

Effect of chitosan and SiO₂ nanoparticles on germination of wheat (*Triticum aestivum* L.)

Faride Behboudi, PhD Student, Department of Agronomy, Faculty of Agriculture, Tarbiat Modares University, Email: f.behboudi@modares.ac.ir

Zeinolabedin Tahmasebi Sarvestani, Associate Professor, Department of Agronomy, Faculty of Agriculture, Tarbiat Modares University, Email: tahmaseb@modares.ac.ir

Mohamad Zaman Kassae*, Professor, Department of Chemistry, Collage of Sciences, Tarbiat Modares University, Email: kassaeem@modares.ac.ir

Seyed Ali Mohamad Modares Sanavi, Professor, Department of Agronomy, Faculty of Agriculture, Tarbiat Modares University, Email: Modaresa@modares.ac.ir

Ali Sorooshzadeh, Associate Professor, Department of Agronomy, Faculty of Agriculture, Tarbiat Modares University, Email: soroosh@modares.ac.ir

Seyed Badreddin Ahmadi, Educational instructor, Department of Arts, Faculty of Architecture, Tarbiat Modares University, Email: sbahmadi@modares.ac.ir

Abstract

In order to study the effect of chitosan and SiO₂ nanoparticles (NPs) on germination and growth of wheat seeds, a factorial experiment (2 × 4) was performed based on a completely randomized design in four replications. The experimental factors included the above NPs types, with concentrations at 0, 30, 60 and 90 ppm. Seeds were separately soaked for six hours, along with the concentrations of both NPs. The types and concentration of NPs did not show any significant effect on parameters such as Germination Percentage (GP), germination rate (GR), Mean Germination Time (MGT), Mean Daily Germination (MDG), Pick Value (PV), Germination Value (GV) and coleoptile fresh weight. Yet, increase in the concentration of NPs, had significant effects on shoot, seedling, coleoptile, root length, fresh and dry weight, as well as vigor index of seedlings. Also, in most measured traits, effect of chitosan NPs was better than SiO₂ NPs. Finally, direct exposure to the above mentioned NPs have produced both positive and negative effects on wheat growth.

Key words: Germination, Nanoparticles, Growth parameters, Vigor Index

Introduction

Nowadays, nanotechnology has been significantly developed and expanded to various fields, such as food processing, water purification, environmental remediation, wastewater treatment, industrial, household purposes, etc (Khot et al, 2012; Jisha et al, 2014). SiO₂ NPs is one of the most popular nanomaterial which has been used in these fields.

On the other hand, silicon is the second most abundant element in soil, which accounts for approximately 32% of the total weight of soil. 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). Also it regulates physiological activities in plants (Bao-shan et al, 2004). Study on the influence of metal NPs (Si, Pd, Au, and 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, measured in terms of shoot to root ratio and growth of the seedling (Shah and Belozerovala, 2009). Some researchers reported that application of SiO₂ and TiO₂ 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 (Lu et al, 2002).

Chitosan is a linear β-(1, 4)-glucosamine polymer produced by de-acetylation of chitin and is an important structural component of several plant fungi cell walls (Radman et al, 2003). Chitosan has

great potentials for being used in a wide range of industries such as pharmacology, medicine, and agriculture (Bautista-Banos et al, ۲۰۰۴). Chitosan has promoted growth of various crops such as, soybean sprouts (Lee et al, ۲۰۰۵) and sweet basil (Kim et al, ۲۰۰۵). It was also reported to increase wheat seed resistance to certain diseases and improve their quality and ability to germinate (Reddy et al, ۱۹۹۹). Similarly, peanut seeds soaked in chitosan were reported to exhibit an increased rate of germination, lipase activity, gibberellic acid and indole acetic acid levels (Zhou et al, ۲۰۰۲). Ruan and Xue (۲۰۰۲) showed that rice seeds coated with chitosan could accelerate their germination and improve their tolerance to stress conditions. This study probes the effects of chitosan and SiO₂ NPs on seed germination of wheat (*Triticum aestivum* L.).

Materials and Methods

Germination condition

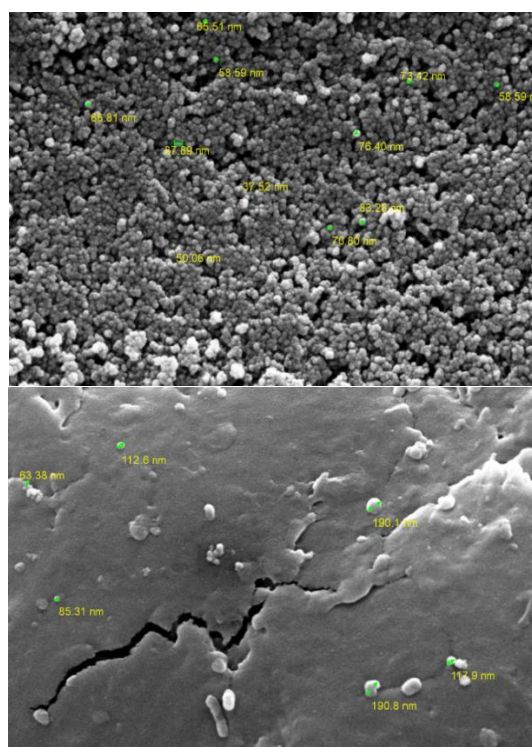
Experiments were carried out to assess the effects of different concentrations of SiO₂ and chitosan NPs on wheat seed germination in a completely randomized design in factorial experiment with four replications. The experimental factors included NPs concentrations (۰, ۲۰, ۶۰ and ۹۰ ppm) and the type of NPs (SiO₂ and chitosan). The experiment was performed in a germinator with an average temperature of ۲۱ ± ۱°C at the College of Agriculture, Tarbiat Modares University, Tehran, Iran (May, ۲۰۱۵).

Plant materials

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

NPs

Two types of NPs were used in this study. Specific surface area of SiO₂ NPs was > ۸۰ m² g⁻¹, average primary particle size was > ۱۰۰ nm and purity was > ۹۹.۵%. Specific surface area of chitosan NPs was > ۸۰ m² g⁻¹, average primary particle size was > ۱۰۰ nm and purity was > ۹۹%. The size of SiO₂ and chitosan NPs (Fig. ۱) were determined through Field Emission-Scanning Electron Microscope (FE-SEM) in Central Laboratory of Deypetronic Company of Iran. The mean diameter of the chitosan NPs (in the dry state) was less than ۱۰۰ nm, which is higher in suspension due to the nanoparticles' swelling ability.



Element	Weight%	Atomic%
O K	36.42	50.13
Si K	63.58	49.87
Totals	100.00	

Element	Weight%	Atomic%
C K	51.44	58.52
O K	48.56	41.48
Totals	100.00	

Fig ۱. Field Emission-Scanning Electron Microscope (FE-SEM) image and X-act of SiO₂ NPs (top) and chitosan NPs (bottom).

SiO₂ NPs solution preparation

The SiO₂ NPs were suspended directly in distilled water 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). Different doses of SiO₂ NPs suspensions (0, 30, 60 and 90 ppm) were prepared for the germination experiment.

Chitosan NPs solution preparation

Chitosan NPs was solubilized in deionized water with 1% acetic acid under constant stirring until complete dissolution of the chitosan NPs. Then solution was alkalized to pH 7.0 with 1 N NaOH (Li et al, 2008).

Treatments

The seeds of wheat were immersed in a 0% sodium hypochlorite solution for 10 min to ensure surface sterility. Then they were soaked in distilled water, SiO₂ and chitosan NPs solution for about 7 hours after being rinsed three times with distilled water. Treated wheat seeds were shade-dried for 1 hour (USEPA, 1996). Then the 20 seeds were placed in a Petri dish (100 mm x 100 mm) with one piece of sterilized filter paper (Whatman No. 1) and 20 mL of distilled water was added (ISTA, 2009). Watering was done for all Petri dish. Then they were covered and placed in a germinator at 21 ± 1°C for seven days.

Measurements

Germination tests were performed according to the rule issued by the International Seed Testing Association (ISTA, 2009). Number of germinated seeds was noted daily for 7 days. Seeds were considered germinated when the radicle showed at least 2 mm in length (USEPA, 1996; ISTA, 2009). On 7th day (after germination), morphological parameters like root, coleoptile and seedling length were measured. Subsequently, they were weighed (initial fresh weight), then oven-dried at 70 °C overnight to estimate their dry weight with a sensitive scale (Gubbins et al, 2011). Also, we used the following germination parameters: Germination percentage (GP), Germination rate (GR) and Mean germination time (MGT).

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

$$MGT = \frac{\sum(F \times X)}{\sum F} \quad (1)$$

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

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

$$\text{Germination rate} = (a/1) + (b-a/2) + (c-b/3) + \dots + (n-n-1/N) \quad (2)$$

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):

$$\text{Vigor index I} = \text{Germination\%} \times \text{Seedling length (cm)} \quad (3)$$

$$\text{Vigor index II} = \text{Germination\%} \times \text{Seedling weight (g)} \quad (4)$$

Evaluations of Mean Daily Germination (MDG), Pick Value (PV) and Germination Value (GV) were calculated by the following equations 5, 6 and 7 (Hartmann et al, 1990):

$$MDG = \text{Germination\%} / \text{total experiment days} \quad (5)$$

$$PV = \text{Maximum germinated seed number at one day} / \text{day number} \quad (6)$$

$$GV = PV \times MDG \quad (7)$$

Germination percentage were calculated by the following equation (Hosseini et al, 2013):

$$GP = \frac{\sum n}{N} \times 100 \quad (8)$$

Where GP is the germination percent, 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.

Result and Discussion

Germination Parameters

Analysis of variance showed that concentration and types of NPs had not significant effect on GP, GV, MDG, MGT, PV and GR (Table 1).

Table 1. Analysis of variance on some germination traits of wheat affected by concentration and types of NPs.

S.O.V	DF	GP	GR	MGT	MDG	PV	GV	Mean Square			
								Seedling	Coleoptile	Root	Shoot
REP	2	0,06	0,00	0,00	0,34	0,04	0,63	0,33	0,00	0,10	0,10
NPs. con (A)	3	12,30 ns	0,00 ns	0,01 ns	0,77 ns	0,20 ns	184,74 ns	2,08 **	0,07 **	1,86 **	0,76 **
NPs types (B)	1	7,41 ns	0,00 ns	0,01 ns	0,46 ns	0,01 ns	0,30 ns	34,46 **	0,04 **	20,00 **	1,90 **
A×B	3	2,47 ns	0,00 ns	0,00 ns	0,10 ns	0,22 ns	101,26 ns	10,87 **	0,00 ns	1,09 **	0,73 **
Error	14	9,79	0,00	0,01	0,61	0,13	96,18	0,08	0,00	0,06	0,06
CV (%)	-	0,18	6,13	9,60	3,19	11,10	11,96	4,01	8,00	0,91	6,08

*, ** and ns: significant at 0,05, 0,01 probability level and no significant, respectively

Seed germination and root elongation is a rapid and widely used acute phyto-toxicity test with several advantages like sensitivity, simplicity, low cost, and suitability for unstable chemicals or samples (Wang et al, 2000). Different concentration of SiO₂ or chitosan NPs (0, 30, 60 and 90 ppm) led to 97-100% germination. This probably could be due to the incompatibility between the seed pores and the size of NPs, which could have prevented easy entrance of the latter in the seed coats. This result was supported by several reports including the following ones. Adhikari et al (2012) found copper NPs not affecting seed germinations of *Glycine max* L. and *Cicer arietinum* L. Similarly, Feizi et al (2012) reported no significant influence on the percentage of germination of wheat seeds in the presence of TiO₂ NPs. Finally, Metzler et al (2011) reported that treatment of pea seeds with TiO₂ NPs (30 nm in size) was inconsequential in terms of germination. Interestingly, these results appeared in contrast to the findings of Shao et al (2000) who has reported that seeds soaked with chitosan increased the germination percentage of maize seedlings.

Seedling, Shoot, Root and Coleoptile Lengths

Analysis of variance showed that the two-way interaction between NPs concentration and NPs types was significant for seedling, shoot and root length (Table 1). Also, main effects were significant for coleoptile length.

Results in table 3 showed that the lowest shoot length (6,05 cm) was obtained in treated seeds with 90 ppm chitosan NPs. In contrast, treated seeds with 60 ppm chitosan NPs was gave the highest shoot length (9,40 cm). Also, the lowest seedling and root length was observed with applying 60 ppm SiO₂ NPs; whereas, the highest these parameters was achieved in the control group (NPs= 0 ppm). Moreover, in most cases, chitosan NPs demonstrated a more conspicuous effect than SiO₂ NPs on seedling, root and shoot growth. Treatment of wheat seeds with chitosan NPs showed 9,11%, 16,78%, 12,86 % increase in the shoot, root and seedling length, compared to SiO₂ NPs respectively. On the other hand, treatment of seeds with SiO₂ NPs showed 13% decrease in the coleoptile length, compared to chitosan NPs (Figure 3a). The latter [30 ppm], gave the best growth response compared to other NPs concentrations (Figure 3b).

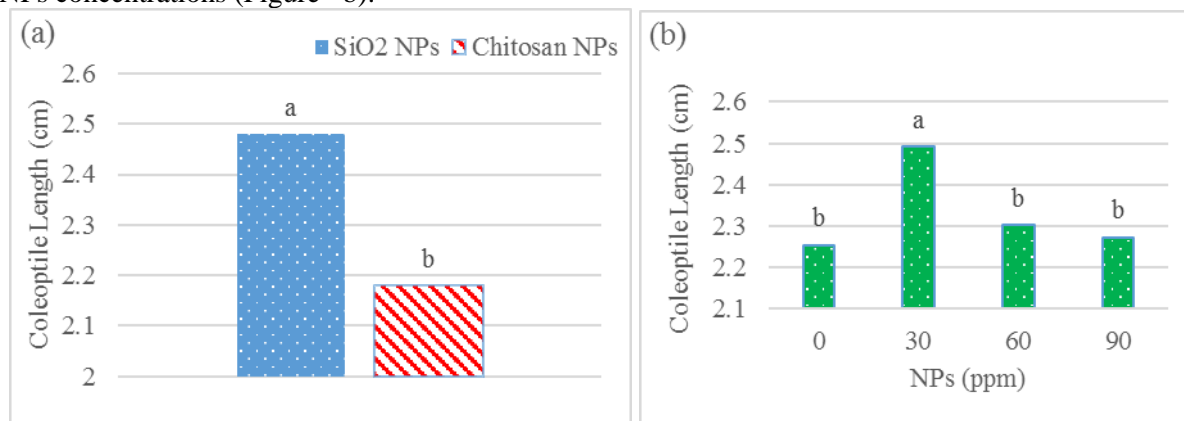


Figure ۲. Effect of SiO_۲ and chitosan NPs on coleoptile length. Means by the uncommon letter in each row and column are significantly different according to Duncan tests (p<۰,۰۰۵).

In this study, germination of seeds occurred normally but the toxic effects of NPs were more pronounced in the shoot, seeding and especially roots, probably due to the seed coatings which can act as a protector for the embryo, but cannot totally guard the whole seed. Therefore, radicles, after penetrating the seed coatings, could contact the NPs directly. So, for root tissues often appear as the first targets to confront the excess concentrations of pollutants. These results seemed consistent with a report by Yang and Watts (۲۰۰۵) who found alumina NPs (nano-Al_۲O_۳) inhibiting root elongation of five plant species at ۲۰۰ mg L^{-۱}. Moreover, Lee et al (۲۰۰۵) stated that the exposure of bean (*Phaseolus radiates* L.) and wheat (*Triticum aestivum* L.) plants to copper NPs decreased the growth and seedling lengths. Also, there was a report on toxicity of SiO_۲ NPs on *Arabidopsis thaliana* (Lee et al, ۲۰۱۰).

Seedling, Shoot, Root and Coleoptile Weight

Analysis of variance showed that concentration and types of NPs had not significant effect on coleoptile fresh weight (Table ۲). On the other hand, the two-way interaction between concentration and types of NPs was significant for seedling, shoot and root fresh weight as well as seedling, shoot, coleoptile and root dry weight.

Table ۲. Analysis of variance on some growth traits of wheat affected by concentration and types of NPs.

S.O.V	D F	Mean Square								Vigor Index I	Vigor Index II
		Fresh Weight				Dry Weight					
		Seedling	Shoot	Coleoptile	Root	Seedling	Shoot	Coleoptil e	Root		
REP	۲	۷۷۶,۲۹	۴,۱۶	۰,۶۵	۷۹۱,۷۳	۰,۳۶	۰,۲۵	۰,۰۵	۰,۰	۴۶۲,۳۸	۰,۰۰
NPs. con (A)	۳	۱۳۴۷,۶۲ **	۴,۴۹ ns	۱۵,۱۰ ns	۹۵۶,۲۳ **	۱,۰۱۷ **	۴,۱۲ **	۰,۷۵ **	۲,۸۱ **	۳۵۲۰۵,۵۷ **	۰,۱۰ **
NPs types (B)	۱	۳,۵۹ ns	۰,۰۲ ns	۳۷,۵۰ ns	۴,۱۶ ns	۱۴,۸۱ **	۷,۵۹ **	۰,۵۷ **	۱,۱۹ **	۳۸۵۳۷۳,۲۶ **	۰,۱۷ **
A×B	۳	۸۴۹,۶۲ * **	۱۵۶,۴۸ **	۳۳,۸۱ ns	۳۵۱,۱۱*	۱۱,۸۴ **	۴,۳۲ **	۱,۸۸ **	۲,۳۵ **	۱۱۱۷۵۵,۷۸ **	۰,۱۱**
Error	۱۴	۱۷۰,۸۷	۲۱,۲۹	۱۱,۲۲	۸۹,۸۸	۰,۲۲	۰,۱۹	۰,۰۴	۰,۰۸	۳۵۰۰,۷۴	۰,۰۰
CV (%)	-	۱۰,۶۴	۸,۴۶	۱۱,۱۹	۱۳,۸۷	۹,۰۹	۷,۲۷	۵,۰۶	۳,۹۷	۶,۰۷	۴,۱۵

*, ** and ns: significant at ۰,۰۵, ۰,۰۱ probability level and no significant, respectively

The lowest seedling (۹۶,۰۴ mg) and shoot fresh weight (۴۵,۳۳ mg) were found when ۶۰ ppm chitosan NPs was employed (Table ۲). Use of both NPs greatly decreased root fresh weight, compared to the control (۰ ppm NPs); whereas, in most levels, applying both NPs had not significant effect on shoot and seedling fresh weight of treated seeds compared to control. On the other hand, the lowest seedling, shoot and coleoptile dry weight were found when ۳۰ ppm SiO_۲ NPs was employed. The greatest seedling (۱۷,۳۲ mg) and root dry weight (۸,۴۷ mg) were achieved from concentrations of ۳۰ ppm chitosan NPs.

Although root weights are not standardized in toxicity tests, they may be helpful in comparing the toxicity effects after seeds exposure to NPs. The increase in biomass to certain concentrations provides the optimum dose limit for the growth of seedlings. However, 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. Chibu and Shibayama (۲۰۰۱) found that dry weight of dry-land rice grown with both ۰,۱ and ۰,۵% of chitosan were increased more than the control. Some researchers demonstrated that treatment of Changbai larch seedlings by ۵۰۰ μL L^{-۱} nanostructured silicon dioxide produced the best result, for mean height, root collar diameter, main root length, and the number of lateral roots were increased compared to those of the control (Lin et al, ۲۰۰۴). Suriyaprabha et al (۲۰۱۲) reported that SiO_۲ NPs significantly enhanced plant dry weight. Seed soaked with chitosan increased germination rate, length and weight of hypocotyls and radicle in rapeseed (Sui et al, ۲۰۰۲).

Vigor Index

Analysis of variance showed that main effects and two-way interaction between concentration and types of NPs was significant for vigor index I and II (Table ۲).

Pretreatment of seeds with ۶۰ ppm SiO_۲ NPs demonstrated the lowest value of vigor index I (۱۱۹۶,۲۰ cm), while the highest (۱۷۶۱,۳۰ cm) was encountered in ۳۰ ppm of chitosan NPs (Table ۳). Exposure of seeds to ۶۰ ppm SiO_۲ NPs decreased seed vigor index I by ۱۰٪ compared to the control. Also, exposure of seeds to ۳۰ ppm SiO_۲ NPs decreased seed vigor index II by ۳۰٪ compared to the control. Using chitosan NPs increased seed vigor index I compared to the control; whereas, using SiO_۲ NPs decreased seed vigor index I and II compared to the control. Between the studied two NPs, chitosan NPs gave the best vigor index value that of the SiO_۲ NPs.

Emergence potential of a seedling is often indicated by its vigor index. Use of SiO_۲ NPs appeared toxic to seeds and adversely affected their performance; but the other NPs appeared no-toxic effect on seeds. It has been stated that the biological activity and bio kinetics of NPs depends on parameters such as size, shape, type, chemistry, crystallinity, surface properties, agglomeration state, bio-persistence, and dose (Casals et al, ۲۰۰۸). Similar inhibitory effects of NPs were reported on radish, rape, and rye grass [۳۴]. It was demonstrated that using TiO_۲ NPs in low concentration (۲ and ۱۰ ppm) could encourage seed germination of wheat in comparison to bulk TiO_۲ and untreated control groups, but in high concentrations (۱۰۰ and ۵۰۰ ppm) it had an inhibitory or no effect on wheat seed (Feizi et al, ۲۰۱۲). Also, the highest vigor index was for under ۲ and ۱۰ mg L^{-۱} TiO_۲ NPs. Manjunatha et al (۲۰۰۸) reported that seed priming with chitosan enhances seed germination and seedling vigor in pearl millet. Also, it was reported that seed priming with two different acidic chitosan solutions improved the vigor of maize seedlings (Shao et al, ۲۰۰۵).

Table ۲. Mean comparison interaction effects of types and concentration of NPs on some wheat traits

NPs (ppm)	Shoot Length/cm		Root Length/cm	
	SiO _۲ NPs	Chitosan NPs	SiO _۲ NPs	Chitosan NPs
۰	۸,۶۸ b	۹,۰۴ ab	۸,۱۰ a	۸,۰۷ a
۳۰	۷,۳۵ d	۸,۲۲ c	۵,۷۹ c	۶,۱۶ c
۶۰	۷,۳۴ d	۹,۴۵ a	۵,۱۸ d	۶,۹۵ b
۹۰	۷,۴۸ d	۶,۵۲ e	۶,۰۷ c	۶,۷۹ b
Seedling length/cm				
۰	۱۶,۷۴ ab	۱۷,۶۱ a	۵۶,۰۰ ab	۵۸,۶۸ a
۳۰	۱۳,۵۲ d	۱۶,۴۲ b	۴۷,۶۵ bc	۵۹,۶۷ a
۶۰	۱۲,۵۲ e	۱۶,۴۱ b	۵۷,۳۰ a	۴۵,۳۳ c
۹۰	۱۴,۸۶ c	۱۳,۳۲ d	۵۷,۳۱ a	۵۴,۰۰ ab
Root Fresh Weight/mg				
۰	۸۶,۰۵ a	۸۲,۶۷ ab	۱۴۲,۳۳ a	۱۴۱,۳۲ a
۳۰	۵۰,۰۰ d	۶۳,۰۱ cd	۹۷,۰۰ b	۱۲۲,۶۷ a
۶۰	۶۹,۶۴ abc	۵۱,۰۰ d	۱۲۷,۰۱ a	۹۶,۰۴ b
۹۰	۶۶,۳۳ bcd	۷۸,۶۳ abc	۱۲۳,۰۵ a	۱۳۲,۰۹ a
Shoot dry weight (mg)				
۰	۸,۳۰ b	۹,۰۰ b	۷,۲۱ bc	۷,۷۰ b
۳۰	۵,۳۳ c	۹,۰۰ b	۶,۰۰ d	۸,۴۷ a
۶۰	۹,۰۰ b	۸,۰۱ b	۶,۸۲ c	۵,۸۳ d
۹۰	۸,۶۵ b	۱۰,۰۰ a	۷,۶۵ b	۷,۵۰ b
Coleoptile Dry Weight/mg				
۰	۴,۵۶ bc	۴,۲۰ cd	۱۵,۳۶ c	۱۶,۳۱ abc
۳۰	۳,۱۰ e	۴,۸۴ ab	۱۱,۳۳ e	۱۷,۳۲ a
۶۰	۴,۸۱ ab	۴,۰۳ d	۱۵,۶۰ bc	۱۴,۰۱ d
۹۰	۴,۵۲ bc	۵,۱۹ a	۱۶,۶۴ ab	۱۷,۰۷ a
Seed Vigor Index I/cm				
۰	۱۳۲۷,۳۳ d	۱۳۰۲,۸۹ d	۱۶۰۳,۳۰ bc	۱۶۶۳,۳۷ ab
۳۰	۱۳۲۲,۲۴ d	۱۷۶۱,۳۰ a	۱۱۲۰,۰۱ f	۱۷۳۳,۳۲ a
۶۰	۱۱۹۶,۲۵ e	۱۶۰۵,۲۷ b	۱۳۶۳,۳۳ e	۱۴۸۰,۰۰ d
۹۰	۱۴۵۲,۲۷ c	۱۶۴۲,۳۴ b	۱۵۴۶,۶۶ cd	۱۶۷۰,۰۴ ab
Seed Vigor Index II (mg)				

Means by the uncommon letter in each row and column are significantly different according to Duncan tests (p<۰,۰۵).

Conclusion

The both positive and negative effects of NPs was observed on germination and growth of wheat. The results suggest that the micro nutrients, silica and chitosan can be delivered into seeds of wheat through NPs. So, nanoscale nutrients can be supplied to the crops either through seed dressing with much decreased doses to get the desired results. Further, detailed studies have to be performed to understand the mechanism of action of nanoscale materials and evaluated the most efficient method of NPs application.

References

Ananta Vashisth and Shantha Nagarajan. (۲۰۱۰). Effect on germination and early growth characteristics in sunflower (*Helianthus annuus*) seeds exposed to static magnetic field. *Journal of Plant Physiology*. ۱۶۷, ۱۴۹-۱۵۶.

Bhaskara Reddy, Joseph Arul, Paul Angers and Luc Couture. (۱۹۹۹). Chitosan treatment of wheat seeds induces resistance to Fusarium graminearum and improves seed quality *J. Agric. Food. Chem.* ۴۷. ۱۲۰۸-۱۲۱۶.

Bin Li, Xiao Wang, Ruoxia Chen, Weiguo Huangfu and Guanlin Xie. (۲۰۰۸). Antibacterial activity of chitosan solution against Xanthomonas pathogenic bacteria isolated from Euphorbia pulcherrima. *Carbohydr. Polym.* ۷۲. ۲۸۷-۲۹۲.

Chang Woo Lee, Shaily Mahendra, Katherine Zodrow and Pedro Alvarez. (۲۰۱۰). Developmental phytotoxicity of metal oxide NPs to Arabidopsis thaliana. *Environmental Toxicology and Chemistry*. ۲۹. ۶۶۹-۶۷۵.

Hiroko Chibu and Hidejiro Shibayama. (۲۰۰۱). Effects of Chitosan applications on the growth of several crops in: T. Uragami, K. Kurita, T. Fukamizo (ed.) *Chitin and Chitosan in life science* Yamaguchi. p ۲۳۹.

David M. Metzler, Minghua Li, Ayca Erdem and C.P. Huang. (۲۰۱۱). Responses of algae to photo-catalytic nano-TiO₂ particles with an emphasis on the effect of particle size. *Chemical Engineering Journal*. ۱۷۰. ۵۳۸-۴۶.

Eudald Casals, Socorro Vázquez-Campos, Neus Bastús and Victor Puntes. (۲۰۰۸). Distribution and potential toxicity of engineered inorganic NPs and carbon nanostructures in biological systems. *Trends in Analytical Chemistry*. ۲۷. ۶۷۲-۶۷۹.

Eva Gubbins, Lesley C Batty and Jamie R Lead. (۲۰۱۱). Phyto-toxicity of silver NPs to Lemna minor L. *Environ Pollution*. ۱۵۹, ۱۵۵۱-۹.

Manjunatha G, Roopa KS, Geetha Prashanth and Shekar Shetty. (۲۰۰۸). Chitosan enhances disease resistance in pearl millet against downy mildew caused by clerospora graminicola and defence-related enzyme activation. *Pest Management Science*. ۶۴. ۱۲۵۰-۱۲۵۷.

Hamid Reza Hosseini, Mehrangiz Chehrizi, Mohammad Mahmoodi Sorestani, Darush Nabati Ahmadi and Karim Sorkhe. (۲۰۱۳). Autotetraploidy induction and seed quality comparison between diploid and tetraploid Madagascar periwinkle (*Catharanthus roseus* cv. rosea) seedlings. *International journal of Agronomy and Plant Production*. ۴. ۲۱۲-۲۱۶.

Hassan Feizi, Parviz Rezvani Moghaddam, Nasser Shahtahmassebi and Amir Fotovat. (۲۰۱۲). Impact of bulk and nanosized titanium dioxide (TiO₂) on wheat seed germination and seedling growth. *Biological Trace Element Research*. ۱۴۶. ۱۰۱-۱۰۶.

Hudson Hartmann, Dale Kester, Fred Davies and Robert Geneve. (۱۹۹۰). *Plant propagation: principles and practices* Prentice Hall Englewood Cliffs New Jersey. p ۶۴۷.

ISTA, ISTA rules ۲۰۰۹ International Seed Testing Association. Zurich, Switzerland.

James Maguire. (۱۹۸۲). Speed of germination Aid in selection and evaluation for seedling emergence and vigor. *Crop Science*. ۲۲, ۱۷۶-۱۷۷.

Jian Feng Ma and Naoki Yamaji. (۲۰۰۶). Silicon uptake and accumulation in higher plants *Trends in Plant Science*. ۱۱(۸). ۳۹۲-۳۹۷.

Jisha Jayadevan Pillai, Arun Kumar Theralikattu Thulasidasan, Ruby John Anto, Devika Nandan Chithralekha, Ashwanikumar Narayanan and Gopalakrishnapillai Sankaramangalam Vinod Kumar. (۲۰۱۴). Folic acid conjugated cross-linked acrylic polymer (FA-CLAP) hydrogel for site specific delivery of hydrophobic drugs to cancer cells. *Journal Nano biotechnology*. ۱۲. ۲۵-۳۵.

Hyun-Jin Kim, Feng Chen, Xi Wang and Nihal Rajapakse. (۲۰۰۵). Effect of chitosan on the biological properties of sweet basil (*Ocimum basilicum* L.) *J. Agric. Food. Chem.* ۵۳. ۳۶۹۶-۳۷۰۱.

Lav Khot, Sindhuja Sankaran, Joe Mari Maja and Reza Ehsani. (۲۰۱۲). Applications of nanomaterials in agricultural production and crop protection: A review. *Crop Protection*. ۳۵. ۶۴-۷۰.

Lin Wang, Chaoyong Yang and Weihong Tan. (۲۰۰۵). Dual-luminophore-doped silica NPs for multiplexed signaling. *Nano Letters*. ۵(۱). ۳۷-۴۳.

Lin Bao-shan, Diao shao-qi, Li Chun-hui, Fang Li-jun, Qiao Shu-chun and Yu Min. (۲۰۰۴). Effects of TMS (nanosized silicon dioxide) on growth of Changbai larch seedlings. *Journal of Forestry Research*. ۱۵, ۱۳۸-۱۴۰.

Ling Yang and Daniel Watts. (۲۰۰۵). Particle surface characteristics may play an important role in phytotoxicity of alumina NPs. *Toxicology Letters*. ۱۵۸. ۱۲۲-۱۳۲.

Lu Changmei ,Zhang Chaoying , Wen Junqiang , Wu Guorong and Tao Mingxuan. (۲۰۰۲). Research of the effect of nanometer materials on germination and growth enhancement of *Glycine max* and its mechanism. Soybean Science. ۲۱. ۱۶۸-۱۷۲.

Nusrat Parven and muhamad Ashraf. (۲۰۱۰). Role of silicon in mitigating the adverse effects of salt stress on growth and photosynthetic attributes of two maize (*Zea mays* L.) cultivars grown hydroponically Pak. J. Bot. ۴۲. ۱۶۷۵-۱۶۸۴.

Romeo Radman, Treza Saez, Christopher Bucke and Tajalli Keshavarz. (۲۰۰۳). Elicitation of plants and microbial cell systems. Biotech Appl Biochem. ۳۷. ۹۱-۱۰۲.

Ruan Songlin and Xue Qingzhong. (۲۰۰۲). Effects of chitosan coating on seed germination and salt-tolerance of seedlings in hybrid rice (*Oryza sativa* L.). Acta Agronomica Sinica. ۲۸. ۸۰۳-۸۰۸.

Silvia Bautista-Baños, Mónica Hernández-López and E. Bosquez-Molina. (۲۰۰۴). Growth inhibition of select fungi by chitosan and plant extracts. Mexican Journal Phytopathology. ۲۲. ۱۷۸-۱۸۶.

Stanley Matthews and Khajeh-Hosseini. (۲۰۰۷). Length of the lag period of germination and metabolic repair explain vigor differences in seed lots of maize (*Zea mays*). Seed Science and Technology. ۳۵. ۲۰۰-۲۱۲.

Sui X Y, Zhang W Q, Xia W and Wang Q. (۲۰۰۲). Effect of chitosan as seed coating on seed germination and seedling growth and several physiological and biochemical indexes in rapeseed. Plant Physiology Communications. ۳۸. ۲۲۵-۲۲۷.

Suriyaprabha R, Karunakaran G, Yuvakkumar R, Prabu P, Rajendran V and Kannan N. (۲۰۱۲). Growth and physiological responses of maize (*Zea mays* L.) to porous silica NPs in soil. Journal of Nanoparticle Research. ۱۴. ۱۲۹۴-۱۲۹۶.

Tapan Adhikari, S. Kundu and A. Subba Rao. (۲۰۱۳). Impact of SiO_۲ and Mo Nano Particles on Seed Germination of Rice (*Oryza Sativa* L.). International Journal of Agriculture and Food Science Technology. ۴ (۸). ۸۰۹-۸۱۶.

Tapan Adhikari, Sam Kundu, Ashis Kumar Biswas and Annangi Subba Rao. (۲۰۱۲). Effect of Copper Oxide Nano Particle on Seed Germination of Selected Crops Journal of Agricultural Science and Technology A. ۲. ۸۱۵-۸۲۳.

U. S. Environmental Protection Agency (USEPA) ۱۹۹۶ Ecological effects test guidelines: Seed germination/root elongation toxicity test. OPPTS ۸۵۰. ۴۲۰۰. EPA ۷۱۲-C-۹۶-۱۵۴. Washington DC. Salama p ۱۹۷.

Vishal Shah and Irina Belozerovala. (۲۰۰۹). Influence of metal NPs on the soil microbial community and germination of lettuce seeds. Water Air and Soil Pollution. ۱۹۷(۱). ۱۴۳-۱۴۸.

Ya-jing Guan, Jin Hu, Xian-ju Wang and Chen-xia Shao. (۲۰۰۵). Effects of seed priming with chitosan solutions of different acidity on seed germination and physiological characteristics of maize seedling. Journal Zhejiang University Agriculture and Life Science. ۳۱(۶). ۷۰۵-۷۰۸.

Young-Sang Lee, Yong-Ho Kim and Sung-Bae Kim. (۲۰۰۵). Changes in the respiration, growth, and vitamin C content of soybean sprouts in response to Chitosan of different molecular weight. Hort Science. ۴۰. ۱۳۳۳-۱۳۳۵.

Zhou Y G, Yang Y D, Qi Y G, Zhang Z M, Wang X J and Hu X J (۲۰۰۲). Effects of chitosan on some physiological activity in germinating seed of peanut. Journal Peanut Science. ۳۱(۱). ۲۲-۲۵.