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Formulation of Essential Oils and Yeast for Controlling Postharvest Decay of Tomato Fruits

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ABSTRACT

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The yeast, *Saccharomyces cerevisiae*, *Candida tenuis* and the commercial backing yeast of *Saccharomyces cerevisiae* mixture (CBY) and/or peppermint, melon and rose essential oils were evaluated for their in vitro activity against the fungal growth of *Botrytis cinerea*, *Rhizopus stolonifer* and *Alternaria alternate* the causal agents of tomato fruit decay. *S. cerevisiae mixture* (CBY) proved it self to have the highest inhibitory effect on the growth of the pathogenic tested fungi followed by the two other yeast isolates *S. cerevisiae* and *C. tenuis*. All the tested concentrations of peppermint oil had not negative effect against the viability of tested yeasts, while significant reduction in the populations of all yeast isolates was observed at melon and rose oils treatments even at the lowest concentration tested. Peppermint oil showed superior inhibitory effect against the growth of tested pathogenic fungi followed by rose and melon oils, respectively. Mixtures of peppermint oil with any of yeast isolate showed high inhibitor effect against the pathogenic fungal growth compared with rose and melon oils mixtures. Under storage conditions, application of carnauba wax formula containing either *S. cerevisiae* or *S. cerevisiae* (CBY) combined with peppermint oil (1%) had more superior effect for reducing gray mould, soft rot and black rot incidence as well as disease development of tomato fruits, reaching up to 100% under artificial inoculation of decay pathogenic fungi. On the light of the obtained results in the present study, it could be concluded that the application of carnauba wax containing 1% peppermint oil combined with *S. cerevisiae* or *S. cerevisiae* (CBY), could control several post-harvest diseases of tomato fruit without affecting tomato fruit quality under storage conditions.

Keywords: Antagonistic yeasts, essential oils, tomato fruit decay

INTRODUCTION

Fresh-market tomatoes (*Lycopersicon esculentum* Mill.) are grown in most countries around the world. Throughout the harvest season, tomato fruits ripen in flushes or waves of production. Under warm environments, fruits can be picked once or twice a week at close to full color. In Egypt, tomatoes are available around a year, and the average yield is 30-35 ton per feddan (4200 m^2), therefore it is considered one of the most important vegetables crop for exportation purpose. Fresh market tomatoes are harvested by hand into plastic or palm tree

ashes boxes when fruits are between the mature green, breaker and pink stages of color development. Harvesting is done frequently to avoid over ripe fruit. Fruits are picked one to three times per week depending on weather or harvest period. Fruits are transported to packinghouses where they are washed and graded for size and color, then packed in cardboards. Cartons of fruits are typically stored in a temperature-controlled chamber (4-10 ℃) up to 15 days prior to shipment to markets. Preliminary standard tomato quality is based on uniform shape and freedom from defects. Tomato is susceptible to postharvest diseases caused by various pathogenic fungi. *Botrytis cinerea* [Pers., Ex. Fr.](gray mould), *Rhizopus stolonifer* [Fhrenb., Fr. Will] (soft rot) and *Alternaria alternata* [(Fr.) Keissl.] (Black mould or Alternaria rot) are the most important decay pathogens of tomato causing post harvest losses at high frequency [2].

Postharvest diseases affect a wide variety of crops particularly in developing countries which lack sophisticated postharvest storage facilities [21]. Losses caused by postharvest diseases are greater than generally realized because the value of fresh fruits and vegetables increases several-fold while passing from the field to the consumer [12]. Postharvest losses are estimated to range from 10 to 30% per year despite the use of modern storage facilities and techniques [17].

With the continued loss of currently used postharvest decay control measures (*i.e.* fungicides), there is a perpetual need to search for alternatives. The increasing recognition of the importance of fungal infections and the difficulties encountered in their treatment have stimulated the search for synthetic chemical fungicides alternative.

Biological control has been advanced as an alternative to synthetic fungicides and considerable success in laboratory and pilot scale tests has been realized utilizing antagonistic microorganisms to control postharvest diseases. Several antagonistic yeasts and bacteria have been isolated and shown to have a broad spectrum of activity against a number of postharvest pathogens on a variety of fruit [11].

Recently, interest has been shown in combining microbial biocontrol agents with other chemical components to increase their activity against post-harvest pathogens [9]. Essential oils are also considered a promising alternative with many having antifungal properties. However, very high concentration is needed when applied to real food systems [16,1]. Application of essential oil is a very attractive method for controlling postharvest diseases. Essential oils and their components are gaining increasing interest because of their relatively safe status, their wide acceptance by consumers, and their exploitation for potential multi-purpose functional use [26]. Essential oils have been used successfully in combination with a variety of treatments, such as antibacterial agents, mild heat and salt compounds [22].

The objectives of this study were to evaluate the effectiveness of peppermint, melon and rose oils and/or antagonistic yeast isolates to inhibit the mycelia growth of *Botrytis cinerea*, *Rhizopus stolonifer* and *Alternaria alternata* under *in vitro* conditions. Pre-storage approach formula of these bio-agents and essential oils was also evaluated for their ability to minimize decay incidence of tomato fruits during storage under natural and artificial inoculation conditions with the disease incidents.

MATERIALS AND METHODS

Pathogens and antagonists

One of each virulent pathogenic fungal isolates of *Botrytis cinerea*, *Rhizopus stolonifer* and *Alternaria alternata* and isolates of the antagonistic yeast, *i.e. Saccharomyces cerevisiae* [Meyen ex E.C. Hansen] and *Candida tenuis* [Berkh] were obtained from Plant Pathology Department of the National Research Centre, Giza, Egypt. These microorganisms were isolated from various healthy and decayed fruits, and their high pathogenic or antagonistic ability was examined during previous work at the same department. In addition, one mixture containing isolates of the backing yeast *Saccharomyces cerevisiae* (CBY) was also used in the present work. This yeast mixture produced commercially by The "Sugar and Complementary Industries Company, Hawamdia, Giza, Egypt" for the purpose of backing and food industries.

Growth media

Potato dextrose agar (Difco Laboratories, Detroit, MI) and NYDB [8 g of nutrient medium (Difco Laboratories, Detroit, MI), 5 g of yeast extract, and 10 g of

dextrose in 1 liter of water] were used for growing fungal and yeast isolates tested in the present work. Fungal and yeast cultures were maintained on PDA and NYD agar slant media at 5 ± 1 ℃ as stock cultures until use. All isolates were activated by growing at the optimum growth conditions at the beginning of the present experiments.

Preparation of fungal spores and yeast cells suspensions

Pathogenic fungal inocula were grown on PDA medium at 25 ± 2 ℃ until an abundant heavy growth of conidia was evident. Conidia were harvested by scraping the surface of the colonies with a spatula, transfered to sterilized distilled water and filtered through nylon mesh. All spore solutions were adjusted with sterile water to give a spore concentration of 10^6-10^7 spores per milliliter. Meanwhile, antagonistic yeast bio-agents were grown on NYDB medium and incubated in a rotary shaker at 200 rpm for 24 h at 28 ℃. The yeast cells were harvested by centrifugation at 6,000 rpm for 10 min, washed twice with 0.05 M phosphate buffer at pH 7.0, and re- suspended in distilled water. The concentrations of yeast cells in the suspensions were adjusted to 3×10^8 cells per milliliter. Concentrations of both yeast cells and fungal spores suspensions were adjusted with the aid of a haemacytometer slide.

Essential oils

Pure-grade of essential oils, *i.e.* peppermint (*Mentha piperita* L.); melon (*Citrallus lanatus* Thunb.) and rose oils (*Rosa damascena* Mill.) were obtained from Cairo Company for oils and aromatic extractions CID, Egypt. The essential oils were stored in dark glass bottles at 4℃.

In vitro growth inhibition of tested microorganisms

The inhibitory effect of essential oils and/or antagonistic yeast isolates on the growth of decay fungi was evaluated *in vitro*. In addition, the inhibitory effect of essential oils on the growth of antagonistic yeast isolates was also evaluated.

Essential oils *i.e.* peppermint, melon and rose oils at concentrations of 0, 0.25, 0.5 and 1% and yeast isolates (3×10^8 cell/mL) either as individual treatment or in combination were evaluated for their inhibitory effect against the linear growth of each of *B. cinerea*, *R. stolonifer* and *A. alternata*.

Emulsified stocks at high concentration of tested essential oils were prepared by dissolving in sterilized distilled water. Few drops of the emulsifier Tween 20 (Sigma Co.) were added to essential oil volumes to obtain emulsion feature. Different volumes of the essential oils emulsion were added to conical flasks containing 100 ml of sterilized PDA medium before its solidification to obtain the proposed concentrations. The supplemented media were poured into Petri-dishes (9 cm) about 20 ml each. Control check treatment was PDA medium free of essential oils.

Disks (5 mm-diameter) of each pathogenic fungi taken from seven days-old cultures were placed on the centre of Petri-dishes. All plates were incubated at 25 ± 2 ℃ until the tested fungi reach full growth in check treatment. Reduction in mycelial growth was calculated as percentage of fungal growth diameter in treatment relatively to the growth diameter in control.

The inhibitory effect of peppermint, melon and rose oils at the same previous concentrations on colony formed by antagonistic yeast isolates was assayed in NYPD broth using a modified method of[28]. Aliquots of 100 μL of the yeast cell suspension (3×10^8) were transferred to glass tubes (180×16 mm) containing 5 mL sterilized distilled water, then the tested emulsion essential oils were added individually to each tube to achieve the proposed concentration. All tubes were left for 6 h, then shaking well using magnetic stirrer for 5 min. One ml of each test tube was dispensed into Petri dish and about 20 mL of semi-solidifying sterilized NYPD agar medium were poured into the inoculated plates and rotated gently to ensure equal distribution of the yeast inocula. Control check treatment was the yeast cell suspension free from essential oils. All plates were incubated for 72 h and then examined. Percent of yeast isolates formed colonies was calculated by comparing with their counts in check treatment. All treatments consisted of three replicates, and experiments were repeated three times.

The interaction between yeast isolates and pathogenic fungi was evaluated as fungal growth inhibition *in vitro*. Dual culture technique after[14] was followed. Yeast isolates (48-h-old) were streaked individually on one side of 9 cm Petri dishes containing PDA medium, while 5 mm disks of

each individual fungal pathogen were placed on the opposite side of the yeast inoculated plates. Both tested microorganisms were placed 2 cm from the plate edges. A set of only fungal inoculated plates was used as control treatment. All plates were incubated at 25 ± 2 ℃ until full fungal growth occurred in check plates. Percentage of fungal growth reduction was calculated in yeast treatments relative to the fungal growth in check treatment.

The efficacy of combined formula between essential oils and yeast isolates against the growth of pathogenic fungi was also evaluated. This test was carried out using Petri dishes containing PDA media supplemented with the above mentioned essential oils concentrations. Growth inhibition of pathogenic fungi affected by Yeast isolates in the presence of essential oils in the growth medium was evaluated following the dual culture technique [14]. All the procedures of PDA supplementation with essential oils concentrations, plates inoculation with the isolates of yeast and fungal inocula, plates incubation and growth reduction measurement were carried out as stated before.

In vivo incidence of postharvest decay of tomato fruits

Since the peppermint oil as well as the yeast isolates *S. cerevisiae* and *S. cerevisiae* (CBY) showed high inhibitor effect against the *in vitro* fungal growth comparing with the other factors tested, therefore, the efficacy of combined formula between peppermint oil at 1% and/or yeast isolates was evaluated against the decay incidence of tomato fruits storage conditions.

Carnauba or paraffin waxes were used as the basic carrier solutions for the mixtures of peppermint oil and yeast isolates. Apparently healthy fruits of tomato (*Lycopersicon esculentum* Mill.) cv. Kasel Rock recently harvested were collected from El-Ebour market, the principle commercial market for vegetables and fruits, at Cairo, Egypt. Tomato fruits were graded into uniformity of size and maturity (pink stage of red color development), then surface disinfested by dipping them into 2% sodium hypochlorite solution for 2 min, rinsed three times with sterilized distilled water and left for air-dried onto filter paper prior to use.

The tomato fruits were wounded by a 1 mm diameter needle at one marked point and dipped individually into the solution of a tested chemical. After 2 h, the treated fruits were artificially inoculated individually by spraying with a fungal (1×10^6 spores/mL) spore suspension. Check treatment consisted of tomato fruits sprayed with sterilized distilled water. Thereafter, all treated fruits were air dried, placed into carton boxes (50 fruits per each), covered with plastic sheet to maintain a relative humidity at 100% and stored in cold room at 10 ± 2 ℃ for four weeks. Three boxes as replicates were used for each particular treatment as well as the control. At the end of storage period the decayed fruits were counted and then the percentage of disease incidence calculated in relative to control treatment. Furthermore, evaluation of the applied formula on the fruit rot development expressed as percentage of fruit rotted tissue weight was recorded relative to the whole infected fruit weight.

Statistical analysis

Tukey test for multiple comparisons among means was utilized [25].

RESULTS AND DISCUSSION

Postharvest biological control is a relatively new approach and offers several advantages over conventional biological control [30, 37]. Several biological control agents have been developed in recent years, and a few have actually been registered for use on fruit crops. Yeasts such as *Pichia guilliermondii* [39] and *Cryptoccocus laurentii*, a yeast that occurs naturally on apple leaves, buds, and fruit [31] were the first to be applied for control of postharvest decay on fruit. The yeast, *Candida oleophilia* has been registered for control of postharvest decay on fruit crops. The yeasts, *Cryptococcus infirmo-minutus* and *Candida sake* successfully control brown rot and blue mold on sweet cherry [33], and three diseases of apple [36], respectively, and may be developed into commercial products. In the present study the efficacy of yeast isolates was evaluated against the growth tomato decay fungi *in vitro*. Results showd that *S. cerevisiae* (CBY) mixture has the highest inhibitory effect on the growth of the pathogenic tested fungi followed the yeast isolates *S. cerevisiae* and *C. tenuis* (Table 1). Reduction in the growth of *B. cinerea*, *R. stolonifer* and *A. alternata* was recorded as 55, 53.8 and 56.1%, respectively, when the yeast *S. cerevisiae* (CBY) was inoculated in the growth medium.

Meanwhile, *C. tenuis* showed the lowest inhibitory effect on fungal growth which recorded as 31.9, 23.9 and 25% in respective order. Although there is no doubt that biocontrols are effective, they do not always give consistent results. This could be because biocontrol efficacy is so directly affected by the amount of pathogen inoculums present or antagonistic ability of the bio agent itself[32].

Compatibility with chemicals used during handling is also important. Indications are that biological control agents must be combined with other disease control strategies if they are to provide acceptable control. Several proposed non- fungicidal approaches, including the use of biological control with antagonistic microorganisms, heat treatment, induction of resistance, natural fungicides and plant extracts and essential oils have been extensively studied. Unfortunately, none of them, when used alone, can provide satisfactory levels of decay control when compared with synthetic fungicides[8, 10, 20, 40]. Thus, an integrated disease control strategy has been investigated, in the present study which is expected to provide high efficacy to biocontrol yeast agents. Peppermint, melon and rose essential oils were evaluated for their effect on viability of yeast isolates as well as the growth of pathogenic fungi. Furthemore, combinations between essential oils and yeast isolates were also evaluated against the decay fungal growth.

Data presented in Table 2 showed that all the peppermint oil at concentrations of 0.25, 0.5 and 1% have no inhibitory effect against the tested yeast isolates, while significant reduction in the populations of all yeast isolates was observed in melon and rose oils treatments even at the lowest concentration of 0.25%. This reduction was significantly different when compared with either peppermint or control treatments. Other investigators have also reported the inhibitory effect of some essential oils on viability of different beneficial or harmful yeast isolates[3, 7].

Furthermore, the individual inhibitory effect of tested essential oils against the linear growth of pathogenic fungi was shown in Tables 3, 4 and 5. Peppermint oil at

Table 1. Reduction in fungal growth in response to antagonistic effect of yeast isolates

Yeast isolate	Fungal growth reduction (%)					
	Botrytis cinerea		*Rhizopus stolonifer*		*Alternaria alternata*	
	Linear growth (mm)	Reduction (%)	Linear growth (mm)	Reduction (%)	Linear growth (mm)	Reduction (%)
C. tenuis	61.3 b[1]	31.9	68.5 a	23.9	67.5 a	25.0
S. cerevisiae	48.5 b	46.1	49.5 b	45.0	41.5 bc	53.8
S. cerevisiae (CBY)	40.5 bc	55.0	41.5 bc	53.8	39.5 bc	56.1
Control	90.0 a	-	90.0 a	-	90.0 a	-

[1] Data in each column with the same letter are not significant difference (P=0.05) according to Tukey test[25].

Table 2. Counts of yeast colonies affected by different concentrations of various essential oils *in vitro*

Treatment	Conc. of essential oil (%)	Number of colonies 10^6 (cfu/mL)					
		Candida tenuis		*Saccharomyces cerevisiae*		*Saccharomyces serevisea* (CBY)	
		Colony numbers	Reduction (%)	Colony numbers	Reduction (%)	Colony numbers	Reduction (%)
peppermint	0.25	292 a[1]	2.7	291 a	3.0	289 a	3.7
	0.50	289 a	3.7	288 a	4.0	284 a	5.3
	1.00	278 a	7.3	282 a	6.0	280 a	6.7
Melon	0.25	202 b	32.7	217 b	27.7	254 b	15.3
	0.50	175 c	41.7	204 b	32.0	231 b	23.0
	1.00	139 c	53.7	166 c	44.7	207 b	31.0
Rose	0.25	203 b	32.3	207 b	31.0	204 b	32.0
	0.50	177 c	37.7	178 c	40.7	188 c	37.3
	1.00	146 c	51.3	158 c	47.3	151 c	49.7
Untreated control		300 a	-	300 a	-	300 a	-

[1] Data in each column with the same letter are not significant difference (P=0.05) according to Tukey test[25].

Table 3. Growth reduction in response to peppermint oil in combination with yeast isolates *in vitro*

Treatment	Conc. of essential oil (%)	Fungal growth reduction (%)					
		Botrytis cinerea		*Rhizopus stolonifer*		*Alternaria alternata*	
		Linear growth (mm)	Reduction (%)	Linear growth (mm)	Reduction (%)	Linear growth (mm)	Reduction (%)
peppermint	0.25	82.1 a[1]	8.7	65.0 b	27.7	78.7 a	12.5
	0.50	65.0 b	27.7	53.3 c	40.7	64.5 b	28.3
	1.00	40.0 e	55.5	45.0 d	50.0	48.6 d	46.0
peppermint+	0.25	30.5 f	66.1	35.1 f	61.0	45.2 d	49.7
Canadida tenuis	0.50	31.0 f	65.5	29.5 fg	67.2	33.3 f	63.0
	1.00	21.3 g	76.3	18.0 eg	80.0	28.4 fg	68.4
peppermint+	0.25	30.0 f	66.6	21.5 g	67.1	35.2 f	60.8
Saccharomyces	0.50	24.2 fg	73.1	23.0 g	74.4	26.8 fg	70.2
cerevisiae	1.00	11.6 h	87.1	10.0 h	88.8	10.0 h	88.8
peppermint+	0.25	50.0 c	44.4	46.9 d	47.8	45.5 d	49.4
Saccharomyces	0.50	45.3 d	49.7	32.8 f	63.5	35.0 e	61.1
cerevisiae (CBY)	1.00	33.3 f	63.0	16.4 eg	81.7	28.0 fg	68.8
Untreated control		90.0 a	-	90.0 a	-	90.0 a	-

[1] Data in each column with the same letter are not significant difference (P=0.05) according to Tukey test[25].

Table 4. Growth reduction in response to melon oil in combination with yeast isolates *in vitro*

Treatment	Conc. of essential oil (%)	Fungal growth reduction (%)					
		Botrytis cinerea		*Rhizopus stolonifer*		*Alternaria alternata*	
		Linear growth (mm)	Reduction (%)	Linear growth (mm)	Reduction (%)	Linear growth (mm)	Reduction (%)
Melon	0.25	82.7 a[1]	8.1	80.0 a	11.1	89.0 a	1.1
	0.50	80.5 a	10.5	78.0 a	13.3	82.3 a	8.5
	1.00	77.0 b	14.4	74.6 b	17.1	76.2 b	15.3
Melon +	0.25	88.0 a	2.2	87.0 a	3.3	83.0 a	7.7
Canadida tenuis	0.50	88.0 a	2.2	85.5 a	5.0	78.6 b	12.6
	1.00	80.0 b	11.1	83.2 b	7.5	69.0 d	23.3
Melon +	0.25	85.1 a	5.4	83.0 a	7.7	74.4 b	17.3
Saccharomyces	0.50	65.6 d	27.1	80.0 a	11.1	68.5 d	23.8
cerevisiae	1.00	64.3 e	28.5	78.6 b	12.6	62.3 e	30.7
Melon +	0.25	76.6 b	14.8	84.0 a	6.6	78.0 b	13.3
Saccharomyces	0.50	65.2 d	27.5	81.0 a	10.0	70.6 c	21.5
cerevisiae (CBY)	1.00	60.4 e	32.8	73.0 b	18.8	67.0 d	25.5
Untreated control		90.0 a	-	90.0 a	-	90.0 a	-

[1] Data in each column with the same letter are not significant difference (P=0.05) according to Tukey test[25].

concentration of 1% showed superior inhibitor effect against the tested pathogenic fungi calculated as 55.5, 50.0 and 46.0% reduction in the growth of *Botrytis cinerea*, *Rhizopus stolonifer* and *Alternaria alternata*, respectively. As for rose and melon oils they showed lesser inhibitor effect at the same concentration (1%) on the fungal growth. Percentages of the growth reduction were calculated as 38.8, 16.6, 28.6 and 14.4, 17.1, 15.3, in respective order. On the other hand, the synergistic or antagonistic effects of yeast and essential oils combination against the fungal growth were shown in Tables 3, 4 and 5. The data revealed that combination of yeast isolates and peppermint oil showed synergistic effect for inhibiting fungal growth, while antagonistic effect was observed when yeast isolates were combined with melon or rose oils.

Since no alternative to chemical control alone is as consistently effective as fungicides in reducing postharvest decay, promising alternatives of biological control with beneficial yeasts and plant essential oils treatments were tested to develop a strategy to provide satisfactory control of postharvest decay on tomatoes fruit in storage. In order to enhance biocontrol activity of antagonists against fungal pathogens, certain strategies, such as adding calcium salts, carbohydrates, amino acids and other nitrogen compounds to biocontrol treatments, were suggested [18, 19].

To achieve a suitable efficacy and avoid antagonistic effect that could be happened in the essential oil-yeast formula, melon and rose oils were neglected to be tested as combined factor with yeast isolates referring to their inhibitor effect on the viability of yeast isolates (Table, 2).

The results in the present work indicate that the applied formula containing combination between yeast and peppermint oil enhanced the efficacy of decay incidence of tomato fruits during storage better than each individual component. Results in Table 6 showed that application of carnauba wax containing *S. cerevisiae* and peppermint oil has superior effect for reducing the percentage of tomatoes fruits decay incidence caused by all the tested pathogenic fungi. It caused 100% reduction of all tested fruit decay incidence. *R. stolonifer* showed more sensitivity to most tested treatments than the others. Decay incidence caused by *R. stolonifer* was completely controlled by formula of carnauba and paraffin wax supplemented with peppermint oil plus *S. cerevisiae* or *S. cerevisiae* (CBY), while either *S. cerevisiae* or *S. cerevisiae* (CBY) alone gave 82.6 and 77.8% reduction of soft rot incidence. As for gray rot caused by *B. cinerea*, application of carnauba wax containing *S. cerevisiae* and peppermint oil resulted in a complete control of decay incidence. *S. cerevisiae* and *S. cerevisiae* (CBY) combined with peppermint oil also reduced decay incidence by 85.1 and 71.1%, respectively. Also application of carnauba or paraffin wax supplemented with *S. cerevisiae* and *S. cerevisiae* (CBY) and peppermint oil resulted in high reduction of black rot incidence caused by *A. alternatai*, which recorded as 69.6, 77.8, 75.5 and 74.6%, respectively.

The applied formula not only could decreased the incidence of tomato fruits decay but also delaying the decay development on the infected fruits. Results in Table 7 indicate that the applied formula of carnauba and paraffin wax containing *S. cerevisiae* and *S. cerevisiae* (CBY) combined with peppermint oil have a suppressive effect on decay development of the infected fruits resulted in significant reduction in the rotted tissues weight compared with the other tested factors when applied individually. Many workers also successfully used different yeast isolates for controlling post harvest diseases

Table 5. Growth reduction in response to rose oil in combination with yeast isolates *in vitro*

Treatment	Conc. of essential oil (%)	Fungal growth reduction %					
		Botrytis cinerea		*Rhizopus stolonifer*		*Alternaria alternata*	
		Linear growth (mm)	Reduction (%)	Linear growth (mm)	Reduction (%)	Linear growth (mm)	Reduction (%)
Rose	0.25	79.5 b [1]	11.6	80.0 a	11.1	78.0 b	13.3
	0.50	65.3 d	27.4	78.6 b	12.6	72.5 c	19.4
	1.00	55.0 e	38.8	75.0 c	16.6	64.2 e	28.6
Rose + *Candida tenuis*	0.25	77.5 b	13.8	80.0 a	11.1	77.6 b	13.7
	0.50	66.5 d	26.1	78.5 b	12.7	65.0 d	27.7
	1.00	63.0 de	30.0	68.6 d	23.7	58.3 e	35.2
Rose + *Saccharomyces cerevisiae*	0.25	65.1 d	27.6	82.0 a	8.8	75.0 b	16.6
	0.50	57.0 e	36.6	79.6 b	11.5	64.5 c	28.3
	1.00	50.0 f	44.4	76.5 c	15.0	52.0 e	42.2
Rose + *Saccharomyces cerevisiae* (CBY)	0.25	60.0 d	33.3	84.0 a	6.6	65.5 d	27.2
	0.50	55.3 e	38.5	77.9 b	13.4	55.0 e	38.8
	1.00	53.3 ef	40.7	66.8 d	25.7	51.0 ef	43.3
Untreated control		90.0 a	-	90.0 a	-	90.0 a	-

[1] Data in each column with the same letter are not significant difference (P=0.05) according to Tukey test [25].

Table 6. Effect of peppermint oil alone or in combination with antagonistic yeasts on decay incidence of tomato fruits

Treatment	Decay incidence of tomato fruits (%)		
	Gray mould (*Botrytis cinerea*)	Soft rot (*Rhizopus stolonifer*)	Black rot (*Alternaria alternate*)
Saccharomyces cerevisiae (CBY)	22.3 c [2]	11.9 d	26.6 c
Saccharomyces cerevisiae	26.7 c	15.2 d	21.4 c
Peppermint oil [1]	38.5 b	22.5 c	26.8 c
Paraffin wax	35.0 b	34.1 b	32.5 b
Carnauba wax	35.6 b	32.9 b	30.0 b
S. cerevisiae (CBY) + peppermint oil	19.5 d	11.0 d	14.5 d
S. cerevisiae (CBY) + Carnauba wax + peppermint oil	18.2 d	0.0 e	16.0 b
S. cerevisiae (CBY) + Paraffin wax + peppermint oil	20.5 c	0.0 e	16.6 d
S. cerevisiae + peppermint oil	10.0 d	10.6 d	13.3 d
S. cerevisiae + Carnauba wax + peppermint oil	0.0 e	0.0 e	0.0 e
S. cerevisiae + Paraffin wax+ peppermint oil	20.0 c	0.0 e	20.0 c
Control	67.5 a	68.5 a	65.5 a

[1] Peppermint oil was used at concentration of 1%.
[2] Data in each column with the same letter are not significant difference (P=0.05) according to Tukey test [(25)].

Table 7. Effect of peppermint oil alone or in combination with antagonistic yeasts on of tomato rotted tissue

Treatment	Tomato rotted tissues (%)		
	Gray mould (*Botrytis cinerea*)	Soft rot (*Rhizopus stolonifer*)	Black rot (*Alternaria alternate*)
Saccharomyces cerevisiae (CBY)	26.4 d [2]	32.7 c	41.0 b
Saccharomyces cerevisiae	18.1 e	26.3 d	19.0 e
Peppermint oil [1]	24.7 d	31.6 c	44.0 b
Paraffin wax	36.4 c	36.9 c	42.5 b
Carnauba wax	32.0 c	33.7 c	30.7 c
S. cerevisiae (CBY) + peppermint oil	21.2 d	28.4 d	28.3 d
S. cerevisiae (CBY) + Carnauba wax + peppermint oil	13.4 e	0.0 f	27.6 d
S. cerevisiae (CBY) + Paraffin wax + peppermint oil	16.7 e	0.0 f	13.8 e
S. cerevisiae + peppermint oil	21.0 d	26.7 d	24.9 d
S. cerevisiae + Carnauba wax + peppermint oil	0.0 f	0.0 f	0.0 f
S. cerevisiae + Paraffin wax+ peppermint oil	12.8 e	0.0 f	18.2 e
Control	75.5 a	74.5 a	78.0 a

[1] Peppermint oil was used at concentration of 1%.
[2] Data in each column with the same letter are not significant difference (P=0.05) according to Tukey test [(25)].

during storage. They reported that treatment of fruit with yeast microbial agents was an efficient method for control of several postharvest decays [(6, 13, 20, 34, 38)]. Essential oils which have potential antifungal properties also have the possibility for use as alternatives to synthetic fungicides [(4, 15, 24, 35)]. Moreover, many studies have concluded that the overall antifungal activity of essential oils is due to a synergistic effect between their various components [(5, 27, 29)]. The usage of fruit coating with wax is also recommended. Waxes coat the plant surface and function to limit water loss and impede the invasion of pathogens. Fruit that is damaged during or after harvest, or has had the cuticle removed by detergents is more susceptible to insect and fungal damage if it is not waxed. Waxes also serve as a physical barrier to reduce gas exchange and decrease shrinkage and spoilage. Finally, fruit waxes serve cosmetic purposes, causing many fruits to have a shiny high-gloss surface [(23,26)].

The obtained results in the present study revealed that the potential of using carnauba or paraffin wax supplemented with *S. cerevisiae* or *S. cerevisiae* (CBY) and peppermint oil to control artificially-inoculated tomato fruits resulted in significant reduction in gray mould, soft rot and black rot incidence and decay development of

infected fruits as well. On the light of these results the usage of the proposed formula could be suggested for application against decay of tomato fruits and such postharvest diseases in backing houses for storage or exportation purposes.

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摘 要

Abd-Alla, M. A.[1], El-Mougy, N. S.[1, 2], and El-Gamal, N. G.[1] 2009. 利用植物精油和酵母菌製劑防治貯藏期番茄果腐病. 植病會刊 18: 23-33. ([1] 埃及國家研究中心 植物病理系；[2] 聯絡作者，電子郵件：nehal_nrc@yahoo.com)

評估酵母菌 (*Saccharomyces cerevisiae* 與 *Candida tenuis*)、商業之酵母混合液 (*S. cerevisiae* mixture, CBY) 或薄荷葉、甜瓜和玫瑰精油對 *Botrytis cinerea*、*Rhizopus stolonifer* 及 *Alternaria alternate* 菌絲生長之影響，結果顯示 CBY 液體抑制三種植物病原菌生長的效果最佳，其次是 *S. cerevisiae* 和 *C. tenuis*。測試不同濃度的薄荷、甜瓜和玫瑰精油對酵母菌菌株的生長，得知薄荷精油不會影響酵母菌菌株的生長，然而甜瓜和玫瑰精油在低濃度下，會顯著抑制酵母菌族群生長。測試薄荷、甜瓜和玫瑰精油對三種植物病原菌的生長，得知薄荷精油抑制病原菌生長的效果優於甜瓜和玫瑰精油的處理。薄荷精油混合任何一種酵母菌菌株，其抑菌效果明顯高於甜瓜和玫瑰精油混合任何一種酵母菌的處理。將包含 *S. cerevisiae* 菌株或 CBY (含有 1% 薄荷精油)的棕櫚蠟 (carnauba wax) 應用於防治儲藏期番茄果實灰黴病、軟腐病及黑腐病，結果顯示棕櫚蠟混合 *S. cerevisiae* 菌株或棕櫚蠟混合 CBY (含有 1% 薄荷精油)可有效降低此三種病害的發生。根據上述結果得知，應用棕櫚蠟混合 *S. cerevisiae* 菌株或棕櫚蠟混合 CBY (含有 1% 薄荷精油) 於儲藏期的番茄果實時，除可有效降低儲藏期病害的發生外，番茄果實的品質亦不會受到影響。

關鍵詞：酵母菌、植物精油、番茄果腐病