Effect of Ethanol Vapour and Mineral Oil to Reduce or Prevent Gray Mold During Postharvest Life of Table Grapes

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ABSTRACT

This investigation was conducted on grapes in two seasons 2007 and 2008 to study the effect of ethanol vapour and mineral oils (CAPL2) to reduce decay of table grapes.

Weight loss, showed lower significancy with ethanol vapour treatment. Titratable acidity decreased significantly with mineral oil CAPL2 (1.5%) and ethanol vapour treatments. TSS% and pH were significantly higher with mineral oils (0.5 and 1%). Decay was decreased significantly with CAPL2.

INTRODUCTION

Fungal decay is the major cause as rapid and extensive postharvest deterioration of table grapes. Two major organisms of concern are the fungi *Botrytis cinerea*, and *Aspergillus niger* (Nelson, 1969).

Relative efficient control of these pathogens in grapes was achieved by application of sulfur dioxide gas, either by frequent fumigation in storage rooms or by packing the grapes with SO_2 generator pads (Luvisi *et al.*, 1992). This technology is very efficient but fruit taste may be compromised and it may cause damage to the berry which is manifested as cracks and leaching. In addition, hypersensitivity was reported in humans justifying the search for alternative technologies. One of the technologies that have been developed recently is based on surface sanitation of the berry which is dipped in ethanol solution or ethanol vapor which are very efficient (Lichter *et al.*, 2002).

Postharvest gray mold, caused by *Botrytis cinerea* Pers., is a major cause of decay of table grape (Barkai-Golan, 2001, Lichter *et al.*, 2002 and Nelson, 1985). 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 troublesome because it can grow at cold temperatures and spread rapidly causing aerial mycelia growth among the fruit.

CAPL2 (E.C, 96.62%) a new mineral oil material (which is used for scale insect control) (Mesbah *et al.*, 2010) was tested to evaluate its efficacy against postharvest gray mold caused by *Botrytis*

cinerea compared with the ordinary grapes gray mold control material which is called Ethanol.

Postharvest disease of perishable fruits such as table grape renders heavy losses during transit and storage. Use of chemical fungicides such as SO_2 generator pads are basic means of controlling postharvest diseases of table grape. Public awareness about chemical residues in food and interest to use of organic crops intended search to find a new safe alternatives of synthetic fungicides. The main reason for examine the possibility use of essential oil is that they are of plant origins, safe, no side-effects and their acceptability for consumers. Consequently, recently the interest to use of essential oils to preserve of fruits and vegetables has increased. The goal of this research was to examine the possibility of using mineral oils to maintain postharvest quality of table grapes (Abbas *et al.*, 2011).

MATERIALS AND METHODS

Present study was carried out over two successive grape seasons (2007 and 2008) on Thompson seedless c.v.

Grapes were harvested from a private orchard in Nobaria Farm. Grape berries were delivered to the postharvest Center of Horticulture Crops, Faculty of Agriculture (Al-Shatby), Alexandria University. The grapes initial qualities are shown in Table (1).

Table (1) : Initial quality of berries and test (three replicates each season).

Replicates	Weight	Length	Width	Volume of juice	Weight of juice	TSS (%)	V.C	Acidity	pH
	Season 1								
1	452.4	1.00	0.02	440	443.6	13.5	0.90	0.80	11.60
2	467.2	0.08	0.02	444	438.6	15.0	0.90	0.84	11.32
3	550.6	0.04	0.02	540	543.0	15.5	0.89	0.80	11.52
Means	490.06	0.37	0.02	474.66	475.06	14.66	0.89	0.81	11.48
	Season 2								
1	398.2	0.06	0.02	380	377.8	15.5	0.9	0.81	11.66
2	496.2	0.08	0.02	500	488.6	15.5	0.9	0.82	11.62
3	517.2	0.08	0.02	520	516.4	15.0	0.89	0.85	11.60
Means	470.53	0.073	0.02	466.66	460.93	15.33	0.89	0.83	11.63

Fruits :

- Thompson Seedless grape (Vitis vinifera L.) were harvest from a private orchard at Nobaria, EL-Behera governorate.

Tested materials:

- CAPL2 oil® 96.62% E.C. (light/ summer mineral oil) which was produced by Central Agricultural Pesticides Laboratory (CAPL), Agriculture Research Center, Ministry of Agriculture

- Ethanol (96%) from El-Gomhouria CO. For Trading Chemicals And Medical Appliances.

Inoculum preparation:

- *Botrytis cinerea* (Family : Sclerotiniaceae) isolate from grape [provided by Faculty of Agriculture (Saba Basha), Alexandria University] was grown on potato dextrose agar medium for 2 weeks at 23°C. Spores were rubbed from the agar surface with a glass rod after adding a small volume of sterile water plus surfactant 0.05% (wt/vol) Triton X-100. The spore suspension was vigorously shaken and then filtered through four layers of cheesecloth. The suspension was diluted with sterile water to an absorbance of 0.25 at 425 nm as determined by a US 7-100 double beam spectrophotometer. This density contained 1.0 × 106 conidia/ml and was diluted with sterile water to obtain the desired spore concentrations. A volume of 50 ml of inoculuae was applied with an air-brush sprayer.

Grapes treatment:

An experiment, was carried out to evaluate the effect of four treatments [Ethanol 96% and three concentrations (0.5, 1.0 and 1.5%) of CAPL2 (96.6%) mineral oil] on gray mold incidence on Seedless grape after inoculation 2, 4, 6, 8 and 10 weeks. Grapes were sprayed by the *B. cinerea* suspension as described previously. Inoculated grapes were air dried then immersed for 1 min in treatments material solutions and dried by ear then incubated at 5°C in a covered plastic sacks in carton boxes until treated. After 2, 4, 6, 8 and 10 weeks gray mold incidence was determined by counting the number of infected grapes. Statistical analysis of variance and LSD value for comparing the mean effects of each treatment were adopted.

Treatment of table grape clusters:

Thompson Seedless grape were divided into small clusters of approximately 100 similar beads each at random so that a portion of each cluster was represented in each replicate. Each replicate consisted of three bags of grape fruit and each treatment was applied to four replicates. After the treatment, the grape clusters were air dried on a wire rack, then repacked in new ventilated polyethylene bags and arranged in commercial corrugated fiberboard boxes. Boxes were loosely covered with large polyethylene bags to retard moisture loss and placed in storage at 0.5°C. The incidence of infected grapes and the quality of the grape fruit were determined.

Clusters were packed in carton boxes at the dimensions of 50× 30× 20 for length, width and height respectively and divided into six sections each one consists of 3 clusters, each group were sampled for the following tested treatments:

1) Stored clusters of groups at 0°C (T_1) non inoculation with fungi or chemicals (control).
2) Clusters were inoculated with *Botrytis cinerea* and stored at 0°C (T_2) (control).
3) Inoculcated clusters with *Botrytis cinerea* were dipped in mineral oil (0.5%) for 1 min. (T_3).
4) Inoculcated clusters with *Botrytis cinerea* were dipped in mineral oil (1%) for 1 min. (T_4).
5) Inoculcated cluster with *Botrytis cinerea* were dipped in mineral oil (1.5%) for 1 min. (T_5).
6) Inoculcated cluster with *Botrytis cinerea* were evaporated with ethanol (50%) (50 ml ethanol + 50 ml water) for 30 min for 1 kg fruits (T_6).

Clusters were taken to determine the initial physio-chemical properties which were followed up in 2 weeks intervals throughout the experimental working period as follows :

A) Weight loss:

- Clusters of the treatments were initially weighted to calculate cluster weight loss percent during the storage period in relation to its original weight as the following equations:

$$\text{Weight loss \%} = \frac{\text{Initial weight - sample weight}}{\text{Initial weight}} \times 100$$

B) Soluble solids content % (SSC) for 100 berries:

- SSC of berries were determined using hand refractometer according to Chen and Mellenthin (1981). The total soluble solids were expressed as percent (%).

C) Titratable acidity (TA):

- Five (ml) of juice of fruits were used to determine the titratable acidity. For the titration 0.1N sodium hydroxide was used in presence of phenolphthalein as an indicator according to Chen and Mellenthin (1981).
- The titratable acidity was expressed as gms of citric acid per 100 ml of grapes juice.
- All data were statistically analyzed according to Sendecor and Cochran (1980).

Statistical analysis:

The incidence of gray mold was analyzed by a three-way analysis of variance and L.S.D value for comparing the mean of each treatment. Means were separated by Fisher's Protected least significant difference (P = 0.05, Super ANOVA, according to Cohort, Software, Inc. (1986).

RESULTS AND DISCUSSION

Laboratory experiment was carried out to evaluate the effect of two materials (Ethanol and CAPL2 (96.62%) E.C mineral oil). Samples of three replicates for six treatments (untreated, infected control, ethanol and three concentrations of CAPL2 mineral oil) were taken within five times intervals (2, 4, 6, 8 and 10 weeks) where it was stored under 5°C and 80-90% R.H.

The results data in Fig. (1) indicated that CAPL2 with (1%) concentration was the most effective one of the tested treatments with significant differences in between during different tested times, followed by CAPL2 (1.5%), Ethanol and CAPL2 (0.5%).

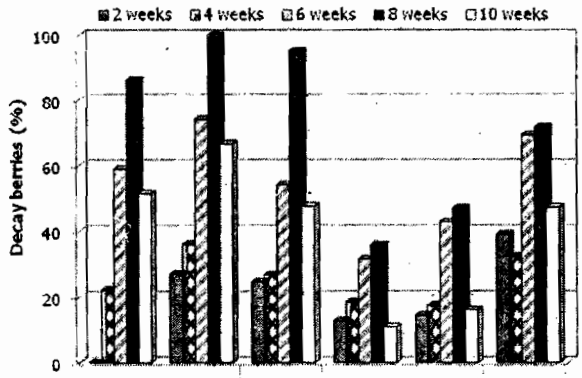
There was no significant differences between CAPL2 (1.0%) and CAPL2 (1.5%), but there were significant differences among the other treatments. Decay percentages increased with sampling time intervals where it reached after 10 weeks to (67%) for untreated grapes, (87%) for infected control, (63%) for CAPL2 (0.5% conc.), (15%) for CAPL2 (1.0% conc.), (22%) for CAPL2 (1.5% conc.) and (61%) for ethanol.

Weight loss (%) of grapes:

Effects of mineral oil and ethanol vapour on weight loss percentages of grapes in both 2007 and 2008 seasons during storage at 0°C were tabulated in Table (2). It was noticed that cluster weight loss percentages had significantly decreased with clusters treated with ethanol vapour compared with all another treatments. The obtained results were in agreement with those found by Ozgur *et al.* (2004) who reported that ethanol vapour treatment caused weight loss.

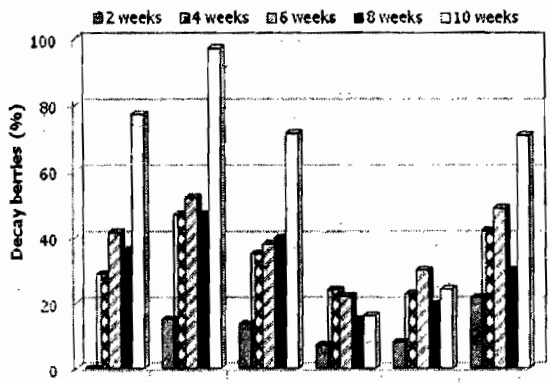
Weight loss increased slightly and gradually from the beginning of cold storage till the end of storage period Table (2)

The weight loss is mainly due to the result of water loss from the fruit tissues and partially of the respiration process and the higher storage temperature (EL-Yaten and Kader, 1984) and (EL-Saedy and EL-Nagar, 2005).



	Untreated	Infected control	CAPL2 oil 0.5%	CAPL2 oil 1.0%	CAPL2 oil 1.5%	Ethanol
2 weeks	0	27	24	13	14	39
4 weeks	22	36	27	18	17	32
6 weeks	59	74	54	31	43	69
8 weeks	86	112	95	36	47	72
10 weeks	51	67	47	11	16	47

Treatments 2007



treatment	Untreated	Infected control	CAPL2 oil 0.5%	CAPL2 oil 1.0%	CAPL2 oil 1.5%
2 weeks	0	15	14	7	8	22
4 weeks	29	47	35	24	23	42
6 weeks	41	52	38	22	30	49
8 weeks	36	47	40	15	20	30
10 weeks	77	97	71	16	24	71

Treatments 2008

Fig. (1) : Effect of ethanol vapour and CAPL2 mineral oil against gray mold, caused by *Botrytis cinerea* on Postharvest Thompson seedless grapes in 2007 and 2008 seasons

Total Soluble Contents (SSC):

The data in Table (3) showed that in the first season, there was higher significant effect on clusters that were untreated with fungi and chemicals (T_1 and T_2) compared with all another treatments whereas in the second season there were higher significant differences between berries which were treated with mineral oil (0.5 and 1%) and berries were untreated with chemicals and treated with fungi (T_2), these results agree with those observed by Baiano *et al.* (2004).

The trend of total soluble solids percentage was decreasing until the end of storage, these results were in agreement with the results of Yun Deng *et al.* (2005) and Arie (2002) who reported that SSC percentage was decrease during storage.

Grapes are non-climacteric fruits which characterized by a lack of starch as a carbohydrate reserve, (Lakshminarayana *et al.*, 1979).

pH value:

The data presented in Table (4) declared the effect of ethanol and mineral oil on pH values of juice of grapes during cold storage. The results clearly indicated that in the first season, there was high significant differences between clusters which were treated with mineral oil (0.5 and 1%) and all other remaining treatments.

In the second season, there was high significant differences between clusters which were treated with mineral oil (0.5%) and those subjected all treatments.

The obtained results in both seasons were in line with those found by Sholberg (1996).

As for the effect of storage period on pH values, the data in Table 4 indicated that, in both seasons, the pH values decreased with storage period. These results agree with that of Baiano *et al.* (2004).

Titratable acidity (%):

Organic acids found in grapes include citric, malic and tartaric acids; however, the major acid accounting for titratable acidity in grapes is tartaric acid. (Soyer *et al.*, 2003).

Effect of ethanol vapour and mineral oils on titratable acidities of grapes in both seasons are presented in Table (5).

The data of both seasons indicated that acidity content decreased significantly as a result of treating berries by ethanol vapour and mineral oil (1.5%) compared with other treatments. This result agreed with that of Lana and Edna (2008) who studied the control of postharvest decay grapes using acetaldehyde vapours and Chervin *et al.* (2005). In addition, the storage period showed the acidities content decreased gradually until 70 days while it increased in the end storage in both seasons (Tables 5). The

results obtained in both seasons of study are in agreement with (Yohanan Zutahy *et al.*, 2008).

Table (2): Effect of ethanol vapour and mineral oils on weight loss (%) of cv. Thompson seedless grapes in 2007 and 2008 seasons.

Treatments	1	2	3	4	5	6	Means
	Season 2007						
T_1	0	3.51	5.90	9.47	14.75	16.41	8.34
T_2	0	5.93	8.47	10.93	13.47	15.59	9.07
T_3	0	2.45	4.75	10.60	12.21	13.55	7.26
T_4	0	3.00	5.93	8.27	10.61	12.07	6.65
T_5	0	3.18	5.95	8.51	10.31	12.34	6.72
T_6	0	2.02	5.37	6.70	11.61	14.19	6.64
Means	0	3.35	6.06	9.07	12.16	14.03	
	Season 2008						
T_1	0	4.05	6.83	10.17	13.35	13.30	7.95
T_2	0	7.83	10.55	11.79	13.49	14.40	9.68
T_3	0	3.38	6.34	9.24	12.05	15.25	7.71
T_4	0	2.93	5.58	7.61	9.39	12.51	6.34
T_5	0	1.83	5.01	9.63	11.26	14.62	7.06
T_6	0	5.87	8.18	8.53	9.05	9.50	6.86
Means	0	4.31	7.08	9.50	11.43	13.26	

Season 2007 L.S.D$_{0.05}$ = 1.522
Season 2008 L.S.D$_{0.05}$ = 1.558

Table (3): Effect of ethanol vapour and mineral oils on SSC (%) of cv. Thompson seedless grapes in 2007 and 2008 seasons.

Treatments	1	2	3	4	5	6	Means
	Season 2007						
T_1	14.67	15.00	13.73	14.33	14.67	14.67	14.511
T_2	14.67	14.66	14.33	14.67	14.33	14.33	14.50
T_3	14.67	14.33	14.0	14.33	14.00	14.0	14.22
T_4	14.67	10.00	14.40	14.47	14.33	14.86	13.79
T_5	14.67	12.00	14.33	14.27	14.33	13.67	13.88
T_6	14.67	14.33	13.87	14.33	14.07	13.20	14.07
Means	14.67	14.11	13.39	14.40	14.29	14.12	
	Season 2008						
T_1	15.33	14.33	14.67	14.00	15.47	15.00	14.80
T_2	15.33	14.67	14.80	14.67	13.33	14.87	14.61
T_3	15.33	14.33	14.00	12.53	14.00	15.27	14.24
T_4	15.33	14.00	14.67	13.60	14.00	14.33	14.32
T_5	15.33	13.67	14.00	12.93	14.33	13.00	13.88
T_6	15.33	13.67	13.67	14.33	14.00	13.87	14.14
Means	15.33	14.11	14.30	13.67	14.19	14.39	

Season 2007 L.S.D$_{0.05}$ = 0.815, Season 2008 L.S.D$_{0.05}$ = 0.466

Table (4): Effect of ethanol vapour and mineral oils on pH (%) of cv. Thompson seedless grapes in 2007 and 2008 seasons.

Treatments	1	2	3	4	5	6	Means
	Season 2007						
T_1	11.48	4.68	3.57	4.62	4.13	4.12	5.43
T_2	11.48	4.40	3.22	4.52	4.07	4.01	5.28
T_3	11.48	4.40	4.47	4.55	4.12	3.86	5.48
T_4	11.48	4.53	4.54	4.19	3.99	4.00	5.46
T_5	11.48	4.36	3.99	4.07	3.96	3.89	5.29
T_6	11.48	4.41	4.09	3.99	3.90	3.81	5.28
Means	11.48	4.46	3.98	4.32	4.03	3.95	
	Season 2008						
T_1	11.63	4.48	4.32	4.53	4.00	3.94	5.48
T_2	11.63	4.36	4.09	4.55	4.03	4.05	5.45
T_3	11.63	4.43	4.34	4.33	4.11	4.06	5.48
T_4	11.63	4.62	3.41	4.10	4.03	4.04	5.31
T_5	11.63	4.47	4.13	3.84	3.94	3.90	5.32
T_6	11.63	4.54	4.11	3.98	3.86	3.78	5.32
Means	11.63	4.49	4.22	4.07	3.99	3.96	

Season 2007 L.S.$D_{0.05}$ = 0.085
Season 2008 L.S.$D_{0.05}$ = 0.0793

Table (5): Effect of ethanol vapour and mineral oils on titretable acidity (%) of cv. Thompson seedless grapes in 2007 and 2008 seasons.

Treatments	1	2	3	4	5	6	Means
	Season 2007						
T_1	0.81	0.61	0.63	0.49	0.56	0.65	0.63
T_2	0.81	0.56	0.64	0.56	0.49	0.63	0.62
T_3	0.81	0.64	0.68	0.53	0.53	0.65	0.64
T_4	0.81	0.55	0.61	0.61	0.50	0.58	0.61
T_5	0.81	0.53	0.47	0.55	0.47	0.60	0.57
T_6	0.81	0.60	0.51	0.49	0.48	0.60	0.58
Means	0.81	0.58	0.59	0.54	0.51	0.62	
	Season 2008						
T_1	0.83	0.48	0.69	0.59	0.59	0.59	0.63
T_2	0.83	0.50	0.74	0.59	0.51	0.58	0.63
T_3	0.83	0.55	0.74	0.57	0.56	0.64	0.65
T_4	0.83	0.55	0.73	0.57	0.48	0.59	0.62
T_5	0.83	0.63	0.46	0.48	0.49	0.57	0.58
T_6	0.83	0.59	0.48	0.50	0.48	0.68	0.59
Means	0.83	0.55	0.64	0.55	0.52	0.61	

Season 2007 L.S.$D_{0.05}$ = 0.0308
Season 2008 L.S.$D_{0.05}$ = 0.0318

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الملخص العربى

تأثير بخار الإيثانول والزيوت المعدنية لتقليل أو منع العفن الرمادى خلال فترة بقاء العنب بعد الحصاد

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*** محطة بحوث الصبحية بالإسكندرية- معهد بحوث البساتين- مركز البحوث الزراعية**

**** محطة بحوث الصبحية بالإسكندرية- المعمل المركزى للمبيدات- مركز البحوث الزراعية**

أجريت هذه الدراسة خلال موسمى ٢٠٠٧ ، ٢٠٠٨ على ثمار العنب وذلك لدراسة تأثير بخار الإيثانول والزيت المعدنى (كابل ٢) على تقليل العفن وتحسين القدرة التخزينية للعنب.

وأظهرت النتائج أن الثمار المعاملة ببخار الإيثانول يكون نسبة الفقد في الوزن للحبات أقل من المعاملات الأخرى وكذلك نسبة الحموضة تقل مع المعاملة ببخار الإيثانول بالزيت المعدنى (كابل ٢ بتركيز ١,٥%).

تجانس المواد الصلبة الذائبة يكون أعلى مع المعاملة بالزيت المعدنى (كابل ٢ بتركيزات ٠,٥% ، ١,٠%) وكذلك رقم الحموضة، ويقل العفن فى حبات العنب مع المعاملة بالزيت المعدنى (كابل ٢ بتركيزات ١,٠%، ١,٥%).