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Antifungal Potentialities of Chitosan and Trichoderma in Controlling *Botrytis cinerea*, Causing Strawberry Gray Mold Disease

Awad, H. M.
Agric. Botany Dept., Fac. of Agriculture, Menoufia Univ., Egypt

ABSTRACT

Strawberry gray mold disease caused by *Botrytis cinerea* (Pers.) is one of the most critical diseases attacking strawberry fruits especially at the post-harvest stage. This study compared the effectiveness of two types of Chitosan, bioagent's filtrates and fungicides in controlling the gray mold of strawberry. The commercial and synthesized *in vitro* Chitosan were tested on radial growth of *B.cinerea* on PDA agar plates and on infected four fruits cultivars strawberry. The results showed that the two types of Chitosan were significantly decreased infection of *B. cinerea* especially on un-wounded fruits that have been treated by dipping method. Biological control agents were used as culture filtrates for control strawberry gray mold pathogen both on wounded and unwounded fruits, the disease incidence was inhibited significantly in comparing to control treatment. Chemical fungicides were also used in these trials for controlling the gray mold disease. The results obtained confirm the superiority of fungicides in controlling the disease in comparing the commercial and synthesized Chitosan well as biocontrol agents.

Keywords: Strawberry gray mold disease, *Botrytis cinerea*, Chitosan, Biological control, Fungicides.

INTRODUCTION

Strawberry (*Fragaria* x *ananassa* Duch.) is one of the most important crops of exported vegetables in Egypt with 283471 ton in 2015 season with average 18.291 ton/feddan (Ministry of Agriculture, Economic sector, 2015). Egypt is considered the third largest producer of strawberry worldwide after the United States and Spain (Wu *et al*, 2012). Strawberry plants are attacked by several diseases which cause considerable losses in fruits yield. Strawberry fruits are perishable fruits during postharvest storage where they susceptible to dryness and mechanical injuries in addition to decay and physiological disorders. Gray mold disease caused by *Botrytis cinerea* is one of the most important diseases where it infected worldwide more than 200 plant species (Williamson *et al*, 2007). In strawberry cultivations, the stage of overwintering of *Botrytis cinerea* could be find as sclerotia or vegetative mycelium in all strawberry wintering parts (Braun and Sutton, 1987 and Sutton, 1998). *B. cinerea* could be attack strawberry plants during growth stages of flowering, fruiting and storage periods causing destructive effects (Sesan, 2003; Droby and Lichter, 2004). Also, *B. cinerea* is consider the main causal pathogen of strawberry post-harvest decay (Maas, 1998). There are many different fungicides anti-Botrytis thus, the occurrence of fungicidal resistance in the fields is not surprising (Weber, 2011), for example, and Carbendazim were discarded from use against *B. cinerea*, the causal agent of gray mold disease, because the resistance for them (Leroux *et al*, 2002) as well as their high toxicity. On the other side, the use of synthetic chemicals to control fungal diseases is restricted due to their high toxicity, where the most gray mold fungicides are often applied at least 7 days pre harvesting which is considered unacceptable. On the other hand, natural ingredients and resistant inducers which can increasing plant defense could be used in controlling strawberry gray mold disease. In this respect, chitosan and some bio-control agents are highly effective for this purpose. Chitosan is derived from glucan with chitin repeating units, Chitins is an abundance mucopolysaccharides are found in arthropods such as shrimps, craps and inferior plants such yeasts. It has been reported that chitosan has a fungicidal action against several fungi (Hirrano and Nagao 1989; Kendra *et al*, 1989). The inhibitory effect of chitosan was also demonstrated against many of soil born fungi (Stossel and Leuba; 1984; El Ghouth *et al*, 1990) and *Rhizopus stolonifer* (Yarahamdi *et al*, 2014). Also, chitosan was used to control post-harvest diseases of many fruits such as Pear (Yu *et al*, 2008) and Strawberry (Ge *et al*, 2010; Bhaskara *et al*, 2000). Chitosan is known to be a potential elicitor of many plant defense responses, including the accumulation of chitinases (Mauch *et al*, 1984) and synthesis of proteinase inhibitors in tomato leaves (Pearce and Ride, 1982). Chitosan has been approved by the U.S Environmental Protection Agency (EPA) and food additive approved by the U.S Food and Drug Administration (FDA).

This research aimed to throw a light on the antifungal potentialities of two types of chitosan (Commercial and synthetic) in controlling the gray mold disease on fruits of four strawberry cultivars in vitro and in vivo comparing with the antifungal potentialities of some *Trichoderma* spp. as bio-agents and some fungicides.

MATERIALS AND METHODS

1. Source of *Botrytis cinerea* as a causal pathogen of strawberry gray mold disease:

Botrytis cinerea was isolated as a causal pathogen of strawberry gray mold disease from diseased samples of naturally infected strawberry fruits showing gray mold symptoms which collected from three localities of strawberry fields *i.e.,* Shebin El-kom, El-Bagour and El-Shohada which belonging to Minufiya governorate. The infection percentage was estimated as diseased fruits in relation to the total healthy ones. The gray mold pathogen (*Botrytis cinerea*) isolates infecting strawberry fruits were isolated on Potato Dextrose Agar (PDA) medium. The resultant cultures were purified using the single spore culture or hyphal tip techniques according to Dhingra and Sinclair, (1985). The growing fungal colonies were transferred to slant tubes of PDA medium and then incubated for 7 days at 24ºC. The pure cultures of *Botrytis cinerea* isolates were examined microscopically and identified based on their morphological features at Agricultural Botany Department, Faculty of Agriculture, Minufiya University using the methods adopted by Neergard, (1945); Barnett, (1960) and Domsch *et al*., (1980) in addition to the key of imperfect fungi of Barnett and Hunter, (1972).

2. Source of *Trichoderma* spp. isolates as antagonists:

Trichoderma fungi were isolated from soil and rhizosphere samples of grown strawberry in the previously

mentioned fields by uprooting the infected plants with great care to obtain most of the intact root system. The dilution plate method (DPM) was used for isolation of *Trichoderma* spp. The isolated Trichoderma fungi were cultured onto 20% malt extract agar, incubated for two days at 25°C then, identified according to Rifai, (1969) and Bissett, (1991). Stock cultures of isolated *Trichoderma* spp. were maintained on PDA slants then kept in a refrigerator at 5°C and they repeatedly sub-cultured every 4 weeks on fresh PDA slants.

Preparation of *Trichoderma* spp. culture filtrates:

Trichoderma culture filtrates were prepared by inoculating disk of the fungus onto liquid potato dextrose medium in flasks (100/200 ml), then incubated by shaking at 25°C for 3 days then incubated for 7 days. Trichoderma culture filtrates were prepared by eliminating the mycelial mates, then the filtrates were centrifuged at 8000g for 10 min., and filtered through a Hydrophobic filter (type A/E, Gelman Sciences, Ann Arbor, M1) (Harman *et al*, 1992).

3. Source of chitosan:

Two types of chitosan were used in this trail against *B. cinerea* where the first one is commercial chitosan (Ch.C.) which was purchased from Pharo-Pharma for Pharmaceuticals Address Plot 9, 3rd Industrial Zone, Block 16, Borg El Arab City, Alexandria, Egypt) while, the second one is synthesized Chitosan (Ch.S.) *in vitro.*

The production process of the synthesized chitosan *in vitro* involved three steps *i.e.,* demineralization (DM), deproteinization (DP) and deacetylation (DA) as described by Youn *et al*., (2007). In this respect, the shrimp shells were demineralized with 1 mol/L HCl for 30 min at ambient temperature with a solid/solvent (HCl) ratio of 1:15 (w/v). Following the DM step, the demineralized shells were collected on a 100-mesh sieve, washed to neutrality in running tap water, rinsed with deionized water, and filtered to remove excess moisture. The DP step was accomplished by treating the demineralized shells with 3 g/100 mL NaOH for 15 min at 15 psi (atmospheric pressure)/121ºC and a solid/solvent (NaOH) ratio of 1:10 (w/v). The residue was then washed, filtered as mentioned above, and dried at 60ºC for 4-hrs in a forced-air oven. The DA step was achieved by treating chitin under conditions of 15 psi/121ºC with 45 g/100 mL NaOH for 30 min and a solid/solvent ratio of 1:10 (w/v). The resulting chitosan was collected, washed as mentioned above and dried at 60 ºC for 4-hrs in a forced-air oven.

Deacetylation degree (D.d):

It was determined for the five chitosan samples by the potentiometric titration method described by Brous – signac, reported by Tolaimate *et al*., (2000).

Chitosan solution in a known excess of HCl was titrated with 0.1 M NaOH solutions, a curve with two inflexion points was obtained. The degree of deacetylation was determined through equation:

$$\% NH2 = 16.1 (V2 – V0) \times Mb/W.$$

Where V0 and V2 are the base volumes (ml) referred to first and second inflexion points respectively, Mb is the base molarity in g/mol, W is the original weight of the polymer in g.

The degree of deacetylation for prepared Chitosan was 85%.

Controlling *B. cinerea* using chitosan *in vitro*:

In this trail, the two types of previously mentioned chitosan were used with 3ml of each one. They were applied at different concentrations *i.e.*, 0.5, 1.0, 2.0 and 2.5%. The used chitosan solutions were dropped onto the surface of poured solid PDA medium in petri dish (9cm), and then the drops were spread well till the complete absorption into media. The plates were inoculated with the inoculum disk (4mm) of the tested fungal pathogen at the center of the dish.

Controlling *B. cinerea* using chitosan *in vivo*:

Both types of chitosan were applied by dipping and spraying at different concentrations *i.e.* 0.5, 1.0, 2.0 and 2.5 % on wounded and un-wounded strawberry fruits. In case of dipping method, the fruits were soaked individually in the targeted concentration for five minutes, then raised and leaved for air drying. Then, the fruits (wounded and unwounded) were inoculated at the surface with an equal disk (4mm) of the pathogen and left in foam plates then covered with stretch film till appearance of gray mold symptoms. The developed symptoms were daily investigated.

Controlling *B. cinerea* using *Trichoderma* spp. *in vitro:*

The antagonistic ability of tested three isolates of *Trichoderma* spp. was assessed against the pathogenic isolate *B. cinerea* that isolated from strawberry rotted fruits in dual culture according to the method described by Fokkema (1973). Three days old cultures of *Trichoderma harzianum, T. hamatum* and *T. viride* were used as sources of antagonistic inocula. An equal disc of each one of tested Trichoderma isolates (4mmØ) was placed at 20 mm far from the edge of PDA plates (9 cmØ). A disc of pathogen was placed 50 mm away from the biocontrol fungal disc. Cultures were incubated in the dark at 25ºC until the growth of the pathogen covered completely the check plates. The biological control agent inhibits the pathogen growth as a result of producing antagonistic metabolites, which decrease the rate of the pathogen growth.

The growth reduction percentage that pooled out was calculated using the following formula:

$$\% \text{ Growth reduction} = \frac{\text{Control - Tretment}}{\text{control}} \times 100$$

Controlling strawberry gray mold infection using *Trichoderma* spp. *in vivo:*

Three culture filtrates of tested Trichoderma isolates were assessed against the pathogenic isolate *B. cinerea* by two methods (dipping and spraying) on wounded and un-wounded strawberry fruits. In dipping method, the fruits were soaked individually in each filtrate for five minutes, then raised and left for air drying. The treated fruits (wounded and unwounded) were inoculated on surface with disk of the pathogen inoculum and left in foam plates then covered with stretch film till appearance of gray mold symptoms. The developed symptoms were daily investigated.

Controlling strawberry gray mold using fungicides *in vivo:*

Three different fungicides i.e., Switch 62.5% WG (Cyprodinil-Fludioxonil), Rovral 50% WP (Iprodione) and Topsin-M70% WP (Thiophanate-methyl) were used at three different concentrations *i.e.,* 100, 150 and 200 ppm based on their active ingredients for controlling the gray infection on wounded and un-wounded strawberry fruits in case of spraying application only. Additionally, using

results of the tested fungicidal treatments in in comparison with those of chitosan and Trichoderma treatments.

Disease assessment

Disease parameters were determined on rotted fruits according to the disease index rating which was made to determine the average diameter of the infected areas on fruit surface after seven days of inoculation. The following numerical rates were suggested to facilitate visual determination and to give a satisfactory comparison:

0 = No rot.
1 = Scattered small rot.
2 = Rots coalescing and including about 25-50 % fruit area.
3 = More than 50% of the fruit area was infected.

Readings were converted to disease index according to the equation suggested by (Townsend and Heuberger, 1943) as follows:

$$\text{Disease Severity \%} = \frac{\sum(n\times r1)+(n\times r2)+(n\times r3)}{3N\times 100}$$

Where (n) is the number of fruits in each numerical rate; r1, r2 and r 3 are ratings and (N) is the total number of inoculated fruits multiplied by the maximum numerical rate 3.

Also the percentage of infected fruits was estimated.

Statistical analysis:

The collected data were subjected to statistical analysis using the F-test and means were compared by the LSD at 0.05 levels of probability as described by Snedecor and Cochran (1967) using Costat Software (1985).

RESULTS AND DISCUSSION

Pathogenicity test:

Data in Table (1) showed that the most virulent isolate of *B. cinerea* from Shebin El-kom district according to the pathogenicity test. This virulent isollate was used to achieve this study.

Table 1. Pathogenicity test for three Botrytis cineria isolates on different cvs. of strawberry fruits under controlled laboratory conditions.

Botrytis cineria Isolates	D.S % on tested strawberry cvs. Sana	Fortuna	Florida	Festival	Mean
Wounded fruits					
Shebin El-kom	85.3	84.9	84.5	84.5	84.8
El-Bagour	79.5	81.1	78.9	80.2	79.9
El-Shohada	78.4	79.4	81.2	79.9	79.7
Un-wounded fruits					
Shebin El-kom	76.2	77.4	77.2	76.4	76.8
El-Bagour	74.1	73.8	74.3	73.6	73.9
El-Shohada	74.2	72.3	73.5	73.4	73.5

Controlling *B. cinerea* using chitosan *in vitro*:

In plant disease management, natural products i.e. chitosan were increased in usage as disease control agent. Chitosan as a naturally polysaccharide, having inhibitory activity against many plant pathogenic fungi (Rabea *et al*., 2003; Xu *et al*., 2007). This direct antifungal activity of Chitosan *in vitro* in other findings confirmed with our studies (Allan and Hadwiger, 1979; Ait Barka *et al*., 2004).

Furusaki *et al.*, 1996 reported that Chitosan is composed from three forms of reactive functional groups (an amino group, a primary hydroxyl group and secondary hydroxyl group) at the C-2, C-3, and C-6 positions of the glucosamine residue; respectively. A lot of useful materials had been provided in many fields of application as a result of the chemical modifications of these groups. (Kurita, 1986; Sugimoto *et al*., 1998). Chitosan is thought to have its antimicrobial effect due to the existence of (Ocarboxymethylated (O-CM)) and its exchange of the hydroxyl group in the C-6 position of Chitosan with the acetyl group, which enhances the protonation of the amine group in the C-2 position in the presence of the new carboxyl ion (Liu *et al*., 2001).

Data in Table (2) indicate that all tested chitosan concentrations were effective in inhibiting fungal growth of *B. cinerea* in all experimental trials in petri dishes. In this respect, the commercial chitosan (Ch.C.) was more effective in inhibiting the growth of *B. cinerea* than the synthesized one (Ch.S.). The highly effective concentration of the two tested types of chitosan *in vitro* was 2.5% while, the least effective concentration was 0.5% of both tested chitosan types. On the other hand, all tested concentrations of the two chitosan types were effective in inhibiting the growth of *B. cinerea* compared with control treatment. There was a clear gradually increase in in inhibiting the growth of *B. cinerea* in vitro for both types of chitosan with increasing the tested concentrations.

Table 2. Effect of two types of chitosan *in vitro* on growth of *B. cinerea* the causal organism of strawberry gray mold disease.

Chitosan	Concentration %	Linear Growth (mm) T1	T2	T3	T4	Mean
Ch.S.	0.5	55.2	54.3	54.6	56.1	55.05
	1	44.2	45.7	44.8	45.6	45.08
	2	29.7	28.8	28.7	28.8	29.00
	2.5	17.2	15.9	15.4	16.2	16.18
Ch.C.	0.5	50.7	50.2	49.4	49.7	50.00
	1	41.2	40.8	42.1	40.6	41.18
	2	20.4	23.1	20.5	22.4	21.60
	2.5	12.5	11.4	11.9	13.2	12.25
Control		90	90	90	90	90.00
LSD at 0.05%		Ch=0.46335		Con.=0.73262		Ch×Con.= 1.0360

***T= Trial Ch= Chitosan Con.= Concentration**

In this study the treatment with two types of Chitosan showed positive effect against *B .cinerea* radial growth in petri dishes. Also we showed that the growth reduction level is correlated with the concentration of Chitosan .Du *et al*., (1997) mentioned that Chitosan inhibited the growth of the *B .cinerea* significantly on artificial media .Siti *et al*., (2009) reported that after 3-day culture the pathogen colony diameter in P.D.A medium containing 0%, 0.05% and 0.20% Chitosan were 77.4 mm 65.4 mm and 25.6 mm respectively .El Ghaouth *et al.,* (1992) explained that 0.6% Chitosan inhibited radial growth of *B .cinerea* and *Rhizopus stolonifer* by 95.5% and 71.5%.

2. Controlling strawberry gray mold infection using chitosan *in vivo*:

Data in Table (3) show that both types of chitosan (Ch.C. and Ch.S.) with different concentrations which applied as dipping treatment decreased disease severity% on wounded and unwounded strawberry fruits which inoculated with *B. cinerea* when compared with control treatment (fruits treated with pathogenic fungi only). In this respect, increasing the concentration of chitosan decreased gradually the determined disease severity %. However, the tested concentrations of Ch.C. were generally more effective than those of Ch.S. in controlling strawberry gray mold infection. Also, no one of the four-tested strawberry cultivars exhibited clear resistant to gray mold infection more than others with the two types of chitosan at all tested

concentrations on wounded and un-wounded fruits. There were no significant differences between the four strawberry cultivars in disease index on the same level of each concentration of Chitosan types.

Table 3. Effect of chitosan as dipping application on gray mold infection caused by *B. cinerea* on wounded and un-wounded strawberry fruits of different cvs.

Chitosan	Concentration %	D.S % on tested strawberry cvs.			
		Sana	Fortuna	Florida	Festival
		Wounded fruits			
Ch.S.	0.5	51.8	52.6	51.2	50.8
	1	44.8	46.7	48.8	46.8
	2	31.4	32.1	32.5	31.9
	2.5	21.8	22.1	23.1	22.5
Ch.C.	0.5	45.4	46.7	44.8	46.1
	1	36.4	35.7	35.1	34.9
	2	24.8	25.4	25.7	26.1
	2.5	16.8	18.4	18.1	17.9
Conrtol		84.9	83.9	84.7	83.5
		Un-wounded fruits			
Ch.S.	0.5	39.9	41.2	38.5	38.2
	1	33.4	34.6	36.7	35.2
	2	20.4	19.8	20.6	18.9
	2.5	10.5	9.6	9.9	10
Ch.C.	0.5	33.5	35.2	33.4	33.8
	1	24.5	23.7	22.8	23.2
	2	12.7	13.1	13.4	14.1
	2.5	5.3	6.2	6.1	5.7
Conrtol		77.1	78.4	76.9	76.8

	LSD at 5% for wounded fruits			
	Sana	Fortuna	Florida	Festival
Verities	0.82665486966	0.54174032845	0.82456998647	0.70713452784
Concentration	1.3070561135	0.85656666914	1.30375962373	1.11807786006
V×C	1.8484564825	1.21136820057	1.84379454195	1.58120087348

	LSD at 5% for wounded fruits			
	Sana	Fortuna	Florida	Festival
Verities	0.90231784291	0.80929397205	0.63938255395	0.71439979935
Concentration	1.4266897785	1.27960612417	1.01095258332	1.12956526295
V×C	2.01764403405	1.80963633529	1.42970285425	1.59744651445

As for the sprayed strawberry fruits with the two types of chitosan, data in Table (4) indicate that the sprayed strawberry fruits with Ch.C type of chitosan were more effective in reducing the strawberry gray mold infection at all tested concentrations than those sprayed with Ch.S type of chitosan. The highest effective concentration in reducing the gray mold infection was 2.5% while, the least effective one was 0.5% with either of Ch.C or Ch.S types of chitosan on wounded and un-wounded strawberry fruits. The effect of 2.5% concentration of Ch.C or Ch.S types of chitosan was better on un-wounded fruits than wounded ones where the least DS% was recorded on fruits of cv. Sana followed by fruits of cvs. Festival, Florida and Fertona. In general, application with chitosan on strawberry fruits either by spraying or dipping was effective in decreasing DS% when compared with control of wounded or un-wounded fruits.

According to the bio-compatible and nontoxic properties of Chitosan (Wu *et al*., 2005), because of this chitosan occupy the place of fungicides in agriculture fields (Bautista-Baños *et al*., 2006). Chitosan activity against *B. cinerea* have been demonstrated in other findings (Ait Barka *et al*., 2004; Trotel-Aziz *et al*., 2006 and Aziz *et al*., 2006) the antifungal effect of chitosan had been verified on grape plantlets downy mildew (Aziz *et al*., 2006), Romanazzi *et al*., 2002, 2006 and 2007 have proven the effective of chitosan against botrytis bunch rot on grapes when applied as a postharvest spray or dipping. In our study both types of Chitosan; whether in dipping or spraying application on un-wounded fruits, showed a great activity against gray mold disease caused by *B. cineria*. Chitosan has greatly contributed to reducing the progression of the disease and its spread to fruits throughout the storage period, also kept the fruits in a fresh and vibrant form. This leads us to the other characteristics of Chitosan as a strong elicitor instead of an antimicrobial agent, In addition to the effect in plant disease control.

The induced plant defense response is greatly correlated with the enzymatic response; many findings demonstrate that Chitosan is external elicitors that improving host defense such as in tomato, Chitosan induced the production of phenolic and phytoalexine compounds; this explain is precedes the role of hydrolytic enzymes of *Fusarium oxysporium* f. sp. *radicislycopersici* (Benhamou and theriault, 1992). Other report showed the induced resistance of Chitosan against *Fusarium oxysporium* in susceptible tomato plants when applied as a seed, root coating or plant spraying, these treatments inhibit Fusarium growth to the external root tissues and can improve some defense reaction including structural barriers (Benhamou *et al*., 1998). On the contrary of our results Ben-shalom *et al*., 2003 reported that, the anti-fungal activity of chitosan was not enough in cucumber plants to inhibit the pathogen of gray mold disease.

In our study, on PDA medium the highly concentrations of Chitosan (2.5%) (Table 1) was the only concentration that inhibits fungal growth, while it was strongly inhibitory on fruits at the same concentrations (Table 2); because this reason various reactions were activated in strawberry fruits, this effect is likely to be referenced to the Chitosan-induced resistance to fungi

strongly results from the stimulation of the plant's natural defense metabolism.

Certainly there were differences between the synthesized *in vitro* Chitosan (Ch.S.) and the commercial Chitosan (Ch.C.) formulation, this could be arises because the preparation techniques. Indeed, dissolving the Chitosan in the acids, it is necessary to prepare the solutions more than one day in advance and to monitor and adjust the pH; in contrast, the commercial one can be prepared before the application by 1–2 h, by dissolving the powder in water directly. Moreover, the final commercial Chitosan solution with formulation has a lower viscosity than the Chitosan acetate, for this reason the application with commercial chitosan more easily.

Table 4. Effect of chitosan as spraying application on gray mold infection caused by *B. cinerea* on wounded and un-wounded strawberry fruits of different cvs.

Chitosan	Concentration %	D.S % on tested strawberry cvs.			
		Sana	Fortuna	Florida	Festival
		Wounded fruits			
Ch.S.	0.5	63.6	64.9	62.2	61.9
	1	57.1	58.3	60.4	58.9
	2	44.1	43.5	44.3	42.6
	2.5	34.2	33.3	33.6	33.7
Ch.C.	0.5	57.2	58.9	57.1	57.5
	1	48.2	47.4	46.5	46.9
	2	36.4	36.8	37.1	37.8
	2.5	29	29.9	29.8	29.4
Control		84.9	83.9	84.7	83.5
		Un-wounded fruits			
Ch.S.	0.5	45.6	47.1	44.4	45.1
	1	39.3	42.6	40.0	40.4
	2	26.1	26.5	25.4	24.8
	2.5	15.5	16.4	15.8	15.9
Ch.C.	0.5	39.4	41.1	39.3	39.7
	1	30.4	29.6	28.7	29.1
	2	18.6	19.9	19.3	20.0
	2.5	11.2	12.1	12.0	11.6
Control		77.1	78.4	76.9	76.8

	LSD at 5% for wounded fruits			
	Sana	Fortuna	Florida	Festival
Verities	0.77715613942	0.75709624431	0.76794143538	0.60051102987
Concentration	1.22879174907	1.19707426998	1.21422202271	0.94949130722
V×C	1.73777395687	1.69291866778	1.71716925224	1.34278348402

	LSD at 5% for wounded fruits			
	Sana	Fortuna	Florida	Festival
Verities	0.91885816938	0.52561347844	0.66511315357	0.73400859235
Concentration	1.45284233095	0.83106788038	1.05163623351	1.16056948698
V×C	2.05462932842	1.17530746769	1.48723822411	1.64129310856

3. Controlling *B. cinerea* using *Trichoderma* spp. *in vitro:*

Data in Table (5) indicate that *T. harzianum* isolate was the most effective one in reducing the growth of *B. cinerea* comparing with *T. hamatum* and *T. viride*. On the other and, the highest growth reduction% was recorded with *T. harzianum* with clear inhibition zone with appearance of over growthing on growth of *B. cinerea* followed by *T. hamatum* which exhibited wide inhibition zone comparing with *T. viride*. *T. harzianum* isolate was the only one exhibiting over growthing on growth of *B. cinerea* without appearance of inhibition zone.

Table 5. Effect of *Trichoderma* spp. on growth of *B. cinerea* the causal organism of strawberry gray mold *in vitro*.

Bioagents	*Botrytis cinerea*		Bio-interaction	
	Linear growth (mm)	Growth reduction %	Over growth (mm)	Inhibition zone (mm)
T. hamatum	20	73.77	—	4
T. harzianum	18	80.00	12	—
T. viride	23	74.44	—	1
LSD at 0.05%	lg=3.44500556847	gr=3.42080553082	og=1.40641763397	iz=1.08940641483

*** lg= Linear growth gr= Growth reduction og= Over growth iz= Inhibition zone**

Zimand *et al*., 1996 showed that the isolate *T. harzianum* T39 reduced the *B.cinerea* biomass by 20 to 50%, this reduction was due mainly to the effect on germ-tubes elongation and the germination rate was partially affected. The effect of the rate of *T. harzianum* T-39 on control efficacy varies with the host and disease system. In earlier studies the lower rate was superior for control *B.cinerea* the causal of tomato grey mould, similar to the findings in this study, but was inferior in controlling cucumber white and grey mould in commercial greenhouses (Elad, 2000).

4. Controlling strawberry gray mold infection using *Trichoderma* spp. *in vivo*:

Data in Table (6) show that dipping or spraying the wounded and un-wounded strawberry fruits in or with three sterilized culture filtrates of three Trichoderma isolates pre-inoculation with *B. cinerea* in vivo affected greatly the gray mold infection on the four-tested strawberry cvs. where the recorded DS% were lesser than those recorded with control treatment. In this respect, dipping treatment was more effective in reducing gray mold infection than spraying treatment with the four-tested strawberry cvs. with superiority of them on un-wounded

fruits than wounded ones. On the other hand, *T. harzianum* treatment was the most effective one followed by *T. hamatum* and *T. viride* respectively whether in case of dipping or spraying treatments with the four-tested strawberry cvs. Furthermore, the least DS% was recorded on treated strawberry fruits cv. Florida by dipping on un-wounded and wounded fruits respectively. Whereas, the highest DS% was recorded in case of sprayed fruits of cv. Fertona with culture filtrates of *T. viride*.

In addition, the culture filtrate of the antagonistic fungus, *T. harzianum* was found to be more efficiency than the other culture filtrates in decreasing the growth of *B.cinerea*, but with less level than Chitosan. Similar results were observed where *T.harzianum* decreased the growth of *Fusarium oxysporium*, *Verticillium albo-atrum* and *Fusarium culmorum* (Ibrahim, 1997 and Hemeida, 1992). Abdulrahman (2005) reported that the bioagent culture filtrate of *T.harzianum* effected on radial growth of *B.cinerea*, *Fusarium oxysporium* and *Rhizoctonia solani*, but this effect was less than the action of some plant extracts.

Table 6. Effect of Trichoderma culture filtrates as dipping or spraying applications on strawberry gray mold infection caused by *B. cinerea* on wounded and unwounded fruits.

Culture filtrate	Treatment	D.S % on tested strawberry cvs. Sana	Fortuna	Florida	Festival
		Wounded fruits			
T. hamatum		61.3	63.9	58.7	61.1
T. harzianum	dipping	54.9	54.4	53.4	54.4
T. viride		65.2	66.4	64.8	64.9
T. hamatum		70.4	71.1	68.7	73.9
T. harzianum	spraying	66.1	64.8	62.9	65.5
T. viride		70.9	72.9	68.6	71.2
Conrtol		84.9	83.9	84.7	83.5
		Un-wounded fruits			
T. hamatum		52.6	55.2	49.8	51.1
T. harzianum	dipping	44.2	45.1	42.7	45.7
T. viride		55.3	55.7	54.6	56.4
T. hamatum		60.4	61.1	59.4	66.2
T. harzianum	spraying	56.4	55.1	53.2	55.8
T. viride		61.2	63.2	58.9	61.5
Conrtol		77.1	78.4	76.9	76.8

	LSD at 5% for wounded fruits Sana	Fortuna	Florida	Festival
Culture filtrate	1.02159313568	0.84002370528	0.67713566481	0.9044171234
Treatment	1.4447508677	1.18797291672	0.95761444075	1.27903896195
C×T	2.04318627135	1.68004741055	1.35427132963	1.80883424679

	LSD at 5% for wounded fruits Sana	Fortuna	Florida	Festival
Culture filtrate	0.56626190059	0.48883292176	0.50627837675	0.55257103033
Treatment	0.80081525967	0.69131414769	0.71598574674	0.78145344527
C×T	1.13252380119	0.97766584352	1.0125567535	1.10514206067

- **Controlling strawberry gray mold infection using fungicides *in vivo*:**

Data in Table (7) show that the three tested fungicides with different concentrations as spraying treatment decreased clearly the determined DS% on wounded and unwounded strawberry fruits infected with *B. cinerea* comparing to control (inoculated fruits with *B. cinerea* only). Also, results indicate that increasing the concentration of each one of the tested fungicides decreased gray mold infection on treated fruits. The best concentration for use was 200 µg/L where the recorded DS% of Switch and Topsin-M fungicides was 0.0% on all wounded fruits of the four-tested strawberry cvs. As for un-wounded fruits, Switch at 150 and 200 µg/L was the best one where the recorded DS% was 0.0% on all treated fruits of the four-tested strawberry cvs. Meanwhile, Topsin-M and Rovral fungicides at 200 µg/L only came in the second rank where the recorded DS% was 0.0% on all un-wounded treated fruits of the four-tested strawberry cvs.

Generally, spraying strawberry fruits with fungicides pre-inoculation with *B. cinerea* was more effective in controlling the gray mold infection on un-wounded fruits than wounded ones. There were slight differences between the four strawberry cultivars in the determined DS% at the same level of each concentration (using of fungicides in these study to find alternative control treatment instead of toxicity effects of chemicals).

Table 7. Effect of three fungicides as spraying treatment on strawberry gray mold infection caused by *B. cinerea* on wounded and unwounded fruits of four cvs.

Fungicides	Concentration (µg/L)	D.S % on tested strawberry cvs. Sana	Fortuna	Florida	Festival
		Wounded fruits			
Switch 62.5 WG	100	8.6	7.4	7.9	8.1
	150	1.2	1.1	2.1	1.6
	200	0	0	0	0
Rovral 50 WP	100	12.3	10.5	9.8	11.2
	150	5.5	5.4	5.8	5.4
	200	1.2	1.5	1.3	1.4
Topsin M70 WP	100	7.8	8.4	8.2	8
	150	1.2	2.5	3.4	3.5
	200	0	0	0	0
Conrtol		84.9	83.9	84.7	83.5
		Un-wounded fruits			
Switch 62.5 WG	100	3.2	3.1	3.5	2.9
	150	0	0	0	0
	200	0	0	0	0
Rovral 50 WP	100	5.4	4.6	5.7	3.9
	150	1.1	1.6	1.4	1.1
	200	0	0	0	0
Topsin M70 WP	100	3.5	4.1	3.9	3.7
	150	1.1	0.9	1.2	1.1
	200	0	0	0	0

In this study, we tried to shed light on the alternatives to fungicides, as long as they give very close results in resistance to many plant diseases. The efforts to find naturally- occurring products having antimicrobial and activities in controlling pathogens actions in hosts like chitosan and its derivative has been getting more attention in these studies. We can outline, that the chitosan can be used on plant hosts to reduce or control disease levels and stopping the development of the pathogen action and minimizing the yield losses in quality and quantity. However, chitosan needs to understand the mechanism and action of disease resistance inducer.

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إمكانية إستخدام الكيتوزان والترايكودرما كمضادات للفطريات فى مقاومة البوترايتس سيناريا المسبب لمرض العفن الرمادى فى الفراولة

حسام محمد عوض

قسم النبات الزراعى – كلية الزراعة – جامعة المنوفية – مصر

يعتبر مرض العفن الرمادى على الفراولة والمتسبب عن الفطر بوترايتس سيناريا واحدا من الأمراض الخطيرة التى تهاجم ثمار الفراولة خاصة فى مرحلة مابعد الحصاد. تهدف هذه الدراسة لمقارنة تأثير نوعين من الكيتوزان وكذلك راشح فطريات التضاد الحيوى وبعض المبيدات الفطرية المتخصصة والموصى بها وذلك فى مقاومة مرض العفن الرمادى فى الفراولة. تم إختبار تأثير الكيتوزان التجارى والتخليقى على نمو الفطر بوترايتس سيناريا معمليا على بيئة البطاطس والدكستروز فى أطباق بترى وكذا على ثمار اربعة أصناف من الفراولة منزرعة تجاريا فى مصر؛ وقد دلت النتائج أن إستخدام نوعى الكيتوزان فى عملية المقاومة للفطر قد قلل شدة الإصابة بالمرض معنويا خاصة فى الثمار الغير مجروحة (السليمة) والتى عوملت بطريقة الغمر فى محلول الكيتوزان. أما راشح مزارع فطريات التضاد الحيوى فقد ثبت أن المعاملة بالراشح على الثمار السليمة أو المجروحة قد ثبطت الإصابة بالفطر معنويا مقارنة بالثمار الغير معاملة (الكنترول) ، أما تجربة المقاومة بالمبيدات الفطرية فقد ثبت تفوقها فى مقاومة حدوث المرض مقارنة بالمعاملة بنوعى الكيتوزان المختبرين الكيميائى والتخليقى بالإضافة الى راشح مزارع فطريات التضاد الحيوى.