Effect of Chitosan Loaded NPK Nanoparticles and Conventional Fertilizers on Wheat Plant Growth Parameters and Production Under Sandy Soil Conditions

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Received :24/7/2024

Abstract: The current study was carried out to investigate the effect of chitosan nanoparticles and loaded N, P and K chitosan nanoparticles on wheat plant growth parameters and productivity. A field experiment was conducted during the winter season 2021/2022 cultivated with wheat (*Triticum aestivum* L.) on sandy soil at the experimental unit of the Faculty of Agriculture farm- Suez Canal University- Ismailia- Egypt. The obtained results indicated that application of chitosan nanoparticles resulted in 25% reduction of the life span of wheat plant. This effect could be related to the capacity of nanochitosan to improve cellular division through the modulation of genes responsible for auxin production. These results may be indicated that the application of foliar chitosan NPs. - loaded nitrogen in different concentrations and chitosan loaded NPK nanoparticles has been employed to enhance plant growth and productivity. Generally, the following sequence was observed of treatments (nano-chitosan NPK fertilizers> conventional NPK fertilizer > nanochitosan > control (except for the root length).

Keywords: *Chitosan NPK - Nano fertilizers – Wheat – Soil Fertility*

INTRODUCTION

Nanofertilizers are defined as materials in the nonmetric scale, usually in the form of nanoparticles, containing macro and micronutrients that are delivered to crops in a controlled mode (DeRosa *et al.,* 2010; Adisa *et al.,* 2019; Shang *et al.,* 2019). Nano-fertilizers enhance growth parameters (plant height, leaf area, number of leaves per plant) dry matter production, chlorophyll production, rate of the photosynthesis which result more production and translocation of photosynthesis to different parts of the plant compare with traditional fertilizers (Ali and AlJuthery, 2017; Singh *et al.,* 2017). Wheat growth parameters, yield parameters and yield were improved by foliar application with combination of N, P and K nano-fertilizers at lower concentrations (Abdel-Aziz *et al.,* 2016). The objective the current stud was aimed to determine the effect of foliar feeding of Chitosan nanoparticle loaded NPK fertilizer and combination of traditional fertilizer compare with control on wheat yield under sandy soil conditions.

MATERIALS AND METHODS

-The comparison between chitosan nanoparticles (Chitosan NPs) loaded NPK and the conventional fertilizers:

To study the effect of chitosan nanoparticles and loaded N, P and K chitosan nanoparticles on wheat growth and productivity. A field study was conducted during the winter season 2021/2022 cultivated with wheat (*Triticum aestivum* L.) on sandy soil at the experimental unit of the Faculty of Agriculture farm- Suez Canal University- Ismailia-Egypt. The treatments were: control (without any fertilizers), chitosan NPs., chitosan NPs. loaded NPK (500-50-60ppm) and conventional fertilizers (178kg.ha-1 N, 71 kg. ha-1 P2O5 and 119 kg. ha-1 K2O). The treatments were arranged in a randomized complete block design having four replicates using flood irrigation (Basin irrigation) system was used each plot was 2m2.Treatments of wheat plants with conventional fertilizers or NPK chitosan nanoparticles solutions were carried out after 15 days of the planting date. All treatments were foliarly sprayed at wheat plants and applied to plants every two weeks intervals.

-Preparation of chitosan nanoparticles:

Amodified ionic gelation method for producing chitosan nanoparticles was described by (Gan *et al.,* 2005) and (Ha *et al.,* 2019).

-NPK Fertilizers loading with chitosan nanoparticles:

Urea, calcium phosphate, and potassium sulphate were used as sources of N, P, and K, respectively. Each substance was used individually. To load these NPK fertilizers into chitosan nanoparticles, the appropriate amount of NPK was dissolved into 50 ml of chitosan nanoparticle solution under magnetic stirring for 8 hours at 25 Co. This resulted in final concentrations of 500 ppm of N, 60 ppm of P, and 400 ppm of K in each solution. The final solutions had pH of 4.5 (Hasaneen *et al.,* 2014).

-Characterization of nanoparticles:

Transmission electron microscopy (TEM) was used to determine the morphology and size of the nanoparticles. For TEM analysis, just a drop of the nanoparticle solution is spread on a carbon-coated copper grid and dried. The grid is subsequently loaded into the TEM, and images are captured using a high-energy electron beam. The data were analyzed using one-way Anova with the origin software.

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RESULTS AND DISCUSSION

-Growth parameters:

The changes in wheat growth parameters, differently treated with conventional NPK fertilizer and nano chitosan- NPK fertilizer in sandy soil are recorded at **(**Table **1)**. The results showed that the root length is generally higher in the conventional fertilizers (13.66 cm), nano chitosan NPK fertilizer, chitosan nanoparticles, and treatments compared to the control treatment. The highest shoot length (84.13 cm) was observed at the nano chitosan NPK treatment. The data showed that the conventional Fertilizers treatment has a higher shoot length of 82.15 cm, showing a significant improvement compared to the control. According to the findings, the shoot length for Chitosan Nanoparticles treatment was 76.93 cm, which is higher than the control but lower than the conventional fertilizers. Also, the results at **(**Table **1)** showed that plant height has a similar pattern of shoot length. The nano chitosan NPK treatment showed the highest plant height (97.21cm), followed by conventional fertilizers, chitosan nanoparticles then control. With regard to the data showed that the nano chitosan NPK treatment have highly significant spike length (10.15cm), Number of spikelet / main spike (17.30) and grain number/main spike (41.53) more than the conventional fertilizers treatment. The control treatment has the lowest values of spike length, number of spikelet / main spike and grain number/main spike. There was no significant differences between treatments on the 1000 grains weight. In general, the following sequence was observed of treatments (nano-chitosan NPK fertilizers > conventional NPK fertilizer > nanochitosan > control) except for the root length, the sequence of treatments was (conventional NPK fertilizer > nanochitosan NPK fertilizers > nanochitosan>control). The nano chitosan NPK treatment significantly improved straw yield compared to conventional fertilizers and chitosan nanoparticles **(**Table **1)**. The control group showed the lowest straw yield at 3433 $kg.ha^{-1}$. The application of chitosan nanoparticles resulted in a significantly higher straw yield of 4705 $kg.ha^{-1}$, compared to 3433 $kg.ha^{-1}$ for the control. Also, the results indicate that the use of conventional fertilizers and nano chitosan NPK may significantly improve the production of straw compared to the control. The nano chitosan NPK treatment showed a significant increase in straw production ($kg.ha^{-1}$) compared to the control, with a percentage increase of 111.59%. The results indicated that the straw yield ($kg.ha^{-1}$) increased by 51.84% with conventional fertilizers, and by 37.05% with chitosan NPs compared to the control.

The study compared conventional fertilizers and chitosan nanoparticles, finding no significant difference in grain yield as shown at **(**Table **1)**. However, a significant difference was observed between the control and Nano chitosan NPK treatments. The chitosan nanoparticles treatment resulted in a grain yield of 7966 $kg.ha^{-1}$, while the control and conventional fertilizers treatments showed no significant difference. The nano chitosan NPK treatment resulted in a grain yield of 8414 $kg.ha^{-1}$, indicating a significant difference between the other treatments. The nano chitosan NPK treatment showed a significant increase in grain production ($kg.ha^{-1}$) in contrast to the control treatment, with a percentage increase of 97.46%. The results indicated that the grain yield ($kg.ha^{-1}$) increased by 35.27% with conventional fertilizers, and by 86.95% with chitosan NPs compared to the control. Regarding to biological harvest, the application of nano chitosan NPK resulted in the maximum biological yield of 15678 $kg.ha^{-1}$, exceeding the control and chitosan nanoparticles treatments **(**Table **1)**. Chitosan nanoparticles increased biological yield by 12671 $kg.ha^{-1}$ compared to the control, but not significantly different from conventional fertilizers and nano chitosan NPK treatments. The conventional fertilizers treatment yielded 10990 $kg.ha^{-1}$, statistically different from the control but not significantly different from chitosan nanoparticles and nano chitosan NPK treatments, while the control had the lowest biological yield. The nano chitosan NPK treatment showed a significant increase in biological yield ($kg.ha^{-1}$) compared to the control, with a percentage increase of 103.76%. The results indicated that the biological yield ($kg.ha^{-1}$) increased by 42.83% with conventional fertilizers, and by 64.68% with chitosan NPs compared to the control.

In general, the study shows that conventional fertilizers, chitosan nanoparticles, and nano chitosan NPK significantly improve biological yield compared to the control. The nano chitosan NPK treatment resulted in maximum biological yield, demonstrating its capacity to enhance total crop productivity, including grain and straw yield.

Obtained findings could indicate to the highly effective treatment for increasing high grain productivity. This result is in agreement with (Wu, 2013) who found that after using slow-release nano-fertilizer, rice grain yield increased by 11.3% in comparison to conventional fertilizers. These results may be due to that Nano-NPK enhances yield attributes by promoting soil water and nutrient absorption and improved photosynthesis (Wu, 2013). (Abdel-Aziz *et al.,* 2018) found that all yield parameters may be significantly increased by applying various concentrations of conventional fertilizer and nanofertilizers to wheat plants cultivated in clay sandy soils. Also, Abdel-Aziz *et al.,*(2016) reported that foliar application by nanocomposite-NPK nanoparticles at low concentration (10%) to wheat plants grown in sandy soils increased different growth parameters. These findings may

Table (1) Effect of Chitosan loaded NPK nanoparticles and Conventional Fertilizers on wheat plant growth parameters

Treatments	Root length (cm)	Shoot length (cm)	Plant height (cm)	Spike length (cm)	Number of spikelet's / main spike	Grain number / main spike	1000 grains weight (g)	Grain yield kg.ha^{-1}	Straw yield kg.ha^{-1}	Biological yield kg.ha^{-1}	Harvest index	Crop index
Control	11.49^{c}	73.26^{c}	84.76^{c}	5.94^{d}	10.88^{c}	18.03 ^{c}	86.17	4261^{b}	3433^{c}	7694^{c}	1.22^{ab}	0.54^{ab}
Conventional Fertilizers	13.66^{a}	82.15^{ab}	95.81^{ab}	7.58^{b}	13.35^{b}	27.93^{b}	91.31	5777^{ab}	5213^{b}	10990^{bc}	1.11^{b}	0.52^{b}
Chitosan Nanoparticles	12.16^{bc}	76.92^{bc}	89.08^{bc}	6.83^{c}	12.45^{bc}	26.18b^{c}	130.23	7966^{a}	4705^{bc}	12671^{ab}	1.69^{a}	0.61^{a}
Nano chitosan NPK	13.08^{ab}	84.13^{a}	97.21^{a}	10.15^{a}	17.30^{a}	41.53^{a}	92.64	8414^{a}	7264^{a}	15678^{a}	1.18^{ab}	0.54^{ab}

Mean in each column followed by the same letter(s) did not significantly at $p \leq 0.05$

be interpreted on the basis that the sprayed nanocomposite-NPK nanoparticles may have been absorbed through the stomata of wheat leaves and transferred throughout the plant (Dhoke *et al.,* 2013). Additionally, Drostkar *et-al.,* (2016) found that Chickpea growth is impacted by foliar application of NPK nano-fertilizers, which improves yield and yield components. The increase in the activity of growth hormone or the activity of the photosynthetic system could be the reason for such a beneficial role, or it could be due to the active role of these nutrients in metabolic processes of plants and photosynthesis, which tended to increase flowering and grain formation, which ultimately increased yield properties. Also, Al-Juthery *el al.,*(2018) found that the highest value of harvest index of wheat plants was obtained in the super micro plus nano-fertilizer treatment, followed by nano mixture fertilizer (N+P+K) and (N+P) while the lowest at (P+K) (44.96, 43.18, 37.65%) respectively.

- Nutrients Content and uptake:

The results at Table **(2)** and Fig**. (1)** represented the effect of the chitosan loaded NPK nanoparticles and conventional fertilizers on nutrients contents at straw and grains of wheat plants. The data showed that the nitrogen and phosphorus content in wheat straw significantly increased at conventional fertilizers, chitosan nanoparticles and nano chitosan NPK compared to control. On the other hand, potassium content showed the highest value (25.37gkg^{-1}) at nano chitosan NPK treatment. Also, the results showed that nitrogen, phosphorus, and potassium were the highest at nano chitosan NPK treatment compared to control. Moreover, the data showed that there was not a significant difference between nitrogen content in grains at chitosan NPs. and nano chitosan NPK treatments**.** The observed data may be attributed to the high absorption rate of Chitosan NPs by leaves, their ability to pass through the plant via stomata, their subsequent transportation through the phloem, and their contribution to the nutritional needs of various plant parts (Abdel-Aziz *et al.,* 2018). According to the study conducted by Abdel-Aziz *et al.* (2018), there was an observed increase in the potassium element concentration in grains of wheat plants that were fertilized with Nano10 and cultivated on clay-sandy soils.

Table (2): The effect of chitosan loaded NPK nanoparticles and conventional fertilizers on nutrients content in straw and grains of wheat plants

Treatments	Straw			Grains		
	N	P	K	N	P	K
			gkg^{-1}			
Control	6.55^{b}	1.74^{b}	10.77^{c}	13.45^{b}	7.12^{b}	7.76^{c}
Conventional Fertilizers	9.85^{a}	4.57^{a}	16.17^{bc}	17.02^{ab}	7.97^{ab}	10.0^{b}
Chitosan Nanoparticles	10.37^{a}	4.94^{a}	19.97^{ab}	18.45^{a}	8.47^{ab}	10.87^{b}
Nano chitosan NPK	10.15^{a}	3.77^{a}	25.37^{a}	20.25^{a}	8.91^{a}	12.37^{a}

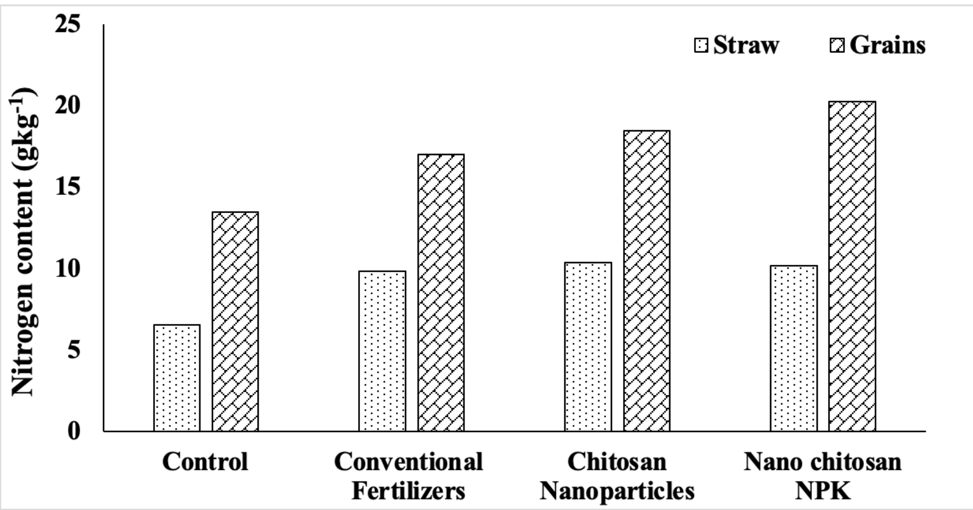

Fig. (1): Effect of chitosan loaded NPK nanoparticles and conventional fertilizers on nitrogen content in straw and grains of wheat plants

The study conducted by (Van *et al.,* 2013) focused on investigating the biophysical properties of chitosan NPs. and their impact on the growth of Robusta coffee in a greenhouse environment. The application of chitosan NPs. resulted in a significant enhancement in the absorption of nitrogen, phosphorous, and potassium, with increases ranging from 9.8% to 27.4%, 17.3% to 30.4%, and 30% to 45%, respectively. Additionally, the utilization of chitosan NPs exhibited a notable influence on the growth of coffee seedlings. The results at **(**Table **3)** represented the effect of the chitosan loaded NPK nanoparticles and conventional fertilizers on nutrients uptake at straw, grains and total uptake of nutrients in wheat plants. The results show that conventional fertilizers, chitosan nanoparticles, and nano chitosan NPK increased straw nutrient uptake for all three of the essential elements compared to the control **(**Fig**.** 2**)**. The application of conventional and chitosan nanoparticles fertilizers resulted in a nitrogen uptake in straw of 50.78 $kg.ha^{-1}$ and 48.9 $kg.ha^{-1}$, respectively, which was higher than that of the control. The straw's nitrogen absorption reached its highest level at 73.5 $kg.ha^{-1}$ when using nano chitosan NPK. The application of nano chitosan NPK treatment resulted in a significant rise in the absorption and accumulation of nitrogen in straw. The use of conventional chitosan nanoparticles (NPs) and nano chitosan NPK fertilizers resulted in a considerable increase in straw phosphorus absorption. amounts recorded were 24.1, 23.2, and 27.07 $kg.ha^{-1}$, respectively, which were much greater compared to the control group. Furthermore, there were no significant differences observed in the phosphorus uptake in wheat straw between the Conventional, chitosan NPs, and nano chitosan NPK fertilizers. Conventional fertilizers increased straw potassium uptake to 84.5 $kg.ha^{-1}$, significantly more than the control group. Chitosan nanoparticles increased straw potassium uptake to 91.9 $kg.ha^{-1}$, greater than that of the control treatment and conventional fertilizers. Nano chitosan NPK treatment increased straw potassium uptake to 188.7 $kg.ha^{-1}$. In general, the results showed that conventional fertilizers, chitosan nanoparticles, and nano chitosan NPK increased nutrient uptake and accumulation in wheat straw, with nano chitosan NPK showing the highest uptake values, indicating its effectiveness. Conventional fertilizers caused grains to take up 97.3 $kg.ha^{-1}$ nitrogen. Nitrogen uptake increased compared to the control group **(**Table **3)** and **(**Fig**.** 3**)**. Chitosan nanoparticles increased grain nitrogen absorption to 151.6 $kg.ha^{-1}$, greater than that of the control treatment and conventional fertilizers. Nano chitosan NPK treatment resulted in the highest grain nitrogen uptake at 196.9 $kg.ha^{-1}$. The highest grain nitrogen absorption and accumulation was with nano chitosan NPK treatment. Conventional fertilizers increased grain phosphorus uptake to 46.8 $kg.ha^{-1}$, A significant increase to the control treatment. Chitosan nanoparticles increased grain phosphorus absorption to 66.6 $kg.ha^{-1}$, greater than that of the control treatment and conventional fertilizers.

Table (3): The effect of chitosan loaded NPK nanoparticles and conventional fertilizers on nutrients uptake in straw and Treatments grains of wheat plants

Treatments	**Straw**			**Grains**			**Total Uptake**		
	N	**P**	**K**	**N**	**P**	**K**	**N**	**P**	**K**
	$Kgha^{-1}$								
Control	22.7^{c}	5.8^{b}	36.4^{b}	56.6^{c}	30.1^{c}	33.1^{c}	79.30^{c}	35.90^{c}	69.50^{c}
Conventional Fertilizers	50.7^{b}	24.1^{a}	84.5^{b}	97.3^{bc}	46.8^{bc}	58.5^{bc}	148.0^{bc}	70.90^{b}	143.0^{b}
Chitosan Nanoparticles	48.9^{b}	23.2^{a}	91.9^{b}	151.6^{ab}	66.6^{ab}	84.7^{ab}	200.5^{ab}	89.80^{b}	176.60^{b}
Nano chitosan NPK	73.5^{a}	27.07^{a}	188.7^{a}	169.9^{a}	75.1^{a}	103.6^{a}	243.4^{a}	102.17^{a}	292.3^{a}

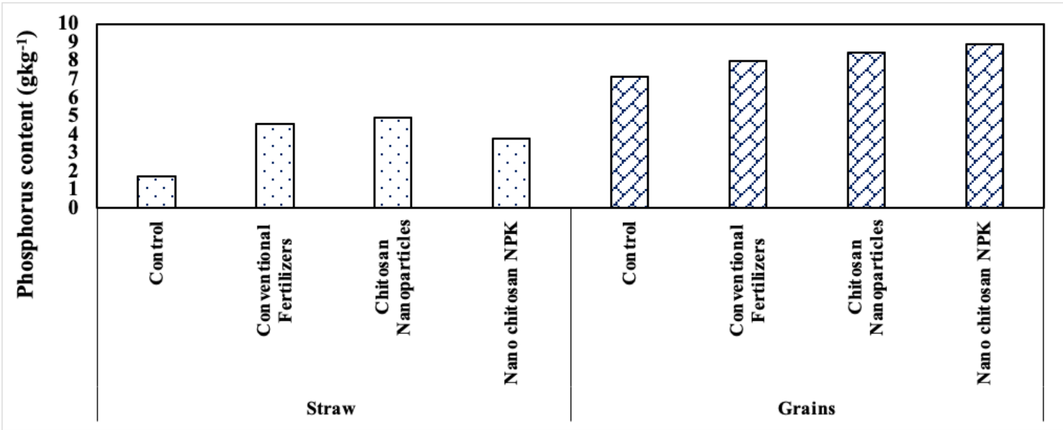

Fig. (2): Effect of chitosan loaded NPK nanoparticles and conventional fertilizers on phosphorus content in straw and grains of wheat plants

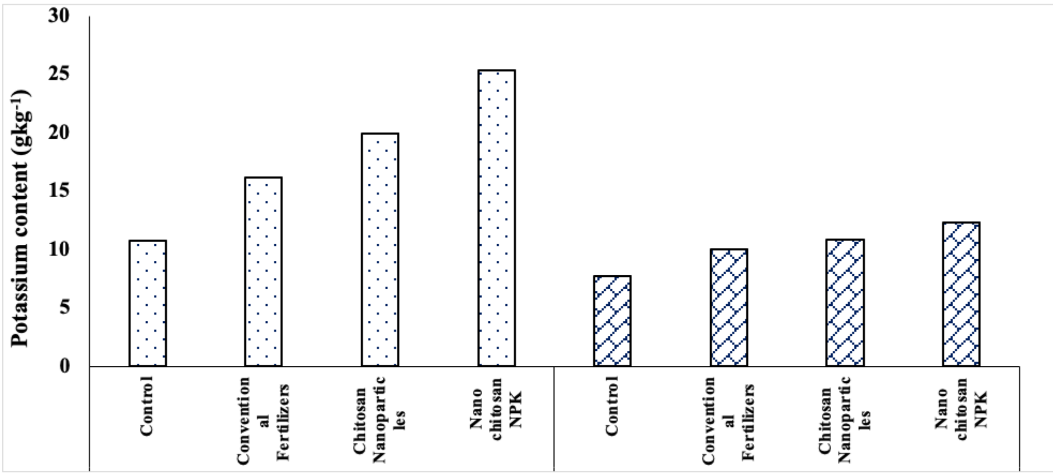

Fig. (3): Effect of chitosan loaded NPK nanoparticles and conventional fertilizers on potassium content in straw and grains of wheat plants

Nano chitosan NPK treatment increased grain phosphorus uptake to 75.1 kg.ha^{-1}. in contrast to the control, conventional fertilizers increased grain potassium absorption to 58.5 kg.ha^{-1}. Chitosan nanoparticles increased grain potassium absorption to84.7 kg.ha^{-1}, in excess of the control and conventional fertilizers. Nano chitosan NPK treatment yielded the highest grain potassium uptake, 103.6 kg.ha^{-1}. For the total nutrients uptake, the results at (Table 3) and (Fig. 4) showed that total nitrogen uptake was 148.0 kg.ha^{-1} using conventional fertilizers. Conventional fertilizers increased nitrogen uptake significantly compared to the control. Chitosan nanoparticles absorbed 200.5 kg.ha^{-1} nitrogen. These findings suggest that chitosan nanoparticles absorbed nitrogen more effectively than the control group and standard fertilizers. The greatest nitrogen was absorbed by nano chitosan NPK at 243.4 kg.ha^{-1}. The same trend was observed at total phosphorus uptake. Conventional fertilizers increased phosphorus uptake to 70.90 kg.ha^{-1}, increasing the control. Chitosan nanoparticles increased phosphorus uptake to 89.80 kg.ha^{-1}, increasing than the control group and conventional fertilizers. Nano chitosan NPK treatment increased total phosphorus uptake by 102.17 kg.ha^{-1}, compared to other treatments. Also, the results showed that the application of conventional fertilizers increased total potassium uptake to 143.0 kg.ha^{-1}, significantly higher than the control. Chitosan nanoparticles increased total potassium uptake to 176.60 kg.ha^{-1}, more than the control and conventional fertilizers. Nano chitosan NPK had the maximum total K uptake of 292.3 kg.ha^{-1}. In general, the results showed that conventional fertilizers, chitosan nanoparticles, and nano chitosan NPK increased total nutrient uptake for all three nutrients (N, P, and K) compared to the control (no treatment). The nano chitosan NPK treatment also has the highest total uptake values for all three nutrients, confirming its effectiveness in enhancing wheat plant nutrient absorption.

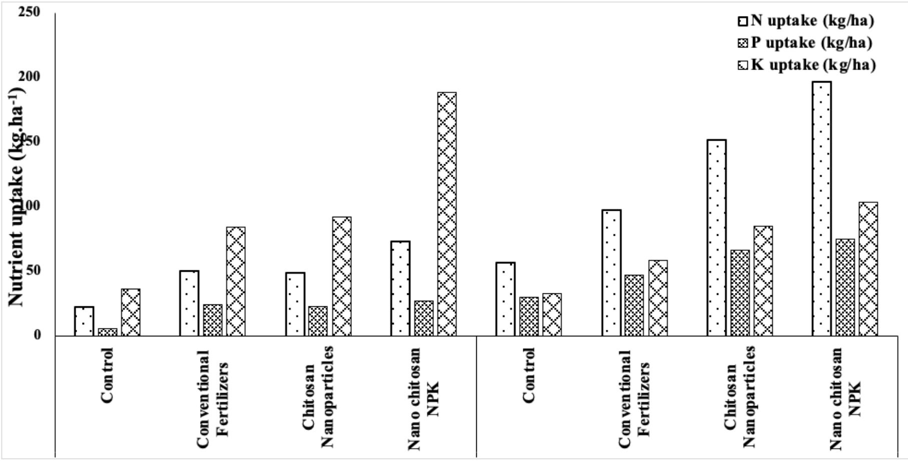

Fig. (4) Effect of chitosan loaded NPK nanoparticles and conventional fertilizers on nutrients uptake in straw and grains of wheat plants

-Assessing the Influence of Chitosan Nanoparticles , Chitosan loaded NPK Nanoparticles and Conventional Fertilizers on The Life Span of Wheat Plant:

Obtained results revealed that wheat plant reached harvesting after 160 days from the sowing under both control and conventional fertilizer treatments. On the other hand, wheat plants treated with chitosan nanoparticles and chitosan loaded NPK nanoparticles reaching the harvesting stage after 120 days of the sowing (Table 4). nanoparticles exposure can regulate the plant growth with their regulatory responses in auxin in-planta biosynthesis and transportation. Previous many studies have been reported the enhance content of auxin under metallic nanoparticles exposure (Zahedi *et al*., 2019; Jonapa *et al*., 2020). The obtained results indicated that the application of chitosan nanoparticles resulted in a reduction of the life span of wheat plants by about 25%. This effect could be related to the capacity of nanochitosan to improve cellular division through the modulation of genes responsible for auxin production. Also, nano chitosan has been found to increase the efficiency of photosynthetic processes and improve the absorption of nutrients (Abdel-Aziz *et al.,* 2016).

Table (4) Effect of chitosan nanoparticles, chitosan loaded NPK nanoparticles and conventional fertilizers on the life span of wheat plant.

Treatments	Life Span	
	Days	Reduction%
Control	160	0.0
Conventional Fertilizers	160	0.0
Chitosan Nanoparticles	120	25.0
Nano chitosan NPK	120	25.0

This data agreed with that obtained byAbdel-Aziz *et al.* (2018) who found that wheat plants grown on sandy soil with NPK fertilized with chitosan-NPK nanofertilizers had a shorter life span of 130 days from sowing, reducing their lifespan by 23.5% compared to the control and normal NPK fertilized crops. There are also many studies that have demonstrated the positive effect of nanomaterials in plant growth. nanomaterials having the potential to improve the plant's growth and development with the modulation in seed priming, photosynthesis enhancement, soil quality, protection against biotic and abiotic stress, etc. (Fiol *et al*., 2021). Hu and Xianyu (2021) elucidated nanotechnology's role as a plant growth regulator and stated that it can be a rising approach to improving plant yield and prosperity. nanomaterials can not only work as plant growth regulators, but they can also regulate the in-planta biosynthesis of plant growth hormones (Jiang *et al*., 2021; Hao *et al*., 2016).

CONCLUSIONS

Based on the study findings, in Comparison between nano chitosan -NPK fertilizer and conventional fertilizers. The nano chitosan NPK treatment showed a significant increase in grain production (kg.ha-1) compared to the control, and conventional fertilizers. The obtained results which indicated that the application of chitosan nanoparticles resulted in a reduction of the life span of wheat plants by about 25%. This effect could be related to the capacity of nanochitosan to improve cellular division through the modulation of genes responsible for auxin production. These results may be indicated that the application of foliar chitosan NPs. -loaded nitrogen in different concentrations and chitosan loaded NPK nanoparticles has been employed to enhance plant growth and productivity**.**

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تأثير استخدام الشيتوزان النانوى المحمل بالعناصر NPK والسماد التقليدى على نمو وانتاجية القمح تحت ظروف الاراضى الرملية

طارق صلاح احمد سالم،السيد محمد السخرى،عبدالمنعم محمود على زايد، احمد ابراهيم محمد، نهى عادل محجوب

قسم الاراضى والمياه - كلية الزراعة - جامعة قناة السويس - مصر

المستخلص : دراسة تأثير جسيمات الشيتوزان النانوية والمحملة بـالعناصر N,P K مقارنة بالسماد التقليدى على نمو وإنتاجية محصول القمح حيث أجريت هذه التجربة لدراسة تأثير السماد التقليدى و جسيمات الشيتوزان النانوية والمحملة بـ العناصر N,P K على نمو وإنتاجية القمح وذلك خلال الموسم الشتوي 2022/2021 بمزرعة كلية الزراعة جامعة قناة السويس- الإسماعيلية - مصر.حيث أشارت النتائج إلى أن معاملة القمح بجسيمات الشيتوزان النانوية بالرش الورقى ادى إلى قصر موسم نمو نباتات القمح بحوالي 25%. كما اشارت النتائج الى ان التسميد الورقى بالنانوشيتوزان او بالنانوشيتوزان المحمل بالعناصر NPKأدت الى تحسين صفات نمو وإنتاجية محصول القمح .وبشكل عام لوحظ التسلسل التالي للمعاملات (أسمدة النانو تشيتوسان > NPK سماد NPK التقليدي > النانوشيتوزان > الكنترول (ما عدا طول الجذر).