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Evaluation the efficiency of chitosan and salicylic acid in controlling gray mold disease caused by *Botrytis cinerea* on eggplant

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Abstract

The study was conducted to isolate and identify the causal agent of gray mold disease on eggplant and evaluate the efficiency of chitosan (CH) and salicylic acid (SA) to control the disease. The morphological characteristics proved that the causal agent of gray mold on eggplant is *Botrytis cinerea* . Three isolates of the pathogen, from sample showing gray mold at different location of Baghdad area, were obtained and designated as BC1, BC2, BC3. The pathogenicity tests on wounded eggplant fruits revealed that the 3 isolates were highly pathogenic. The disease severity was found to be 90.25 , 83.21 , 69.52% followed by BC2 with disease severity , 72.20 , 66.39 , 57.20 % and BC1 , 56.20 , 56.72 , 39.59 % after 14 days of inoculation on wounded fruits inoculates on upper third , lower third , and on unwounded fruits respectively. The addition of chitosan at 300 mg / L and SA at 125 mg / L separately or in combination into PDA caused high reduction in *B. cinerea* growth. The combination of SA + CH was the more effective with growth inhibition percentage 95.29% compared with 87.84% and 87.83% with SA and CH respectively. The application of SA and Chitosan on the plants 7 days before leaves inoculation with BC3 caused signification reduction in disease severity to 4.23% compared with 55.6% in control.

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Introduction

Eggplant is one of the most vegetable crops in Solanaceae, cultivated at wide areas in Iraq. Eggplant fruits contains many vitamins and minerals including, P, Fe, Ca (Al-Rekabi *et al.,* 1981). Eggplant are subjected to infection with several pathogens in both protected and open fields cultivation .Of these pathogens, *Botrytis cinerea*, causal agent of gray mold disease, reported to be the more important (Zhang, 2009, Al-Maamoory, 2013). *B.cinerea* is an air borne fungi infecting Tomato, grape, Strawberry, in addition to eggplant causing high losses in yield worldwide (Freeman *et al.*, 2004, Nicot, 2008, Zhang *et al.*, 2009, Dean *et al.*, 2012).

It has been reported that *B. cinerea* infects eggplant at All stages of growth inducing symptoms on leaves, stem, flowers and fruits (Karabulut *et al.,* 2004, Mosbach *et al.*, 2011, Martin *et al.*, 2011). Several methods were adapted to control the disease among those chemical fungicide was the more effective (Rosslenbroish and Stuebler, 2000). But due to the enormous problems created by the excessive use of fungicides to ecosystem , human health and animals , the effort of searchers were oriented toward searching for alternative to fungicide, safe and effective, capable to activate defense mechanisms in the plants (Induced systemic resistance) against the pathogene (Grabke *et al.*, 2013,2014).

Numerous substances were reported to induce systemic resistance in the plants against *B.cinerea* including chemical and biological agents (Benitez *et al.*, 2004, Yin *et al.*, 2010, Saleh and Al-Mansoury, 2015) . Many previous studies indicated to the activity of Chitosan and Salicylic acid against many pathogens including *B. cinerea*, directly or through inducing systemic resistance in many host plants (Vlot *et al.*, 2008, Reglinski *et al.*, 2010, Silva *et al.*, 2014, Soliman *et al.*, 2015, Li and Zou, 2017). The study was conducted to evaluate the efficiency of Chitosan and Salicylic acid to restrict *B. cinerea* growth directly on culture medial and indirectly through induce systemic resistance in the plants.

Materials and methods

Isolation and Identification of B. cinerea

Eggplant fruits showing gray mold were collected from plastic houses cultivated with eggplant in different locations of Baghdad area (Fig 1). Parts of fungal growth (mycelium and spores) were transferred into 9 cm dim petri plates containing potato dextrose agar (PDA) amended with tetracyclin (200 mg / L) and maintained at 17 C º for 7 days. The growing fungus was purified and identified as described by Ellis (1971) and conserved in small flasks containing PDA.

Pathogencity tests

The Pathogencity of three isolates of *B.cinerea* , BC1 , BC2, BC3 was carried out on healthy eggplant fruits . The fruits were surface sterilized with 1% sodium hypochlorite for 2 mins, rinsed with distilled water and dried on filter papers. The fruits of a treatment were wounded in the upper third, while the fruits of the second treatment were wounded in the lower third with sterilized needle . The wounded fruits were inoculated with 2 mm discs from the border of *B. cinerea* isolates culture on PDA, 7 days old, placed on the middle of wounds. Unwound fruits were inoculated with the fungus isolates and others were left without inoculation as control. The fruits were placed on a layer of sterile humidified cotton in 19 cm dim petriplates (2 fruits / plate) and maintained at 17 C º. Five plates for each treatment were used. The disease severity for each isolate was determined after 14 days of inoculation as described by Rachandram and Rao (1980) , using a scale of 6 degrees , where 0= healthy fruits , 1= 1-10% , 2= 11-25% , 3= 26-50% , 4= 51-75% , 5= 76-100% of the fruit area were infected.

Inhibition activity of Chitosan and Salicylic acid against B. cinerea growth on culture media

Chitosan at 32, 62.5 , 125 mg / L amended with 0.1% acetic acid, and salicylic acid at 100, 200, 300 mg / L were added separately and in combination into 250 ml flasks containing 100 ml PDA. The medium was homogenized and poured into 9 cm dim petri plates.

The center of each plate was inoculated with 5 mm dim disc from the border of fungal colony, 7 days old, on PDA. The plates were maintained at 17 C ° for 7 days. The radial growth of fungus was calculated and the inhibition percentage of fungal growth was determined by the equation;

% growth inhibition=

$$\frac{\text{mean dim of fungal growth in control} - \text{mean dim of fungal growth in treatment}}{\text{mean dim of fungal growth in control}}$$

Three replication of each concentration were used.

Efficiency of Chitosan and Salicylic acid to control gray mold disease in eggplant

Ten ml of sterilized distilled water were added into BC3 culture on PDA, 7 days old, and spread with fine brush. The spores suspension was passed through two layers of sterilized muslin cloth and collected in flask. The spores suspension was amended with two drops of tween 20 and the spores concentration was adjusted to 1×10^6 spores / ml by haemocytomrter. Plastic pots of 2 kg soil were filled with sterilized soil (at 121 C ° and 1-5 kg / cm^2 for one hour, twice in two successive days) . The pots were seeded with eggplant seeds (2 seeds / pot), watering and maintained in plastic house. The plants emerged were treated , at 30 days old, with Chitosan at 125 mg / L, and Salicylic acid at 300 mg / L separately and in combination through spraying plant foliage and inoculated by BC3 isolate by spraying the leaves with the spores suspension , as fallows;

1-plants treated with Chitosan before 7 days of inoculation.

2-plants treated with Chitosan at inoculation time.

3-plants treated with Chitosan after 7 days of inoculation.

4-plants treated with SA before 7 days of inoculation.

5-plants treated with SA at inoculation time.

6-plants treated with SA after 7 days of inoculation .

7-plants treated with SA+Chitosan before 7 days of inoculation.

8-plants treated with SA+Chitosan at inoculation time.

9- plants treated with SA+Chitosan after 7 days of inoculation .

10-plants modulated with BC3 only/control.

11-plants sprayed with water/control.

The disease severity was determined using a scale of 5 degree , where, 0= healthy leaves, 1= 1-10% of leaves infected, 2= 11-25% of leaves infected , 3= 26-50% of leaves infected , 4= 51-75% of leaves infected , 5= 76-100% of leaves infected.

Results and discussion

Isolation and Identification of B. cinerea

Three different isolates from infected eggplant fruits showing gray mold were obtained. The isolates showed different ability to form sclerotia and intense mycelium (Fig 2). The microscopic observation showed septate hyphae with long branched sporophores bearing cluster of single cell ovale spores. These results were found in accordance with chareteristics stated by Ellis (1971) concerning *B.cinerea* indicating that the isolates are belong to *B.cinerea* and designated as BC1, BC2, BC3. Several previous studies reported that *B.cinerea* can infect eggplant, tomato, strawberry and grape causing gray mold (Zhang *et al.*, 2009, Reglinski *et al.,* 2010, Dean *et al.*, 2012, Al-Maamoory , 2013 , Silva Junior ,2014, Hassan *et al.*, 2018). Al-Mansoory (2015) obtained 4 different isolates of *B.cinerea* from eggplants at Baghdad and Basra areas.

Pathogenicity of B. cinerea

Results obtained showed that the three isolates, BC1, BC2, BC3, isolated from infected eggplant fruits were highly pathogenic as proved by the intensive gray mold development representing the fungal mycelium and spores.

The disease severity was found to be between 37.69-90.28 compared with zero in control. Different in pathogenicity was observed between the isolates. The isolate BC3 was found the more pathogenic with disease severity, 90.28, 87.21, 69.52% followed by BC2 with disease severity, 72.20, 66.59 , 57.20 % and BC1with disease severity, 56.20, 51.72, 37.69 % on the fruits wounded in the upper third, lower third, and unwounded fruits respectively (Table 1).

Table 1. Pathogenicity of *B. cinerea* isolates on eggplant fruites.

Treatment	Disease severity%
	After 14 days
Inoculation with Bc1 on unwounded fruits	37.69
Inoculation with Bc1 on the upper third of wounded fruits	56.20
Inoculation with Bc1 on the lower third of wounded fruits	51.72
Inoculation with BC2 on unwounded fruits	57.20
Inoculation with BC2 on the upper third of wounded fruits	72.20
Inoculation with BC2 on the lower third of wounded fruits	66.59
Inoculation with BC3 on unwounded fruits	69.53
Inoculation with BC3 on the upper third of wounded fruits	90.28
Inoculation with BC3 on the lower third of wounded fruits	87.21
Non-inocilation fruits	0.00
L.S.D 0.05	1.242

Each value represent mean of 5 replication.

The pathogenic effects of the isolates may result from their penetration the fruits through natural opening and produce hydrolytic enzymes that hydrolyze cell wall constituents. It was reported that *B.cinerea* produce many enzymes capable to hydrolyze plant cells wall including lipase, pectinases, xylanases , cellulases and proteases (Choquer *et al.*, 2007, Kubicek *et al.*, 2014, Shah *et al.*, 2006, Elad and Fillinger, 2016).

Activity of Chitosan and Salicylic acid in B. cinerea growth inhibition on PDA

Different in *B. cinerea* growth inhibition induced by Chitosan and Salicylic acid on PDA were observed. The more efficient treatment was the mixture of 125 mg/L Chitosan and 300 mg/L SA with inhibition percentage of 95.29 % compared with 87.83% and 87.84% with Chitosan and SA, at the same concentrations, separately (Table 2).

Table 2. Effect of different concentrations of Salicylic acid and Chitosan in growth inhibition of Botrytis cinerea on culture media PDA.

Treatment	Concentration Mg / L	Inhibition percentage
	32	57.64
Chitosan + *B.cinerea* (BC3)	62.5	79.27
	125	87.84
	100	54.1
Salicylic acid + B.cinerea(Bc3)	200	77.52
	300	87.83
	32+100	65.09
Chitosan +Salicylic acid +BC3	62.5+200	85.87
	125 +300	95.29
Control	_____	0

Each value represent mean of 3 replications

LSD, p= 0.05 between concentrations =1.85, LSD, P=0.05 between treatments =3.71.

The fungal growth inhibition by the other treatments was ranged between 54.1 -85-87 %. The inhibition effects of Chitosan and SA on fungal growth may resulted from their entrance into fungal cells with other nutrients and affect some biological activities in the cells leading to reduction in the growth. It has been reported that Chitosan affect all the parameters growth of *B. cinerea* and reduced the viability of spores at high concentration (El-Ghaouth *et al*, 1992). Some previous studies reported that the effect of Chitosan on *B. cinerea* is correlated with concentration (Dipiero and Grada, 2008, Elmer *et al.*, 2010). Positive correlation between *pythium aphanidematum* and *B.cinerea* growth inhibition and

SA concentration was reported (Hassan, 2005, Vlot *et al.*, 2008 Hayfa *et al.*, 2015) .

Effiency of Chitosan and Salicylic acid for controling gray mold disease in eggplant

High reduction in disease symptoms development was achieved upon treatment of inoculated plants with Chitosan and Salicylic acid. The application of SA+Chitosan combination , 7 days before inoculation of BC3 isolate was found the more effective , with disease severity 4.23% compared with 55.61% in control (Fig 3).

Fig. 1. Symptom of gray mold disease caused by Botrytis cinerea on eggplant fruits.

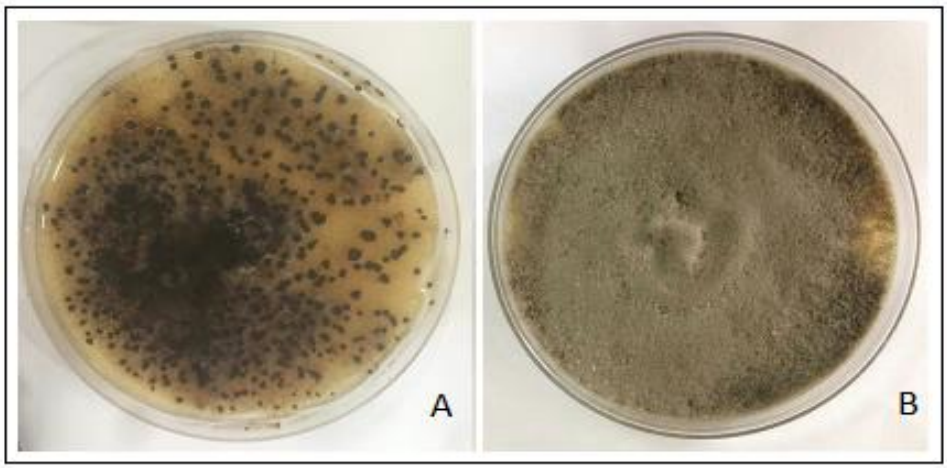

Fig. 2. *Botrytis cinerea* growth on PDA; A- *B.cinerea* sclerotia B- Fungus colony.

The reduction of disease development by the control agents may resulted from the activation of plant defence mechanisms leading to produse antifungal subtances including enzymes and proteins that restrict *B.cinerea* growth and disease symptom development. It was reported that treatment of grape leaves with Chitosan reduced gray mold disease severity by 55% (Romanazzi *et al.*, 2002). Aziz *et al.,* (2006), Trotel-Aziz *et al.,* (2006) found that treatment of grape leaves with Chitosan enhanced plant defense mechanisms and induce systemic resistance against infection with *B. cinerea*. Similar results were reported about the role of SA in pathogen control. The infection of some host plants, Bean,

Tobacco, Tomato, Arabidopsis with *B. cinerea* induced the accumulation of SA at infection position and systemically away from infection position associated with activation of certain genes encoding for proteins having antifungal activity that indicate the responsibility of SA in inducing systemic resistance in plants (Pinninckx *et al.*, 1996, Zimmerli *et al.,* 2001, Metrau, 2001, Farhan and Al-Juboory, 2018).

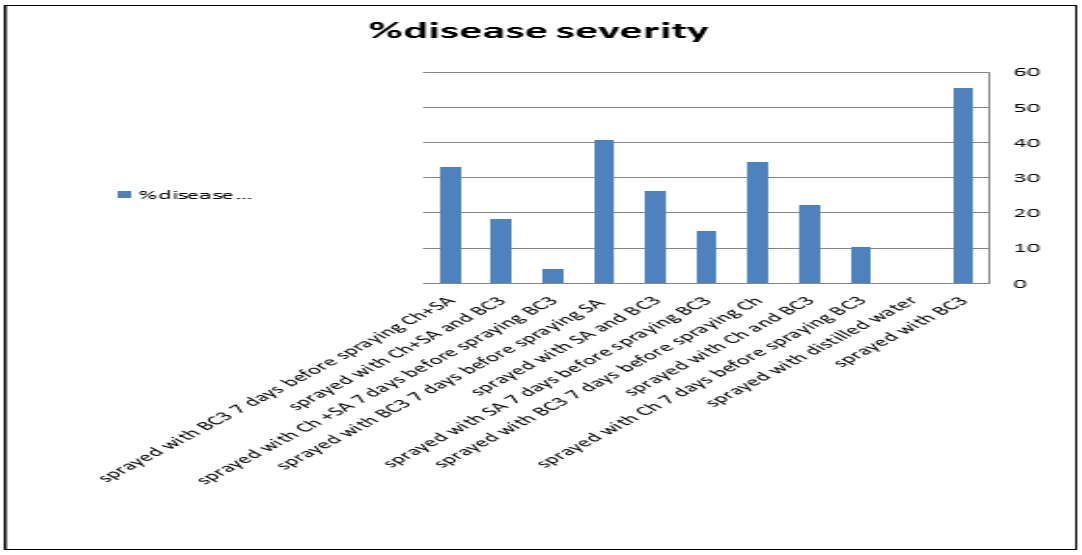

Fig. 3. Effect of spraying eggplant with Chitosan and Salicylic acid on *B.cinerea* disease severity.

The results of the study showed that eggplant are very susceptible to infection with *B. cinerea*.

The treatment of eggplant with SA and Chitosan separately or in combination induced high reduction in disease development as proved by decreases in disease severity. SA and Chitosan may be promising in disease management.

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