ISSN 1110-0419

Annals Of Agric. Sc., Moshtohor,
Vol. 46(2): Bo. 23-34, (2008).

PRE-HARVEST APPLICATION OF KOMBUCHA FILTRATE TO CONTROL POSTHARVEST BUNCH ROT OF TABLE GRAPES

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ABSTRACT

Botrytis cinerea Pers., is responsible for significant losses of table grape berries both before and after harvest, and is a major obstacle to long-distance transport and storage. So, this study aims to evaluate non-chemical substances to control Botrytis rot through these conditions. During 2006 and 2007 growing seasons, in the grape vineyard of the three Seedless table grapes in El-Nobariya area, at Beheira Governorate. Laboratory trials were performed to evaluate the effect of Kombucha filtrate on the spore germination. Suppression the detached berries decay after being inoculated with the spore suspension and immersed in Kombucha for 30 sec, then stored at 15°C for 7 days. Inoculated and non-inoculated entire clusters of the three tested grapes were immersed in the Kombucha filtrate for 30 sec and stored for 4 weeks at 0 – 1°C (RH >90%), number of decayed berries per kilogram were recorded after storage quality parameters of both groups were also evaluated. These treatments was compared with traditional commercial method to control this decay using sulfur dioxide. Kombucha reduced the spore germination at low concentrations and prevented it at 80 or 100%. Highly reducing percentage of the decayed berries or the number of decayed berries per kilogram when entire clusters were treated with both Kombucha or SO_2. Finally, it is suggested that Kombucha is a natural alternative antifungal which could be used as near-harvest dipping application without need to any chemical or fungicidal applications pre- and post-harvest especially for exportation grapes. Grapes quality were not negatively affected as result to using a new tested substance.

Key words: Seedless grapes, Kombucha, cooling storage, SO_2

INTRODUCTION

Gray mold, caused by *Botrytis cinerea* Pers., is the most economically important postharvest disease of table grapes (*Vitis vinifera* L.) (Cappellini *et al.*, 1986). It is responsible for significant losses of table grape berries both before and after harvest, and is a major obstacle to long-distance transport and storage. Control of the disease is especially important in storage because it develops at low temperatures (−0.5°C) and spreads quickly among berries (Karabulut *et al.*, 2004). Currently, postharvest diseases of table grapes are controlled by postharvest application of SO_2, either by weekly fumigation in storage rooms or by packing grapes in polyethylene-lined boxes with SO_2 generator pads. These are used worldwide for the control of gray mold, caused by *Botrytis cinerea*, during long-term cold storage and export shipment of table grapes. Problems associated with SO_2 use include the following: (1) SO_2 residues that exceed the tolerance of 10mg/kg of most countries, which can occur if the gas dosage is too high; (2) unsightly bleaching injuries that can occur to berries after numerous or high dosage fumigations; (3) SO_2 cannot be used on organically certified grapes (Mlikota and Smilanick, 2001); and (4) because of sulfite hypersensitivity in some people, the dietary hazard of SO_2 was recognized and it was removed from the US Food and Drug Administration 'generally regarded as safe' classification in 1986 (Zahavi *et al.*, 2000). However, because

of the emergence of pesticide-resistant pathogen strains (Leroux *et al.*, 1999). Therefore, the development of alternative strategies to control postharvest decay of table grapes that are safe, effective, economical, and compatible with commercial handling is of interest.

Kombucha (the tea fungus) is a symbiotic colony of acetic and lactic acids bacteria with yeasts which can be divided for multiplication. Kombucha is mainly cultivated in sugared green tea to produce a slightly acidulous beverage including *Acetobacter xylinum* as a characteristic species and various yeasts (Mayser *et al.*, 1995 and Sreeramulu *et al.*, 2000). Acetic acid concentration may rise to levels as high as 20 g/L if the tea is allowed to ferment for up to 30 days (Asai, 1968 and Greenwalt *et al.*, 1998).

Recent research on Kombucha has proved that its antimicrobial activity against pathogenic microorganisms is largely due to acetic acid. Shehata and Lila (2005) reported that fermented tea beverage has antimicrobial activity against a wide spectrum of organisms including some phytopathogenic fungi (*i.e. Fusarium oxysporum, Alternaria solani, Aspergillus niger, Penicillium*).

Antioxidant and antimicrobial activeties were achieved after fermenting sugared black tea, green tea or tea manufacture waste with tea fungus (Kombucha) for 12 to 15 days (Jayabalan *et al.*, 2007).

The purpose of this study was to evaluate efficacy of near-harvest vineyard applications with Kombucha as a biocontrol agent, compared with commercial anhydrous sodium metabisulfite ($Na_2S_2O_5$) antifungal to control *B. cinerea* associated with pre- and post-harvest decay of table grapes in Egypt. This approach could accomplish control of table grape postharvest diseases without the need of additional harvest or postharvest handling procedures, and it could reduce or eliminate the need for SO_2 fumigation or release.

MATERIALS AND METHODS

Fruits

During 2006 and 2007 growing seasons and near-harvest applications to control post-harvest gray mold caused by *Botrytis cinerea* Pers.:Fr. in table grapes were conducted in the Laboratory of Plant Pathology, Agric. Botany Dept., Faculty of Agriculture, Moshtohor, Banha University and Commercial Vineyards located in El-Nobariya area, at Beheira Governorate, Egypt.

Three Seedless table grapes (*Vitis vinifera* L.) cultivars were used. Two white cultivars namely 'Early Superior Seedless' and 'Thompson Seedless' and one red cultivar 'Flame Seedless'.

All the experiments were conducted on healthy and mature clusters of table grapes which were harvested when soluble solids content recorded 14% or higher. The soluble solids content of the berries was determined by a Portable Refractometer with Brix 0 - 32% ATC (Huake Instrument Co., Ltd., Shenzhen, China). The grapes were harvested in the early morning and used on the day of harvest.

Source of inoculum

Botrytis cinerea cultures were isolated from naturally infected grape berries. Cultures were maintained on PDA slants at 5°C, and identified by culture characteristics and microscopic observations in accordance to Mirzaei *et al.* (2007) in Plant Pathology Lab., Fac. Agric., Moshtohor, Banha University.

A spore suspension was created by flooding 2-weeks plates with a small volume of sterile distilled water containing 0.05% (v/v) Tween-80, and spores were removed by gently scraping with a glass spatulum. The resulting spore suspension was filtered through four layers of cheesecloth to remove mycelial fragments and was diluted with sterile water to obtain an absorbance of 0.25 at 425 nm as determined by a spectrophotometer (SPECTRONIC® 20-D). The density was about 1.2×10^6 conidia/ml as determined by a

hemacytometer. Further dilutions with sterile water were made to obtain the desired spore concentration (Karabulut *et al.*, 2005).

Kombucha

Kombucha (kombu=seaweed, cha= tea in Japanese) *i.e.*, tea fungus, has been consumed worldwide as a healthy drink for a very long time especially in China, Russia, Denmark, Poland and Germany (Dipti *et al.*, 2003).

Starter culture:

It was obtained from Günther W. Frank, Genossenschafts Str. 19, D-75217 Brikenfeld, Germany through Dr. Ahmed Abbass Nowair, Lecturer of Genetic Engineering & Biotech. Inst., Sadat City, Menofyia Univ.

Kombucha is prepared by fermenting sweetened green tea (100 g sucrose, 10 g Chinese green tea per litre of water) preparations with a symbiotic colony of yeasts and bacteria (starter). After 12 days at 28°C, mother culture was omitted and extract was kept to self-refermented for additional 21 days, extract was collected, centrifuged for 10 min. at 1000 rpm to separate any debris, then sterilized using sintered glass (G4) funnel (Betsy and Sonford, 1996). Crude Kombucha extract was used either as its (100%) or after being diluted with distilled water to produce the desired concentrations.

Pathogenicity test:

The pathogenicity of the causal pathogen isolated from grapes and identified as *Botrytis cinerea* was tested on 90 detached grape berries of the three tested cultivars, with soluble solids concentration between 14 and 16%. Berries with their pedicels attached were surface disinfected (5min in 1% w/v NaOCl), and aseptically injured with a sterile hypodermic needle that delivered 10µL of a conidial suspension (10^5–10^6 conidia mL^{-1}) obtained from 15-day-old PDA cultures. Inoculated and non-inoculated, but equally injured, berries were incubated in a humid chamber at >97% RH by moist paper towels (determined by a hydrometer) for over 6 days at 20°C. Typical Botrytis gray mold symptoms were appeared. Lesion diameter was measured. *Botrytis* conidia was reisolated from injured berries and cultured on fresh PDA plates.

Effect of Kombucha concentrations on *B. cinerea* spores viability

Botrytis cinerea spores (10^4 conidia/ml) were mixed with various Kombucha concentrations (20, 40, 60, 80, 100%) or distilled water (as control) under laboratory conditions (22–24°C) in a final volume of 2ml. After 30 sec, the spore suspensions were diluted 100-fold in sterile water and 100µl were distributed on PDA plates. After 48 h incubation at 24°C, the colonies per plate were counted. Data were expressed as the percentage of viable spores. The experiment was performed twice.

2. Kombucha treatments of table grape

2.1. Effect on detached berries:

The effectiveness of treatments with Kombucha (as antifungal agents) on gray mold incidence of three Seedless "Early Superior, Thompson and Flame" table grape berries (at 14°Brix) 24 h before harvest was sprayed with a spore suspension of *B. cinerea* was determined. Berries were either cut from the rachis with pedicel intact, or pulled from the rachis (pedicel detached), which exposed the berry flesh and enabled wound inoculation to occur. The berries were injured their pedicel with aseptically sterile hypodermic needle then all sprayed with the spore suspension of about 1×10^5 spores/ml then air dried for 30 min as described by Mlikota *et al.* (2005). A volume of 50ml of spore suspension (inoculum) was enough for spraying on about 1200 berries. Inoculated berries were kept at 15°C in a covered plastic box for 2, 12, 24, or 48 h before being immersed separately for 30 sec in 20, 40, 60, 80 and 100% Kombucha or distilled water (as a control check) at 25°C, then kept at 15°C for 7 days in covered plastic boxes lined with moist paper towels (>90% RH). Inoculated and non-inoculated, but equally injured, berries were treated. At the end of storage % decayed berries was recorded. The experiment was performed twice. Each replicate consisted of 20 single berries and four replicates were used for each treatment.

2.2. Effect on entire clusters inoculated:

Mature entire clusters from the three Seedless table grapes cultivars "Early Superior, Thompson and Falme" were harvested. Only healthy clusters with soluble solids content of 14% or higher were randomly selected and labeled 48 h prior harvesting according to refractometer index. After two days, labeled clusters were harvested and arranged in a single layers (out of bags) on metal racks and whole clusters were inoculated by spraying 150 ml of a spore suspension (10^5 spore/ml) of *B. cinerea* with a compressed air sprayer, then left to air dry at 25°C for 48 hours before treatments. A volume of 100ml of inoculum was sprayed on to 40 kg clusters. Each replication, about 5 kg and a total of 10 bags of grape clusters per carton box, three replications were used for each treatment. Clusters were enclosed in individual perforated polyethylene bags (6.5% vented area) according to Karabulut *et al.* (2004). Clusters were distributed in three homogeneous batches:

1-**Control**: 30 Clusters (500 g) were placed individually in 30 punnets enclosed in secondary perforated polyethylene bags (80 x 60 cm) with 5-mm-aperture mesh, and packaged in 3 carton boxes (10 for each) which wrapped with polyethylene stretch film (20μm) to minimize weight loss during storage. This batch was used as control.

2-**Control with SO_2**: Similar batch as in 1, but after 48 hrs from inoculation SO_2-dual-release generator pads containing 5.5 g of anhydrous sodium metabisulfite 97.5% $Na_2S_2O_5$ (G3, Osku-vid, Osku S.A., Santiago, Chile) were place on top of punnets inside the secondary perforated polyethylene bags before packaging in carton boxes and wrapped with polyethylene stretch film (20μm).

3-**Kombucha treatment**: The same batch 1, but after 48 hrs from inoculation, entire clusters were immersed in 5-litres crude extract of Kombucha (100%) for 30 sec, the air-dried for about one hour, and placed in a new punnets which arranged in the secondary perforated polyethylene bags and packaged in 5 kg carton boxes and wrapped with polyethylene stretch film (20μm).

Treated clusters with SO_2 (as positive control) or without SO_2 (as negative control) as well as Kombucha treated ones were stored for 4 weeks at 0 – 1°C (RH >90%). After cold storage, the polyethylene stretch film was removed and the fruits were stored at 20°C for an additional 48 h to stimulate the environmental conditions that occur during market display. Experiments were repeated twice. The number of decayed berries per kg of fruits was recorded after storage.

2.3. Effect on entire clusters in the vineyard:

Mature entire clusters from the three Seedless table grapes cultivars with soluble solids content of 14% or higher were randomly selected and labeled 48 h prior harvesting. Thirty attached clusters from each cultivar were selected and distinguished with different labels. Then were completely immersed for 30 sec in 5 litre crude extract of Kombucha and left to dry. After two days, all the labeled clusters were harvested. Each replication was about 5 kg and a total of 10 bags of grape clusters per carton box, three replicates (15 kg) were used for each treatment. Clusters were enclosed in individual perforated polyethylene bags (6.5% vented area) according to Karabulut *et al.* (2004). Clusters were distributed in three homogeneous batches:

1-**Kombucha treatment**: 30 Clusters were placed individually in 30 punnets enclosed in secondary perforated polyethylene bags (80 x 60 cm) with 5-mm-aperture mesh, and packaged in 3 carton boxes (10 for each) wrapped with polyethylene stretch film (20μm) to minimize weight loss during storage.

2-**SO_2 treatment:** Non-inoculated, untreated patch, but SO_2- dual-release generator pads containing 5.5 g of anhydrous sodium metabisulfite 97.5% $Na_2S_2O_5$ (G3, Osku-vid, Osku S.A., Santiago, Chile) were place on the top of punnets inside the secondary perforated polyethylene bags before packaging in carton boxes and wrapped with polyethylene stretch film (20μm).

3- **Control**: This patch were exactly packaged as previously without inoculation or any treatments.

Naturally infected clusters were used in this experiment without inoculation and packaged as the ideal exportation protocols in three patches as mentioned. All patches, treated and non-treated were stored for 4 weeks at 0 – 1°C (RH >90%). After cold storage, the polyethylene stretch film was removed and the patches were stored at 20°C for an additional 48 h to stimulate the environmental conditions that occur during market display. Experiments were twice repeated. The number of decayed berries per kg of fruits was recorded after storage.

3. Quality evaluation

Sometimes, excellent antimicrobial bioagents were affected negatively on some quality parameters of the sensitive treated fruits like grapes. So, at the end of storage, five clusters of the treated and untreated patches were sampled and tested for quality specifications using facilities of the International for Economic Development, El-Aguizy Co. Laboratory, Sadat City, Menofyia Governorates. The quality of the fruit was examined by evaluation of berry and rachis appearance, incidence of cracked and shattered berries, flavour and weight loss by a panel of five trained judges. The visual characteristics including berry and rachis appearance, were scored under daylight. The flavour of the grapes was evaluated under red light in a taste room with individual booths in order to avoid the interference of visual judgment. The detailed evaluation methods and the units employed are described in Table (1). The quality experiment was repeated twice

Table (1): Quality specifications and methods used.

Quality parameters	Methods of evaluation and unit
Weight loss	Percentage differences between the weight of clusters before and at the end of storage.
Cracking	Number of cracked berries/kg.
Shatter	Number of shattered berries/kg.
Flavour	Flavour acceptability, using a five-point scale: excellent, 1; good, 2; acceptable, 3; poor, 4; unacceptable, 5.
Berry appearance	Visual index of clusters: excellent, 1; good, 2; slightly dull, 3; <50% brownish and soft berries, 4; >50% brownish and soft berries, 5.
Rachis appearance	Visual index of clusters: fresh and green, 1; green, 2; semi-dry, 3; 50% dry, 4; completely dry, 5.

4. Statistical analysis

Data were subjected to analysis of variance (ANOVA) using MSTAT-C Software version 2.1 (Michigan State University, USA). Incidence data were transformed (arcsine of the square root of the proportion of affected fruit) before analysis. Statistical significance was assessed at $P < 0.05$ and Duncan's multiple range test was used to separate means.

RESULTS

1. Effect of Kombucha concentrations on *B. cinerea* spores viability

Exposure of *B. cinerea* spores for 30 sec to Kombucha (as antifungal filtrate) at various concentrations then germinated in PDA plates under laboratory conditions was gradually reduced the average percentage of spore viability. The highest efficacy in inhibiting the spore germination was achieved by exposure spores to high concentrations of Kombucha (80 and 100%) (Fig. 1).

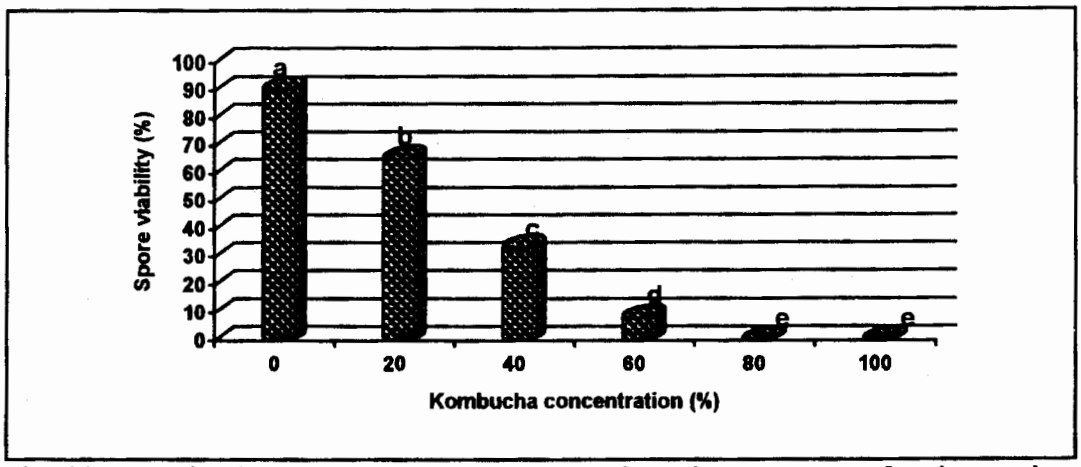

Fig. (1): Germination on potato dextrose agar of *B. cinerea* spores after immersion in various Kombucha concentrations or distilled water (0) and 48 hrs incubation at laboratory conditions.
Column with unlike letters differ significantly according to Duncan's multiple range test at P ≤0.05.

2. Kombucha treatments of table grape

2.1. Effect on detached berries:

The decay incidence of detached berries of three cvs. of seedless table grapes are recorded in Table (2). Berries without pedicel were more sensitive to decay with Botrytis inoculation than those with pedicel. Also, results showed that Thompson Seedless cv. was the most susceptible to infestation, followed by Early Superior Seedless then Flame Seedless cvs. High concentration of Kombucha was most effective in the reduction of detached berries decay than the lower concentrations. Decay percentages were highest after 48 h after inoculation than lowest periods. Then Kombucha was sub-lethal or nearly lethal agent to *Botrytis cinerea* spores especially if applied after 48 hours from inoculation.

Table (2): Incidence percentage of gray mold on detached 'Flame Seedless' (F), Early Superior Seedless (E) and 'Thompson Seedless' (T) berries inoculated with spores of *B. cinerea* prior to immersion for 30 s in various concentrations of Kombucha or distilled water (0) as control and storage for 7 days at 15°C.

Berries	Kombucha cons(%)	Percentage of decayed detached berries after inoculation by 2 hours			12 hours			24 hours			48 hours		
		F	E	T	F	E	T	F	E	T	F	E	T
Pedicel intact	Control (0)	68	72	76	79	83	87	84	88	92	88	93	98
	20	33	35	37	36	38	40	41	43	45	51	54	57
	40	17	18	19	29	30	32	33	35	37	37	39	41
	60	7	7	7	13	14	15	16	17	18	20	21	22
	80	0	0	0	3	3	3	4	4	4	6	6	6
	100	0	0	0	0	0	0	0	0	0	0	0	0
Pedicel detached	Control (0)	81	85	87	89	94	96	92	97	99	93	98	100
	20	41	43	44	51	54	55	55	58	59	73	77	79
	40	24	25	26	38	40	41	46	48	49	52	55	56
	60	10	10	10	17	18	18	22	23	23	26	27	28
	80	1	1	2	5	5	6	6	6	7	7	8	9
	100	0	0	0	1	2	2	2	1	3	2	3	4

2.2. Effect on entire clusters inoculated:

Fig. (2) show that Kombucha was similar to SO_2 in antifungal efficacy as they induced high reduction in the number of decayed berries/kg comparing with the control (untreated patch).

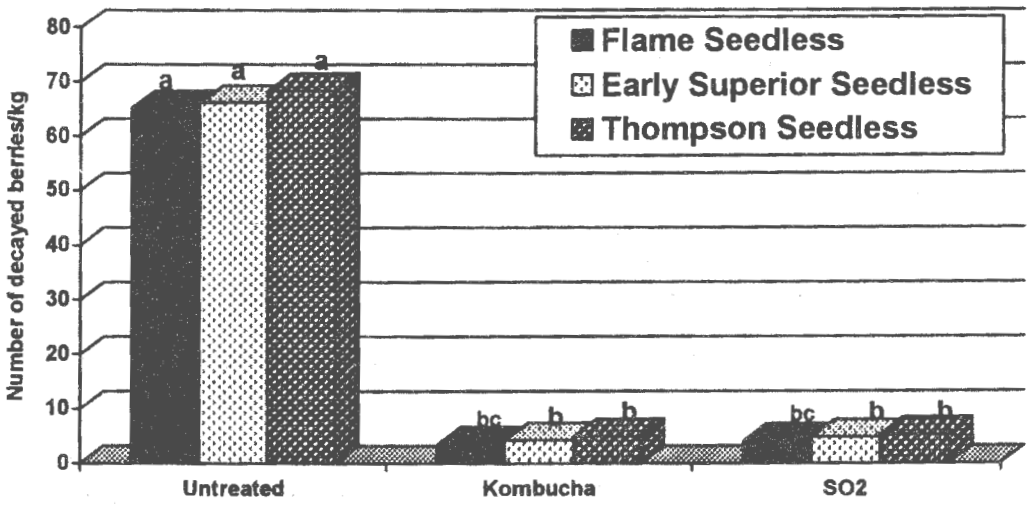

Fig. (2): Incidence of gray mold on 'Flame', 'Early Superior' and 'Thompson' seedless clusters inoculated with spores of *B. cinerea* prior to immersion for 30 sec in filtrate of Kombucha or treated or not with SO_2 and storage for 30 days at 1°C (RH >90%).
Column with unlike letters differ significantly according to Duncan's multiple range test at P ≤0.05.

2.3. Effect on entire clusters naturally infested:

Natural infection of grapes by *Botrytis cinerea* was significantly reduced by Kombucha or SO_2 as compared with the water control (Fig. 3). Kombucha treatment was almost similar to SO_2 in antifungal efficacy, they induced high reduction in the number of decayed berries/kg as compared with control (untreated patch).

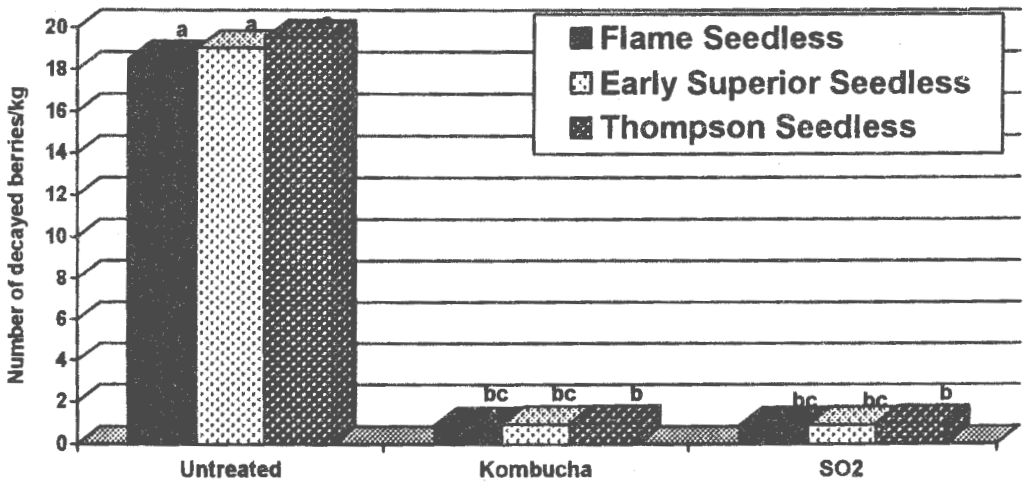

Fig. (3): The natural incidence of decayed berries of 'Flame', 'Early Superior' and 'Thompson' seedless clusters after treatment and storage for one month at 0 – 1°C (RH >90%).
Column with unlike letters differ significantly according to Duncan's multiple range test at P ≤0.05.

3. Sensory quality

Sensory analyses of the berries treated with Kombucha and SO_2 resulted in high scores for all analyzed parameters as compared with untreated control (Table, 3). Control grapes lost 7–9% of their initial weight during cold storage. Meanwhile, Kombucha and SO_2 treatments were significantly reduced the weight loss than control. The lowest number of cracking or shattering berries per kilogram were recorded as a result to Kombucha treatment and almost similar to SO_2 treatment. Kombucha treatment recorded the lowest score of berry and rachis appearance, followed by SO_2 treatment. Flavour in Kombucha treatment was superior than SO_2 or control especially with Thompson cv. Change in skin color of the grapes increased more slowly in Kombucha and SO_2-treated fruit than in the controls. At the end of cold storage, color of Flame Seedless cv. fruits was more reddish in the than Kombucha and SO_2-treated fruits, showing characteristics of over-ripped fruit, considered to be detrimental to color quality. The beneficial effect of Kombucha and SO_2 coating was also clear in delaying rachis dehydration and browning, which is associated with decayed berries.

Table (3): Effect of Kombucha filtrate, sulfur dioxide or water treatments on the quality attributes of inoculated 'Early Superior Seedless (E), Flame Seedless (F) and Thompson Seedless (T)' table grapes after storage for 4 weeks at 0 – 1°C (RH >90%).

Parameters / Treatments	Weight Loss (%)			Cracking (No. of cracked berries/kg)			Shatter (No. of drop berries/kg)		
	E	F	T	E	F	T	E	F	T
Control	7.2a	7.5a	8.1a	8.4a	8.6a	8.7a	46.7a	47.1a	48.2a
Kombucha	2.1d	2.3d	2.4de	1.4e	1.8e	1.6e	4.8e	5.2e	6.1e
SO_2	3.2c	2.8bc	2.2e	2.3	2.7	2.5	7.2d	6.4d	8.3d

Parameters / Treatments	Flavour (score index 1 - 5)			Berry appearance (score index 1 - 5)			Rachis appearance (score index 1 - 5)		
	E	F	T	E	F	T	E	F	T
Control	3.8a	3.9a	4.0a	3.3a	3.4a	3.5a	3.4a	3.5a	3.5a
Kombucha	1.5c	1.8c	1.2c	1.8d	1.9d	2.1c	2.1c	2.2c	1.8c
SO_2	2.3bc	2.2bc	2.6bc	2.2c	1.9d	2.4c	2.2bc	2.6b	2.4bc

* Values in column followed by unlike letters differ significantly according to Duncan's multiple range test at $P < 0.05$.

DISCUSSION

Decaying disease seems to become increasingly important in Egyptian fruit-production and cause economically important damage on all table grape cultivars. Post-harvest gray mold, caused by *Botrytis cinerea* Pers., is a major cause of decay of table grape berries. It develops during both commercial cold storage, typically at –0.5 to 0°C, and subsequent transport and marketing at warmer temperatures. *B. cinerea* is trouble some because it can grow at cold temperatures and spread rapidly by aerial mycelial growth among the fruit (Mlikota *et al.*, 2005). Although, its defects, sulfur dioxide generating pads are used worldwide for the control of gray mold, caused by *Botrytis cinerea*, during long-term cold storage and export shipment of table grapes. So, this study designed to using a natural (non-chemical) alternative antifungal bioagent to control bunch rot of grapes.

Kombucha is an Asian-East name for sweetened tea fermented using a macroscopic solid mass colony of microorganisms usually consisting principally of Acetobacter-species and yeast cultures.

Antimicrobial activity of fermented tea (Kombucha) against a number of pathogenic microorganisms was due to the organic acids, primarily acetic acid (Greenwalt *et al.*, 1998).

According to the literature on Kombucha, acetic acid is considered to be responsible for the inhibitory effect towards a number of microbes tested. This finding suggests the presence of antimicrobial compounds other than acetic acid and large proteins in fermented tea (Sreeramulu *et al.*, 2000). Levels of acetic acid and gluconic acid were found to increase with fermentation time (Sreeramulu *et al.*, 2001).

In this study, exposure of *B. cinerea* spores for 30 sec to Kombucha filtrate at high concentrations (80 and 100%) completely prevented the spore germination on PDA plates under laboratory conditions.

The decay incidence of detached berries without pedicel were more sensitive to decay with *Botrytis* than those with pedicel. Also, Thompson Seedless was the most susceptible cv. to infection, followed by Early Superior Seedless then by Flame Seedless. High concentration of Kombucha was most effective in the reduction of detached berries decay than lower concentrations. Decay percentages were highest especially after 48 h from inoculation than lowest periods. Then Kombucha was sub-lethal or nearly lethal agent to *Botrytis cinerea* spores.

Near-harvest, naturally entire clusters of 'Flame', 'Early Superior' and 'Thompson' seedless grapes were dipped in Kombucha for 30 sec 48 h prior harvesting. Other group was covered with SO_2-generator pads before packages. Kombucha has high significantly reduced the number of decayed berries per kilogram, similarly almost with SO_2 as compared with the untreated control. The same trend was achieved with inoculated postharvest entire clusters of the three tested table grape cvs. at 16°Brix which immersed in Kombucha for 30 sec and fumigated with SO_2-generator pads and stored in the same conditions.

Sensory analyses of the berries treated with Kombucha and SO_2 resulted in high scores for all analyzed parameters as compared with untreated control. Kombucha enhanced all of the tested quality specifications, in addition to its excellent role as antifungal bioagent, so it is increased the shelf-life of treated table grapes.

In other studies, there are no prior reports describing the use of Kombucha to control postharvest decay of table grapes.

As acetic acid is considered to be responsible for the inhibitory effect towards a number of microbes (Sreeramulu *et al.*, 2000). Acetic acid vapours completely inhibit growth and spore germination of *B. cinerea, Penicillium* sp. and *Rhizopus stolonifer* at 4 and 6 µl/L, respectively. It is also, at different concentrations significantly reduced gray mould incidence in table grapes (Thompson, Flame 'seedless' and Red Roomy cvs.) artificially inoculated with the spore suspention (10^6 spore/ml) of *B. cinerea* then stored at 20°C for 21 days. Disease incidence decreased by increasing concentrations of acetic acid vapours (Abd-El-Kareem, 2001). Similar results was recorded by Morsy *et al.* (1999) who found that spore germination was completely inhibited after immersion *B. cinerea* spore in 0.75 ml/L acitic acid. Strawberry fruits inoculated with *B. cinerea* spore suspention (10^6 ml/L) were fumigated with acetic acid vapours at 20 and 30 µl/L and gray mould incidence reduced was by 80.1 and 77.2%.

Cuticular fractures have been associated with increased susceptibility of grape berries to infection with *B. cinerea* (Mlikota *et al.*, 2003). Therefore, the higher incidence of decay in berries that were inoculated after their pedicel was removed was expected (Coertze and Holz, 1999).

Shehata and Lila (2005) found that Kombucha filtrate preventing *Alternaria solani* growth which is responsible for *Alternaria* tomato rot especially during storage in the refrigerator at 5°C and at room temperature (25°C). This may implies the

active antimicrobial component produced by Kombucha against *Botrytis cinerea* the causal of grape bunch rot.

Table grapes encounter severe problems during postharvest storage and retailing. The loss of quality is based on weight loss, color changes, accelerated softening and rachis browning, and high incidence of berry decay (Crisosto *et al.*, 2002), which leads to a reduction of shelf-life. Change in skin color of the grapes increased more slowly in Kombucha and SO_2-treated fruit than in the controls. Table grapes, such as 'Flame Seedless', are rich in anthocyanin compounds, which account for their red color, and ripening of berries has been correlated with the anthocyanin content in 'Red-globe' table grapes (Cantos *et al.*, 2002).

Browning symptoms firstly appeared on pedicels followed by lateral branches and finally on the central axis, as has been reported for 'Flame Seedless' table grapes, due to increased polyphenol oxidase activity (Carvajal-Millan *et al.*, 2001). In addition to antimicrobial activities, Kombucha has and antioxidant components which increased, as acetic acid, by increasing the fermentation period (Jayabalan *et al.*, 2007).

These results may be explained by noting that Kombucha containing, acetic acid, ethanol, and gluconic acid are the major components of the liquid broth (Roussin, 1996), other minor constituents such as lactic acid, glucuronic acid, phenolic acid, groups of vitamin B and enzymes are also present (Blanc, 1996), forms a film on the surface of the berries, which can act as antioxidant, a physical barrier to infection, reducing conidial germination and mycelial growth of *B. cinerea* and resulting in long-lasting protection of grape berries against gray mold.

CONCLUSION

Kombucha filtrate, expensive consumption as a healthful beverage as it is easily and safely produced at home, but scrimpy in the cost, can be useful as commercial applicable alternative antifungal to control table grape bunch rot near-harvesting without need to any chemical or fungicide applications and improving grape quality. Kombucha produced many vitamins, enzymes, organic acids *etc.*, so can be used on organically certified grapes.

REFERENCES

Abd-El-Kareem, F. (2001): Fumigation of table grapes with acetic acid vapours for controlling gray mould decay. Egypt. J. Phytopathol., 29(1):89-98.

Asai, T. (1968): Acetic acid Bacteria: Classification and Biochemical Activities. University of Tokyo Press, Tokyo, pp. 27–32.

Betsy, P. and H. Sonford (1996): Kombucha Phenomenon. The Miracle Health tea how to safely make and use kombucha. 2nd ed sierra sunrise Publishing Inc.

Blanc, P.J. (1996): Characterization of the tea fungus metabolites. Biotechnology Letters, 18(2):139-142.

Cantos, E.; Espın, J.C. and Tomas-Barberan, F.A. (2002): Varietal differences among the polyphenol profiles of seven table grape cultivars studied by LC-DAD-MS-MS. J. Agric. Food Chem. 50, 5691–5696.

Cappellini, R.A.; Ceponis, M.J. and Lightner, G.W. (1986): Disorders in table grape shipments to the New York market, 1972–1984. Plant Dis., 70: 1075–1079.

Carvajal-Millan, E.; Carvallo, T.; Orozco, J.A.; Martínez, M.A.; Tapia, I.; Guerrero, V.M.; Chu, A.R.; Llamas, J. and Gardea, A.A. (2001): Polyphenol oxidase activity, color changes, and dehydration in table grape rachis during development and storage as affected by N-(2-chloro-4-pyridyl)-N-phenylurea. J. Agric. Food Chem. 49, 946–951.

Coertze, S. and Holz, G. (1999): Surface colonization, penetration, and lesion formation on grapes inoculated fresh or after cold storage with single airborne conidia of Botrytis cinerea. Plant Dis. 83:917-924.

Crisosto, C.H.; Garner, D. and Crisosto, G. (2002): Carbon dioxide-enriched atmospheres during cold storage limit losses from Botrytis but accelerate rachis browning of 'Redglobe' table grapes. Postharvest Biol. Technol. 26,181–189.

Dipti, P.; Yogesh, B.; Kain, A.K.; Pauline, T.; Anju, B.; Sairam, M.; Singh, B.; Mongia, S.S.; Kumar, G.I. and Selvamurthy, W. (2003): Lead induced oxidative stress: beneficial effects of Kombucha tea. Biomed Environ Sci., 16(3):276-82.

Greenwalt, C.J.; Ledford, R.A. and Steinkraus, K.H. (1998): Determination and characterization of the antimicrobial activity of the fermented tea Kombucha. Lebensmittel Wissenschaft und Technologie, 31(3):291-296.

Jayabalan, R.; Marimuthu, S. and Swaminathan, K. (2007): Changes in content of organic acids and tea poly-phenols during kombucha tea fermentation. Food Chemistry, 102(1): 392-398.

Karabulut, O.A.; Gabler, F.M.; Mansour, M. and Smilanick, J.L. (2004): Postharvest ethanol and hot water treatments of table grapes to control gray mold. Postharvest Biol. Technol. 34, 169–177.

Karabulut, O.A.; Romanazzi, G.; Smilanick, J.L. and Lichter, A. (2005): Postharvest ethanol and potassium sorbate treatments of table grapes to control gray mold. Postharvest Biology and Technology, 37: 129–134.

Leroux, P.; Chapeland, F.; Desbrosses, D. and Gredt, M. (1999): Patterns of cross-resistance to fungicides in *Botryotinia fuckeliana* (*Botrytis cinerea*) isolates from French vineyards. Crop Protection, 18, 687–697.

Mayser, P.; Fromme, S.; Leitzmann, C. and Grunder, K. (1995): The yeast spectrum of the tea fungus Kombucha. Mycosis, 38:289-295.

Mirzaei, S.; Goltapeh, E.M. and Shams-Bakhsh, M. (2007): Taxonomical studies on the genus *Botrytis* in Iran. J. Agric. Tech., 3(1): 65-76.

Mlikota Gabler, F. and Smilanick, J.L. (2001): Postharvest control of table grape gray mold on detached berries with carbonate and bicarbonate salts and disinfectants. Am. J. Enol. Vitic. 52:12-20.

Mlikota Gabler, F.; Smilanick, J.L.; Mansour, M.; Ramming, D.W. and Mackey, B.E. (2003): Correlations of morphological, anatomical, and chemical features of grape berries with resistance to *Botrytis cinerea*. Phytopathology 93:1263-1273.

Mlikota, G.F.; Smilanick, J.L.; Ghosoph, J.M. and Margosan, D.A. (2005): Impact of postharvest hot water or ethanol treatment of table grapes on gray mold incidence, quality, and ethanol content. Plant Dis. 89:309-316.

Morsy, A.A.; Abd-El-Kareem, F. and Abd-Alla, M.A. (1999): Effect of acetic acid on postharvest decay of strawberry fruits. Egypt. J. Phytopathol., 27:117-126.

Roussin, M.R. (1996): Analysis of Kombucha Ferments: Report on Growers. Salt Lake City, UT: Information Resources, LC.

Shehata, Sawsan F. and Lila Abdel-Aty (2005): Antimicrobial activity of the fermented Kombucha. Annals Agric. Sci., Ain-Shams Univ., Cairo, 50(2):467-477.

Sreeramulu, G.S.Y.; Zhu, Y. and Knol, W. (2000): Kombucha fermentation and its antimicrobial activity. J. Agric. Food Chem., 48:2589-2594.

Sreeramulu, G.S.Y.; Zhu, Y. and Knol, W. (2001): Characterization of Antimicrobial Activity in Kombucha Fermentation. Acta Biotechnologica, 21(1):49-56.

Zahavi, T.; Cohen, L.; Weiss, B.; Schena, L.; Daus, A.; Kaplunov, T.; Zutkhi, J.; Ben-Arie, R. and Droby, S. (2000): Biological control of *Botrytis*, *Aspergillus* and *Rhizopus* rots on table and wine grapes in Israel. Postharvest Biol. Technol. 20:115-124.

استخدام راشح الكمبوشا قبل الحصاد لمقاومة عفن عناقيد عنب المائدة بعد الحصاد

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يعتبر عفن البوترايتس سينيرا في عناقيد العنب من أخطر أمراض ما بعد الحصاد التي تصيب العنب وخاصة أثناء التخزين والتصدير عبر البحار. لذلك أجريت هذه الدراسة في موسمي النمو ٢٠٠٦ – ٢٠٠٧ على ثلاث أصناف من العنب البناتي، بمزارع العنب التجارية بمنطقة النوبارية، محافظة البحيرة. ومن خلال التجارب المعملية أختبر نقع جراثيم الفطر في الكمبوشا بتركيزات مختلفة وزراعة محلول الجراثيم المخفف جداً على بيئة نمو ثم تسجيل عدد المستعمرات الناتجة بعد التحضين على درجة حرارة المعمل لمدة ٢٤ ساعة. كذلك اختبر نقع حبات العنب المنفصلة عن العناقيد في نفس تركيزات الكمبوشا السابقة بعد عدواها وهي متصلة بعنقها أو منفصلة عنه بمعلق جراثيم الفطر رشاً. نقع عناقيد أصناف العنب الثلاثة الكاملة سواء على كرومها أو في المعمل في راشح الكمبوشا لمدة ٣٠ ثانية بعد عدواها أو عدم عدواها بجراثيم الفطر رشاً ثم التخزين لمدة ٤ أسابيع في درجة حرارة من صفر وحتى واحد درجة مئوية ودرجة رطوبة أكبر من ٩٠%. وتمت مقارنة تلك المعاملات باستخدام مولدات ثاني أكسيد الكبريت المستخدمة عالمياً وعلى نطاق واسع في مقاومة هذا المرض. وتركت مجموعة بدون معاملات للمقارنة. ولمعرفة هل هناك تأثير سلبي للكمبوشا على صفات جودة العنب أجريت اختبارات الصفات النوعية للعنب وشملت لون الثمر ولون العنقود ونسبة حبات العنب المنفصلة عن العناقيد والصغيرة الحجم وغيرها على نفس المجموعات والمعاملات السابقة. وقد أدت معاملة جراثيم الفطر بالكمبوشا إلى خفض حيويتها ونسبة إنباتها وقضت عليها تماماً بالتركيزات العالية. كما أدى نقع العناقيد الكاملة لمدة ٣٠ ثانية في الكمبوشا المركزة إلى حدوث خفض شديد يصل إلى حد المنع في النسبة المئوية لحبات العنب التالفة أو عدد الحبات التالفة من كل كيلو جرام عناقيد. من نتائج الدراسة وجد أن نتائج المعاملة بالكمبوشا المركزة يقترب من نتائج المعاملة بثاني أكسيد الكبريت بل ويتفوق عليها في بعض المعاملات إلا أن الكمبوشا (كائن حيوي يتكون من بكتيريا حمض الخليك والاكتيك و بعض الخمائر في معيشة تكافلية، يتخمر على الشاي المُحلى ويقوم بتحليل الشاي والسكر منتجاً عدة تحولات حيوية مفيدة إلى جانب إفرازه نواتج الأيض الثانوية له في المحلول) مادة طبيعية زهيدة الثمن تصلح لمعاملة العنب المنتج في الزراعة العضوية ويمكن باستعماله في نقع العناقيد قبل الحصاد بيومين أو أكثر وهي على كرومها من تجنب حدوث المرض بعد الحصاد أو أثناء التصدير والاستغناء على استعمال المبيدات الفطرية أو الكيماويات قبل وبعد الحصاد لمقاومة هذا المرض.