

## Evaluation of Chitosan Efficacy on Tomato Growth and Control of Early Blight Disease

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### ABSTRACT

Tomato (*Solanum lycopersicom* L.) is one of the world's most widely cultivated crops. Among the inflicting disease, early blight caused by *Alternaria alternata* is a major yield-limiting factor in Sudan. Management of the disease has been hampered by use of ineffective fungicides. Chitosan is a natural nontoxic biopolymer derived by deacetylation of chitin. The effects of chitosan at different concentrations against the early blight pathogen *A. alternata* and growth promotion of tomatoes were evaluated *in vitro* and under field conditions. Three chitosan concentrations (1, 3 and 5mg/ml) were used *in vitro*. Chitosan at 3mg/ml and 5mg/ml, was found to have strong antifungal activity against *A. alternata*, and was selected for further studies. In addition, chitosan at the above concentrations and the commercial fungicide **Ortiva** (250g/l Azoxystrobin) were used *in vivo* as treatments and distilled water as control. Chitosan at 5mg/ml applied as foliar spray alone, or in combination with seed treatment or seed treatment alone, reduced disease severity by 46%, while fungicide spraying reduced severity by 46% compared to control. In general, regardless of the application method, tomato growth parameters were significantly increased by chitosan treatments compared to control. Application of chitosan at 5mg/ml as foliar spraying combined with 5mg/ml as seed treatment increased plant height and it is fresh and dry weight by 16%, 36%, 24% respectively; and when applied at 5mg/ml as foliar spraying combined with 3mg/ml as seed treatment increased plant height, fresh and dry weight by 16%, 13%, 14%, respectively. This is the first study on the use of chitosan for control of tomato early blight and promotion of growth of tomato plants in Sudan. In conclusion, chitosan shows promise for enhancement of plant growth and control of *A. alternata* in tomato particularly when applied at 5mg/ml.

**Keywords:** *Alternaria alternata*, chitosan, ortiva, tomato growth promotion.

### INTRODUCTION

Tomato (*Solanum lycopersicom* L. Syn. (*Lycopersicon esculentum* Miller) belongs to the family Solanaceae and is grown for its edible fruits (Jones 2008; Caicedo and Peralta, 2013). Early blight disease, caused by the necrotroph fungus

*Alternaria* sp., is one of the most destructive diseases that affect quality and/or quantity of tomato product. In Sudan, Abbo (2009) reported that *A. alternata* is the main causal agent of early blight disease. The lack of effective fungicides and/or resistant varieties incited the need for the development of alternative strategies for the management of this disease. Biological control involving microbial agents or biochemicals offers an eco-friendly and cost-effective alternative as an important component of an integrated disease management program (Li *et al.*, 2007).

Chitosan is a natural nontoxic biopolymer derived by

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deacetylation of chitin, a major component of the shells of crustacean such as crab, shrimp, and crawfish. In recent years, applications of chitosan in the fields of medicine, food, chemical engineering, pharmaceuticals, nutrition, environmental protection and agriculture have received considerable attention (Li *et al.*, 2007 and ElHadrami *et al.* 2010). Applications of chitosan in environmental protection and agriculture include its use as a biocontrol agent for controlling plant disease (Khan *et al.*, 2006). Algam *et al.* (2010) reported that chitosan successfully controlled *Ralstonia* wilt in tomato as well as promotes tomato growth. Application of chitosan to tomato plants increases growth parameters and reduces diseased severity (EL-Tantawy, 2009). El-Mougy *et al.* (2006) reported that tomato root rot pathogens were controlled using chitosan, and increased yield by 66.7%. Moreover, chitosan application reduced severity of downy mildews in pearl millet (Sharathchandra, *et al.* 2004; Manjunatha *et al.* 2008; Nandeeshkumar, *et al.*, 2008). On the other hand, Jayaraj, *et al.*, (2009) stated that *Alternaria radicina* and *Botrytis cinerea* in carrot were well controlled by chitosan application. The objectives of this study were to examine the effect of chitosan on mycelial growth of *Alternaria alternata in-vitro*, development of early blight in tomato and plant growth promotion.

## MATERIALS AND METHODS

### Preparation of chitosan

Chitosan extracted from crab shell degree of N-deacetylation (75%, Sigma Aldrich USA) was solubilized in 1% acetic acid aqueous solution to obtain a concentration of 10 mg/ml (stock solution). The solution was alkalized to pH 5.6 with 1 N NaOH and autoclaved at 121°C for 15 min (Algam *et al.*, 2010).

### Source of the fungus

The fungus *Alternaria alternata* was obtained from the Plant Pathology Laboratory, Faculty of Agriculture,

University of Khartoum, and was maintained on PDA medium.

### Antifungal effect of chitosan

Using agar dilution method (Pillai *et al.*, 2005; Hanlon *et al.*, 2007), sterilized chitosan stock solution was incorporated into sterilized cool melted PDA at 50 °C (Muñoz *et al.*, 2009; Laflamme *et al.*, 1999) to obtain concentrations of 1, 3 and 5mg/ml of chitosan-amended media; then poured into 9-cm diameter sterilized Petri dishes. Plates receiving no chitosan served as controls. The plates were then inoculated with 6-mm diameter fungal-plugs, fungus-side down, from the margin of 7-days old colony, then incubated at 27±2°C. Two colony radii were measured daily up to ten days. Inhibition percentage was determined by the following equation (Abd-Elkareem *et al.*, 2006; Guo, *et al.* 2006):

% of Inhibition =  $[(X_c - X_i) / X_c] \times 100$ , where

X<sub>c</sub>: mean of colony diameter in control plates.

X<sub>i</sub>: mean of colony diameter of test plates.

### Effect of chitosan on tomato growth

To study the effect of chitosan concentrations and methods of application on plant growth and disease development, a pot-experiment was carried out. Two chitosan concentrations (3 and 5mg/ml) and two application methods (seed treatment (ST) and foliar spraying (FS) and their combinations were examined. Treatments were comprised of the following: 3 mg/ml and 5 mg/ml foliar spraying (FS3 and FS5), 3 mg/ml and 5mg/ml seed treatment (ST3 and ST5), and the following combinations (ST3+ FS3), (ST3 + FS5), (ST5 + FS3) and (ST5+FS5). Pots receiving one spray with the fungicide Ortiva (Azoxystrobin) at 1.5ml/l served as standard and distilled water (D.W.) as control. Each treatment was replicated four times. The experiment was conducted under field conditions at the nursery of the Department of Horticulture, Faculty of

Agriculture, University of Khartoum, Sudan.

### Seed treatment

As the pathogen *A. alternata* considered as soil and/or seed-borne pathogen and causes seed rot, pre- and post-emergence damping off, and seedling blight, therefore seed treatment is necessary. Moreover, application of chitosan as seed treatment might enhance plant resistance against foliar disease. However, tomato (cv. peto-86) seeds were, surface sterilized by 2% sodium hypochlorite for two min, then thoroughly rinsed in sterilized distilled water several times. Seeds were soaked into the two concentrations of chitosan (3 and 5mg/ml) for two hr. (Algam *et al.*, 2010). Pre-germinated seeds were transferred to 30-cm ø plastic pots containing sand: clay (1:3) soil. Ten seeds were sown per pot, and then thinned to 3 plants /pot three weeks later.

### Effect of chitosan on seed germination

To study the effect of chitosan concentrations on seed germination, germination test was carried out as described by Lyall *et al.* (2003) and NandeeshKumar *et al.* (2008).

### Foliar spraying with chitosan

Five-week old plants were sprayed with chitosan at 3 and 5 mg/ml (20 ml/pot). Spraying was carried out 24 hr. before inoculation using a 1000-ml capacity hand-held sprayer.

### Inoculum preparation

The fungus was periodically inoculated and re-isolated from ripened tomato fruits. Fourteen days old culture plates were each flooded with 10 ml sterilized distilled water containing 0.01%Tween20 (v/v) (Schaefer *et al.*, 2005). Spore concentration was adjusted to 3×10<sup>4</sup> spores/ml by sterilized distilled water using a Neubaur counting chamber. (Fontenell *et al.*, 2011).

### Inoculation of tomato plants with *A. alternata*

Chitosan-treated plants were sprayed with spore suspensions of 50 or 100ml/plant until run-off (Sathiyabama and Balasubramanian, 1998; Chaerani *et al.*, 2007). To maintain plants humid for a long time, inoculation was carried at dusk, then the plants were covered with transparent plastic bags overnight (Schaefer *et al.*, 2005; Vloutoglou *et al.*, 2000; Algam *et al.*, 2010).

### Disease severity assessment:

Disease severity was recorded weekly up to the 4th week from appearance of the first symptoms according to the scale described by Pandey *et al.*, (2003), and the scale was then converted into percentages of early blight index (PEBI) using the following formula:

$$PEBI = \frac{\text{sum of all ratings}}{\text{No. of leaf samples} \times \text{maximum dis. scale}} \times 100$$

Then reduction percentage in disease severity was calculated according to the following formula:

$$\text{Reduction\%} = \frac{\text{severity index of control} - \text{severity index of treatment}}{\text{severity index of control}} \times 100$$

The calculated severity index was subjected to angular transformation before analysis.

### Plant growth promotion

This was measured in terms of plant height at 21 day intervals, while plant fresh and dry weights were recorded once. For dry weight, plants were dried in an oven at 60 C for 3 days and weight was evaluated for each treatment. The relative growth promotion efficacy was estimated as follows (Algam *et al.* 2010):

$$GPE\% = \frac{\text{Plant parameter of chitosan treatment} - \text{Plant parameter of control}}{\text{plant parameter of control}} \times 100$$

### Statistics

Both laboratory and pot experiments were laid out in

completely randomized design. Data obtained were subjected to analysis of variance (ANOVA) and means were separated using Duncan's Multiple Range Test at the 5% level of significance (Gomez and Gomez, 1984). Analysis of variance and mean separation were performed by the statistical software SPSS 16.0.

## Results

### Effect of chitosan on mycelial growth of *A. alternata*

Mycelial growth of *A. alternata* was significantly reduced on media amended by the tested chitosan concentrations (1, 3 and 5 mg/ml) compared to control. At the chitosan concentrations of 3mg/ml and 5mg/ml fungal growth was fully inhibited while fungal growth at the chitosan concentration of 1mg/ml was inhibited by 89% (**Table.1**)

**Table 1. Effect of chitosan concentration on growth of *A. alternata***

Chitosan concentrations	Mycelial growth (mm)	Inhibition (%)
5 mg/ml	0 a	100
3mg/ml	0 a	100
1mg/ml	10 b	88
Control	83 c	0
Probability	0.00**	
Coefficient of variation	1.17%	

Means followed by the same letter(s) are not significantly different at  $P < 0.05$ .

\*\*Highly significant differences ( $P < 0.01$ )

### Effect of chitosan on seed germination

The chitosan concentrations of 3 and 5mg/ml did not affect seed germination, which was 94% and 95%, respectively compared to the control (95%). ( Data not shown in table).

### Disease severity

All plants were infected within three weeks after inoculation in varied degrees. It was observed that fungicide spraying or chitosan spraying alone or in combination with seed treatment delayed disease onset for a week. Regardless of the method of application, all

treatments significantly reduced early blight severity compared to the control ( $P < 0.05$ ).

Results in **Table 2** revealed that the highest reduction percentage was achieved by spraying plants with **FS5** (46%) followed by fungicide **Ortiva** treatment which reduced severity by 46% and then **ST5 + FS5** (46%), **ST5** (46%), **ST3 + FS5** (43%) and **ST5 + FS3** (43%). Less reduction in disease severity percentage was that in case of, **ST3** (37 %), **ST3 + FS3** (36%) without statistical significant differences. The less significant reduction percentage was that of plants treated with **FS3** (32).

**Table 2. Effect of chitosan and methods of application or fungicide on early blight severity under field conditions.**

Treatments	Severity index (%)	Reduction (%)
FS5	29 a	46
FS3	37 b	32
S T5	29 a	46
ST3	34 ab	37
ST5 + FS5	29 a	46
ST5 + FS3	31 a	43
ST3 + FS5	31 ab	43
ST3 + FS3	34 ab	37
Fungicide	29 a	46
control	54 c	0.00
C.V.	10.56%	

Means followed by same letter(s) are not significantly different at 0.05 level of significance. (ST= seed treatment; FS= foliar spraying); (0= No chitosan application. // 3= 3mg/ml chitosan and 5= mg/ml chitosan)}

### Growth promotion

Regardless of the application method and tested concentrations, chitosan treatments or fungicide (Ortiva) spraying resulted in plant growth promotion, i.e. plant height and plant fresh and dry weights, compared to control.

Results in **Table 3** revealed that the **ST5 + FS5** treatment increased plant height; plant fresh and dry weights by 16%, 36% and 24% respectively while in case of **ST5 + FS3** treatment, plant height, plant fresh and dry weights were increased by 9%, 9% and 4%, respectively. Application of chitosan as **ST3 + FS5** increased plant height, plant fresh and dry weights by 16%, 13% and 14% respectively, and in case of **ST3 +**

**FS3**, plant height, and plant dry weight were increased by 6%, and 4%, respectively, while plant fresh weight did not affected. In case of **ST5** treatment increased plant height, plant fresh and dry weights by 12%, 9% and 9%, respectively. Meanwhile **ST3**, treatment increased plant dry weights by 4%, whereas plant height and plant fresh weight were not affected. Treatment tomato plants with **FS5** increased plant height, plant fresh and dry weights by 9%, 3% and 4%, respectively. **FS3** treatment increased plant height, plant fresh and dry weights by 9%, 4% and 4%, respectively. Moreover application of the fungicide increased plant height by 12%, plant fresh weight by 12% and plant dry weight by 14% as compared to control.

**Table 3. Effect of chitosan concentrations and methods of application; and fungicide on height, and fresh and dry weights of tomato plant.**

<u>Treatment</u>	<b>Plant height</b>		<b>Plant weight</b>			
	<b>cm</b>	<b>GPE%</b>	<b>Fresh</b>		<b>Dry</b>	
			<b>g</b>	<b>GPE%</b>	<b>g</b>	<b>GPE%</b>
<b><u>Chitosan alone</u></b>						
<b><u>Foliar spray</u></b>						
FS5	34abc	9	123b	3	22abc	4
FS3	34abc	9	124b	4	22bc	4
<b><u>Seed treatment</u></b>						
ST5	35ab	12	130b	9	23ab	9
ST3	31bc	0	120b	0	22abc	4
<b><u>Combined treatments</u></b>						
ST5 + FS5	36 a	16	162a	36	26a	24
ST5 + FS3	34 abc	9	130b	9	22ab	4
ST3 + FS5	36 a	16	135b	13	24ab	14
ST3 + FS3	33 abc	6	120 b	0	22abc	4
<b><u>FUNGICIDE</u></b>	35 ab	12	133 b	12	24c	14
<b><u>CONTROL</u></b>	31c	0.00	119 b	0.00	21bc	0.00

\*Means followed by the same letter(s) are not significantly different ( $P < 0.05$ ).

(ST=Seed treatment / FS= foliar spraying).

### Discussion

At the present time, the methods of plant protection based on stimulation of natural mechanisms of resistance to fungal diseases, acquired great significance. Triggering of plant innate resistance using chitosan against most plant pathogens including fungi, bacteria and even some viruses has been well documented. This phenomenon are based on the fact that genes in the plant genome, which operate natural mechanisms of resistance, often do not function completely because of absence of appropriate external or internal inducer (Tiuterev, *et al.* 1996). Hence promising results on the effect of chitosan against phytopathogenic fungi have

been documented which may lead to reduction in use of fungicides.

In this study, application of chitosan gave promising results in both in-vitro and in-vivo experiments. In the in-vitro experiment, mycelial growth of the fungus *A. alternata* was successfully inhibited by chitosan treatment, varying from partial inhibition at low concentration to full inhibition at high concentrations, which is in agreement with the result of Reglinski, *et al.*(2010), Abd-Alla and Haggag (2010), El Hassni *et al.* (2004), Muñoz and Moret (2010) and Prapagdee *et al.* (2007).

It was observed that chitosan treatment did not affect tomato seed germination. This result is consistent with

Lizárraga-Paulín *et al.* (2011) as they did not find differences in germination rates in maize seeds treated with chitosan, while it contrasted to what was reported by Reddy *et al.* (1999) who found an increase in germination rates of wheat seeds treated with chitosan.

In the in-vivo experiment, disease development was greatly decreased by chitosan treatments and fungicide application. Chitosan application reduced disease severity in whatever concentration it was applied. This result agrees with many researchers' findings, where it was reported that application of chitosan as foliar spray and/or seed treatment successfully controlled foliar diseases; viz: severity of downy mildew in pearl millet was decreased upon chitosan treatments (Manjuatha *et al.*, 2008; Sharathchandra, *et al.*, 2004). Moreover, downy mildew in sunflower was kept in check by chitosan application (Nandeeshkumar *et al.*, 2008).

The plant growth promoting effects of chitosan observed in the present study are consistent with the results of many workers who reported that application of chitosan could enhance the growth of many crops. Algam *et al.* (2010) found that chitosan was able to enhance the growth of tomato plants. Saad and Al-

Malki, (2011) reported that bean growth parameters were positively enhanced upon chitosan treatment. Moreover, the presence of chitogel (a formulated chitosan solution) in culture medium of *Vitis vinifera* L. plantlets at 1.75 % (V/V), increased shoot and root dry weights, stem length and number of nodes (Ait Barka *et al.*, 2004). Boonlertnirun *et al.* (2008) reported that chitosan application as seed soaking and/or soil drench, increased rice growth parameters in terms of plant height, plant dry matter as well as grain yield. Spraying tomato plants with chitosan increased all vegetative parameters expressed in plant height, number of branches, number of leaves and plant fresh and dry weight (El-Tantawy, 2009). On the other hand, in this study all chitosan concentrations did not show any phytotoxic effect on treated plants even at the concentration of 5mg/ml.

According to these results, it could be concluded that chitosan at 5mg/ml showed strong antifungal effect against *Alternaria alternata*. Moreover, when applied as foliar spray and/or as seed treatment, it markedly reduced early blight severity as well as promoted tomato plant growth.

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## تقييم كفاءة المبيد الفطري (شيتوسان) على نمو البندورة ومكافحة مرض اللفحة المبكرة

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### ملخص

الطماطم واحدة من أكثر محاصيل الخضر التي تزرع علي نطاق واسع عالميا. ومن بين الأمراض المدمرة، يعد مرض اللفحة المبكرة الذي يسببه فطر *Alternaria alternata* العامل الرئيس المحدد لإنتاجية الطماطم في السودان، كما أن استخدام المبيدات غير الفاعلة أعاق مكافحة المرض . الكيتوسان هو مشتق كيتيني منزوع مجموعة الأستيل. لذا هدفت هذه الدراسة إلي تقييم تأثير تراكيز مختلفة من الكيتوسان علي فطر *A. alternata* تحت الظروف المعملية وتطور مرض اللفحة المبكرة وتشجيع النمو في نباتات الطماطم تحت ظروف الحقل . كلا التجريبتين اجريتا بإتباع التصميم العشوائي الكامل. استخدمت في التجربة المعملية ثلاثة تراكيز من الكيتوسان 1 و 3 و 5 ملجم/مل. أوضحت النتائج أن تركيزي الكيتوسان 3 و 5 ملجم/مل لهما نشاط قاتل قوي علي الفطر. استخدم في التجربة الحقلية التراكيزين 3 و 5 ملجم/مل بالإضافة إلي المبيد الفطري ذا الاسم التجاري ortiva بالجرعة الموصى بها والماء المقطر كشاهد. ضمت المعاملات رش ورقي ومعاملة البذور ورش ورقي مع معاملة البذور. أوضحت ان استخدام الكيتوسان بتركيز 5 ملجم/مل (كرش ورقي أو كرش ورقي مع معاملة البذور أو معاملة البذرة) أدى إلى انخفاض شدة المرض بنسبة 46 % ، بينما أدى رش المبيد الفطري إلي خفض شدة المرض بنسبة 46% مقارنة بالشاهد. بغض النظر عن طريقة التطبيق ،ازدادت مقاييس نمو الطماطم معنويا بمعاملات الكيتوسان مقارنة بالشاهد. تطبيق الكيتوسان بتركيز 5 ملجم/مل كرش ورقي مع 5ملجم/مل كمعاملة للبذور أدى إلى زيادة في طول النبات والوزن الرطب والوزن الجاف للنبات بنسبة 16%، 36% و 24% على التوالي. وعند تطبيقه بتركيز 5 ملجم/مل كرش ورقي مع 3ملجم/مل كمعاملة للبذور أدى إلى زيادة في طول النبات والوزن الرطب والوزن الجاف للنبات بنسبة 16%، 13% و 14% على التوالي. تعد هذه الدراسة هي الأولى عن استخدام الكيتوسان في مكافحة مرض اللفحة المبكرة وتشجيع نمو نبات الطماطم في السودان. ونتيجة لذلك نجد أن المعاملة بالكيتوسان تعطي تأثيرات واعدة في تشجيع نمو النبات ومكافحة مرض اللفحة المبكرة خصوصا إذا استخدم بتركيز 5ملجم/مل كرش ورقي مع/أو معاملة البذور.

الكلمات الدالة: الكيتوسان، الطماطم، مرض اللفحة المبكرة.

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