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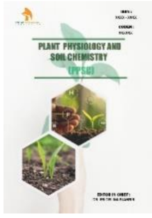

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RESEARCH ARTICLE

BIO-FERTILIZATION BY CHITOSAN WITH MINERAL N-FERTILIZATION AT DIFFERENT RATES AND THEIR EFFECT ON THE WHEAT YIELD UNDER SANDY SOIL CONDITIONS

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ABSTRACT

This study aims to compare between the effects of soaking and/or spraying by chitosan (Chs) solution in combinations with the mineral N-fertilization. Two field experiments were carried out in a split–plot design with three replicates including a control treatment (without application). The main factor (F1) was the mineral nitrogen (N) fertilization in the ammonium nitrate form applied at the rates: 50%, 75%, and 100% of the recommended dose (RD). The sub-factor (F2) was the Chs soak and/or spray treatments. Aqueous Chs solution was prepared (20 mL Chs diluted in 10 L water was used for a 24 h soaking time of the grains. A similar solution was sprayed on the soil 50 and 70 days after cultivation. Wheat grains (*Triticum aestivum* L.) were sown during November 2021 and 2022. After harvesting and analysis of soil and plant samples, the results have indicated that soaked grains combined with the 75% rate has showed the most significant increase in the soil available N by 120.4%, P by 1128.9%, and K by 68.1% compared to the control. Spraying the Chs has maximally increased the yield (ton kg^{-1}) of the wheat grains significantly by 59.4%, 71.8% and 84.7%, respectively, with the nitrate rates 50, 75, and 100% RD compared to the control. Spraying also showed the most significant increase in N, P, and K uptake (kg ha^{-1}) by grains was by 245.1, 194.6 and 192.6%, respectively at 75% N rate. The N, P, and K use efficiency (NUE) showed maximum values by soaking the wheat grains in the Chs solution before cultivation with the 75% N rate. The AE values of the Chs spraying are greater than the Chs soaking being linked to the wheat yield that may be attributed to repeating the spraying after cultivation during the plant growth stages rather than one soaking stage.

KEYWORDS

Chitosan; Sandy soil, Spray fertilization; Soaking grains

1. INTRODUCTION

Plant nutrition under sandy soil conditions needs a repetitive concentrated fertilization mainly by the inorganic chemicals that quickly dissolve to provide available nutrients and satisfy the crop production increase requirements. However, the nutrients' use efficiency (NUE) is usually inhibited due to the fast loss of a fraction of the applied fertilizer by quick leaching from the destructive texture of the soil. Currently, the global interest is directing towards the agricultural sustainability by the use of the environment-friendly bio-degradable fertilizers out of bio-resources, which enhance the NUE (Pandey et al., 2018).

On another hand, physical and chemical seed treatments before sowing have been studied because seeds' storing is frequently resulting in certain degradation of their components due to the moisture, formation of oxidizing agents, and the reactive oxygen species (ROS). Such techniques are easy and low cost practice to alleviate the effects of several stresses on the seed germination as it induces the water stress and simulate osmotic stress effects. Treatments have included seed soaking, coating, pelleting, thermal, and magnetic field MF, laser and gamma irradiation treatments (Mohaseb et al., 2023; Rashad, 2020; Rashad et al., 2022). Studies on the wheat had used distilled water, dilute solutions of $CaCl_2$, $ZnSO_4$, nano-particles' suspensions of TiO_2, chitosan, and plant growth regulators (PGRs). Water is a prerequisite to start the germination and soaking wheat seed in water before sowing had increased the seed yield.

Seeds' treatment by Chs may accelerate their germination and can increase their resistance to stress factors perhaps due to the increase in a chitosan-induced catalase activity of antioxidant role eliminate the free radicals (Faqir et al., 2021). It has also increased the growth characters, and yield of barley and soybean plants (Hafez et al., 2020). Seed treated by 0.05–0.4% Chs solution showed a reduced enzyme activity and stimulated seedling growth, increase the chitinase activity in seedlings by 30–50%, and tolerance to oxidative stress in the Safflower (*Carthamus tinctorius* L.). It improved the chlorophyll content, growth parameter and antioxidant enzymes of the wheat (*Triticum aestivum* L.) Seed treatment with 40 mg/L solution of oligomeric chitosan had induced the drought resistance of rice (*Oriza sativa* L.) Seed treated with 0.2% chitosan increased shoot and root length and adjusted salt toxicity of the Ajowan (*Carum copticum*) while seed soaked with oligochitosan at 0.0625% for 5 h, led to significant increase in proline level (Hidangmayum et al., 2019).

Chitosan (Chs) is a natural biodegradable and biocompatible biopolymer obtained from the marines' wastes that has a high nitrogen content and low C/N ratio (Ahing and Wid, 2016; Burrows et al., 2007; Ibrahim et al., 2019; Takarina and Fanani, 2017). It can play the role of a growth promoter, antimicrobial agent, carrier for nutrients slow-release, increasing water use efficiency and as an absorbent of some heavy metals (Ali et al., 2021; Jayanudin et al., 2021).

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Chitosan has been widely applied as a bio-fertilizer, coating films for seeds, a soil conditioner, foliar spray, and seed soaking to activate plant natural metabolism. A maximum rice yield was achieved after seed soaking and soil application. Seed priming and direct exposure were studied using Chs nanoparticles on barley (*Hordeum vulgare cv. Reyhan*) and wheat (*Triticum aestivum cv. Pishtaz*). Seeds priming at low Chs nanoparticle concentrations (30 ppm) had exhibited positive effects on seedling, root and shoot lengths as it protected crops from downy mildew. At high concentrations (e. g. 90 ppm), negative effects were observed on growth characteristics in both application methods. In barley studies, the application of 1, 3, and 6 g/L of Chs resulted in a significant improvement in plant height (Kocięcka and Liberacki, 2021).

Its application as a soil amendment at 0.05%, 0.10%, and 0.15% (w/w) has enhanced the lettuce (*Lactuca sativa*) growth and increased the leaf fresh weight from 28.6 to 39.4, 39.1, and 39.8 g, respectively. Chitosan is capable to be a soil coagulant that increases the water stability of the soil aggregates with improving its water-holding capacity depending on its physicochemical characteristics (Adamczuk et al., 2021).

Foliar spray of the Chs might stimulate the stomatal conductance, abscisic acid content and reduced the transpiration. It increased the activity of the leaf nitrate reductase in the Indian spinach and okra. The foliar spray alone or along with the soil application has significantly affected the growth, yield and biochemical features of the tomato fruits. It has been improved the macronutrient (N and P) uptake for wheat, potatoes, melon, begonia, chili fruits and seeds. Chitosan concentration at 2-4 g/L has affected the endogenous hormone content, alpha-amylase activity and chlorophyll content in the maize leaves (Quynh et al., 2020; Waly et al., 2020; Waly et al., 2019).

A proposed action mechanism of the Chs is the metal-chelation interaction between the poly-cationic Chs and the poly-anionic structures like lipo polysaccharides, proteins, and metal ions existent in the cell wall and plasma membrane. The Chs cationic groups perhaps bind the negatively charged phosphate groups of the DNA leading to particular modifications in the protein expression (Burrows et al., 2007). This study aims to compare the effects of seed soaking and/or foliar spraying by the Chs aqueous solution as an organic bio-fertilizer in combination with different application rates of the nitrogen (N) mineral fertilizer on the yield and quality of the wheat crop cultivated in sandy soil. The nutrients use efficiency (NUE) was calculated and discussed.

2. MATERIALS AND METHODS

2.1 The experimental field work

This study aims to estimate the effect of the Chs bio-fertilizer used as a solution for soaking grains as well as a foliar spraying solution along with mineral fertilization on the wheat yield in sandy soil. The field experiment was carried out under the sandy soil conditions (*Typic Torripsamment; Entisol* "Arenosol AR") at the Ismailia Agricultural Research Station, Agricultural Research Center (ARC) - Egypt (31° 37' 42.8" N 33°19'52" E elevation 3.1 m) during the two successive winter seasons of 2021 and 2022. Some physical and chemical properties of the experiment soil before cultivation were presented in Table 1 (Page et al., 1982; Jackson 1973; Piper 1950).

<table>
<tr><td colspan="7">Table 1: Some physical and chemical properties of the studied experiment soil before cultivation</td></tr>
<tr><td colspan="7">Particle size distribution (%)</td></tr>
<tr><td colspan="3">Sand</td><td colspan="2">Silt</td><td>Clay</td><td>Texture class</td></tr>
<tr><td colspan="3">94.00</td><td colspan="2">2.00</td><td>4.00</td><td>Sandy</td></tr>
<tr><td colspan="7">Moisture properties w/w (%)</td></tr>
<tr><td>SP</td><td>Field capacity</td><td>Wilting point</td><td>Available water</td><td>Bulk density (g cm^{-3})</td><td>Total porosity (%)</td><td><b>Hydraulic Conductivity (cm hr^{-1})</td></tr>
<tr><td>30.00</td><td>18.64</td><td>3.61</td><td>14.83</td><td>1.64</td><td>37.36</td><td>18.64</td></tr>
<tr><td>pH†</td><td colspan="3">Electrical Conductivity EC (dS m^{-1})‡</td><td>$CaCO_3$ (%)</td><td colspan="2">Organic Matter OM (%)</td></tr>
<tr><td>8.00</td><td colspan="3">2.25</td><td>0.68</td><td colspan="2">0.26</td></tr>
<tr><td colspan="7">Soluble ions (mmol$_c$ L^{-1}) ‡</td></tr>
<tr><td>Na^+</td><td>K^+</td><td>Ca^{2+}</td><td>Mg^{2+}</td><td>HCO_3^-</td><td>Cl^-</td><td>SO_4^{2-}</td></tr>
<tr><td>12.30</td><td>0.30</td><td>5.90</td><td>4.00</td><td>1.20</td><td>17.20</td><td>4.10</td></tr>
<tr><td colspan="7">Available macronutrients (mg kg^{-1})*</td></tr>
<tr><td colspan="2">N</td><td colspan="3">P</td><td colspan="2">K</td></tr>
<tr><td colspan="2">22.90</td><td colspan="3">2.97</td><td colspan="2">109.33</td></tr>
</table>

† (1:2.5 soil : water suspension)

‡ (Saturated soil extract i.e. soil paste extract)

*Critical levels of the available nutrient (mg kg^{-1}) according to Soltanpour and Schwab (1977): N ≤ 40.0 Low; 40.0 – 80.0 Medium; > 80.0 High, P ≤ 5.0 Low; 5.0 – 10.0 Medium; > 10.0 High, K ≤ 85 Low; 85 –170 Medium; >170 High

The experimental area was plotted in a split–plot design with three replicates including a control treatment (without application). The main factor (F1) was the mineral nitrogen (N) fertilization in the ammonium nitrate form (NH_4NO_3, 33% N) applied at the rates: 50%, 75%, and 100% of the recommended dose RD that are equivalent to 357.1, 535.7, 714.3 kg NH_4NO_3 ha^{-1} containing 235.7, 176.8, 117.9 kg N ha^{-1}, respectively. The sub-factor (F2) was the Chs soak and/or spray treatments.

The chitosan (Chs) used in the study was a commercial liquid product Chito-Grow® of pH 6 – 7 and density 1.4 g mL^{-1} (5.5% chitosan, 15% organic nitrogen N, total organic matter OM > 10%) contains ~ 20% sea-algae extract aligned with acetate and citrate carboxylic acids. It was applied in the form of a diluted aqueous solution (2 mL L^{-1}) that was prepared by well mixing of 2 mL of the stock Chs solution with 1 L of water. The Chs was used for soaking the wheat grains before cultivation and/or for spraying on soil after cultivation. For the plot area, a Chs aqueous solution prepared by 20 mL Chs diluted in 10 L water was used for soaking the specified amount of grains to be sown for 24 h. After that, the soaked grains were separated from the soaking solution and air-dried without washing. Another similar solution was prepared then sprayed on the soil after cultivation at 50 and 70 days of the plant age.

Wheat grains (*Triticum aestivum* L.) Sakha 98 variety was hand sown in 10.5 m^2 (3.0 m × 3.5 m) plot area during November 2021 and 2022. The mineral fertilization was as follows: the desired doses of the ammonium nitrate were applied in two equal doses before the 1st and 2nd irrigation. The phosphorus (P) and potassium (K) fertilization was respectively by the super phosphate (15.5% P_2O_5, 238.1 kg ha^{-1} = 16.13 kg P ha^{-1}) applied during the soil tillage before planting and by the potassium sulphate (178.6 kg K_2O ha^{-1} = 148.21 kg K ha^{-1}) repeated in two equal application doses 30 and 55 days after sowing.

2.2 Soil and Plant Sampling and Analyses

The wheat plants were harvested after 150 days of planting and representative samples were chosen for analysis (Black, 1965; Black, 1982). The yields (ton ha^{-1}) of grains and straw as well as some yield components have been calculated based on the plot area data and mean of the two seasons was obtained. The available N-P-K in surface soil samples (0-30 cm layer) were extracted by 1% K_2SO_4, 0.5 N $NaHCO_3$, and 1 N NH_4OAc (pH 7.0), respectively (Jackson, 1973). Plant samples were dried at 70 ºC for 48 h and ground. A half gram of the ground grains and/or straw was wet digested using the $H_2SO_4/HClO_4$ acid mixture (1:1) (Chapman and Pratt, 1961). The concentrations of N, P and K in plant and soil extracts were measured by distillation (Kjeldahl apparatus), colorimetric (UV-Vis Spectrophotometer), and by flame photometer, respectively to calculate the macronutrients uptake by the grains and straw (Piper, 2019; Rayment and Lyons, 2010).

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Nutrient Use Efficiency Indices: Use efficiency (UE) and agronomic efficiency (AE) were calculated for treatments as follows (Roozbeh et al., 2011):

$$\text{Nutrient Use Efficiency (UE)} = \frac{(Pn_f - Pn_0)}{Fertilizer\ rate\ (N\ or\ P, kg\ ha{-}1\ )} \times 100$$

$$\text{Agronomic Efficiency (AE)} = \frac{Y_f - Y_0}{Fertilizer\ rate\ (N\ or\ P\ ,kg\ ha{-}1)}$$

P_n = grain (N) and/or (P) and/or (K), g kg^{-1}

P_{nf} = grain N in fertilized plots **f =** fertilized plots

P_{n0} = grain N in non-fertilized plots **0** = non-fertilized plots (control treatments)

Y = grain yield (kg ha^{-1})

2.3 Statistical Analysis

The statistical significance (LSD) of the data at *P ≤ .05* was calculated by the two-way analysis of variance (ANOVA) test using the Co-State software Package - Ver. 6.311, Cohort software Inc., Berkley-California (Gomez and Gomez, 1984).

3. Results and Discussion

3.1 Effect of soaking and/or spraying by the Chs solution on some properties of the experimental soil

Sandy soil is usually losing a fraction of the applied N-fertilizer applied to it especially in the mineral form like the nitrate. Partial replacement of the applied mineral nitrate by an organic form of the N-fertilizer such as Chs may enhance the N nutrient immobilization in the sandy soil so that its availability and uptake by plant is improved. Soaking the wheat grains before sowing as well as spraying after sowing by Chs solution have significantly affected some properties of the cultivated soil as indicated by Table 2 according to the LSD values. Limited variations in the soil pH and EC perhaps controlled by the soil buffering action and/or fast leaching loss of soluble ions under sandy conditions. The available concentrations of N, P, and K (mg kg^{-1}) in soil were increased significantly at *p≤ .05* for the applied nitrate rates 50, 75, and 100% RD and for the Chs treatments compared to the control. The 100% RD nitrate treatment without Chs has significantly increased the soil available N, P, and K by 91.7%, 701.2%, 60.2%, respectively.

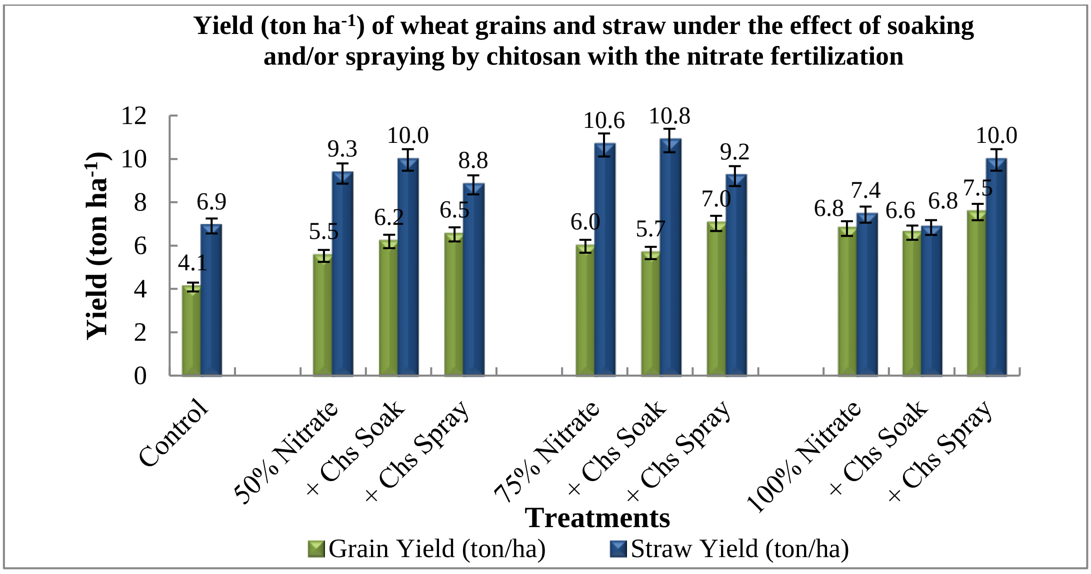
Table 2: Soil pH, EC and available macro-micronutrients content after harvest (mean values of the two seasons)

Treatment			pH†		EC (dS m^{-1})‡		Available nutrients (mg kg^{-1}) N		P		K	
			Soak	Spray	Soak	Spray	Soak	Spray	Soak	Spray	Soak	Spray
Control			7.99		2.30		37.97		0.55		82.00	
Nitrate	50%	– Chs.	8.03		1.98		41.90		1.07		90.33	
		+ Chs.	8.0	8.01	1.90	1.84	64.73	62.57	1.80	1.67	130.33	126.33
	75%	– Chs.	8.01		1.90		55.03		1.23		93.80	
		+ Chs.	8.00	8.03	1.83	1.88	83.67	82.83	6.80	5.10	137.83	134.37
	100%	– Chs.	8.01		1.86		72.80		4.43		131.33	
		+ Chs.	8.07	8.01	1.95	1.88	77.23	76.50	4.93	4.73	135.33	135.40
LSD		F1	SD = 0.02		SD = 0.14		1.34		0.15		1.67	
		SL					***		***		***	
		F2					1.34		0.24		1.40	
		SL					***		***		***	
		F1 * F2					***		***		***	

SD (Standard Deviation), † (1:2.5 Soil: Water suspension), ‡ (Soil paste extract)

Soaking the grains in the Chs solution combined with the 75% rate has showed the most significant increase in the soil available N by 120.4%, P by 1128.9%, and K by 68.1% compared to the control. Combination between the nitrate mineral fertilization (F1) and the Chs fertilization (F2) was significantly affecting the estimated properties of the sandy soil as their interaction (F1 × F2).

3.2 Effect of the applied nitrate with and without Chs treatments on the wheat yield (ton kg^{-1}) and some yield components



		Grains yield	Straw yield
LSD	**F1**	0.11	0.17
	SL	***	**
	F2	0.08	0.07
	SL	***	***
	F1 * F2	***	***

Figure 1

According to Figure 1, spraying the Chs has maximally increased the yield (ton kg^{-1}) of the wheat grains significantly by 59.4%, 71.8% and 84.7%, respectively, with the nitrate rates 50, 75, and 100% RD compared to the control. But soaking in Chs with 75% nitrate has showed the most significant increase of the straw yield by 57.1% and the 1000 grains wt (g) in Table 3 by 87.8%. However, the 75% RD without Chs was the most significant treatment that increased the weight of grains (g) per plant and the plant length (cm) by 286.5 and 52.7%, respectively, compared to the control (Table 3). The effects of the studied factors F1 and F2 can be complementary to each other because their interaction (F1 × F2) was significant for the wheat yield and the estimated yield parameters.

Table 3: Some yield components of the wheat plant as affected by different treatments after harvest (average values of the two seasons)

Treatment			**Wt. of grains/plant (g)**		**Shelling (%)**		**Plant length (cm)**		**1000 grains wt. (g)**	
			Soak	**Spray**	**Soak**	**Spray**	**Soak**	**Spray**	**Soak**	**Spray**
Control			9.40		46.11		73.12		30.22	
Nitrate	**50%**	**– Chs.**	24.21		49.29		95.37		43.30	
		+ Chs.	22.90	30.38	44.76	50.36	88.59	90.34	46.98	48.85
	75%	**– Chs.**	36.33		49.40		111.67		52.59	
		+ Chs.	28.01	20.06	45.42	49.23	97.53	95.00	56.75	44.93
	100%	**– Chs.**	24.82		47.91		84.00		45.44	
		+ Chs.	26.63	35.68	47.98	49.71	93.12	110.67	42.37	46.93
LSD		**F1**	0.64		0.77		1.58		0.36	
		SL	***		***		***		***	
		F2	0.36		0.78		0.86		0.70	
		SL	***		***		***		***	
		F1 * F2	***		***		***		***	

3.3 Macro-nutrients concentration (g kg^{-1}) and uptake (kg ha^{-1}) by the wheat plant as affected by the studied treatments

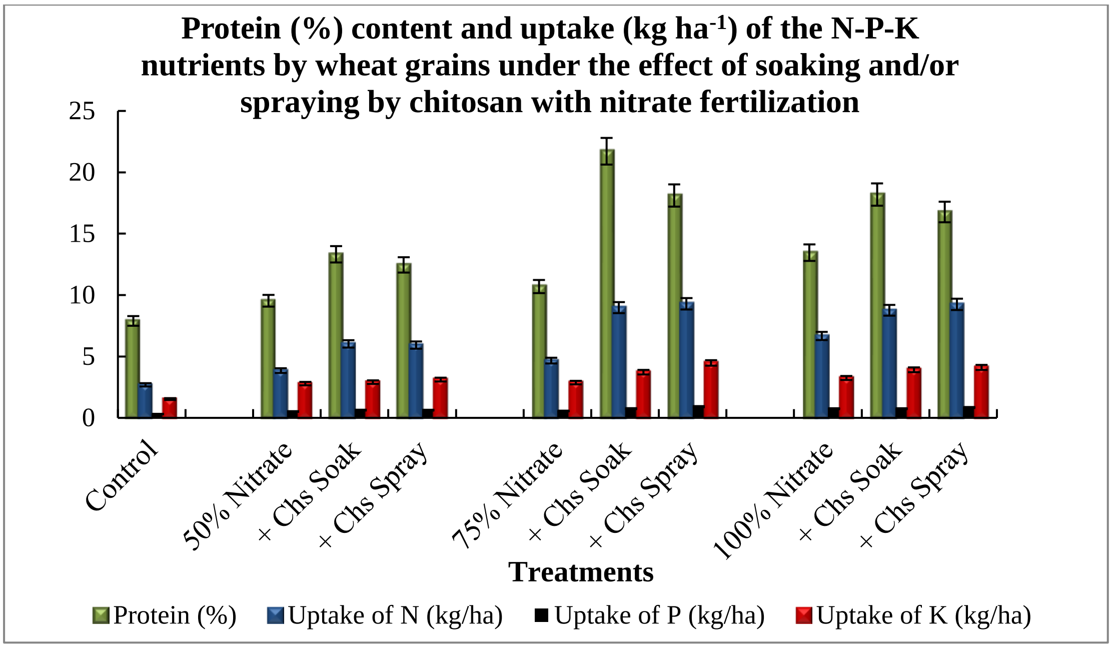
The nitrate application rates 50, 75, and 100% RD with and without the Chs treatments have increased the concentration (g kg^{-1}) significantly of the N, P, and K nutrients as well as their uptake (kg ha^{-1}) for the wheat plant as presented in Table 4 and Figure 2 compared to the zero control at $p \leq .05$. The 75% rate combined with soaking in the Chs has resulted in the most significant increase in the grains concentration of N by 175%, P by 75.9%, K by 76%, and the protein (%) content by 175% followed by the Chs spray treatments at the same nitrate rate compared to the control. Also, the most significant increase in N, P, K uptake (kg ha^{-1}) by grains was by 245.1, 194.6 and 192.6%, respectively (Figure 2) obtained by the Chs spray treatments at the same nitrate rate (75% RD).

Table 4: Concentrations of macro-micro nutrients content in wheat grains after harvest (average values of the two seasons)

Treatment			**Total nutrients (g kg^{-1})**					
					P		**K**	
			Soak	**Spray**	**Soak**	**Spray**	**Soak**	**Spray**
Control			13.73		1.80		8.90	
Nitrate	**50%**	**– Chs.**	16.57		2.23		12.00	
		+ Chs.	23.17	22.00	2.43	2.30	11.20	11.40
	75%	**– Chs.**	18.60		2.23		11.47	
		+ Chs.	37.77	31.50	3.17	3.10	15.67	15.17
	100%	**– Chs.**	23.40		2.63		11.40	
		+ Chs.	31.63	29.17	2.70	2.67	14.13	12.97
LSD		**F1**	1.02		0.27		0.79	
		SL	***		**		**	
		F2	0.50		0.12		0.77	
		SL	***		***		***	
		F1 * F2	***		***		***	

4. Discussion

A partial replacement of the quickly soluble ammonium nitrate fertilizer by an organic form like chitosan (Chs) for the N-fertilization in sandy soil perhaps enhances the availability and uptake of the N, P, and K nutrients in sandy soil. this is because the organic moiety of the Chs acts as a ligand that capture the ionic forms of the nutrients either by an electrostatic attraction or complexation mechanisms and immobilize them from fast leaching within the soil matrix (Faqir et al., 2021; Kocięcka and Liberacki, 2021). Additionally, the organic N form can be a more compatible and absorbable by plant than the mineral nitrate form. So that, the plant nutritional role played by the Chs both by soaking and spraying its solution was significantly effective and complementary to the nitrate fertilization role as indicated by the present study (Parvin et al., 2019; Rashad, 2020).



		Protein (%)	Uptake (kg ha-1)		
			N	P	K
LSD	F1	0.54	0.21	0.08	0.27
	SL	***	***	**	**
	F2	0.27	0.17	0.03	0.16
	SL	***	***	***	***
	F1 * F2	***	***	***	***

Figure 2

The mentioned role may be reflected in the calculated nutrients use efficiency (NUE) and agronomic efficiency (AE) illustrated in Figure 3. The N, P, and K use efficiency showed maximum values by soaking the wheat grains in the Chs solution before cultivation with applying a 75% RD of nitrate fertilization. It was increased by 393.8% (N), 215.4% (P), 163.6% (K) compared to the NUE calculated for the single addition of nitrate at 75% RD without Chs followed by the Chs spraying treatment at the same nitrate rate. This behaviour can be linked to an improved N-P-K availability and uptake by the wheat plant (Table 2, 4, Figure 2). It can be said that the grains soaked in the organic Chs solution were subjected to some type of the osmotic pressure, which simulates the grains' chemical and biological content and affected their equilibrium as well as their membrane permeability. The nutrients uptake behaviour during the germination was affected (Waly et al., 2020). Presence of a thin layer of the soaking solution covering the grain when sown may also play an additional role during the cultivation season. On another hand, it is observed that the AE values of the Chs spraying are greater than the Chs soaking. Being linked to the wheat yield (ton ha-1) in Figure 1, the greater AE of spraying may be attributed to repeating the spraying after 50 and 70 days from cultivation during the plant growth stages rather than one soaking stage. Utilization and uptake of the organic and mineral forms by the developed plant 50 and 70 days age perhaps increased the harvested grain yield more than a soaking stage far from the final mature plant (Waly et al., 2019).

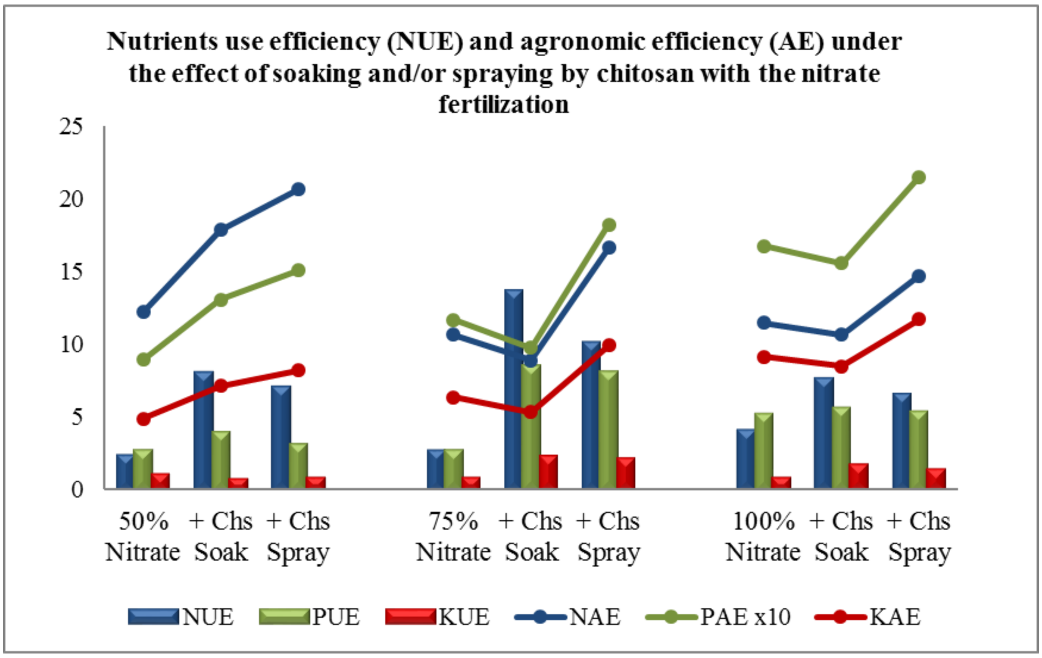


Figure 3

5. Conclusion

The organic N form can be a more compatible and absorbable by plant than the mineral nitrate form. The plant nutritional role played by the Chs both by soaking and spraying its solution was significantly effective and complementary to the nitrate fertilization role as indicated by the present study. The N, P, and K use efficiency showed maximum values by soaking the wheat grains in the Chs solution before cultivation with applying a 75% RD of nitrate fertilization. This behaviour can be linked to an improved N-P-K availability and uptake by the wheat plant. The greater AE of spraying may be attributed to repeating the spraying after 50 and 70 days from cultivation during the plant growth stages rather than one soaking stage.

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Compete of Interest

The authors declare no compete of interest.

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