Application of enzymatic hydrolyzate of chitosan as a plant growth promoter

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Abstract. The natural polymer chitosan is widely used in various fields of agriculture, industry and medicine. The properties of a polymer largely depend on its molecular weight and supramolecular structure. In particular, to enhance the beneficial properties of chitosan, its low-molecular derivatives are obtained. The presented work shows the possibility of using enzymatic chitosan hydrolysates as a biostimulator of plants. It has been established that pretreatment of pea seeds (*Pisum sativum*) variety "Zhegalova 112" with solutions of chitosan hydrolyzate in all studied concentrations increases the seeds and soil germination. In the course of the work, the highest efficiency of chitosan hydrolysate was confirmed when growing pea seeds in the soil, namely higher soil germination, significantly faster growth of green biomass of plants, acceleration of leaf formation. A pronounced effect of chitosan hydrolysates on plants under stress conditions was discovered. So, cultivation of pretreated with chitosan hydrolysate cucumber seeds (*Cucumis sativus*) in an unsuitable period for growth promotes their germination. At the same time, seed germination is completely absent in the control group.

1 Introduction

The use of chemical protection agents and plant fertilizers negatively affects the environment, and therefore human health. The search and study of effective and at the same time safe bio-fertilizers and growth biostimulants is an urgent problem. According to literature data [1-5], chitosan supports the immune system of plants in unfavorable environmental conditions and provides crop protection from pathogenic microorganisms. The effectiveness of using chitosan on plant seeds depends on its properties - molecular weight, supramolecular structure, the presence of impurities and some others. It is known that the effectiveness of chitosan increases with a decrease in its molecular weight [6-8].

To obtain low molecular weight chitosan and its oligomers, various methods are used: chemical, among which the most common are hydrolysis of high molecular weight chitosan with hydrogen peroxide and hydrolysis with highly concentrated acids, physical and

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biological (enzymatic). Currently, the attention of researchers is focused on enzymatic methods for the destruction of chitosan, since they are more consistent with the requirements of environmental safety of production, less affect the original natural structure of the polymer and are comparatively more profitable from a production point of view.

The aim of the work was to study the effect of enzymatic chitosan hydrolysates on seed germination and soil germination of plant seeds.

2 Methods

Objects of research. Pea seeds (*Pisum sativum*) variety “Zhegalova 112”, cucumber seeds (*Cucumis sativus*) variety “Rodnichok F1”.

It was used food-grade low-molecular acid-soluble chitosan (manufacturer: Bioprogress LLC). Molecular weight ranges from 1 kDa to 30 kDa. The degree of deacetylation is 75-95 %.

Hydrolysis of chitosan was carried out with the complex enzyme preparation "CelloLux A," the producer of which is mycelial fungi *Trichoderma viride*. The cellulase activity of the preparation is 2000 U/g.

Preparation of chitosan hydrolysates. Chitosan gels based on 1 % acetic acid were prepared for hydrolysis. The chitosan concentration was 3 %. Chitosan gels were subjected to freezing (T = -18 ºC, 12 hours) and subsequent thawing. Further, chitosan was hydrolyzed with the enzyme preparation "CelloLux A" at a temperature of 50 ºC for 60 minutes.

Pre-sowing treatment and plant growth research. Pea seeds (*Pisum sativum*) of the “Zhegalova 112” variety were soaked in aqueous solutions of chitosan hydrolysate with a concentration of 0; 0.2; 0.4; 0.6; 0.8; 1.0 % within 30 minutes. The growth activity of the pretreated seeds was studied upon following procedure:

Stage I. The seeds were germinated in laboratory conditions on a bed of filter paper in sterile Petri dishes for 6 days.

Stage II. On day 7 after seed germination in Petri dishes, seeds were placed in soil (in trays) to a depth of 2.5-3 cm. During the next 8 days, the following indicators of ontogenesis were evaluated: soil germination, the number of sprouts, the number of sprouts with expanded cotyledon leaves; the number of sprouts with the first and second main leaves.

Growing of plants under stress conditions. The study was carried out in October, a month unsuitable for germination. Cucumber seeds (*Cucumis sativus*) were soaked with chitosan hydrolysate solutions with concentrations ranging from 0 to 1.2 % for 30 minutes before planting. Germination was carried out at a temperature of about 20 °C directly in the ground.

3 Results and discussion

Low molecular weight chitosan compounds have potential applications in agro-industry as a natural fungicide and plant growth stimulant and can be used to stimulate growth and increase yield of many crops.

The presented study proposes the use of enzymatic hydrolyzate of chitosan in various concentrations as a plant growth biostimulator. To obtain low-molecular-weight chitosan derivatives, chitinases and chitosanases specific for this process and quite expensive in cost were replaced with an inexpensive and non-specific domestic enzyme preparation "CelloLux A". The chitosan hydrolysate used in further work was a mixture of low molecular weight fractions.

The study of the effect of chitosan hydrolysates on plant growth was carried out on pea seeds (*Pisum sativum*) variety “Zhegalova 112” and on cucumber seeds (*Cucumis sativus*) variety “Rodnichok F1”.

Studies with pea seeds were carried out in several stages. The seeds were pre-soaked in aqueous solutions of chitosan hydrolysate for 30 minutes, the content of the additive (chitosan hydrolysate) was 0; 0.2; 0.4; 0.6; 0.8; 1.0 %.

At the first stage, the seeds were germinated in laboratory (ideal) conditions on a layer of filter paper in sterile Petri dishes for 6 days. At the second stage, the germinated seeds were placed in the soil (in trays), that is, the growing conditions were brought closer to real ones. The germination process in Petri dishes is shown in Figure 1.

Fig. 1. Germination of pea seeds in Petri dishes

The results of germination of pea seeds in Petri dishes were evaluated according to the following indicators: the number of germinating seeds, the number of non-germinating seeds, the percentage of germination.

Germinating seeds include normally sprouted seeds.

Dissimilar seeds include: rotten seeds with a soft decomposed endosperm, with a rotted or blackened embryo, with rotted roots; hard seeds that have not swollen and have not changed their appearance; abnormally sprouted seeds: with ugly roots or sprouts; which, in the presence of a sprout, have no roots; having roots with swellings and by the time germination counts that have not developed additional roots; seedlings, roots or seed sprouts have cracks and interceptions reaching conductive tissues; seed seedlings have abnormally enlarged cotyledons and shortened roots.

The results of the studies are shown in Table 1.

Table 1. The effect of chitosan hydrolysate on the germination of pea seeds in Petri dishes (day 6 of germination).

Concentration of chitosan hydrolisate, %	Number of seeds, units			
	Total	Germinating ones	Ungerminating ones	Germination, %
0.0	60	51	9	85.0 ±4.2
0.2	60	55	3	91.6 ±0.0
0.4	60	52	5	86.7±4.4
0.6	60	54	4	90.0±3.6
0.8	60	56	1	93.3±3.5
1.0	60	56	2	93.3±3.5

As can be seen from the presented data, seed pretreatment with chitosan hydrolysate solutions at all test concentrations increases seed germination and soil seed germination by 5-9 %. The findings suggest that chitosan hydrolysate seed pretreatment has little efficacy on growth rates.

Interesting results were obtained when planting pea seeds in the soil. Growth and development of plants were evaluated within a week of planting. The evaluated indicators were:

- Soil germination - the number of seeds that germinated from the total number of sown seeds, expressed as a percentage.
- Number of plants with unfolded cotyledon leaves.
- Number of first and second true leaves.

The data is presented in Table 2.

Table 2. Germination of pea seeds treated with chitosan hydrolysate solution after planting in soil (fourth day after planting in soil). The date of sowing seeds in soil 23.01.23. Data received 27.01.23.

Concentration of chitosan hydrolisate, %	Total, units	Number of sprouts, units	Number of sprouts with cotyledon leaves, units	Soil germination, %
0.0	60	14	8	23.3±3.6
0.2	60	51	43	85.0±3.6
0.4	60	50	24	83.3±0.0
0.6	60	36	19	60.0±3.4
0.8	60	43	18	71.7±4.3
1.0	60	51	27	85.0±3.6

As can be seen from the data presented in Tables 1 and 2, the effect of chitosan hydrolyzate after planting peas into the soil is much more pronounced. Soil germination of seeds treated with chitosan hydrolysate increases 2.5-3.5 times. This is especially evident in the early stages of cultivation. When seeds are pretreated with chitosan hydrolysate, plant development is also accelerated. The number of plants with cotyledon leaves has increased several times.

When further assessing the growth and development of the plant, such indicators as the rate of formation of the first and second true leaves were additionally evaluated. The research data is shown in Table 3.

Table 3. Development of pea sprouts after planting in the soil.

Data	Concentration of chitosan hydrolisate, %	Total, units	Number of sprouts, units	Number of sprouts with leaves, units			Soil germination, %
				sprouts with cotyledon leaves	first true leaves	second true leaves	
Six day of attendance since sowing	0.0	60	26	22	13	7	43.3±3.5
	0.2	60	54	52	52	52	90.0±3.6
	0.4	60	58	57	56	46	96.7±5.0
	0.6	60	44	43	21	15	73.3±4.7
	0.8	60	49	44	43	36	81.7±4.1
	1.0	60	59	59	59	58	98.3±4.3
Seven day of attendance since sowing	0.0	60	37	24	20	11	61.7±4.4
	0.2	60	54	52	52	52	90.0±3.6
	0.4	60	58	57	57	56	96.7±5.0
	0.6	60	44	41	41	17	73.3±4.7
	0.8	60	50	50	45	42	83.3±0.0
	1.0	60	59	59	59	58	98.3±4.3
Eight day of	0.0	60	37	28	21	20	61.7±4.4
	0.2	60	54	52	52	52	90.0±3.6

attendance since sowing	0.4	60	58	57	56	46	96.7±5.0
	0.6	60	45	43	41	18	75.0±0.0
	0.8	60	50	47	45	43	83.3±0.0
	1.0	60	59	59	59	59	98.3±4.3

The data of the studies show that even with the further growth and development of peas, the positive effect of chitosan hydrolysate remains. Plants whose seeds have been treated with hydrolysate accelerate ontogenesis, which is expressed in faster formation of the plant, foliage development, accelerated growth of biomass. During the entire observation period, the ground germination of seeds treated with solutions of chitosan hydrolysates was 1.2-1.5 times higher compared to untreated seeds.

Thus, it was found that the highest efficiency of chitosan hydrolysates is manifested when growing pea seeds (*Pisum sativum*) in soil. After treating seeds with hydrolyzate, higher soil germination is observed, a significantly faster increase in green biomass occurs, and leaf formation is accelerated.

Chitosan and its hydrolysate are known to be most effective in seed germination under stress conditions, such as during the uncharacteristic planting season of the culture. To study plant growth under stress conditions, cucumber seeds (*Cucumis sativus*) were pretreated with chitosan hydrolysate. The study took place in October, a month unsuitable for germination. Solutions in the range of chitosan hydrolysate concentrations from 0 to 1.2 % were used for cucumber seed pretreatment. Cucumbers were soaked in solutions for 30 minutes before planting. Germination took place at a temperature of about 20 °C directly in the soil. The results obtained 55 days after planting are presented in Table 4.

Table 4. The effect of additives on the germination of cucumber seeds after planting in the soil.

Concentration of chitosan hydrolisate, %	**Total, units**	**Germinants, units**	**Number of sprouts with one leaves, units**	**Number of sprouts with two leaves, units**	**Number of sprouts with three leaves, units.**
0.0	12	-	-	-	-
0.2	12	-	-	-	-
0.4	12	3	3	2	-
0.6	12	2	2	1	-
1.0	12	4	4	3	-
1.2	12	2	2	2	1

4 Conclusion

It has been found that pretreatment of seeds with solutions of low molecular weight chitosan in all tested concentrations increases the germination efficiency of cucumber seeds under stress conditions.

Thus, chitosan hydrolysate has been shown to be an effective plant growth promoter. The highest efficiency is observed in soil cultivation of plants, and especially in the cultivation of plants under stress conditions.

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