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BIOLOGICAL CONTROL OF PRE AND POSTHARVEST DISEASES OF TOMATO AND GRAPE FRUITS.

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ABSTRACT

From seventy five yeast isolates, only eleven were selected as most effective isolates against the main causal fungal rot of tomato fruits, i.e. *Alternaria alternata*. These isolates which are considered biocontrol strains reduced *A. alternata* (1×10^6 conidia/ml) infection by more than 90% . Five isolates from those eleven isolates were found to be the most effective ones since they inhibited the infection of the causal organism (1×10^5 and 1×10^6 conidia/ml) after seven days of incubation at 21 ± 0.5^oC. The yeast isolate Ap638 (*Arthroacus* sp.) completely inhibited the rot infection causd by *A. alternata* (1×10^5 conidia/ml) when this isolate was used at high concentration (1×10^9 cfu/ml) after 18 days of incubation at $13 \pm l^oC$.

In the test of different biocontrol agents (40 isolates) against the causal organism of grapes *Penicillium crustosum* and *Botrytis cinerea,* one bacterial isolate (*Bacillus* sp.) and about ten yeast isolates were found to be more effective in reducing the percentage of infection between 50-90%. Pre-and post harvest application of the above mentioned selected isolates were used to control and reduce the percentage of decay, weight loss, shatter, total wastage and frequency of the isolated fungi of Flam and Thompson seedless grapes.

INTRODUCTION

Limitations of disease control in plants by conventional methods (e.g. chemical control or breeding for resistance) have become apparent in recent years (Janisiewicz, 1991). Many postharvest pathogens have become resistant to fungicides in current use (Ogawa *et al*., 1977; Rosenberger, 1980; Eckert and Wild, 1983 and Wild, 1994). Most fruits and vegetables storage diseases are caused by about thirty pathogen species while only a limited number of chemicals are registered for their control. Janisiewicz (1998) reported that there are few adequate fungicides replacements when pathogens develop resistance. Meanwhile, recent health concerns over pesticide contamination of food and the presence of chemical residues in the food chain are took place. The National Academy of Science reported that the fungicides pose more a carcinogenic risk than insecticides and herbicides (Anonymous, 1987). All these factors together have generated an urgent need for the developoment of safer alternative technologies. In this regard, the biological control of post harvest diseases is considered one of the most promising alternatives (Wilson and Pusey, 1985). The control of post harvest diseases using epiphytic antagonists was successful by pre-and postharvest treatments of various crops; grapes (Mclaughlin *et al*., 1992), beans and tomatoes (Elad *et al*., 1994).

Biocontrol of tomato fruit diseases has received little attention although potential for success might be even greater than other fruits. Chalutz *et al*., (1991) stated that the yeast antagonist *Pichia guilliermondii* was effective in reducing incidence of *Botrytis cinerea , Rhizopus stolonifer* and *Alternaria alternata* decay of tomato fruits by 90%, they also reported that this reduction was affected by concentration of both the yeast cells and the spore suspension of the pathogen. The same authors found that nutrient competition between the yeast and the pathogen is involved in the mode of action of *P. guilliermondii* in reducing gray mold of tomato fruits. Mari *et al.,* (1996) reported that gray mold was reduced in fresh market tomatoes treated with the antagonist (*Bacillus amyloliquefaciens*) and artificially inoculated with *B. cinerea* and stored at 20ºC for at least seven days.

Biocontrol of gray mold disease of grapes resulting from *B. cinerea* has been successfully studied by many investigators using different fungal and yeast isolates as bioagents. Dubos *et al.,* (1978) and Dubos (1984) found that the colonization of floral parts of grape plants by *Trichoderma harzianum* significantly reduced subsequent colonization by *B. cinerea.* Three additional sprays with *T. harzianum* ; one at the bunching stage of grapes, one at the onset of ripening and another 3 weeks preharvest, significantly reduced incidence of grey mold. However, Ben-Arie *et al*., (1991) found that the yeast *Pichia guilliermondii* and *Hauseniaspora uvarum* were effective in the control of *Rhizopus stolonifer and Botrytis cinerea* rots and of other postharvest diseases of grapes. They decided that the dipping injured or non-injured berries in a 48 hours culture of the antagonists protected the berries against subsequent inoculation with the pathogenes.

Lima *et al.* (1995) reported that the yeast isolates of *Aureobasidium pullulans* (yest-like fungi), *Candida vanderwaltii* and *C. oleophila* were effective agaisnt grape *B. cinerea* infection as the infection was reduced by more than 85% in comparison with control.

The aim of this work is screening different bio-agents isolated from surface of tomato, apple and grape fruits and testing them against the most important pre-and posthervest pathogens of these fruits.

MATERIALS AND METHODS

Isolation of bio-control agents:

Organisms, which tested for their antagonistic activity, were isolated from tomato, apple and grape fruits in the field throughout the growing season near harvest. Each fruit, along with its stem, was submerged in a 1000ml beaker containing sterile phosphate buffer (0.005M,pH6.5) and 0.05% Tween 20. Beakers containing the fruits were shaken on a rotary shaker at 120 rpm for 10 min. Serial 0.1 ml dilution's were plated on various media, mainly on nutrient yeast dextrose agar (NYDA) and malt yeast glucose peptone agar (MYGP) which are a universal media for yeast. Plates were incubated at 21 ± 0.5ºC for 48 h. After colonies appeared, isolation was made at random based on the visual characteristics of the colonies. Purification of isolated microorganisms were made by triple restreaking and

isolates were transferred to NYDA slants and stored under phosphate buffer at 4°C until use.

I- Tomatoes Primary Screening:

Primary screening was made to select organisms which were capable of reducing disease development, i.e. inhibition of rot expansion by more than 90%. To conduct such massive screening, each isolate from the selected organisms was tested only on four wounded fruits. Fruits were surface sterilized with 1000 ppm sodium hypochlorite for two minutes, rinsed with running water then air dried. Two wounds per fruit were used (wound was done by the removal of a tissue block 3 x 3 x3 mm). The culture of microorganisms were activated on fresh slants and after 24 hours were transferred to 250 ml Erlenmeyer flasks containing 50 ml nutrient yeast dexterous broth (NYDB) medium. The inoculated flasks were placed on a rotary shaker at 120 rpm for 48 hours.

Twenty five µl of each microbial suspension was applied to each wound. This was followed by applying 25µl of the pathogen spore suspension (1 x 10^6 conidia/ml of *A. alternata*) to each wound within 30-60 minutes. Incubation was made in individual plastic box for each fruit at 21 ± 0.5°C and high humidity (>84% RH). Fruits were evaluated for rot development after 7 days. Lesion diameter was measured = A; infected area mm^2 was calculated as $(A/2)^2$ x 3.14 = B; and percent of infected area as compare with control was calculated as B for treatment/B for control x 100.

II. Tomatoes Secondary Screening:

A: Phase one:

Secondary screening (phase one) was used to determine the effectiveness and the antagonistic potential of bioagents selected in primary screening. Two concentrations from each yeast isolate were used. Two fruits were used for each replicate and three replicates for each concentration. Tomato fruits were treated as described previously. Twenty-five µl of washed cells from each concentration was applied to each wound. This was followed by applying 25 µl of the *A. alternata* suspension (1 x 10^5 or 1 x 10^6 conidia/ml), as the main pathogen for tomato, within 1 h. Incubation was made in individual plastic box for each fruit at 21 ± 0.5°C and at high humidity (more than 84% RH). The fruits were evaluated for rot development after 7 days, lesion diameter and percent of infected area as compare with control were measured as described previously.

B: Phase two:

Secondary screening (phase two) was used to determine the effectiveness and potential antagonists selected by secondary screening (phase one). Four concentrations of each yeast isolate mainly 1 x 10^9, 2 x10^8, 1 x 10^8 and 6.6 x 10^7 were used. These desired concentrations were obtained by adjusting the suspension after cell yeast concentrations were determined with a hemacytometer and confirmation were made by plate dilution methods on the basis of colony forming unit (CFU/ml). Three fruits

for each replicate and three replicates for each concentration were used. Tomato fruits were treated by *A. alternata* (1 x 10^5 conidia/ml) as described previously. The fruits were evaluated for rot development after 18 days incubation in individual plastic box for each fruit at 13 ± 1°C and high humidity (more than 84% RH).

III. Grapes screening:

The antagonists were incubated in 50 ml nutrient yeast dextrose broth (NYDB) in 250 ml Erlenmeyer flasks on a rotary shaker (120 rpm) at 28 ± 2°C for 48 h . Freshly harvested grapes of Thompson seedless cultivar as individual berries, which, had been removed from the stems by pulling, thereby causing a wound. One hour after the berries were dipped momentarily in the antagonistic preparation and air dried, they were inoculated by spraying with pathogen spore suspension (1 x 10^5 conidia/ml of *Penicillium crustosum* and *Botrytis cinerea*) and air-dried. Three replicates for each treatment and 20 berries for each replicate were used. Each replicate was placed in individual plastic box. Incubation was carried at 21 ± 0.5°C and high humidity (>84% RH). Decay incidence was determined by counting the number of infected berries.

IV- Application of biocontrol agent on grapes:

A- Preharvest application:

Different isolates of antagonists yeast were used to test their effectiveness in inhibiting rot development in grapes, the following procedure was used: The yeast isolates were incubated in 50 ml NYDB in 250 ml Erlenmeyer flask on a rotary shaker (120 rpm) at 28 ± 2°C for 48 h. Then, yeast-NYDB culture (5 x 10^7 CFU/ml) were sprayed on clusters of Thompson seedless grapes in the field, 4 days before harvest time, and air dried . Three replicates for each isolate, each replicate consisted of 3 clusters were used. After harvest the grapes clusters were incubated at 0°C and high humidity (> 84 % RH). Percent of decay, frequency of different isolated fungi, fruit weight loss and percent of shattering were recorded every 10 days up to 30 days. Total wastage was calculated after 30 days of storage.

B - Post harvest application:

The effectiveness of antagonistic bacterial isolates in inhibiting rot development in grapes, was determined as follows: The bacteria were incubated in 100 ml NYDB in 250 ml Erlenmeyer flask on a rotary shaker (120 rpm) at 28 ± 2°C for 48h. Freshly harvested grapes of the Flame seedless as whole clusters (4 cartons, each carton consisted of 9-12 cluster) were sprayed by bacteria-NYDB culture (1 x 10^9 CFU/ml) and air dried, then incubated at 0°C and > 84% RH. Percent of decay, frequency of different isolated fungi, weight loss and percent of shattering were recorded every 10 days up to 50 days. The total wastage was calculated after 50 days of storage.

Determinations:

*** Decay percent of grapes was determined as follows:**

$$\frac{\text{Weight of decayed berries}}{\text{Initial weight of grape bunches}} \times 100$$

Decay % = x 100

*** Weight loss (WL%):**

$$WL\% = \frac{\text{Initial weight - weight at sampling date}}{\text{Initial weight}} \times 100$$

*** Shattering percent:**

This value was determined as follows:

$$\text{Shatter \%} = \frac{\text{weight of shattered berries}}{\text{Initial weight of the grape bunches}} \times 100$$

*** Wastage percent:**

This was demonstrated as follows:

Wastage % = Decay % + Weight loss% + Shatter % , (Wassel, 1985)

*** Identification of the biocontrol isolates:**

The identification of the promising isolates was made by Dr: A.E. Abdel-Hafez, Department of Microbiology, Faculty of Agriculture, Ain Shams University.

RESULTS AND DISCUSSION

Tomatoes Primary Screening:

Seventy five yeast isolates which were isolated from surfaces of apple, grape and tomato fruits were tested to biocontrol rot development in tomato caused by *Alternaria alternata*. The infected area and the percent of infected area as compared with control were calculated. Data in Table (1) show that from all tested yeast isolates there were only 23 isolates which had antagonistic effectiveness. Six isolates of them completely inhibited growth of *Alternaria alternata* (1 $x10^6$ conidia/ml) infected tomato fruits. These isolates are: TR7, TR4, AP638, GF 1, GF12 and GF15. Mean while there were 17 isolates that inhibited the infection by more than 90%. Chalutz *et al*., (1991) reported that the yeast antagonist *Pichia giulliermondii* was effective in reducing incidence of *Botrytis cinerea, Rhizopus stolonifer* and *A. alternata* decay of tomato fruits. They also decided that a water suspension of the yeast cells applied to wounds of the surface of the fruit prior to inoculation with spore suspension of the pathogens reduced disease by 90%.

To determine whether the mode of action of potential yeast is antibiosis, the supernatant of each yeast culture under study was tested to control rot lesion development and excluded isolates which their supernatant protected the wounds of tomato from infection by *A. alternata.*

The most effective biocontrol yeasts (11 isolates) were selected to test their different dilutions against the fungus *A. alternata* (1 $x10^5$,1 x 10^6 conidia/ml). Data shown in table (2) cleared that the most effective isolates

against this fungus were: Ap 638, TG4 , TG3, TG2 and TR6 which reduced the percentage of infection to 0.0% at some different concentrations of bio-control agents using lower fungal spore suspension(1 x 10^5 conidia /ml).

The above five yeast isolates which were found to be the most effective to reduce the tomato *A. alternata* fungal infection percent, were identified. These isolates are related to four yeast genera, i.e. *Debaromyces* sp. (TG2, TR6), *Torulospora* sp. (TG3), *Bretanomyces* sp. (TG4) and *Arthroacus* sp. (Ap638).

The effect of different concentrations of these five yeast isolates against 1 x 10^5 couidia/ ml of *A. alternata*, was shown in table (3). The data indicate that all yeast isolates reduced the percentage of rot as compared with control but at different levels. It was clear from the data that the isolate Ap 638 was the most effective isolate at the high concentration used, i.e. 1 x 10^9 cfu/ml, as the rot percent was 0.0%. Mean while the rot percent were 0.05, 0.09, 15.45 and 16.22% for the isolates TR6 , TG2, TG3 and TG4 , respectively at the same concentration of antagonist. The data also indicate that the efficacy of yeast isolates for bio-controlling the rot development decreased by decreasing the concentration of the tested yeast. Chalutz *et al*., (1991) stated that the efficacy of the yeast antagonist *Pichia guilliermondii* in reducing the gray mold disease of tomato was affected by the concentration of both the yeast cells and the fungal *B. cinerea* spore suspension. They added that dipping tomato fruits in yeast cell suspension, right after harvest, did not reduce the decay development on the fruits. Finally, they concluded that nutrient competition between the yeast and the pathogen is involved in the mode of action of *P. guilliermondii* in reducing gray mold of tomato fruits.

Table (1):Effect of selected isolates challenged with *Alternaria alternata* (1 x 10^6 cfu) on tomato fruits after 7 days of incubation at 21 ± 0.5°C.

Isolate Number	Diameter of Infected area in (mm)	Infected area $(mm)^2$	% of infected area compared with control	Isolate Number	Diameter of Infected area in (mm)	Infected area $(mm)^2$	% of infected area compared with control
Control	24.4	467.4	100.0	Control	24.6	475.1	100.0
Ap 611	2.9	6.6	1.4	Ap 621	7.4	43.0	9.1
TG 2	3.0	7.1	1.5	Ap 638	0.0	0.0	0.0
TR 7	0.0	0.0	0.0	TG 7	5.6	24.6	5.2
TG 4	6.8	36.3	7.8	TG 3	3.9	11.9	2.5
Control	28.5	637.6	100.0	Control	31.4	774.0	100.0
GF 11	7.3	41.8	6.6	Ap 619	4.0	12.3	1.6
GF 1	0.0	0.0	0.0	Ap 617	2.5	4.9	0.6
Ap636	8.0	50.2	7.9	TY 6	9.9	76.9	9.9
GF 4	8.6	58.0	9.1	TR 4	0.0	0.0	0.0
GF 15	0.0	0.0	0.0	TR 6	2.0	3.1	0.4
GF 10	9.0	63.5	9.9	TY 3	1.6	2.0	0.3
GF 12		0.0		0.0		0.0	
GF 5		8.8		60.8		9.5	
GF 8		7.6		45.3		7.1	

Table (2): Effect of most effective biocontrol isolates challenged with *Alternaria alternata* (1 x 10^5 and 1 x 10^6 cfu) on tomato fruits after 7 days of incubation of 21± 0.5°C.

Concentr-ations	Diameter of Infected area in (mm)	Infected area $(mm)^2$	% of infected area compared with control	Concentr-ations	Diameter of Infected area in (mm)	Infected area $(mm)^2$	% of infected area compared with control
TR7				**TG3**			
Cont.1 x 10^6	27.6	598.0	100.0	Cont. 1 x 10^6	29.8	697.1	100.0
1 x 10^8	4.8	18.1	3.0	1.2x10^9	10.0	78.5	11.3
3.3x10^7	12.8	128.6	21.5	3.9x10^8	13.3	138.9	19.9
Cont. 1 x 10^5	21.0	346.2	100.0	Cont. 1 x 10^5	24.1	455.9	100.0
1 x 10^8	2.5	4.9	1.4	1 x 10^9	0.0	0.0	0.0
1 x10^7	13.5	143.1	41.3	1 x10^8	14.8	171.9	37.7
AP 617				**TY 6**			
Cont. 1 x 10^6	24.9	486.7	100.0	Cont.1 x 10^6	23.8	444.7	100.0
3 x 10^9	12.7	126.6	26.0	1.2x10^8	13.3	138.9	31.2
1 x10^9	15.9	198.6	40.8	3.9x10^8	16.0	201.0	45.2
Cont. 1 x 10^5	22.3	390.4	100.0	Cont. 1 x 10^5	20.3	323.5	100.0
3 x 10^9	10.7	89.9	23.0	1 x 10^8	0.0	0.0	0.0
3 x10^8	21.4	359.5	92.1	1 x10^7	18.0	254.3	78.6
TY 3				**GF 12**			
Cont. 1 x 10^6	27.0	572.3	100.0	Cont. 1 x 10^6	24.9	486.7	100.0
1 x 10^9	16.8	221.6	38.7	7 x10^8	13.1	134.7	27.7
3.3 x10^8	21.0	346.2	60.5	2.8x10^8	16.5	213.7	43.9
Cont. 1 x 10^5	20.5	329.9	100.0	Cont. 1 x 10^5	22.3	390.4	100.0
1 x 10^9	0.0	0.0	0.0	7 x 10^8	11.2	98.5	25.2
1 x10^8	9.0	63.6	19.3	7 x10^7	20.6	3331.	85.3
GF 1				**TG 2**			
Cont. 1 x 10^6	29.8	697.1	100.0	Cont. 1 x 10^6	29.3	673.9	100.0
1.2x 10^9	4.8	18.1	2.6	1.4 x10^9	3.6	10.2	1.5
3.9x10^8	15.3	183.8	26.4	4.6x10^9	4.3	14.5	2.2
Cont. 1 x 10^9	24.1	455.9	100.0	Cont. 1x 10^5	21.5	362.9	100.0
1.2x 10^9	0.0	0.0	0.0	1.4x 10^9	0.0	0.0	0.0
1.2x10^8	17.9	251.5	55.2	1.4x10^8	4.1	13.2	3.6
TR 6				**TG 4**			
Cont. 1 x 10^6	29.3	663.9	100.0	Cont. 1 x 10^6	29.8	697.1	100.0
6 x 10^8	0.0	0.0	0.0	1.4 x10^9	0.0	0.0	0.0
1.9x10^8	0.0	0.0	0.0	4.6x10^8	5.2	21.2	3.0
Cont. 1 x 10^5	21.5	362.9	100.0	Cont. 1x 10^5	24.1	455.9	100.0
6 x 10^8	0.0	0.0	0.0	1.4x 10^9	0.0	0.0	0.0
6 x10^8	8.7	0.0	0.0	1.4x10^8	8.3	54.1	11.9
AP 638							
Cont. 1 x 10^6	29.8	697.1	100.0				
1.1 x 10^9	0.0	0.0	0.0				
3.8x10^8	0.0	0.0	0.0				
Cont. 1 x 10^5	24.1	455.9	100.0				
1.1 x 10^9	0.0	0.0	0.0				
1.1 x10^8	0.0	0.0	0.0				

Table (3): Infected area (mm^2) and percentge of rot, after tomato wounds were inoculated with different concentrations of washed cells of five yeast isolates, challenged with *Alternaria alternata* 1 x10^5 conidia/ml and stored for 18 days at 13°C ± 1.

Isolates	TG2		TH3		TH4		TR6		Ap638	
Concentrations (CFU/ml)	Area (mm^2)	% of Rot	Area (mm^2)	% of Rot	Area (mm^2)	% of Rot	Area (mm^2)	% of Rot	Area (mm^2)	% of Rot
1 x 10^9	0.601	0.09	102.61	15.45	107.67	16.22	0.306	0.05	0.0	0.00
2 x 10^8	19.15	2.88	185.49	27.94	142.91	21.52	83.3	12.55	0.11	0.02
1 x 10^8	67.84	10.22	265.89	40.05	218.85	32.96	134.40	20.24	55.47	8.35
6.6 x 10^7	201.87	30.40	317.07	47.76	312.99	47.14	240.04	36.15	218.85	32.96
Control (1x10^5)	663.94	100.00	663.94	100.00	663.94	100.00	663.94	100.00	663.94	100.00

(TG2, TR6) ⟶ ***Debaromyces* sp** **(TG3)** ⟶ ***Torulospora* sp.**

(TG4) ⟶ ***Bretanomyces* sp.** **(AP638)** ⟶ ***Arthroacus* sp.**

The effectiveness of 22 bacterial isolates and 18 yeast isolates (isolated from grapes surface) to reduce the grape infection percent as compared with control, was studied. The data in table (4) show that only one bacterial isolate (TC 100) was most effective against *Penicillium crustosum* and *Botrytis cinerea* which infected grape berries. This bacterial isolate inhibited the growth of the above pathogenic fungi to 10 and 13.3% respectively in comparison with control. This isolate was identified and was found to be *Bacillus sp.*

Table (5): Effect of pre-harvest application of bio-control agents on the percentage of decay of Thompson seedless grapes during storage at 0°C.

Storage Periods (Days)	Biocontrol agents										
	Cont.	GF 1	GF 2	GF 7	GF 8	GF 10	GF 11	GF 12	GF 13	GF 14	GF 15
10	1.1	0.5	0.0	0.1	1.9	0.1	0.2	0.5	1.1	0.6	1.2
20	1.9	0.7	0.2	0.3	3.2	0.2	0.3	0.8	1.8	0.9	1.8
30	3.5	1.6	0.5	0.7	4.2	0.5	0.4	2.7	2.7	2.5	2.6
Total	6.5	2.8	0.7	1.1	9.3	0.8	0.9	4.0	5.6	4.0	5.6

Table (6): Effect of pre-harvest application of bio-control agents on the frequency % of different isolated fungi from Thompson seedless grapes during storage at 0°C.

Bio-control agents	Storage period (days)	Isolated Fungi			
		Alternaria alternata	*Botroytis cinerea*	*Cladosporium herbarum*	*Penicellium crustosum*
Control	10	14.1	61.8	9.5	14.5
	20	9.7	60.1	7.3	22.9
	30	10.9	63.4	4.0	21.7
GF2	10	0.0	0.0	0.0	0.0
	20	39.0	0.0	39.0	22.0
	30	47.5	0.0	38.0	14.5
GF7	10	100	0.0	0.0	0.0
	20	100	0.0	0.0	0.0
	30	100	0.0	0.0	0.0
GF10	10	79.0	0.0	21.0	0.0
	20	87.5	0.0	12.5	0.0
	30	100	0.0	0.0	0.0
GF11	10	89.5	0.0	10.5	0.0
	20	100	0.0	0.0	0.0
	30	100	0.0	0.0	0.0

Table (7): Effect of pre-harvest application of bio-control agents on the weight loss % , shatter % and total wastage % of Thompson seedless grapes during storage at 0ºC.

Bio-control Periods (Days)	Control	Bio-control Agents			
		GF2	GF7	GF10	GF11
		Weight loss % (WL %)			
10	1.90	0.35	0.46	0.57	0.58
20	2.10	0.50	0.55	0.69	0.76
30	3.30	0.79	0.91	0.94	0.98
		Shatter % (Sh %)			
10	1.90	0.46	0.50	0.52	0.55
20	2.60	0.53	0.56	0.58	0.62
30	4.90	0.58	0.61	0.63	0.70
Total	9.40	1.57	1.67	1.73	1.87
		Total Decay (D%)			
	6.50	0.70	1.10	0.80	0.90
		Total Wastage (WL% + Sh% + D%)			
	19.20	3.06	3.68	3.47	3.75

Table (8): Effect of post-harvest application of bacterial bio-control agent (TC 100) on the percentage of decay of Flam seedless grapes during storage at 0ºC.

Storage Periods (days)	Percentage of decay	
	Control	Bio-control-agent
10	2.2	1.2
20	3.3	1.3
30	3.3	1.8
40	3.4	2.3
50	4.8	2.9
Total	17.0	9.5

Table (9): Effect of post-harvest application of bio-control agent (TC100) on the frequency % of different isolated fungi from Flam seedless grapes during storage at 0ºC

Storage period (days)						
	A	B	C	P	S	Others
		Control				
10	36.6	9.5	23.1	17.3	4.1	9.4
20	37.6	10.8	25.9	13.1	3.8	8.8
30	48.8	10.2	27.3	7.5	2.8	3.4
40	48.8	12.2	27.1	7.8	2.0	2.0
50	51.4	11.2	26.8	10.1	0.5	0.0
Mean	44.6	10.8	26.0	11.2	2.6	4.7
		Bio-control agent (TC100)				
10	72.5	6.9	12.7	7.9	0.0	0.0
20	72.9	6.8	11.9	8.4	0.0	0.0
30	73.3	6.3	11.0	9.0	0.0	0.4
40	74.5	6.2	10.7	8.5	0.0	0.1
50	75.7	6.4	9.9	8.0	0.0	0.0
Mean	73.8	6.5	11.2	8.4	0.0	0.1

A = *A .alternata* ,B= *B .cinerea*, C= *Cladosporium herbarum*
P= *P .crustosum* , S= *Stemphylium herbarum*.

Table (10): Effect of post-harvest application of bio-control agent (TC100) on the weight loss % shatter % and total wastage % of Flam seedless grapes during storage at 0°C.

Storage period (days)	Control	Bio-control agent
	Weight loss % (WL%)	
10	1.7	0.94
20	2.6	1.03
30	3.1	1.23
40	3.5	1.34
50	3.9	1.55
	Shatter % (Sh %)	
10	3.3	0.9
20	5.1	1.02
30	6.8	1.30
40	7.4	1.42
50	8.6	1.51
Total	31.2	6.15
	Total Decay % (D%)	
50	17.0	9.50
	Total Wastage (WL% + Sh% + D%)	
50	52.1	17.20

Data in table (4) also show that from the tested 18 yeast isolates there were 4 isolates caused more than 80% protection to the grapes fruits against *P. crustosum* and *B. cinerea*. These isolates were GF2, GF7, GF10 and GF11, whereas the infection percent was 10, 16.7, 16.7 and 15%, respectively by *P. crustosum* and was 8.3, 13.3, 11.7 and 13.3 respectively by *B. cinerea*. The data in table (4), also cleared that there were six yeast isolates caused more than 50% protection to grape fruits from infection by *P. crustosum* and *B. cinerea*, these isolates were GF1, GF8, GF12,GF13, GF14 and GF15. Similar results were shown by lima *et al*., (1995) who isolated more than 200 yeast (including yeast-like fungi) from fresh or stored fruits (table grapes, kiwifruit and strawberry) to test their activity against *B. cinerea* Pers. They found that the isolates *Aureobasidium pullulans*, *Candida vanderwaltii* and *C. oleophila* were most effective against the pathogen and reducing gray mold infection by more than 85% in comparison to the control. These isolates were also effective in controlling *Rhizopus stolonifer* Erhenb and *Apergillus niger* Van. Tiegh.

The aforementioned 10 bioagent yeast isolates which were found to be the most biocontrol isolates to reduce the fungal infection percent of grape fruits were used (as preharvest treatment) to test their efficiency for reducing the decay percent of grape fruits (Thompson seedless). The data presented in table (5) show that all the tested isolates were effective to reduce the decay % in comparison with control. But from these isolates, four isolates were more active than others they were GF2, GF7, GF10 and GF11 as the total decay percent with them were: 0.7, 1.1, 0.8 and 0.9% , respectively compared with 6.5% for control. They were identified to be *Phaffia sp*. (GF7), *Saccharomyses sp*. (GF10, GF11) and *Rhodotorula sp*.(GF2) Ben-Arie *et al*., (1991). found that the yeast *Pichia guilliermondii* was effective in

reducing decay grapes when applied as a preharvest spray , 3 days before harvest.

For preharvest application, the effect of abovementioned 4 yeast isolates, i.e. GF2, GF7, GF10 and GF11 on the frequency percentage of isolated fungi from grape fruits was presented in table (6). The data indicate that the isolated fungi from control samples were *Alternaria alternata, Botrytis cinerea*, *Cladosporium herbarum* and *Penicllium crustosum* with different frequency percent during 30 days of storage. The data also showed that the four bioagents completely controlled *B. cinerea*. From the data it was also clear that the bioagents GF7, GF10, and GF11 showed highly promising effect for controlling *B. cinerea, C.herbarum* and *P. crustosum* during storage till 30 days at 0^{o}C. The antagonistic activity of yeast and yeast-like fungi against *Botrytis cinerea* and other causal fungal rots of grapes was recorded by many investigators (Ben-Arie *et al*., 1991 and Lima *et al*., 1995, 1996, 1997).

The data in table (7) indicate that the percent of weight loss (WL%) shatter (Sh%) , decay (D%) and subsequently total wastage (WL% + Sh% + D%) was affected by preharvest application of yeast biocontrol agents (GF2, GF7, GF10 and GF11) as the total wastage was 3.06%, 3.68%, 3.47% and 3.75% for GF2 , GF7, GF10 and GF11, respectively compared with 19.20% for control. This finding was confirmed by Lima *et al*., (1997).

From above our results it is worth to mention that only one bacterial isolate (TC 100) protected grape berries from infection by *P. crustosum* and *B. cinerea* by 90% and 86.7% respectively. This isolate was used for the following postharvest application tests. Data in table (8) indicate that the decay percent of Flam seedless grape has less values with the biocontrol agent compared with control, as the total decay decreased from 17.0% for control to 9.5% with the biocontrol agent after 50 days of storage at 0^{o}C.

The effect of (TC100) on the frequency percent of postharvest isolated fungi was presented in table (9). This bacterial isolate decreased the frequency percent of all isolated fungi except *A. alternata*. This may be due to that the competition between the isolated fungi was only suitable for *A. alternata* even in the presence of the biocontrol agent.

Data in table (10) cleared the effect of bacterial isolate (TC100) on the weight loss %, shatter% and total wastage % (WL% + Sh% + D%) , as these values were 3.9% , 31.2% and 52.1% for control treatment compared with 1.55% , 6.15% and 17.20% with the biocontrol agent TC100 after 50 days of storage.

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المقاومة الحيوية لأمراض ما قبل و ما بعد الحصاد فى ثمار الطماطم والعنب

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**** قسم الميكروبيولوجى- المركز القومى لبحوث وتكنولوجيا الإشعاع- القاهرة**

أجرى فى هذا البحث اختبار عدد 75 عزلة خميرة معزولة من ثمار مختلفة من حيث قدرتها على تثبيط المسبب الرئيسي لعفن ثمار الطماطم (التروناريا الترناتا) وتوصلت النتائج إلى وجود 11 عزلة منها، كان لها كفاءة عالية فى تثبيط هذا المسبب الرئيسي عند استخدامه بتركيز 1 x 10^6 خلية/مل. وعند استخدام هذه الإحدى عشر عزلة فى اختبار قدرتها على تثبيط المسبب المرضى عند استخدام تركزين منه (1 x10^6، 1x10^5 خلية/مل) وذلك بعد 7 أيام من التحضين على درجة 21°م ± 0.5 أظهرت النتائج وجود عزلة خميرة (Ap638 والتى عرفت على أنها من أصناف الأرثرواكاس) كان لها قدره تثبيطية تامة على الفطر ألترناريا الترناتا عند استخدامها بتركيز عالى (1 x10^9 خلية/مل) إذ أدت هذه العزلة إلى حماية تامة للثمار المجروحة والمعدية بهذا الفطر لمدة 18 يوم على درجة حرارة 13°م ±1.

أجرى أيضا اختبار 40 عزلة ميكروبية مختلفة من حيث قدرتها على تثبيط المسببات الرئيسية لأعفان ثمار العنب (بوتريتس سيناريا وبنسيليوم كرستوزم بتركيز 1 x10^5 خلية/مل) وأظهرت النتائج وجود

عزلة بكتيرية واحدة من نوع بسيلس و10 عزلات من الخمائر لها قدرة على تثبيط الإصابة بنسبة تتراوح بين 50-90 %. أوضحت النتائج أيضا أن استخدام هذه العزلات سواء ما قبل أو ما بعد الحصاد فى ثمار الطماطم والعنب إلى تقليل نسبة التالف والفقد فى الوزن ونسبة التفريط فى العنب وكذلك الخسائر الكلية ونسبة وجود الفطريات.

Table (4): Effect of different bacterial and yeast isolates on the grape infection (*P. crustosum* and *B. cinerea*) percent.

	Bacterial isolates																						
	Control	GF21	GF22	GF 23	GF 25	GF 26	GF 28	GF 29	GF 32	GF 34	GF36	GF37	GF38	GF39	GF40	GF46	GF100	TC100	TC3	TC23	OC 3	BG 4	BG32
P. crustosum	100	63.3	50.0	73.3	80.0	53.3	70.0	63.3	60.0	83.3	61.7	71.7	53.3	55.0	40.0	46.7	35.0	10.0	73.3	36.7	36.7	85.1	53.3
B.cinerea	100	43.3	70.0	61.7	70.0	55.0	61.7	80.0	70.0	90.0	35.0	36.7	36.7	35.0	36.7	40.0	33.3	13.3	56.7	31.7	40.0	53.3	40
	Yeast isolates																						
	Control	GF1	GF2	GF3	GF4	GF5	GF6	GF7	GF8	GF9	GF10	GF11	GF12	GF13	GF14	GF15	GF16	GF17	GF18				
P. crustosum	100	30.0	10.0	61.7	53.3	55.5	61.7	16.7	48.3	56.7	16.7	15.0	33.3	38.3	31.7	40.0	60.0	61.7	71.6				
B.cinerea	100	35.0	8.3	55.0	66.7	68.3	58.3	13.3	46.7	66.7	11.7	13.3	41.7	41.7	38.3	43.3	58.3	63.3	65.0				