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Chitosan and Citral Alone or in Combination For Controlling Early Blight Disease of Potato Plants Under Field Conditions.

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ABSTRACT

Early blight caused by *Alternaria solani* is the most important disease attacking potato plants. Chitosan or Citral alone or in combinations were tested to study their effect against early blight disease of potato plants under field conditions . Different concentrations of chitosan and Citral were tested to study their inhibitory effect on linear growth of *Alternaria solani* in vitro . Both treatments significantly reduced the linear growth of *Alternaria solani.* Complete inhibition was obtained with chitosan at 5.0 g / L and Citral at 5.0 mL/ L. The highest reduction was achieved with chitosan at 4.0 g / L and Citral at 4.0 mL/ L. which reduced the linear growth by 93.3 and 95.6 % respectively. Moreover, under field conditions results indicated that chitosan and Citral alone or in combination significantly reduced the disease incidence. The highest reduction was obtained combined treatments between chitosan at 2.0 g/ L. and Citral at 4.0 or 5.0 ml / L which reduced the early blight incidence more than 76.6 and 83.3 % respectively during two growing seasons as compared with untreated plants. As for tuber yield the highest increase was obtained with combined treatments between chitosan at 2.0 g/ L. and Citral at 4.0 or 5.0 ml / L which increased the tuber yield more than 88.6 and 80.0 % during two growing seasons respectively as compared with untreated plants. These treatments also increased chitinase activity which increased the activity by 124.2 and 127.3 % respectively as compared with untreated plants. It could be suggested that using a combination treatments of chitosan and Citral may be used for controlling early blight disease of potato plants under field conditions.

Keywords: Early blight – Chitosan – Citral – Potato- Control

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INTRODUCTION

Potato crop (*Solanum tuberosum* L.) is one of the most important vegetable crops in Egypt. Early blight caused by *Alternaria solani* is the most important disease attacking potato plants (Waals, *et al.*, 2004 ; Pasche, *et al.*, 2004 and 2005 , El-Gamal *et al.,* 2007 and Abd-El-Kareem , 2007). Controlling this disease depends mainly on fungicidal treatments (Pasche, *et al.,* 2005).

Induced resistance more effective for controlling several plant diseases (Guan, *et al.,* 2009 ; Suprakash and Chatterjee, 2012 ; Abd-El-Kareem and Abd-El- Latif, 2012 and Abd-El-Kareem, *et al.*, 2013) .

Chitosan deacetylated chitin, is currently obtained from the outer shell of crustaceans such as crabs, krills and shrimps. Chitosan exhibits a variety of antimicrobial activities (Badawy, *et al*., 2003 and El-Mohamedy, *et al.,* 2013) . On the other hands, chitosan induce host defense responses against several plant diseases (Uppal, *et al.,* 2008 ; Elwagia and Algam, 2014 and Mishra, *et al*., 2014).

Chitosan has been extensively utilized as a foliar treatment to control the growth, spread and development of many diseases involving viruses, bacteria, fungi and pests (Badawy, *et al.,* 2003, Faoro, *et al.*, 2008 and Elwagia and Algam, 2014) . It has also been used to increase yield and tuber quality of potatoes (Kowalski, *et al.,* 2006).

Citral is a commercial product of the citrus essential oil fractions it was reported to have antifungal activity against several plant diseases (El-Mohamedy *et al.,* 2002 and Abd-El-Kareem and Abd-El- Latif, 2012).

The main objective of the present research are studying the effect of chitosan and Citral alone or in combination against early blight disease of potato plants under field conditions.

MATERIALS AND METHODS

Source of pathogenic fungi and potato tubers

Pathogenic isolate of *Alternaria solani* the causal agent of early blight diseases was kindly provided by Plant Pathology Department , National Research Centre , Giza , Egypt. Potato tubers cv. Nigola were obtained from the Department ., of Vegetable Crop Research, Agricultural Research Centre, Giza, Egypt.

Laboratory experiments

Testing of different concentrations of chitosan and Citral on linear growth of Alternaria solani

Different concentrations of chitosan and Citral were tested to study their inhibitory effect on linear growth of *Alternaria solani* in vitro . Six concentrations of Chitosan and Citral *i,e*. 0.0 , 1.0, 2.0, 3.0, 4.0 and 5.0 g/ L or mL- L for chitosan and Citral respectively were added individually to conical flasks containing sterilized PDA medium to obtain the proposed concentrations, then mixed gently and dispensed in sterilized Petri plates (9 cm – diameter). Plates were individually inoculated at the center with equal disks (6-mm- diameter) of 10-days old culture of *Alternaria solani* . Five plates were used as replicates for each particular treatment . Inoculated plates were incubated at 25 ± 2 °C .The average linear growth of fungus was calculated after 10 days .

Field experiments

Testing of chitosan and Citral alone or in combination on early blight disease of potato plants under field conditions

Field experiments were carried out, at Research and Production Station of National Research Centre at El-Noubareia, Behera governorate, Egypt during two growing seasons under sandy soil and drip irrigation system.

Field experiments were conducted under natural infection in plots (4x8 m) each comprised of 8 rows (32 holes / row) in a randomized complete block design with three replicates (plots) for each treatment.

Treatments

Chitosan at 1.0 ,2.0 g/ L. and Citral ant 4.0, 5.0 mL/L. alone or in combination in addition to the Fungicides (Redomil – plus at 2 g / l) were tested to study their effect on early blight disease incidence of potato plants

Treatments were applied as follow

Treatment	
Single	Combined
1- Chitosan 1.0 g/ L	1- Chitosan 1.0 g/ L + Citral 4.0 ml/L
2- Chitosan 2.0 g/ L	2- Chitosan 1.0 g/ L + Citral 5.0 ml/L
3- Citral 4.0 ml/L	3- Chitosan 2.0 g/ L + Citral 4.0 ml/L
4- Citral 5.0 ml/L	4- Chitosan 2.0 g/ L + Citral 5.0 ml/L
5- Fungicide (Redomil plus 2 g / L)	
6- Un-treated plants (control)	

Application

All treatments were applied as foliar application on potato plants which had 4-5 compound leaves and every 15 days up to 90 days of planting.

Disease assessment

Early blight scale from 0 to 4 according to Cohen *et al.*, (1991) based on the leaf area infected was used , as follows

0 = No leaf lesions.
1 = 25 % or less .
2 = 26 to 50
3 = 51 to 75
4 = 76 to 100 % infected leaf area .

Diseases was recorded up to 90 days of planting.

Determination of tuber yield

Tuber yield of potato (kg /m^2) for each treatment was determined.

Effect of chitosan and Citral alone or in combination on chitinase activity of potato plants

Chitosan at 1.0 ,2.0 g/ L. and Citral ant 4.0, 5.0 mL/L. alone or in combination were tested to study their effect on chitinase activity of potato plants

Extraction of chitinase enzyme

Chitinase activity was determined after 60 days of planting . Extraction of enzyme from potato leaves was done according to method of Tuzun *et al.,* (1989).

Chitinase assay

Chitinase activity was determined by colourimetric method of **Boller and Mauch , (1988)** . Colloidal chitin was used as a substrate and dinitrosalicylic acid as reagent to measure reducing sugars.

Chitinase activity was expressed as mM N-acetylglucose amine equivalent released / gram fresh weight tissue / 60 minutes.

Statistical analysis

Tukey test for multiple comparisons among means was utilized **(Neler *et al.* 1985).**

RESULTS

Testing of different concentrations of chitosan and Citral on linear growth of Alternaria solani

Different concentrations of chitosan and Citral were tested to study their inhibitory effect on linear growth of *Alternaria solani* in vitro . Six concentrations of Chitosan and Citral *i,e*. 0.0 , 1.0, 2.0, 3.0, 4.0 and 5.0 g/ L or mL- L for chitosan and Citral respectively were tested to study their inhibitory effect against linear growth of *Alternaria solani* in vitro. Results in Table 1 indicate that both treatments significantly reduced the linear growth of of *Alternaria solan.* Complete inhibition was obtained with chitosan at 5.0 g / L and Citral at 5.0 mL/ L. High reduction was achieved with chitosan at 4.0 g / L and Citral at 4.0 mL/ L. which reduced the linear growth by 93.3 and 95.6 % respectively. Other treatments showed moderate effect.

Table 1: Linear growth (mm) of *Alternaria solani* as affected with different concentrations of chitosan and Citral

Treatment	Conc.	Linear growth (mm)	Reduction %
Chitosan (g/L.)	1.0	62.3 b[(1)]	30.7
	2.0	44.0 cd	51.0
	3.0	22.5 e	75.0
	4.0	6.0 f	93.3
	5.0	0.0 f	00
Citral (ml/L)	1.0	c 51.4	42.9
	2.0	41.2 d	54.2
	3.0	20.1 e	77.7
	4.0	4.0 f	95.6
	5.0	0.0 f	0.0
Control		90.0 a	——

1- Figures with the same letter are not significantly different (P= 0.05)

Field experiments

Effect of chitosan and Citral alone or in combination on early blight disease of potato plants under field conditions

Chitosan at 1.0 ,2.0 g/ L. and Citral ant 4.0, 5.0 mL/L. alone or in combination in addition to the Fungicides (Redomil – plus at 2 g / l) were tested to study their effect on early blight disease incidence of potato plants under field conditions. Results in Table 2 reveal that all treatments receiving chitosan and Citral alone or in combination significantly reduced the disease incidence. The highest reduction was obtained combined treatments between chitosan at 2.0 g/ L. and Citral at 4.0 or 5.0 ml / L which reduced the early blight incidence more than 76.6 and 83.3 % respectively during two growing seasons as compared with untreated plants. Moderate effect was obtained with combined treatments between chitosan at 1.0 g/ L. and Citral at 4.0 or 5.0 ml / L which reduced the early blight incidence more than 61.8 % during two growing seasons. Meanwhile, single treatments of both treatments were less effective.

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Table 2: Early blight incidence on potato plants as affected with different concentrations of chitosan and Citral alone or in combination under field conditions

Treatment	First season		Second season	
	Disease incidence	Reduction %	Disease incidence	Reduction %
Single treatment				
Chitosan 1.0 g/ L	2.5 b (1)	26.5	2.6 b	27.8
Chitosan 2.0 g/ L	2.3 b	32.4	2.5 b	30.6
Citral 4.0 ml/L	2.6 b	23.5	2.4 b	33.3
Citral 5.0 ml/L	2.4 b	41.2	2.5 b	30.6
Combined treatment				
Chitosan 1.0 g/ L + Citral 4.0 ml/L	1.2 c	64.7	1.3 c	63.9
Chitosan 1.0 g/ L + Citral 5.0 ml/L	1.3 c	61.8	1.3 c	63.9
Chitosan 2.0 g/ L + Citral 4.0 ml/L	0.8 d	76.5	0.6 d	83.3
Chitosan 2.0 g/ L + Citral 5.0 ml/L	0.7 d	79.4	0.6 d	83.3
Redomyl – plus 0.2	1.2 c	64.7	1.0 c	72.2
Control	3.4 a	——	3.6 a	——

(1) Figures with the same letter are not significantly different (P= 0.05)
(2) Early blight scale from 0 to 4 according to Cohen *et al*.,(1991).

Effect of chitosan and Citral alone or in combination on tuber yield of potato plants under field conditions

Chitosan at 1.0 ,2.0 g/ L. and Citral ant 4.0, 5.0 mL/L. alone or in combination in addition to the Fungicides (Redomil – plus at 2 g / l) were tested to study their effect on tuber yield of potato plants under field conditions. Results in Table 3 illustrate that all treatments receiving both chitosan or Citral and fungicide significantly increased the tuber yield . A considerable increase was obtained with combined treatments between chitosan at 2.0 g/ L. and Citral at 4.0 or 5.0 ml / L which increased the tuber yield more than 88.6 and 80.0 % during two growing seasons respectively as compared with untreated plants. Moderate effect was obtained with combined treatments between chitosan at 1.0 g/ L. and Citral at 4.0 or 5.0 ml / L which increased the tuber yield more than 40.0 % during two growing seasons. Meanwhile, single treatments of both treatments were less effective.

Table 3: Tuber yield of potato plants as affected with different concentrations of chitosan and Citral alone or in combination under field conditions

Treatment	First season		Second season	
	Yield (kg /m^2)	Increase %	Yield (kg /m^2)	Increase %
Single treatment				
Chitosan 1.0 g/ L	3.1 c (1)	29.2	3.1 c	24.0
Chitosan 2.0 g/ L	3.2 c	33.3	3.4 c	36.0
Citral 4.0 ml/L	3.0 c	25.0	3.1 c	24.0
Citral 5.0 ml/L	3.0 c	25.0	3.2 c	33.3
Combined treatment				
Chitosan 1.0 g/ L + Citral 4.0 ml/L	3.4 b	41.7	3.5 b	40.0
Chitosan 1.0 g/ L + Citral 5.0 ml/L	3.6 b	50.0	3.8 b	52.0
Chitosan 2.0 g/ L + Citral 4.0 ml/L	a 4.4	88.6	4.5 a	80.0
Chitosan 2.0 g/ L + Citral 5.0 ml/L	4.6 a	91.7	4.6 a	84.0
Redomyl – plus 0.2	3.4 b	41.7	3.5 b	40.0
Control	2.4 d	——	2.5 d	——

1- Figures with the same letter are not significantly different (P= 0.05)

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Effect of chitosan and Citral alone or in combination on chitinase activity of potato plants

Chitosan at 1.0 ,2.0 g/ L. and Citral ant 4.0, 5.0 mL/L. alone or in combination were tested to study their effect on chitinase activity of potato plants. Results in Table 4 indicate that all treatments receiving chitosan or Citral significantly increased chitinase activity. The most effective treatment was combined treatments between chitosan at 2.0 g/ L. and Citral at 4.0 or 5.0 ml / L which increased the chitinase activity by 124.2 and 127.3 % respectively as compared with untreated plants. Moderate effect was obtained with combined treatments between chitosan at 1.0 g/ L. and Citral at 4.0 or 5.0 ml / L which increased the chitinase activity 97.0 %. Meanwhile, single treatments of both treatments were less effective.

Table 4: Chitinase activity on potato as different concentrations of chitosan and Citral alone or in combination under field conditions

Treatment	Chitinase activity	Increase %
Single treatment		
Chitosan 1.0 g/ L	5.5 d[(1)]	66.7
Chitosan 2.0 g/ L	6.0 c	81.8
Citral 4.0 ml/L	5.2 d	57.6
Citral 5.0 ml/L	5.4 d	63.6
Combined treatment		
Chitosan 1.0 g/ L + Citral 4.0 ml/L	6.5 b	97.0
Chitosan 1.0 g/ L + Citral 5.0 ml/L	6.8 b	106.1
Chitosan 2.0 g/ L + Citral 4.0 ml/L	7.4 a	124.2
Chitosan 2.0 g/ L + Citral 5.0 ml/L	7.5 a	127.3
Redomyl – plus 0.2	5.0 e	51.5
Control	3.3 f	——

(1) Figures with the same letter are not significantly different (P= 0.05)

(2) Chitinase activity expressed as mM N-acetyl glucose amine equivalent released/ gram fresh weight/ 60 min.

DISCUSSION

Early blight caused by *Alternaria solani* is the most important disease attacking potato plants (Waals, *et al.*, 2004 ; Pasche, *et al.*, 2004 and 2005 , El-Gamal *et al.,* 2007 , Haggag, Wafaa, and Abd-El Khair, 2007 and Abd-El-Kareem , 2007). Controlling this disease depends mainly on fungicidal treatments (Pasche, *et al.,* 2005).

Chitosan deacetylated chitin, is currently obtained from the outer shell of crustaceans such as crabs, krills and shrimps. Chitosan exhibits a variety of antimicrobial activities (Badawy, *et al*., 2003 and El-Mohamedy, *et al.,* 2013). In the present study, in laboratory trail results indicated that complete inhibition in linear growth of *Alternaria solani* was obtained with chitosan at 5.0 g / L and Citral at 5.0 mL/ L. The highest reduction was achieved with chitosan at 4.0 g / L and Citral at 4.0 mL/ L. which reduced the linear growth by 93.3 and 95.6 % respectively. . In this respect, Kulikov *et al.,* (2006) reported that the antimicrobial activity increased with the increase in chitosan molecular weight and seems to be faster on fungi and algae than on bacteria. Fungicidal activity of chitosan has been documented against various species of fungi and oomycetes (Vasyukova, *et al.,* 2005 and El-Mohamedy, *et al.,* 2013). The minimal growth-inhibiting concentrations varied between 10 and 5,000 ppm (Rabea, *et al.* 2005). Some of the derivatives also repressed spore formation at rather high concentrations (Badawy, *et al*., 2005). Recently, Palma-Guerrero, *et al.* (2009) demonstrated that chitosan is able to permeabilize the plasma membrane of *Neurospora crassa* and kills the cells. In general, chitosan is able to reduce the *In Vitro* growth of a number of fungi and oomycetes (Palma-Guerrero , *et al.,* 2008). For instance, chitosan was reported to exert an inhibitory action on the hyphal growth of numerous pathogenic fungi, including root and necrotrophic pathogens, such as *Fusarium oxysporum*, *Botrytis cinerea*, *Monilina laxa*, *Alternaria alternata* and *Pythium aphanidermatum* (El Hassni, *et al.,* 2004). The mechanism by which chitosan affects the growth of several pathogenic fungi has not been fully elucidated, but several hypotheses have been postulated , first: its polycationic nature, it is believed that chitosan interferes with negatively charged residues of macromolecules exposed on the fungal cell surface. This interaction leads to the leakage of intracellular electrolytes and proteinaceous constituents (El Hassni, *et al.,*2004)). Second the

interaction of diffused hydrolysis products with microbial DNA, which leads to the inhibition of mRNA and protein synthesis (Vasyukova, *et al.,* 2005), third the chelating of metals, spore elements and essential nutrients (Rabea, *et al.* 2005). Forth : the interaction of chitosan with fungal DNA and RNA (Palma-Guerrero , *et al.,* 2008) . Five : Malformation of fungal mycelial . Chitosan is not only effective in inhibition the growth of the pathogen fungi, but also induces marked morphological changes, structural alterations and molecular disorganization of fungal cells (El Ghaouth *et al.,* 2002 and Ait Barka *et al.,* 2004). Moreover, El Hassni *et al.,* (2004) reported that, chitosan caused morphological changes such as large vesicles or empty cells devoid of cytoplasm in the mycelium of B. cinerea. Furthermore, Banos *et al.,* (2006) revealed that by microscopic observation of fungi treated with chitosan, it can affect the morphology of the hyphae.

On the other hands, chitosan induce host defense responses against several plant diseases (Uppal, *et al.,* 2008 ; Elwagia and Algam, 2014 and Mishra, *et al*., 2014). In the present study, under field conditions results revealed that both treatments of chitosan and Citral alone or in combination significantly reduced the disease incidence. The highest reduction was obtained combined treatments between chitosan at 2.0 g/ L. and Citral at 4.0 or 5.0 ml / L which reduced the early blight incidence more than 76.6 and 83.3 % respectively during two growing seasons. As for tuber yield the highest increased was obtained with combined treatments between chitosan at 2.0 g/ L. and Citral at 4.0 or 5.0 ml / L which increased the tuber yield more than 88.6 and 80.0 % during two growing seasons respectively as compared with untreated plants. In this respect chitosan has been extensively utilized as a foliar treatment to control the growth, spread and development of many diseases involving viruses, bacteria, fungi and pests (Badawy, *et al.,* 2003, Faoro, *et al.*, 2008 and Elwagia and Algam, 2014) . It has also been used to increase yield and tuber quality of potatoes (Kowalski, *et al.,* 2006). Citral is a commercial product of the citrus essential oil fractions it was reported to have antifungal activity against several plant diseases (El-Mohamedy *et al.,* 2002 and Abd-El-Kareem and Abd-El- Latif, 2012).

Chitosan had different properties *i.e.* had inhibitory effect against pathogenic fungus and had ability to be potent elicitors of plant defense resistance . In the presents study , the role of chitosan could be acting as antifungal activity and induced resistance against early blight disease . Chitosan significantly increased the chitinase activity in potato plants in this research, the most effective treatment was combined treatments between chitosan at 2.0 g/ L. and Citral at 4.0 or 5.0 ml / L which increased the the chitinase activity by 124.2 and 127.3 % respectively as compared with untreated plants. In this respect, chitosan (ch) induce host defense responses in both monocotyledons and dicotyledons. These responses include lignification, cytoplasmic acidification, membrane depolarization and protein phosphorylation , chitinase and glucanase activation , phytoalexin biosynthesis , generation of reactive oxygen species (Kuchitsu, *et al.,* 1995), biosynthesis of jasmonic acid (Nojiri, *et al.,* 1996), and the expression of unique early responsive and defense-related genes (Takai, *et al*., 2001). In addition, chitosan was reported to induce callose formation and proteinase inhibitors (Conrath, *et al*., 1989). It could be suggested that combined treatments between chitosan and Citral might be used for controlling late blight disease of potato plants under field conditions.

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