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EFFICIENCY OF NANOPOLYMER APPLICATION ON THE GROWTH, DEVELOPMENT AND YIELD OF SOYBEAN

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ABSTRACT

The current study was carried out to analyze the effects of agro-nanopolymer preparations application on the seed germination, seedling and root sprout lengths, growth, development, yield, and resistance to *Fusarium* wilt in Baraka and Selecta-302 soybean variety under laboratory condition. For encapsulation of soybean seeds and nanopreparation UZHITAN, nanochitosan, chitosan (initial), polymer metallocomplexes (PMCs) of copper and silver ions were used at various ratios for nanoascorbate chitosan, ascorbate chitosan, polymer metallocomplex (PMC) Cu²⁺:Ag 7:3 and PMC Cu²⁺:Ag 8:2 preparations. Results of the study revealed a significant effect of these agronanopolymers on the studied soybean varieties; application of this nanoascorbate chitosan, ascorbate chitosan, polymer metallocomplex (PMC) Cu²⁺:Ag 7:3 and PMC Cu²⁺:Ag 8:2 showed 17 g, 14.6 g, 13.5 g, and 11.9 g weight of the 1000-grains respectively which was significantly higher than the Gaucho treatment. Further, these agronanopolymers also have a significant effect on the various yield attributes such as number of beans per plant, number of seeds per bean, and seed mass per bean. Application of chitosan and PMC agronanopolymers increased productivity by 4.5-6.1 kg/ha and 3.3-5.1 kg/ha, respectively, compared to the control. Also, the effect of these agro nanopolymer preparations on enzyme activity and protein content was studied in the current study. Among the various tested nanopreparation, the greatest effect on total soybean yield was observed when seeds were treated with nano ascorbate chitosan. All nanopreparation treated seeds has a significant effect on the accumulation of macro and micronutrient accumulation in Soybean seed and except few one rest are the superior to the control and UZHITAN.

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1 Introduction

In recent decades, the introduction of nanotechnology for agricultural applications has been widespread throughout the world. Over the past 10 years, the worldwide production of chitosan has reached more than 3,000 tons and the USA, Japan, India, China, Italy, and Russia are the leaders of chitosan production. More than 15% of the chitosan is globally produced by Primex while approximately 25% of the chitosan is produced and sold by Japanese firms.

Worldwide, researchers from various countries such as the USA, Japan, India, China, Vietnam, Germany, Russia, and Uzbekistan are researching to study the effect of nano polymer preparations on the seed sowing quality, growth, development, and yield of crops. Research is being conducted to study the effects of nanomaterials on seeds and plants and the resulting products affecting metabolic processes in plants.

Albumin-rich soy is a worldwide one of the main industrial crops. Various biotic and abiotic factors including pathogenic microbe's affect soybean seed's survival and germination. To overcome such adverse situations, soybean seeds are usually treated with various preparations before sowing (Ge et al., 2013; Chau et al., 2019; Coman et al., 2019).

Nanotechnology can play a vital role in increasing agricultural production. The properties of nanoparticles not only depend on their physical nature, production method, size, and structure but also depend on the biological model on which the tests are carried out (Tikhonov et al., 2000; Fedorenko et al., 2006; Cheng et al., 2016; Cheng et al., 2017; Sendra et al., 2017).

Nanotechnology products, such as engineering nanomaterials (ENM), are among the new and exciting discoveries of the current century. These ENMs are with sizes that ensure biological interactions, have contributed to many revolutionary developments, from energy production and storage to agriculture and environmental management to targeted delivery systems (Tong et al., 2007; Johansen et al., 2008; Ghormade et al., 2011; Nowack et al., 2012; Priester et al., 2013; Ge et al., 2013). It has been established that plants can use the surface energy of these nanoparticles. Further, pre-sowing treatment of seeds with nanoparticles has a prolonged effect on seedling growth and cellular level development (Акрафьрова et al., 2011; Didenko et al., 2005; Hernandez-Viezas et al., 2013).

Chitosan is a multifunctional polymer, which is derived from chitin, and it is known for its unique microbial degradability, biocompatibility and nontoxicity (Zeng & Zhang, 2010). Chitin, chitosan and their derivatives, which have several valuable physicochemical and biomedical properties, have been the subject

of numerous studies form last 200 years. The solubility of chitosan and its derivatives in aqueous media opened up new possibilities for creating unique polymer systems that can be used in the field of agriculture (Tikhonov et al., 2000). Further, Chitosan also possessed fungicidal (Kulikov et al., 2006a), virucidal (Kulikov et al., 2006b; Stevanoic et al., 2012), bactericidal (Chirkov, 2002; Gerasimenko et al., 2004; Didenko et al., 2005; Rashidova & Milusheva, 2009) and immunomodulatory (Pomogaylo et al., 2000; Zorin, 2004) properties. This material also has anti-radiation effects (Ilyin et al., 2004; Varlamov et al., 2005) and are used as sorbents (Aktuganov et al., 2003), and flocculants (Bykova et al., 2004) in the creation of nematode-resistant preparations (Vasyukova et al., 2001; Kandrashina, 2006) and growth regulators (Rashidova & Milusheva, 2009).

In the Republic of Uzbekistan, for the first time, the role of polymers in the creation and use of polymer preparative forms of chemical plant protection products was identified by Rashidova et al. (2013) and Rashidova et al. (2008). In Uzbekistan, waste from the natural silk production in the form of silkworm pupae is a source of chitin and chitosan (Rashidova & Milusheva, 2009). The use of polymers for crops seed encapsulating has been scientifically substantiated by targeted selection and polymer synthesis (Milusheva et al., 2002; Rashidova et al., 2005; Rashidova et al., 2006; Rashidova & Milusheva 2009; RUz patent No. IAP 03956, 2009). Based on the "Method for encapsulating seeds" (No. IAP03956) and "Composition for pre-sowing treatment of seeds of crops and method for its preparation" (No. IAP 2013 0271), the Intellectual Property Agency of the Republic of Uzbekistan issued two patents (IAP 03956, 2009). UZHITAN is a mixture of chitosan and CMC polymers and has water-soluble properties (Rashidova & Voropaeva, 2006). Chitosan with a certain degree of deacetylation is used as part of the polymer UZHITAN preparation (a disinfectant with a stimulatory effect), which is registered with the State Chemical Commission of the Republic of Uzbekistan for use in the agricultural sector (Fedorenko et al., 2006).

Zeng & Zhang (2010) used a new preparation 55-F-1 to encapsulate soybean variety seeds, which includes carboxymethylchitosan as the main component, microelements as a supplement, and growth regulators to stimulate the germination and growth. Results of this study revealed that the yield of 55-F-1 treated soybean seeds increased by 17.95%. Further, this preparation also has a significant effect on the antifeedant effect activities and provides fundamental protection to the crops from pest damage.

Similarly, various researchers reported a highly significant stimulatory effect of nanoformulation application on the germination and growth of soybean seedlings. Further, among these Co- and Mo-containing nanoscales formulations led to the

highest growth indices (excess of approximately 10%). Enzymatic analysis of shoots from nano-processed soybean plants also revealed a similar result (Chau et al., 2019).

Positive effects of engineering nanomaterials on germination and growth of soybeans were recorded when low concentrations of ZnO (50 mg/kg) and iron oxides were applied to the soil (both to the soil and through topdressing). Further, TiO₂ had an almost neutral effect, while CuO and Cr₂O₃ negatively affected the soybean plant development (Coman et al., 2019). Further, at higher concentration of ZnO nanoparticles (500 mg/L - 2000 mg/L) have an inhibitory effect on the root growth of soybean root (Lin & Xing, 2007; López-Moreno et al., 2010; Yoon et al., 2014; Tamuly et al., 2014).

To obtain high and guaranteed soybean crops, the use of promising technologies for the preparation of sowing seeds, which involves the use of a wide range of environmentally friendly plant protection products, is important. The current study was aimed to evaluate or study the effect of nano preparation application on germination, seedling growth, root lengths, yield development, enzymatic activities, protein content, micro and macronutrient concentration, and *Fusarium wilt* resistance in the Baraka and Selecta-302 varieties of soybean under laboratory conditions. Additionally, the behavior of the nano preparation when interacting with biological materials during the metabolic processes of plants was also examined.

2 Materials and methods

In the current study seeds of two soybean varieties viz., Baraka and Selecta-302 were used, these two are included in the State Register of Agricultural Crops and recommended for sowing in the Republic of Uzbekistan. Before the showing, these seeds were encapsulated/treated with various active nanoagropolymer

preparations such as UZHITAN, nanochitosan, polymer metallocomplexes of copper and silver ions at various ratios. Further, nanoascorbate chitosan and ascorbate chitosan based on chitosan and its derivatives are synthesized in the Laboratory of Advanced Polymers Synthesis, Institute of Chemistry and Physics, Republic of Uzbekistan. As a standard reference treatment, soybean seeds were dressings with Gaucho/Dalbron/Uzgumi, which are approved agro-industrial complex by the State Chemical Commission of the Republic of Uzbekistan for use agriculture while untreated seeds were used as a control.

The germination of soybean seeds was determined according to GOST 12038-86 "germination determination methods for crops seeds." Under laboratory conditions, micro-and macronutrients concentrations were determined (mg/kg) in seeds and one-month-old soybean plants as well as during the flowering and ripening stage by using an ECO and Optima 240 0DV US ICP instrument. For the estimation of *fusariosis* incidence on the soybean leaves and roots, the method of Syrmolot (2015) was used. The obtained results were analyzed using the SPSS program.

3 Results and Discussion

Influence of various nanopolymer preparations i.e. PMC Cu²⁺:Ag 7:3, PMC Cu²⁺:Ag 8:2 (0.5%); chitosan ref. (0.5%), nanochitosan(90 kDa), ascorbate chitosan, nanoascorbate chitosan @ 0.5% (4:1), and UZHITAN on the germination of soybean seeds was studied in the laboratory of Biochemistry and Plant Physiology at the Cotton Breeding, Seed Production and Agrotechnologies Research Institute, Tashkent, Uzbekistan. Seeds treated with Dalbron were used as a reference, and untreated seeds were used as a control. The laboratory germination of Baraka soybean seeds was studied on 3 and 7 days of inoculation (Table 1). Among the various tested nano preparations, maximum seed germination was reported with the

Table 1 Effect of various nanopreparation applications on the germination of soybean variety Baraka seeds in 2019

№	Treatment	Germination (%)				
		After 3 days	Oksuv Farm On 7th day	+ relative to the control	Moygir Yusufkhon Farm After 3 days	+ relative to the control
1	Control	81.0±0.6	85.2±0.5	0	81.4±0.4	86.2±0.4
2	Dalbron (standard)	82.7±0.7	86.3±0.4	+1.1	80.0±0.3	86.5±0.5
3	UZHITAN (standard)	83.5±0.4	88.0±0.6	+3.2	80.5±0.2	88.0±0.6
4	PMC Cu ²⁺ :Ag 7:3	85.2±0.3	89.0±0.6	+3.8	83.2±0.5	84.5±0.4
5	PMC Cu ²⁺ :Ag8:2	83.0±0.2	87.2±0.3	+2.0	80.7±0.5	86.7±0.2
6	Chitosan source (0.5%)	84.7±0.5	90.2±0.4	+5.0	86.4±0.4	88.2±0.4
7	Nanochitosan (0.5%)	86.0±0.4	92.5±0.2	+5.3	84.7±0.6	90.5±0.5
8	Ascorbate chitosan	85.6±0.5	90.4±0.4	+5.2	86.5±0.6	91.2±0.4
9	Nanoascorbate chitosan	88.6±0.4	92.5±0.5	+7.3	82.0±0.4	92.0±0.3
		NSR ₀₅ =2.23%		NSR ₀₅ =2.88%		

nanochitosan @ 0.5% (90 kDa) and nanoascorbate chitosan @ 0.5% (4:1), with a germination rate of 92.5% and 90.5%, respectively, and these two are 4.3-7.3% exceeding than the control and 3.2-6.2% than the standard agrochemicals Dalbron.

Results of the study revealed that encapsulation of soybean seeds with nanoascorbate chitosan @ 0.5% (4:1) and nanochitosan @ 0.5% (90 kDa) positively affect the laboratory germination of soybean seeds and can be recommended to the farmers for better soybean production.

Effect of nanopreparation on the seedling growth of Baraka soybean seeds obtained from Oksuv Farm of Kurgantepa and Maygir Yusufkhon Farm of the Izboskan district of Andijan region was studied. Seeds obtained from these two farms were encapsulated by the above-said nanopreparation and sown in phytotron vessels and the length of the seedlings and roots were measured after 10 days of germinations. The aboveground and below-ground parts of the seedlings treated with nano preparation were longer than those in the control by 0.9 mm to 4.4 mm. Results

are in agreement with the findings of Shah et al. (2016).

Among the various tested combinations, best seedling and root growth was reported in the seeds encapsulated with nanoascorbate chitosan @ 0.5% (4:1). Specifically, this treatment has 10.9 – 11% longer seedlings than the control. A similar result was obtained for the below-ground part (Table 2). Further, the results of study revealed that various other nano preparations i.e. nanoascorbate chitosan @ 0.5% (4:1), $\text{PMCCu}^{2+}:\text{Ag}$ 7:3, and $\text{PMCCu}^{2+}:\text{Ag}$ 8:2 also have high significant biological activities and increases the length of the above and below ground parts of tested seedlings. Plants from seeds treated with UZHITAN were taller than those in the control.

Under laboratory conditions, the concentrations of macro - and microelements (mg/kg) were determined in seeds and one-month-old soybean plants (Tables 3). Results of the study revealed that the concentrations of micro-and macroelements in the soybean seeds are higher than the one-month-old plants except for calcium and Sodium. Among the tested element, copper was reported in the

Table 2 Effect of nano preparation application of the seedling and root growth of Baraka soybean seedlings

№	Options	Oksuv Farm						Moygir Yusufkhon Farm					
		Aboveground part (mm)	C	V	Belowground part (mm)	C	V	Aboveground part (mm)	C	V	Belowground part (mm)	C	V
1.	Control	34.8±0.4	1.14	3.2	86.6±0.4	1.2	1.4	35.3±0.3	0.9	2.6	88.4±0.4	1.3	3.0
2.	Dalbron (standard)	35.6±0.5	1.4	4.0	89.7±0.4	1.1	1.3	35.8±0.3	1.0	2.9	89.6±0.5	1.7	1.9
3.	UZHITAN (standard)	36.0±0.3	1.0	2.7	89.6±0.3	1.0	1.2	35.9±0.3	1.0	2.8	89.7±0.3	1.1	2.8
4.	PMC $\text{Cu}^{2+}:\text{Ag}$ 7:3	38.0±0.4	1.4	3.7	97.2±0.3	0.9	0.9	37.8±0.4	1.5	3.8	94.8±0.4	1.3	3.4
5.	PMC $\text{Cu}^{2+}:\text{Ag}$ 8:2	37.6±0.2	0.9	2.4	96.5±0.4	1.1	1.2	39.2±0.6	2.1	5.3	99.1±0.5	1.6	4.5
6.	Chitosan source (0.5%)	35.3±0.3	1.15	3.3	89.7±0.3	1.1	1.2	36.8±0.5	1.5	4.3	92.4±0.3	1.1	1.2
7.	Nanoascorbate chitosan (0.5%)	38.6±0.4	1.1	2.9	97.4±0.4	1.4	1.4	39.7±0.4	1.2	2.9	99.4±0.5	1.4	4.2
8.	Nanochitosan (0.5%)	37.9±0.3	0.8	2.3	91.7±0.3	1.0	1.2	37.9±0.3	1.1	2.9	96.6±0.4	1.2	2.9
		NSR ₀₅ =3.49%		NSR ₀₅ =2.24%		NSR ₀₅ =3.59%		NSR ₀₅ =2.76%					

Table 3 Effect of various nano preparation applications on the contents of micro-and macroelements in Baraka soybean seeds and plants

№	Preparation	Micro & Macroelements (Mg/Kg)									
		Copper		Iron		Potassium		Calcium		Sodium	
		Seed	Plant	Seed	Plant	Seed	Plant	Seed	Plant	Seed	Plant
1.	Control	11.91	4.24	113.92	31.12	11942.4	5856.17	2524.85	6187.38	306.47	652.16
2.	UZHITAN (Standard)	11.12	4.24	91.88	24.71	11026.5	4271.15	2241.25	5879.3	201.54	691.22
3.	Ascorbate chitosan	16.90	7.05	126.06	25.43	11992.7	6869.73	2687.25	5589.71	303.52	526.24
4.	Nanoascorbate chitosan (0.5%)	12.04	7.16	93.43	31.22	12841.2	5289.41	2684.82	5427.32	262.85	557.40
5.	Chitosan source (0.5%)	11.92	5.13	92.33	22.33	12940.8	6068.19	2727.61	6068.49	260.13	489.49
6.	Nanochitosan (0.5%)	11.33	6.13	91.28	26.38	13823.8	4227.42	2281.38	4237.58	292.63	519.43
7.	PMC $\text{Cu}^{2+}:\text{Ag}$ 7:3	13.99	5.19	116.43	30.10	10231.5	5242.13	2276.41	5465.84	275.82	608.57
8.	PMC $\text{Cu}^{2+}:\text{Ag}$ 8:2	15.96	5.04	118.22	32.77	11470.7	6085.49	2513.28	5923.24	268.48	591.46

lowest concentration while potassium was available in the highest concentration. In seeds treated with nano preparation, the concentration of micro and macroelements are higher than the one-month-old plants (Table 3).

The concentration of copper and iron in soybean seeds treated with ascorbate chitosan was 16.90 and 126.032 mg/kg respectively while this concentration was 7.05 and 25.43 mg/kg for the one-month-old plants respectively. Compared to the control and UZHITAN the concentration of copper, iron, potassium, calcium, and sodium was significantly higher in $\text{PMCCu}^{2+}:\text{Ag } 8:2$, $\text{PMCCu}^{2+}:\text{Ag } 7:3$ and ascorbate chitosan was higher.

The contents of the macronutrients potassium and calcium are significantly higher in Ascorbate chitosan, Chitosan source (0.5%), and Nanochitosan (0.5%) treated seeds and one-month-old soybean plants. This shows that plants in the initial period require an increase in these macronutrients for development.

Seeds and plants of the soybean variety Selecta-302 were obtained from Durdona Farm, Pakhtakor district of the Jizzakh region and the contents of micro-and macroelements were determined during the flowering and fruiting periods (Table 4).

All seeds except those are treated with nanochitosan (24.80 mg/kg) showed a lower copper content than the control (16.46 mg/kg). Among the various tested combinations, the lowest copper content was reported in ascorbate chitosan (4.17 mg/kg) which was 12.29 mg/kg lower than control (Table 4). In the case of plants, all nano preparation treatments have a lower value than the controls. In the case of standard treatments UZHITAN and Uzgumi, these two have lower concentrations of all micro and macroelements as compared to the control. Further, the amount of seed iron and

potassium was also reported lower than the control while in the case of calcium and Sodium concentration, all treatments except Chitosan source (31241.52 & 673.37 respectively) and PMC $\text{Cu}^{2+}:\text{Ag } 7:3$ (30260.15 & 949.69 respectively) have a lower concentration than the control (Table 4).

An increase in iron during the fruiting period was observed under treatment ascorbate chitosan, nanoascorbate chitosan, and PMC $\text{Cu}^{2+}:\text{Ag } 8:2$ while in the rest treatments iron content decreased as compared to that in seeds. The results of potassium concentration at the fruiting period also showed the highest concentration of potassium ion in seed treated with ascorbate chitosan and this is significantly different from the control. Compared to the seeds, the potassium content in fruiting plants grown from seeds treated with PMC $\text{Cu}^{2+}:\text{Ag } 7:3$ treatment increased by 15.4%. Determination of the calcium content showed that the seeds treated with the ascorbate chitosan and UZHITAN preparations had 11.2 and 7.1 times lower calcium content than the control plants, respectively. The calcium content during the fruiting period has been decreased as compared to the seeds treated with nanoascorbate chitosan, chitosan, nanochitosan, PMC $\text{Cu}^{2+}:\text{Ag } 7:3$, and PMC $\text{Cu}^{2+}:\text{Ag } 8:2$. The data obtained from the study revealed that the use of nano preparations for the treatment of soybean seeds significantly affects the contents of micro-and macroelements.

The results of the micro and macro elements concentration at flowering and fruiting stage in various nano preparations treated plants are presented in table 5. Except for Chitosan source and Nanochitosan, all other nano preparations have lower copper concentrations as compared to the standard treatment. For other preparations, a decrease in the concentration of copper during the period of fruit formation was observed (Table 5). In terms of iron

Table 4 Effect of various nano preparations on the micro-and macroelements in the seeds and plants at the fruit formation stage of soybean variety Select-302

№	Preparation	Micro & Macroelements (Mg/Kg)									
		Copper		Iron		Potassium		Calcium		Sodium	
		Seed	Plant	Seed	Plant	Seed	Plant	Seed	Plant	Seed	Plant
1.	Control	16.46	19.95	364.43	726.91	14052.12	12032.14	26089.63	39113.36	630.64	570.84
2.	UZHITAN (Standard)	9.60	26.14	98.72	982.74	11312.20	10087.38	3551.87	44351.62	495.81	389.11
3.	Ascorbate chitosan	4.17	15.26	79.73	182.34	635.37	13546.98	2243.16	26001.46	412.88	452.17
4.	Nanoascorbate chitosan (0.5%)	11.37	11.35	198.38	348.29	7508.49	6106.06	20971.30	19758.91	688.19	165.92
5.	Chitosan source (0.5%)	14.29	9.68	296.30	281.56	9146.84	6444.67	31241.52	19062.19	673.37	118.76
6.	Nanochitosan (0.5%)	24.80	7.29	192.83	127.16	12613.36	4298.78	25292.38	12545.19	307.65	65.69
7.	PMC $\text{Cu}^{2+}:\text{Ag } 7:3$	12.59	10.02	317.01	100.97	10925.21	16536.68	30260.15	10165.21	949.69	137.68
8.	PMC $\text{Cu}^{2+}:\text{Ag } 8:2$	9.85	10.03	111.75	124.19	13654.45	11568.13	23056.40	9366.15	626.97	77.56
9.	Uzgumi (Standard)	14.079	11.456	267.165	170.419	12440.083	14214.57	20631.00	13356.76	637.846	67.633

Table 5 Determination of the micro- and macroelements contents in Selecta-302 soybean cultivar at the beginning of flowering and during fruiting stage

№	Preparation	Micro & Macroelements (Mg/Kg)									
		Copper Flowering	Flowering	Iron Flowering	Flowering	Potassium Flowering	Flowering	Calcium Flowering	Flowering	Sodium Flowering	Flowering
1.	UZHITAN (Standard)	11.72	9382.7	189.35	196.1	11752.4	10186.3	16142.3	23246.7	894.4	637.34
2.	Gaicho (Standard)	10.37	9.46	301.56	214.52	11482.7	10177.7	16078.6	10934.3	807.5	232.7
3.	Ascorbate chitosan	5.23	9.38	287.19	176.32	12537.04	12113.32	21417.37	25318.7	641.63	308.38
4.	Nanoascorbate chitosan (0.5%)	10.69	11.032	177.94	343.92	7704.72	6203.62	24179.40	20683.64	617.09	234.19
5.	Chitosan source (0.5%)	12.92	9.58	269.59	198.71	8264.72	5882.17	22141.21	16620.11	614.40	108.67
6.	Nanochitosan (0.5%)	16.71	8.27	187.32	172.50	9442.60	5117.87	23311.70	11455.73	278.81	117.54
7.	PMC Cu ²⁺ :Ag 7:3	11.49	9.21	301.01	127.31	9529.11	13126.8	25062.17	11651.12	862.92	273.81
8.	PMC Cu ²⁺ :Ag8:2	10.32	9.16	121.46	125.30	11546.51	9693.19	2056.4	11661.58	546.46	107.45

Table 6 Determination of the enzymes activity in seven-day-old seedlings of the Selecta-302 soybean variety treated with various nanopolymer preparations

S. №	Preparation	Peroxidase (mg/kg)	Polyphenol oxidase (mg/kg)	Proteins	Relative to control (%)		
					Peroxidase	Polyphenoloxidase	Proteins
1	Control	6.65	2.98	106.34	-	-	-
2	Uzgumi	13.90	3.93	55.14	209.0	131.9	51.8
3	UZHITAN	4.94	2.40	165.42	74.3	80.5	155.6
4	PMC Cu ²⁺ :Ag 7:3	6.49	2.82	106.34	97.6	94.6	0
5	PMC Cu ²⁺ :Ag 8:2	6.77	2.65	118.16	101.8	88.9	111.1
6	Chitosan source	5.92	2.06	118.1	89.0	69.1	111.1
7	Nanochitosan	7.89	3.42	86.65	118.6	112.8	81.5
8	Ascorbate chitosan	11.85	2.26	157.54	178.2	75.8	148.1
9	Nanoascorbate chitosan	8.46	3.62	70.89	127.2	121.5	66.7

concentration, the highest iron content was observed during flowering in plants grown from seeds treated with Gaicho, and it was 1.60 times higher than the plants treated with the second standard UZHITAN. While the lowest iron concentration was observed during the period of fruit formation in plants grown from seeds treated with PMC Cu²⁺:Ag 8:2.

In terms of potassium concentration, seeds treated with ascorbate chitosan exceeded than those treated with the standard UZHITAN and Gaicho and this was 1.1- and 1.2 folds higher than the standard during flowering and fruit formation respectively. All the tested nano preparations except PMC Cu²⁺:Ag8:2 have higher calcium concentrations than the both tested standard during flowering. The greatest calcium content during flowering was observed in the seeds treated with PMC Cu²⁺:Ag 7:3, which was 1.6 times greater than that in seeds treated with the standard during fruit formation.

While at the fruiting stage, the highest calcium content was observed under Ascorbate chitosan treatment. The sodium content during flowering and fruit formation decreased in all samples as compared to the standard. Thus, the contents of micro- and macroelements in plants from seeds treated with nanopolymer preparations exhibited an uneven distribution in terms of concentration.

During the current study, enzymatic activities were also determined in the seven-day-old seedlings of soybean variety Selecta-302. The data in table 6 showed that the highest activity of the enzyme peroxidase occurred in soybean seeds treated with Uzgumi (13.90) and this was more than 2 times higher than that in the control. Further, seedlings treated with UZHITAN, chitosan, nanochitosan, PMCCu²⁺:Ag 7:3, and PMCCu²⁺:Ag 8:2 also exhibited higher peroxidase activity than control seedlings.

Similarly, the activity of polyphenol oxidase was also reported highest in seeds treated with Uzgumi (3.93) while it was lowest in seeds treated with chitosan (2.06, or 1.9 times lower).

Among the tested nano-preparations, the lowest protein activity was reported in seeds treated with standard Uzgumi (- 55.14), and this was almost 3 times lower than that in seeds treated with UZHITAN (- 165.42) and almost 2 times lower than that in seeds treated with ascorbate chitosan, chitosan, and PMCCu²⁺:Ag 8:2.

Soybean is susceptible to many fungal, bacterial, and viral diseases. Some most common soybean diseases and pathogens are charcoal rot (*Macrophomina phaseolina*), *Fusarium wilt*, *Rhizoctonia*, *anthracnose*, brown stem rot (*Cadophora gregata*), *Phomopsis*, stem rot (*Sclerotinia*), stem cancer (*Diaporthe phaseolorum*), soybean cyst nematodes, and Asian soybean rust (*Phakopsora pachyrhizi*). These diseases can cause significant crop losses. Under harsh conditions, soybean nematodes or Asian soybean rust could destroy the crop. In the current study, the incidence of *Fusarium strain* on soybean leaves and roots was estimated at the Maigir Yusufkhon farm (Figure 1). Similar types of results was reported by Brooker et al. (2007).

The results of the study showed that soybean treated with nanoascorbate chitosan, ascorbate chitosan, and PMCCu²⁺:Ag 7:3

was less susceptible to disease. The most susceptible plants were those treated with Gaucho (standard), UZHITAN (standard), and PMCCu²⁺:Ag 8:2, where the incidence of lesions was significantly higher than the standard one.

At the same farm during the growing season of experimental crops, phenological and biometric observations of soybean plant development were carried out (Tables 7-8). The data in table 7 revealed that plants treated with the nano preparations were superior to those treated with the standards UZHITAN and Gaucho at all phases of development. Harvesting of plants from seeds treated with ascorbate chitosan, nanoascorbate chitosan, and nanochitosan was 5-6 days earlier than that of plants from seeds treated with the standard. Similar types of findings were reported by Egorov et al. (2008).

Biometric measurements and observations are presented in Table 8. Plants treated with the nanoascorbate chitosan, ascorbate chitosan, PMC Cu²⁺:Ag 7:3, and PMC Cu²⁺:Ag 8:2 nanopolymer preparations were superior to those treated with other preparations. The weights of 1000-grain under these preparations were greater than those treated with the Gaucho standard by 17 g, 14.6 g, 13.5 g, and 11.9 g, respectively. Productivity under the chitosan and PMC treatments was 4.5-6.1 c/ha and 3.3-5.1 c/ha higher than the control respectively.

Figure 1 Susceptibility to *Fusarium wilt* of the Selecta-302 soybean variety in various nano-preparation

Table 7 Phenological observations of soybean variety Select-302 crops at Moygir Yusufkhon Farm

No	Preparation	Date of sowing	Appearance of seedlings	Phase with 2-3 leaves	Buddingphase	Branchingphase	Floweringphase	Height of the first bean from the ground	Ripeningphase	Harvest
1	Gaucho (standard)	03.04	15.04	01.05	14.05	21.05	07.06	16.06	12.08	17.09
2	UZHITAN	03.04	14.04	01.05	13.05	19.04	06.06	15.06	10.08	15.09
3	Ascorbate chitosan	03.04	13.04	28.04	10.05	17.05	04.06	13.06	08.08	11.09
4	Nanoascorbate chitosan	03.04	12.04	27.04	10.05	17.05	04.06	13.06	08.08	11.09
5	Chitosansource	03.04	13.04	28.04	11.05	18.05	05.06	14.06	09.08	13.09
6	Nanochitosan	03.04	13.04	28.04	10.05	17.05	04.06	13.06	08.08	12.09
7	PMC Cu ²⁺ :Ag 8:2	03.04	14.04	01.05	11.05	18.05	05.06	14.06	08.08	13.09
8	PMC Cu ²⁺ :Ag 7:3	03.04	13.04	28.04	11.05	18.05	05.06	14.06	08.08	13.09

Table 8 Biometric observations of the nano preparation application on the Selecta-302 soybean variety at Moygir Yusufkhon Farm

No	Options	Plant height (cm)	Height of the first bean from the ground (cm)	Number of beans per plant (pcs.)	Number of grains per pod (pcs.)	Grains / plant and their mass	Weight of seeds from one plant (g)	Weight of 1000 grains (g)	Number of plants per thousand hectares	Productivity (t/ha)
1	Gaucho(standard)	133.5	21.9	56.2	2.4	138.6	19.2	138.8	210	29.1
2	UZHITAN	135.6	21.2	59.1	2.3	142.3	20.9	142.7	215	30.6
3	Chitosansource	137.4	19.7	59.9	2.5	155.5	24.1	146.3	207	32.2
4	Nanochitosan	136.6	19.4	60.3	2.7	157.3	25.2	152.0	204	32.1
5	Ascorbate chitosan	136.8	19.4	60.8	2.7	160.2	25.4	153.4	210	33.6
6	Nanoascorbate chitosan	144.5	19.6	62.8	2.8	174.4	27.8	155.8	202	35.2
7	PMC Cu ²⁺ :Ag 8:2	143.3	19.8	62.2	2.7	168.3	26.7	150.7	193	32.4
8	PMC Cu ²⁺ :Ag 7:3	143.5	19.9	62.3	2.8	169.9	27.1	152.3	201	34.2

Thus, this research has shown that nanopolymer preparations not only have stimulatory and fungicidal properties but also have a significant effect on the micro and microelements. The applied nano preparations had a stimulatory effect on development and can be recommended for use in encapsulating seeds after a few more studies under different environmental conditions. Similar types of results were reported by various researchers who evaluated the effect of various nano preparation on the soybean crop (Priester et al., 2012; Yoon et al., 2014; Chau et al., 2019; Coman et al. (2019).

Conclusion

Results of the study can be concluded that various nanopolymer preparations such as nanoascorbate chitosan (4:1) @ 0.5% and nanochitosan (90 kDa) @0.5% positively affect the laboratory

germination of soybean seeds. Further, the preparations, nanoascorbate chitosan (4:1) @ 0.5%, PMCCu²⁺:Ag 7:3, and PMCCu²⁺:Ag 8:2 have higher biological activity than the control and standard. Similarly, various nano polymer preparations have left their effect on the content of micro and macro elements. Among these PMCCu²⁺:Ag 8:2 encapsulation had almost 1.5 times higher concentrations than that in one-month-old plants

The use of nano preparation for the treatment of Selecta-302 soybean seeds favorably affects the contents of micro-and macroelements and contributes to a more uniform distribution of the concentration of these elements. In the case of enzymatic and protein concentration, various nano preparations showed superiority over the recommended standard preparation Uzgumi and UZHITAN, the lowest protein activity was found in seeds

treated with Uzgumi (- 55.14), which was almost 3 times lower than that in seeds treated with UZHITAN (- 165.42) and almost 2 times lower than that in seeds treated with ascorbate chitosan, chitosan and PMCCu²⁺:Ag 8:2. Further, the plant treated with various nano preparations such as nanoascorbate chitosan, ascorbate chitosan, and PMCCu²⁺:Ag 7:3 were less susceptible to disease.

Conflict Of Interest

Authors would hereby like to declare that there is no conflict of interests that could possibly arise.

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