Effect of chitosan and SiO₁ nanoparticles on germination of wheat (*Triticum aestivum L.*)

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Abstract

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In order to study the effect of chitosan and SiO^{τ} nanoparticles (NPs) on germination and growth of wheat seeds, a factorial experiment ($^{\gamma}\times^{\xi}$) was performed based on a completely randomized design in four replications. The experimental factors included the above NPs types, with concentrations at \cdot , $^{\tau}\cdot$, $^{\tau}\cdot$ and $^{q}\cdot$ 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 SiO^{τ} 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, $7 \cdot 17$; Jisha et al, $7 \cdot 15$). SiO₇ 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 $\[mathbb{n}\]''$ 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, $\[mathbb{n}\]''$. Parven and Ashraf, $\[mathbb{n}\]''$. Also it regulates physiological activities in plants (Bao-shan et al, $\[mathbb{n}\]''$. 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 Belozerova, $\[mathbb{n}\]'\cdot \[mathbb{n}\]' \cdot \[mathbb{n}$

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





great potentials for being used in a wide range of industries such as pharmacology, medicine, and agriculture (Bautista-Banos et al, $\uparrow \cdot \cdot \cdot \uparrow$). Chitosan has promoted growth of various crops such as, soybean sprouts (Lee et al, $\uparrow \cdot \cdot \circ$) and sweet basil (Kim et al, $\uparrow \cdot \cdot \circ$). It was also reported to increase wheat seed resistance to certain diseases and improve their quality and ability to germinate (Reddy et al, $\uparrow \cdot \cdot \uparrow$). 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, $\uparrow \cdot \cdot \uparrow$). Ruan and Xue ($\uparrow \cdot \cdot \uparrow$) 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 SiO₇ 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 SiO₇ and chitosan NPs on wheat seed germination in a completely randomized design in factorial experiment with four replications. The experimental factors included NPs concentrations ($\cdot, \, {}^{\tau} \cdot, \, {}^{\tau} \cdot$ and ${}^{q} \cdot$ ppm) and the type of NPs (SiO₇ and chitosan). The experiment was performed in a germinator with an average temperature of ${}^{\tau} \cdot \pm {}^{\circ}C$ at the College of Agriculture, Tarbiat Modares University, Tehran, Iran (May, ${}^{\tau} \cdot {}^{\circ}$).

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

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Two types of NPs were used in this study. Specific surface area of SiO₇ NPs was > $\wedge \cdot m^{\gamma}$ g- $\wedge m^{\gamma}$ g- $\wedge \cdot m^{\gamma}$ g- \wedge

92.51cm	Element	Weight%	Atomic%
Alicel na seusonais. Alice a			
- Tradium Anna State	ОК	36.42	50.13
	Si K	63.58	49.87
	Totals	100.00	
The second se			
112.0 mm.	Element	Weight%	Atomic%
-112.0 mm -123.35 pm	Element C K	Weight%	Atomic% 58.52
B3 38 gm B3 38 gm B5 31 nm	Element C K O K	Weight% 51.44 48.56	Atomic% 58.52 41.48

Fig '. Field Emission-Scanning Electron Microscope (FE-SEM) image and X-act of SiO^x NPs (top) and chitosan NPs (bottom).

SiO^v NPs solution preparation





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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 $3,\circ$ with 1 N NaOH (Li et al, 5.5%).

Treatments

The seeds of wheat were immersed in a \circ ' sodium hypochlorite solution for $\cdot \cdot$ min to ensure surface sterility. Then they were soaked in distilled water, SiO₁ and chitosan NPs solution for about \neg hours after being rinsed three times with distilled water. Treated wheat seeds were shade-dried for \cdot hour (USEPA, \uparrow 9 \uparrow). Then the $\uparrow \circ$ seeds were placed in a Petri dish ($\uparrow \cdot \cdot mm \times \uparrow \circ mm$) with one piece of sterilized filter paper (Whatman No. \uparrow) and \circ mL of distilled water was added (ISTA, $\uparrow \cdot \cdot \uparrow$). Watering was done for all Petri dish. Then they were covered and placed in a germinator at $\uparrow \uparrow \pm \uparrow \circ C$ for seven days.

Measurements

Germination tests were performed according to the rule issued by the International Seed Testing Association (ISTA, $\uparrow \cdot \cdot \uparrow$). Number of germinated seeds was noted daily for \lor days. Seeds were considered germinated when the radicle showed at least \uparrow mm in length (USEPA, $\uparrow \uparrow \uparrow \uparrow$); ISTA, $\uparrow \cdot \cdot \uparrow$). On \lor th day (after germination), morphological parameters like root, coleoptile and seedling length were measured. Subsequently, they were weighed (initial fresh weight), then oven-dried at $\lor \cdot \circ C$ overnight to estimate their dry weight with a sensitive scale (Gubbins et al, $\uparrow \cdot 1 \uparrow$). 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 \uparrow (Matthews and Khajeh-Hosseini, $\uparrow \cdot \cdot \lor$): $\Sigma(F \times X)$ (1)

MGT= **DF**

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 Υ (Maguire, Υ):

Germination rate = $(a^{\prime}) + (b - a^{\prime}) + (c - b^{\prime}) + \dots + (n - 1^{\prime})$

Where a, b, c, ..., n are numbers of germinated seeds after 1, 7, 7, ..., N days from the start of imbibition.

Seedling vigor index were computed based on equations r and ϵ (Vashisth and Nagarajan, r.): Vigor index I = Germination% × Seedling length (cm) (r)

Vigor index II = Germination $\% \times$ Seedling weight (g)

Evaluations of Mean Daily Germination (MDG), Pick Value (PV) and Germination Value (GV) were calculated by the following equations °, ⁷ and ⁷ (Hartmann et al, ¹⁹⁹.): MDG = Germination% / total experiment days (°)

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

$$GV = PV \times MDG$$

Germination percentage were calculated by the following equation (Hosseini et al, Y ·) "):

 $GP = \frac{\sum n}{N} \times \cdots$

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, 9,1,7). The significance of differences among treatment means were compared by Duncan's multiple range tests at P<••••.

Result and Discussion



Germination Parameters

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

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						Me	an Square				
S.O.V	DF	GP	GR	MGT	MDG	PV	GV	Length			
								Seedling	Coleoptile	Root	Shoot
REP	۲	०,०٦	۰,۰۰	۰,۰۰	۰,٣٤	۰,۰٤	0.,1٣	۰,۳۳	• , • •	۰,۱۰	۰,۱۰
NPs. con (A)	٣	17,70 ns	• , • • ^{ns}	• , •) ^{ns}	•, YY ns	•, Y ons	۱۸٤,V٤ ns	۲,•۸ **	۰,۰۷**	۱,۸٦**	•, ٧٦ **
NPs types (B)	١	۲,٤١ ^{ns}	• , • • ^{ns}	• , •) ^{ns}	۰,٤٦ ns	•,•) ^{ns}	•,70 ns	۳٤,٤٦ **	•,0ź**	** ۲۰,۰۰	1,90 **
A×B	٣	۲, ٤ ۷ ^{ns}	• , • • ^{ns}	• , • • ^{ns}	•, \o ns	•, 77 ns	101,77 ns	۱۰,۸۷ **	• , • • ^{ns}	** ۱٬۰۹	0,77 **
Error	١٤	۹,٧٩	• , • •	۰,۰۱	۰,٦١	۰,۱۳	97,17	•,•٨	•,••	۰,۰٦	۰,۰٦
CV (%)	-	0,11	٦,١٣	9,70	۳,۱۹	11,1.	11,97	٤, • ١	٨,٥.	0,91	٦,•٨

Tahle '	Analysis of	variance on so	me germination	traits of wheat	affected by a	concentration and	d types of	NPc
Labic	•• Analysis 01	variance on se	me ger mination	trans or wheat	anceicu by v	concentration and	a types of	141 0.

** and ns: significant at ..., 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, $\forall \cdot \cdot \circ$). Different concentration of SiO_Y or chitosan NPs ($\cdot, \forall \cdot, \forall \cdot and \vartheta \cdot ppm$) led to $\vartheta \cdot$ $\cdot \cdot \cdot$ 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 $(\uparrow \cdot \uparrow \uparrow)$ found copper NPs not affecting seed germinations of *Glycine max* L. and *Cicer arietinum* L. Similarly, Feizi et al (۲۰۱۲) reported no significant influence on the percentage of germination of wheat seeds in the presence of TiO_Y NPs. Finally, Metzler et al $(\gamma \cdot \gamma)$ reported that treatment of pea seeds with TiO_Y NPs $(\gamma \cdot nm in$ size) was inconsequential in terms of germination. Interestingly, these results appeared in contrast to the findings of Shao et al $(\uparrow \cdot \cdot \circ)$ 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 \mathbb{T} showed that the lowest shoot length (1,07 cm) was obtained in treated seeds with $9 \cdot$ ppm chitosan NPs. In contrast, treated seeds with *i*, ppm chitosan NPs was gave the highest shoot length (^{9, £ o} cm). Also, the lowest seedling and root length was observed with applying [¬] · ppm SiO[↑] NPs; whereas, the highest these parameters was achieved in the control group (NPs= \cdot ppm). Moreover, in most cases, chitosan NPs demonstrated a more conspicuous effect than SiO⁷ NPs on seedling, root and shoot growth. Treatment of wheat seeds with chitosan NPs showed 9,11%, V1,VA%, 17,A7 % increase in the shoot, root and seedling length, compared to SiO₁ NPs respectively. On the other hand, treatment of seeds with SiO_{τ} NPs showed $\gamma\gamma$ decrease in the coleoptile length, compared to chitosan NPs (Figure γ a). The latter [γ , ppm], gave the best growth response compared to other NPs concentrations (Figure ⁷b).





Figure ^{γ}. Effect of SiO^{γ} and chitosan NPs on coleoptile length. Means by the uncommon letter in each row and column are significantly different according to Duncan tests (p<·,·°).

In this study, germination of seeds occurred normally but the toxic effects of NPs were more pronounced in the shoot, seeding and epesially 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 ($\gamma \cdot \cdot \circ$) who found alumina NPs (nano-Al₁O₇) inhibiting root elongation of five plant species at $\gamma \cdot \cdot$ mg L- γ . Moreover, Lee et al ($\gamma \cdot \cdot \circ$) 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 SiO₇ NPs on Arabidopsis thaliana (Lee et al, $\gamma \cdot \gamma$).

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 γ). 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 γ . Analysis of variance on some growth traits of wheat affected by concentration and types of NPs.

						Mean S	quare				
S.O.V	D	Fresh Weight			Dry Weight				Vigor	Vigor	
	F	Seedling	Shoot	Coleoptile	Root	Seedling	Shoot	Coleoptil	Root	Index I	Index II
								e			
REP	۲	٧٧٦,٢٩	٤,١٦	۰,٦٥	٧٩١,٧٣	۰,۳٦	•,70	•,•0	۰,۰ ۱	277,87	•,••
NPs. con (A)	٣	۱۳٤٧,٦٢ **	٤•,٤٩ ns	10,1. ns	907,78 **	۱۰,۱۷ **	٤,17 **	•,70 **	۲,۸۱**	Tot.o,ov **	•,1• **
NPs types (B)	١	۳,09 ns	•,•Y ns	۳۷,0. ns	٤,١٦ ns	۱٤,٨١ **	** ۷,0۹	•,07 **	۱,۱۹ **	TNOTVT,TI **	۰,۱۷ **
A×B	٣	٨٤٩,٦٢ *	۱٥٦,٤٨ **	۳۳,۸۱ ns	۳0۱,۱۱*	11,42 **	£,87 **	۱,۸۸ **	7,70 **	111100,77 **	۰,۱۱**
Error	١٤	۱۷۰,۸۷	21,29	11,77	Λ٩, ΛΛ	•, ٢٢	۰,۱۹	۰,۰٤	۰,۰۸	۳0,٧٤	• , • •
CV (%)	-	۱۰,٦٤	٨,٤٦	11,19	١٣,٨٧	۹,.۹	٧,٢٧	0,.7	٣,٩٧	٦,.٧	٤,١٥

*, ** and ns: significant at •, • °, •, •) probability level and no significant, respectively

The lowest seedling $({}^{\eta}{}^{\tau}, {}^{t}$ mg) and shoot fresh weight $({}^{t}\circ, {}^{\eta}{}^{r}$ mg) were found when ${}^{\tau}\circ$ ppm chitosan NPs was employed (Table ${}^{\eta}$). Use of both NPs greatly decreased root fresh weight, compared to the control (${}^{\circ}$ 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 ${}^{\eta}\circ$ ppm SiO₇ NPs was employed. The greatest seedling (${}^{\eta}{}^{\eta}{}^{r}{}^{r}$ mg) and root dry weight (${}^{\Lambda}{}^{t}{}^{\gamma}$ mg) were achieved from concentrations of ${}^{\eta}\circ$ 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 ($\gamma \cdot \gamma$) found that dry weight of dry-land rice grown with both \cdot, γ and \cdot, σ ? of chitosan were increased more than the control. Some researchers demonstrated that treatment of Changbai larch seedlings by $\circ \cdot \cdot \mu L L - \gamma$ 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, $\gamma \cdot \cdot \hat{\gamma}$). Suriyaprabha et al ($\gamma \cdot \gamma$) reported that SiO₇ 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, $\gamma \cdot \gamma$).



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 γ).

Pretreatment of seeds with $\overline{} \cdot$ ppm SiO₇ NPs demonstrated the lowest value of vigor index I ($\underline{}$, $\underline{} \circ$, cm), while the highest ($\underline{}$, $\underline{} \circ$, cm) was encountered in $\underline{} \circ$, ppm of chitosan NPs (Table $\underline{}$). Exposure of seeds to $\overline{} \circ$, ppm SiO₇ NPs decreased seed vigor index I by $\underline{} \circ \underline{}$, compared to the control. Also, exposure of seeds to $\underline{} \circ$, ppm SiO₇ NPs decreased seed vigor index II by $\underline{} \circ \underline{}$, compared to the control. Also, exposure of seeds to $\underline{} \circ \underline{}$, ppm SiO₇ NPs decreased seed vigor index II by $\underline{} \circ \underline{}$, compared to the control. Using chitosan NPs increased seed vigor index I compared to the control; whereas, using SiO₇ 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 SiO₇ NPs.

Emergence potential of a seedling is often indicated by its vigor index. Use of SiO_Y 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, biopersistence, and dose (Casals et al, $\uparrow \cdot \cdot \land$). Similar inhibitory effects of NPs were reported on radish, rape, and rye grass [\uparrow^{ϵ}]. It was demonstrated that using TiO_Y NPs in low concentration (\uparrow and $\uparrow \cdot$ ppm) could encourage seed germination of wheat in comparison to bulk TiO_Y and untreated control groups, but in high concentrations ($\uparrow \cdot \cdot$ and $\circ \cdot \cdot$ ppm) it had an inhibitory or no effect on wheat seed (Feizi et al, $\uparrow \cdot \cdot \uparrow$). Also, the highest vigor index was for under \uparrow and $\uparrow \cdot$ mg L– \uparrow TiO_Y NPs. Manjunatha et al ($\uparrow \cdot \cdot \land$) 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, $\uparrow \cdot \cdot \circ$).

NPs (ppm)	SiO ^r NPs	Chitosan NPs	SiOr NPs	Chitosan NPs
	Sho	ot Length/cm	R	oot Length/cm
•	۸,٦٨ ^b	۹,.٤ ^{ab}	۸,۱۰ ^a	Λ,0Υ a
۳.	V,70 d	1,77 °	0,79 c	٦,١٦ °
٦.	٧,٣٤ d	9,20 a	0,11 d	٦,٩० b
۹.	٧, ٤٨ d	7,07 e	٦,•Y ^с	٦.٧٩ b
	Seedling length/c	em	Shoot Fresh We	ight/mg
•	17,V£ ab	۱۷,٦١ a	07, ab	01,71 a
۳.	18,08 d	۱٦, ٤٢ ^b	٤٧,٦٥ bc	09,77 a
٦٠	17,07 e	۱٦,٤١ b	07,7° a	20,77 c
۹.	۱٤, ۸٦ °	۱۳,۳۲ d	07,71 a	02, ab
	Root F	resh Weight/mg	Seedlin	g Fresh Weight/mg
•	۸٦,.º a	AT, TY ab	127,88 a	1 £ 1, TT a
۳.	o.,d	٦٣,٠١ ^{cd}	97, b	177,7V a
٦٠	٦٩,٦٤ ^{abc}	01,d	177,•1 a	۹٦,•٤ ^b
۹.	77,77 bcd	٧٨,٦٣ abc	178,00 a	187,.9 a
	Shoot dry weight	(mg)	Root Dry Weigh	nt/mg
•	۸,۳۰ ^b	9,b	V, Y) bc	V,V. b
۳.	0,87° c	۹,b	٦,•• d	A, ÉV a
٦.	9, b	۸,۰۱ b	٦,٨٢ ٢	0,18 d
۹.	Л,70 b	۱., a	Y,70 b	V,0. b
	Coleoptile Dry W	/eight/mg	Seedling Dry W	eight/mg
•	٤,07 bc	٤,٢. cd	10,77 0	17, 71 abc
۳.	۳,1. e	£,A£ ab	11,77 e	17,77 a
٦٠	٤, ۸۱ ab	٤,.٣ ^d	10,7. bc	۱٤,•۱ d
۹.	2,07 bc	0,19 a	۱٦,٦٤ ab	۱۷,•۷ ^a
	Seed Vigor Index	x I/cm	Seed Vigor Inde	ex II (mg)
•	1877,88 d	۱۳۰۲, ۸۹ d	17 bc	1777,77 ab
۳.	1377,7£ d	1771, T. a	117.,.1 f	1777,77 a
٦.	1197,70 e	17.0,7V b	۱۳٦٣,٣٣ e	۱٤٨., d
۹.	1207,77 °	1727,72 b	1057,77 cd	۱٦٧.,.٤ ab

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					by peo ana		01111001		ene er mres

Means by the uncommon letter in each row and column are significantly different according to Duncan tests $(p < \cdot, \cdot \circ)$.

Conclusion

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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.

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