

Research Article

The Effect of Chitosan Hydrolysate on *Solanum Lycopersicum* Plant Growth

Tatiana Timofeeva*, Daria Shtan'ko, Balzhima Shagdarova, Anton Zakurin, Anastasiya Kamionskaya, and Alla Il'ina

The Federal Research Centre "Fundamentals of Biotechnology" of the Russian Academy of Sciences, Russia

ORCID

Tatiana Timofeeva; 0000-0001-6458-7371

Abstract. Chitosan is a well-known subject of research because of its beneficial properties, including its antibacterial and fungicidal activity, as well as its effect on plant physiology. One of the main difficulties in agricultural chitosan implementation is the poor solubility of high molecular chitosan in water. Reducing the molecular weight of chitosan by acid hydrolysis increases its solubility. This research studied the effect of chitosan hydrolysate on the germination and development of *Solanum lycopersicum* L. plants. The effects of chitosan hydrolysate on seed germination, shoot development in the first week of development and in the first month of development were evaluated. According to the results, high concentrations of chitosan hydrolysate completely inhibited seed germination. However, short-term treatment by high concentrations of chitosan hydrolysate stimulated the development of seedlings, leading to substantially longer roots. Regular root application of high concentrations of chitosan hydrolysate inhibited plant development.

Keywords: chitosan hydrolysate, chitosan, plant growth regulators, plant germination

Corresponding Author: Tatiana Timofeeva; email: timofeyka2033@mail.ru

Dates

Published 13 January 2022

Publishing services provided by Knowledge E

© Tatiana Timofeeva et al. This article is distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use and redistribution provided that the original author and source are credited.

Selection and Peer-review under the responsibility of the 8th Scientific and Practical Conference Conference Committee.

1. Introduction

Currently, agriculture sector faces major challenges due to the global uncontrolled use of pesticides and other agrochemicals. Despite significant progress in crop productivity, this can have a negative impact on the environment. The regular use of agrochemicals causes many adverse effects, including negative effects on non-target organisms, and soil degradation.

To overcome such challenges, new approaches to agriculture are being sought. Ways to reduce the use of synthetic compounds and introduce biodegradable agrochemicals based on biomaterials for efficient and safe use in agriculture are being explored. Chitosan is a promising compound which may have prospects for application in environmentally friendly agriculture systems. Chitosan is a non-toxic, biocompatible and biodegradable biopolymer derived from chitin. Chitosan have a wide range of biological properties, such as antibacterial and antimycotic activities, film-forming ability [1,2].

OPEN ACCESS

Due to the prospects of its use in agriculture sector, the physiological effects of chitosan are being actively studied. Chitosan causes serious physiological changes in plants, affects the growth and differentiation of plant tissues, seed germination, enhances the rate of photosynthesis, acts as a causative agent of the synthesis of secondary plant metabolites [3].

A large number of studies describe chitosan as a polymer that promotes plant growth and development, affecting on germination [4], root elongation [5], seedling elongation, and increasing root activity [6].

The main reason which hinders the widespread use of chitin biopolymers, including chitosan, is their poor solubility at neutral pH. A wide range of chitosan derivatives helps to solve the problem of their solubility in water or weakly acidic aqueous solutions [7,8].

Another way to avoid this problem is the use products of chitosan depolymerization. A decrease in the molecular weight of chitosan promotes its better solubility in water, increases its bioavailability, which affects its biological properties [9]. Earlier, it was shown the fungicidal activity of chitosan hydrolysate [10]. The aim of this study is to study the effect of chitosan hydrolysate on plant development.

2. Materials and methods

2.1. Chitosan hydrolysate

Chitosan hydrolysate (CH), average molecular weight 33 kDa ($I_p = 2.1$), at a concentration 10 mg / ml, was obtained by hydrolysis of high molecular weight chitosan using nitric acid. The pH was adjusted at 5.5 with NH_4OH solution [10].

The effect of chitosan hydrolysate was compared with a solution (control solution - CS) of HNO_3 and NH_4OH respectively. Distilled water was used as a negative control.

2.2. Plants

Tomato seeds (*Solanum lycopersicum* L.) varieties Cherry Rosovy and Viking were used in experiments. The seeds were germinated in Petri dishes on moistened filter paper. The seeds were sterilized by incubation in 70% ethanol (1 min.), 10% sodium hypochlorite solution (3 min.) then washed three times with sterile distilled water.

TABLE 1: Experimental variants of the experiment to study the effect of chitosan hydrolysate on the development of shoots in the first week of growth.

Active substance (initial concentration)	Seed soaking duration	Concentration (pH 5,5)[dilution]				
		[1/2]	[1/5]	[1/50]	[1/500]	[1/5000]
CS: NH ₄ OH (14mM) + HNO ₃ (200mM)	constantly	[1/2]	[1/5]	[1/50]	[1/500]	[1/5000]
	22 hours	[1/2]	[1/5]	[1/5]	[1/50]	[1/500]
CH (10 mg/ml), containing 14mM NH ₄ OH □ 200mM HNO ₃	constantly	5 mg/ml [1/2]	2mg/ml [1/5]	0,2mg/ml [1/50]	0,02 mg/ml [1/500]	0,002mg/ml [1/5000]
	22 hours	5mg/ml [1/2]	2mg/ml [1/5]	0,2mg/ml [1/50]	0,02mg/ml [1/500]	0,002mg/ml [1/5000]
H ₂ O (control)	constantly	-	-	-	-	-

2.3. Study of chitosan hydrolysate effect on seed germination and seedling development in the first week of growth

The effect of various concentrations CH and CS (Table 1) was checked by short-term soaking (a) and constant incubation in a solution (b).

a) Tomato seeds were soaked in 15 ml plastic vials for 22 hours in 2 ml CH and CS of various concentrations. 50 seeds were used in each test. After 22 hours, 10 seeds were transferred to Petri dishes on filter paper soaked with 2 ml of distilled water.

b) Tomato seeds were placed on Petri dishes (10 seeds in 1 Petri dish) on filter paper soaked with 2 ml of CH and CS in appropriate dilutions. 50 seeds were used in each test.

Germination conditions: 20-24 °C, 16-hour photoperiod.

Germination energy was measured on the 4th day from the beginning of the experiment. The results were recorded on the 8th day. Seedling length and root length were measured.

2.4. Study of the tomato plants response on regular root CH irrigation in the first month of growth

Cherry tomato seeds were germinated for 4 days then the seedlings were replanted into 250 cm³ individual containers filled with soil. Starting from the 7th day from the moment of germination, the plants were irrigated three times a week with CH solution (at concentrations of 2 mg/ml, 0.2 mg/ml, 0.02 mg/ml), CS (in appropriate dilutions) and distilled water as a control. Each variant was tested in 4 replicates. On the 25th day, the

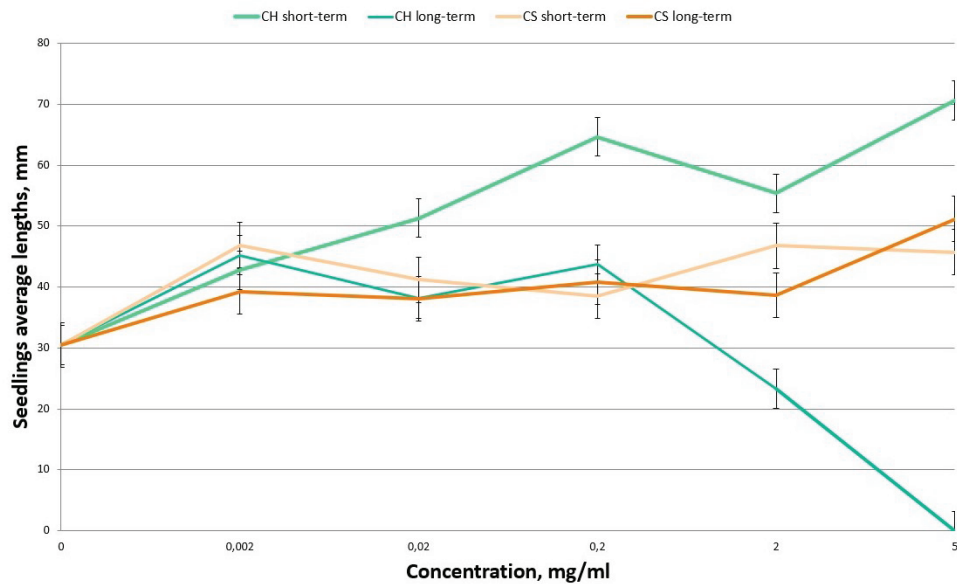


Figure 1: Comparison of the Cherry tomato seedlings average lengths on the 8th day of germination depending on solutions concentration. Light green line - short-term soaked CH, dark green line - constant incubation with CH, orange and yellow – CS constant incubation and short-term soaked respectively. Author’s own work.

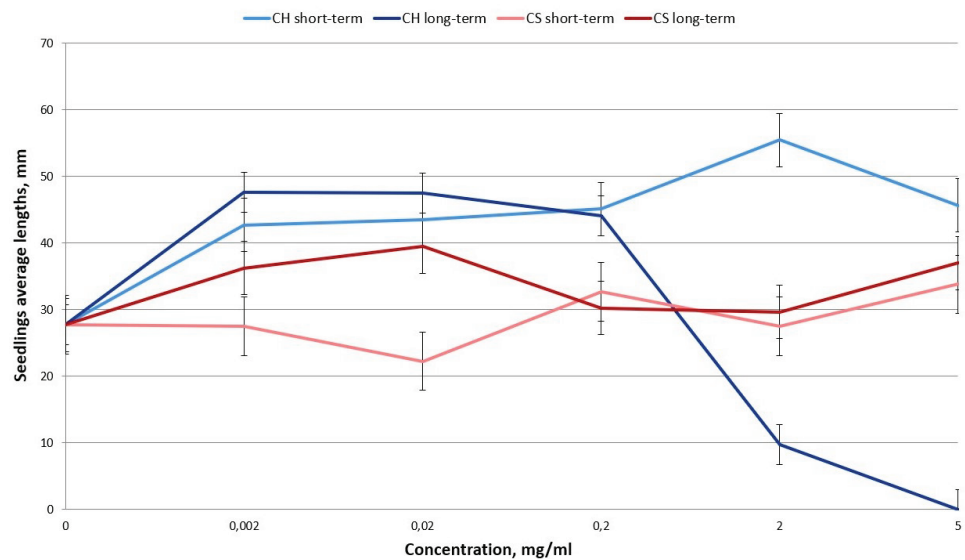


Figure 2: Comparison of the Viking tomato seedlings average lengths on the 8th day of germination depending on solutions concentration. Light blue - short-term soaked CH, blue line - constant incubation with CH, red and pink – CS constant incubation and short-term soaked respectively. Author’s own work.

plants were removed from soil, roots were washed with running water. The maximum plant length, maximum root length, plant weight, root weight, and number of true leaves were measured per plant.

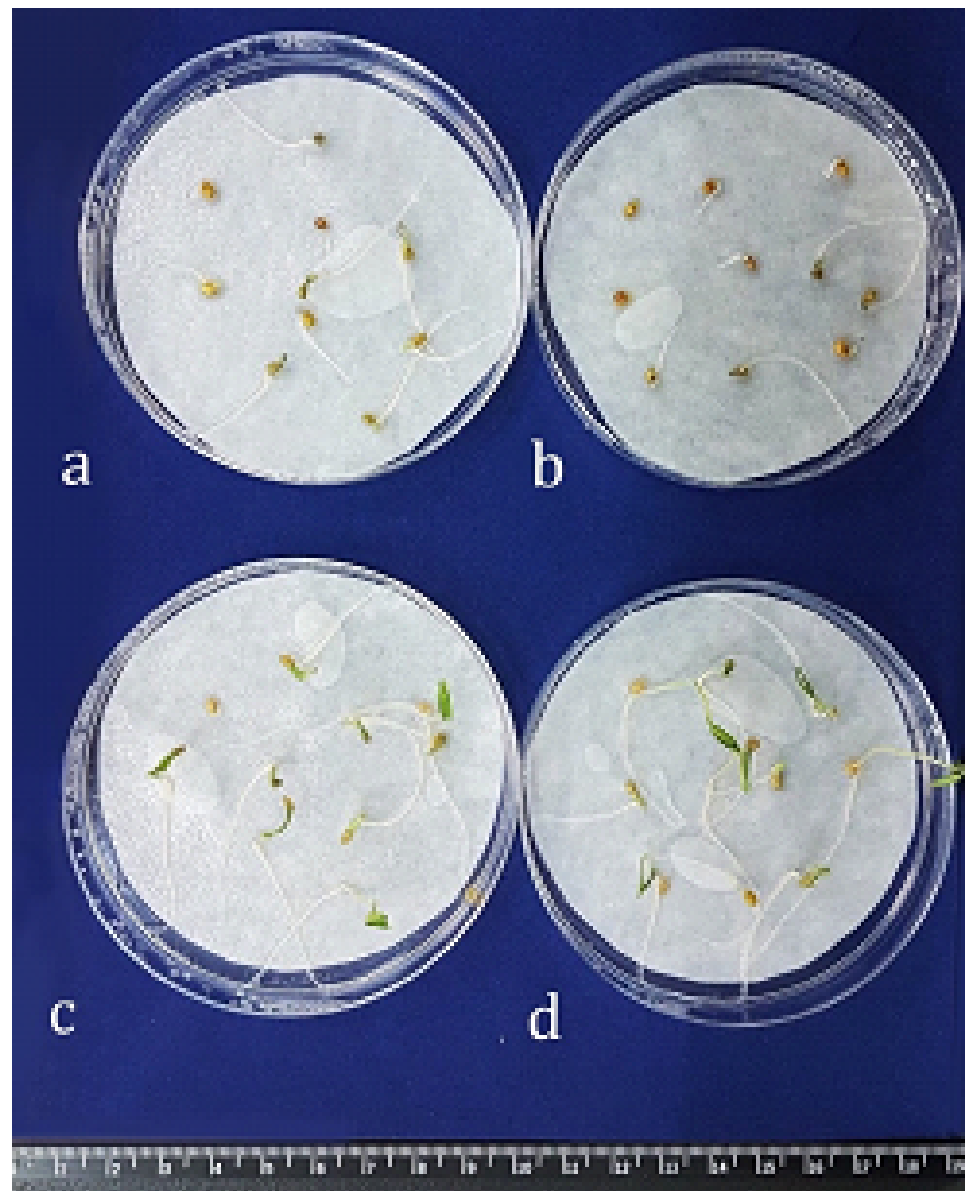


Figure 3: Short-term soaked variants CH 5 mg/ml (c,d) compared with the control (a,b).

3. Results and discussion

Chitosan hydrolysate concentration 5 mg/ml completely inhibited seed germination. Lower concentrations of chitosan hydrolysate did not affect the germination energy. In all other variants, the germination energy index did not statistically differ from the control.

Short-term soaking Cherry tomato seeds for 22 hours in a CH solution with a concentration 5 mg/ml, followed by transferring the seeds to distilled water showed the greatest average length of seedlings (Figure 1). Soaking tomato seeds in chitosan hydrolysate with a concentration 5 mg/ml, 0.2 mg/ml and the subsequent transfer of seeds to distilled

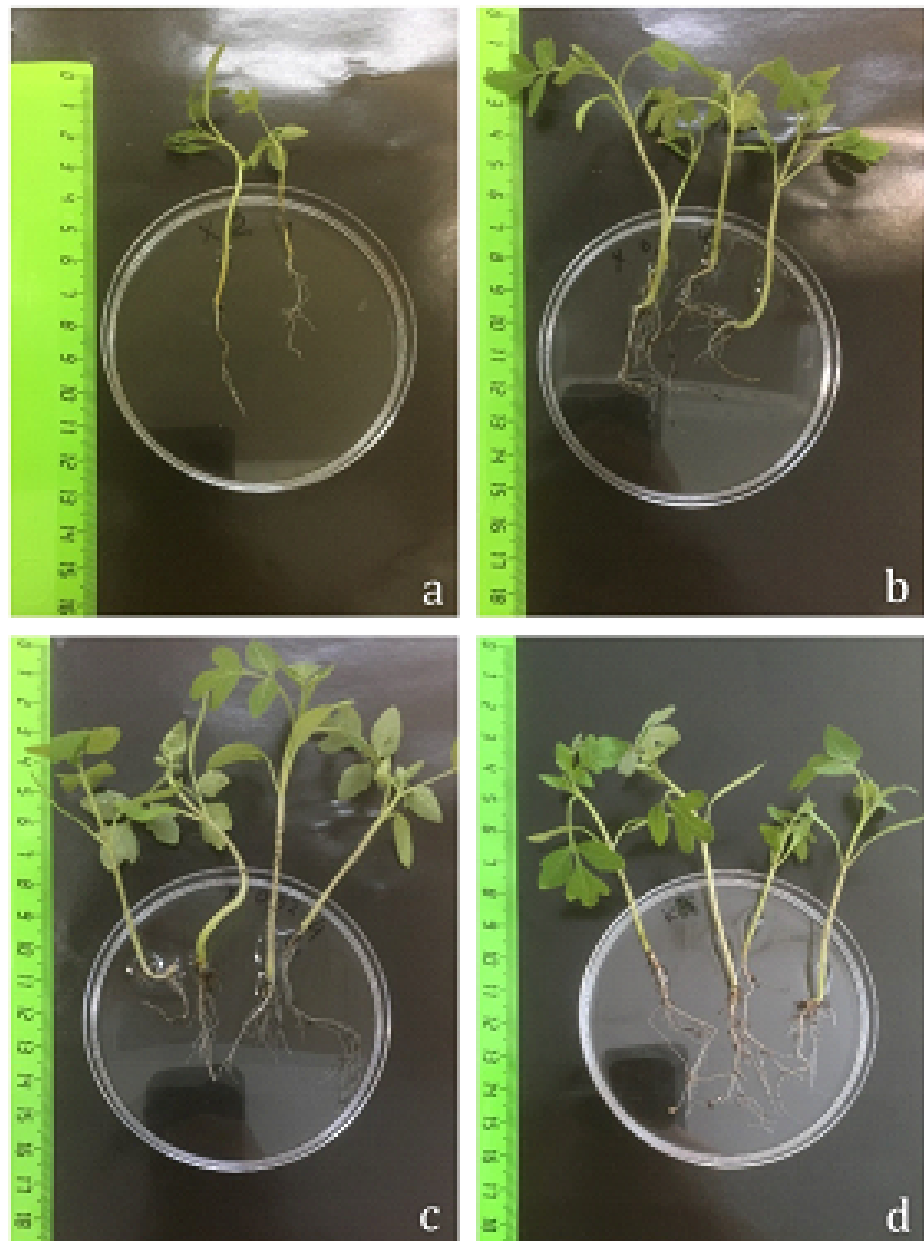


Figure 4: Cherry tomato plants irrigated with a) 2 mg/ml CH; b) 0.2 mg/ml CH; c) 0.02 mg/ml CH, and d) distilled water as control -.

water allowed, on average, to increase the length of the roots by more than two times (respectively 231%, 201%) compared to the control. The length of hypocotyls and roots pre-soaked samples in chitosan hydrolysate with concentrations 5 mg/ml, 2 mg/ml, and 0.2 mg/ml and transfer to distilled water was significantly higher than the length in all controls.

The statistical analysis of experimental data for the Viking tomato variety showed similar results to Cherry tomato (Figure 2). The average length of seedling roots when

soaked for 22 hours is also significantly longer than the control. Chitosan hydrolysate at a concentration of 5 mg / ml completely inhibited seed germination under constant exposure, and the transfer of seeds to distilled water in a case of short-term soaking results in an increase in root length by 178% (Figure 3).

As a result of both experiments the effect of a CS (which contained nitric acid and NH_4OH) were observed but the maximum increase in root length were observed at CH with the same content of nitric acid and NH_4OH .

Regular root irrigation of a CH solution with a concentration 2 mg/ml significantly inhibited growth of the root system and led to inhibition of plant growth in general. The inhibitory effect of a CH solution with a concentration 0.2 mg/ml was less significant (Figure 4). Lower concentration of chitosan hydrolysate, as well as control solutions, did not show statistically significant effect on plant development

Based on the obtained results, it can be concluded that chitosan hydrolysate has a physiological effect on plant growth. The constant presence of high concentrations of chitosan hydrolysate slows down plant development. At the same time, a short-term treatment has a stimulating effect. The difference between long-term and short-term exposure in chitosan hydrolysate paves the way for further research. It is necessary to study in detail the effects of chitosan hydrolysate on plants at the physiological and molecular levels.

4. Acknowledgement

This study was partially supported by Russian Foundation for Basic Research grant N^o 20-016-00205.

References

- [1] Katiyar, Deepmala & Hemantaranjan, A. & Singh, Bharti. Chitosan as a promising natural compound to enhance potential physiological responses in plant: A review. *Advances in Plants Agricultural Research*. 2015;20(1). 1-9 doi.org/10.1007/s40502-015-0139-6.
- [2] Zeng D, Luo X. Physiological effects of chitosan coating on wheat growth and activities of protective enzyme with drought tolerance. *Journal of Soil Science*. 2012;2:282-288. doi.org/10.4236/ojss.2012.23034.
- [3] Lopez-Moya F, Escudero N, Zavala-Gonzalez EA et al Induction of auxin biosynthesis and WOX5 repression mediate changes in root development in *Arabidopsis* exposed

- to chitosan. *Scientific Report*. 2017;7: 16874-16875 doi.org/10.1038/s41598-017-16874-5.
- [4] Guan YJ, Hu J, Wang XJ, Shao CX Effects of seed priming with chitosan solutions of different acidity on seed germination and physiological characteristics of maize seedling. *Journal of Zhejiang University (Agriculture and Life Science)*. 2005;31(6):705-708.
- [5] Batool M, Asghar, R. (2013) Seed priming with chitosan improves the germination and growth performance of ajowan (*Carum copticum*) under salt stress. *Eurasia Journal of Bioscience*. 2013;7:69-76. oi.org/10.5053.
- [6] Manjunatha G, Roopa KS. Chitosan enhances disease resistance in pearl millet against downy mildew caused by *Sclerosporagraminicola* and defence-related enzyme activation. *Pest Management Sciences*. 2008;64:1250-1257. doi.org/10.1002/ps.1626.
- [7] Mourya VK, Inamdar NN. Chitosan-modifications and applications: Opportunities galore. *Reactive & Functional Polymers*. 2008;68:1013–1051.
- [8] Varlamov VP, Il'ina AV, Shagdarova BT, Lunkov AP, Mysyakina IS. Chitin/Chitosan and Its Derivatives: Fundamental Problems and Practical Approaches. *Biochemistry (Mosc)*. 2020. 85(1): 154-176. doi: 10.1134/S0006297920140084. P
- [9] Badawy MEI, Rabea EI. Potential of the biopolymer chitosan with different molecular weights to control postharvest gray mold of tomato fruit. *Postharvest Biology and Technology*. 2009;51:110–117.
- [10] Shagdarova, Balzhima & Ilyina, A. & Lopatin, S. et al. Study of the protective activity of chitosan hydrolysate against septoria leaf blotch of wheat and brown spot of tobacco. *Applied Biochemistry and Microbiology*. 2018;54(1):71-75.