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EFFECT OF CHITOSAN AND CALCIUM CHLORIDE APPLICATION ON TUBER YIELD AND VEGETATIVE PARAMETERS AGAINST POTATO GANGRENE UNDER FIELD CONDITIONS

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Abstract

Phoma dry rot of potato is considered a serious quarantine agent. Tuber yield losses by *Phoma* reach 80% in Russia. In this work, the influence of calcium chloride ($CaCl_2$) and chitosan, alone or in combination, on plant growth and tuber yield under artificial infestation of *Phoma* in the field was studied. Soil inoculation with *Phoma* conducted before planting. Potato tubers were treated with 0.05 or 0.1 kg/t $CaCl_2$, and chitosan 0.05 kg/t, with solution volume 10 l/t. The foliage was sprayed twice with 2 or 4 kg/ha $CaCl_2$, and 0.4 kg/ha chitosan, with solution volume 400 l/ha. Tubers and plants treated with water served as control. During the season, growth indicators such as germination (%), plant height (cm), and branches number per plant were measured. At harvesting, the total and marketable number of tubers, and tuber yield were calculated. Results indicated that combined pre-planting application with 0.1 kg/t $CaCl_2$ and 0.05 kg/t chitosan with 2 h intervals, then, spraying foliar twice with 4 kg/ha $CaCl_2$ and 0.4 kg/ha chitosan with 10 days intervals starting at 40 days after planting was most effective in maintaining the germination, plant height, and branches number. Although in Arosa variety, the branches number wasn't affected by any of the treatments. Also, the combined application of $CaCl_2$ and chitosan had a significant effect on the marketable number of tubers, increasing reached 111.1% and 123.8 % in Neveske and Arosa varieties, respectively. In addition, the combined application of $CaCl_2$ and chitosan increased the marketable tuber yield by 42.3-77.1 % in Arosa and Neveske varieties, respectively.

Keywords: Chitosan, Calcium chloride, Potato gangrene, *Phoma*.

Introduction

Potato gangrene (*Phoma* dry rot) is a storage disease which caused mainly by the soil-borne fungus *Phoma exigua* var. *foveata* (Foister) Boerema, (1967). *Phoma* dry rot can infect tubers through mechanical wounds arising at harvest or sorting. *Phoma exigua* var. *exigua*, the less aggressive pathogen, is also associated with potato gangrene (Boerema, 1967). The fungus can infect tubers in the soil, but the infections remain hidden until the late storage period when some potatoes turn to rot (Todd and Adam, 1967). In Russia, potato crop losses due to *Phoma* rot often exceed 25% (Anisimov *et al*., 2009) and maximum losses exceed 60-80% (Konyayeva and Cheprasova, 1987). Primary control of gangrene is carried out by post-harvest application of fungicides, such as Thiabendazole. Due to appearance new pathogenic races, which are resistant to Thiabendazole (Desjardins, 1993), and public concerns about food safety require research on new fungicides that are potentially less harmful to human health and the environment (Tripathi and Dubey, 2004).

Chitosan (poly-β - (1→4) N-acetyl-d-glucosamine) is a natural, safe and cheap biopolymer obtained from chitin, the main component of the cell walls of the exoskeleton of arthropods and fungi and the second renewable source of carbon after lignocellulose biomass (Kurita, 2006). The fungicidal activity of chitosan has been well documented in *in vitro* and *in vivo* studies (Bautista-Baños *et al*., 2006). Chitosan treatment not only effectively stops the growth of pathogens, but also leads to noticeable morphological changes, structural changes, and molecular disorganization of fungal cells (Ben-Shalom *et al*., 2003; Ait Barka *et al*., 2004). Chitosan also increases the activity of peroxidase, polyphenol oxidase, phenylalanine ammonia-lyase, chitinase, and β-1,3-glucanase in tomatoes, strawberries, raspberries and potato (Liu *et al*., 2007; Zhang and Quantick, 1998; Mohammed *et al*., 2019). Chitosan can improve the plant growth and development by enriching enrich photosynthesis and chloroplast enlargement. Also, it influences plant nutrition through improving soil fertility, increasing fixation of nitrogen and enhancing minerals uptake (Nguyen Van *et al*., 2013; Katiyar *et al*., 2015) .

Calcium (Ca) is considered a vital secondary nutrient. It can participate in: metabolic processes of uptake other elements; plant cell elongation; reinforcing cell wall structure and help plants to overcome the diseases (Mengel and Kirkby, 2001). Higher calcium content in potato foliage resulted in greater resistance against *Phytophthora infestans* infection (Subhani *et al*., 2015). Moreover, the application of calcium can also increase the yield, shelf life, weight, and quality of tubers (Ozgen *et al*., 2003; Hamdi *et al*., 2015). Treatment of potato plants with calcium chloride ($CaCl_2$) reduced the spread of early blight disease by 64.2 % and increased tuber productivity by 50 % (El-Mougy and Abdel-Kader, 2009).

The aim of recent study is to evaluate the effect of tubers treatment and foliar application with calcium chloride and chitosan alone or in combination on the potato plant growth and tuber yield, under artificial infection with *Phoma exigua* var. *foveate.*

Materials and Methods

Plant Materials

Tow potato varieties (Arosa and Neveske) were planted during two successive growing seasons, in 2017 and 2018 (May-August), at Moiseev farm, Bazarnyi Karabulak District, Saratov Oblast, Russia.

Pathogen

Naturally infected tubers with typical symptoms of *Phoma* rot were used to isolate the pathogen according to

(Logan and Khan, 1969), with some modifications. Diseased tissues were cut into small slices (1 cm^2) and superficially disinfected for in 70% ethanol for 30 s, followed by 2% sodium hypochlorite for 2 min. These slices were dried between sterilized filter papers and placed on Petri plates containing Potato Dextrose Agar medium (PDA) supplemented with streptomycin sulphate 5 mg L^{-1}. The plates were incubated at 20°C for 2 weeks, and single tips of fungal mycelium were transferred to new PDA for more purification. Isolates were identified morphologically as described by (Dennis, 1946; Malcolmson, 1958; Boerema and Höweler, 1967).

Fungal inocula preparation and soil infection

Barley grains (1 kg) were soaked overnight with 500 mL of sterile distilled water, then the excess water was discarded. Grains were placed in mushroom grow bags and autoclaved at 121 °C twice for 30 min each. After the cooling of grains, they inoculated with 3 discs (5 mm diameter) from a 2 weeks *Phoma exigua* var. *foveate* culture. The inoculated bags were incubated for three weeks at 20 °C. Soil inoculation was conducted at planting by placing the inoculum under the seed tubers at a rate of 50 g/m. The control traits did not receive any *Phoma exigua* var. *foveate* inoculum.

Chemicals

A medium molecular weight chitosan (150 kDa) with 80% degree of deacetylation, was purchased from Chitosan Technologies company, Engels city, Saratov region, Russia. Preparation of chitosan concentrations were obtained by dissolving desired amount of chitosan in 0.5% acetic acid, and the pH was adjusted to 5.5 by using 1 M NaOH. Calcium chloride ($CaCl_2$), colorless crystals, was obtained from Russkaya dymka company, Saratov city, Russia. Fungicide Maxim SC (Suspension concentrate) contains Fludioxonil as an active ingredient (Syngenta Crop Protection Inc, Russia) for pre-planting seed tubers treatment.

Experimental Design and Treatments

The field experiment was conducted during (May-August) in 2017 and 2018 to assess the effectiveness of seed tuber treatment and foliar application with chitosan and calcium chloride, and their interactions on plant growth and tuber yield under soil artificial infection. The experiment was conducted in a completely randomized block design (for each variety) and consisted of plots 28 m^2, with three replicates for each treatment in addition to the untreated control. The used treatments were as follow: 1- chitosan 0.05 kg/t and 0.4 kg /ha; 2- $CaCl_2$ 0.05 kg/t and 2 kg /ha; 3- $CaCl_2$ 0.1 kg/t and 4 kg /ha; 4- $CaCl_2$ 0.05 kg/t, then chitosan 0.05 kg/t, and $CaCl_2$ 2 kg /ha, then chitosan 0.4 kg /ha; 5- $CaCl_2$ 0.1 kg/t, then chitosan 0.05 kg/t, and $CaCl_2$ 4 kg /ha, then chitosan 0.4 kg /ha; 6- Maxim, SC 0.4 l/t; 7- control treated with water. For the pre-planting combined application of calcium chloride and chitosan, the tubers were firstly treated with $CaCl_2$, then chitosan with 2 h intervals. The solution volume was 10 l/t for pre-planting application and 400 l/ha for the foliar spraying. The foliar application was conducted twice with 10-days intervals and started at 40 days after planting.

Measurement of morphological parameters and tuber yield

During the growing season, germination (%), plant height (cm) and number of branches were measured. During harvest, calculation of the total and marketable number of tubers was conducted, and tubers from each plot were weighed.

Data 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 morphological parameters and tuber yield

The results shown in table 1 indicate the effect of pre-planting and during vegetation treatment with calcium chloride and chitosan alone or in combination, and fungicide Maxim on the growth and development of potato plants (with artificial infection with *Phoma* dry rot). Germination of plants increased in all variants by 0.7-100 % (table 1) and the increasing value was greater in the combined application of $CaCl_2$ and Chitosan than single applications.

Table 1: Influence of pre-planting and during vegetation treatment on the growth and development of potato plants infected with *Phoma exigua* var. *foveate*, during two successive seasons 2017/2018*.

Variety	Treatments	Germination		Plant height (cm)	Branches number/Plant
		(%)	Increasing (%)		
Arosa	Control	44.6	0	32.6^{b}	2.6^{a}
	Chitosan (A), (B)	54	21	34.3^{b}	3^{a}
	$CaCl_2$ (C), (E)	46.6	4.4	33.6^{b}	3.3^{a}
	$CaCl_2$ (D), (F)	47.2	5.8	34^{b}	3.3^{a}
	$CaCl_2$ (C), (E) + Chitosan (A), (B)	70	56.9	41.3^{a}	4^{a}
	$CaCl_2$ (D), (F) + Chitosan (A), (B)	89.2	100	42.3^{a}	4^{a}
	Maxim, SC 0.4 l/t	66.6	49.3	33^{b}	3.3^{a}
$LSD_{0.05}$				6.7	1.3
Neveske	Control	53.2	0	33^{b}	2.3^{b}
	Chitosan (A), (B)	58.6	10.1	34^{b}	3^{ab}
	$CaCl_2$ (C), (E)	53.6	0,7	34.6^{b}	3^{ab}
	$CaCl_2$ (D), (F)	62	16.5	35.6^{ab}	3.6^{ab}
	$CaCl_2$ (C), (E) + Chitosan (A), (B)	80.6	51.5	37.3^{ab}	4^{a}
	$CaCl_2$ (D), (F) + Chitosan (A), (B)	92	72.9	42.3^{a}	4.3^{a}
	Maxim, SC 0.4 l/t	62.2	16.9	35.6^{ab}	3.6^{ab}
$LSD_{0.05}$				7.1	1.5

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

(A)- Chitosan 0.05 kg/t; (B)- Chitosan 0.4 kg/ha; (C)- $CaCl_2$ 0.05 kg/t; (D)- $CaCl_2$ 0.1 kg/t; (E)- $CaCl_2$ 2 kg/ha; (F)- $CaCl_2$ 4 kg/ha.

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

Most of all, germination increased by 72.9 and 100% in Neveske and Arosa varieties respectively, when treated with ($CaCl_2$ 0.1 kg/t, 4 kg/ ha; chitosan 0.05 kg/t, 0.4 kg/ha) in (Table 1). Also, for plant height and branches number, treatment with ($CaCl_2$ 0.1 kg/t, 4 kg/ ha; chitosan 0.05 kg/t, 0.4 kg/ha) was more significant in comparison to the control (table 1). Although in Arosa variety, there were no significant differences between all variants of the experiment for the number of branches (Table 1).

For the total and marketable number of tubers, the best results were obtained by using the combined application of calcium chloride and chitosan ($CaCl_2$ 0.1 kg/t, 4 kg/ ha; chitosan 0.05 kg/t, 0.4 kg/ha), maintaining reached 62.5% and 85.3% for the total number of tubers in Neveske and Arosa varieties respectively, 111.1% and 123.8 % for the marketable number of tubers in Neveske and Arosa varieties, respectively (tables 2, 3). Also, the combined application of $CaCl_2$ and chitosan increased the marketable tuber yield by 42.3-77.1 % in Arosa and Neveske varieties, respectively (Tables 2, 3).

Table 2: Influence of pre-planting and during vegetation treatment on the number of tubers and yield, Arosa variety, infected with *Phoma exigua* var. *foveate*, during two successive seasons 2017/2018*.

Treatments	Tubers number/plant				Yield, t/ha		Marketability, (%)
	Total		Marketable		Total	Marketable	
	Tuber/ plant	Increasing, (%)	Tuber/ plant	Increasing, (%)			
Control	3.4	0	2.1	0	9.2^{c}	7.8^{d}	85.3
Chitosan (A), (B)	3.7	8.8	2.1	0	10.1^{b}	8.9^{c}	88.9
$CaCl_2$ (C), (E)	3.5	2.9	2.9	38.1	9.8^{bc}	9.3^{c}	94.5
$CaCl_2$ (D), (F)	3.9	14.7	3.3	57.1	10.2^{b}	9.6^{bc}	94.4
$CaCl_2$ (C), (E) + Chitosan (A), (B)	5.6	64.7	4.7	123.8	11.1^{a}	10.3^{b}	92.2
$CaCl_2$ (D), (F) + Chitosan (A), (B)	6.3	85.3	4.7	123.8	11.7^{a}	11.1^{a}	95
Maxim, SC 0.4 l/t	4.1	20.6	3.3	57.1	9.9^{bc}	8.2^{d}	83.1
$LSD_{0.05}$					0.7	0.7	

* The showing data of the two successive seasons were presented as average.
(A)- Chitosan 0.05 kg/t; (B)- Chitosan 0.4 kg/ha; (C)- $CaCl_2$ 0.05 kg/t; (D)- $CaCl_2$ 0.1 kg/t; (E)- $CaCl_2$ 2 kg/ha; (F)- $CaCl_2$ 4 kg/ha.
Mean values within columns followed by the same superscripts are not significantly different at $p < 0.05$

Table 3: Influence of pre-planting and during vegetation treatment on the number of tubers and yield, Neveske variety, infected with *Phoma exigua* var. *foveate*, during two successive seasons 2017/2018*.

Treatments	Tubers number/plant				Yield, t/ha		Marketability, (%)
	Total		Marketable		Total	Marketable	
	Tuber/ plant	Increasing, (%)	Tuber/ plant	Increasing, (%)			
Control	4	0	2.7	0	9.9^{c}	8.3^{d}	83.6
Chitosan (A), (B)	4.5	12.5	3	11.1	10.3^{c}	9.2^{c}	89.8
$CaCl_2$ (C), (E)	4.1	2.5	3.4	25.9	10.1^{c}	9.1^{c}	90.5
$CaCl_2$ (D), (F)	4.7	17.5	4	48.1	10.3^{c}	9.4^{c}	91.8
$CaCl_2$ (C), (E) + Chitosan (A), (B)	6.1	52.5	5	85.2	13.1^{b}	12.3^{b}	93.9
$CaCl_2$ (D), (F) + Chitosan (A), (B)	6.5	62.5	5.7	111.1	15.5^{a}	14.7^{a}	94.8
Maxim, SC 0.4 l/t	4.9	22.5	3.7	37	10.5^{c}	9.6^{c}	91.4
$LSD_{0.05}$					0.9	0.6	

* The showing data of the two successive seasons were presented as average.
(A)- Chitosan 0.05 kg/t; (B)- Chitosan 0.4 kg/ha; (C)- $CaCl_2$ 0.05 kg/t; (D)- $CaCl_2$ 0.1 kg/t; (E)- $CaCl_2$ 2 kg/ha; (F)- $CaCl_2$ 4 kg/ha.
Mean values within columns followed by the same superscripts are not significantly different at $p < 0.05$

Phoma exigua var. *foveate* incites disease on stems and tubers of the potato, the tuber disease being called gangrene (Entwistle, 1972). Fungicides could effectively control most of plant diseases, although they have negative effects on human health and environmental pollution, so alternatives of these fungicides are needed (El-Gamal *et al*., 2007). There are many fungicides alternatives used for the plant resistance induction against diseases. In this concern, 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). Optimization of the nutritional status of potato with calcium nutrients has been reported to reduce different diseases, improve tuber yield and quality (El-Gamal *et al*., 2007; Hamdi *et al*., 2015; Karlsson *et al*., 2006; Ozgen *et al*., 2003; Seifu, 2017).Chitosan has been also considered as a valid alternative to synthetic fungicides (El-Ghaouth, 1997). Chitosan at 4.0 g/l applied as soil drench showed significantly 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). It can induce defense activity in potato tubers against *Fusarium* dry rot (Sun *et al*., 2008) and *Rhizoctonia solani* (Mohammed *et al*., 2019). Also, 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 and hydrolyzed chitosan enhanced potato yield between 15-30%. 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 shelf life of 'Early Swelling' peach (Gayed *et al*., 2017).

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.

To our recent knowledge, this is the first report on the combined effect of chitosan and calcium chloride on the plant growth and potato tuber yield under the infection of *Phoma exigua* var. *foveate* which considered a serious quarantine agent.

In conclusion, pre-planting with calcium chloride at 0.1 kg/t and chitosan at 0.05 kg/ t with 2 h intervals, followed by foliar application twice with $CaCl_2$ at 4.0 kg/ha and chitosan at 0.4 kg/ ha enhanced plant growth and potato yield under infection of *Phoma* dry rot pathogen. Therefore, the combined application of $CaCl_2$ and chitosan pre-planting and during vegetation may be recommended for potato producers to augment about 42.3-77.1% yields.

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