The Open Agriculture Journal

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RESEARCH ARTICLE

Combined Influence of Chitosan and Calcium Chloride on Fusarium Dry Rot Disease Under Field Conditions

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Abstract:

Background:

Fusarium dry rot disease caused by *Fusarium sambucinum* Fuckel (*F. sambucinum*) can infect the potato tubers in the field and during storage. Yield losses by *F. sambucinum* reach 60%. Traditional methods to control Fusarium dry rot are fungicides application, which led to developing many isolates resistant to these fungicides.

Objective:

The aim of this study is to evaluate the effect of calcium chloride ($CaCl_2$) and chitosan, alone or in combination, on plant development, tuber yield, and Fusarium dry rot disease incidence under field conditions.

Methods:

Soil inoculated with *F. sambucinum* before planting. We treated the seed tubers with $CaCl_2$ (0.5 or 1%), chitosan 0.5%, or both. The foliage was sprayed twice with $CaCl_2$ (0.5 or 1%), 0.1% chitosan, or both. 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. In addition, during storage, we assessed the incidence of Fusarium dry rot disease on tubers.

Results:

Results revealed that combined pre-planting application with 1% $CaCl_2$ and 0.5% chitosan with 2 hours intervals, then spraying foliar with 1% $CaCl_2$ and 0.1% chitosan twice with ten days intervals starting at 40 days after planting resulted in: a) increasing the germination, enhancing the growth parameters such as plant height and branches number per plant; b) enhancing the marketable tuber yield by 75.2 and 97.6% in Sante and Kolobok varieties, respectively; c) reducing Fusarium dry rot disease incidence by 61.9-72.7%.

Conclusion:

The work highlighted that the combined pre-planting and foliar application of $CaCl_2$ and chitosan might be recommended for potato producers to reduce the incidence of Fusarium dry rot disease and augment yields.

Keywords: Chitosan, Calcium chloride, Fusarium dry rot, *Fusarium sambucinum*, Potato, Disease incidence.

Article History	Received: July 10, 2020	Revised: September 28, 2020	Accepted: October 29, 2020

1. 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 [1]. In terms of potato production, Russia ranks third globally (after China and India) (FAOSTAT, **2018**).

Fusarium dry rot is one of the most devastating postharvest diseases of potato worldwide and is caused by several *Fusarium* species such as *Fusarium sambucinum* Fuckel [2 - 4]. During the vegetative period, *Fusarium sambucinum* can reduce crop establishment by affecting the potato sprouts development, resulting in poor emergence [4, 5]. During

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storage, losses associated with Fusarium dry rot range from 6 to 25%, and occasionally as great as 60% [3, 6]. Fusarium dry rot also can threaten human and animals' life by producing toxins such as trichothecenes [7].

Traditional methods to control Fusarium dry rot are fungicides application such as thiabendazole and benzimidazole. However, *F. sambucinum* developed many isolates resistant to these fungicides [8]. Consequently, alternative control strategies are required to achieve efficient, long-term, and eco-friendly management of Fusarium dry rot disease. 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 [9].

Chitosan (β-1,4-D-glucosamine), is a natural biopolymer obtained by deacetylation of chitin, the second most plentiful natural polymer in the world [10]. Chitosan is a resistance activator and an antifungal agent [11, 12]. Many studies showed that chitosan could increase productivity, reduce transpiration, and induce more resistance to fungal, bacterial, and viral infections [13, 14]. Chitosan stimulates various physiological features of growth and development. It can significantly enrich photosynthesis, chloroplast enlargement, and photosynthetic pigments. It directly influences plant nutrition by improving soil fertility, increasing nitrogen fixation, and enhancing mineral uptake through regulating cell osmotic pressure [15, 16]. Chitosan can promote the vital mechanisms of plants at the level of single cells, tissues, and molecular changes based on gene expression [17, 20]. 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 [18]. Chitosan with high and low molecular weight and a hydrolyzed chitosan derivative applied on 31, 45, and 59 days after planting increase the size of tubers in two different potato varieties [19]. Mohammed *et al.* (2019) [12] stated that chitosan could inhibit the mycelial growth of *Rhizoctonia solani* AG-3 and induce a 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 and 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 [20]. Calcium nutrition suppresses the severity of early blight and late blight of potato [21 - 23] and brown rot of peach [24]. Besides, the calcium application can reduce internal tuber damage and increase yield, shelf life, tuber weight, size, and quality [25, 26].

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 incidence of Fusarium dry rot disease, plant growth, and tuber yield in the field under artificial infection of *Fusarium sambucinum*.

2. MATERIALS AND METHODS

2.1. Plant Materials

Two potato varieties (Sante and Kolobok) were planted during two successive growing seasons, in 2016 and 2017 (May-August), at Moiseev farm, Bazarnyi Karabulak District, Saratov Oblast, Russia.

2.2. Pathogen

Fusarium sambucinum was initially isolated from diseased potato tubers showing typical dry rot symptoms, according to Gachango *et al*. (2012a) [27], and identified as described by Leslie *et al*. (2006) [28], and Gachango *et al*. (2012a) [27].

2.3. 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 *F. sambucinum* culture. The inoculated bags were incubated for 20 days at 20±5°C. At planting, the soil was inoculated by placing 10 g of inoculum under each of the seed tubers.

2.4. 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 ($CaCl_2$), colorless crystals, contain 27% calcium, were purchased from the Russkaya dumka company, Saratov city, Russia.

2.5. Experimental Design and Treatments

2.5.1. Experimental Design

The field experiment was conducted at Moiseev farm, Bazarnyi Karabulak District, Saratov Oblast, Russia (Latitude 52° 16′ 37.56″ N, Longitude 46° 24′ 41.04″ 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 Fusarium dry rot disease, plant growth, and tuber yield under artificial infection with *F. sambucinum*. The experiment consisted of 10.5 m^2 plots and was conducted in a completely randomized block design (for each variety), with three 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.

2.5.2. Treatments

The pre-planting and foliar treatments are 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 $CaCl_2$ and Chitosan on tubers was conducted as

follows: treatment tubers firstly with $CaCl_2$, and after two hours with chitosan. The control traits 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 began with $CaCl_2$, and after ten days with chitosan. The control traits served plants sprayed with water.

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

№	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% 0.1%
6	$CaCl_2$ + Chitosan	1% 0.5%	1% 0.1%

2.6. Data Collection

2.6.1. 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.

2.6.2. The Incidence of Fusarium Dry Rot Disease

We assessed the Fusarium dry rot disease incidence, according to Kirk *et al*. (2013) [29], with some modifications. Briefly, after two months of storage, a sample of 100 tubers from each treatment was washed with water and cut longitudinally into four slices, and evaluated for the presence of dry rot symptoms. Tubers with symptoms of dry rot were counted, and disease incidence was determined as a percentage of symptomatic tubers relative to the number of tubers in each replicate.

2.7. 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.

3. RESULTS AND DISCUSSION

3.1. 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 Fusarium dry rot disease). The germination enhanced in all variants by 9.3-61.9% (Table **2**), and the increasing value was higher in the combined application of calcium chloride and chitosan than single applications. Most of all, germination increased by 46.8 and 61.9% in Sante and Kolobok varieties, respectively, when the tubers and foliage were treated with calcium chloride and chitosan at the highest concentrations (Table **2**).

In addition, for plant height and branches number, treatment with calcium chloride and chitosan at the highest concentrations was more significant than the control (Table **2**).

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

Variety	Treatments	Germination		Plant Height, (cm)	Branches number/Plant
		(%)	Increase, (%)		
Sante	Control	51.2^{c}	0.0	30.0^{c}	2.6^{b}
	Chitosan (A), (B)	59.2^{bc}	15.6	32.6^{bc}	3.3^{ab}
	$CaCl_2$ (C), (E)	56.0^{bc}	9.3	33.0^{bc}	3.0^{ab}
	$CaCl_2$ (D), (F)	59.2^{bc}	15.6	33.0^{bc}	3.6^{ab}
	$CaCl_2$ (C), (E) + Chitosan (A), (B)	62.6^{b}	22.3	38.0^{b}	3.6^{ab}
	$CaCl_2$ (D), (F) + Chitosan (A), (B)	75.2^{a}	46.8	46.0^{a}	4.0^{a}
$LSD_{0.05}$	-	9.7	-	5.4	1.2
Kolobok	Control	45.2^{d}	0.0	29.3^{d}	2.3^{b}
	Chitosan (A), (B)	59.2^{bc}	30.9	41.6^{b}	3.0^{ab}
	$CaCl_2$ (C), (E)	52.6^{cd}	16.3	37.0^{c}	2.6^{ab}
	$CaCl_2$ (D), (F)	55.2^{c}	22.1	42.3^{b}	2.3^{b}
	$CaCl_2$ (C), (E) + Chitosan (A), (B)	66.0^{ab}	46.0	39.3^{bc}	3.0^{ab}
	$CaCl_2$ (D), (F) + Chitosan (A), (B)	73.2^{a}	61.9	46.6^{a}	3.6^{a}
$LSD_{0.05}$		8.4		4.2	1.0

*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)- $CaCl_2$ 0.5% tubers treatment; (D)- $CaCl_2$ 1% tubers treatment; (E)- $CaCl_2$ 0.5% foliar application; (F)- $CaCl_2$ 1% foliar application.

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

For the total and the marketable number of tubers, the best results were obtained when the tubers and foliage were treated with calcium chloride and chitosan at the highest concentrations, maintaining reached 48.7 and 63.6% for the total number of tubers in Sante and Kolobok varieties, respectively, 62.8 and 64.8% for the marketable number of tubers in Sante and Kolobok varieties, (Tables **3**, **4**). Besides, the combined application of $CaCl_2$ and Chitosan significantly increased the total and marketable tuber yield in both varieties (Tables **3**, **4**).

Optimization of the nutritional status of potato with calcium nutrients can improve tuber yield quality and reduce different diseases [21, 23, 25, 26, 30]. Chitosan has a positive effect on the growth and potato tuber yield. Falcón-Rodríguez *et al.* (2017) [19] reported that foliar application with high molecular weight chitosan enhanced potato yield between 15-30%. However, the mechanism through which the chitosan causes the increase in potato yields is not known [19]. 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 [31, 32]. Morales *et al*. (2015) [33] elucidated that foliar application of chitosan on potato plants increased the leaves number per plant. From a greater leaf area, it can be inferred a higher photosynthetic activity that may lead to an increase in tuber yield in the plant.

3.2. Dry Rot Disease Incidence

During the storage, there were significant differences in the disease incidence observed due to the treatments. The combined application of calcium chloride and chitosan significantly reduced Fusarium dry rot disease incidence on tubers compared to the control. The disease incidence reduced by 61.9 and 72.7% in Sante, and Kolobok varieties, respectively, with the combined treatment of calcium chloride and chitosan at the highest concentrations (Table **5**).

Calcium chloride was reported as a plant resistance inducer and an essential nutritional element in many species, such as tomato against powdery mildew [34], potato against late and early blight [21, 23, 35]. Arfaoui *et al*. (2016) [36] 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 is also considered a valid alternative to synthetic fungicides [37]. 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 [38] and tomato [39]. Chitosan can induce defense activity in potato tubers against *Fusarium* dry rot [40] and *Rhizoctonia solani* [12]. Pre-harvest application with $CaCl_2$ 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 [41].

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

Treatments	Tubers number/Plant				Yield, t/ha		Marketability, (%)
	Total		Marketable		Total	Marketable	
	Tuber/ Plant	Increase, (%)	Tuber/ plant	Increase, (%)			
Control	4.1^{c}	0.0	3.5^{c}	0.0	11.9^{e}	8.5^{e}	71.4
Chitosan (A), (B)	4.7^{bc}	14.6	4.2^{bc}	20.0	13.5^{d}	9.7^{d}	71.8
$CaCl_2$ (C), (E)	4.3^{c}	4.8	4.0^{bc}	14.2	13.0^{d}	9.6^{d}	73.8
$CaCl_2$ (D), (F)	4.3^{c}	4.8	4.0^{bc}	14.2	14.1^{c}	10.4^{c}	73.8
$CaCl_2$ (C), (E) + Chitosan (A), (B)	5.3^{b}	29.2	4.9^{ab}	40.0	18.1^{b}	15.5^{b}	85.6
$CaCl_2$ (D), (F) + Chitosan (A), (B)	6.1^{a}	48.7	5.7^{a}	62.8	18.6^{a}	16.8^{a}	90.3
$LSD_{0.05}$	0.68	-	1.1	-	0.46	0.60	-

***** 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)- $CaCl_2$ 0.5% tubers treatment; (D)- $CaCl_2$ 1% tubers treatment; (E)- $CaCl_2$ 0.5% foliar application; (F)- $CaCl_2$ 1% foliar application.
Mean values within columns followed by the same superscripts are not significantly different at $p \leq 0.05$

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

Treatments	Tubers number/Plant				Yield, t/ha		Marketability, (%)
	Total		Marketable		Total	Marketable	
	Tuber/Plant	Increase, (%)	Tuber/ plant	Increase, (%)			
Control	4.4^{b}	0.0	3.7^{b}	0.0	13.3^{f}	12.1^{e}	90.9
Chitosan (A), (B)	4.9^{b}	11.3	4.1^{b}	10.8	17.5^{de}	16.2^{d}	92.5
$CaCl_2$ (C), (E)	4.7^{b}	6.8	4.2^{b}	13.5	16.9^{e}	15.9^{d}	94.0
$CaCl_2$ (D), (F)	4.9^{b}	11.3	4.2^{b}	13.5	17.8^{d}	16.1^{d}	90.4
$CaCl_2$ (C), (E) + Chitosan (A), (B)	6.6^{a}	50.0	5.9^{a}	59.4	20.4^{b}	19.6^{b}	96.0

(Table 4) contd.....

$CaCl_2$ (D), (F) + Chitosan (A), (B)	7.2[a]	63.6	6.1[a]	64.8	22.0[a]	21.2[a]	96.4
$LSD_{0.05}$	1.0	-	0.88	-	0.63	0.57	-

*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)- $CaCl_2$ 0.5% tubers treatment; (D)- $CaCl_2$ 1% tubers treatment; (E)- $CaCl_2$ 0.5% foliar application; (F)- $CaCl_2$ 1% foliar application.

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

Table 5. Dry rot disease incidence on potato tubers in storage during two successive seasons 2016/2017*, varieties Sante and Kolobok.

Treatments	Dry Rot Disease Incidence, (%)			
	Sante		Kolobok	
	%	Reduction, %	%	Reduction, %
Control	19.2[a]	0.0	22.0[a]	0.0
Chitosan (A), (B)	14.0[bc]	-27.0	16.6[b]	-24.5
$CaCl_2$ (C), (E)	17.2[ab]	-14.4	19.2[ab]	-12.7
$CaCl_2$ (D), (F)	16.0[ab]	-16.6	18.0[b]	-18.1
$CaCl_2$ (C), (E) + Chitosan (A), (B)	10.6[cd]	-44.7	10.6[c]	-51.8
$CaCl_2$ (D), (F) + Chitosan (A), (B)	7.3[d]	-61.9	6.0[d]	-72.7
$LSD_{0.05}$	5.0	-	3.5	-

*Dry rot disease incidence was assessed after two months of storage.

**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)- $CaCl_2$ 0.5% tubers treatment; (D)- $CaCl_2$ 1% tubers treatment; (E)- $CaCl_2$ 0.5% foliar application; (F)- $CaCl_2$ 1% foliar application.

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

CONCLUSION

In conclusion, pre-planting with 1% calcium chloride followed by 0.5% chitosan with two h intervals, then foliar application twice with 1% $CaCl_2$ and 0.1% chitosan with ten days intervals enhanced plant growth and potato yield and decreased incidence of *Fusarium* dry rot disease. Therefore, the combined pre-planting and foliar application of $CaCl_2$ and Chitosan may be recommended for potato producers to reduce *Fusarium* dry rot disease incidence by 61.9-72.7%, and augment about 75.2-97.6% yields.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No animals/humans were used for studies that are the basis of this research.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

Not applicable.

FUNDINGS

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

Declared none.

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