Available online freely at www.isisn.org

Bioscience Research

Print ISSN: 1811-9506 Online ISSN: 2218-3973

Journal by Innovative Scientific Information & Services Network

RESEARCH ARTICLE BIOSCIENCE RESEARCH, 2017 14(2): 128-138. **OPEN ACCESS**

Formulation of essential oils based on various carriers for controlling blue mold of lemon fruits

Nehal Samy El-Mougy and Mokhtar Mohamed Abdel-Kader*

Plant Pathology Dept., National Research Centre, Dokki, Giza, 12622, **Egypt**

***Correspondence:** mokh_nrc@yahoo.com **Accepted:** 30 Mar. 2017 **Published online:** 25 Apr. 2017

Different applied formula of essential oils based on different carriers as fruit coating against the postharvest disease, blue mold of lemon were evaluated. The coated lemon fruits were artificially inoculated individually with blue mold pathogen *Penicillium italicum* under laboratory conditions. In descending order the best superior protective effects of essential oils are lavender, carrot, medulla followed by wheat germ, grape, sweet almond and olium, respectively. Data also showed that, in general, all tested essential oils reduced disease incidence ranged between 0.0 up to 66.6% when formulated on the different used carriers. It is interesting to note that fruit coating with only the carrier formula of sodium alginate (2%), arabic gum (2%), gelatin (4%), and glycerol (1%) individually or in combination with the rest of used carriers showed similar mold incidence records compared with control treatment which recorded 100% infection. The improved formulation of essential oils and carrier materials mixtures provided an effective control for lemon fruit against postharvest pathogen infections by *P. italicum* with values of decay reduction started at 33.3%.and increased to reach 100%. These results demonstrated that the commercialization of some of formulated essential oils to control post-harvest decay of such fruits appears to be feasible and may present an alternative to synthetic pesticides.

Keywords: Blue mold, carrier materials, essential oils, fruits coating, lemon, postharvest diseases, *Penicillium italicum*.

INTRODUCTION

In Egypt, lemon fruits are available around a year, therefore it is considered one of the most important fruit crop for exportation purpose. Fresh market lemon is harvested by hand into plastic or palm tree ashes boxes when fruits are between the mature green and yellow stages of color development. Harvesting is done frequently to avoid over ripe fruit. Lemon fruits are susceptible to postharvest diseases caused by various pathogenic fungi. *Penicillium digitatum* (green mold), *P. italicum* (blue mold) and *Geotricum candidum* (sour rot) are the most important decay pathogens of lemon causing postharvest losses at high frequency (Akhtar et al. 1994). 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 (Eckert and Sommer, 1967).Generally, postharvest losses are estimated to range from 10 to 30% per year despite the use of modern storage facilities and techniques in developed and developing countries (Harvey, 1978). However, particularly in developing countries postharvest diseases affect a wide variety of crops due to lack sophisticated storage facilities (Jeffries and Jeger, 1990).

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 has stimulated the search for synthetic chemical fungicides

alternative. 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 (Hammer et al. 2003; Ahmet et al. 2005). 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 (Ormancey et al., 2001). Essential oils have been used successfully in combination with a variety of treatments, such as antibacterial agents, mild heat and salt compounds (Karatzas et al., 2000).

The aim of the present study was to evaluate the efficacy some essential oils as single treatment or formulated on different carrier on the fungal growth. Further, coating Lemon fruit with formula containing some essential oils carried individually on different carrier materials against postharvest fruit molds incidence under artificial inoculation with the fungal pathogen of blue mold incidence caused by *P. italicum* was also evaluated under *in vivo* conditions.

MATERIALS AND METHODS

Essential oils:

Essential oils used in the present study were obtained from CID Company, Egypt. The essential oils tested in present study were lavender, carrot, sweet almond, wheat germ, grape, olium (*aloexylon agallochum*) and medulla.

Carrier materials:

The used essential oils based on various carrier materials in the present study are shown in the Table 1.

Growth inhibition:

Seven essential oils at concentrations of 1, 2 and 4% were evaluated for their inhibitory effect on *P. italicum* fungal radial growth, through *in vitro* tests. Emulsified stocks at high concentrations of tested essential oils were prepared by dissolving in sterilized distilled water. A few drops of the emulsifier Tween 20 (Sigma Co.) were added to the essential oil volumes to obtain an emulsion feature. Different volumes of the essential oil 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. The control treatment was PDA medium which was free of essential oils. Disks (5 mm-diameter) of *P. italicum* growth taken from seven day-old culture were placed on the centre of Petri dishes. All plates were incubated at 25±2°C until the fungal reached full growth in the control treatment. Linear growth was measured (mm) and mycelial growth reduction (%) was calculated as follows:

$$\text{Growth reduction \%} = \left[\frac{C-T}{C} \text{ X}100\right]$$

Where:

C = linear fungal growth in control.

T = linear fungal growth in treatment.

Lemon fruits coating:

Collected apparently healthy lemon fruits (*cv.* Balady) were used in the present *in vivo* experiments. All the fruits were disinfected (Lopez-Reyes et al., 2010) in sodium hypochlorite solution (2.5%) for 2min then washed with distilled water 3 times and air dried. The fruits were placed in sterile commercial packages. Different tested essential oils were prepared at concentration of 4% individually or formulated on carrier materials which dissolved in water at different concentration (carrier : water, v:v) as stated in Table (2).

Lemon fruits were arranged by groups according to the applied essential oil treatments. Lemon fruits were wounded (0.5 cm deep and 1.0 cm long and 3 wounds per fruit) using sterile scalpel. After wounding, coating process for lemon fruits were carried out by immersing immediately in solutions of different tested essential oils based on various carriers (as stated in Table 2). The treated fruits were left to air dry for 2 h at ambient temperature (24-26^{o}C), and then artificially inoculated. The wounded treated fruits were inoculated with spore suspension (Lopez-Reyes et al. 2010). Conidia of fungal pathogen *P. italicum* were recovered from 2-week old cultures by adding 10 mL of sterile water to each plate. The conidial suspension was filtered through three layers of sterile cheesecloth. The concentration of the conidial suspension was adjusted to 10^5 conidia per mL and a drop of Tween 80 was added to the suspension. Each fruits group was inoculated with *P. italicum.* The fruits were artificially inoculated by dipping the wounded treated fruits into the prepared fungal suspensions. The inoculated treated fruits were air dried, after each individual essential oils treatment, for 2 h in a laminar flow. The inoculated treated fruits were placed into egg carton tray

(containing 30 holes) with a capacity of 30 fruit tray and stored in a cold room at (20±2^{o}C) for 30 days, then examined. Three egg trays were used as replicates for each particular treatment (essential oil on various carriers) and laid in carton package having moistened blotters at the bottom. Percentage of infected fruits was calculated after the storage period.

Statistical analysis

Tukey test for multiple comparisons among means was utilized as described by Neler et al. (1985).

RESULTS

The efficacy of growth inhibitor was evaluated against the growth of Lemon blue mold fungus *P. italicum in vitro*. Results in Table (2) show that all tested essential oils had inhibitor effect on the fungal growth. Inhibition in fungal growth increased with increasing in concentration of essential oils tested. Lavender, sweet almond and carrot oils showed the highest inhibitory effect on the fungal growth followed by wheat germ, grape and olium oils. The lowest inhibitor effect was observed at Medulla oil treatment. Data also showed that the highest reduction in the growth of *P. italicum* was recorded as 46.6, 43.3, 37.7, 31.1, 25.5 and 26.6% at the concentration of 4% of lavender, carrot, sweet almond, wheat germ, grape seed, olium and medulla essential oils, respectively.

The present study conducted with evaluation of different applied formula of essential oils carried on various carriers as fruit coating against the postharvest disease, blue mold of lemon. The coated Lemon fruits with essential oils formulas were artificially inoculated individually with blue mold pathogen under *in vivo* conditions. Molds incidence was recorded after 30 days of incubation. Presented data in Table (3) revealed that the used treatments had protected effect against blue mold incidence ranged between 33.3 to 66.6% comparing with 100% infection in control treatment.

The best protective effect of essential oils was observed with lavender, carrot, medulla followed by wheat germ, grape, sweet almond and olium treatments, respectively.

Complete protective effect against blue mold incidence was observed on lemon fruits coated with different formulated essential oils. That achieved with [s-ar] carrier mixed with essential oil of lavender, carrot, sweet almond, wheat germ, olium and medulla oils. That was followed by the carrier [CMC-ar-] mixed with essential oils of lavender, carrot, sweet almond and wheat germ as well as the carrier [ge-ar] mixed with essential oils of lavender, sweet almond, grape, olium and medulla, respectively. Data also showed that, in general, all tested essential oils showed disease incidence ranged between 0.0 up to 66.6% when formulated on the different used carriers. It is interesting to note that fruit coating with only the carrier formula of sodium alginate (2%), CMC (2%), arabic gum (2%), [CMC-ar], [ar-gl], [s-CMC-ge-al], [s-ge-al-ar], [s-CMC-gl-ar], [s-ge-gl-ar], [s-gl-al-ar], [ge-gl-ar-CMC], [ge-gl-ar-al] and [gl-ar-al-CMC]. Meanwhile, the rest of used carriers showed mold incidence recorded as 33.3% and 66.6% compared with control treatment which was 100% infection. The obtained results in the present study are confirmed with other previous reports by several investigators. Recorded results in the present study concerning the antifungal efficacy of different essential oils, lavander, carrot, sweet almond, wheat germ and grape in agreement of several cited reports. Meanwhile, in literature very lack or even not found reports concerning olium (*Aloexylon agallochum*) and Medulla essential oils, although, in present work they proved their antifungal activity against blue mold incidence of lemon fruits caused by the fungus *P. italicum* (Tables 2 and 3).

DISCUSSION

Application of essential oil is a very attractive method for controlling postharvest diseases. Essential oils have been used successfully in combination with a variety of treatments, such as antibacterial agents, mild heat and salt compounds (Karatzas et al. 2000). Recently, there has been considerable demand for the discovery of new natural antimicrobials. Plant products with antimicrobial properties have notably obtained attention as possible applicants in order to prevent bacterial and fungal growth (Lanciotti et al. 2004).

Plant products are characterized as having a wide range of volatile compounds. This means that essential oils that some plant extracts could be used as alternative anti-bacterial and anti-fungal treatments (Karapinar 1985; Nanir and Kadu 1987; Nirmala et al. 1988; Kumar and Tripathi 1991; Jenny 2000; Juglal et al. 2002).

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 uses (Ormancey et al. 2001; Devkatte et al. 2005).

Table 1: The used single and combined carrier materials

Sr. No.	Treatments	Abbreviation
	Single treatments	
1	Sodium alginate (2%)	[s]
2	Carboxy Methyl Cellouse (2%)	[CMC]
3	Gelatin (4%)	[ge]
4	Aloe (10%)	[al]
5	Arabic gum (2%)	[ar]
6	Glycerol (1%)	[gl]
	Combined treatments	
1	Sodium alginate (2%) + (CMC) (2%)	[s-c]
2	Sodium alginate (2%) + gelatin (4%)	[s-ge]
3	Sodium alginate (2%) + aloe (10%)	[s-al]
4	Sodium alginate (2%) + arabic gum (2%)	[s-ar]
5	Sodium alginate (2%) + glycerol (1%)	[s-gl]
6	CMC (2%) + gelatin (4%)	[CMC-ge]
7	CMC (2%) + aloe (10%)	[CMC-al]
8	CMC (2%) + arabic gum (2%)	[CMC-ar]
9	CMC (2%) + glycerol (1%)	[CMC-gl]
10	Gelatin (104%) + aloe (10%)	[ge-al]
11	Gelatin (4%11) + arabic gum (2%)	[ge-ar]
12	Gelatin (4%) +12 glycerol (1%)	[ge-gl]
13	Aloe (10%) + ara13bic gum (2%)	[al-ar]
14	Aloe (10%) + glycerol (1%)	[al-gl]
15	Arabic gum (2%) + glycerol (1%)	[ar-gl]
16	Sodium alginate (2%) + CMC (2%) + gelatin (4%) + aloe(10%)	[s-CMC-ge-al]
17	Sodium alginate (2%) + gelatin (4%) + aloe (10%) + arabic gum (2%)	[s-ge-al-ar]
18	Sodium alginate (2%) + (CMC) 2% + glycerol (1%) + arabic gum (2%)	[s-CMC-gl-ar]
19	Sodium alginate(2%) + gelatin(4%) + glycerol(1%) + arabic gum (2%)	[s-ge-gl-ar]
20	Sodium alginate (2%) + glycerol (1%) + aloe (10%) + arabic gum (2%)	[s-gl-al-ar]
21	Gelatin (4%) + glycerol (1%) + arabic gum (2%) + CMC (2%)	[ge-gl-ar-CMC]
22	Gelatin (4%) + glycerol (1%) + arabic gum (2%) + aloe (10%)	[ge-gl-ar-al]
23	Glycerol (1%) + arabic gum (2%) + aloe (10%) + CMC (2%)	[gl-ar-al-CMC]

Table 2: Effect of some essential oils on the growth of *Penicillium italicum in vitro*

Essential oil	Concentration (%)	*P. italicum*	
		linear growth (mm)	Growth reduction (%)
Lavender ***Lavandul aspica***	1	78 c	13.3
	2	64 d	28.8
	4	48 e	46.6
Carrot ***Daucus carota***	1	82 bc	8.8
	2	69 d	23.3
	4	51 e	43.3
sweet almond ***Prunus dulcis***	1	80 bc	11.1
	2	68 d	24.4
	4	56 e	37.7
wheat germ ***Triticum durum***	1	83 b	7.7
	2	71 c	21.1
	4	62 d	31.1
grape seeds ***Vitis vinifera***	1	84 b	6.6
	2	72 c	20.0
	4	61 d	32.2
Olium ***Aloexylon agallochum***	1	85 b	5.5
	2	76 c	15.5
	4	67 d	25.5
Medulla ***medulla oblongata***	1	86 b	91.1
	2	75 c	16.6
	4	66 d	26.6
Control		90 a	-------

Figures with the same letter are not significantly different (P= 0.05).

Table 3: Effect of different essential oils formulated on different carriers against blue mold incidence of lemon fruits *in vitro*

Carrier material (Carrier : water, V:V)	Treatment (essential oil at 4%)								
	lavender	carrot	sweet almond	wheat germ	grape	olium	medulla	carrier only	control (fungus only)
Sodium alginate (2%)	33.3 c	0.0 e	66.6 b	0.0 e	33.3 c	33.3 c	66.6 b	0.0 e	100 a
CMC (2%)	33.3 c	33.3 c	33.3 c	33.3 c	33.3 c	66.6 b	33.3 c	0.0 e	100 a
Gelatin (4%)	0.0 e	0.0 e	33.3 c	0.0 e	0.0 e	66.6 b	0.0 e	66.6 b	100 a
Aloe (10%)	33.3 c	33.3 c	66.6 b	33.3 c	33.3 c	33.3 c	66.6 b	33.3 c	100 a
Arabic gum (2%)	33.3 c	66.6 b	66.6 b	33.3 c	0.0 e	33.3 c	0.0 e	0.0 e	100 a
Glycerol (1%)	0.0 e	0.0 e	33.3 c	66.6 b	0.0 e	0.0 e	33.3 c	33.3 c	100 a
sodium alginate (2%) + (CMC) 2%	66.6 b	66.6 b	0.0 e	33.3 c	0.0 e	66.6 b	0.0 e	33.3 c	100 a
Sodium alginate (2%) + gelatin (4%)	0.0 e	66.6 b	33.3 c	0.0 e	66.6 b	33.3 c	33.3 c	33.3 c	100 a
Sodium alginate (2%) + aloe (10%)	0.0 e	0.0 e	33.3 c	33.3 c	33.3 c	66.6 b	33.3 c	66.6 b	100 a
Sodium alginate (2%) + arabic gum (2%)	0.0 e	0.0 e	0.0 e	0.0 e	33.3 c	0.0e	0.0 e	33.3 c	100 a
Sodium alginate (2%) + glycerol (1%)	0.0 e	0.0 e	33.3 c	0.0 e	33.3 c	33.3 c	0.0 e	33.3 c	100 a
(CMC) 2% + gelatin (4%)	0.0 e	66.6 b	33.3 c	0.0 e	66.6 b	33.3 c	33.3 c	33.3 c	100 a
(CMC) 2% + aloe (10%)	0.0 e	0.0 e	33.3 c	33.3 c	33.3 c	66.6 b	33.3 c	66.6 b	100 a
(CMC) 2% + arabic gum (2%)	0.0 e	0.0 e	0.0 e	0.0 e	33.3 c	33.3 c	33.3 c	0.0 e	100 a
(CMC) 2% + glycerol (1%)	66.6 b	66.6 b	0.0 e	33.3 c	0.0 e	66.6 b	0.0 e	33.3 c	100 a
Gelatin (4%) + aloe (10%)	0.0 e	66.6 b	33.3 c	0.0 e	66.6 b	33.3 c	33.3 c	33.3 c	100 a
Gelatin (4%) + arabic gum (2%)	0.0 e	33.3 c	0.0 e	33.3 c	0.0 e	0.0 e	0.0 e	33.3 c	100 a
Gelatin (4%) + glycerol (1%)	0.0 e	0.0 e	33.3 c	33.3 c	33.3 c	66.6 b	33.3 c	33.3 c	100 a
Aloe (10%) + arabic gum (2%)	33.3 c	0.0 e	33.3 c	66.6 b	66.6 b	33.3 c	0.0 e	33.3 c	100 a
Aloe (10%) + glycerol (1%)	0.0 e	0.0 e	33.3 c	66.6 b	33.3 c	33.3 c	33.3 c	33.3 c	100 a
Arabic gum (2%) + glycerol (1%)	33.3 c	0.0 e	66.6 b	33.3 c	33.3 c	0.0 e	0.0 e	0.0 e	100 a

Table 3: Continue….

Carrier material (Carrier : water, V:V)	Treatment (Essential oil at 4%)								
	lavender	carrot	sweet almond	wheat germ	grape	olium	medulla	carrier only	control (fungus only)
Sodium alginate (2%) + (CMC) 2% + gelatin (4%) + aloe (10%)	0.0 e	0.0 e	33.3 c	66.6 b	0.0 e	0.0 e	0.0 e	0.0 e	100 a
Sodium alginate (2%) + gelatin (4%) + aloe (10%) + arabic gum (2%)	0.0 e	66.6 b	66.6 b	0.0 e	0.0 e	33.3 c	0.0 e	0.0 e	100 a
Sodium alginate (2%) + (CMC) 2% + glycerol (1%) + arabic gum (2%)	0.0 e	33.3 c	0.0 e	0.0 e	0.0 e	33.3 c	0.0 e	0.0 e	100 a
Sodium alginate (2%)+ gelatin (4%) + glycerol (1%) + arabic gum (2%)	33.3 c	33.3 c	33.3 c	33.3 c	33.3 c	33.3 c	0.0 e	0.0 e	100 a
Sodium alginate (2%) + glycerol (1%) + aloe (10%) + arabic gum (2%)	0.0 e	0.0 e	0.0 e	33.3 c	0.0 e	0.0 e	0.0 e	0.0 e	100 a
gelatin (4%) + glycerol (1%) + arabic gum (2%) + (CMC) 2%	33.3 c	33.3 c	66.6 b	66.6 b	33.3 c	66.6 b	33.3 c	0.0 e	100 a
Gelatin (4%) + glycerol (1%) + arabic gum (2%) + aloe (10%)	0.0 e	33.3 c	0.0 e	33.3 c	33.3 c	33.3 c	33.3 c	0.0 e	100 a
Glycerol (1%) + arabic gum (2%) + aloe (10%) + (CMC) 2%	0.0 e	33.3 c	33.3 c	0.0 e	33.3 c	0.0 e	33.3 c	0.0 e	100 a

Total No. of tested fruits = 30 per each treatment
Figures with the same letter are not significantly different (P= 0.05).

Lavender, as a kind of medicinal herb, can be effectively used in the treatment of vaginal discharges, and has a wide number of applications in supplementary medicine. Investigations conducted on lavender in both Iran and Italy have proved the fact that this medicinal herb has antifungal effects on different fungal species (Shin and Lim, 2004; Mahboubi et al., 2008).Moreover, Behmanesh et al. (2015) suggested that with attention to the significant antifungal activity of lavender plant, it can be suggested that lavender could serve as a source of compounds with therapeutic potential, and can be used against Candida-related infections.

Furthermore, Carrot seed oil is the source of the carotane sesquiterpenes carotol, daucol and *β*-caryophyllene. These sesquiterpenic allelochemicals were evaluated against *Alternaria alternate* isolated from the surface of carrot seeds cultivar Perfekcja, a variety widely distributed in horticultural practice in Poland. *A. alternata* is one of the most popular phytotoxic fungi infesting the carrot plant. The strongest antifungal activity was observed for the main constituent of carrot seed oil, carotol, which inhibited the radial growth of fungi by 65% (Jasicka-Misiak et al., 2004). Analyses of seed essential oil show that sabinene (40.9%) and *α*-pinene (30.1%), followed by *β*-bisabolene (6.2%), *β*-pinene (5.7%) and *trans*-caryophyllene (5.3%) are the dominant compounds (Aćimović *et al*., 2016). The major constituents of essential oil from cultivated carrot seeds were reported as carotol (22.0%), sabinene (19.6%) and *α*-pinene (13.2%). The essential oil from carrot seeds possesses strong antimicrobial activity against fungi such as *Candida albicans* and *A. alternate*, as well as bacteria *Staphylococcus aureus* (Imamu et al., 2007).

Grape seed is a well-known dietary supplement and contains vitamins, minerals, and polyphenols. The abundant phenolic compounds from grape seed are catechins, epicatechin, procyanidin, and some dimmers and trimers. The grape seed is shown to exhibit bioactivities such as antioxidant, antiflammatory, anti-bacterial, anti-cancer, antiviral, cardioprotective, hepatoprotective, neuroprotective, antiaging and anti-diabetic. The oil extracted from grape seeds is used in cosmetic, culinary, pharmaceutical and medical purposes (Baydar et al., 2007; Mendes et al., 2013).

In the present study Wheat germ oil showed antifungal effect against *P. italicum*. One of the benefits of wheat germ oil for skin is reducing inflammation. The possible reason may be that it contains vitamin E which has anti-inflammatory properties. Furthermore, wheat germ oil has a plenty of vitamins and nutrients for rejuvenation such as vitamin E, vitamin A, vitamin D, thiamine, riboflavin, nicotine acid, pantothenic acid, pyridoxine, folic acid. Mineral in wheat germ oil include calcium, potassium, phosphorus, manganese, iron, zinc, selenium, copper. Rizzello et al. (2011) stated that, methanol and water/salt-soluble extracts from wheat germ (SFWG) showed antifungal activity against various fungi isolated from bakeries. The antifungal activity was attributed to a mixture of organic acids and peptides which were synthesized during fermentation. Formic (24.7mol/L) acid showed the highest antifungal activity. Four peptides, having similarities with well-known antifungal sequences, were identified and chemically synthesized.

Recently, interest has been shown in combining microbial biocontrol agents with other chemical components to increase their activity against plant pathogens.

Also, Pre-storage approach formula of bio-agents and essential oils was successfully minimize decay incidence of tomato fruits during storage under natural and artificial inoculation conditions with the disease incidents (Abd-Alla et al., 2009). Abd-Alla *et al*. (2014) reported that under storage conditions, artificially inoculated bananas with *Fusarium semitectum* showed reduction in both crown rot disease incidence and severity when treated with cinnamon, thyme bitter and sweet almond oils. Almonds have many essential vitamins such as vitamin D, vitamin E and minerals such as calcium, magnesium.

The advantage of edible films over other traditional synthetic films is that can be consumed with the packaged products. The films can function as carriers for antimicrobial and antioxidant agents. Antimicrobial edible films may supply an effective way to control food-borne pathogens and spoilage microorganisms to thus enhance food safety and reduce product spoilage. The use of edible films as antimicrobial carriers represents an interesting approach for the external incorporation of plant extract onto food system surfaces (Behbahani et al., 2014). The advantages of using an edible film with extract plant for food products are that it may be easy to use and it may be able to enhance quality and extend the shelf life while reducing packaging waste. Moreover, in this regard, extract plant is a valuable component for processing biodegradable packaging which can extend shelf-life and inhibit pathogens and spoilage.

In the present study, sodium algenate, gelatin CMC and aloe were used individually or in combination as edible film (carrier material) for the tested essential oils forming a test solution used for coating lemon fruits in purpose of protecting fruit against mold decay infection.

Rawdkuen et al. (2012) investigate the effect of the addition of an ethanolic propolis extract (EPE) to gelatin-based films plasticized with acethyltributhyl citrate (AC) on the mechanical properties, solubility and the antimicrobial activity against food spoilage microorganism. The films kept their antimicrobial activities during 177 days of storage. These results demonstrated the antimicrobial capacity of gelatin-based films added of propolis, and their stability over time. Moreover, Khalil et al. (2013) reported that Soy-starch and gelatin edible films were prepared and incorporated with *Myrtus communis* and *Ziziphus spina-christi* essential oils separately and as a mixture in different concentrations. The films were characterized for their antimicrobial activity and their physico-chemical properties. The films were studied on different food applications (orange, apple, lemon, tomato, pizza dough, chicken salami, meat salami, artificial cheese, mayonnaise, yoghurt and skimmed cheese). Their results showed that, the films extended the shelf-life of the food products depending on the effective chemical compounds of the essential oils α-pinene and limonene. Further, Behbahani et al. (2014) studied the antimicrobial properties of CMC films containing different concentrations of the extracted Eucalyptus tree leaves against *Streptococcus pyogenes*, *Pseudomonas aeruginosa* and *Staphylococcus epidermidis*. As a result, aqueous and ethanloic extracts of *Eucalyptus camaldulensis* leaves, have been strong antimicrobial activity against many food pathogen bacteria.A work of Angelova et al. (2015) has been demonstrated that silicon dioxide (SiO_2/CMC hybrid materials with included silver nanoparticles *(*AgNPs*)*showed fungistatic behavior against *A. niger*. They added that the composites prepared using metal nanoparticles and polymers can be more effective due to their enhanced antimicrobial activity.

Another work aimed to formulate sodium alginate nanospheres of amphotericin B by controlled gellification method and to evaluate the role of the nanospheres as a “passive carrier” in targeted antifungal therapy. *In vivo* studies showed that the nanosphere-bound drug produced a higher antifungal efficacy than the free drug. They conclude that the formulated sodium alginate nanospheres containing amphotericin B was found to have better antifungal activity when compared to the free drug and also yielded sustained *in vitro* release. Chemically, alginates are naturally occurring polycarbohydrates consisting of co-polymers of α-L-glucuronic acid (G) and β-D-mannuronic acid (M). The relative amounts of these two building blocks influence the total chemistry of this bio-polymer, where G/M ratio determines the permeability properties of the swollen alginate gel which envelops the essential oils (Amsden, 1998). Soliman et al. (2013) reported that the antifungal activity of vapors of microencapsulated and non-microencapsulated oils was evaluated against two of pathogenic fungi species for stored grains: *Aspergillius niger* and *Fusarium verticillioides*. They found that encapsulation in calcium alginate microspheres may effectively reduce the evaporation rate of essential oils, thus increase the potential antifungal activity. Furthermore, *Aloe vera*was used in the present study as based formula for coating lemon fruit with tested essential oils. The antifungal activity of Aloe (*A. vera*)has been reported against postharvest fruit pathogens, such as *P. digitatum*, *P. expansum*, *B. cinerea* and *A. alternate* (Rodridus et al.,2005) and was based on the suppression of germination and the inhibition of mycelia growth (Ferro et al., 2003). Some individual components found in *A. vera* gel, such as saponins, acemannan and anthraquinones derivatives, are known to have antibiotic activity and could be responsible for its antibacterial activity. The fresh leaves of *A. vera* are used to obtain two components: a bitter, yellow liquid fraction (exudates) and a mucilaginous pulp from the parenchymatous tissue. The liquid fraction constituents are largely phenolic in nature (Reynolds, 1985). It also has a high content of 1,8-dihydroxianthraquinone derivatives (*A. emodin*) and their glycosides (aloins), which are used as cathartics (Morton, 1977). The pulp contains carbohydrate polymers (glucomannans or pectic acid) and other organic and inorganic components (Grindlay and Reynolds, 1986). *A. vera* is reported to contain mono- and polysaccharides, tannins, sterols, organic acids, enzymes, saponins, vitamins and minerals (Newall et al., 1996). The main active constituent of *A. vera* plant extract is aloine, an anthraquinone heteroside (Bruneton, 1993).

CONCLUSION

In conclusion, the improved formulation of essential oils and carrier materials mixtures

provided an effective control for lemon fruit against postharvest pathogen infections and artificial infections of *P. italicum* with values of decay reduction started at 33.3%.and increased to reach 100%. These results demonstrated that the commercialization of some of formulated essential oils to control postharvest decay of such fruits appears to be feasible and may present an alternative to synthetic pesticides. This study also provides an insight into expanding these strategies, partly or fully, for the control of other postharvest infections. Actually, despite the distinctive features of these alternative methods, several reasons hinder the commercial use of such treatments. Consequently, research should emphasize the development of appropriate tools to effectively implement these alternative methods to commercial citrus production. It is hoped that this study would lead to the establishment of some compounds that could be used to formulate new and more potent antimicrobial agent of natural origin.

ACKNOWLEGEMENT

This research was supported in part by In-House project No. 10120606 Entitled "New safe approaches for controlling postharvest diseases of some economic Fresh fruits and vegetables crops in Egypt".

REFERENCES

Abd-Alla, M.A., El-Mougy, N.S. & El-Gamal, N.G. 2009. Formulation of Essential oils and Yeast for Controlling Postharvest Decay of tomato fruits. Plant Pathol. Bull. 18: 23-33.

Abd-Alla, M.A., El-Gamal, N.G., El-Mougy, N.S. & Abdel-Kader, M.M. 2014. Post-harvest treatments for controlling crown rot disease of Williams banana fruits (*Musa acuminata* L.) in Egypt. Plant Path. & Quar. 4 (1): 1–12.Doi: 10.5943/ppq/4/1/1

Aćimović, M., Stanković, J., Cvetković, M., Ignjatov, M. & Nikolić, L. 2016. Chemical characterization of essential oil from seeds of wild and cultivated carrots from Serbia. *Botanica Serbica* 40 (1): 44-60.

Ahmet, C., Saban, K., Hamdullah, K. & Ercan, K. 2005. Antifungal properties of essential oil and crude extracts of Hypericum linarioides Bosse. Biochem. Syst. Ecol. 33: 245- 256.

Akhtar, K.P., Matin, M., Mirza, J.H., Shakir, A.S. & Rafique, M. 1994. Some studies on the postharvest diseases of tomato fruits and their chemical control. Pakistanian J. Phytopathol. 6: 125-129.

Amsden, B., 1998. "Solute Diffusion within Hydrogels," Mac-romolecules, Vol. 31, No. 23, pp. 8382-8395. doi:10.1021/ma980765f

Angelova, T., Uzunova, V., Rangelova, N., Tzoneva, R. & Georgieva, N. 2015. Antifungal effect of silver doped hybrid materials based on silica and carboxymethyle cellulose against *Aspergillus niger*. Scientific works of University of food, Volume XII, 505-509.

Baydar, N.G., Özkan, G. & Çetin, E.S. 2007. Characterization of grape seed and pomace oil extracts. *Grasas Y Aceites*, 58(1): 29-33.

Behbahani, B.A., Yazdi, F.T., Mortazavi, A., Gholian, M.M., Zendeboodi, F. & Vasiee, A. 2014. Antimicrobial effect of Carboxy Methyl Cellulose (CMC) containing aqueous and ethanolic *Eucalyptus camaldulensis* L. leaves extract against *Streptococcus pyogenes*, *Pseudomonas aeruginosa* and *Staphylococcus epidermidis.* J. Paramed. Sci. (JPS), 5(2): 59-69.

Behmanesh, F., Pasha, H., Sefidgar, A.A., Taghizadeh, M., Moghadamnia, A.A., Rad, H.R. & Shirkhani, L. 2015. Antifungal Effect of Lavender Essential Oil (*Lavandula angustifolia*) and Clotrimazole on *Candida albicans*: An *In Vitro* Study. *Hindawi Publishing Corporation Scientifica* Volume 2015, Article ID 261397, 5 pages. http://dx.doi.org/10.1155/2015/261397.

Bruneton, J.1993. *Pharmacognosie. Phytochimie. Plantes médicinales*, Tec Doc, Paris p. 363.

Devkatte, A.N., Zore, G.B. & Karuppayil, S.M., 2005. Potential of plant oils as inhibitors of *Candida albicans* growth. FEMS Yeast Res 5(9): 867- 873.

Eckert, J.W. & Sommer, N.F. 1967. Control of diseases of fruits and vegetables by postharvest treatment. Ann. Rev. Pl. Pathol. 5: 391-432.

Ferro, V.A., Bradbury, F., Cameron, P., Shakir, E., Rahman, S. R. & Stinson, W.H. 2003. *In vitro*

susceptibilities of *Shigella flexneri* and *Streptococcus pyogenes* to inner gel of *Aloe barbadensis* Miller. Antimicrob. Agents Chemother. 47: 1137–1139.

Grindlay, D. & Reynolds, T. 1986. The *A. vera* phenomenon: a review of the properties and modern uses of the leaf parenchyma gel. J. *Ethnopharmacol.* 16: 117–151.

Hammer, K.A., Carson, C.F. & Riley, T.V. 2003. Antifungal activity of the components of *Melaleuca alternifolia* (tea tree) oil. J. Appl. Microbiol. 95: 853-860.

Harvey, J.M. 1978. Reduction of losses in fresh fruits and vegetables. *Annual* Rev. Pl. Pathol.16:321-341. doi:10.1146/annurev.py.16.090178.001541

Imamu, X., Yili, A., Aisa, H.A., Maksimov, V.V, Veshkurova, O.N. & Salikhov, Sh.I. 2007. Chemical composition and antimicrobial activity of essential oil from *Daucus carota sativa* seeds. Chem. Nat. Compounds 43(4): 495-496.

Jasicka-Misiak, I., Lipok, J., Nowakowska, E.M., Wieczorek, P.P., Młynarz, P., Kafarski, P., 2004. Antifungal activity of the carrot seed oil and its major sesquiterpene compounds. Zeitschrift für Naturforschung C. 59: 791-796.

Jeffries, P. & Jeger, M.J. 1990. The biological control of postharvest diseases of fruit. BNI International - Business Networking and Referrals.11: 333-336.

Jenny, J. 2000. Essential oils: A new idea for postharvest disease control. Good Fruit and Vegetables magazine 11 (3): p. 50. Available online:http://postharvest.com.au/GFV_oils.PDF

Juglal, S., Govinden, R. & Odhav, B., 2002. Spices oils for the control of co-occurring mycotoxin- producing fungi. J. Food Protec. 65: 638–687.

Karapinar, M. 1985. The effects of citrus oil and some Turkish spices on growth and aflatoxin production by *Aspergillus parasiticus* NRRL 2999.Int. J. Food Microbiol.12, 239–245.

Karatzas, A.K., Bennik, M.H., Smid, E.J. & Kets, E.P. 2000. Combined action of S- carvone and mild heat treatment on *Listeria monocytogenes* Scott A. J. Appl. Microbiol. 89:296-301.DOI:10.1046/j.1365-2672.2000.01110.x

Khalil, M.S., Ahmed, Z.S. & Elnawawy, A.S. 2013. Evaluation of the Physicochemical Properties and Antimicrobial Activities of Bioactive Biodegradable Films. Jordan J. Biol. Sci. (*JJBS*) 6 (1): 51 – 60.

Kumar, A. & Tripathi, S.C. 1991. Evaluation of the leaf juice of some higher plants for their toxicity against soilborne pathogens. Plant and Soil 132: 297–301.

Lanciotti, R., Gianotti, A., Patrignani, N., Belleti, N., Guerzoni, M.E. & Gardini, F. 2004. Use of natural aroma compounds to improve shelf-life of minimally processed fruits. Trends Food Sci. & Tech. 15: 201–208.

Lopez-Reyes, J.G., Spadaro, D., Gullinoa, M.l. & Garibaldia, A. 2010. Efficacy of plant essential oils on postharvest control of rot caused by fungi on four cultivars of apples *in vivo*. Flavour and Fragrance Journal. 25:171- 177.

Mahboubi, M., Feizabadi, M.M. & Mahin Safara, M. 2008. Antifungal activity of essential oils from *Zataria multiflora, Rosmarinus officinalis, Lavandula stoechas, Artemisia sieberi Besser* and *Pelargonium graveolens* against clinical isolates of Candida albicans. Pharmacognosy Magazine 4(15): 15-18.

Mendes, J.A.S., Prozil, S.O., Evtuguin, D.V. & Lopes, L.P.C. 2013.Towards comprehensive utilization of winemaking residues: Characterization of grape skins from red grape pomaces of variety *Touriga Nacional*. Indust. Crops & Prod. 3, 43: 25-32.http://dx.DOI:org/10.1016/j.indcrop.2012.06.047

Morton, J.F. 1977. *Aloe*. In: Thomas, C.C. (Ed.), Major Medicinal Plants-Botany, Culture and Uses. Springfield, IL, pp. 46–50.

Nanir, S.P. & Kadu, B.B., 1987. Effect of some medicinal plants extract on some fungi. Acta Botanica India 15, 170–175.

Neler, J., Wassermann, W. & Kutner, M.H. 1985. Applied Linear Statistical Models. Regression, analysis of variance and experimental design: 2nd Ed. Richard, D. Irwin Inc. Homewood Illionois.

Newall, C.A., Anderson, L.A. & Phillipson, J.D. 1996. *Herbal medicines.* The Pharmaceutical Press, London. p. 25.

Nirmala Kishore Singh, S.K. & Dubey, N.K. 1988. Fungitoxic activity of essential oil of *Juniperus communis.* Indian Perfunm 33: 25–29.

Ormancey, X., Sisalli, S. & Coutiere, P. 2001. Formulation of essential oils in functional perfumery. Parfums, Cosmetiques, Actualites 157: 30-40.

Rawdkuen, S., Suthiluk, P., Kamhangwong, D. & Benjakul, S. 2012. Mechanical, physico-chemical, and antimicrobial properties of gelatin-based film incorporated with catechin-

lysozyme. Chem. Cent. J. 6: 131.DOI: 10.1186/1752-153X-6-131.

Reynolds, T. 1985. The compounds in *Aloe* leaf exudates: a review. Bot. J. inn. Soc. 90: 157–177.

DOI:10.1111/j.1095- 8339.1985.tb00377.x

Rizzello, C.G., Cassone, A., Coda, R. & Gobbetti, M., 2011. Antifungal activity of sourdough fermented wheat germ used as an ingredient for bread making. Food Chem. 1127(3): 952-959.DOI: 10.1016/j.foodchem.2011.01.063

Rodrigus, J.D., Castillo, H.D., Garc´ıa, R.R. & Sa´nchez, A.J.L., 2005. Antifungal activity in vitro of *Aloe vera* pulp and liquid fraction against plant pathogenic fungi. Indust. Crops *&* Prod. 21: 81–87.

Shin, S. L. & Lim, S., 2004. Antifungal effects of herbal essential oils alone and in combination with ketoconazole against *Trichophyton* spp. J. Appl. Microbiol. 97(6):1289-1296. DOI: 10.1111/j.1365-2672.2004.02417.x

Soliman, E.A., El-Moghazy, A.Y., Mohy El-Din, M.S. & Massoud, M.A. 2013. Microencapsulation of Essential Oils within Alginate Formulation and *in Vitro* Evaluation `of Antifungal Activity. Journal of Encapsulation and Adsorption Sciences (JEAS) 3: 48-55. DOI: 10.4236/jeas.2013.31006