

Priming Seeds with Urea-Loaded Nanocellulose to Enhance Wheat (*Triticum aestivum*) Germination

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ABSTRACT

The objective of this study was to investigate the effect of priming wheat seeds (*Triticum aestivum*, Giza171 local wheat genotype) with bulk cellulose (BC) and nanocellulose crystals (NC) aqueous solutions and their urea-loaded formulations on different germination parameters. BC was extracted from rice straw. NC was prepared from BC through bleaching, acid hydrolysis, and flocculation processes. Priming treatments were prepared using six BC and NC concentrations, three urea concentrations, and their combinations in a completely randomized design with three replicates. Seeds were soaked in the treatment solutions for 3 hrs, and then germinated on wet filter papers in Petri dishes for 7 days at 28°C. Results revealed that NC treatments significantly increased root length and surface area, and vigor index compared to BC treatments which had significant positive effect on Germination percentage, root fresh weight, and root radius. The concentration of 0.5% BC or 0.3% NC recorded the significantly highest values of all parameters except root radius. Although application of 0.5% urea significantly increased root fresh weight and root radius, significant reduction in Germination percentage, root length, and vigor index was observed. According to the statistical analysis of the vigor index data obtained, priming wheat seeds using 0.3% NC without urea loaded is the most efficient treatment to enhance germination of wheat seeds.

Keywords: Bulk-cellulose, nano-cellulose, urea, germination, vigor index, wheat.

INTRODUCTION

The germination process represents the most important physiological function of seeds and is considered a precondition for successful cultivation of most crops (Tajbakhsh and Ghiyasi, 2009). It is controlled by several eco-physiological factors, such as temperature, oxygen, and water availability. In particular, the water potential of soil is an important factor in arid and semiarid climates, where seeds are often sown into the soil with inadequate water for rapid germination (Ghiyasi *et al.*, 2015). Conventional seed priming mainly employs water (hydro priming) or solutions containing substances (nutrients, hormones, or biopolymers) which form seed coatings or dressing (Shukla *et al.*, 2019). As one of the priming seed treatments, seed coating has been widely applied for

many crops worldwide. The seed coating agents are generally composed of active (i.e., pesticide and plant growth regulator) and inactive (film-forming agents, suspension concentrates, and pigments) components (Mnasri *et al.*, 2017).

Seed nano-priming uses nanomaterials, mainly nanoparticles. In seed nano-priming, the media used are suspensions or nano formulations, where the nanoparticles may be absorbed by seeds (Acharya *et al.*, 2019). Even when nanoparticle absorption occurs, the greatest fraction is retained on the seed surface as a coating (Savassa *et al.*, 2020). Nano-priming can be applied to seeds to provide protection for seeds during storage, improve germination, germination synchronization, and plant growth, as well as to increase the resistance of crops to abiotic or biotic stress conditions (Elkhatib *et al.*, 2019, 20007A20). This can help to reduce the required quantities of pesticides and fertilizers (Malik *et al.*, 2021). Studies showed that seed nano-priming can activate different genes during the germination, especially those related to plant stress resistance (An *et al.*, 2020). Nano-priming can also be used for aiming bio-fortification of seeds to promote the increase in food quality and production (Ye *et al.*, 2020).

The rapidly increasing importance of urea fertilizer in the world agriculture has stimulated research activities to find methods of reducing the problems associated with the use of this fertilizer. One of these problems is that urea has adverse effects on seed germination, seedling growth, and early plant growth. These adverse effects are caused largely by urea itself, biuret, or other impurities in urea fertilizers (Bremner, 1990).

Cellulose (general formula, $C_6H_{10}O_5$) is only soluble in liquids which can break the associative forces between its chains and, at the meantime, combines with the chains to prevent their re-association. Cellulose is assumed to be isomeric with starch since it undergoes hydrolysis to yield glucose (Achmat, 2018). Cellulose has a strong affinity for water and most reactions start when it is in the fibrous state (Orhan *et al.*, 2018). The fibrous nature gives it the large surface area which makes it easy for sorption on many substances. Several

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studies have been focused on the development of new biodegradable products and understanding of the properties of hydroxyl-cellulose derivative in aqueous solutions from different wastes (Crini *et al.*, 2019 and Abdullah *et al.*, 2021).

Nanocellulose is classified into 2 major categories, 1) nanostructured materials (cellulose microcrystals and polysaccharide microfibrils) and 2) nanofibers (cellulose nanofibrils, polysaccharide nanocrystals, and microorganism cellulose) (Hussin *et al.*, 2019) variety of nanocellulose forms is made exploitation completely different ways and from varied plastic sources (Salimi *et al.*, 2019). The morphology, size, and different characteristics of every nanocellulose category rely on the polysaccharide origin, the isolation and process conditions similarly because the attainable pre- or post-treatments. the advantages of the 3-D class-conscious nanostructure of nanocellulose and its chemical science characteristics at nano scale open new prospects in many applications (Köse *et al.*, 2020). The rising demand and also the employment of recent applications (e.g., nanocomposite materials, medical specialty merchandise, supercapacitors, chemical change supports, electroactive polymers, fibers and textiles, food coatings, barrier/separation membranes, antimicrobial films, paper merchandise, cosmetic, cements) have driven the researchers and also the trade to take advantage of additional employment of nanocellulose (Coelho *et al.*, 2018). The objective of this study was to investigate the effects of bulk- and nano-cellulose loaded urea on wheat seeds germination.

MATERIALS AND METHODS

Preparation of Bulk Cellulose (BC): Rice straw was obtained from the farm of faculty of agriculture, Alexandria University. Bulk cellulose was extracted following three steps namely, dewaxing, delignification, and purification according to Hasan *et al.* (2014).

Preparation of Nano-cellulose (NC): Extracted BC was used to prepare NC through three main processes; bleaching, acid hydrolysis, and flocculation according to Hossain *et al.* (2018). Nano particle size of the prepare nanocellulose was confirmed by XRD examination (data not shown).

Six concentrations of BC and NC were prepared as shown in Table 1.

BC and NC-Loaded urea: urea stock solution was prepared by dissolving 50g urea in 100 ml DW (50 % w/v) and BC and NC solutions were loaded by 0, 0.25 and 0.5% of the N-urea solutions.

Table 1. Prepared concentrations of BC and NC.

Carrier	Concentration %					
	C1	C2	C3	C4	C5	C6
BC	0.00	0.10	0.25	0.50	1.00	1.50
NC	0.00	0.05	0.10	0.30	0.40	0.50

Wheat germination: wheat seeds (*Triticum aestivum L.*) of an Egyptian local genotype (Giza171) were used for the germination experiment. Seeds were initially washed with distilled water and then soaked in a combination of BC, NC, and N-urea solutions (0, 0.25 and 0.5 N %) in Erlenmeyer flasks for 3 hrs. All treatments were in triplicates. After soaking, ten seeds were germinated on a filter paper moistened with distilled water in Petri dishes for seven days at room temperature (28 ± 2 °C). On the 7th day, germinated seeds were counted, and the length of root (L, cm) was measured by a ruler (± 0.1 cm). A seed was considered germinated when radicle was larger than 2 mm long.

Germination percentage (GP, %) was calculated and the **Vigor index (VI, cm)** was calculated using the GP and root length (cm) as follows: Elouaer and Hannachi (2012)

$$VI = \frac{GP \times \text{Root length}}{100}$$

Root length Rate (RLR, %) was calculated using the following equation: Elouaer and Hannachi (2012)

$$RLR = \frac{(RL_{Sx} - RL_{S0})}{RL_{S0}} \times 100$$

Where; RL_{Sx} is the root length for any treatment; RL_{S0} is the root length for the control treatment.

Volume of root (V, cm³) was measured by the volume displacement method using a 25-mL graduated cylinder. The **root radius (R_r, cm)** was then calculated using the following equation Hallmark and Barber (1984).

$$R_r = \sqrt{\frac{V}{\pi L}}$$

Root surface area (RSA) was calculated using the following equation according to Hallmark and Barber (1984).

$$RSA = 2 \pi R_r L$$

The treatments were set up according to the completely randomized design with three replicates. Statistical analysis of experimental data was carried out using CoStat Software package (CoHort, 2004). Differences between means of treatments were tested using the least square difference technique of Student-Newman-Keuls at 5% significance level ($LDS_{.05}$).

RESULTS AND DISCUSSION

Data in Table 2 and Figs.1-3 show that the highest significant seed germination percentage (GP, %) of primed wheat seeds was recorded at 0 and 0.25 % of

urea-N concentration compared to 0.5% urea-N. Mean GP of bulk-cellulose treatments (BC) was significantly higher than for nano-cellulose treatments (NC).

Table 2. Effect of priming with different BC and NC concentrations and levels of loaded N-urea on germination percentage (GP, %) of wheat seeds.

N levels, %	Carrier type	Carrier concentrations						Mean Carrier ²	Mean N Levels ¹
		C1	C2	C3	C4	C5	C6		
GP (%)									
0.0	BC	80.00	83.33	96.67	100.00	83.33	80.00	84.81 A	88.61 a
	NC	80.00	80.00	83.33	90.00	90.00	90.00		
0.25	BC	90.00	80.00	93.33	100.00	90.00	70.00	86.39 a	
	NC	90.00	70.00	90.00	90.00	100.00	100.00		
0.50	BC	60.00	70.00	80.00	90.00	100.00	80.00	82.41 B	75.83 b
	NC	60.00	60.00	70.00	70.00	80.00	90.00		
Mean Carrier Conc. ³		76.67 c	73.89 c	85.55 b	90.00 a	90.55 a	85.00 b		

¹LSD_{0.05} =2.67 ²LSD_{0.05} = 2.18 ³LSD_{0.05} =3.78

Means with the same letters are not significantly different

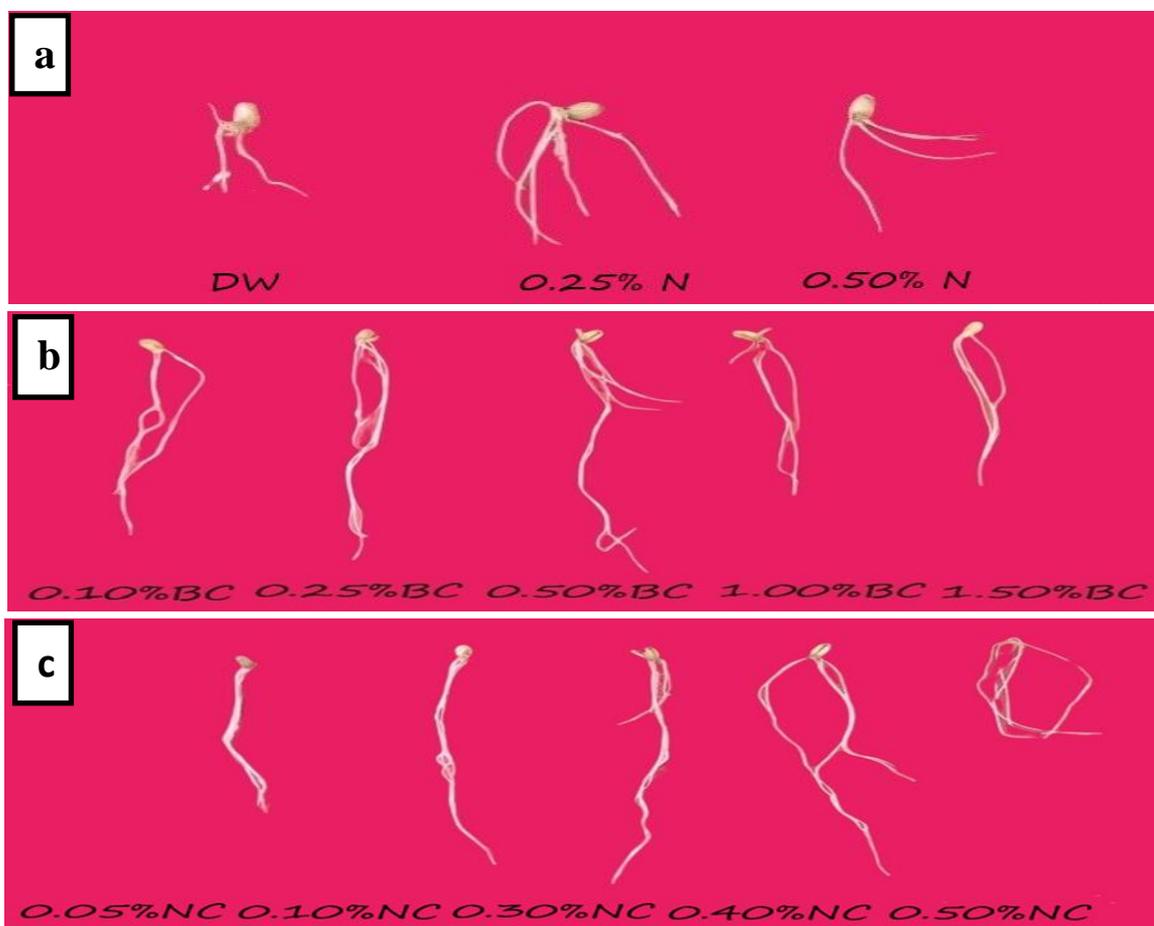


Fig. 1. Effect of priming wheat seeds with urea (a), bulk-cellulose (b), and nano-cellulose (c) on seed germination.



Fig. 2. Effect of priming wheat seeds with BC and BC-loaded with 0.25%N (a), and 0.5%N (b) of on root length.

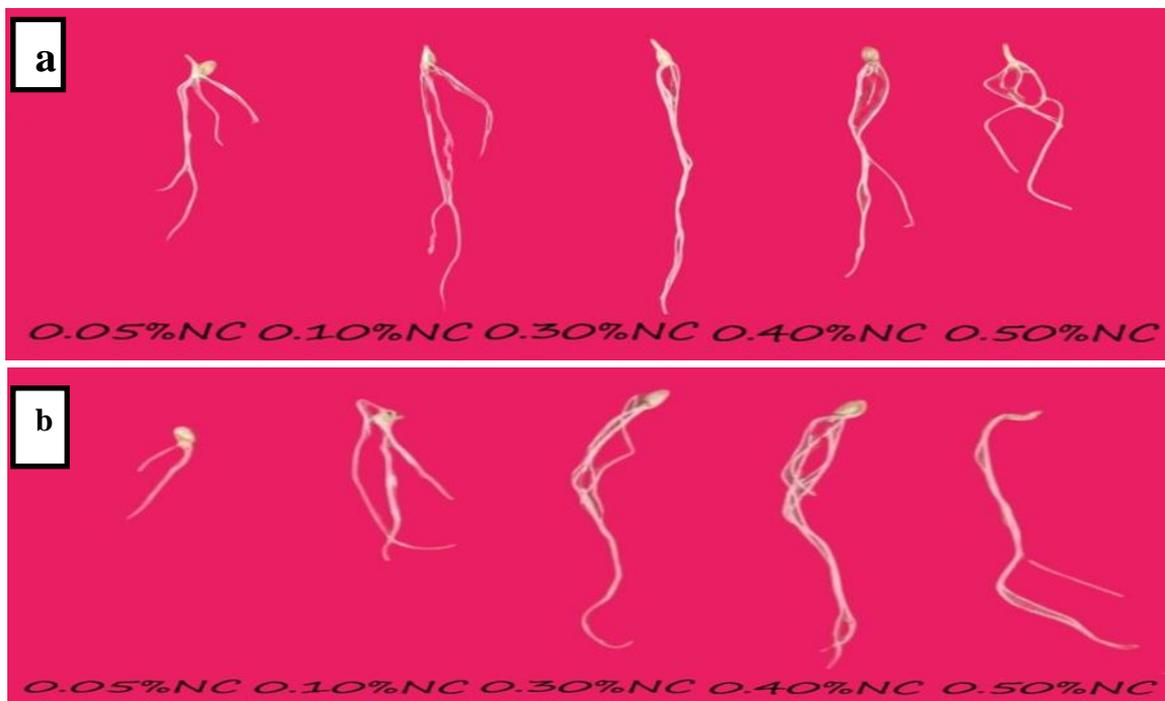


Fig. 3. Effect of priming wheat seeds with NC and NC-loaded with 0.25%N (a), and 0.5%N (b) of primed wheat seeds on root length.

Increasing BC concentration in the absence of urea increased GP up to 0.5 % BC (C4). At 0.25% urea-N the highest GP was observed at 0.5 % BC (C4) compared to the control (C1) and GP decreased with increasing of BC concentration. At 0.50% urea-N the highest GP was obtained at 1.0% BC (C5) compared to control (C1) and then it decreased with the increase of BC concentration. On the other hand, increasing NC concentration loaded with 0.50% urea-N increased GP. The highest GP observed at C6 of NC may be explained by the amelioration of NC of urea adverse effect on seed germination. The mean GP of BC treatments was significantly higher compared to NC at a significance level 95%. Although both BC and NC treatments showed the highest mean GP of wheat seeds at C4 and

C5, increasing their concentrations to C6 significantly decreased GP.

Means of root length (RL, mm) and root fresh weight (RFW, mg) were significantly affected by all the investigate treatments (Tables 3 and 4) Increasing urea-N concentration significantly reduced mean root length to 87.8 and 93.5% of the control at 0.25 and 0.5 % urea-N, respectively (Table 3). NC treatments had significantly higher mean RL compared to BC. Although mean RL values were increased with increasing BC and NC up to C4, values were significantly decreased at C5 and C6. In the absence of urea, the highest was obtained at C4 for the BC and NC treatments. However, the increase was 82, and 72% for BC and NC, respectively relative to the control.

Table 3. Effect of priming with different BC and NC concentrations and levels of loaded N-urea on wheat root length (RL, mm).

N levels, %	Carrier type	Carrier concentration., g/100 ml						Mean Carrier ²	Mean N Levels ¹
		C1	C2	C3	C4	C5	C6		
RL (mm)									
0	BC	57.67	286.67	423.33	478.33	223.33	240.00	260.25 B	296.08 a
	NC	57.67	290.00	393.33	421.67	404.33	276.67		
0.25	BC	183.33	191.67	199.33	305.00	241.33	199.00	259.83 c	
	NC	183.33	231.00	359.33	430.00	368.67	226.00		
0.5	BC	131.33	307.00	215.00	453.33	359.00	190.00	294.9 A	276.83 b
	NC	131.33	100.00	200.00	420.00	475.00	340.00		
Means of Carrier Conc. ³		124.11 f	234.38 e	298.38 c	418.05a	345.27 b	245.27 d		

¹LSD_{0.05} = 2.20 ²LSD_{0.05} = 1.79 ³LSD_{0.05} = 3.11

Means with the same letters are not significantly different.

Table 4. Effect of priming with different BC and NC concentrations and levels of loaded N-urea on wheat root fresh weight (RFW, mg).

N levels, %	Carrier type	Carrier concentration., g/100 ml						Mean Carrier ²	Mean N Levels ¹
		C1	C2	C3	C4	C5	C6		
RFW (mg)									
0	BC	106.67	376.67	473.33	666.67	230.00	290.00	340.92 A	330.28 b
	NC	106.67	333.33	353.33	393.33	360.00	273.33		
0.25	BC	263.33	300.00	273.33	393.33	320.00	300.00	298.89 c	
	NC	263.33	270.00	323.33	390.00	230.00	200.00		
0.5	BC	190.00	326.67	393.33	540.00	403.33	290.00	314.63 B	359.17 a
	NC	190.00	206.67	270.00	500.00	696.67	303.33		
Mean Carrier Conc. ³		186.67 f	302.22 d	347.78 c	480.55 a	373.33 b	276.11 e		

¹LSD_{0.05} = 3.29 ²LSD_{0.05} = 2.69 ³LSD_{0.05} = 4.65

Means with the same letters are not significantly different.

Mean Root fresh weight values were significantly increased with increasing urea-N concentration (Table 4) and the highest mean value was obtained at 0.5% urea-N (108.8% relative to 0% urea-N). However, increasing the concentration of either BC or NC significantly increased RFW with the highest mean RFW at C4 (257.4% relative to C1). On contrast with RL data (Table 3), mean RFW was significantly lower for NC treatments (92.3%) compared to BC treatments. It is remarkable that mean RL values were the least for the control of all urea-N and cellulose treatments.

Analysis of variance showed that urea stress with BC and NC had significant effects on root radius (R_r , mm) and root surface area (RSA, cm^2) of wheat seedlings (Tables 5 and 6). From Table 5, Mean R_r was

significantly increased with urea-N concentration and the highest mean obtained was at 0.5% urea-N (106.5% of the control). BC had significantly larger mean R_r than NC. Increasing concentrations of either BC or NC had a negatively significant effect on R_r , the treatment C5 showed the lowest R_r . Priming wheat seed in NC solutions significantly increased mean RSA compared to BC treatments. Increasing the concentration of either BC or NC significantly increased mean RSA of wheat roots up to C4 where mean RSA obtained represented 293.9% of the control). However, further increment in the concentration to C5 and C6 led to significant reduction in RSA. As shown in Table 6, loading BC and NC with urea-N caused fluctuated effect on RSA.

Table 5. Effect of priming with different BC and NC concentrations and levels of loaded N-urea on wheat root radius (R_r , mm).

N levels, %	Carrier type	Carrier concentration., g/100 ml						Mean Carrier ²	Mean N Levels ¹
		C1	C2	C3	C4	C5	C6		
R_r (mm)									
0.00	BC	0.08	0.07	0.06	0.07	0.06	0.06	0.066 A	0.062 b
	NC	0.08	0.06	0.05	0.06	0.05c	0.06		
0.25	BC	0.07	0.07	0.07	0.06	0.07	0.07	0.062 b	
	NC	0.07	0.06	0.05	0.05	0.05	0.05		
0.50	BC	0.07	0.06	0.08	0.06	0.06	0.07	0.061 B	0.066 a
	NC	0.07	0.08	0.07	0.06	0.07	0.05		
Mean of Carrier Conc. ³		0.071 a	0.067 b	0.063 c	0.061 d	0.058 e	0.061 d		

¹LSD_{0.05} =0.0005 ²LSD_{0.05} =0.0004 ³LSD_{0.05} =0.001

Means with the same letters are not significantly different

Table 6. Effect of priming with different BC and NC concentrations and levels of loaded N-urea on wheat root surface area (RSA, cm^2).

N levels, %	Carrier type	Carrier concentration., g/100 ml						Mean Carrier ²	Mean N Levels ¹
		C1	C2	C3	C4	C5	C6		
RSA (cm^2)									
0	BC	28.07	117.69	160.32	202.25	81.17	94.49	106.39 B	111.49 a
	NC	28.07	111.35	133.52	145.86	136.64	98.49		
0.25	BC	78.69	89.45	122.07	146.67	104.29	76.15	98.05 b	
	NC	78.69	89.45	122.07	146.67	104.29	76.15		
0.5	BC	56.58	113.41	104.15	177.21	136.29	84.07	108.39 A	112.35 a
	NC	56.58	51.47	83.23	164.13	206.03	115.02		
Mean Carrier Conc. ³		54.45 f	94.88 d	114.48 c	160.03 a	127.33 b	92.62 e		

¹LSD_{0.05} =0.90 ²LSD_{0.05} =0.74 ³LSD_{0.05} = 1.27

Means with the same letters are not significantly different.

Figs. 4 and 5 show that treatment means of root length rate (RLR) of germinated seeds had significant differences under different concentrations of BC or NC at the three levels of urea-N. The highest RLR was observed at 0.5% BC at 0& urea-N. In general, priming of wheat seeds in various concentrations of BC or NC significantly ($p < 0.05$) increased the RLR at all urea-N levels. With the highest urea level (0.5%), NC at 0.4% concentration recorded the highest rate. Moreover, 0.5% BC showed the highest effect compared with different concentration of BC. Therefore, priming in either BC or NC ameliorated the adverse effect of urea on germination.

Vigor index (VI) is a measure of the extent damage that accumulates as viability declines, and the damage accumulates in seeds until the seeds are unable to germinate and eventually die (Copeland and McDonald, 2012). From Fig. 5, urea-N, carrier type (BC or NC) and concentration of carriers had significant effects on VI of wheat seedlings. There was a significant ($p < 0.05$) reduction in VI of wheat seedlings caused by increasing urea-N concentration. NC primed seeds had significantly higher mean VI compared to BC. The highest mean VI was obtained at C4 of either BC or NC carriers. These results are comparable with those reported by (Itrotwar *et al.*, 2020 and Rather *et al.*, 2022).

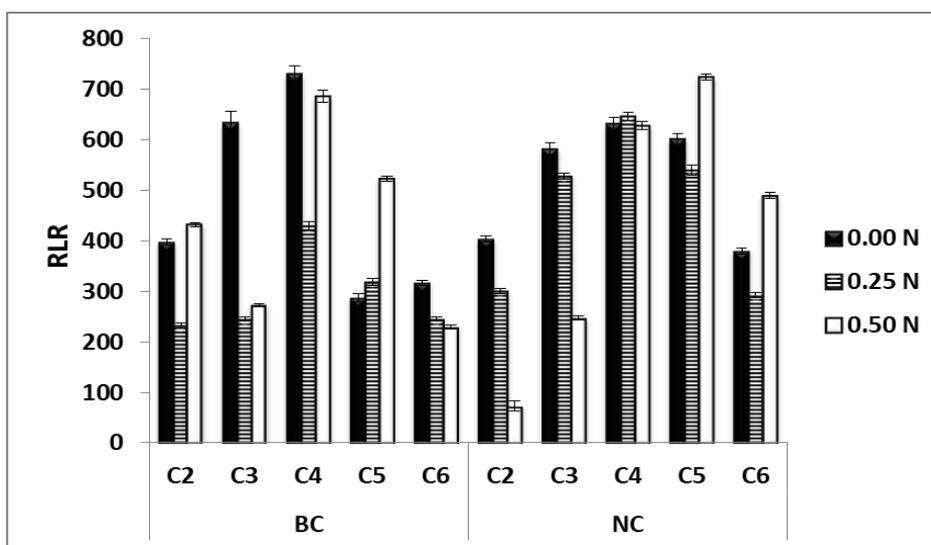


Fig. 4. Effect of priming wheat seeds with BC and NC at different concentrations, and level of loaded N-urea on root length rate (RLR).

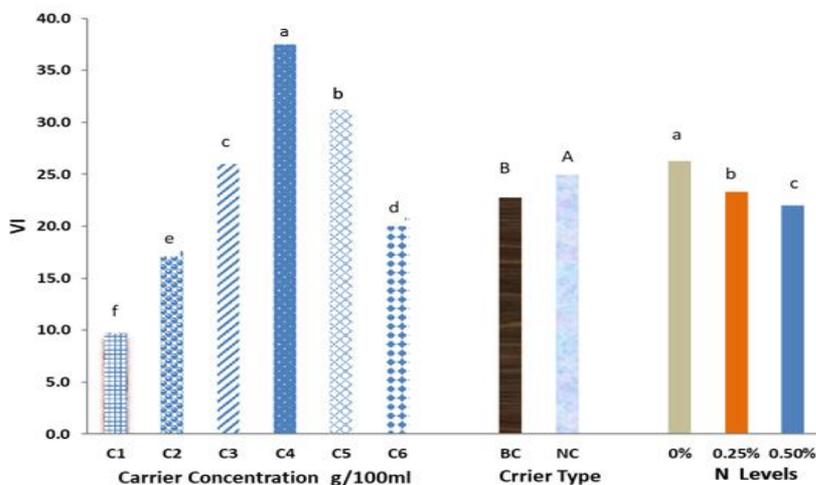


Fig. 5. Treatment means of vigor index (VI) as affected by priming wheat seeds using different loaded urea-N levels, carrier types and concentrations.

The negative effects of urea on seed germination agreed with the results obtained by Mikkelsen (1990) who concluded that growing tomatoes in hydroponic media having urea as the sole nitrogen source may be explained by the accumulation of urea in germinated seeds that adversely affected the activity of some enzymes such as peroxidases and phosphatases which inhibit directly or indirectly the synthesis of proteins. Gerendas and Sattelmacher (1997) also suggested that the reduced growth of urea-grown plants could also be related to alteration in the osmotic homeostasis. Urea-grown seedlings had long primary roots with only a few branches, which are symptomatic of N deficiency. This is like adult plants, which showed signs of N deficiency when transferred from sufficient ammonium nitrate nutrition to urea (Me'rigout *et al.*, 2008). Increasing the loaded urea to 0.25% increased lateral roots, while a further increase to 0.5% had no obvious effect on the morphology of roots Figs.1-3. This result suggested that either the uptake capability or the metabolism of urea was the limiting factor (Yangi *et al.*, 2015).

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The enhancing effect of cellulose on seed germination may be due to the ability of BC to hold more water that help increasing water uptake and enhanced enzyme activity, which begins very quickly after hydration, and cell elongation resulted in the emergence of the root by (Nonogaki, 2014). Cellulose coated seeds might also be protected from the harmful of urea.

NC crystals are expected to have higher specific surface area of that help absorbing more water by seeds and increased seed germination activities and cell elongation (Nonogaki, 2014 and Awadallah, 2019). Milewska-Hendel *et al.* (2019) reported that nanoparticles will simply enter the cell membrane depending on the diameter of pore (< twenty nm) and afterward the cytomembrane. Nair *et al.* (2010) additionally advised that the mode of action of nanoparticles is also the induction of pore size enlargement or improving formation of latest pores to induce NC uptake carrying a lot of water into the cells. Nanoparticles is also transported into the plant cell

through formation a complex with root excretions (Larue *et al.*, 2011). Hussain, *et al.* (2013) reported the doorway of silicon nanoparticles within the foundation of Arabidopsis thaliana and emotional up via vascular tissue tissues. The presence of magnetite nanoparticles in plant tissues of pumpkin was additionally reported by Tombuloglu *et al.* (2020). Lee *et al.* (2008) indicated the entrance of copper nanoparticles in mung (*Phaseolus radiatus* L.) and wheat.

CONCLUSION

Extracted cellulose from rice straw was successfully used to prepare nanocellulose crystals investigated in this study. Priming wheat seeds (*Triticum aestivum*, Giza171 local wheat genotype) using bulk cellulose and nano-cellulose suspensions proved to significantly improve many germination parameters of wheat seeds at different concentrations. Nanocellulose had the same effect on seed germination but at lower concentrations compared to bulk cellulose. In addition, nanocellulose mitigated the negative effect of priming wheat seeds in the presence of urea and different concentrations.

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الملخص العربي

نقع البذور في النانو سليولوز المحملة باليوريا لتحسين إنبات القمح (*Triticum aestivum*)

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استخدام NC زادت بشكل كبير من طول الجذر، مساحة السطح ومؤشر الحيوية مقارنة باستخدام BC بينما كانت الأخيرة لها تأثير إيجابي معنوي على نسبة الإنبات، الوزن الرطب للجذر ونصف القطر سجل تركيز 0,5% BC أو 0,3% NC أعلى قيم معنوية لجميع القياسات باستثناء نصف القطر. وعلى الرغم من أن تطبيق 0,5% من اليوريا أدى إلى زيادة ملحوظة في الوزن الرطب للجذر ونصف القطر، فقد تم تسجيل انخفاض كبير في نسبة الإنبات وطول الجذر ومؤشر الحيوية. ووفقاً للتحليل الإحصائي لبيانات مؤشر الحيوية، فإن نقع بذور القمح باستخدام 0,3% NC بدون تحميل اليوريا هو المعاملة الأكثر كفاءة لتعزيز إنبات بذور القمح.

الكلمات المفتاحية: السليولوز الخام، السليولوز النانوي، اليوريا، الإنبات، ومؤشر الحيوية، القمح.

الهدف من هذه الدراسة هو معرفة تأثير نقع البذور باستخدام السليولوز الخام (BC) والسليولوز النانوي (NC) وثلاثة مستويات من النيتروجين مصدره اليوريا وخليط منهما على معايير إنبات بذور القمح *Triticum aestivum*، صنف جيزة 171. تم تحضير NC من BC من قش الأرز من خلال عمليات التبييض، التحلل المائي الحمضي، والتجميع. تم تحضير التركيزات الأولية باستخدام ست تركيزات من BC (0,00، 0,10، 0,25، 0,50، 1,00، 1,50% (وزن/ حجم)، ستة تركيزات من NC (0,00، 0,05، 0,10، 0,30، 0,40، 0,50% (وزن/ حجم)، وثلاثة تركيزات من اليوريا (0,00، 0,25 و 0,50% نيتروجين وتوليفات منها. تم تنفيذ التجربة طبقاً للتصميم الإحصائي العشوائي الكامل من ثلاث مكررات. تم نقع البذور في هذه التركيزات لمدة 3 ساعات. ثم نبتت على أوراق ترشيع مبللة بالماء المقطر في أطباق بتري لمدة 7 أيام عند 28 درجة مئوية. أظهرت النتائج أن