Novochizol™ Seed Treatment: Effects on Germination, Growth and Development in Soft Spring Wheat

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Abstract

Novochizol™ (chitosan nanospheres) treatment of seeds of Spring Soft Wheat at concentrations of 0.1% and 0.05% favorably impacted both germination and growth processes. The effects on growth at the initial stages of organogenesis observed with the two concentrations depended on the growth conditions. When seeds were germinated and allowed to grow on filter paper in a germination chamber, the lower concentration Novochizol™ treatment (0.05%) was most effective in accelerating sprout appearance. In contrast, larger biomass accumulation and more uniform development of growth parameters were observed after the higher concentration Novochizol treatment (0.1%). The 0.1% Novochizol™ treatment had a significant stimulating effect on germination in soil substrate, with a 27.1% increase in sprout appearance after one day, and subsequent 100% germination. The 0.05% Novochizol™ treatment in the same experiment led to a significant increase in both root biomass (1.5-fold increase) and total seedling biomass (1.8-fold increase). Based on the results obtained in our experiments, and given its physicochemical characteristics. Novochizol™ appears to be a promising, practical candidate for pre-sowing seed treatment, leading to enhanced germination and growth at the initial stages of organogenesis.

Keywords: Novochizol™• Chitosan • Soft spring wheat• Seed infestation • Growth processes

Introduction

Plant growth and development are adversely affected by different stressors that are either biotic (pests, diseases, weeds) or abiotic (lack or excess of moisture, low or high temperatures, salinity, heavy metals, other toxic substances, etc.). The resulting loss in crop yield may be substantial. Among the various approaches that have been developed to increase plant resistance to different stressors, chemical agents play a preeminent role. They can act as highly effective pesticides, fertilizers and growth regulators. However, because they accumulate in the soil, water, and inside living organisms, their widespread use in agriculture has an adverse effect on the environment. There is growing pressure from the public to reduce if not ban altogether chemical pesticides, as exemplified by the popular initiative "For a Switzerland without artificial pesticides" that the Swiss electorate shall be voting on June 13, 2021. Importantly, both the proponents and the opponents of this initiative agree that the reduction in the use of synthetic pesticides is unavoidable. More ecological solutions to control the mechanisms that cause plant stress are thus desperately needed.

Chitin and its derivative chitosan are promising alternatives to chemical agents [1-3]. Chitin is a long-chain, high molecular weight polysaccharide composed of N-acetyl-D-glucosamine and D-glucosamine. It is the main component of the exoskeleton of arthropods and the cell wall of mushrooms. Chitosan is derived from chitin by deacetylation. Different factors impact the effectiveness of chitosan-based treatments: molecular weight, chemical modifications, pH, concentration, chelating potential, and the type of microorganism that is targeted. The main limiting factor in the use of chitosan is its poor solubility [4-10]. The use of chitosan in various crops is actively studied in Russia and worldwide. Chitosan biofilm formulations have been developed to treat seeds before sowing and to treat fruit after harvest [11-14]. Low molecular weight chitosan treatments have been shown to increase seed germination energy, viability, biomass, and crop yield, and to help control *alternariosis* [15, 16]. Chitosan, complexed with a humic preparation, has been shown to inhibit scabs, without affecting the development of phomose [17]. In grapevine, chitosan elicits plant defense mechanisms and confers protection against *Botrytis cinerea* and *Plasmopara viticola* when complexed with $CuSO_4$ [18]. It reduces the rate of development of viral diseases in vegetable crops, and its effectiveness in controlling *Colletotrichum sp.* in cucumber is comparable to that of chemical fungicides [19, 20]. Chitosan treatment of wheat leaves results in an increase in lignin in the cell walls and the levels of phenols and peroxidase, in a lower leaf susceptibility to obligate phytopathogens, and in enhanced growth, leading to higher crop yields [21-23]. Chitosan and chitosan-based nanoparticles are effective against *Fusarium graminearum,* the causal agent of Fusarium head blight. In in vitro experiments, both mycelium growth and spore germination were inhibited, with observable deformation and destruction of hyphae and a decrease in the rate of infection of the treated ears. Treatment effectiveness was found to depend on concentration and time of incubation [24]. Chitosan-based formulations were also found to inhibit the development of root rot, caused by Bipolaris sorokiniana [25]. Earlier, chitosan had been shown to increase the yields of spring wheat, in the absence of any control of root rot [26]. Recently, chitosan was found to elicit the growth of *Triticum aestivum L.* Chitosan oligosaccharide treatment of seeds or leaves resulted in the development of plants of increased height, with larger ear size and an increased length of the first internodes. The observed responses depended on the tested varieties and irrigation conditions.

The objective of the current work was to study the effects of seed treatment with the chitosan derivative Novochizol™ on the germination, growth, and development of seedlings of Spring Soft Wheat.

Materials and Methods

Novochizol™ base, having a degree of deacetylation of 90% or above and a molecular weight in the 500 kD range, was provided by NOVOCHIZOL SA, Monthey, Switzerland (www.novochizol.ch). In contrast to chitosan, which is a linear polymer, Novochizol™ has a globular, near-spherical shape, owing to intramolecular cross-linking. Such a molecular design confers to Novochizol™ several advantages over chitosan, both on its own and as a carrier of active ingredients (Table 1).

Novochizol™ aqueous suspensions were obtained by dissolving succinic acid (500 mg per 100 ml sterile water), gradually adding Novochizol™ (1000 mg per 100 ml succinic acid solution) under sonication, and sonicating the mixture for one hour, using model UZTA-0,4/22-OM sonicator (U-sonic, www.u-sonic.com) at maximum power. Sterile water was added to compensate for evaporation caused by prolonged sonication. The solution was filter-sterilized using 0.45 μm apyrogenic acetate cellulose filters (Minisart®, Sartorius Stedim Biotech Gottingen, Germany), stored as a 1% stock at+4°C and used within one week. Dilutions were performed immediately before seed treatments.

The effects of Novochizol™ solutions were studied under laboratory conditions, in two sets of experiments, using size-calibrated seed of Spring Soft Wheat, Novosibirskaya 31 variety. Each experiment included three conditions: 1) water, untreated control; 2) high concentration Novochizol™ treatment (0.1%); 3) low concentration Novochizol™ treatment (0.05%). After wetting each batch (10 liters per ton of seed), the seeds were stored in closed plastic containers.

Experiment No 1 was designed to assess the growth-promoting effects of Novochizol™ at the initial stages of organogenesis of wheat, in a germination chamber. After a 24 hours treatment with either water or Novochizol™ solutions, seeds were laid in Petri dishes, lined with two layers of filter paper, and interspersed with cotton (4 Petri dishes, with 12 seeds per dish).

The Petri dishes were kept under natural light, at a temperature between 20°C and 22°C. Germination energy and germination capacity were measured after 1 day and 3 days, and after 7 days, respectively. Growth indicators at the initial stages of organogenesis were determined after 3 days (length of roots of each seedling, total length of seedlings) and after 7 days (number of roots per seedling, length of seedlings, total mass of roots and shoots per seedling, and mass of each root).

Experiment No 2 was designed to assess the effects of Novochizol™ on the growth and development of seedlings of spring wheat in soil substrate. Seeds were treated 6 days before sowing in leached chernozem (4 duplicate sets of 12 pots, n=48, each containing 500 grams of soil), covered with a single layer of gauze. The pots were kept under natural light, at a temperature between 20°C and 22°C, under conditions of controlled soil humidity. During the nighttime, the pots were covered with plastic shields. Growth parameters were recorded during the first 7 days, including germination energy, germination capacity, height, and biomass. The seedlings were also monitored for symptoms of any lesion to the roots or rootstocks. Experimental data were analyzed using Statistics 7.0 and Excel 13 software programs.

Results and Discussion

A positive effect of Novochizol™ on seedling development was established in the treated seeds laid out on humid paper in Petri dishes (Figure 1). Higher rates of sprout appearance were apparent within one day of Novochizol™ treatment. The strongest effect was observed with the low concentration solution (22.9% increase for 0.05% Novochizol; 14.6% increase for 0.1% Novochizol, with a 70.8% baseline in the untreated control). Considering the higher increase in the germination rate conferred by the low concentration Novochizol treatment, it may be the preferred modality in the seed treatment of spring wheat.

The rate of growth of seedlings was increased by Novochizol™ treatment at both concentrations is shown in Table 2.

After 3 days, the total length of roots was 10.9% and 30.9% higher for 0.1% Novochizol™ and 0.05% Novochizol™, respectively. At the same time, the increase in root length was more uniform in plants treated with 0.1% Novochizol™ (coefficient of uniformity=81%) than in those treated with 0.05% Novochizol™ (78.2%) or in the control (68.9%). The same trend was observed when measuring the height of the seedlings. The increase after 0.05% Novochizol™ treatment (46.2% higher than the control=1.84 cm) was more significant than after 0.1% Novochizol™ treatment (24.4% higher than the control). However, the coefficient of uniformity was superior after 0.1% Novochizol™ treatment (75.4%, compared to 72.8% for 0.05% Novochizol™ and 61,7% in the control).

Based on measurements made 7 days after the beginning of the experiment, the low concentration Novochizol™ treatment was more effective in enhancing root formation (0.05% Novochizol™: 9.5% increase; Novochizol™ 0.1%: 6.1% increase) and overall seedling length (7.7% increase). In contrast, the highest increase in biomass was observed following treatment with the more concentrated Novochizol™ solution both for roots (Novochizol™ 0.1%: 17.1% increase; Novochizol™ 0,05%: 14.6% increase) and for the entire seedling (Novochizol™ 0.1%: 12.9% increase; Novochizol™ 0.05%: 8.7% increase).

The growth-stimulating effects of Novochizol™ on spring soft wheat were confirmed in seedlings sown and grown in soil substrate (Table 3). A higher rate of sprout appearance was observed after 24 hours for seeds treated with Novochizol™, especially at the higher concentration (Novochizol™ 0.1%: 27.1% increase; Novochizol™ 0.05%: 23% increase). Complete germination (100%) was achieved when the high concentration Novochizol™ solution was used.

Growth processes were favorably affected by Novochizol™ at both concentrations. In the first 7 days after setting grains on the soil substrate, the growth of seedlings from seeds treated with 0.1% concentration of Novochizol™ was significantly increased (Figure 2).

The high initial rate of growth stimulation resulting from 0.1% Novochizol™ treatment (56.3% after 2 days) was reduced 3-fold after 3 days (for Novochizol™, 0.05%, by 11.6 fold), and by 2.3 fold, after 7 days. The increase in biomass after 7 days (compared to the control, 16.63 mg) was higher in both the 0.1% Novochizol™-treated group (14% higher for total biomass and 23.8% for roots) and the 0.05% Novochizol™-treated group (25.7% higher for total biomass and 35.1% for roots). The 0.05% Novochizol™ treatment led to a 1.3-fold increase in total biomass and in root biomass.

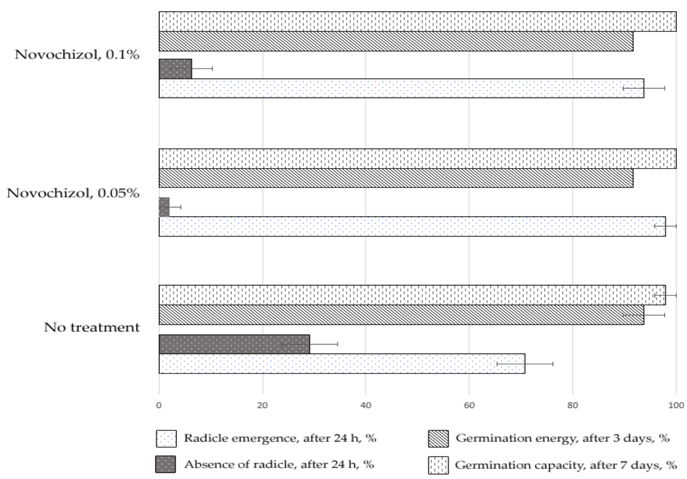

Figure 1. Effects of Novochizol™ on germination of spring wheat in Petri dishes.

Table 1. Characteristics of Novochizol™ of importance in plant treatment, in comparison with chitosan.

Characteristic	Chitosan	Novochizol™
Solubility (pH < 6)	Yes	Yes
Solubility (pH > 6)	No	Yes (dispersion)
Viscosity	High	Low
Biodegradability	Fast	Slow
Chemical stability	Low	High
Frost resistance	No	Yes
Physical states	Modifiable only through chemical reactions with other compounds.	Modifiable by changing the degree of intramolecular cross-linking.

Table 2. Effects of Novochizol™ on seedling development in Petri dishes.

Growth duration	Growth indicators	Control	Novochizol 0.1%	Novochizol 0.05%
3 days	Number of roots per seedling	2.9	3.0	3.0
	Root length per seedling, cm	7.79±0.27	8.64±0.46	10.20±0.94
	Length of individual root, cm	2.60±0.12	2.86±0.08	3.4±0.11
	Length of shoot, cm	1.84±0.09	2.29±0.03	2.69±0.30
7 days	Number of roots per seedling	4.41	4.68	4.83
	Length of shoot, cm	10.05±0.75	10.11±0.69	11.27±0.26
	air-dried biomass of roots, per seedling, mg	8.20±0.07	9.60±0.56	9.40±0.20
	air-dried biomass of 1 root, mg	1.86±0.02	2.06±0.15	1.95±0.08
	air-dried biomass of 1 seedling, mg	15.13±0.13	17.25±0.19	16.45±0.41

Table 3. Effects of Novochizol™ on seedling development at the initial stages of organogenesis of Spring Wheat in leached chernozem soil substrate.

Indicators			Experimental groups		
			Control	Novochizol 0.1%	Novochizol 0.05%
Germination energy, %	After 1 day	Sprout appearance	70.8	97.9	93.8
	After 2 days	Normally germinated seedlings %	95.9	93.8	100
	After 3 days		97.9	100	83.4
Germination, %		After 7 days	93.8	100	83.4
Length of normally developed shoot, cm		After 2 days	0.71±0.05	1.11±0.05	0.85±0.03
		After 3 days	2.87±0.13	3.40±0.13	2.92±0.06
		After 7 days	18.95±0.30	20.48±0.24	19.31±0.28
air-dried biomass, mg		Total seedling	16.65±0.32	18.95±0.41	20.90±0.37
		Roots, per seedling	6.93±0.14	8.53±0.23	9.33±0.22

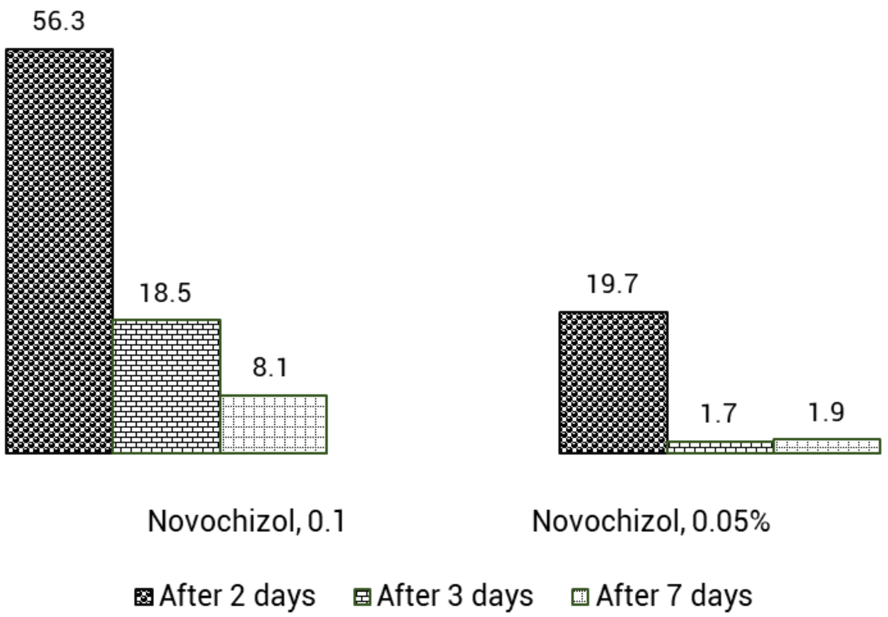

Figure 2. Growth increase (% increase over control) of aerial parts of Spring Wheat seedlings at the initial stages of organogenesis after Novochizol™ treatment of seeds.

Conclusion

Novochizol™ treatment of seeds of Spring Soft Wheat at concentrations of 0.1% and 0.05% favorably impacted both germination and growth processes. The effects on growth at the initial stages of organogenesis observed with the two concentrations depended on the growth conditions. When seeds were germinated and allowed to grow on filter paper in a germination chamber, the lower concentration Novochizol™ treatment (0.05%) was most effective in accelerating sprout appearance. At the same time, larger biomass accumulation and more uniform development of growth parameters were observed after the higher concentration Novochizol™ treatment (0.1%).

The 0.1% Novochizol™ treatment had a significant stimulating effect on germination in soil substrate, with a 27.1% increase in sprout appearance after one day, and subsequent 100% germination. At the same time, the 0.05% Novochizol™ treatment in the same experiment led to a 1.3-fold increase in both root biomass and total seedling biomass.

While chitosan is known to exert antifungal and growth elicitor effects upon seed treatment in different plants, it suffers from practical limitations: poor solubility, high viscosity, and rapid degradability. Novochizol™ overcomes these limitations. Because it can also be used in a variety of co-formulations (V.V.F, personal communication), and based on the results obtained in our experiments, Novochizol™ appears to be a promising candidate for pre-sowing seed treatment, leading to enhanced germination and growth at the initial stages of organogenesis. This section is not mandatory but can be added to the manuscript if the discussion is unusually long or complex.

Author Contributions

In vitro investigation, O.I.T., N.G.V.; chemistry investigation, V.V.F.; methodology, N.F.S., and N.G.V.; project administration, N.G.V.; supervision, V.V.F.; writing—original draft O.I.T., N.G.V., and V.V.F.; writing—review and editing, N.F.S. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

The authors O.I.T., N.F.S., N.G.V. declare no conflict of interest. V.V. Fomenko is the inventor of patent covering Novochizol™ technology and uses and owns stock in Novochizol SA.

References

1. Malerba, M. & Cerana, R. "Chitin- and Chitosan-Based Derivatives in Plant Protection against Biotic and Abiotic Stresses and in Recovery of Contaminated Soil and Water." *Polysaccharides* 1 (2020): 21–30.
2. Ruiz-García, Y. & Gómez-Plaza, E. "Elicitors: A Tool for Improving Fruit Phenolic Content" *Agriculture* 3.1 (2013): 33-52.
3. Chakraborty, M., et al. "Mechanism of Plant Growth Promotion and Disease Suppression by Chitosan Biopolymer" *Agriculture* 10.12 (2020): 624.
4. Zimina, Y., et al. "Application of biopreparations on the basis of chitosan in agriculture." *Nat Syst Resour* 8.3 (2018): 22-27.
5. Badawy, M.E.I. & Rabea, E.I. "A Biopolymer chitosan and its derivatives as promising antimicrobial agents against plant pathogens and their applications in crop protection" *Int J Carbohydr Chem* (2011): 1-29.
6. Deepmala, K., et al. "A future perspective in crop protection: chitosan and its oligosaccha-rides" *Adv Plants Agric Res* 1.1(2014): 23-30.
7. Morozova, E.A., & Vereschagin A.L. "The use of chitosan preparation on

woody grapevine cuttings at the stage of rooting (in Russian)" *Vestnik AGAU* 2. 64 (2010): 41-43.

8. Salachna, P., & Zawadzińska, A. "Effect of chitosan on plant growth, flowering and corms yield of potted freesia" *J Ecol Eng* 15.3 (2014): 97-102.
9. Dubin, A., et al. "Effect of chitosan formulations of different biological origin on tobacco (nicotiana tabacum l.) pr-genes expression" *J. Microbiol Biotech Food Sci* 9.6 (2020): 1141-1144.
10. Veliz, E.A., et al. "Chitinase-producing bacteria and their role in biocontrol" *AIMS Microbiol* 3.3 (2017): 689-705.
11. Bazunova, M.V., et al. "Biological activity of citrus pectine, chitosan succinate and their derivatives as film-making components in formulations for pre-sowing seed treatment (in Russian)" *Vestn Vogogr Gos Un-ta Ser* 11, *Yestyev. Nauki* 1.11(2015): 8-14.
12. Bazunov, A.A, et al. "The use of chitosan-glycerin complexes as a binding base in for-mulations for microencapsulation and seed pelleting in agriculture (in Russian)" *Vestnik tekhnologicheskogo universiteta* 19.5 (2016): 12-14.
13. Boeva, T.V., et al. "Biostimulator on the basis of low-molecular weight chitosan taken from crayfish shell for presowing processing of water melon seeds (in Russian)" *Vestnik AGTU. Ser.: Rybnoe khozyajstvo* 2 (2011): 133-136.
14. Adiletta, G., et al. "Chitosan Coating to Preserve the Qualitative Traits and Improve Antioxidant System in Fresh Figs (Ficus carica L.)" *Agriculture* 9.4 (2019): 84.
15. El Guilli, M., et al. "Effectiveness of Postharvest Treatment with Chitosan to Control Citrus Green Mold" *Agriculture* 6.2 (2016): 12.
16. Bairambekov, S.B., et al. "Biostimulator on the basis of low-molecular weight chitosan from the crayfish crust for preplant processing of tomato seeds (in Russian)" *Vestnik AGTU. Ser.: Rybnoe khozyajstvo* 2012. 1 (2012): 181-184.
17. Antonova, O.I., & Komyakova, Ye.M. "Effectiveness of different application techniques of Tellura bio, Novosil and Lariksin bio-logic products in the cultivation of potatoes (in Russian)" *Vestn Krasnoyarsk Gosud Agrar Univ* 8.154 (2017): 48-53.
18. Aziz, A., et al. "Chitosan oligomers and copper sulfate induce grapevine defense reactions and resistance to gray mold and downy mildew" *Dis. Control Pest Mana* 96.11 (2006): 1188-1194.
19. Firmansyah, D., et al. "Chitosan and plant growth promoting rhizobacteria application to control Squash mosaic virus on cucumber plants" *Asian J. Plant Pathol* 11.3 (2017): 148-155.
20. Dodgson, J.L.A., & Dodgson, W. "Comparison of effects of chitin and chitosan for control of Colletotrichum sp. on cucumbers" *J Pure Appl Microbiol* 11.1(2017): 87-93.
21. Vander, P., et al. "Comparison of the ability of partially n-acetylated chitosans and chitooligosaccharides to elicit resistance reactions in wheat leaves" *Plant Physiol* 118.4 (1998): 1353-1359.
22. Wafaa, M.H., et al. "Enhancement of wheat resistant to diseases by elicitors" *Int J Sci Res* 3.11 (2014): 1526-1530.
23. Chandra, S., et al. "Chitosan-induced immunity in Camellia sinensis (L.) against blister blight disease is mediated by nitric-oxide" *Plant Physiol Biochem* 115 (2017): 298-307.
24. Kheiri, A., et al. "Application of chitosan and chitosan nanoparticles for the control of Fusarium head blight of wheat (Fusarium graminearum) in vitro and greenhouse" *Int J Biol Macromol* 93 (2016): 1261-1272.
25. Vereschagin, A.L., et al. "Development and tests of liquid chitosan formulations based on potassium phosphate and chitosan Artemia sp. (in Russian)" *Vestnik AGAU* 9.83 (2011): 31-34.
26. Borisenko, S.I., & Lutkova, Je.F. "The efficiency of biostimulators in the fight against root rot of spring wheat (in Russian)" *Vestnik AGAU* 2(2003): 27-29.

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