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INFLUENCE OF NANOPREPARATIONS ON LABORATORY SEED GERMINATION, GROWTH, DEVELOPMENT AND YIELD OF COTTON (*Gossypium hirsutum* L.)

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Abstract

Currently, an important role is given to technologies based on a wide range of environmentally friendly plant protection products. The nanopolymer bioactive preparations based on chitosan from the silkworm (*Bombyx mori* Linnaeus, 1758) pupae with fungicidal and bactericidal properties are well suited for pre-sowing treatment of crop seeds. Here, for the first time, it was revealed that the seed treatment with nanopolymer preparations containing copper and silver ions accelerates the growth and development of seedlings and increases the yield of cotton cultivar Andijan 36. Our aim was to evaluate the effect of biologically active nanopolymer complexes based on chitosan and its derivatives on the morphophysiological and sowing parameters of seeds, the economically valuable qualities of raw cotton, and the yield of cotton in lab tests and under field conditions (the Research Institute of Selection, Seed Production and Agro-Technology of Cotton Growing, 2018-2020). In the experiments, we used nanopolymeric preparations PMC (polymer-metal complex) Cu²⁺:Ag 7:3, PMC Cu²⁺:Ag 8:2, Nano-chitosan (NanoChS, 0.5 %, 90 kDa), Nanoascorbachitazan (NanoAChS, 0.5 %, chitosan:ascorbic acid 4:1) and polymer preparations Chitosan initial 0.5 %, Kuprimhit 0.5 %, Ascorbatchitosan (AChS). Preparations UZKHITAN (Institute of Chemistry and Physics of Polymers of the Academy of Sciences of the Republic of Uzbekistan) and Dalbron (Dalston associated SA, Panama) served as standards, seeds without processing served as control. Seeds ($n = 36$) of cotton (*Gossypium hirsutum* L.) cultivar Andijan 36 were soaked in preparations (at the rate of 20 l/t seeds) at least 3-4 days before sowing. In each variant of the experiment, 6 kg of cotton seeds were treated. In lab tests, the seeds were germinated in sand at 25 °C and a 60-65 % air humidity. Germination energy was determined on day 4, germination on day 12. The length of the aboveground and underground parts of the seedlings was measured on days 3, 5, 7, 9, and 10. Field experiments were carried out at the experimental farm of NIISAVKh (Tashkent region, Kibray district, Salar settlement). The emergence of seedlings was registered, field germination was assessed, and phenological observations were made over the plant growth and development period from June 1 to September 1. To assess the economically valuable characteristics of raw cotton (fiber yield, weight per box, fiber length), test samples were collected before harvesting. Yields were recorded on September 15, October 1, October 15, and November 1. In lab tests, the seeds treated with PMC Cu²⁺:Ag 8:2, NanoAChS 0.5 %, 4:1 or PMC Cu²⁺:Ag 7:3 had the best germination rate, the 96.0, 96.0, and 97.0 %, respectively, that exceeded the control by 4.0-5.0 % and the Dalbron standard by 2.0-3.0 %. The aboveground part of the seedlings with NanoAChS treatment turned out to be 0.6-4.6 mm longer than that with AChS, the underground part was 0.1-2.1 mm longer. The differences between NanoChS and Chitosan were 0.3-2.1 mm and 0.8-2.3 mm, respectively, between PMC Cu²⁺:Ag 8:2 and Kuprumhit — 0.3-4.1 mm and 0.1-2.7 mm. On days 3, 5, 7, 9, and 10, plants from seeds treated with nanopolymer preparations outperformed those treated with polymer preparations. PMC Cu²⁺:Ag 8:2, NanoAChS and NanoChS had a more pronounced effect on seed germination and length of the aboveground and underground parts of the seedlings than their polymer counterparts. In field tests, the best indicators of growth and development were recorded for PMC

Cu²⁺:Ag 8:2, PMC Cu²⁺:Ag 7:3, and NanoAChS 0.5 %, specifically, the plant height on September 1 exceeded the control by 7.4; 8.0; 7.7 cm, the number of sympodial branches by 1.5; 1.2; 0.6 pcs, the number of boxes by 2.4; 1.8; 2.5 pcs. Also, upon seed treatments with PMC Cu²⁺:Ag 8:2 and PMC Cu²⁺:Ag 7:3, the cotton yields exceeded the control by 4.0 and 3.7 c/ha, respectively, and the Dalbron standard by 3.3 and 3.0 c/ha. A trend towards higher yields was observed from the first crop count. Therefore, nanopolymeric preparations PMC Cu²⁺:Ag 7:3 and PMC Cu²⁺:Ag 8:2 can be used for cotton seed encapsulation.

Keywords: *Gossypium hirsutum* L., cotton, seeds, variety, nanopreparations, germination, seedling length, phenological observation, growth, development, raw cotton, yield

Nanotechnology is a promising area of interdisciplinary research with great opportunities in medicine, pharmaceuticals, electronics, and agriculture. Advances in nanotechnology can be used to control insect pests using pesticides and insecticides based on nanomaterials, to increase crop productivity through the use of bioconjugated nanoparticles (encapsulation) to slowly release nutrients and water, for nanoparticle-mediated gene or DNA transfer in plants when creating varieties resistant to pests [1, 2].

In many countries (in Russia, Japan, India, China, USA, Vietnam, Germany, Uzbekistan), the effect of nanopolymer preparations on the sowing qualities of seeds, growth, development and productivity of agricultural crops, and metabolic processes in plants is being studied. Methods are being developed to obtain this information. In the Republic of Uzbekistan, work is also being carried out on the use of domestic nanomaterials. Nanopolymer preparations based on chitosan and its derivatives are being investigated. It has been shown that nanoparticles can have both positive and negative effects on plants depending on their size, concentration, chemical composition, stability and shape [3]. In addition, thanks to nanomaterials, it is possible to increase the efficiency of the restoration of soil contaminated with metals and metalloids [4]. Engineering nanomaterials with particle sizes of approximately 100 nm, where biological interactions are inevitable, have contributed to many revolutionary developments in various fields, including agriculture [5-10].

Encapsulation of seeds with preparations of chitosan and its derivatives has a positive effect on the process of photosynthesis in cotton plants even against a wilt background [11]. Chitosan nanoparticles are a natural material with excellent physicochemical, antibacterial and biological properties. Chitosan nanoparticles have a beneficial effect on the environment, have biological activity and do not adversely affect the human body [12-15]. They are widely used in agriculture, especially for crop protection, aided by their size, high surface area to volume ratio, and unique optical properties [16, 17]. The use of chitosan nanoparticles increases the content of chlorophyll and improves the absorption of nutrients by plants, affects germination, seedling growth and wheat yield [18, 19]. Chitosan nanopurges have an effect at lower concentrations than chitosan, in particular, they can enhance the growth of wheat, which reduces the use of fertilizers [20].

Since the end of the 20th century, the Research Institute of Breeding, Seed Production and Agricultural Technology of Cotton Growing (Republic of Uzbekistan) has been studying the physicochemical and biologically active properties of chitin and chitosan isolated from silk-reeling production waste, the silkworm (*Bombyx mori* Linnaeus, 1758) pupae [21]. A technology has been developed and production of *Bombyx mori* chitosan and its various derivatives for agricultural use has been established [21].

In this work, it was revealed for the first time that the treatment of cotton seeds of the Andijan 36 variety with preparations containing copper and silver ions

based on chitosan nanoparticles from silkworm pupae accelerates the growth and development of seedlings, and also increases the yield of cotton.

The purpose of the work is to evaluate the effect of biologically active nanopolymer complexes based on chitosan and its derivatives on the morphophysiological and sowing parameters of seeds, the economically valuable qualities of raw cotton, and the yield of cotton.

Materials and methods. The studies were carried out in laboratory and field conditions in 2018-2020 at the Breeding, Seed Production and Agricultural Technology of Cotton Growing (NISSAVKh). Environmentally safe biologically active nanopolymer preparations based on chitosan and its derivatives were synthesized at the Institute of Chemistry and Physics of Polymers of the Republic of Uzbekistan Academy of Sciences (ICPPRUAS). In the experiments, we used nanopolymer preparations of PMC (polymer metal complex) Cu²⁺:Ag 7:3, PMC Cu²⁺:Ag 8:2, Nanochitosan (NanoChZ, 0.5%; 90 kDa), Nanoascorbitchitosan (NanoAChZ, 0.5%, ratio of chitosan to ascorbic acid 4:1) and polymer preparations Chitosan initial 0.5%, Kuprimhit 0.5%, Ascorbitchitosan (AChZ). Preparations UZKHITAN (ICPPRUAS) and Dalbron (Dalston associated SA, Panama) served as standards. Control seeds were not treated with preparations.

We used cotton (*Gossypium hirsutum* L.) seeds of the Andijan 36 variety which is included in the State Register of crops recommended for sowing on the territory of the Republic of Uzbekistan.

Seeds were soaked in preparations (20 l/t of seeds) at least 3-4 days before sowing. In each option, 6 kg of cotton seeds were processed. In a lab tests, the seeds were germinated in a thermostat in sand at 25 °C and the air humidity of 60-65%. Germination energy was determined on day 4, germination rate on day 12. The length of the aboveground and underground parts of the seedlings was measured on days 3, 5, 7, 9, and 10.

Field trials were carried out at the experimental farm of NISSAVKh (Tashkent region, Kibray district, Salar village) as accepted [22] on 50-hole plots, sowing was carried out manually, 5 seeds per hole.

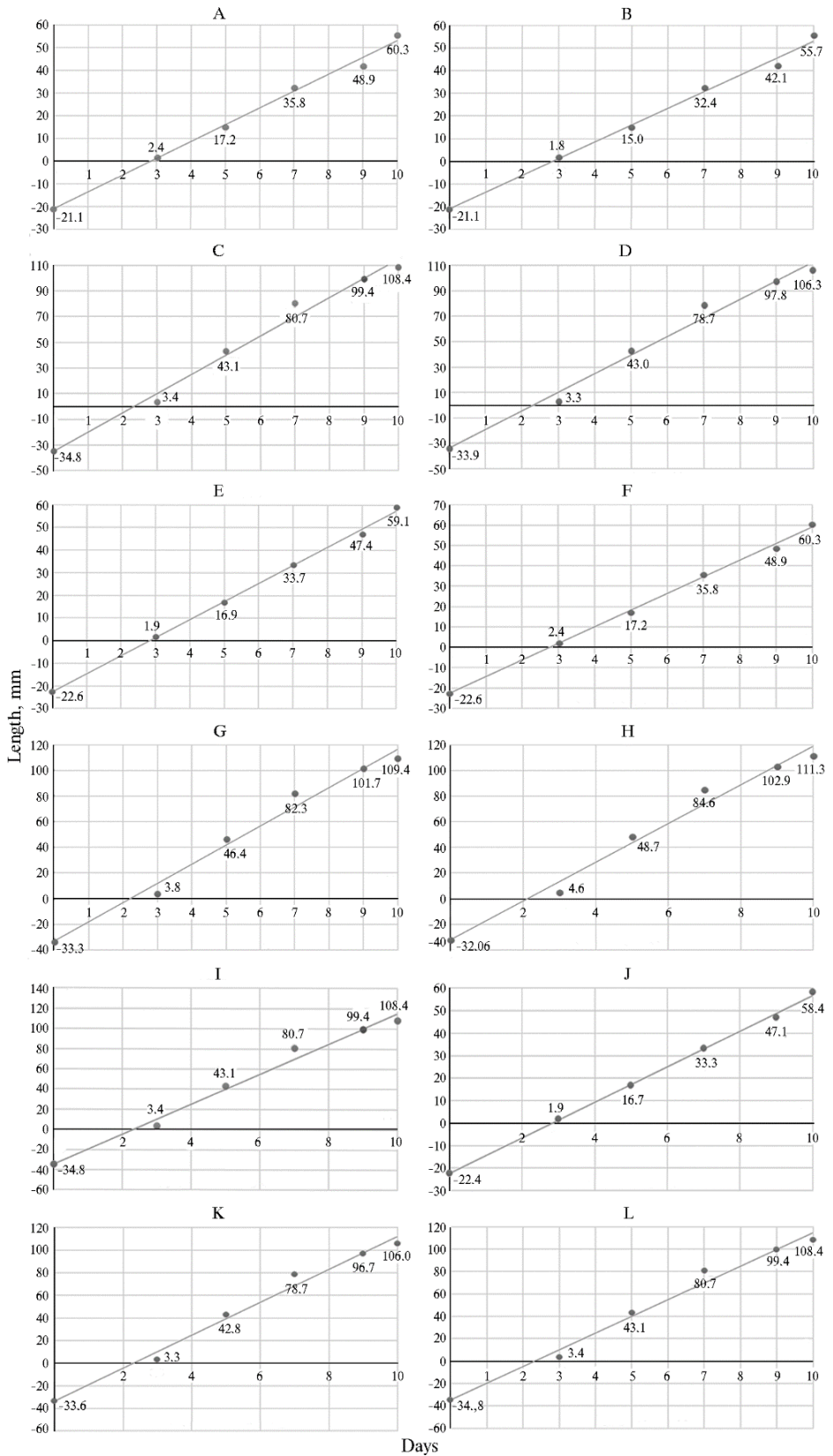
Seedling emergence was recorded, and field germination was calculated. Phenology of plant growth and development was examined from June 1 to September 1. To assess the economically valuable characteristics of raw cotton (fiber yield, weight of one box, fiber length), test samples were collected before harvesting. Yields were assessed on September 15, October 1, October 15 and November 1 in accordance with the current instructions. Sowing and varietal qualities were determined based on existing standards [23-25].

Mathematical processing of the obtained results was carried out according to B.A. Dospikhov [26]. Means (*M*) and standard errors of means (\pm SEM) were calculated, and the least significant difference for the 5% significance level (LSD₀₅) was calculated. The least squares method [27] was also used for analysis.

Results. For high and guaranteed raw cotton yields, the use of a wide range of environmentally friendly plant protection products for pre-sowing seed processing are promising. Metal nanopowders and nanoparticles easily penetrate cells and actively influence enzymes and physiological and biochemical reactions, increase laboratory and field germination [28, 29]. Analysis of variance showed that pre-sowing treatment of seeds with nanopreparations has a significant effect on most plant traits: the length of seedlings, roots and shoots, their wet and dry weight, resistance to pathogens and pests, as well as on indicators of crop structure and yield [30].

1. Germination and length of cotton (*Gossypium hirsutum* L.) cv. Andijan 36 seedlings upon seed treatment with nanopolymer and polymer preparations (lab tests, $N = 4$, 2018-2020)

Treatment	Germination energy/germination rate, %			Length, μmm									
				day 3		day 5		day 7		day 9		day 10	
	day 4	day 12	to control	AG	UG	AG	UG	AG	UG	AG	UG	AG	UG
Control (no treatment)	90.0±0.95	92.0±0.6	0	1.4±0.06	3.0±0.05	14.6±0.11	39.4±0.25	31.7±0.22	76.8±0.21	40.6±0.30	90.2±0.51	49.7±0.32	101.5±0.31
Dalbron (reference)	92.0±0.2	94.0±0.9	+2.0	1.5±0.04	3.1±0.05	15.1±0.09	42.1±0.37	32.1±0.25	77.2±0.29	42.3±0.31	91.9±0.37	50.3±0.31	103.7±0.35
UZKHITAN (standard)	94.3±0.4	95.0±0.2	+3.0	1.7±0.05	3.4±0.04	14.8±0.12	42.8±0.29	31.9±0.33	78.4±0.19	43.4±0.25	94.2±0.39	54.1±0.32	105.2±0.38
Chitosan original 0.5%	94.2±0.7	95.0±0.2	+3.0	1.9±0.04	3.8±0.06	16.9±0.13	46.4±0.41	33.7±0.30	82.3±0.28	47.4±0.26	101.7±0.56	59.1±0.38	109.4±0.39
NanoChZ 0.5%	94.8±0.6	95.0±0.2	+3.0	2.4±0.06	4.6±0.07	17.2±0.14	48.7±0.23	35.8±0.25	84.6±0.31	48.9±0.34	102.9±0.41	60.3±0.47	111.3±0.52
PMC Cu ²⁺ :Ag 7:3	94.9±0.1	97.0±0.3	+5.0	1.6±0.05	3.5±0.04	16.1±0.09	43.2±0.33	33.1±0.23	79.8±0.28	43.1±0.33	98.8±0.54	56.8±0.41	107.9±0.45
PMC Cu ²⁺ :Ag 8:2	95.1±0.2	96.0±0.4	+4.0	1.9±0.03	3.4±0.05	16.7±0.10	43.1±0.27	33.3±0.28	80.7±0.30	47.1±0.18	99.4±0.37	58.4±0.47	108.4±0.42
Kuprumhit	93.6±0.3	95.0±0.2	+3.0	1.6±0.04	3.3±0.03	15.7±0.13	42.8±0.36	32.3±0.19	78.7±0.23	43.0±0.21	96.7±0.38	55.6±0.39	106.0±0.42
AChZ	93.7±0.8	95.0±0.2	+3.0	1.8±0.06	3.3±0.03	15.0±0.08	43.0±0.28	32.4±0.29	78.7±0.33	42.1±0.32	97.8±0.42	55.7±0.37	106.3±0.38
NanoAChZ 0.5%, 4:1	94.5±0.5	96.0±0.4	+4.0	2.4±0.04	3.4±0.04	17.2±0.17	43.1±0.42	35.8±0.31	80.7±0.25	48.9±0.27	99.4±0.45	60.3±0.35	108.4±0.43
LSD ₀₅ = 2.21 %													
Note. For a description of the preparations and the experimental design, see the Material and methods section; AG — aboveground part, UG - underground part.													



The length of roots and seedlings of cotton (*Gossypium hirsutum* L.) cv. Andijan 36 on days 3-10 upon

seed treatment with nanopolymer and polymer preparations: A — NanoAChZ, seedlings ($k = 7.68$), B — AChZ, seedlings ($k = 7.42$), C — NanoAChZ, roots ($k = 14.74$), D - AChZ, roots ($k = 14.66$), E — Chitosan original, seedlings ($k = 8.00$), F — NanoAChS, seedlings ($k = 8.16$), G — Chitosan original, roots ($k = 15.01$), H — NanoChZ, roots ($k = 15.07$), I — Kuprumhit, seedlings ($k = 7.46$), J — PMC Cu²⁺:Ag 8:2, seedlings ($k = 7.92$), L — Kuprumkhit, roots ($k = 14.58$), M — PMC Cu²⁺:Ag 8:2, roots ($k = 14.98$) (lab test, 2018-2020). For a description of the preparations and the experimental design, see the Material and methods section.

In our lab tests, the seeds treated with PMC Cu²⁺:Ag 8:2 (96.0%), NanoAChZ 0.5%, 4:1 (96.0%) and PMC Cu²⁺:Ag 7:3 (97.0%) had the best germination which was 4.0-5.0% higher than in the control and 2.0-3.0% higher compared to Dalbron (reference) (Table 1).

Previously, we found that the nanopolymer preparations Nanoascorbic acid, PMC Cu²⁺:Ag 7:3 and PMC Cu²⁺:Ag 8:2 have a positive effect on the laboratory germination of soybean seeds. In addition, the yield of Selecta 302 and Baraka varieties when treated with these preparations was higher than that for Gaucho WS 70 standard (wetting powder, Bayer Crop Science, Germany) by 6.1-4.5 and 5.1-3.3 c/ha, respectively [31]. The effectiveness of the action of nanopolymer preparations on the activity of peroxidase and polyphenol oxidase enzymes and protein content in 7-day-old soybean seedlings has also been proven [31].

V. Saharan et al. [32] revealed higher values of germination, length of shoots and roots, number of roots, length of seedlings, wet and dry weight of corn seedlings treated with Cu-chitosan nanoparticles (NP) at concentrations of 0.04-0.12% compared to water, CuSO₄ and loose chitosan. Cu-chitosan NPs at the same concentrations induced the activity of α -amylase and protease enzymes. Cu-chitosan NPs at a concentration of 0.16% had an inhibitory effect on the growth of seedlings, which can be explained by the toxicity of copper excess [32].

In this work, we compared the effectiveness of the use of nano-preparations with polymer preparations on cotton. When measurements were carried out at all times, the length of the above-ground part of the seedlings in the variant with NanoAChZ turned out to be 0.6-4.6 mm longer than in the variant with ACP, and the length of the underground part by 0.1-2.1 mm. When comparing NanoChZ with Chitosan, the differences were 0.3-2.1 mm and 0.8-2.3 mm, respectively; when comparing PMC Cu²⁺:Ag 8:2 and Kuprumkhit, by 0.3-4.1 mm and 0.1-2.7 mm (see Table 1, Fig.). In all measurements on days 3, 5, 7, 9, 10, plants from seeds treated with nanopolymer preparations outperformed the variants where polymer preparations were used. PMC Cu²⁺:Ag 8:2, NanoAChZ and NanoChZ had a more effective effect on the germination and length of the above-ground and underground parts of seedlings than their polymer counterparts.

An analysis of the results obtained using the least squares method [27] and summing up the influence of various composite compositions is given in the form of the dynamics of biological effects over time (five points, days 3, 5, 7, 9 and 10). It seems that the most realistic comparative indicator of these dependencies can be linear:

$$y = kx + b,$$

where y is an indicator characterizing the growth of the object, x are the observation periods, k and b are the internal parameters of the phenomenon under study. As can be seen from the figure, when using nanopreparations, the value of the coefficient k , which was considered as a collective indicator of the effect of preparations on the efficiency of plant growth, was higher than in the variants with polymer preparations.

In 2018-2020, in the experimental farm of NIISAVKh, we studied the effect of nanopolymer preparations on the sowing qualities of seeds, the growth and development of plants, as well as the yield of cotton.

2. Yield and economically important traits of cotton (*Gossypium hirsutum* L.) cv. Andijan 36 upon seed treatment with nanopolymer and polymer preparations ($N = 4$, experimental farm NIISAVKh, Republic of Uzbekistan, 2018-2020)

Вариант	The number of plants per ha	Yield, c/ha					Economically important traits					
		10/01	10/15	11/01	total	± to control	fiber output, %	± to control	pod weight, g	± to control	fiber length, mm	± to control
Control (no treatment)	66.7±0.2	27.3±0.4	6.4±0.2	3.4±0.2	37.1±0.4	0	38.5±0.4	0	5.65±0.06	0	34.8	0
Dalbron (reference)	71.2±0.3	28.1±0.5	6.2±0.2	3.5±0.3	37.8±0.3	+0.7	38.6±0.5	+0.1	5.76±0.06	+0.11	34.7±0.3	-0.1
UZKHITAN (standard)	73.4±0.3	29.4±0.4	6.5±0.3	2.7±0.2	38.6±0.4	+1.5	38.5±0.4	0	5.72±0.07	+0.07	34.8±0.3	0
AChZ	74.3±0.3	29.7±0.5	5.9±0.3	2.9±0.3	38.5±0.3	+1.4	38.4±0.3	-0.1	5.76±0.06	+0.11	34.9±0.2	+0.1
PMC Cu ²⁺ :Ag 7:3	76.6±0.3	32.1±0.5	6.1±0.2	2.6±0.3	40.8±0.3	+3.7	38.7±0.4	+0.2	5.83±0.10	+0.18	35.0±0.3	+0.2
PMC Cu ²⁺ :Ag 8:2	77.1±0.4	33.4±0.5	5.8±0.3	1.9±0.2	41.1±0.3	+4.0	38.7±0.5	+0.2	5.84±0.07	+0.29	35.0±0.3	+0.2
Chitosan original	72.4±0.4	29.3±0.4	5.5±0.2	3.3±0.3	38.1±0.4	+1.0	38.5±0.4	0	5.87±0.06	+0.22	34.8±0.2	0
NanoChZ	75.5±0.2	32.2±0.4	5.2±0.3	2.1±0.2	39.5±0.4	+2.4	38.6±0.3	+0.1	5.71±0.05	+0.06	34.9±0.3	+0.1
NanoAChZ 0.5%	76.8±0.3	32.9±0.3	5.8±0.3	1.8±0.3	40.5±0.4	+3.4	38.6±0.4	+0.1	5.83±0.07	+0.18	34.9±0.2	+0.1

LSD₀₅ = 2.34 c/ha

Note. For a description of the preparations and the experimental design, see the Material and methods section.

The germination of the Andijan 36 cv. seeds treated with NanoChZ, PMC Cu²⁺:Ag 8:2, PMC Cu²⁺:Ag7:3, Chitosan original was higher by 15.3; 15.1; 13.6 and 13.0%, respectively, and exceeded the values obtained with the UZKHITAN standard by 11.9; 11.7; 10.2 and 9.6%. The best effect on growth and development were recorded when PMC Cu²⁺:Ag 8:2, PMC Cu²⁺:Ag 7:3 and NanoAChZ 0.5% were treated. On September 1, the plants were higher than the control ones by 7.4, 8.0, 7.7 cm and exceeded the control by 1.5, 1.2, 0.6 sympodial branches and 2.4, 1.8, 2.5 boxes per plant.

The yield forecast carried out on September 1 showed that PMC Cu²⁺:Ag 8:2 and PMC Cu²⁺:Ag 7:3 preparations had an advantage over the control. The yield upon the treatments was higher by 4.8 and 5.2 c/ha, respectively. It was also found that the yield of raw cotton is more affected by seed treatment with nanopolymer preparations than with polymer ones (Table 2). In almost all variants, where the seeds were treated with nanopolymer preparations, the yield was higher vs. control and vs. the standard. For example, with PMC Cu²⁺:Ag 8:2, the yield exceeded the control by 4.0 c/ha, with the Dalbron standard by 3.3 c/ha, and with the UZKHITAN standard by 1.6 c/ha. It should be noted that a trend towards higher yields occurred from the first count, which indicates an earlier ripening of cotton when seeds were treated with nanopolymer preparations.

Based on test samples, economically valuable features of raw cotton were determined. The yield of fiber, the mass of raw cotton in one box and the length of the fiber corresponded to the author's description of the varieties. We did not find a wide variation in parameters between the treatments (data not shown), that is, nanopolymer preparations did not affect the economically valuable traits but had a positive effect on the overall yield.

Our results are consistent with the data obtained by M. Wang et al. [33] on four irrigated and rainfed wheat varieties upon treatment with chitosan oligosaccharides (COS). The preparations were used for seed treated and foliar spraying at different stages of plant growth. In varieties of irrigated wheat, the number of grains per ear increased significantly when seeds were treated, and the number of spikelets when leaves were sprayed at tillering and heading. COS significantly affected the grain yield in all irrigated varieties, while the effect of chitosan oligosaccharides on rainfed varieties was insignificant [33]. D.-F. Zeng et al. [34] treated soybean seeds using a new chitosan-based preparation with carboxymethylchitosan as the main component supplemented with trace elements and growth regulators. Upon treatment, the soybean yield increased by 17.95%. The drug had an excellent antifidant effect.

Thus, cotton Andijan 36 cv. seeds treated with nanopreparations PMC (polymer metal complex) Cu²⁺:Ag 7:3, PMC Cu²⁺:Ag 8:2 and NanoAChZ 0.5% 4:1 had a high laboratory germination rate and exceeded the control by 4-5% and Dalbron standard by 2-3%. High biological activity of these preparations at the first stage of seed germination also leads to an increase in the length of the aboveground and underground parts of the seedlings and activates plant growth. In field trials, plants from seeds treated with PMC Cu²⁺:Ag 8:2 and PMC Cu²⁺:Ag 7:3 outperformed the control by 4.0 and 3.7 c/ha, respectively, and the Dalbron standard by 3.3 and 3.0 c/ha. From the first record, there was a trend towards higher yields. Therefore, nanopolymer preparations PMC Cu²⁺:Ag 7:3, PMC Cu²⁺:Ag 8:2 which are recommended for all regions of the Republic of Uzbekistan, can be used to encapsulate cotton seeds.

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