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APPLIED APPROACH FOR PRESERVATION AND PROLONGED STORAGE PERIODS OF THOMPOSN SEEDLESS GRAPEVINES

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ABSTRACT: *The aim of the present study is to determine the losses from postharvest wastage of Thompson seedless grape. Possibilities to control these wastages by safe methods such as fumigation by vaporus acetic acid, and chitosan coated. Moreover, screening new bio-agents isolated from surface of fruits and testing them in cold storage against the most important postharvest decay of grape was also investigated.*

The main causal organisms of postharvest fruit rots of Thompson grape were Botrytis cinerea and Pencillium expensum during storage periods in two seasons.

Fumigation treatments with acetic acid vapour 30 µl/L. reduced the total wastage to 6.11 and 7.45% in two seasons respectively, as compared with 31.57 and 35.70% for control after 30 days of storage at 0°C. It could be suggested that acetic acid vapours might be safely used commercially as a new approach for prolongate of storage periods of grapes.

Coated Thompson seedless grape with chitosan after 30 days of storage at 0°C, the most effective concentration was 3%, reduced percentage of total wastage to 4.85 and 6.08% in the two seasons respectively, as compared with uncoated fruits. Chitosan could be used as a substitute of natural product to protect, preserve and prolongate of storage period of grapes clusters against postharvest decay, weight loss, fruit shatter and total wastage.

Three different isolates of yeast postharvest application to concentration (5×10^7 CFU/ml) reduced the total wastage of Thompson grape. The most effective isolated Rhodotorula sp. reduced the total wastage as 13.40 and 14.17% in the two seasons, compared with 31.57 and 35.70% for control.

Key words: *Grape, Postharvest, Cold storage, vapour acetic acid, Chitosan coated, Biocontrol agent, Total wastage.*

INTRODUCTION

Grapes (*Vitis vinifera*, L.) is the largest fruit crop in the world which approximately produced 58.466 million tons (FAO, 1998). However, table grapes are among the most important fruits in Egypt. Grape is considered to be the second largest fruit crop by area and value of production (NARP, 1998).

The approximate total harvested acreage for grapes is 164,000 feddan and the production 985600 tons according to the latest statistics of Ministry of Agriculture (2002).

Vine grapes are among the most suitable fruit crops for sandy soil (newly reclaimed areas) as well as for old lands. Egyptian economic progress is in great need to increase the agricultural export especially of grape, which has had a great progress to productivity in the last year. Grapes are exported to Arab countries and some European countries, especially at the beginning of the season, when the European grapes are not yet available in their markets.

Deterioration of table grapes during harvesting to consuming occurs at any stage of product handling. Loss in Thompson seedless reached 35.3% (NARP, 1998). The highest percentage of losses in Thompson seedless were at the wholesale and retail level and was more equally distributed throughout the marketing channel. In 1992, a total of 108780 tons of Thompson seedless did not reach the consumer (pre-and post harvest loss) (NARP, 1998).

Recently a great attention has been made up by many investigators all over the world concerning the growing need to develop alternative approaches for controlling postharvest decay.

Acetic acid is a universal metabolic intermediary and occurs in plants and animals. It was commonly used by food manufactures as antimicrobial preservative or acidulants in a veriety of food products (Davidson and Juneja, 1990) and safe to environment. Acetic acid vapours were extremely effective for killing spores of postharvest fungi which cause decay to various fruit. Fumigation with acetic acid prevented postharvest decay of apple, grapes, kiwifruit, pear and tomato inoculated with *Botrytis cinerea* and apple, orange and pears inoculated with *Penicillium* spp. (Sholberg and Gaunce, 1996). Acetic acid fumigation is a viable alternative to sodium hypochlorite dips for sterilizing fruit surfaces (Sholberg *et. al.*, 1996). There are several advantages to using acetic acid fumigation to control postharvest fruit decay: it is a natural compound found throughout the biosphere, pousing little or no residual hazard at the low levels required to kill fungal spores; it does not require rigorous registration procedures; it is inexpensive and can be in airtight storage rooms without handling the product (Sholberg *et. al.*, 1998).

Chitosan, which is a by-product in sea food industries, has been shown to have fungicidial activities against several fungi (Du *et. al.*, 1997). It is a safe material as indicated by toxicological studies (Hirano *et. al.*, 1990). Coating fruits with chitosan decreased postharvest decay of tomato, strawberries and grapes resulting from fungal infection (El-Gaouth *et. al.*, 1992 a and b). Du *et. al.*, (1997) reported that chitosan can be a potent elicitor of plant defense reactions, and has been widely used in medicine, agricultural production, and food industry (Benhamou *et. al.*, 1998).

As well as, recent health concerns over pesticide contamination of food public concerns over of chemical residues in the food chain, especially after

1986 National Academy of Sciences report (Anonymous, 1987a) on pesticides residues which indicated that fungicides pose more of a carcinogenic risk than insecticidies and herbicides. All those factors together have generated an urgent need for the development of safer alternative technologies.

In this regard, biocontrol agents of postharvest decay emerges as one of the most promising alternatives. Promising postharvest biocontrol agents have been identified, and significant efforts are being made to develop them for commercial use.

Different approaches to the microbial control of postharvest decay of fruits have been tried in recent years.

MATERIALS AND METHODS

I.Isolation and identification of the causal decay:

Grape berries of Thompson seedless were stored at 0°C with 4 replicates for each treatment, each replicate consisted of 4kg ± 250g and examined during storage at 10 days intervals up to 30 days. The berries which showed rotten symptoms, were used for isolating the causal organisms of the grape rots according to Waller (1981) and identified according to Barnett & Hunter (1987).

Identification test:

The growing fungi were examined microscopically. Either hyphal tips or single spores were carefully transferred to slopes of PDA medium. Pure culture of each isolate on PDA slants and kept at 5°C for further experiments.

Identification studies were made on 7-10 days old culture, according to (Barnett & Hunter, 1987) for genus.

Pathogenicity test:

Grapes were harvested at commercial maturity, apparently healthy mature clusters, were used for testing pathogenicity of isolated fungi. Berries sprayed with spore suspension of the tested fungi containing (1×10^6 conidia/ml). Berries were air dried and incubated at 25 ± 1°C under high humidity enclosed plastic container containing 5 ml sterile water. Percent of infected berries was measured after 7-10 days of incubation.

II. Physical properties during storage:

1- Percentage of decayed:

This analysis was carried out at 10 days intervals up to 30 days during storage. Grape berries showed any symptoms of decay were counted and the % of decayed was calculated by weight as follows:

$$\text{Decay\%} = \frac{\text{weight of decayed barriers}}{\text{initial weight of grapes bunches}} \times 100$$

2- Weight loss percentage:

The initial weight of grapes were recorded at zero-time. Weight loss was calculated by weighing the same grapes bunches every 10 days during storage, using the following formula:

$$\text{weight loss\%} = \frac{\text{initial weight - weight of sampling date}}{\text{initial weight of the bunches (gm)}} \times 100$$

3- Shattering percentage:

The value was determined as follow:

$$\text{Shatter\%} = \frac{\text{weight of shattered barriers}}{\text{initial weight of grapes bunches}} \times 100$$

4- Wastage percentage:

This value was demonstrated as follow :

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

III. Chemical properties during storage:

1- Total Soluble Solids:

TSS was determined by using an abbe' refractometer.

2- Total Acid Content:

Total acid content was determined by using a standard solution of sodium hydroxide (0.1 N) and phenolphthaline as an indicator. Treatments were expressed as percentage of anhydrous tartaric acid. (1 ml of N/0.1 alkali = 0.0075 gm of anhydrous tartaric acid).

3- Reducing Sugars:

The sugars were extracted from the juice according to Murphy (1958).

Reducing sugars were determined calorimetrically (Spectronic 21D) using phenol, sulfuric acid method according to Dubois *et. al.*, (1956). The contents were pointed out from a standard curves of glucose as gm/100 ml of fresh juice.

IV. Post harvest treatments during storage:

1- Fumigation with acetic acid:

Fresh bunches of Thompson seedless were fumigated with vapour acetic acid 10, 20 and 30 µl/L. (v/v) in air in closed glass container with continuous air circulation for 30 min. Fumigated bunches were air dried for 2 hour, and baked in 40×30×10 cm carton boxes and directly stored at 0°C. Each replicate was represented by 5-6 bunches (about 2Kg ± 250g) and four replicates were used for each treatments comparison with untreated grapes. (RH more than 84%).

2- Coating with chitosan:

Fresh bunches of Thompson seedless grapes apparently free of physical damage and diseases, were used. Bunches were dipped in 1,2 or 3% chitosan solutions. Control bunches were dipped in sterilized water. Tested

bunches were air dried for 2 hour and baked in carton boxes and directly stored at 0°C. Each treatment included 4 replicates, and 5-6 bunches (about 2Kg) were used for each replicate.

Chitosan used in this investigation was purchased from Sigma chemicals.

3- Bio-agents treatment:

Different isolates of yeast i.e. *Phaffia* sp., *Saccharomyces* sp. and *Rhodotorula* sp., were used as follow: the yeast isolates were incubated in 50ml nutrient yeast dextrose broth (NYDB) in 250 ml Erlenmeyer flask on a rotary shaker (120 rpm) at 28 ± 2°C for 48h. Then yeast-NYDB culture (with 5 × 10^7CFU/ml) were sprayed on clusters of Thompson grapes, and air dried. Three replicates of each isolate, each replicate consisted of 3 cluster were used, and stored at 0°C.

Department of Microbiology, Faculty of Agriculture, Ain Shams University, kindly identified the promising isolates.

Percent of decay, fruit weight loss, percent of shattering and total wastage recorded every 10 days up to 30 days of storage.

RESULTS

I- Frequency and Identification of isolated fungi from decayed grape berries during storage:

a- Frequency of isolated fungi:

Thompson seedless grape harvested at commercial stage were stored at 0°C and examined during storage at 10 days intervals up to 30 days. Berries, which showed rot symptoms, were subjected to isolating and culturing the associated fungi. Frequency of different isolated fungi during storage periods were presented in Table (1).

Date in Table (1), show that *Botrytis* (42.8, 43.3%)and *Penicillium* (39.0, 41.8%), were the most frequent isolated fungi from decayed grape berries during storage for 30 days at 0°C in two seasons respectively.

Table (1): Frequency of different isolated fungi (%) on Thompson seedless grapes during storage at 0°C in 2002 and 2003 seasons.

Storage period (days)	1st season 2002						2nd season 2003					
	Isolated fungi (%)											
	A*	B	C	P	S	Other	A	B	C	P	S	other
10	12.7	39.2	6.1	36.3	5.7	0.0	10.3	40.1	10.0	34.5	4.4	0.7
20	13.7	41.3	7.3	37.7	0.0	0.0	11.0	41.8	9.9	37.3	0.0	0.0
30	14.0	42.8	4.2	39.0	0.0	0.0	11.6	43.3	3.3	41.8	0.0	0.0
L.S.D. at 0.05%	0.70	0.92	0.55	0.96	-	-	0.65	0.73	0.42	0.83	-	-

** A = Alternaria alternata* *B = Botrytis cinerea.*
C = Cladosporium herbarum *P = Penicillum expensum.*
S = Stemphylium herbarum.

Results indicate that the percentage of frequency of fungi *Botrytis* and *Penicillium* increased by increasing storage periods, while it decreased for other fungi.

b- Identification of isolated fungi:

Data in Table (2), indicate that the fungi *B. cinerea* and *P. expensum* were more frequent, their percentages were 44.8 and 36.4% at the first season and 47.7 and 39.7% at the second season. However, both fungi *A. alternate*, *C. herbarum* and *S. herbarum* were isolated at lower frequency at the two seasons.

Table (2): Identification of different isolated fungi from decayed grape berries during 2002 and 2003 seasons.

Seasons	No. of decayed berries	Isolated fungi (%)				
		Alternaria alternata	*Botrytis cinerea*	*Cladosporium herbarum*	*Penicillium expensum*	*Stemphyllium herbarum*
2002	245	10.7	44.8	6.0	36.4	2.1
2003	287	8.3	47.7	2.3	39.7	2.0

c- Pathogenicity test:

Pathogenicity tests were carried out on isolated fungi. Tested fungi, were *Alternaria alternate*, *Botrytis cinerea*, *Penicillium expensum* and *Cladosporium herbarum*.

Data in Table (3), show that all the isolated fungi, were pathogenic to grapes berries, percentages of infection for *B. cinerea* and *P. expensum* were 46.6, 43.8% respectively. Results indicate that the two fungi were high pathogenic for Thompson grape berries under storage at 0°C or 25°C.

Table (3): Pathogenicity of different isolated fungi from Thompson seedless grapes berries, incubated at 25 + 1°C

Infection (%) of isolated fungi			
Alternaria alternata	*Botrytis cinerea*	*Penicillium expensum*	*Cladosporium herbarum*
7.1	46.6	43.8	2.5

II- Post harvest treatments:

1- Effect of fumigation with acetic acid:

Results in Table (4) indicate that all concentrations reduced the percentage of decay, weight loss, fruit shatter and total wastage from 31.57% for control to 22.65%, 15.97% and 6.11% at concentrations , 10, 20 and 30 µl/L. respectively. The reduction % than control were 28.25, 49.41 and 80.65% for the same concentrations during the first season. The same results at the second season, the percentage of total wastage reduction than control were 28.43, 54.90 and 79.13%, respectively.

The most effective concentration was 30 µl/L. reduced the total wastage than control to 80.65% at the first season and 79.13% at the second season after 30 days from storage at 0°C.

Meanwhile treatment with acetic acid 20 µl/L. showed moderate effect when compared with other treatments.

Table (4): Effect of fumigation with vapours acetic acid on decay, fruit weight loss, fruit shatter, total wastage and reduction than control (%) of Thompson seedless grapes during storage periods at 0°C.

Treatment	Con. µl/L	Storage periods (days)	1st season 2002					2nd season 2003				
			Decay %	Weight loss %	Fruit shatter %	Total wastage %	Reduction than control %	Decay %	Weight loss %	Fruit shatter %	Total wastage %	Reduction than control %
Control (untreated)	-	0	0	0	0	0	-	0	0	0	0	-
	-	10	2.20	1.70	3.30	7.20	-	2.50	1.80	3.50	7.80	-
	-	20	3.30	2.60	5.10	11.00	-	3.60	2.30	5.30	11.20	-
	-	30	3.97	3.10	6.90	13.37	-	5.70	3.90	7.10	16.70	-
	-	Total	-	-	-	31.57	-	-	-	-	35.70	-
Fumigation with vapours acetic acid	10	0	0	0	0	0	-	0	0	0	0	-
		10	1.80	1.25	2.75	5.80	-	1.70	1.10	2.60	5.40	-
		20	2.10	1.80	3.90	7.80	-	2.90	1.25	3.00	7.15	-
		30	2.75	2.30	4.00	9.05	-	4.20	3.00	5.80	13.00	-
		Total	-	-	-	22.65	28.25	-	-	-	25.55	28.43
	20	0	0	0	0	0	-	0	0	0	0	-
		10	0.97	1.00	1.80	3.77	-	0.90	0.80	1.75	3.45	-
		20	1.30	1.75	2.45	5.50	-	1.10	0.75	1.90	3.75	-
		30	1.70	1.90	3.10	6.70	-	3.80	1.95	3.75	9.50	-
		Total	-	-	-	15.97	49.41	-	-	-	16.10	54.90
	30	0	0	0	0	0	-	0	0	0	0	-
		10	0.53	0.36	0.49	1.38	-	0.45	0.55	0.90	1.90	-
		20	0.56	0.57	0.78	1.85	-	0.60	0.65	0.95	2.20	-
		30	0.81	0.87	1.20	2.88	-	1.50	0.85	1.30	3.35	-
		Total	-	-	-	6.11	80.65	-	-	-	7.45	79.13
L.S.D. at 0.05%			0.06	0.07	0.09	0.57	-	0.05	0.06	0.08	0.45	-

$$\text{Reduction \% than control} = \frac{\text{Total wastage of treatment - control}}{\text{control}} \times 100$$

2- Effect of chitosan coating:

Three concentrations of chitosan solution i.e. 1, 2 or 3% were tested as cluster coating for preservative agent for controlling postharvest decay of Thompson grapes. Results in Table (5) indicate that all tested concentrations of chitosan significantly reduced the total wastage of both two seasons. The percentage of decay, weight loss, fruit shatter and total wastage increased by storage periods. Similar trends were observed at all used concentrations of chitosan as well as control fruits in two seasons.

Moreover, the percentage of total wastage decreases with increases in chitosan concentrations.

Chitosan concentrations of 2 and 3% resulted in the highest reduction in total wastage. Both chitosan concentrations differed significantly from the 1% concentration. The highest percentage of total wastage was observed after 30 days of storage. Uncoated control showed 31.57 and 35.70% in the two seasons, respectively. Fruits treated with 2 and 3% chitosan showed 10.30 & 4.85% and 16.45 & 6.08% in the two seasons respectively. Reduction in total wastage incidence of fruit coating with 2 and 3% chitosan as compared with control could be calculated as 67.37 & 84.64% in the first season and 53.92 & 82.97% in the second season.

The most effective concentration was 3% it could reduced the total wastage than control to 84.64% at the first season and 82.97% at the second season after 30 days for storage.

Table (5): Effect of Chitosan coating on decay, fruit weight loss, fruit shatter, total wastage and reduction than control (%) of Thompson seedless grapes during storage periods at 0°C.

Treatment	Con. %	Storage periods (days)	1st season 2002					2nd season 2003				
			Decay %	Weight loss %	Fruit shatter %	Total wastage %	Reduction than control %	Decay %	Weight loss %	Fruit shatter %	Total wastage %	Reduction than control %
Control (untreated)	-	0	0	0	0	0	-	0	0	0	0	-
	-	10	2.30	1.70	3.30	7.30	-	2.50	1.80	3.50	7.80	-
	-	20	3.30	2.60	5.10	11.00	-	3.60	2.30	5.30	11.20	-
	-	30	3.57	3.10	6.90	13.37	-	5.70	3.90	7.10	16.70	-
	-	Total	-	-	-	31.57	-	-	-	-	36.70	-
Chitosan Coated (%)	1	0	0	0	0	0	-	0	0	0	0	-
		10	1.05	1.00	1.35	4.00	-	1.05	1.30	2.15	5.30	-
		20	2.00	1.80	3.60	7.40	-	2.10	1.85	3.60	7.55	-
		30	2.35	2.18	4.85	9.38	-	2.55	2.90	4.00	8.45	-
		Total	-	-	-	20.75	34.27	-	-	-	22.30	37.54
	2	0	0	0	0	0	-	0	0	0	0	-
		10	0.85	0.55	0.65	2.05	-	1.35	1.00	1.85	4.20	-
		20	1.30	0.95	1.35	3.50	-	1.90	1.25	2.75	5.80	-
		30	1.75	1.20	1.80	4.75	-	1.70	1.60	3.15	6.45	-
		Total	-	-	-	10.30	67.37	-	-	-	16.45	53.92
	3	0	0	0	0	0	-	0	0	0	0	-
		10	0.45	0.35	0.40	1.20	-	0.55	0.45	0.45	1.45	-
		20	0.65	0.40	0.55	1.60	-	0.75	0.65	0.73	2.13	-
		30	0.85	0.85	0.65	2.05	-	0.90	0.85	0.75	2.50	-
		Total	-	-	-	4.85	84.64	-	-	-	6.08	82.97
L.S.D. at 0.05%			0.04	0.05	0.07	0.43	-	0.05	0.01	0.06	0.51	-

3- Effect of bio-agents treatments:

The effectiveness of postharvest application of bio-control agents (yeast isolates) i.e. *Phaffia* sp., *Saccharomyces* sp. and *Rhodotorula* sp. on Thompson grape during storage at 0°C for 30 days at the two seasons on the percentage of decay, subsequent fruit weight loss (%), subsequent fruit shatter (%) and total wastage (%) are presented in Table (6).

Data in Table (6), reveal that the percentage of decay, fruit weight loss, fruit shatter and total wastage increased with the progress of storage period. On the other hand, the total wastage decreased from 31.57% for control to 24.95 & 17.85 and 13.40% for biological control treatments. However, the reduction of total wastage than control increased to 20.97 & 43.46 and 57.55% for three isolates of yeast i.e. *Phaffia* sp., *Saccharomyces* sp. and *Rodotorula* sp. respectively at the first season. In the second season, the same trend was the total wastage decreased in biocontrol treatment and reduction percentage increased compared with control.

The most effective of biocontrol agent, isolate yeast *Rhodotorula* sp. at concentration (5×10^7 CFU/ml), redcued the total wastage than control to 57.55% at the first season and 60.31% at the second one, followed by isolate yeast *Saccharomyces* sp. when compared with the other treatments after 30 days for storage.

Table (6): Effect of spraying with some bicontrol agents (yeast) on decay, fruit weight loss, fruit shatter, total wastage and reduction than control (%) of Thompson seedless grapes during storage periods at 0°C.

Treatment	Con. CFU/ml	Storage periods (days)	1st season 2002 Decay %	Weight loss %	Fruit shatter %	Total wastage %	Reduction than control %	2nd season 2003 Decay %	Weight loss %	Fruit shatter %	Total wastage %	Reduction than control %
		0	0	0	0	0	-	0	0	0	0	-
(spraying with water)	-	10	2.20	1.70	3.30	7.20	-	2.50	1.80	3.50	7.80	-
	-	20	3.30	2.80	5.10	11.00	-	3.60	2.30	5.30	11.20	-
Control	-	30	3.37	3.10	6.90	13.37	-	5.70	3.90	7.10	16.70	-
		Total	-	-	-	31.57	-	-	-	-	35.10	-
		0	0	0	0	0	-	0	0	0	0	-
Spraying with yeast *Phaffia* sp.	$5 \cdot 10^7$	10	1.85	1.50	2.35	5.70	-	1.90	1.55	2.60	6.05	-
		20	2.60	1.90	4.60	9.10	-	2.35	2.00	4.35	8.70	-
		30	2.45	2.35	5.35	10.15	-	2.50	2.75	5.15	10.40	-
		Total	-	-	-	24.95	20.97	-	-	-	25.15	29.55
		0	0	0	0	0	-	0	0	0	0	-
Spraying with yeast *Saccharomyuces* sp.	$5 \cdot 10^7$	10	0.95	0.75	1.95	3.65	-	1.00	0.85	1.70	3.55	-
		20	1.70	0.95	3.35	6.00	-	1.35	1.15	3.20	5.70	-
		30	2.00	1.40	3.80	7.20	-	1.90	1.25	3.60	6.75	-
		Total	-	-	-	17.85	4346	-	-	-	16.00	55.18
		0	0	0	0	0	-	0	0	0	0	-
Spraying with yeast *Rhodotorula* sp.	$5 \cdot 10^7$	10	0.85	0.55	1.60	3.00	-	0.85	0.75	1.55	3.15	-
		20	1.45	0.70	2.15	4.30	-	1.10	0.97	2.90	4.97	-
		30	1.75	1.25	3.10	6.10	-	1.65	1.15	3.25	6.05	-
		Total	-	-	-	13.40	57.55	-	-	-	14.17	60.31
L.S.D. at 0.05%			0.07	0.08	0.06	1.05	-	0.05	0.06	0.08	1.20	-

III- Chemical properties during storage:

1- Total soluble solid percentage:

Date in Table (7), demonstrate that there was an increase in the content of total soluble solids with prolonging the storage periods and decrease than control.

Regardless of the effect of the storage period, data obtained that show all treatments reduced the increasing rate of total soluble solids in the stored grape than the control.

Bio-control agents treatments were the most effective in limiting the increase in TSS contents in the stored Thompson seedless grape in the two seasons. There was no clear trend for the effect of different contents in the stored Thompson seedless grapes in both seasons during storage. In biocontrol agents represented the lowest total soluble solid values during the two seasons.

Table (7):Effect of vaprous acetic acid, chitosan coated and spraying of bicontrol agents on total soluble solids (%) of Thompson seedless grapes during storage periods at 0°C.

Treatment	Con.	1st season 2002 Storage periods (days) Zero time	10	20	30	2nd season 2003 Storage periods (days) Zero time	10	20	30
Control	-	19.12	19.23	19.90	20.95	19.70	19.75	20.50	21.75
Vaprous acetic acid (µl/L)	10	18.71	18.10	18.90	19.11	18.33	18.35	18.40	18.71
	20	18.75	19.00	19.20	19.60	19.10	19.15	19.25	19.45
	30	19.13	19.20	19.40	20.35	19.65	19.50	20.00	20.13
Chitosan coated (%)	1	18.60	18.90	19.10	19.25	18.35	18.40	18.20	18.63
	2	18.81	19.10	19.00	19.35	18.21	18.33	18.40	18.52
	3	19.45	19.50	19.80	20.73	20.23	19.80	20.33	21.15
Spraying with *phaffia* sp.	5×10^7 (CFU/ml)	18.70	18.70	18.60	18.88	18.51	18.70	18.60	19.62
Saccharomyuces sp		18.75	18.90	19.30	19.41	19.30	19.40	19.80	19.75
Rhodotorula sp		19.10	19.13	19.70	20.11	19.35	19.20	19.40	19.71
L.S.D. at 0.05%		0.17	0.20	0.25	0.43	0.15	0.19	0.12	0.11

2- Total acidity percentage:

Date in Table (8), demonstrate that, there was a slight decrease in the percent of total acidity to the end of storage. Acidity in Thompson seedless grape decreased gradually with prolonged storage till the end of storage period in two seasons. However, grapes treated with any treatment had higher total acidity contents at compared with control, it were contained lower content of acids at the end of storage period than untreated grapes, especially in fruits treated with chitosan coated during two seasons.

Table (8): Effect of vaprous acetic acid, chitosan coated and spraying of bicontrol agents on total acidity (%) of Thompson seedless grapes during storage periods at 0°C.

Treatments	Con.	1st season 2002				2nd season 2003			
		Storage periods (days)							
		Zero time	10	20	30	Zero time	10	20	30
Control	-	0.57	0.51	0.52	0.53	0.61	0.59	0.57	0.54
Vaprous acetic acid (µl/L.)	10	0.59	0.60	0.56	0.54	0.66	0.64	0.69	0.60
	20	0.61	0.60	0.55	0.53	0.66	0.64	0.67	0.62
	30	0.60	0.60	0.54	0.52	0.67	0.67	0.66	0.57
Chitosan coated (%)	1	0.58	0.55	0.53	0.53	0.69	0.64	0.62	0.61
	2	0.59	0.62	0.59	0.53	0.66	0.61	0.60	0.59
	3	0.60	0.62	0.53	0.60	0.66	0.57	0.56	0.56
Spraying with *phaffia* sp.	$5 \cdot 10^7$ (CFU/ml)	0.59	0.56	0.54	0.58	0.71	0.66	0.69	0.61
Saccharomyuces sp		0.62	0.56	0.57	0.52	0.70	0.67	0.60	0.56
Rhodotorula sp		0.60	0.54	0.56	0.54	0.68	0.65	0.59	0.55
L.S.D. at 0.05%		0.005	0.007	0.003	0.002	0.006	0.006	0.004	0.003

3- Reducing sugars of the juice:

Data illustrated in Table (9), indicate that all treatments increased gradually reducing sugars in the stored grapes with prolonged storage till it reached the maximum percentage after 30 days from storage. Grapes treated with any treatment had higher contents of reducing sugars during storage period compared with control. This increase was in two seasons.

Reducing sugars contents in Thompson seedless grapes treated with chitosan reached the values of 15.8% in the first season and 16.2% in the second season after 30 days for storage, while the percentage of reducing sugar in untreated (control) reached the percentage of 14.1 & 15.00 in two seasons of this study respectively.

Table (9):Effect of vaprous acetic acid, chitosan coated and spraying of bicontrol agents on reducing sugar (%) of the juice of Thompson seedless grapes during storage periods at 0°C.

Treatments	Con.	1st season 2002				2nd season 2003			
		Storage periods (days)							
		Zero time	10	20	30	Zero time	10	20	30
Control	-	13.6	13.8	14.2	14.1	14.1	14.4	14.7	15.0
Vaprous acetic acid (µl/L)	10	14.3	14.0	14.4	14.8	14.1	14.4	14.8	15.9
	20	14.1	14.5	14.7	14.9	14.3	14.8	15.2	15.4
	30	14.7	15.0	15.2	15.7	14.2	14.4	15.2	15.4
Chitosan coated (%)	1	14.0	14.1	14.3	15.5	14.4	14.7	15.0	15.3
	2	14.1	14.5	15.0	15.1	14.0	15.2	15.4	16.0
	3	14.8	14.7	15.2	15.8	14.2	15.1	15.4	16.2
Spraying with *phaffia* sp. *saccharomyucas* sp *Rhodotorula sp*	$5 \cdot 10^7$ (CFU/ml)	14.1	14.3	14.3	14.6	14.4	15.3	15.3	16.3
		14.5	14.7	15.1	15.2	14.2	14.7	15.4	15.8
		14.3	14.5	15.0	15.1	14.5	15.5	15.8	16.1
L.S.D. at 0.05%		0.29	0.34	0.42	0.37	0.21	0.51	0.39	0.29

DISCUSSION

Grapes are one of the largest cultivated fruit crops in the most countries. Fruits are subjected to infection at any stage of production, and during handling transportation and storage, which causes high losses.

For all these reasons, the objective aimed to study this problem and to reach safe methods to preservation and prolongated storage period of grapes.

Fruits of Thompson seedless variety was sorted at 0°C for 30 days. Samples were taken every 10 days. This experiment was replicated for two seasons, i.e. 2002 and 2003.

Data in this experiment revealed that *Botrytis* and *Penicillium* were the most frequent fungi during storage in both seasons followed by *Alternaria* and *Cladosporium*.

Botrytis rot is most widespred and major cause of deterioration of grapes in cold storage (Snowdon, 1990; and Benkhammer *et. al.*, 1992). This fungus is the main decay problem all over the world in grapes exposed in the field to high humidity.

The fungus *Penicillium* needs humid conditions and direct penetration of mature grapes in the absence of rain, but wet weather results in a higher incidence of disease (Snowdon, 1990). This may be due to the variation in the climatic factors in different areas of Egypt where different variety is grown.

As for the decay in berries, weight loss, berries shattering and total wastage were estimated during storage. Four aforesaid factors increased with increase in time of storage.

Decay of grape berries that occurs in storage is largely due to the initial incipient field infection, which is not detectable at harvest. The organisms that cause decay further invade the tissues of individual infected berries during storage and may spread by contact to sound ones. Preventing field infections by applying an appropriate fungicide in the field would, therefore,

greatly reduce the losses from decay, which often occur in stored grapes (Harvey, 1955).

Acetic acid vapour with 20 or 30 µl/L were the most effective treatments for controlling postharvest decay of grapes fruits. Using acetic acid vapours for controlling postharvest decay were reported by Sholberg and Gaunce (1996) Sholberg *et. al.*, (1996) and Sholberg *et. al.*, (1998). Hardenburg *et. al.*, (1986) reported that fumigation of grapes fruits inoculated with conidia of *B. cinerea* gave complete control of postharvest decay.

Activity of acetic acid was related to pH, carbon chain length and inherent susceptibility of the microorganisms and the undissociated part of the acid was primarily responsible for its antimicrobial activity (Banwart, 1981). He added that the inhibitory effect of AA on microorganisms is greater than that due to pH alone and undissociated AA, it can penetrate the microbial cell to exert its toxic effect.

The mechanism of acetic acid that inhibits microorganisms is apparently that it affects the cell membrane interfering with the transport of metabolites and maintenance of membrane potential (Sholberg *et. al.*, 1998).

Acetic acid vapour at low concentrations has many qualities that makes it an excellent biocide, first it kills fungal spores, second it does not injure the fumigated fruits surface, third it is effective at low temperatures which means that fruit in 1°C cold storage could be effectively treated with acetic acid vapour. Forth, it is not flammable at the low concentrations that are required to kill fungal spores (Sholberg and Gaunce, 1996).

There are several advantages of using acetic acid fumigation to control postharvest decay. It is a natural compound found throughout the biosphere possing little or no residual hazard at low levels required to kill fungal spores; it is also generally-regarded as safe compound in the United States and does not require rigorous registration procedures; it is inexpensive, and it can be used to treat products in airtight storage rooms or containers without requiring handling of the products Sholberg *et. al.*, (1998). It could be suggested that acetic acid vapour might be safely used commercially as a new approach for controlling postharvest decay.

Chitosan products from the seafood industry is reported as a safe material based on toxicological studies Hirano *et. al.*, (1990). The mode of action of chitosan was explained by Du *et. al.*, (1997), who reported that coating significantly reduced the respiration rate, ethylene production, and interval O_2 level of peach, pear and kiwifruit. Chitosan coated fruits were markedly firmer and less ripened.

Moreover, chitosan has antifungal activity against several fungi including *B. cinerea* (Hirano and Nagao, 1989). Allan and Hadwiger, (1979) reported that, chitosan (1g/L.) was effective in reducing the radial growth of most fungi tested except those containing chitosan as a major cell wall component. The effectiveness of chitosan solution to inhibit decay of *B. cinerea* was also reported by (El-Gaouth *et. al.*, 1992b). Recently, two models

have been proposed explain the antifungal activity of chitosan: first, the interaction of chitosan with fungal DNA and RNA (Hadwiger and Loschke, 1981) and second, the activity of chitosan is related to its ability to interfere with the plasma membrane function (Leuba and Stossel, 1986).

Chitosan could be used as a substitute of fungicides to inhibit postharvest decay. Chitosan coating decreased postharvest decay of several fruits, (Hirano and Nago, 1989, Du *et. al.*, 1997). El-Gaouth, *et. al.*, (1991 and 1992, b) reported that chitosan coating decreased decay of tomato and strawberry fruits, decay of peach, pears and kiwifruit (Du *et. al.*, 1997). These reports confirming the obtained results that chitosan 2 and 3% were the most effective treatments for preventing postharvest decay of grapes fruits. The appears to be related the fungistatic property of the product as advocated by El-Gaouth *et. al.*, (1992b). It could be suggested that chitosan might be safely used commercially as fruit coating to control postharvest diseases and for prolonging the shelf life of sensitive fruits.

There is an urgent need for new and effective means of postharvest decay control. In this regard, Wilson and Pusey (1985) concluded that biological control is one of the most promising alternatives especially in postharvest decay using epiphytic antagonists was successful for pre- and postharvest treatment of various crop; grapes (Mclaughlin *et. al.*, 1992), apples and pear (Chand-Goyal and Spotts, 1997), beans and tomatoes (Elad *et. al.*, 1994).

Many yeasts isolates were highly effective against *A. alternata*, these isolates were found to produce active antibiotics. The yeast *Pichia anomla* strain "K" and *Candida sake* strain "O" have been reported to effectively inhibit development of rot of apple fruits induced by *B. cincerea* and *P. expensum* at both 5 and 25°C (Jijakli *et. al.*, 1993).

The efficacy of yeast for controlling postharvest decay depend on the number of viable cells per milliliter or per wound (Roberts, 1990; Chand-Goyal and Spotts, 1996; and Filonow *et. al.*, 1996). However, to achieve effective biological control generally it is necessary to treat the fruits with a yeast approaching 1×10^8 CFU/ml or more (Chand-Goyal and Spotts, 1997).

The effective used concentration of the bioagents was relatively low i.e. 1×10^9. Also the tested concentration of the pathogen was 1×10^5 while it ranged from 2-5 $\times 10^5$ (Chand-Goyal and Spotts, 1996, 1997) and 1×10^4 (Mari *et. al.*, 1996) which means isolates related to three genera i.e. *Rhodotorula* sp., *Phaffia* sp., and *Saccharomyces* sp. were isolated from fruit surface and its wounds.

Moreover, results show that the commercial production and application of these yeast isolates as bioproduct for postharvest control of grapes fruits is realistic.

Treatments before storage with vapours acetic acid, chitosan coating and biocontrol agents reduced the increasing rate of total soluble solid contents in the stored grapes. There was no clear trend for the effect of different concentrations on the total soluble solids contents. These results are in

agreement with those reported by Gautam *et. al.*, (1981), Drake & Spayed (1983), Ropson *et. al.*, (1989) and Turky (1996).

Grapes treated with some application and directly storage under cooling conditions were less in acid contents during storage compared with untreated. These results in line with those mentioned by Carl & Conway (1984); Haggag (1987); Gautam, *et. al.*, (1981) and Turky (1996), they indicated that storage decreased total acid content of grapes during storage.

Reducing sugars in the stored grapes increased gradually with prolonged storage compared with control. However, this increasing was significant in one season and was not significant in the other season. These results partially agree with those obtained by Cooper (1976) and Bulatovic & Trialo (1991).

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اتجاه تطبيقي لحفظ وإطالة فترات تخزين صنف العنب تومسون سيدلس

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الملخص العربي

استهدفت هذه الدراسة استخدام طرق آمنة لحفظ وإطالة فترات تخزين محصول العنب تومسون سيدلس وتقليل الفقد الكلي (الخسائر) في المحصول خلال ٣٠ يوم تخزين على درجة حرارة صفر درجة منوية. ومعرفة مسببات أعفان ما بعد الحصاد والتخزين.

كانت الفطريات بوتراتيس سيناريا، بنسيليوم اكسبنزم هي أكثر الفطريات تكراراً بالنسبة لصنف العنب تومسون سيدلس أثناء فترات التخزين لكلا موسمي الدراسة.

تم اختبار ٣ تركيزات من بخار حمض الخليك لبيان تأثيرها على النسبة المئوية للفقد الكلي وكانت أفضل النتائج لتركيز ٣٠ ميكرولتر/لتر حيث أدى لخفض الفقد الكلي إلى ٦,١١% في الموسم الأول، ٧,٤٥% في الموسم الثاني مقارنة بمعاملة المقارنة ٣١,٥٧%، ٣٥,٧٠%. هذه المعاملة أدت إلى اختزال الفقد الكلي بنسبة ٨٠,٦٥%، ٧٩,١٣% في كلا الموسمين على التوالي.

تم اختبار ٣ تركيزات من الكيتوزان بتغطية ثمار صنف العنب بالكيتوزان وكانت أفضل النتائج لتركيز ٣% حيث أدى لخفض الفقد الكلي إلى ٤,٨٥% في الموسم الأول، ٦,٠٨% في الموسم الثاني مقارنة بمعاملة المقارنة. كما أدت هذه المعاملة إلى اختزال الفقد الكلي بنسبة ٨٤,٦٤% و٨٢,٩٧% في كلا الموسمين على التوالي.

وتشير هذه النتائج إلى إمكانية استخدام الكيتوزان في مقاومة أعفان ما بعد الحصاد في ثمار العنب وهو أيضاً آمن بالنسبة للإنسان والبيئة.

أظهرت ثلاث عزلات من الخمائر القدرة على خفض الفقد الكلي حيث أدى استخدام إحدى هذه العزلات بتركيز ٥ × ١٠^{٧} في معاملة ثمار العنب بعد الحصاد وقبل التخزين مباشرة إلى تقليل الخسائر الكلية إلى ١٣,٤٥%، ١٤,١٧% في كل من موسمي الدراسة كما أدت إلى اختزال الخسائر الكلية بنسبة ٥٧,٥٥%، ٦٠,٣١% بالمقارنة بغير المعامل بعد ٣٠ يوم من التخزين.

كانت للمعاملات السابقة قبل التخزين تأثير معنوي على كمية المواد الصلبة الذائبة وقلت الحموضة الكلية في العنب المخزن وزاد محتوى حبات العنب من السكريات الذائبة أثناء التخزين.