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PREVENTIVE EFFECT OF CHITIN AND DIFFERENT BIOAGENTS AGAINST *BOTRYTIS CINEREA* IN STRAWBERRY FRUITS

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ABSTRACT: ***Botrytis cinerea was the most frequent fungus isolated from strawberry fruits either in the field or in the market. Pathogenicity test of the fungus on two varieties of strawberry (Sweetsharly and Kamarosa) indicated that the percentage of infection increased as number of spores increased and Sweetsharly variety is more susceptible than kamarosa under the same number of spores.***

Under laboratory conditions, the inhibitory effect of chitin and different bioagents (Bacillus subtilis, plant guard, Trichoderma harzianum) on the linear growth of B. cinerea was observed. Chitin showed the highest antagonistic effect and highly significant reduction in mycelial growth of B. cinerea (100% reduction) whereas plant guard (commercial product of Trichoderma harzianum) gave the lowest effect (65% reduction) in mycelial growth.

Application the same preparations in sweetsharly and kamarosa varieties in trays during storage under laboratory condition revealed that treatment with chitin gave 40% and 30% of infection whereas Bacillus subtilis gave 45% and 40% and Trichoderma harzianum isolate gave 50% and 35% of infection while plant guard gave 80% and 63% as compared with control (100% of infection) for Sweetsharly and Kamarosa after ten days from treatment, respectively.

Field applications of chitin and bioagent two weeks pr-harvest showed that the percentage of infection depends on different variety and place. Chitin was more effective in controlling gray mold on fruits than bioagents. It decreases percentage of infection to 12% and 18% as compared with control (40% and 55%). Bacillus subtilis gave 15.5% and 26% of infection while, Trichoderma harzianum isolate and plant guard gave the lowest effect against fruit rot disease of both strawberry varieties. The results indicated that chitin could be used to control gray mold on strawberries fruit and decrease the dependent on fungicides used.

Key words: ***Strawberry, gray mold, Botrytis cinerea, Chitin, bioagents.***

INTRODUCTION

Strawberry becomes one of the most important vegetable crops in Egypt for the local consumption and exportation. Strawberry gray mold caused by *Botrytis cinerea* is one of the most destructive pathogen of strawberry fruits.

These fruits are eaten fresh therefore there are restrictions for using fungicides, hence satisfactory control measures as well as fruit protection against such diseases are essential (Morsy *et al*., 1999). Safety material or induced resistances in plants are needed. Chitin as the safe material has antifungal activity against many plant pathogens (El-Mougy *et al*., 2006).

Bharathi *et al.* (2004) found that the mixed bioformulation *Psedomonas fluorescence* + *Bacillus subtilis* + neem + chitin was found to be the best for reducing the chilli fruit rot incidence besides increasing the plant growth and yield parameters under both greenhouse and field conditions. Also Wichitra *et al*. (2008) revealed that *B. subtilis* 155 and its antibiotics are considered to be potent biological control agents to suppress growth of *Penicillium digitatum* in the postharvest protection of citrus. Yacoub (2007) found that the invert emulsion formulation of *Trichoderma harzianum* protects strawberry fruits from infection by *Botrytis cinerea* and is promising treatment to prolong the postharvest shelf life of the fresh fruit.

MATERIALS AND METHODS

1- Isolation and identification of causal organisms:

1.1 Isolation of causal organisms:

Diseased fruits of strawberries showing various types of rots were collected from field and markets (Ismailia and Kalubia Governorates). About one hundred fruits per replicate were rinsed several times in sterilized distilled water. The sterilization was applied using ethyl alcohol of 70% concentration for 2 minutes. Surface sterilized fruits were then washed several times with sterilized distilled water and dried between two sterilized filter papers then cut into small pieces. The cut pieces were placed in Petri dishes containing potato dextrose agar medium (PDA) and incubated 5-7 days at 25^{o}C. The isolated fungi were purified by single spore technique then kept in refrigerator on PDA medium.

1.2. Identification:

Identification of the isolated fungi was carried out according to cultural properties, morphological and microscopically characteristics described by Gilman (1957), Barnett and Hunter (1972) and Singh (1982). Identification was confirmed in the Department of Taxonomy, Plant Pathology Institute, ARC, Egypt.

2- Effect of spore concentrations of *Botrytis cinerea* on reaction of strawberry fruits:

The effect of different concentrations of spore suspension of *B. cinerea* on two different varieties of strawberry namely Sweetsharley and Kamarosa were tested. Using four replicates for each variety consisting of 25 fruits per each replicate in trays. Spore suspension was prepared at different

concentrations *i.e.*, 0.0, $3x10^3$ $6x10^3$ and $9x10^3$ spores/ml using Haemosytometer. Spores suspension were spraying on the fruits using atomizer, and allowed to dry at room temperature for 2 h. All treatments were incubated at 20°C. The percentage of infection was recorded after 3 days of spraying.

3- Effect of chitin and different biopreparations on the linear growth of *Botrytis cinerea*:

Chitin and different biopreparations were subjected to test under laboratory conditions to evaluate their antagonistic effect against *B. cinerea*. Chitin and bioagents obtained from Central Lab. of Organic Agricultural Research Center, Giza-Egypt. Chitin solution prepared by developing four grams of chitin fine powder dissolved in 100ml 0.25N HCl and undissolve particles were removed by centrifuged for 10 min at 3000 rpm at 20 °C. Solution were then neutralize by 1N NaOH (pH 5.6) (El-Neshawy *et al*., 2004). Chitin solution was added to conical flasks containing gliotoxin fermented (GF) medium developed by Brian and Hemming (1945) to obtain the final concentration 4 g/l and mixed gently and then dispensed in sterilized Petri plates 9.0 cm diameter each contains 15 ml of GF medium. Plates were inoculated at the center with equal disks (6-mm-diam.) of *Botrytis cinerea* obtained from the periphery of 7 days old culture. Nutrient glucose agar (NGA) medium prepared by Dowson (1957) were used to detect the effect of *Bacillus subtilis* against growth of *B. cinerea* and GF medium to determined effect of *T. harzianum* and plant guard against *B. cinerea*.

The pathogenic fungus was inoculated at one side and the opposite side was inoculated with loop full of:

1) **Antagonistic *Bacillus subtilis* grown on liquid NG medium for 48 h as first group**
2) ***T. harzianum* grown on liquid GF medium for 9 days as second group.**
3) **Plant guard (3 ml/l) as commercial product as third group**

Other plates inoculated only with the pathogenic fungus served as control. Five plates were used for each treatment. All plates were then incubated at 25°C. When mycelial growth covers all the medium surface in control, the plates treatments were examined and the percentage of reduction in mycelia growth of *B. cinerea* was calculated as follows:

$X = [G_2 / G_1 \times 100] - 100$

Where X: % of reduction

G_1: growth of pathogenic fungus in control plates and

G_2: Growth of pathogenic fungus in treated plates.

4- Effect of chitin and different biopreparations on the controlling gray rot of strawberry fruits in trays during storage under laboratory conditions:

Sweetsharley and Kamarosa varieties of strawberry fruits were placed on trays. Fruits in trays were sprayed with different biological preparations using one hundred fruits for each treatment. Chitin solution prepared as mentioned above and 0.1ml Tween 80 was added to a solution to improve wettability.

***Tricholerma harzianum* was grown on GF medium under complete darkness for 9 days to stimulate toxin production (Abd -El-Moity and Shatla, 1981). The spore suspension of *Trichoderma harzianum* was prepared by adjusting number in the fungal suspension to be 30 x 10^6spore/ml using sterilized water. *Bacillus subtilis* was grown on NG broth for 4-8 hours. The bacterial suspension was adjusted to be containing 30 x 10^6 CFU/ml. Plant guard a commercial product was also used at the rate of 3ml/l water. Treated fruits were then sprayed with spore suspension 9x10^3spores/ml of *B. Cinerea*.**

Fruits were sprayed only with *B. cinerea* serve as control treatment. All trays were incubated at room temperature (20±2^{o}C). The trays were examined after 7and 10 days and percentages of infection were determined. .

5- Effect of chitin and different biopreparations on the controlling fruits rot of strawberry under field conditions:

This study was carried out in two locations in Kalubia and Ismalia governorates in one faddan area. Chitin and different biopreparations (Chitin, *Bacillus subtilis*, plant guard and *Trichoderma harzianum*) were used in this experiment. The used preparations were as follows:

Chitin was prepared at the rat of 4 g/l water. *Trichoderma harzianum* was grown on GFM as mentioned above. While *B. subtilis* was grown on NG broth for 48 h. Both *B. subtilis* and *T. harzianum* were adjusted to contain 30 x 10^6 CFU/ml, in addition to use plant guard (3ml/l) as commercial product. Different biopreparations were diluted to 1: 100 (reparation: water V/V) before plant treatment and two strawberry varieties (Sweetsharley and Kamarosa) were used. Preharvest application of chitin and biopreparations was carried out on the two varieties of strawberry before two weeks of harvest underfield condition. Plants received water only acted as control treatment. Different treatments were examined and percentages of infection were determined.

Statistical analysis:

The data were statistically analyzed using MSTAT-C computer program V.2. (1988).

RESULTS AND DISCUSSION

Research was designed to obtain a safe method to keep environment free of pollution and to protect human health from harmful effects of the fungicides. Accordingly, the efficiency of the chitin was compared with the different bioagents. Data in Table (1) show that different pathogenic fungi were isolated from strawberry rotted fruits which collected from field and market. These pathogenic fungi were identified as *Botrytis cinerea, Rhizopus stolonifer*, *Penicillium* spp. *Rhizoctonia solani* and *Asperagillus* spp., whereas *R. solani* and *Asperagillus* spp. were not isolated from rotted fruits which were collected form market. *Botrytis cinerea* was the most frequently isolated fungus both from filled 52% and 60% and market 75% and 77% in Ismailia and Kalubia, respectively. The range of all other pathogenic fungi was isolated between 0 .0% to 25% so, *Botrytis cinerea* fungus was selected for further study.

Table (1): Frequency of fungi associated with strawberry fruit rot in the field as well as in market

Fungi	% frequency			
	In the field		In the market	
	Ismailia	Kalubia	Ismailia	Kalubia
Asperagillus spp.	5.0	3.0	0.0	0.0
Botrytis cinerea	52.0	60.0	75.0	77.0
Penicillium spp.	10.0	7.0	5.0	4.0
Rhizoctonia solani	8.0	10.0	0.0	0.0
Rhizopus stolanifer	25.0	20.0	20.0	19.0

The pathogenic capabilities and different concentration of spores of *Botrytis cinerea* to inoculate both two strawberry varieties, Sweetsharley and Kamarosa were tested. The percentages of infection of the two tested varieties for each number of spores were presented in Table (2). The data show that all the concentrations of spores (3×10^3, 6×10^3 and 9×10^3) were pathogenic to strawberry fruits. It was also noticed that as the increased number of spores the percentage of infection increased. Inoculation with $3x10^3$ spores gave 55and 45% infection in both varieties Sweetsharley and Kamarosa, respectively, whereas inoculation with $9x10^3$ spores caused 80 and 50 % infection in the same varieties. On the other hand the results indicated that Sweetsharley variety is more susceptible to infect with different concentration of spores than Kamarosa.

Table (2): Effect of different concentrations of *Botrytis cinerea* spore on reaction of strawberry fruits.

Concentration (spores/ml)	% of infection	
	Sweetsharly	Kamarosa
0.0	5.0	0.0
$3x10^3$	55.0	45.0
$6x10^3$	65.0	48.0
$9x10^3$	80.0	50.0
L.S.D at 5%	2.86	2.82

The effect of chitin and the antagonistic microorganism (fungi and bacteria) on the linear growth of *B. cinerea* were studied. The data in Table (3) indicated that chitin showed the highest significant effect in reducing the mycelial growth of the pathogenic fungus and gave 100% of reduction, this may be attributed to its fungistatic effect. (Amborabe *et al.*, 2004). *T. harzianum* gave 95% of reduction and *B. subtilis* gave 85% on the mycelial growth of *B. cinerea*. On the other hand plant guard (as a commercial product) had the lowest effect on mycelial growth and gave 65% reduction as compared with the control treatment. The high potentiality in antagonistic effect may be due to the ability of *T. harzianum* to act through different mechanisms including mycoparastism (Abd -El-Moity and Shatla, 1981), production of antifungal substances (Sanz *et al.*, 2002) and its destructive enzymes *i.e.*, chitenases (Padares *et al.*, 1992 and Bolar *et al.*, 2000). *B. subtilis* followed *T. harzianum* this may be due that *B. subtilis* acts through the production of a number of antibiotics (subtilicin, bacteriocin) (Ferreira *et al.*, 1991 and Aska and Shoda, 1996).

Table (3): Effect of different biopreparutions on the linear growth of *Botrytis cinerea*

Treatments	Reduction % of linear growth of *B. cinerea*
Chitin	100.0
Bacillus subtilis	85.0
Plant guard	65.0
Trichoderma harzianum	95.0
Control	0.0
L.S.D. at 5%	3.15

Effect of chitin and bioagent on gray mold of strawberry fruits under laboratory conditions:

The data obtained in Table 4 indicate that the highest percentage of infection in both varieties (Sweetsharly and Kamarosa) was observed after ten days from treatment under laboratory condition. Data also clear that chitin was the highest effective in controlling gray rot of strawberry fruits. This protection could result from either fungistatic effect of chitin on *B. cinerea* or the elicitation of defense reaction of strawberry fruits (Amborabe *et al.*, 2004). The observed reduction in strawberry fruit rot in present study might be attributed to indirect effect of chitin treatments and its elicit or defense response in plants (Kuchitsu *et al.,* 1993). He reported that chitin fragments appear to elicit host responses through rabid and transient membrane depolarization. El Mougy *et al.* (2006) found that chitin treatments increased tomato yield under field conditions and found the highly reduction of tomato root rot incidence was obtained with 6g/ Kg soil.

***Trichoderma harzianum* and *Bacillus subtilis* occupied the second rank after chitin. *Bacillus subtilis* possibly acts through the production of a number of antibiotics (subtilisin, bacteriocin) (Ferreira *et al.*, 1991 and Maisa *et al.*, 2006) the control effect of *T. harzianum* could be explained as direct mycoparasitsim or through enzyme and/or antifungal substances which induce resistance (Abd- El -Moity and Shatla 1981; Padares *et al.*, 1992 and Sanz *et al*., 2002). Plant guard gave the least effect as compared with control.**

Table (4): Effect of chitin and different biopreparations on the controlling gray rot of strawberry fruits in trays under laboratory conditions

Treatment	Varieties			
	Sweetsharly		Kamarosa	
	% of infection after		% of infection after	
	7 days	10 days	7 days	10 days
Chitin	30	40	25	30
Bacillus subtilis	40	45	36	40
Plant guard	70	80	60	63
Trichoderma harzianum	40	50	34	35
Control	80	100	50	100
L.S.D at 5%	3.35	3.67	2.70	2.68

Effect of chitin and bioagents on fruit rot of strawberry fruits under field conditions

The results in Table (5) show that strawberry plants previously treated with different preparation led to significant control of disease incidence. The data also indicate that chitin was the most effective in controlling fruit rot, this may be due to the inducing effect of chitin on the plant cell and prevent pathogens to invade the plant in addition to fungistatic effect of chitin against *B. cinerea* (Amborabe *et al.,* 2004). *Bacillus subtilis* was effective than *T. harzianum* in controlling gray mold of the fruit, where *B. subtilis* showed a considerable effect in controlling fruit rot disease. This might be due to this bacteria produced more antibiotics which act as inhibitors to pathogenic fungi (Ferreira *et al.*, 1991, Asaka and Shoda, 1996). In addition, *B. subtilis* also grows very fast and occupies the count of infections and consumes all available nutrients and thus prevent pathogens to invade the plant (Wolk and Sorkar, 1994). Plant guard showed the lowest effectiveness treatment against gray mold in strawberry fruits. The obtained data were confirmed with Abdel Rahman *et al*., 2007.

Table (5): Effect of chitin and different biopreparations on the controlling fruit rot of strawberry under field conditions

Treatment	Varieties			
	Sweetsharley % of infection		Kamarosa % of infection	
	Kalubia	Ismailia	Kalubia	Ismailia
Chitin	18	17	15	12
Bacillus subtilis	26	26	16	15.5
Plant guard	35	32	22	21.5
Trichoderma harzianum	30	31	20	20
Control	55	52	40	43
L.S.D at 5%	3.17	2.49	2.30	2.64

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التأثير الواقى للشيتين والمركبات الحيوية المختلفة ضد بوتريتس سيناريا على ثمار الفراولة

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الملخص العربي

فطر بوتريتس سيناريا هو الفطر الأكثر تكراراً في الحقل أو بالأسواق من بين الفطريات المسببة لعفن ثمار الفراولة. وبدراسة القدرة المرضية لفطر بوتريتس سيناريا على صنفي سويت شارلي والكماروزا وجدا أنه بزيادة عدد الجراثيم تزيد نسبة الإصابة وكان الصنف سويت شارلي أكثر إستجابة عن الصنف كماروزا عند نفس عدد الجراثيم. ووجد أن الشيتين و المستحضرات الحيوية (تريكوديرما هارزيانم وباسيلس ساتلس والبلانت جارد) لها تأثير مثبط للنمو الميسليومى للفطر بوتريتس سيناريا تحت الظروف المعملية وتبين أيضاً أن الشيتين له قدرة تضادية وإختزال معنوي للنمو الميسليومى للفطر بنسبة ١٠٠٪ بينما البلانت جارد (مركب تجاري من تريكودرما هارزيانم) أعطى أقل تأثير لإختزال النمو الميسيلومى بنسبة ٦٥٪. وبإختبار نفس المركبات الحيوية على صنفي الفراولة سويت شارلى والكماروزا أثناء التخزين تحت الظروف المعملية وجد أن أعلى نسبة إصابة بعد ١٠ أيام من المعاملة حيث أن الشيتين أعطى نسبة أصابة ٤٠٪ و٣٠٪ بينما البلانت جارد أعطى نسبة إصابة ٨٠٪ و ٦٣٪ ولكن الباسيلس ساتلس أعطى نسبة إصابة٤٥٪ و٤٠٪ على التوالى مقارنة بالكنترول نسبة إصابة١٠٠٪ .

تحت الظروف الحقلية تم استخدام الشيتين والمركبات الحيوية وتبين أن الشيتين أعطى أحسن نتائج ونسبة الإصابة كانت بين ١٢ و ١٨ ٪ وكان ذلك يعتمد على الصنف والمكان مقارنة بالكنترول وأن نسبة الإصابة تتراوح ما بين ٤٠ و ٥٥٪. الباسيلس ساتلس كانت نسبة الأصابة ١٥.٥ و ٢٦٪ بينما تريكوديرما هارزيانم و البلانت جارد كانا أقل تأثير على عفن ثمار الفروالة.

ويتضح من ذلك قد يمكن استخدام الشيتين لمقاومة العفن الرمادى على ثمار الفراولة لتقليل الأعتماد على استخدام المبيدات الفطرية.