



COMBINED EFFECT OF CHITOSAN AND CALCIUM CHLORIDE ON POTATO BLACK SCURF DISEASE UNDER FIELD CONDITIONS

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Abstract

Potato black scurf disease caused by *Rhizoctonia solani* AG-3 (*R. solani*) can infect the underground plant parts. In Russia, annual yield losses by *R. solani* reach 30-45%. In this study, we evaluated the effect of calcium chloride (CaCl₂) and chitosan, alone or in combination, on plant development, tuber yield and black scurf disease severity under field conditions. Soil inoculated with *R. solani* before planting. Seed tubers sprayed with 0.5 or 1% CaCl₂ and/or 0.5% chitosan. The foliage was sprayed twice with 0.5 or 1% CaCl₂ and/or 0.1% chitosan. During the vegetation period, growth parameters, such as germination (%), plant height (cm) and branches number per plant, were measured. At harvest, we calculated the total and the marketable number of tubers and tuber yield. Results revealed that combined pre-planting application with 1% CaCl₂ and 0.5% chitosan with 2 hours intervals, then, spraying foliar with 1% CaCl₂ and 0.1% chitosan twice with ten days intervals starting at 40 days after planting resulted in: a) increasing the germination, plant height and branches number per plant; b) enhancing the marketable tuber yield by 28.2 and 53% in Sante and Romano varieties, respectively; c) reducing black scurf disease severity by 43.9-51.1%.

Key words: Chitosan; Calcium chloride; Black scurf; *Rhizoctonia*; Potato.

Introduction

Potato (*Solanum tuberosum* L.) is one of Russia's strategic products and deservedly called the second bread. This crop is essential for humans, a source of animal feed and a technical raw material for many types of industry. The tuber is the most economical part of the potato plant and it is also a great source of carbohydrates, protein and vitamins (Jansky *et al.*, 2019). In terms of potato production, Russia ranks third globally (after China and India) (FAOSTAT, 2018).

The potato plants are subject to attack by many fungal pathogens, including *Rhizoctonia* species (Dias *et al.*, 2016). Black scurf disease of potato caused by *Rhizoctonia solani* AG-3 can affect roots, stolons, stems and tubers. It also depreciates the product (Kapsa, 2008). *R. solani* can cause severe disease symptoms in different underground plant parts (Lehtonen *et al.*, 2008). Carling *et al.*, (1989) recorded a significant delay of plant emergence, a reduction of tuber yield and a high occurrence of stem canker caused by tuber borne inoculum. Losses of marketable tuber yield due to *R.*

solani can reach 30-45% in Russia (Malyuga *et al.*, 2003).

Traditional methods to control *R. solani* are fungicides application (Pramesh *et al.*, 2017; Ganesha Naik *et al.*, 2017). However, repeated use of fungicides can lead to a decrease in the effectiveness of fungicides due to the gradual loss of sensitivity in the target pathogen population (Farrar *et al.*, 2002). Consequently, alternative control strategies are required to achieve efficient, long-term and eco-friendly management of *Rhizoctonia solani* (Khaing *et al.*, 2014; Yu *et al.*, 2017). Recent research efforts have focused on the use of resistance inducers as alternative management methods. These inducers, such as chitosan and chitin, are environmentally safe and have no adverse effects on fruit quality (Romanazzi *et al.*, 2012).

Chitosan (β -1,4-D glucosamine), a natural biopolymer obtained by deacetylation of chitin, the second most plentiful natural polymer in the world (El Hadrami *et al.*, 2010). Chitosan is a resistance activator and an antifungal agent (Feliziani *et al.*, 2013; Mohammed *et al.*, 2019). Many studies showed that chitosan could increase

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productivity, reduce transpiration and induce more resistance to fungal, bacterial and viral infections (Al-Hetar *et al.*, 2011; Mondal *et al.*, 2012). Chitosan stimulates various physiological features of growth and development. It can significantly enrich photosynthesis, chloroplast enlargement and photosynthetic pigments. Also, it directly influences plant nutrition by improving soil fertility, increasing nitrogen fixation and enhancing mineral uptake through regulating cell osmotic pressure (Nguyen *et al.*, 2013; Katiyar *et al.*, 2015). Chitosan can promote the vital mechanisms of plants at the level of single cells, tissues and molecular changes based on gene expression (Hadwiger, 2013; Nguyen *et al.*, 2013). Some of the distinctive features of chitosan may specifically enhance plant defense reactions and inhibit the growth of microorganisms. The role of chitosan as a plant growth promoter and a pathogen control agent has been evaluated in recent years (Trotel-Aziz *et al.*, 2006). Chitosan with high and low molecular weight, as well as a hydrolyzed chitosan derivative applied on 31, 45 and 59 days after planting, increase the size of tubers in two different potato varieties (Falcón-Rodríguez *et al.*, 2017). Mohammed *et al.* (2019) stated that chitosan could inhibit the mycelial growth of *Rhizoctonia solani* AG-3 and induce defense reaction in potato tubers.

Calcium (Ca) is an essential secondary nutrient and a vital factor in plant growth. Calcium plays various roles in the plant, such as, participating in metabolic processes of other elements uptake; enhancing plant cell elongation; strengthening cell wall structure by forming calcium pectate which helps keep the cell walls sturdy and rigid and bind cells together; protecting the plants against diseases (Mengel and Kirkby, 2001).

Calcium nutrition suppresses the severity of early blight and late blight of potato (El-Gamal *et al.*, 2007; Subhani *et al.*, 2015; Seifu, 2017), brown rot of peach (Elmer *et al.*, 2006). Besides, the calcium application can reduce internal tuber damage and increase yield, shelf life, tuber weight, size and quality (Ozgen *et al.*, 2003; Hamdi *et al.*, 2015).

Therefore, this study aims to determine the effect of pre-planting and foliar application of calcium chloride and chitosan alone or in combination on the severity of black scurf disease, plant growth and tuber yield, in the field under artificial infection of *R. solani*.

Materials and Methods

Plant Materials

Two potato varieties (Sante and Romano) were planted during two successive growing seasons, in 2016

and 2017 (May-August), at Moiseev farm, Bazarnyi Karabulak District, Saratov Oblast, Russia.

Pathogen

Rhizoctonia solani AG-3 was isolated, according to Mohammed *et al.*, (2019) and identified as described by Sherwood (1970).

Fungal inocula preparation and soil infection

To prepare the inoculum in bulk: one kg barley grains were soaked for 12 h with 500 mL of sterile distilled water and the excess water was removed. The Grains were placed in mushroom growing bags (25×50 cm) and autoclaved twice at 121°C for 30 min. The media allowed to cool, then inoculated with three plugs (5 mm diameter) of a 5-day *R. solani* culture. The inoculated bags were incubated for 20 days at 20±5°C. At planting, soil inoculated by placing 15 g of inoculum under each of seed tubers. The control traits did not receive any *R. solani* inoculum.

Chemicals

Chitosan, edible level, with a medium molecular weight (150 kDa) and degree of deacetylation 80%, was obtained from Chitosan Technologies LLC, Engels city, Saratov oblast, Russia. Preparation of chitosan concentrations was obtained by dissolving the desired amount of chitosan in 0.5% glacial acetic acid in a Thermo shaker at 40°C/24 h and the pH was adjusted to 5.5-6 by adding 1 M NaOH.

Calcium chloride (CaCl₂), colorless crystals, contains 27% calcium, was purchased from the Russkaya dumka company, Saratov city, Russia.

Experimental design and treatments

Experimental Design: The field experiment was conducted at Moiseev farm, Bazarnyi Karabulak District, Saratov Oblast, Russia (Latitude 52°16'23.563 N, Longitude 46°24'41.043 E), during two successive seasons (May-September), 2016-2017 to evaluate the efficacy of pre-planting and foliar application with calcium chloride, chitosan and their combinations on the incidence of black scurf disease, plant growth and tuber yield under artificial infection with *R. solani*. The experiment consisted of plots 28 m² and was conducted in a completely randomized block design (for each variety), with three schemes as replicates for each treatment, as well as an untreated control. The planting scheme was an intra-row distance of 30 cm and inter-row distance of 70 cm and a depth of 12 cm.

Treatments: The pre-planting and foliar treatments showed in table 1. The solution volume was 10 l/t for tubers treatment and 400 l/ha for foliar application. The

pre-planting combined application of CaCl₂ and chitosan on tubers was conducted as follows: treatment tubers firstly with CaCl₂ and after two hours with chitosan. The control tubers served tubers sprayed with water. The foliar treatments were conducted twice with 10-days intervals, starting at 40 days after planting. For the combined foliar application, we started with CaCl₂ and after ten days with chitosan. The control tubers served plants sprayed with water.

Data collection

Table 1: Scheme of pre-planting and foliar treatments.

No.	Treatments	Seed tubers treatment	Foliar application
1	Control	water	water
2	Chitosan	0.5%	0.1%
3	CaCl ₂	0.5%	0.5%
4	CaCl ₂	1%	1%
5	CaCl ₂ + Chitosan	0.5%0.5%	0.5%1%
6	CaCl ₂ + Chitosan	1%0.5%	1%0.1%

Table 2: Influence of pre-planting and foliar treatment on the growth and development of potato plants infected with *Rhizoctonia solani*, during two successive seasons 2016/2017*.

Variety	Treatments	Germination		Plant height (cm)	Branches number /Plant
		(%)	Increasing (%)		
Sante	Control	44.6 ^c	0	33.3 ^b	2.3 ^c
	Chitosan (A), (B)	55.2 ^b	23.7	34 ^b	2.6 ^{bc}
	CaCl ₂ (C), (E)	54 ^b	21	34 ^b	3 ^{bc}
	CaCl ₂ (D), (F)	50.6 ^{bc}	13.4	34.3 ^b	3.6 ^{ab}
	CaCl ₂ (C), (E) + Chitosan (A), (B)	58.6 ^b	31.3	38.3 ^{ab}	3.6 ^{ab}
	CaCl ₂ (D), (F) + Chitosan (A), (B)	77.2 ^a	73	45 ^a	4.3 ^a
LSD _{0.05}		8.6		7.7	1.1
Romano	Control	34.6 ^c	0	28 ^c	2.3 ^d
	Chitosan (A), (B)	74.6 ^b	115.6	34 ^{bc}	3.3 ^{bcd}
	CaCl ₂ (C), (E)	77.3 ^{ab}	123.4	33.3 ^{bc}	3 ^{cd}
	CaCl ₂ (D), (F)	76 ^b	119.6	35.3 ^b	4 ^{abc}
	CaCl ₂ (C), (E) + Chitosan (A), (B)	82 ^{ab}	136.9	37.3 ^{ab}	4.6 ^{ab}
	CaCl ₂ (D), (F) + Chitosan (A), (B)	88.6 ^a	156	42 ^a	5 ^a
LSD _{0.05}		11.7		6.4	1.3

* The showing data of the two successive seasons were presented as average.

(A)- Chitosan 0.5% tubers treatment; (B)- Chitosan 0.1% foliar application; (C)- CaCl₂ 0.5% tubers treatment; (D)- CaCl₂ 1% tubers treatment; (E)- CaCl₂ 0.5% foliar application; (F)- CaCl₂ 1% foliar application.

Mean values within columns followed by the same superscripts are not significantly different at $p < 0.05$

Measurement of plant growth parameters and tuber yield

During the growing season, germination (%), plant height (cm) and the number of branches per plant were measured. At harvest, we calculated the total and the marketable number of tubers per plant and tubers (total and marketable) from each plot were weighed and expressed as t/ha.

The severity of black scurf disease

At harvest, we assessed the black scurf disease severity. A sample of 50 tubers from each treatment washed with water and disease severity was assessed based on the percentage of tuber skin covered with sclerotia according to Tanil *et al.*, (1982) and Sharma and KC (2007) using a rating scale from 1 to 5; where one = 1-10%, two = 11- 20%, three = 21-30, four = 31-50%, five < 50% of tuber surface covered with sclerotia. Moreover, the disease severity index was computed using the formula:

Disease severity index = $\frac{(n \times r) \times 100}{5 \times N}$;
Where, n = number of tubers in each rating, r = each rating number, 5 = highest rating, N = Total number of tubers examined.

Statistical analysis

Statistical analysis of the obtained data was performed with CoStat 6.45 software program, using (LSD) test at $p = 0.05$ level, by One-way Analysis of Variance (ANOVA) for each variety.

Results and Discussion

Plant Growth Parameters and Tuber Yield

The results shown in table 2 indicate the effect of pre-planting and foliar treatments with calcium chloride and chitosan alone or in combination on the growth of potato plants (with artificial infection of black scurf disease). The germination enhanced in all variants by 13.4-156% table 2 and the increasing value was higher in the combined application of CaCl₂ and chitosan than single applications. Most of all, germination increased by 73 and 156% in Sante and Romano varieties, respectively, when treated with (1% CaCl₂ for tubers treatment and foliar application; 0.5% chitosan for tubers treatment and 0.1% for foliar application) table 2.

Also, for plant height and branches number, treatment with (1% CaCl₂ for tubers treatment and foliar application; 0.5% chitosan for tubers treatment and 0.1% foliar application) was more

significant in comparison to the control table 2.

For the total and the marketable number of tubers, the best results were obtained by using the combined application of calcium chloride and chitosan at the mentioned above concentrations, maintaining reached 53.8 and 80.5% for the total number of tubers in Sante and Romano varieties respectively, 75 and 103 % for the

marketable number of tubers in Sante and Romano varieties, respectively tables 3, 4. Although in Romano variety treatment with combined application of chitosan and calcium chloride had no significant effect on the total and the marketable number of tubers. Besides, the combined application of CaCl₂ and chitosan significantly increased the marketable tuber yield in both varieties

tables 3, 4.

Table 3: Influence of pre-planting and during vegetation treatment on the number of tubers and yield, Sante variety, infected with *Rhizoctonia solani*, during two successive seasons 2016/2017*.

Treat-ments	Tubers number/plant				Yield, t/ha		Mark-etabi- lity, (%)
	Total		Marketable		Total	Marke- table	
	Tuber/ plant	Increas- ing, (%)	Tuber/ plant	Increas- ing, (%)			
Control	3.9 ^c	0	3.2 ^b	0	17 ^d	13.1 ^d	77.3
Chitosan (A), (B)	4.3 ^c	10.3	4.1 ^b	28.1	18.2 ^{bc}	13.2 ^d	72.5
CaCl ₂ (C), (E)	4.1 ^c	5.1	3.7 ^b	15.6	17.7 ^{cd}	14 ^{cd}	79.3
CaCl ₂ (D), (F)	4.7 ^{bc}	20.5	4.1 ^b	28.1	17.8 ^c	13.8 ^d	77.9
CaCl ₂ (C), (E) + Chitosan (A), (B)	5.6 ^{ab}	43.6	5.1 ^a	59.4	18.9 ^b	15.5 ^b	82.1
CaCl ₂ (D), (F) + Chitosan (A), (B)	6 ^a	53.8	5.6 ^a	75	19.7 ^a	16.8 ^a	84.9
LSD _{0.05}	0.9		0.9		0.7	1.1	

* The showing data of the two successive seasons were presented as average. (A)- Chitosan 0.5% tubers treatment; (B)- Chitosan 0.1% foliar application; (C)- CaCl₂ 0.5% tubers treatment; (D)- CaCl₂ 1% tubers treatment; (E)- CaCl₂ 0.5% foliar application; (F)- CaCl₂ 1% foliar application.

Mean values within columns followed by the same superscripts are not significantly different at p < 0.05.

Table 4: Influence of pre-planting and during vegetation treatment on the number of tubers and yield, Romano variety, infected with *Rhizoctonia solani*, during two successive seasons 2016/2017*.

Treat-ments	Tubers number/plant				Yield, t/ha		Mark-etabi- lity, (%)
	Total		Marketable		Total	Marke- table	
	Tuber/ plant	Increas- ing, (%)	Tuber/ plant	Increas- ing, (%)			
Control	4.1 ^b	0	3.3 ^c	0	16.4 ^c	11.5 ^e	70.1
Chitosan (A), (B)	4.9 ^b	19.5	4.1 ^{bc}	24.2	16.8 ^c	12.3 ^{cd}	73.1
CaCl ₂ (C), (E)	4.6 ^b	12.2	4.1 ^{bc}	24.2	16.6 ^c	11.9 ^{de}	71.9
CaCl ₂ (D), (F)	4.9 ^b	19.5	4.3 ^{bc}	30.3	16.6 ^c	12.4 ^{cd}	74.4
CaCl ₂ (C), (E) + Chitosan (A), (B)	6.9 ^a	68.3	6.2 ^a	87.9	19.5 ^a	16.6 ^b	85
CaCl ₂ (D), (F) + Chitosan (A), (B)	7.4 ^a	80.5	6.7 ^a	103	20.3 ^a	17.6 ^a	86.7
LSD _{0.05}	1.8		1.8		0.8	0.7	

* The showing data of the two successive seasons were presented as average. (A)- Chitosan 0.5% tubers treatment; (B)- Chitosan 0.1% foliar application; (C)- CaCl₂ 0.5% tubers treatment; (D)- CaCl₂ 1% tubers treatment; (E)- CaCl₂ 0.5% foliar application; (F)- CaCl₂ 1% foliar application.

Mean values within columns followed by the same superscripts are not significantly different at p < 0.05.

Optimization of the nutritional status of potato with calcium nutrients has been reported to improve tuber yield quality and reduce different diseases (El-Gamal *et al.*, 2007; Hamdi *et al.*, 2015; Karlsson *et al.*, 2006; Ozgen *et al.*, 2003; Seifu, 2017). Chitosan has a positive effect on the growth and potato tuber yield. Falcón-Rodríguez *et al.*, (2017) reported that foliar application with high molecular weight enhanced potato yield between 15-30%. However, the mechanism through which the chitosan causes the increase in potato yields is not known (Falcón-Rodríguez *et al.*, 2017). Previous studies reported actions of chitosan as fertilizer, considering the amino groups of the polymer or anti-transpirant effect through promoting stomata closure and activation of other physiological processes (Ohta *et al.*, 2004; Iriti *et al.*, 2009). Morales *et al.*, (2015) elucidated that foliar application of chitosan on potato plants, increased the leaves number by plant and from a greater leaf area, it can be inferred a higher photosynthetic activity that may lead to an increase at tuber yield in the plant.

Black Scurf Disease Severity

Under artificial infestation with *Rhizoctonia solani* in the field, there were significant differences in the disease severity observed due to the treatments. The combined application of calcium chloride and chitosan significantly reduced black scurf disease severity on tubers at harvest compared to the control. The disease severity reduced by 43.9 and 51.1% in Sante and Romano varieties, respectively, with treatment (1% CaCl₂ for tubers treatment

and foliar application; 0.5% chitosan for tubers treatment, 0.1% and foliar application) table 5.

Calcium chloride was reported as plant resistance inducer and as an important nutritional element in many species such as tomato against powdery mildew (Ehret et al., 2002), potato against late and early blight (El-Gamal et al., 2007; El-Mougy and Abdel-Kader, 2009; Seifu, 2017). Arfaoui et al., (2016) indicated that pretreatment with calcium enhanced defense responses with higher levels of isoflavone phytoalexins in soybeans, thus reducing infection with *Sclerotinia sclerotiorum* and suggesting an indirect impact on the pathogen. Chitosan also considered a valid alternative to synthetic fungicides (El-Ghaouth, 1997). Chitosan at 4.0 g/l applied as soil drench showed significant levels of protection against soil-borne fungi, for example, *Fusarium* wilt on potato plants (Mejdoub-Trabelsi et al., 2020) and tomato (Khiareddine and El-Mohamedy, 2015). Chitosan can induce defense activity in potato tubers against *Fusarium* dry rot (Sun et al., 2008) and *Rhizoctonia solani* (Mohammed et al., 2019). Pre-harvest application with CaCl₂ and chitosan was effective in minimizing weight loss and decay, as well as in maintaining maximum firmness and lengthening the shelf life of 'Early Swelling' peach (Gayed et al., 2017).

Conclusion

In conclusion, pre-planting with 1% calcium chloride **Table 5:** Black scurf disease severity on potato tuber at harvest, during two successive seasons 2016/2017*, varieties Sante and Romano.

Treat-ments	Black scurf disease severity (%)			
	Sante		Romano	
	%	Reduction, %	%	Reduction, %
Control	38 ^a	0	27.2 ^a	0
Chitosan (A), (B)	30.6 ^{abc}	19.4	24 ^{ab}	11.7
CaCl ₂ (C), (E)	34.6 ^{ab}	8.9	25.2 ^{ab}	7.3
CaCl ₂ (D), (F)	29.3 ^{bcd}	22.8	26.2 ^{ab}	3.6
CaCl ₂ (C), (E) + Chitosan (A), (B)	24.6 ^{cd}	35.2	16.6 ^c	38.9
CaCl ₂ (D), (F) + Chitosan (A), (B)	21.3 ^d	43.9	13.6 ^c	51.1
LSD _{0.05}	8.2		6.8	

* The showing data of the two successive seasons were presented as average.

(A)- Chitosan 0.5% tubers treatment; (B)- Chitosan 0.1% foliar application; (C)- CaCl₂ 0.5% tubers treatment; (D)- CaCl₂ 1% tubers treatment; (E)- CaCl₂ 0.5% foliar application; (F)- CaCl₂ 1% foliar application.

Mean values within columns followed by the same superscripts are not significantly different at p < 0.05.

and 0.5% chitosan with two h intervals, followed by foliar application twice with 1% CaCl₂ and 0.1% chitosan with ten days intervals enhanced plant growth and potato yield and decreased severity of *Rhizoctonia solani* disease. Therefore, the combined pre-planting and foliar application of CaCl₂ and chitosan may be recommended for potato producers to reduce black scurf disease severity by 43.9-51.1% and augment about 28.2-53% yields.

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