreased GP, GR, MDG and seed vigor index II as compared to the control. Also, exposure of barley seeds to relatively high concentration of either SiO_2 or chitosan NPs significantly led to diminished GV, PV and vigor index I. In contrast, application of chitosan NPs with low concentration in wheat seeds led to the height GV (46.96), PV (1.93) and vigor index I (1,877.87 cm). In general, low concentration of both NPs had not significant effects on most studied traits. On the other hand, as a general rule, lower MGT represents a faster germination speed. Exposure of barley seeds to 90 ppm SiO_2 NPs demonstrated the highest MGT (2.50 day).

Seedling, shoot and root length

Analysis of variance showed that two-way interaction between plant species and NPs was significant for seedling, shoot and root length (Table 6). Barley seeds exposed to both NPs exhibited the sharp decline in length compared to the control (Table 7). The lowest seedling, shoot and root length was observed when barley seeds treated with 90 ppm chitosan NPs. Low concentration of both NPs led to increasing wheat seedling length.

Seedling, shoot and root weight

Analysis of variance showed that two-way interaction between plant species and NPs was significant for seedling, shoot and root weight of barley and wheat plants (Table 6). Using both NPs in wheat seeds had not significant effect on seedling, shoot and root fresh weight as well as seedling and root dry weight (Table 7). Whereas high concentration of both NPs significantly decreased dry and fresh weight of barley seeds as compared to the control. The lowest seedling,

Table 4. Analysis of variance for some germination parameters of barley and wheat

		Mean square							
Source	df	GP	GR	GV	PV	MDG	MGT	VI (I)	VI (II)
NPs (A)	6	83.07 *	17.85 **	575.46 **	0.93 **	12.52 *	0.13 **	509,252.43 **	628,655.85 **
Plant species (B)	1	752.08**	45.84 **	802.63**	10.71 **	58.52 **	0.99 **	2,511,601.86 **	164,062.50 ^{ns}
A×B	6	12.30 **	5.28 **	450.58 **	0.78 **	2.07 **	0.08 *	231,653.44 **	461,021.38 **
Error	30	27.66	1.24	4.34	0.02	2.57	0.02	9,137.54	70,916.16
CV (%)	-	5.66	8.63	9.82	16.41	6.94	7.76	7.08	18.29

VI show vigor index. *, ** and ns: significant at 0.05, 0.01 probability level and no significant, respectively

Table 5. Means comparison the influence of application SiO_2 and chitosan NPs on germination parameters of wheat and barley

Traits / Treatments	GP (%)	GR (seed da[1])	GV	PV	MDG	MGT (day)	VI (I) (cm)	VI (II) II (mg)
Control/Wheat	96.66 ^{ab}	15.64 ^{a}	40.56 ^{cb}	1.66 ^{ab}	24.33 ^{ab}	1.71 ^{gh}	1,626.52 ^{bc}	1,468.53 ^{ab}
Control/Barley	95.31 ^{ab}	12.63 ^{bc}	14.75 ^{ef}	0.62 ^{ed}	23.49 ^{ab}	2.01^{bcdef}	1,763.16 ^{ab}	1,913.00 ^{a}
30 SiO_2 NPs/Wheat	100.00 ^{a}	15.22 ^{a}	45.93 ^{a}	1.91 ^{a}	24.00 ^{ab}	1.85 ^{efgh}	1,847.22 ^{ab}	1,675.04 ^{ab}
60 SiO_2 NPs/Wheat	96.00 ^{ab}	15.55 ^{a}	37.01 ^{c}	1.50 ^{b}	24.64 ^{a}	1.80 ^{fgh}	1,751.56 ^{ab}	1,516.74 ^{ab}
90 SiO_2 NPs/Wheat	98.65 ^{ab}	14.66 ^{a}	29.16 ^{d}	1.16 ^{c}	25.00 ^{a}	1.78 ^{gh}	1,545.49 ^{c}	1,587.75 ^{ab}
30 SiO_2 NPs/Barley	90.64 ^{ab}	11.67 ^{bc}	11.23 ^{f}	0.50 ^{def}	22.62 ^{ab}	2.16 ^{bcd}	1,252.71 ^{d}	1,654.31 ^{ab}
60 SiO_2 NPs/Barley	89.32 ^{b}	11.38^{bcd}	7.38 ^{g}	0.32 ^{efg}	22.31 ^{ab}	2.11^{bcde}	1,319.56 ^{d}	1,519.72 ^{ab}
90 SiO_2 NPs/Barley	72.00 ^{c}	7.33 ^{e}	3.78 ^{gh}	0.25 ^{fg}	17.00 ^{c}	2.50 ^{a}	646.44 ^{f}	905.24 ^{c}
30 chitosan NPs/Wheat	98.66 ^{ab}	15.50 ^{a}	46.96 ^{a}	1.93 ^{a}	24.34 ^{ab}	1.74 ^{gh}	1,877.87 ^{a}	1,702.03 ^{ab}
60 chitosan NPs/Wheat	98.68 ^{ab}	12.83 ^{b}	12.26 ^{ef}	0.50 ^{def}	24.65 ^{a}	2.03^{cdefg}	1,335.18 ^{d}	1,369.54 ^{bc}
90 chitosan NPs/Wheat	89.35 ^{b}	12.05 ^{bc}	12.45 ^{ef}	0.55 ^{de}	22.33 ^{ab}	2.28 ^{abc}	1,334.00 ^{d}	1,306.50 ^{bc}
30 chitosan NPs/Barley	92.00 ^{ab}	12.55 ^{cb}	15.30 ^{e}	0.67 ^{d}	23.00 ^{ab}	2.08^{bcdef}	1,335.51 ^{d}	1,890.71 ^{a}
60 chitosan NPs/Barley	88.64 ^{b}	11.05 ^{cd}	5.41 ^{gh}	0.25 ^{fg}	21.32 ^{b}	2.34 ^{ab}	977.69 ^{e}	1,560.56 ^{ab}
90 chitosan NPs/Barley	90.67 ^{ab}	10.00 ^{d}	1.88 ^{h}	0.08 ^{g}	22.68 ^{ab}	2.19 ^{bcd}	442.67 ^{g}	307.59 ^{d}

VI show vigor index. Means by the uncommon letter in each column are significantly different according to Duncan tests ($p<0.05$)

Table 6. Analysis of variance for some growth parameters of barley and wheat

		Mean square								
		L			F.W.			D.W.		
Source	df	Seedling	Shoot	Root	Seedling	Shoot	Root	Seedling	Shoot	Root
NPs (A)	6	45.66 **	5.13 **	24.29 **	12,989.77 **	2,930.30 **	4,303.63 **	88.71 **	25.09 **	24.30 **
Plant species (B)	1	147.39 **	11.16 **	80.09 **	17,272.94 **	9,533.01 **	1,312.78 **	15.55 *	9.67 *	0.19 ^{ns}
A×B	6	20.76 **	3.58 **	7.60 **	1,638.06 **	609.88 **	489.00 **	38.12 **	21.33 **	7.02 **
Error	26	1.25	0.16	0.67	85.45	18.67	32.92	3.28	1.45	0.77
CV (%)	-	7.67	7.31	9.05	6.48	5.42	9.08	11.43	12.16	13.83

L, F.W, D.W show vigor index, length, fresh weight, dry weight, respectively. *, ** and ns: significant at 0.05, 0.01 probability level and no significant, respectively.

Table 7. Means comparison the effect of SiO_2 and chitosan NPs on growth parameters of wheat and barley

Traits / Treatments	L (cm)			F.W. (mg)			D.W. (mg)		
	Seedling	Shoot	Root	Seedling	Shoot	Root	Seedling	Shoot	Root
Control/Wheat	16.72 ^{bc}	6.27 ^{bc}	10.46^{bcd}	119.81 ^{de}	62.45 ^{ef}	57.37^{efg}	15.52 ^{cdef}	8.14 ^{f}	6.92 ^{bcde}
Control/Barley	18.73 ^{a}	6.86 ^{ab}	11.61 ^{bc}	189.50 ^{bc}	110.23 ^{ab}	78.87 ^{bc}	19.82 ^{abc}	11.56^{abcd}	8.92 ^{a}
30 SiO_2 NPs/Wheat	18.47 ^{a}	6.27 ^{bc}	12.20 ^{ab}	129.37 ^{de}	65.25 ^{ef}	64.12 ^{de}	16.66 ^{cdef}	9.50 ^{cdef}	7.34 ^{bc}
60 SiO_2 NPs/Wheat	17.76 ^{ab}	6.26 ^{bc}	11.49 ^{bc}	125.87 ^{de}	70.00 ^{e}	55.87^{efg}	16.04 ^{cdef}	10.71^{bcde}	5.93 ^{cde}
90 SiO_2 NPs/Wheat	16.06 ^{bc}	6.27 ^{bc}	10.13^{cde}	124.29 ^{de}	64.58 ^{ef}	59.87^{def}	15.69 ^{cdef}	9.50 ^{cdef}	6.51 ^{bcde}
30 SiO_2 NPs/Barley	13.74 ^{d}	4.91 ^{e}	8.82 ^{ef}	226.75 ^{a}	117.62 ^{a}	110.02^{a}	18.35 ^{bcd}	12.47 ^{ab}	6.01 ^{cde}
60 SiO_2 NPs/Barley	14.77 ^{cd}	5.36 ^{de}	9.40 ^{def}	176.00 ^{c}	97.25 ^{c}	78.75 ^{bc}	16.37 ^{cdef}	10.79^{abcde}	6.95 ^{bcde}
90 SiO_2 NPs/Barley	10.65 ^{e}	3.59 ^{f}	7.06 ^{g}	128.87 ^{de}	78.50 ^{d}	50.37^{fgh}	14.31 ^{f}	8.62 ^{ef}	5.53 ^{de}
30 chitosan NPs/Wheat	19.33 ^{a}	5.78 ^{cd}	13.13 ^{a}	129.75 ^{de}	65.62 ^{ef}	64.12 ^{de}	16.04 ^{cdef}	11.79 ^{abc}	5.40 ^{e}
60 chitosan NPs/Wheat	13.52 ^{d}	5.56^{cde}	7.96 ^{fg}	125.75 ^{de}	69.75 ^{e}	56.00^{efg}	14.01 ^{f}	8.50 ^{ef}	6.11 ^{cde}
90 chitosan NPs/Wheat	14.94 ^{cd}	5.51^{dce}	9.42 ^{def}	114.77 ^{ef}	64.08 ^{ef}	48.87 ^{gh}	14.03 ^{ef}	9.33 ^{def}	5.63 ^{cde}
30 chitosan NPs/Barley	14.54 ^{cd}	5.85 ^{cd}	8.68 ^{ef}	222.50 ^{a}	118.62 ^{a}	108.02^{a}	22.68 ^{a}	13.04 ^{a}	9.03 ^{a}
60 chitosan NPs/Barley	11.51 ^{e}	5.97 ^{cd}	5.53 ^{h}	132.50 ^{de}	98.70 ^{bc}	34.12 ^{i}	17.61 ^{bcde}	11.75 ^{abc}	6.01 ^{cde}
90 chitosan NPs/Barley	4.90 ^{f}	2.13 ^{g}	2.76 ^{i}	77.75 ^{g}	49.50 ^{g}	28.25 ^{j}	9.13 ^{g}	5.00 ^{g}	4.10 ^{f}

L, F.W, D.W show vigor index, length, fresh weight, dry weight, respectively. Means by the uncommon letter in each row and column are significantly different according to Duncan tests ($p<0.05$).

Table 8. Summary of results for studying effects of NPs on germination parameters as compared to the control

	Experiment 1 (Seeds priming)												Experiment 2 (Direct exposure)											
	SiO_2 NPs						Chitosan NPs						SiO_2 NPs						Chitosan NPs					
	Wheat			Barley			Wheat			Barley			Wheat			Barley			Wheat			Barley		
	30	60	90	30	60	90	30	60	90	30	60	90	30	60	90	30	60	90	30	60	90	30	60	90
GP	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	-	N	N	N	N	N	N
GR	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	-	N	-	-	N	N	-
GV	N	N	N	N	N	N	N	N	N	N	N	N	+	N	-	N	N	N	+	-	-	N	-	-
PV	N	N	N	N	N	N	N	N	N	N	N	N	N	N	-	N	N	-	N	-	-	N	-	-
MDG	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	-	N	N	N	N	N	N
MGT	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	+	N	N	+	N	N	N
VI (I)	N	-	-	+	N	N	N	N	-	+	-	N	N	N	N	-	-	-	+	-	-	-	-	-
VI (II)	Wheat < Barley												N	N	N	N	N	-	N	N	N	N	N	-
L. Seedling	-	-	-	+	+	N	+	+	-	+	-	-	+	N	N	-	-	-	+	-	N	-	-	-
L. Shoot	+	-	-	+	N	N	N	+	-	+	-	-	N	N	N	-	-	-	N	N	N	-	-	-
L. Root	-	-	-	+	+	N	+	N	N	+	N	N	N	N	N	-	-	-	+	-	N	-	-	-
F.W. Seedling	N	N	-	N	N	N	N	N	-	+	N	-	N	N	N	+	N	-	N	N	N	+	-	-
F.W. Shoot	N	N	N	N	N	-	N	N	N	N	N	-	N	N	N	N	-	-	N	N	N	N	N	-
F.W. Root	N	N	-	N	N	N	N	N	-	N	N	N	N	N	N	+	N	-	N	N	N	+	-	-
D.W. Seedling	Wheat < Barley												N	N	N	N	N	-	N	N	N	N	N	-
D.W. Shoot	N	N	-	N	N	N	N	N	N	N	N	-	N	+	N	N	N	-	+	-	-	N	N	-
D.W. Root	No significant effect												N	N	N	-	-	-	N	N	N	N	-	-

L, F.W, D.W show vigor index, length, fresh weight, dry weight, respectively. Also, N, -, + show no significant effect, negative effect, positive effect, respectively

shoot and root weight were achieved at treated barley seeds with 90 ppm chitosan NPs. The highest seedling (226.75 mg) and root fresh weight (110.02 mg) were observed at barley seeds treated with 30 ppm SiO_2 NPs.

Discussion

Type, concentration and consumption methods of NPs play an important role in the behavior, in reactivity and in toxicity of plants. Seed germination and root elongation is widely used as acute phytotoxicity test with several advantages like sensitivity, simplicity, low cost and suitability for unstable chemicals or samples (Wang *et al.*, 2005). In seeds priming with the NPs, there were no toxicity effect on seeds germination parameters. This could be due to the incompatibility between the priming time and permeability of the NPs through seed coat, which could have prevented easy entrance of the latter in the seed coats. Also, in seeds priming, vigor indexes were affected by NPs, that is due to NPs effect on length and weight of seedling. Direct exposure of the seeds to low concentration of NPs (30 ppm) increased some germination parameters that may be due to the absorption and utilization of NPs by seeds (Suriyaprabha *et al.*, 2012). In contrast, in high concentration of NPs (90 ppm), possibly NPs did not fully penetrate the seed coat and endosperm and thus had limited effects on the embryos. Therefore, NPs might find the seed coat and endosperm to be effective barriers, especially when they are agglomerated (Duke and Kakefuda, 1981). These results are supported by similar data of Adhikari *et al.* (2013), Azimi *et al.* (2014), Kananont *et al.* (2010) and Shao *et al.* (2005).

Furthermore, the toxic effects of NPs in high concentrations were more pronounced in seedling, shoot and root length, especially in wheat seeds priming with SiO_2 NPs, as well as direct exposure of barley seeds to the NPs (Table 8). In contrast, seeds priming with 30 ppm NPs (except wheat seeds priming with SiO_2 NPs) gave the highest seedling and root length of the corresponding plants, as well as direct seed exposure of wheat seeds to 30 ppm NPs (Table 8). Different morphological effects depend on the nanomaterial type, size, concentration, surface properties, consumption methods, chemistry, crystallinity, agglomeration state and plant species (Casals *et al*., 2008; Rico *et al*., 2011). Also, root elongation of plant species would have a dose dependent response (Sresty and Rao, 1999). Therefore, radicles, after penetrating the seed coatings could contact the NPs directly. The presence of NPs in large quantities on the root surface could alter the surface chemistry of the root and clog the root openings and both hydraulic and nutrient uptake in roots is inhibited. Therefore, plant growth is negatively affected because of NPs. Siddiqui and Al-Whaibi (2014) found that SiO_2 NPs inhibiting root elongation of tomato at high concentration, whereas, in middle concentration of NPs (8 $g.L^{-1}$) increased root elongation as compared to the control. Also, seeds priming with chitosan solution (0, 0.01%, 0.05%, 0.1%, 0.2% and 0.5%) had not significant effect on shoot length of ajowan (*Carum copticum*), but increased root length as compared to the control (Mahdavi and Rahimi, 2013).

On the other hand, using of 90 ppm NPs in both experiments decreased seedling, shoot and root weight especially in wheat seeds priming, as well as direct exposure of barley seeds to NPs (Table 8). A decrease in biomass beyond such concentrations suggested toxic effects of NPs. It might be probable that increasing the concentration of both NPs could have induced aggregation of particles and resulted in clogging of root pores that interrupted water uptake by seeds (Barrena *et al*., 2009). Saharan *et al*. (2016) found that Cu-chitosan NPs at low concentration had positive effect on fresh and dry weight of maize, whereas in high concentration showed inhibitory effect on seedling growth. In the case of tomato seeds, Haghighi *et al*. (2012) found that high concentration of nano-silicon (2 mM) decreased dry weight of tomato compared to the control and low concentration. Moreover, chickpea seeds treated with chitosan NPs showed positive morphological effects such as enhanced percentage germination, seed vigor index and vegetative biomass of seedlings (Sathiyabama and Parthasarathy, 2016).

Conclusions

The effect of NPs on plants is complex and many factors can affect plant growth and germination. In the current study, both positive and negative effects of NPs were observed for wheat and barley plants respectively. Using SiO_2 and chitosan NPs at low dosage could promote seed germination and growth parameters of both crops, while these parameters decreased following an exposure to high concentrations of both NPs. Therefore, the results obtained in the two experiments suggest that the micro nutrients, SiO_2 and chitosan, can be delivered into seeds of wheat and barley through NPs in suitable concentration and methods.

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