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RESEARCH ARTICLE

Nano chitosan-NPK fertilizer enhances the growth and productivity of wheat plants grown in sandy soil

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Abstract

Nanofertilizers have become a pioneer approach in agriculture research nowadays. In this paper we investigate the delivery of chitosan nanoparticles loaded with nitrogen, phosphorus and potassium (NPK) for wheat plants by foliar uptake. Chiotsan-NPK nanoparticles were easily applied to leaf surfaces and entered the stomata via gas uptake, avoiding direct interaction with soil systems. The uptake and translocation of nanoparticles inside wheat plants was investigated by transmission electron microscopy. The results revealed that nano particles were taken up and transported through phloem tissues. Treatment of wheat plants grown on sandy soil with nano chitosan-NPK fertilizer induced significant increases in harvest index, crop index and mobilization index of the determined wheat yield variables, as compared with control yield variables of wheat plants treated with normal non-fertilized and normal fertilized NPK. The life cycle of the nano-fertilized wheat plants was shorter than normal-fertilized wheat plants with the ratio of 23.5% (130 days compared with 170 days for yield production from date of sowing). Thus, accelerating plant growth and productivity by application of nanofertilizers can open new perspectives in agricultural practice. However, the response of plants to nanofertilizers varies with the type of plant species, their growth stages and nature of nanomaterials.

Additional key words: *Triticum aestivum*; nanofertilizer; crop index

Abbreviations used: CRF (controlled-release fertilizers); CS (chitosan); CS-PMAA-NPK (chitosan polymethacrylic acid nano particles loaded with nitrogen, phosphorus and potassium); EL (electrolyte leakage); PMAA (poly methacrylic acid); SRF (slow release fertilizers); TEM (transmission electron microscopy)

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Introduction

Chitosan is a natural polymer derived from deacetylation of chitin, which may be obtained from crustaceans, insects, fungi, etc. (Boonsongrit *et al*., 2006). A positive effect of chitosan has been observed on the growth of roots, shoots and leaves of various plants including gerbera (Wanichpongpan *et al*., 2001) and several crop plants (Chibu & Shibayama, 2001). However, Walker *et al*. (2004) conducted some trials on chitosan in organic and conventional crops with variable results. While chitosan application resulted in yield increases of nearly 20% in two out of three tomato trials, no significant difference in yield of treatments in the organic carrot trial or in average weight of individual carrots was found. Walker *et al*. (2004) did not find either significant differences among cucumber, capsicum, beet-root or pea plants from any treatment; however, the chitosan foliar treatment had a tendency for greater yield than the other treatments.

Most slow release fertilizers (SRF) are chemical compounds that are only slightly soluble in water or are slowly broken down by microbial action (Sartain *et al*., 2004). On the other hand, controlled-release fertilizers (CRF) are soluble fertilizers coated with materials that limit exposure of the soluble material to water and/or release of the resulting nutrient solution by diffusion. Thus, the rate of nutrient liberation from SRF is related to their water solubility, microbiological degradation, and chemical hydrolysis (Morgan *et al*., 2009).

Xiao *et al*. (2008) demonstrated that NO_3^--N leaching was decreased by applying SRF coated with nanomaterials in a rotation of wheat-maize. Liu *et al*. (2009) indicated increases in grain yields of rice (10.29%), spring maize (10.93%), soybean (16.74%), winter wheat (28.81%) and vegetables (12.34-19.76%) after applying fertilizer together with nano-materials. As reported by Liu *et al*. (2007), nano-materials could promote germination and rooting early for rice seeds and seedings and the growth of rice at tillering stage was affected obviously by nano-composites. They indicated that the grain yield of rice and nitrogen agronomic utilization efficiency was increased after applying nano-carbon-incorporated SRF.

The objective of the present study was to examine the effects of nano chitosan-NPK application on growth and productivity responses of wheat plants grown on sandy soil.

Material and methods

Preparation and characterization of nano chitosan NPK fertilizers

Chitosan poly-methacrylic acid (CS-PMAA) nanoparticles were obtained by polymerization of methacrylic acid (MAA) in chitosan (CS) solution in a two-step process according to Hasaneen *et al*. (2014). Nitrogen, phosphorus and potassium (NPK) were loaded on the CS-PMAA nanoparticles using the following concentrations 500, 60, 400 ppm respectively (100% concentration stands for 500 ppm of N, 60 ppm of P and 400 ppm of K in both nano and normal NPK solutions and other concentrations were made from these stock solutions). All chemicals used were purchased from Sigma Aldrich, Germany.

Plant material and growth conditions

Two experiments were carried out in order to compare growth, development, life span and translocation of chitosan nano-sized NPK fertilizer in two fertilized treatments of wheat with a bulk material NPK (normal fertilizer) (Sigma Aldrich, Germany) or with nanoengineered composite NPK fertilizer (CS-PMAA-NPK) grown on sandy soil. Sandy soil was used as it is a relatively poor type of soil (in case of nutrients), so plants will depend mainly on the fertilizers applied to support their growth.

The first experiment was conducted under outdoor conditions, from November 2012 to April 2013. For confirmation of the results obtained from this experiment, the second experiment of exactly similar design was conducted in the next season in the period from November 2013 to April 2014. Both experiments were carried out with sandy soil, obtained from the Agriculture Research Station of Mansoura, Dakahlia Governorate, Egypt.

Uniformly sized grains of pure strain wheat (*Triticum aestivum*) L. cv. Egypt-1 were kindly supplied by the Agriculture Research Center, Ministry of Agriculture, Giza, Egypt. The grains were washed with tap water and then planted in sandy soil, in pots (30 × 28 × 26 cm). All pots contained equal amounts of homogenous soil (8 kg). We used 14 pots for each treatment and for the control.

Fertilization of wheat plants was carried out 21 days after planting. The appropriate amount (20 mL) of either bulk material (normal) NPK fertilizer or nanofertilizer (CS-PMAA-NPK) was added. The desired concentrations of aqueous NPK solution and NPK-nanoparticle suspension were foliary sprayed by the solution dropping method. The mean leaf area of all plants was 8.20 cm^2 when foliar fertilization started. During application of normal and nanofertilizers the pot surface was covered by plastic cover to prevent redundant bulk material or nanoparticles from entering into the soil system. Foliar application of different fertilizers was done three times at three weeks intervals. All pots were irrigated with tap water, if required, to maintain the soil at the field capacity throughout the experiment. For clarity the following treatments (foliar sprays) were used: C, Control; NPK 10, 10% normal NPK; NPK 25, 25% normal NPK; NPK 100, 100% normal NPK; Nano 100, 10% nano NPK; Nano 25, 25% nano NPK; Nano 100, 100% nano NPK.

Samples representing the adult, reproductive and yield stages were taken after 46, 71 and 96 days from the date of planting, respectively. In each treatment, samples were leaves from plants taken from 2 pots. The allotted samples were used for determination of growth and yield variables, measurement of electrolyte leakage and for assessment of uptake and translocation of nanofertilizer in wheat tissue by transmission electron microscope (TEM).

Measurements

Root length, shoot length, fresh weight, dry weight, water content and leaf area were determined to evaluate the sequence of growth characters of the different treated wheat plants throughout the entire period of the experiment.

Shoot length, spike length, plant height, number of spikelets/main spike, grain number/main spike, 100 kernel weight, grain yield/plant, straw yield/plant, and crop yield/plant were determined to evaluate the sequence of yield characters of wheat plants in response to the administered normal NPK fertilizer (bulk material fertilizer) and chitosan–NPK nanofertilizer (CS-PMAA-NPK). Life span was identified and calculated as the period from the date of sowing until harvest stage of the plant. Furthermore, harvest index, crop index and mobilization index were calculated to evaluate the efficiency of crop production of wheat plants under the present set of experimental conditions:

$$\text{Harvest index} = \frac{\text{Grain yield}}{\text{Straw yield}} \text{ (Beadle, 1993).}$$

$$\text{Crop index} = \frac{\text{Grain yield}}{(\text{Grain yield} + \text{Straw yield})} \text{ (Beadle, 1993).}$$

$$\text{Mobilization index} = \frac{\text{Crop index}}{\text{Straw yield}} \text{ (Ray \& Choudhuri, 1980).}$$

To determine electrolyte leakage, plant leaves were cut into discs (10 mm) and 20 leaf discs were placed in a 50 mL glass test tube, rinsed 3 times with 20 mL distilled water. Tubes were filled with 30 mL distilled water and left in dark for 24 h at room temperature. Electrical conductivity (EL) of the solution was measured at the end of incubation period (EC_1) using EC meter (Shi *et al*., 2006). The tubes were then heated in water bath at 95°C for 20 min and then cooled to room temperature (EC_2). The final EC was measured as following: $EC = (EC_1 / EC_2) \times 100$

To verify the presence of the absorbed chitosan-NPK nanofertilizer inside wheat plants, a TEM analysis of the treated plant leaves was performed. Plant samples were collected after applying chitosan-NPK nanoparticles for 10 days. Small parts (~ 1 mm^2) of freshly harvested leaves were cut with a sharp razor blade under 2.5% (v/v) glutaraldehyde. Leaf tissues were transferred to vials of 2.5% (v/v) glutaraldehyde in 1M phosphate buffer at pH 7.5 at 4°C for 24 h. Following fixation, the specimens were embedded in gelatine capsules and left in an oven at 60°C for 60 h. The gelatine capsules were dissolved in boiling water for 1-2 h.

Ultra-thin sections were cut on a Reichert ultramicrotome using glass knife. Silver or pale gold interference sections were picked up on the dull surface of formvar-coated 100 or 200 mesh copper grids (Juniper *et al*., 1970) and the grids were left on a clean filter paper to dry. Ultra-thin sections were stained by 2% aqueous uranyl acetate (Juniper *et al*., 1970). A drop of stain was put in a clean plastic Petri dish and the grids were gently floated, with the sections facing down the drop. Grids were washed by a stream of distilled water and then were transferred to drops of lead citrate (Reynolds, 1963) which were placed on a wax plate in a Petri dish. Pellets of sodium hydroxide were placed in the Petri dish to remove carbon dioxide. The grids were left in lead citrate for 10-20 min and then rinsed by distilled water, dried under a bench lamp and stored in a grid box. The stained sections were examined and photographed with a JEOL 1010 transmission electron microscope at 80 kV (Regional Center for Mycology and Biotechnology - RCMB, Al-Azhar University, Cairo, Egypt).

Statistical analysis

Experimental data were subjected to one-way analysis of variance (ANOVA) with Post Hoc LSD (least significant difference) test. A p value <0.05 was accepted as statistically significant. Statistical analysis was performed using package SPSS, v 13.0, Chicago, IL, USA).

Results

Treatment of wheat plants with either NPK normal fertilizer or nanocomposite NPK fertilizer led to significant progressive increase in all growth variables (root length, shoot length, fresh weight, dry weight, water content and leaf area), determined throughout the adult and reproductive growth and developmental stages.

At all experimental stages, the values of the different growth variables were higher in nanofertilizer-treated plants than in normal fertilizer-treated plants. The following sequence of treatments (Nano 10 > Nano 25 > Nano 100 > NPK 100 > NPK 25 > NPK 10 > C) was displayed with respect to wheat plants grown on sandy soil throughout the entire period of the experiment (see Fig. 1 a-f).

Examination of the results revealed that the life span of the control and normal NPK fertilized wheat plants grown on sandy soil reaching harvesting stage after 170 days from the date of sowing. On the other hand, wheat plants grown on sandy soil and fertilized with chitosan-NPK nanofertilizers reached the harvesting stage after 130 days from the date of sowing. Thus, in this connection and of particular interest, it is worthy to mention that nanofertilizers treatment resulted in the reduction of life span of wheat crop by 23.5% from the normal life span of respective crop (Table 1).

Table 2 shows the changes of the above mentioned yield variables of wheat plants cultivated on sandy soil under the different treatments. As compared with control values, treatment of wheat plants grown on sandy soil with normal and nano-NPK fertilizer induced

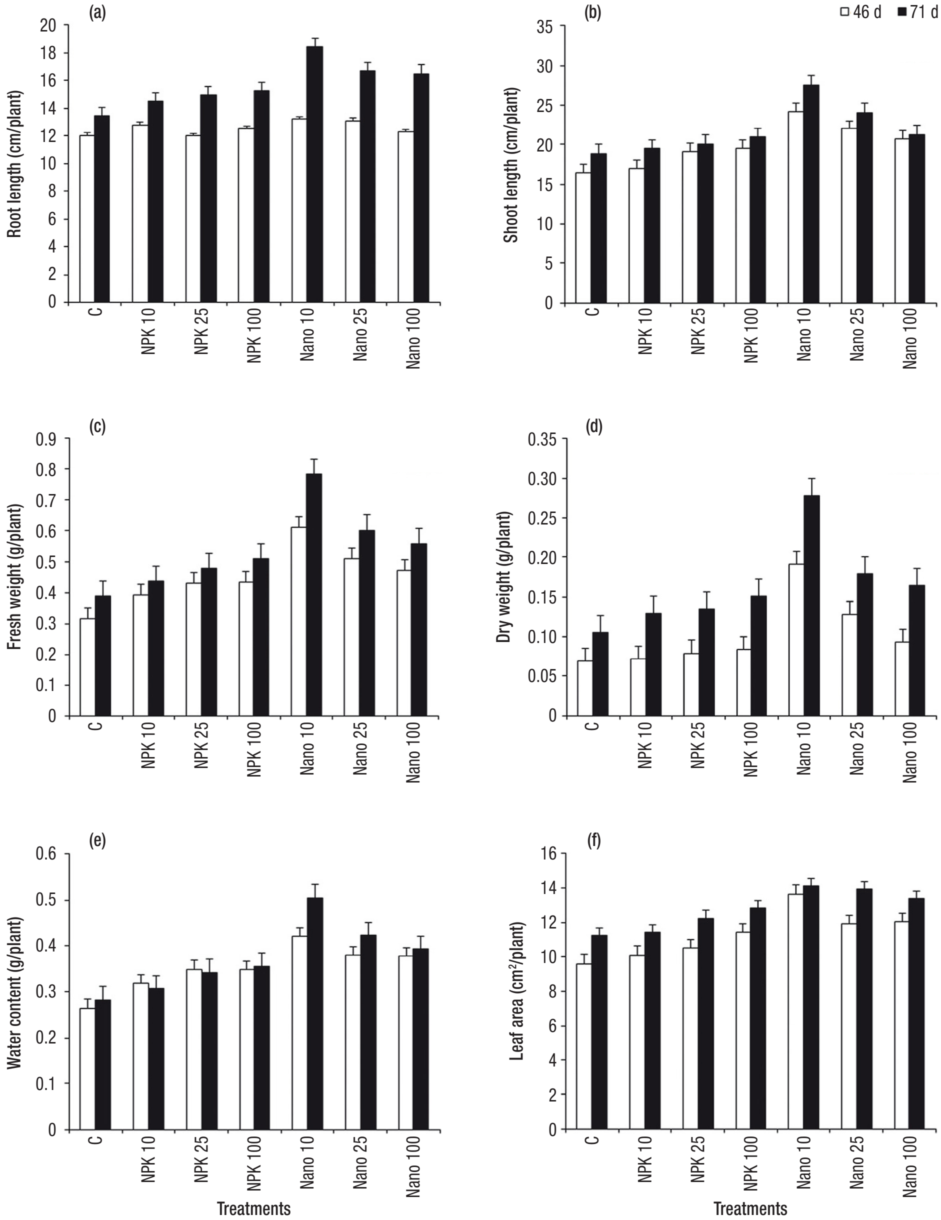

Figure 1. The effect of normal and nanofertilizers on (a) root length, (b) shoot length, (c) fresh weight, (d) dry weight, (e) water content and (f) leaf area of wheat plants grown on sandy soil, 46 and 71 days after sowing. Vertical bars represent the standard error (± S.E.).

significant increases in all yield variables determined. The following sequence of treatments (Nano 10 > Nano 25 > Nano 100 > NPK 100 > NPK 25 > NPK 10 > C) was found for all the yield variables except for number of spikelets/main spike, for which the following sequence of treatments was Nano 10 > Nano 25 > NPK 100 > Nano 100 > NPK 25 > NPK 10 > C. For harvest index, the sequence of treatments was: Nano 25 > Nano 100 > Nano 10 > NPK 10 > C > NPK 100 > NPK 25. And for mobilization index: Nano 10 > Nano 100 > Nano 25 > NPK 25 > NPK 100 > NPK 10 > C. In the case of crop index, all values were the same (0.96) except for NPK 25 which was 0.95.

The observed changes in electrolyte leakage from variously treated wheat plants, throughout the entire period of the experiment are presented in Fig. 2.

Under the present set of experimental conditions, the application of normal NPK fertilizers and nano-NPK fertilizers with increasing concentrations to wheat plants grown on sandy soil throughout the entire period of experiment appeared, in general, to significantly decrease the leakage of ions from the differently treated plants below those of control levels; the response being more operative with the nano-NPK fertilizers.

The following sequence of treatments (Nano 10 > Nano 25 > Nano 100 > NPK 100 > NPK 25 > NPK 10

Table 1. Effects of bulk material NPK fertilizer and nano-engineered composite NPK fertilizer (CS-PMAA-NPK) on life span of wheat plants grown on sandy soil.

Treatments	Life span	
	Days	Changes[1]
Control	170	100
Normal NPK fertilizers	170	100
Nano-NPK fertilizers	130	23.5

[1] Reduction of the life span period in % (100% stands for complete normal life span).

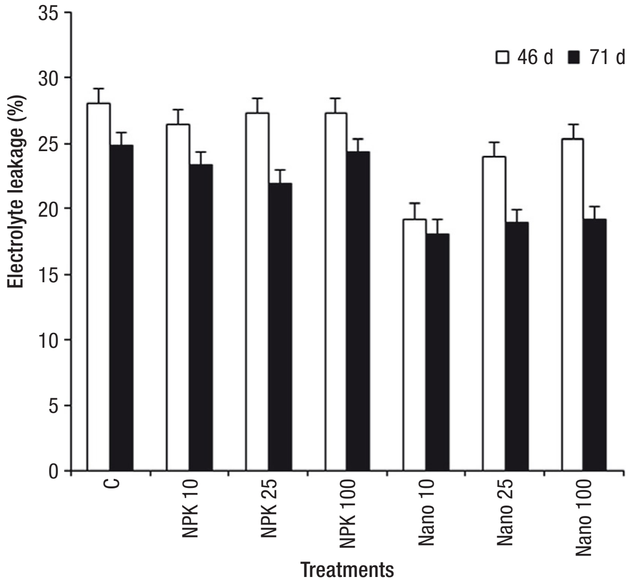

Figure 2. The effect of normal and nanofertilizers on electrolyte leakage of wheat plants grown on sandy soil. Vertical bars represent the standard error (± S.E.).

> C) was displayed with respect to decrease in electrolyte leakage.

Figures 3 and 4 revealed that in all chitosan-NPK nanofertilizer-treated wheat plants, nanoparticles were observed inside the phloem tissue, especially in sieve tubes. After entering the stomata, these nanoparticles were translocated by the phloem system. The phloem consists of living vascular tissues that translocate photosynthetic products including sucrose, protein and some mineral ions for plant growth. The diameter of the nanoparticles present inside sieve tubes showed a mean diameter varying between 26.2 and 30.6 nm. No nanoparticles of chitosan NPK fertilizer were detected in xylem vessels in all samples treated with increasing concentrations of nanoparticles chitosan NPK fertilizer and grown on sandy soil (see Fig. 4).

Discussion

The application of a nano-engineered composite consisting of N, P, K micronutrients, mannose and amino acids enhance the uptake and use of nutrients by grain crops (Jinghua, 2004). In addition, nanofertilizers will combine nanodevices in order to synchronize the release of fertilizer-N and -P with their uptake by crops, so preventing undesirable nutrient losses to soil, water and air via direct internalization by crops, and avoiding the interaction of nutrients with soil, microorganisms, water, and air (De Rosa *et al.*, 2010).

The results herein reported show that foliar application of either normal or nanofertilizer at different concentrations to wheat plants, induced marked significant variable increases in all growth variables determined at fully vegetative and reproductive growth stages. The magnitude of increased growth variables was most pronounced with 10% nano-NPK. Auffan *et al*. (2009) stated that unlike macronutrients nanomaterials have particular properties, such as surface effect, volume ef-

Table 2. Effects of bulk material NPK fertilizer and nano-engineered composite NPK fertilizer (CS-PMAA-NPK) on yield variables of wheat plants grown on sandy soil.

Yield variables	Control	NPK 10	NPK 25	NPK 100	Nano 10	Nano 25	Nano 100
Shoot length (cm)	30.43	31.66*	32.43*	32.86*	43.56*	38.36*	35.06*
Spike length (cm)	5.80	5.90	5.96	5.99*	7.76*	6.40*	6.23*
Plant height (cm)	36.23	37.56	38.39*	38.85*	51.32*	44.76*	41.29*
Main spike wt. (g)	0.125	0.130	0.135	0.136	0.305*	0.185	0.178
No. of spikelets/main spike	3.00	3.33*	3.75*	4.25*	5.50*	4.33*	4.00*
100 kernel wt. (g)	3.30	3.66*	3.69*	3.93*	4.64*	4.03*	3.98*
No. of grains/ main spike	4.00	4.50*	4.80*	5.25*	8.66*	6.40*	5.78*
Grain yield/plant (g)	2.75	2.83	2.85	3.03*	4.28*	4.10*	3.88*
Straw yield/plant (g)	0.127	0.130	0.136	0.140	0.163	0.151	0.144
Crop yield/plant (g)	4.37	5.02*	5.97*	6.13*	8.28*	7.23	6.95*
Harvest index	21.65	21.76	20.59*	21.64	26.25*	27.15*	26.94*
Mobilization index	33.86	38.62*	43.90*	43.79*	50.80*	47.88*	48.26*
Crop index	0.96	0.96	0.95	0.96	0.96	0.96	0.96

* Mean values are significantly different from control at $p \leq 0.05$.

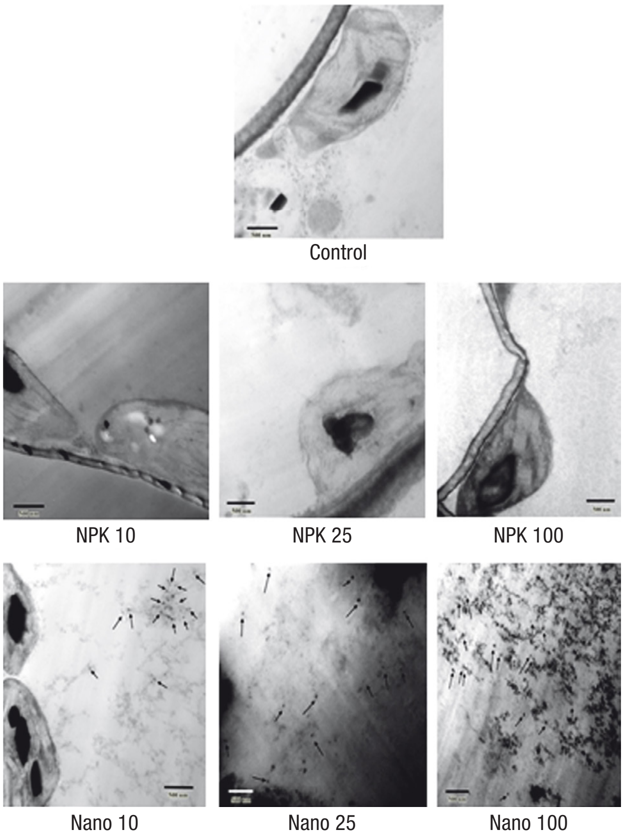

Figure 3. TEM micrograph of phloem tissue of leaves of wheat plants treated with bulk material NPK fertilizer and nano-engineered composite NPK fertilizer grown on sandy soil. Bar: 500 nm. Arrows indicate presence of nano particles.

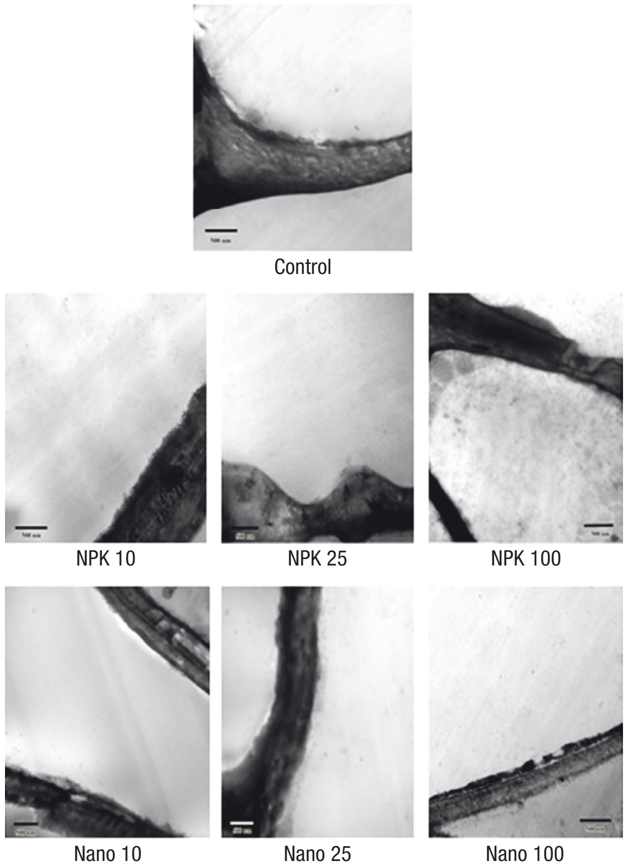

Figure 4. TEM micrograph of xylem tissue of leaves of wheat plants treated with bulk material NPK fertilizer and nano-engineered composite NPK fertilizer grown on sandy soil. Bar: 500 nm.

fect and quantum size effect and so on. Previous studies have investigated the absorption and uptake of nanomaterials by plants and mainly focused on their dubious adverse effects (Lee *et al*., 2008; Rico *et al*., 2011).

Nevertheless, Zheng *et al*. (2005) and Lin *et al*. (2009) found that nanomaterials can enhance crop seed germination and promote plant growth. For example, multi-walled carbon nanotubes (10-40 µg/mL) can penetrate seed coats, stimulate germination and enhance growth of tomato plant (Khodakovskaya *et al*., 2009).

The present results concerning the increased growth variables of wheat plants as influenced by foliar application of nanocomposite-NPK nanoparticles, in particular at low concentration (10%), and grown on sandy soil can be explained on the basis that the sprayed nanocomposite-NPK nanoparticles may got absorbed through the stomata of wheat leaves and be translocated in the plant. The selective uptake, biotransformation, and translocation of various nanoparticles by a model plant have been schematically represented (Dhoke *et al*., 2013). Nanoparticles have high reactivity because of more specific surface area, more density of reactive areas, or increased reactivity of these areas on the particle surfaces. These features in nano-scale simplify their absorption in plants (Dhoke *et al*., 2013). Mahmoodzadeh *et al*. (2013) reported that direct exposure of wheat plants to specific types of nanoparticles cause significant increase in all growth variables determined at optimum concentrations of nanosolution. The application of carbon nanoparticles promoted tobacco plant growth at the resettling growth stage, vigorous growth stage and maturity stage compared with conventional fertilizer (Liang *et al*., 2013). Furthermore, nanoparticles have high surface energy and activated properties. Based on studies on nanoparticles effects on seed germination mechanism, Lu *et al*. (2002), Lei *et al*. (2008) and Feizi *et al*. (2012) concluded that nanoparticles increased water absorption by the seeds; increased nitrate reductase enzyme concentration; promoted seed antioxidant system; reduced antioxidant stress by reducing H_2O_2, superoxide radicals, and malonyldialdehyde content; and increased some enzymes such as superoxide dismutase, ascorbate peroxidase, guaiacol peroxidase and catalase activities, which improved seed germination in some plant species.

Grain yield rice was improved significantly after applying slow-released nanofertilizer by 11.3% compared normal fertilizers (Wu, 2013). The present results indicated that all yield variables of wheat plants treated with increasing concentrations of nanocomposite NPK fertilizer (CS-PMAA-NPK), 11.06% for shoot length, 37.5% for spike length, 15.43% for plant height, 124.64% for main spike weight, 26.98% for number of spikelets/main spike, 83.03% for 100 kernel weight, 26.69% for number of grains/main spike, 101.85% for grain yield/plant, 23.26% for straw yield, 89.37% for crop yield, 63.82% for harvest index, 37.21% for mobilization index and 6.66% for crop index. The possible reasons are: (i) nano-NPK promotes the plant to absorb the water of soil and nutrients, then the photosynthesis is improved (Wu, 2013); (ii) nano-NPK is considered the biological pump for the plants to absorb nutrients and water (Ma *et al*., 2009). As reported by Liu & Liao (2008), the activity of water after adding nano-materials was increased and N, P and K were absorbed by the plants along with the absorbed water, thus the production was also increased.

Furthermore, Moosapoor *et al*. (2013) reported significant effects on the yield of fresh seeds, yield of dry seed, the number of seeds/bush, the number of green pods, the number of mature pods, the number of pods/bush, the yield of pod, total biomass, harvest index and the weight of 100 seeds of pea nut plants treated with Bohr nanofertilizer.

As mentioned in the results section, treatment of wheat plants grown on sandy soil with increasing concentration of either normal (bulk NPK fertilizer) or nanocomposite NPK fertilizer, throughout the experimental growth stages induced significant valuable decrease in electrolyte leakage from treated plants; the magnitude of decreased leakage was more operative with nanofertilized plants (see Fig. 2).

Normal and nanofertilizers appeared to reduce the amount of malonyldialdehyde and ion leakage in treated wheat plants grown on clay, clay-sand or sand soils throughout the entire period of experiment, as reported by Oancea *et al*. (2009), who hypothesized that controlled release of active plant growth stimulators and other chemicals encapsulated in nanocomposites made of layered double hydroxides (anionic clay) could be another feasible option for organic agriculture.

Of interest, these results might indicate that nanocomposite NPK fertilizers mitigated the increase in the plasma membrane permeability and cell mortality under nanoparticle effects in wheat plants (Du *et al*., 2011). Similar results were observed by Wang *et al*. (2013) in watermelon plant after foliar uptake of nanocomposite.

Nair *et al*. (2010) observed that the uptake efficiency and the effect of various nanoparticles on the growth and metabolic functions vary among plants. Foliar uptake (uptake through the leaves) of nanoparticles by plants represents another possible way for this purpose. Leaves are important plant organs primarily for photosynthesis, transpiration and gas exchange (Nadakavukaren & McCracken, 1985).

In the present work, intracellular penetration of NPK nanoparticles applied on wheat plants was tracked using transmission electron microscopy (TEM). The fact that cell wall opens up the possible application of these nanotechnology tools for agronomical purpose, given the special characteristic of the epidemic outer cell wall, specially its considerable thickness, a possible nanoparticle penetration point through the stomata and the substomatal chamber (Corredor *et al*., 2009). In fact, this aperture is a route used by pathogens of different species; water-suspended 43 nm hydrophilic nanoparticles have been described as occasionally penetrating *Vicia faba* leaves through stomatal pores (Eichert *et al*., 2008).

As shown in Figs. 3 and 4 plant cell wall acts as a barrier for easy entry of any external agent including nanoparticles into plant cells. The sieving properties are determined by pore diameter of cell wall ranging from 5 to 50 nm (Fleischer *et al*., 1999). Hence, only nanoparticles or nanoparticle aggregates with diameter less than the pore diameter of the cell wall could easily pass through and reach the plasma membrane (Moore, 2006; Navarro *et al*., 2008). There is also a chance for enlargement of pores or induction of new cell wall pores upon interaction with engineered nanoparticles which in turn enhance nanoparticle uptake (Nair *et al*., 2010).

Further internalization occurs during endocytosis with the help of a cavity like structure that form around the nanoparticles by plasma membrane. They may also cross the membrane using embedded transport carrier proteins or through ion channels. In the cytoplasm, the nanoparticles may bind with different cytoplasmic organelles and interfere with the metabolic processes at that site (Jia, 2005).

When nanoparticles are applied on leaf surfaces, they enter through the stomatal openings or through the bases of trichomes and then are translocated to various tissues (Uzu *et al*., 2010). Studies on the mechanism of uptake and formation of nanoparticles within plants have also led to more investigations on the use of plants as source for nanoparticle synthesis (Nair *et al*., 2010).

Du *et al*. (2011) stated that plants are an important component of the soil ecosystem and may serve as a potential pathway for nanoparticle transport and bioaccumulation into the food chain.

Transported compounds need to penetrate through the cell wall prior to membrane invagination. Plants are able to take up nanoparticles from environment and transport them through the vascular system to various shoot systems (Zhu *et al*., 2008; Corredor *et al*., 2009). Navarro *et al*. (2008) and Du *et al*. (2011) also found that nanoparticles induce formation of new larger pores in the plant cell wall to allow the entrance of large nanoparticles.

Only a few studies support the foliar uptake of engineered nanoparticles (*e.g.,* Birbaum *et al*., 2010). The size exclusion limits of the stomatal foliar uptake of water-suspended nanoparticles by *Vicia faba* were analyzed by Eichert *et al*. (2008). Their results suggested that the stomatal pathway is highly capacitive because of its large size exclusion limit above 10 nm and its high transport velocity (Wang *et al*., 2013). For successful foliar uptake, in addition to particle size, other various factors should also be considered such as working environment (light, water and gas), plant species and nanoparticle application methods.

The present work shows that chitosan-NPK nanoparticles entered in the stomata are translocated by the phloem system (Fig. 3). The phloem consists of living vascular tissues that translocate photosynthetic products including sucrose, proteins and some mineral ions for plant growth (Nadakavukaren & McCracken, 1985; Wang *et al*., 2013). The nanoparticles are carried in this sugar flow through the phloem sieve tubes to shoots and roots as a result of pressure differentials between source (leaves) and sink (*e.g.,* growing shoot apex) based on mass flow or pressure flow hypothesis, which explains the presence of chitosan-NPK nanoparticles inside the phloem tissue of wheat plants and their absence in the xylem tissue. The observed results indicate that phloem tissue is the main and unique pathway for translocation of nanoparticles and in consequence, confirm the penetration of plant leaves and lead to a strong support to the observed changes in growth, development and life span of wheat plants affected by nano-NPK fertilizers.

In conclusion, considering the results obtained in this study, the Nano 10 fertilizer shows the best growth results of all nanofertilizers used. Thus, accelerating plant growth and productivity through the application of nanofertilizers can open new perspectives in agricultural practices, because nanofertilizers promise to be a safe way to enrich nutrients to plants without doing harm to the environment. Nevertheless, further field studies are needed to study the effect of such concentration on growth and metabolism of wheat plants and to ensure the safety of the nano-treated plants for the use of animals and humans.

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