J. Plant Prot. and Path., Mansoura Univ., Vol.9 (11): 755 - 758 , 2018

Chitosan as a Potential Postharvest Treatment for Minimizing Rotscausing Decay of some Vegetable Fruits

Samira Abdelaziz [1]; S. T. El-Afifi [2] and M. El-Sheshtawi[1]

[1] Plant Pathology Dept. Faculty of Agriculture,Mansoura University , Mansoura,35516 Egypt

[2]Vegetableand ornamentals Dept. Faculty of Agriculture,Mansoura University ,Mansoura,35516 Egypt

ABSTRACT

Fungal pathogens such as *Alternaria solani* in tomato,*Rhizopus stolonifer* in strawberry, *Botrytis cineara* in pepper, *Sclerotinia sclerotiorum* in cucumber, and *Sclerotium rolfsii* in carrot causes many losses of postharvest decay of different fruits affect quality and quantity during the storage, marketing and handling. Its control has been and still largely based on the use fungicides. However, it is able to develop resistance to these chemicals very rapidly, so its control has become problematic.Chitosan (poly-*β*-(1-4)*N*-acetyl-d-glucosamine),was applied on maycelial growth of fungi and fruits, by dipping fruits in solution 0.05% and 0.2% concentrations for five minutes. *Invitro,* 0.2% Chitosan was the best concentration for inhibition of mycelial growth of postharvest fungi giving 75.7% inhibition of *A. solani*, followed by 76.9% of *R.stolonfer*,then 94.9% of *S.rolfsii*.While, the 0.05% chitosan gave moderate inhibition of *A. solani* and *R. stolonifer* and gave high inhibition on *S. rofsii*, while the two concentrations achived complete inhibition in *B. cinerea* and *S. sclerotiorum*. Chitosan treatments applied with acetic acid on fruits gave significant decrease in disease incidence and disease severity.Resultes indicate that chitosan treatments gives good treatmentfor reducing the maycelial growth of fungi and increasing control of postharvest decay of different fruits and roots.

Keywords: Chitosan, postharvest, rot fruits, postharvest fungi ,pathogenic fungi

INTRODUCTION

Postharvest diseases caused by various fungal and bacterial pathogens may occur at any stage through postharvest processing and handling that take place from field to consumer. Losses due postharvest decay including the reduction of fruit quality and quantity. Common pathogens of postharvest decay such as *A. solani*(early blight) in tomato (*Solanum lycopersicum*) , *R. stolonifer* (Rhizopus fruit rot) in strawberry (*Fragaria × ananassa*), *B.cineara* (grey mold) in pepper (*Capsicum annuum*), *S. sclerotiorum* (white mold) in cucumber (*Cucumis sativus*) , and *S. rolfsii* (sclerotium rot) in carrot (*Daucus carota subsp. sativus*) could attack these crops in the fieled or after the harvest but the infection is clrearly develop during the storage,handling and marketing.lately, there is increased need for developinga safe and environment friendly alternatives for controling such postharvest fungal diseases.Among potential alternatives chitosan found to be safe, and efficient option. Chitosan which produced from natural sources, has advance treatment through antifungal activity,and increases the plant defense responses (Terry and Joyce, 2004).Previous studies showed that asignificant reduction of postharvest decays of sweet cherry including greymold caused by *B. cinerea* and blue mold caused by *Penicillium expansum* by spraying or dipping ina solution sof chitosan (Romanazzi *et al*., 2003) .Moreover the inhibitory effect of tomato pathogens during storage (Liu *et al.*, 2007).In General, it was reported that chitosan effect is usually due to inducing resistance in the treated fruits rather than merely inhibiting the pathogen directly (Capdville *et al*., 2002).

The aim of this study is testing; effect of chitosan on mycelial growth of some postharvest fungi in addition to evaluate the chitosan coating treatment on the incidence and disease severity occuring at postharvest decay of some vegetable fruits during the cold storage.

MATERIALS AND METHODS

Isolation of pathogenicfungi

Naturally infected vegetable fruits showing a typical postharvest decay symptoms were collected from local markets. In case of samples showing a fungal growth on the surface of decayed fruits a direct transfer of this fungal growth in an Acidified Potato Dextrose Agar plate (APDA) at 20±2°C for 4-7 days. After that according to hyphal tip technique the purification of fungiwere carried out (Teik-Khiang Goh 1999) and identified by Barnett and Hunter, (1998). Slants contain PCA medium were used to kept all isolated fungi at 4°C for this study.

Pathogenicity test for isolated fungi

Prepared the spore suspensions by adding 5-10 ml sterilized distilled water amended with 0.5 ml tween80 to pure culure of *A. solani*, *B. cinerea*, *R. stolonifer, S. sclerotiorum* and *S. rolfsii*.Spores were scraping byusing steril plastic spetula from the cultures surfaces. The spore suspension was filtered through double layers of cheese cloth. spores. The resulted spores suspension was determined by using haemocytometer slide and was adjusted to be approximately 1x 10^6 spores/ml. Whereas, the inocula in case of *S. sclerotiorum* and *S. rolfsii* were done by using sclerotia from 15 days old culture . Sclerotia were harvested using scraping techniques as mentioned before and stored until using (El-Sheshtawi*et al.*, 2016).

Tomato, strawberry, pepper, cucumber fruits and carrot roots were dipped in1%Chlorox® solution to sterilize the fruits surface for 10 min., then washed many times with distilled water and left to air to be dried. Four wounds for each fruit were done by sterilized needle, and then divided to two amounts ,the first to be inoculated by dipping in spores suspension was previously prepared. While, the second for control treatment was dipped in sterilized distilled water and left to dry, then fruits were put in sterilized plastic box amended with wet paper and incubated at20±2°C, lesions diameter were measured after 7 days by mm (Liu *et al.*, 2007).

Fruits

Fresh tomato ,strawberry, pepper, cucumber fruits and carrot roots were obtained from the local market at mature green stage, then selected to be free of injuries or any infection. Before treatments, all fruit were divided into two sets, the first set was sterilized by dipping in 5% sodium hypochlorite solution, where the 2nd set dipped in 1% acetic acid for 2 min. Both sets were then washed with

distilled water and air dried to get rid of any excess droplets of disinfectant.

Effect of chitosan on mycelial growth and spore germination of some postharvest fungi on PDA medium

Chitosan suspension :

Chitosan (fine powder, purchased from a comerial Company Sigma Chemical) was mixed with a 0.5% glacial acetic acid and distilled water, and using 1 N NaOH to adjuste pH near 5.6. Suitable amount of chitosan was mixed with 100 ml medium in flasks to give ,0.05 and 0.2 % concentrations. After that mediawas poured in petriplates,each plate contained 20 ml mediumand then 5mm discs of tested postharvest fungi were inoculated and incubated at 15±2°C for 5 days for measuring the diameter of each colony. Three replicates were used for each treatment and Control treatment (Petri dishes without chitosan).

Effect of chitosan coating on postharvest disease incidence and severity on some vegetable fruits under 15°C:

Each type of fruit was splitted to two groups. The first group was surface sterilized by dipping in 5% sodium hypochlorite solution, and the second group was dipped in 1% acetic acid for 2 min. then rinsed with distilled water and left to dry byair.A half number of fruits was dipped in the solution (0.05%chitosan), and the other half was dipped in (0.2% chitosan) for five minutes, then allowed all fruits to dry for 2 hours at 25°C, Zhao *et al.,* 2009. Each fruit surface was wounded four wounds by sterilized needle, 2mm in depth and 2mm in diameter at the equatorial region. In case of tomato, strawberry and pepper each wound was inoculated with 10µl of spore suspension (10^6 spores/ml) of *A. solani, R. stolonifer* and *B. cinrea*,respectively. But, in case of Cucumber fruits and carrot roots were inoculated with sclerotia of *S. sclerotiorum* and *S. rolfsii* . directly after inoculation, treatments were placed in two incubators where the temperature and the RH inside both incubators were adjusted to 15 ±2 ℃ and 98% respectively. Lesions diameters were measured after 7 days. Disease incidence data were expressed as percentage of fruit showing symptoms out of the total number of fruits in each treatment.(Brix and Zinkernagel, 1992):

$$DI = \left[\frac{C - I}{C}\right] \times 100$$

(DI) = Disease incidence %, (I)=number of healthy fruits in replicates and (C)=mean number of survived fruits in the control

While ,disease severity was scored according to (Zewide *et. al.*2007): where 0=Healthy fruits (0% of fruits surface with symptomatic lesions), 1=1-25%, 2=26-50%, 3=51-75% and 4=76-100% . Disease severity was calculated according to the following formul

$$\text{Disease severity (DS)} = \left[\frac{\text{Sum of all ratings}}{\text{Total number of plant pieces} \times \text{maximum score}}\right] \times 100$$

Statistic alanalysis

Statistical analysis of results was performed using a one-way analysis of variance (ANOVA). Means were separated using the Tukey test ($P < 0.05$) (SPSS commercial software, SPSS Inc., Chicago, IL). The data were analyzed and graphically plotted using Sigma-plot software (Systat Software Inc., Richmond, CA).Duncan's at 5% level of significant adoption.

RESULTS

1- Pathogenicity test for isolated fungi

Data in Table 1 show that all isolated fungi used achived 100% disease incidence at all fruits inoculated, giving 100%disease severity in tomato, strawberry and pepper , 91.6% in cucumber fruits and 77.7% in carrot roots compared with control (non- infected fruits and roots) treatments that gave disease incidence and disease severity 0% at all fruits without strawberry gave 66.67% and 55.56%, respectively.

Table 1. Pathogenicity test for isolated fungi:

Treatment	Tomato		Strawberry			Pepper		Cucumber		Carrot
	Di%	D s%	D i%	Ds%	D i%	Ds%	Di%	Ds%	Di%	Ds%
Control (non-infected)	0b	0b	66.67b	55.56b	0b	0b	0b	0b	0b	0b
Infected Control	100a	100a	100a	100a	100a	100a	100a	91.6a	100a	77.7a

Where

Di = Disease incidence , Ds = Disease severity

Values in each line followed by the same letter are not significantly different p < 0.05

2- Effects of chitosan on mycelial growth of *A. solani, B.cineara, R. stolonifer,S. sclerotiorum* and *S. rolfsii*

Data in Table 2 show that 0.2 chitosan concentration was the best for inhibition of mycelia growth of postharvest fungi giving 75.7% inhibition of *A. solani*, followed by 76.9% of *R.stolonifer*,then94.9% inhibition of *S. rolfsii*. While, 0.05chitosan concentration gave a moderate inhibition on*A. solani* and *R. stolonifer* and gave high inhibition on *S. rofsii*, and both concentrations achived complete inhibition in *B. cinerea* and *S. sclerotiorum* when compared with control.

3- Effects of chitosan on tomato postharvest disease caused by *A. solani*

Data in Table 3 show that hitosan concentrations when treated with acetic acid andchlorox on tomato gave highly significant reduction of disease incidence and disease severity reached 100% reduction. When compared with control ;chlorox and acetic acid gave also 100%, disease incidence and100, 66.7and 66.7% disease severity, respectively

Table 2. Effects of chitosan on mycelial growth of *A . solani,B. cineara,R. stolonifer, S. sclerotiorum* and *S. rolfsii*

fungi treatment	*A . solani*	*B . cinerea*	*R . stolonifer*	*S. sclerotium*	*S. rolfsii*
Control	8.50a	8.50a	8.50a	8.50a	8.50a
Ch 0.05%	3.36c	0c	4.16b	0b	1.16c
Ch 0.2%	2.06d	0c	1.96c	0b	0.43c

Values in each line followed by the same letter are not significantly different p < 0.05

Table 3. Effects of chitosan on tomato postharvest diseases caused by *A. solani*

Treatment	Disease incidence (Di) %	Disease severity (Ds) %
Control (untreated)	100a	100a
Acetic acid	100a	66.7b
Chlorox	100a	66.7b
Chitosan 0.05+ Acetic acid	0b	0c
Chitosan 0.2 + Acetic acid	0b	0c
Chitosan 0.05 + Chlorox	0b	0c
Chitosan 0.2 + Chlorox	0b	0c

Values in each line followed by the same letter are not significantly different p < 0.05

4- Effects of chitosan on strawberry postharvest disease caused by *R. stolonifer*

Data in Table 4 show that chitosan 0.2 concentration when treated with acetic acid on strawberry gave high significant reduction of disease incidence and disease severity reached 100% reduction, when compared with control , while the other treatments gave 100%, disease incidence and100, 100 , 83.3 , 88.8 and 66.6% disease severity, respectively. When compared with controls ,chlorox andacetic acid 100%, disease incidence and100% disease severity, respectively.

Table 4. Effects of chitosan on strawberry postharvest disease caused by *R. stolonifer*.

Treatment	Disease incidence (Di) %	Disease severity (Ds) %
Control (untreated)	100a	100a
Acetic acid	100a	100a
Chlorox	100a	100a
Chitosan 0.05+ Acetic acid	100a	83.3b
Chitosan 0.2 + Acetic acid	0b	0d
Chitosan 0.05 + Chlorox	100a	88.8b
Chitosan 0.2 + Chlorox	100a	66.6 c

Values in each line followed by the same letter are not significantly different p < 0.05

5- Effects of chitosan on pepper postharvest disease caused by *B.cineara*.

Data in Table 5 show that chitosan 0.2 concentration when treated with chloroxon pepper gave moderate reduction of disease incidence at 33.4% reduction. While chitosan 0.2 concentration when treated with acetic acidon pepper gave 23.3% ofdisease severity. When compared with control, chlorox andacetic acid 100%, disease incidence and100, 91.7and 91.7% disease severity, respectively.

Table 5. Effects of chitosan on pepper postharvest disease caused by *B. cineara*.

Treatment	Disease incidence (Di) %	Disease severity (Ds) %
Control (untreated)	100a	100a
Acetic acid	100a	91.7a
Chlorox	100a	91.7a
Chitosan 0.05 + Acetic acid	100a	35.3c
Chitosan 0.2 +Acetic acid	100a	23.3d
Chitosan 0.05 +chlorox	100a	43.3b
Chitosan 0.2 +chlorox	66.67b	35.3c

Values in each line followed by the same letter are not significantly different p < 0.05

6- Effects of chitosan on cucumber postharvest disease caused by *S. sclerotiorum*.

Data in Table 6 show that chitosan concentrations with acetic acid andchlorox on cucumber gave complete inhibition of disease incidence and disease severity at 100% reduction. When compared with control ,chlorox and acetic acid gave 100%,0% disease incidence, 91.6% and 0% disease severity, respectively.

Table 6. Effects of chitosan on cucumber postharvest disease caused by *S. sclerotiorum*.

Treatment	Disease incidence (Di) %	Disease severity (Ds)%
Control (untreated)	100 a	91.6 a
Acetic acid	0b	0b
Chlorox	0b	0b
Chitosan 0.05 + Acetic acid	0b	0b
Chitosan 0.2 +Acetic acid	0b	0b
Chitosan 0.05 +chlorox	0b	0b
Chitosan 0.2 +chlorox	0b	0b

Values in each line followed by the same letter are not significantly different p < 0.05

7- Effects of chitosan on carrot postharvest disease caused by *S. rolfsii*.

Data in Table 7 show that chitosan concentrations when treated with acetic acid and chlorox on carrot roots gave highly significant reduction of disease incidence and disease severity at 100% reduction. When compared with control ,Chlorox and Acetic acid gave 100%, 0% disease incidence, 77.7% and 0% disease severity, respectively.

Table 7. Effects of chitosan on carrot postharvest disease caused by *S. rolfsii*.

Treatment	Disease incidence (Di) %	Disease severity (Ds) %
Control (untreated)	100a	77.7a
Acetic acid	0b	0b
Chlorox	0b	0b
Chitosan 0.05 + Acetic acid	0b	0b
Chitosan 0.2 +Acetic acid	0b	0b
Chitosan 0.05 +chlorox	0b	0b
Chitosan 0.2 +chlorox	0b	0b

Values in each line followed by the same letter are not significantly different p < 0.05

DISCUSSION

Based on the obtained results, chitosan as a natural substance which is biodegradable and non-toxic not inhibition the mycelial growth of fungi ,reduced the diseases severity and induced defense responses in fruits, exoressing a promising substance to control postharvest diseases(Edirisinghe*et al*. 2014).

The antifungal property of chitosan might be related to its forming a physical barrier against infection, reducing the conidial germination and mycelial growth of *B. cinerea* and resulting in the long lasting protection of grape berries against gray mold(Romanazzi *et al.* 2002).Results of mycelial growth study *invitro*, confirmed that the efficacy of chitosan to inhibit or reduce the radial mycelial growth of some postharvest fungi . Chitosan activity was detected at both concentrations and the growth of fungi was reduced as the chitosan concentration increased and the tratment gave complete inhibition in case of *B.cinerea* and *S. sclerotium* at both concentrations . Different results were obtained in a previous study by(El

Ghaouth *et al*.,1992b) who found that chitosan at 6 mg /ml^{-1} did not completely inhibited the radial mycelial growth of *B. cinerea* and *R.stolonifer*. (Krol,2005) also found that chitosan poorly inhibited the mycelial growth of *Phomopsis viticola* sacc. On the other side, several researchers confirmed that chitosan is very effective against postharvest fungi such as *B. cinerea* of tomato (Badawy and Rabea2009) and in other fruits (El Ghaouth *et al*. 1997).

REFERENCES

Badawy, M.E.I., and Rabea, E.I., (2009).Potential of the biopolymer chitosan with different molecular weights to control postharvest gray moldof tomato fruit.*Postharvest Biol Technol* 51:110–117.

Barnett H. L. and Barry B. Hunter (1998). Illustrated Genera of Imperfect Fungi, Fourth Edition. 218 pages. ISBN 978-0-89054-192-0.

Brix, H. D., and V. Zinkernagel. (1992).Effects of cultivation, conditioning and isolate on sclerotium germination in *Sclerotium cepivorum* .*Plant Pathology* (1992) 41, 13-19.

Capdeville,G.; De Wilson, C.L.; Beer, S.V., andAist, J.R., (2002). Alternative disease control agents induce resistance to blue mold in harvested Red Delicious apple fruit. *Phytopathology* 92, 900-908 .

Edirisinghe, M.; Ali, A.; Maqbool, M., and Alderson, P., (2014). Chitosan controls postharvest anthracnose in bell pepperby activating defense-related enzymes.*J Food SciTechnol* 51(12):4078–4083.

El Ghaouth, A.; Arul, J.; Asselin, A., andBenhamou, N., (1992b).Antifungal activity ofchitosan on postharvest pathogens: induction of morphological and cytological alterations in Rhizopus stolonifer. *Mycol Res* 96:769–779.

El Ghaouth, A.; Arul, J.;Wilson, C.,and Benhamou, N., (1997). Biochemical and cytochemical aspects of the interaction of chitosan and Botrytis cinerea in bell pepper fruit. *Postharvest BiolTechnol* 12:183–194

El-Sheshtawi, M. and Saad, A. (2016). New Ways for Controlling Onion White Rot Disease (*Sclerotium cepivorum*) in North Egypt. International Journal of Advanced Research 4(3):1472-1477

Goh, T.K. (1999). Single-spore isolation using a hand-made glass needle. Fungal Diversity 2:47-63.

Krol, E., (2005). Influence of some chemicals on the viability of Phomopsis viticola sacc. spores. *J Plant Prot Res* 45:195–203. .

Liu, J.;Tian, S.P.; Meng, X.H., and Xu, Y.,(2007). Effects of chitosan on control of postharvest diseases and physiological responses of tomato fruit.*Postharvest Biol. Technol.44*, 300–306.

Romanazzi, G., Nigro, F., &Ippolito, A., (2003). Short hypobaric treatments potentiate the effect of chitosan in reducing storage decay of sweet cherries. *Postharvest Biol. Technol.* 29, 73–80.

Terry, L.A., &Joyce, D.C., (2004). Elicitors of induced disease resistance in postharvest horticultural crops: a brief review. *Postharvest Biol.Technol.* 32, 1–13.

Zhao, Z.; Gu, Y.; Kun, M. and Li, X.(2009). Effect of Chitosan Coating on the Antioxidant Enzymes and Quality of "Dashi Early Ripening" Plums. In 3rd International Conference on Bioinformatics and Biomedical Engineering, June 11-13, 2009. China, pp. 1-4

Zewide,Tamire.;Fininsa, Chemeda., andSakhuja,P. K., (2007) .Management of white rot (*Sclerotium cepivorum*) of garlic using fungicides in Ethiopia.*Crop Protection* 26, 856–866.

استخدام الشيتوزان كمعاملة ممكنة لأمراض ما بعد الحصاد لتقليص الأعفان التى تسبب تلف بعض ثمار الخضر

سميرة عبد العزيز[1] ، سمير طه العفيفى[2] و محمد الشيشتاوى عبدربه[1]

[1]قسم أمراض النبات –كلية الزراعة – جامعة المنصورة – المنصورة – مصر

[2]قسم الخضر والزينة - كلية الزراعة – جامعة المنصورة – المنصورة – مصر

تصاب ثمار و جذور الخضر بالعديد من أعفان الثمار بعد الحصاد وأثناء عمليات التخزين والتسويق بالمسببات المرضية مثل فطر الالترناریاسولانی الذى يصيب ثمار الطماطم ، فطر البوتريتيسسنريا يصيب الفلفل ، فطر الريزوبستولونيفر الذي يصيب ثمار الفراولة ، فطر الاسكليروتينياسكليروشيورم الذى يصيب ثمار الخيار وفطر اسكليروشيم رولفسياى الذى يصيب جذور الجزر ،وأستخدمت العديد من طرق المقاومة ومن بينها المبيدات الفطرية الكيماوية التي سرعان ما ظهرت سلالات فطرية مقاومة لها. مما تطلب البحث عن طرق مقاومة جديدة ومنها استخدام الشيتوزانو تطبيقها علي النمو الميسليومى للفطريات الممرضة والثمار عن طريق غمس الثمار في تركيزات مختلفة من الشيتوزان لمدة خمس دقائق. معمليا حيث أعطى تركيز 0.2 من الشيتوزان أفضل نسبة تثبيط للنمو الفطرى وقد كانت 75.7 لفطر الالترناريا سولانى على ثمار الطماطم،يليه 76.9 لفطر الريزوبس ستولونيفر على ثمار الفراولة ثم 94.9 لفطر الاسكليروشيم رولفسياى على جذور الجزر، بينما التركيز 0.05 أعطى تثبيط متوسط لنفس الفطريات وكلا التركيزين حققا تثبيط كامل للنمو الميسليومى لفطر البوتريتسسنريا على ثمار الفلفلوالاسكليروتينيا اسكليروشيورم على ثمار الخيار. وعندما عوملت الثمار محل الاختبار بتركيزات مختلفة من الشيتوزان أعطى تركيز 0.2 من الشيتوزان خفض لنسبة الإصابة وشدة الإصابة ل0% فى بعض الحالات. النتائج تشير الى أن المعاملة بالشيتوزان تعد مؤشرا مبشرا لخفض النمو الميسليومى للفطريات وزيادة مقاومة الثمار و الجذور لبعض أنواع الخضر لأمراض ما بعد الحصاد.