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EFFECT OF SOME ESSENTIAL OILS ON GREY MOULD, CAUSED BY *Botrytis cinerea* ON TABLE GRAPE AT COLD-STORAGE

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ABSTRACT

Essential oils (EOs) from cinnamon (*Cinnamon zylanicum*), clove (*Syzygium aromaticum*) camphor (*Eucalyptus globulus*), and rocket (*Eruca sativa*), were evaluated for their botryocidal effect. *In-vitro*, *Botrytis cinerea* was exposed to 4 different concentrations of EOs, using three different techniques, *i.e.* amended medium, vapourisation, and volatilising. Cinnamon and clove EOs were the highest tested concentrations found to be the most effective in all techniques which completely inhibited 100% of radial growth for *B. cinerea in vitro*. A post-harvest trial to control grey mould on grape bunches of Flame seedless and Superior seedless cvs. were conducted using cinnamon and clove oils in seasons 2014 and 2015. Both of the two EOs were used at concentrations of 25, 50 and 100 µL./L^{-1}air v/v, exposed as vapour treatment significantly suppressed grey mould during the cold-storage. There was not a significance differences observed among both EOs treatments. However, cinnamon at 100 µL.$1L^{-1}$air v/v was the most effective treatment to control grey mould of both grape cultivars

INTRODUCTION

Grape has a great economic importance as cash crop, it was and still one of the most important fruit crops not only in Egypt but also globally. It considered a second fruit crop in Egypt following citrus regarding the cultivated area and yield either for local consumption or exportation. On the other hand, cultivated area of grapes is increasing annually, as it reached this year 2016 more than 196,993 feddans including 178,323 already fruitful areas. The total production of grape in Egypt is 1,686,706 tonnes with an average of 9.459 tonnes per feddan as published in a recent report of **Anonymous (2016)**

Botrytis cinerea is one of a serious plant fungal pathogen causing grey mould on many crops, in our study its causing grey mould on grapes bunch during cold storage as well as shipping by sea for exportation. *B. cinerea* is a fungal pathogen responsible for serious losses in vineyards in conditions of wet weather at critical stages in the season such as flowering and harvest. The grape growers and exporters considered *B. cinerea* is the biggest obstruction in this industry. The seriousness of *B. cinerea* becomes more clear when we expect that it will tolerate and grow well under cold conditions, where it can grow at 0^{o}C and of course grow better at 3-5^{o}C causing severe losses.

In recent years, a research work has been raised up the concept of developing a novel control tools as alternatives to synthetic fungicides; for schematic reasons, these alternatives could be classified into four major groups: 1st, compounds generally recognized as safe (GRAS); 2nd, natural compounds; 3rd, biological control agents (BCAs); and 4th, physical methods alone or the combination of all four groups (**Mari et al 2009 and Romanazzi et al 2012**)

Plant essential oils (EOs) showed antimicrobial activity against a variety of plant pathogens and pests. Several studies demonstrated the potential of essential oils as antifungal agents **(Kurita et al 1981; Grane & Ahmed, 1988; Wilson et al 1997;**

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Cowan, 1999; Abd-Alla et al 2001; Abdolahi et al 2010 and Ramadan et al 2012). A lot of research work reported the inhibition of post-harvest fungi *in vitro* by several plant essential oils **(Hidalgo et al 2002 and Kordali et al 2005).** For instance, essential oils of cinnamon and clove are known to have potent antibiotic activity and their application for controlling postharvest diseases has been suggested **(Feng & Zheng, 2007 and Kishore et al 2007)**.

Li et al (2013) showed that Cinnamaldehyde is the most abundant component of cinnamon oil representing 73.2%, followed by Eugenol (3.62%). On the other hand, clove oil contains mainly Eugenol (63%), (**Makhaik et al 2005**).

Cinnamon oil extracted from *Cinnamomum zeylanicum* Blume (Laureceae), contained Cinnamaldehyde, as well as *β*-Caryophyllene, linalool and other terpenes (**Carmo et al 2008**).

Melgarejo-Flores et al (2013) reported that the table grape berries were exposed to different headspace concentrations of cinnamon vapours. The fruit was placed in 0.62 L polypropylene trays, and cinnamon were added into individual small glass containers. The volatile cinnamon compounds were vaporised inside the containers. The results indicate that cinnamon oil as a vapour at all tested concentrations almost totally inhibited fungal decay of inoculated grape berries with *B. cinerea*. The suppressive effect of cinnamon oil vapour was attributed to its constituents Cinnamaldehyde and Eugenol as cell wall and membrane active antifungal agents.

The present work has been designed to investigate the effect of some essential oils as part of managing the grey mould in grapevine bunches caused by (*B. cinerea*) .*in vitro* experiment by simulation with different techniques, then *in vivo* trial to get the most efficacy to apply with the most efficient techniques.

MATERIALS AND METHODS

In vitro

The pathogen

The *Botrytis cinerea* isolates used in this study were isolated and microscopic identified, from samples of grape bunches were gathered from a commercial orchard in 2013 in Alex. Desert road. The samples were surface sterilised and inoculcated onto potato dextrose agar medium (PDA). The Petri's dishes were incubated at 20°C for 7 days to allow fungi to grow. A severity test done with the fungal isolates which collected previously, all were re-inoculated onto grape bunches to find the pathogenic capability. *B. cinerea* isolates were tested for their virulence by spraying the surface sterilised grape berries of both cultivars Flame seedless and Superior seedless, with spores from a 7-days-old culture were suspended in 0.5% Tween 80®, and spore suspension adjusted at a concentration of 1.4×10^6 ml^{-1} (**Viret et al 2004**) by using a haemocytometer technique. Five replicates of grape bunches were used for each fungal isolate. Tested grape berries were incubated at 0 to1°C for a month (**Rashid, 2001**). After the incubation period, the percentage of diseased berries was determined as follow:

$$\textit{Decay (\%)} = \frac{\textit{Diseased grape berries X 100}}{\textit{Average number of berries per bunch}}$$

Essential oils and plant material

For essential oils, a ready-to-use EOs of four plant species were used: cinnamon (*Cinnamon zylanicum*), clove (*Syzygium aromaticum*) camphor (*Eucalyptus globulus*), and rocket (*Eruca sativa*), *all* were purchased from Haraz Co. Ltd. (Cairo, Egypt).

The Grape bunches used in this study gathered from a farm vary from geographical location road with two cultivars of grapevine *i.e* Flame seedless and Superior seedless.

Evaluation of certain essential oils (EOs) on B. cinerea growth in vitro

Four essential oils (EOs)*, i.e.* cinnamon (*Cinnamon zylanicum*), clove (*Syzygium aromaticum*) camphor (*Eucalyptus globulus*), and rocket (*Eruca sativa*), were evaluated for their capability to suppress the fungal growth of *B. cinerea in vitro*. Three techniques were used to testing their effect on fungal growth, the first one is amended medium technique with the four EOs on PDA medium, the second is vapourisation of tested EOs, and the last one is volatilising of the previous EOs.

Different control measures were tested *in vitro* to assess their efficiency to control grey mould rot on grapes during cold storage to predict which treatments could be investigated *in vivo*.

A mathematical model to correlate the concentration of tested elements of investigated control measures with its efficacy to suppress *B. cinerea*

radial growth *in vitro* were developed. That model were used to calculate the Half maximal effective concentration (EC_{50}), and 90% effective concentration (EC_{90}) for each element. Comparison among treatments and concentrations according to their EC_{50} and EC_{90} supports determining precisely the most effective treatment and its concentration.

Essential oils embedded in medium

The first technique, for each essential oil, was added to PDA medium at final concentrations i.e., 0.25,0.5,1.0 and 2.0%. Stock emulsifiables of EOs were prepared in sterile water containing 0.5% Tween 80®. Either treated or untreated medium with EOs were poured into 5 Petri's dishes per each treatment. After medium solidification, 5 mm discs-cut off from periphery of 7-days-old cultures of *B. cinerea* isolate were seeded in the midpoint on the surface of oil-amended PDA medium, then incubated at 20 to 22 ^{o}C.

Effect of EOs treatments on the diameter of developed colonies was measured when fungal mycelium covered one plate in the control treatment or any treatment. The percentages of Mycelial Growth Inhibition (MGI) were recorded using the formula suggested by **Sirirat et al (2009)** as follows:

$$\boldsymbol{MGI}\ \% = \frac{\boldsymbol{\Delta do} - \boldsymbol{\Delta d}}{\boldsymbol{\Delta do}}\ \boldsymbol{x\ 100}$$

Where: Δ*do* and Δ*d* are the average diameters of the fungal colonies in the control and treatment sets, respectively.

Essential oils used as vapours

The second technique is a vapourisation for each EOs as follows; different concentrations of (25, 50, 75 & 100 mL.L^{-1} air, v/v) were introduced through pipelines into 10L glass jars, each jar has a five replica of PDA plates seeded with 5 mm mycelial-discs-cut off from periphery of 7-days-old cultures of *B. cinerea* isolate. One glass jar was used for each concentration, and each jar was sealed with plastic lid. Petri's dishes let in a jar without essential oils served as a control. EOs vapourisation treatments were used by utilising a Nebulizer pump (Model: A1000230®, Manufacturer: Elettroplastica spa, Italy), then the all plates incubated at 20 to 22^{o}C. The percentages of Mycelial Growth Inhibition (MGI) were recorded using the formula as mentioned earlier.

Essential oils used as volatiles

The third technique, was applied by using a sterilized 5mm discs of Whattman® filter paper no.1 dipped into concentrations of, 0.25, 0.5 and 1.0% with 2.0%, of each essential oil, then placed inside the inner surface of Petri's dish cover. While 5 mm mycelial-discs-cut off from periphery of 7-days-old cultures of *B. cinerea* isolate were seeded in the midpoint of PDA medium, cover dishes were replaced and sealed with thick parafilm, then the all plates were incubated at 20 to 22 ^{o}C. The percentages of Mycelial Growth Inhibition (MGI) were recorded using the formula as mentioned earlier.

Post-Harvest Trials

Efficacy of post-harvest vapourisation with EOs on grey mould rot incidence

In both cultivars Flame seedless and Superior seedless at two seasons 2014/15, the two Essential oils, i.e. cinnamon and clove each at 25, 50 and 100 µL.L^{-1} air v/v vapour, were tested for controlling grey mould in grapevine bunches. the tested treatment subjected for natural infection and artificial inoculation. Fresh samples of bunches were washed thoroughly with tap water, sterilised in 70% ethanol for one minute, and a fresh sample of bunches was used without sterilisation as natural infection then left to dry at room temperature. Sterilised bunches were inoculatedby spraying it separately with spore suspension of *B. cinerea*, with 1.4x10^{6} spores.ml^{-1}. 24 hours after incubation **(Abdel-Rahman, 2015)**. Artificially inoculated and naturally infected bunches, were vapourised separately at a different concentration of tested plant oils treatment. The artificially inoculated and naturally infected bunches were put in punnets while control treatment was vapoured by air and put in punnets too.

A five replicates were used for each singl treatment. They were placed in 10 L glass jars, oils vapours were introduced through pipelines in and out. Each jar along vapourised was sealed with a plastic lid. Control treatments of bunches were vapourised with air only. EOs concentrations were vapourised utilising nebulizer pump. tested EOs and control has been vapoured at one time, the severity of infection and disease percentage were recorded as mentioned before. And stored in commercial cold-rooms, for a month at 0 $^{\circ}$C the transferred to shelf life storage at 12 to 17 $^{\circ}$C for 5 days.

Statistical analysis

Analysis of variance (ANOVA) of all data was performed using the CoStat version 6.400 software (Lighthouse Ave. PMB 320, Monterey, CA, USA, 2008). Results of *in vitro* test were reported as values pointed in regression curve to determine the EC_{50} and EC_{90} values of it. Statistically significant differences ($P < 0.05$) between samples were determined according to Duncan's multiple range test (DMRT). A transformation of decay percentage values was performed prior statistical analysis.

RESULTS

In vitro

Virulence of *B. cinerea* isolates

The *B. cinerea* named H_8 isolated from Bader District in superior seedless cv.,was the most virulent isolate on both grape cultivars. Therefore, as **Table (1)** *B. cinerea* isolate, H_8 was chosen to be used in testing different control measures to be sure that the resulting effective treatment will achieve proper control of the disease whatever the virulence of prevalent *B. cinerea* isolate.

Table 1. Virulence of *B. cinerea* isolates on Flame Seedless and Superior Seedless grape incubated at 0 to1^{o}C for a month

***B. cinerea* isolate code**	**Geo. Location**	**Infection (%)**	
		Flame Seedless	**Superior Seedless**
mulak_31	Wadi Al-Mulak	29.42 ^{cd}	28.13 ^{bc}
Han_25	Bader District	38.58 ^{ab}	33.94 ^{ab}
k_70_4	K70 Alex. Roade	36.65 ^{abc}	34.06 ^{ab}
H_8	Bader District	**40.52** ^{a}	**39.23** ^{a}
Chrouq_17	K75 Alex. Roade	23.23 ^{d}	23.74 ^{c}
kassacin_35	Kassacin District	30.45 ^{bcd}	37.42 ^{a}

Means within a column followed by different letter (s) are statistically differ with DMRT at Significance Level: 0.05 - Geo. Location = Geographical location of the Isolate

Essential oils embedded in medium

Essential oils of cinnamon, clove, camphor and rocket were tested at concentrations of 0.25, 0.5, 1.0 and 2.0% as embedded in PDA medium for their efficiency to suppress *B. cinerea* radial growth *in vitro* as shown in **Table (2)**. It was found that cinnamon and clove were the most effective to suppress *B. cinerea*, where their EC_{50} and EC_{90} of both essential oils were much less than that of rocket and camphor. While the EC_{50} for clove and cinnamon were less than tested concentrations (< 0.25%), the clove showed higher suppressive effect than cinnamon as EC_{90} value was 0.13 and 0.31, respectively**.** Camphor was the least effective oil to suppress *B. cinerea, in vitro*. Higher concentrations of clove and cinnamon oils showed higher suppressive effect. The concentration 0.5% and 1.0% of clove and cinnamon, respectively, completely suppressed *B. cinerea* radial growth on PDA medium.

Table 2. Effect of different concentrations of EOs embedded in PDA medium on radial growth (mm) of *B. cinerea* at 20-22^{o}C for 7 days

Treatment	**EC_{50} %**	**EC_{90} %**	**Y= a + bX**	**Coeff. of Determ. (r^2)**
Cinnamon EO	0.09	0.31	Y=7.58+2.55X	62.27%
Clove EO	0.02	0.13	Y=7.90+1.85X	35.07%
Camphor EO	2496.53	1043643.30	Y=3.34+0.49X	60.90%
Rocket EO	16.57	4892.68	Y=4.37+0.52X	76.34%

Radial growth reached 90 mm in check treatment

Coefficient of Variation = 4.17%

Y: Probit of means the inhibition (%), and X: Log of means the concentration of the tested essential oil

EC_{50}: Half maximal effective concentration; EC_{90}: effective concentration at 90 percent

Coeff. of Determ. (r^2) : Coefficient of determination

Essential oils used as vapours

Vapourisation of essential oils of cinnamon, clove, camphor and rocket to be used in 25, 50, 75 and 100 µL.L^{-1}air v/v to affect *B. cinerea* growth *in vitro* was evaluated. Data in **Table (3)** show that the radial growth of *B. cinerea* was significantly suppressed by percentages more than 84% by clove and cinnamon vapours even at low concentration as 25 µL.L^{-1}air v/v.

The concentration of 75 µL.L^{-1} air v/v of both oils almost completely inhibited the fungal growth. On the other hand, these tested concentrations of rocket and camphor did not achieve remarkable fungus suppression, where maximum inhibition was less than 17% by highest concentration (data not shown). The EC_{50} values of clove and cinnamon essential oils were less than 25 µL.L^{-1}, while the EC_{90} values were 22.60 µL.L^{-1} air v/v and 33.11 µL.L^{-1} air v/v, respectively. It was found that clove essential oil was significantly suppressive oil treatment against *B. cinerea* even at 25 µL.L^{-1} air v/v.

Table 3. Effect of different concentrations of essential oils with vapourisation technique on radial growth (mm) of *B. cinerea* grown on PDA at 20-22 ^{o}C for 7 days

Treatment	EC_{50} µL/L	EC_{90} µL/L	Y= a + bX	Coeff. of Determ. (r^2)
Cinnamon EO	14.78	33.11	Y=0.72+3.66X	83.30%
Clove EO	8.90	22.60	Y=2.00+3.16X	63.54%
Camphor EO	106862.94	39563626.18	Y=2.44+0.05X	87.11%
Rocket EO	867.91	18960.12	Y=2.19+0.96X	64.86%

Radial growth reached 90 mm in check treatment

Coefficient of Variation = 3.83%

Y: Probit of means the inhibition (%), and X: Log of means the concentration of the tested essential oil

EC_{50}: Half maximal effective concentration ; EC_{90}: effective concentration at 90 percent

Coeff. of Determ. (r^2) : Coefficient of determination

Essential oils used as volatiles

Volatiles of the EOs of cinnamon, clove, camphor and rocket from discs impregnated in concentrations of 0.25, 0.5, 1.0 and 2.0% was tested to suppress *B. cinerea* growth *in vitro*. Volatiles of camphor and rocket showed too low efficacy percentages as shown in **Table (4).** On the other hand clove and cinnamon showed high suppressive effect, EC_{50} and EC_{90} of cinnamon and clove were less than 0.25%.

Table 4. Effect of different concentrations of EOs with volatilisation technique on radial growth (mm) of *B. cinerea* grown on PDA at 20-22^{o}C for 7 days

Treatment	EC_{50} %	EC_{90} %	Y = a + bX	Coeff. of Determ. (r^2)
Cinnamon EO	0.08	0.27	Y=7.64+2.44X	48.27%
Clove EO	0.03	0.15	Y=7.84+1.92X	36.63%
Camphor EO	5160.63	1912020.64	Y=3.15+0.50X	86.94%
Rocket EO	56637.64	644300241.6	Y=3.50+0.32X	68.25%

Radial growth reached 90 mm in check treatment

Coefficient of Variation = 1.81%

Y: Probit of means the inhibition (%), and X: Log of means the concentration of the tested essential oil

EC_{50}: Half maximal effective concentration; EC_{90}: effective concentration at 90 percent

Coeff. of Determ. (r^2) : Coefficient of determination

Post-Harvest Trials

Efficacy of post-harvest vapourisation with EOs on grey mould rot incidence

Flame Seedless cv.

Vapours of cinnamon and clove as postharvest treatment of Flame seedless grapes controlled decay development on naturally infected grapes or artificially inoculated ones with *B. cinerea* during seasons 2014 and 2015 **(Table 5).** The vapour of both essential oils tested at concentrations of 25 µL.L^{-1} air v/v, 50 µL.L^{-1} air v/v and 100 µL.L^{-1}air v/v were very effective to control postharvest decay of grapes during cold storage at 0-1^{o}C for 30 days followed by 5 days shelf life at (12-18^{o}C).

Clove oil showed more efficiency to control *B.* infection either on naturally infected Flame seedless grapes or artificially inoculated. The essential oils of concentrations of 50 µL.L^{-1} air v/v and 100 µL.L^{-1} air v/v were the most effective against *B. cinerea*.

Table 5. Effect of postharvest treatment of Flame seedless grapes with tested EOs vapours on incidance of grey mould during cold storage at 0-1 ^{o}C and shelf-life of naturally infected and artificially inoculated grapes, seasons 2014 and 2015

Season 2014					
		Natural Infection		Artificially inoculation	
Treatment	Conc. (in air)	30 Days-Storage	5 Days-Shelf-life	30 Days-Storage	5 Days-Shelf-life
Cinnamon	25 µL/L	7.59^{a}	22.76^{a}	9.20^{bc}	26.90^{b}
	50 µL/L	3.68^{b}	10.34^{b}	5.06^{bcd}	14.48^{c}
	100µL/L	**0.92^{b}**	5 .29^{b}	3.22^{d}	10.80^{c}
Clove	25 µL/L	8.28^{a}	24.83^{a}	10.34^{b}	31.03^{b}
	50 µL/L	2.76^{b}	8.05^{b}	4.14^{cd}	11.03^{c}
	100µL/L	1.61^{b}	**4.60^{b}**	**2.7^{d}**	**10.57^{c}**
Control		10.34^{a}	30.11^{a}	17.93^{a}	44.37^{a}
Season 2015					
		Natural Infection		Artificially inoculation	
Treatment	Conc. (in air)	30 Days-Storage	5 Days-Shelf-life	30 Days-Storage	5 Days-Shelf-life
Cinnamon	25 µL/L	1.38^{b}	5.06^{b}	2.07^{b}	8.97^{bc}
	50 µL/L	1.15^{b}	4.37^{b}	1.38^{b}	6.21^{bc}
	100µL/L	1.15^{b}	**3.22^{b}**	**0.92^{b}**	**4.83^{c}**
Clove	25 µL/L	1.61^{b}	7.13^{b}	2.99^{b}	12.87^{b}
	50 µL/L	1.38^{b}	6.21^{b}	1.84^{b}	8.28^{bc}
	100µL/L	**0.92^{b}**	**3.22^{b}**	1.61^{b}	6.67^{bc}
Control		9.66^{a}	28.05^{a}	17.70^{a}	42.07^{a}

Means within a column followed by different letter (s) are statistically differ with DMRT at Significant Level:0.05 , Conc.: Concentration

Superior seedless cv.

On Superior seedless grapes, cinnamon vapour was more effective than clove vapourisation treatment to control *B. cinerea* on grape bunches of natural infection or artificial inoculation during cold storage and shelf-life as shown in **Table (6).**

The most effective treatment on naturally infected Superior seedless grapes was clove at 100 µL.L^{-1} air v/v, while on artificially inoculated bunches with *B. cinerea*, cinnamon at that concentration was the most effective treatment during cold storage and shelf life.

DISCUSSION

The effect of EOs on mycelial growth of *B. cinerea in vitro* was studied with back information about the impact of each EOs on other fungi as **Plaza et al (2004)** found that clove and cinnamon essential oils added to the medium at concentration of 0.1% completely inhibited *P. digitatum* and *P. italicum* growth.

Table 6. Effect of postharvest treatment of Superior seedless grapes with tested EOs vapours on incidance of grey mould during cold storage at 0-1 ^{o}C and shelf-life of naturally infected and artificially inoculated grapes, seasons 2014 and 2015

Season 2014					
Treatment		Natural Infection		Artificially inoculated	
	Conc. (in air)	30 days	5d shelf-life	30 days	5d shelf-life
Cinnamon	25µL/L	1.27^{b}	5.10^{b}	1.70^{b}	7.22^{b}
	50µL/L	0.85^{b}	3.82^{b}	1.49^{b}	5.73^{b}
	100µL/L	**0.42^{b}**	**2.55**^{b}	**1.06**^{b}	**4.46**^{b}
Clove	25µL/L	1.91^{b}	8.07^{b}	2.34^{b}	9.98^{b}
	50µL/L	1.06^{b}	5.10^{b}	2.12^{b}	8.49^{b}
	100µL/L	0.85^{b}	3.61^{b}	1.70^{b}	6.79^{b}
Control		9.13^{a}	25.90^{a}	16.35^{a}	38.85^{a}
Season 2015					
Treatment		Natural Infection		Artificially inoculated	
	Conc. (in air)	30 days	5d shelf-life	30 days	5d shelf-life
Cinnamon	25µL/L	1.91^{bc}	4.67^{c}	2.55^{b}	6.37^{bc}
	50µL/L	1.06^{cd}	3.61^{c}	2.12^{b}	5.52^{bc}
	100µL/L	**0.00**^{e}	**2.12**^{c}	**1.49^{b}**	**4.25^{c}**
Clove	25µL/L	2.55^{b}	7.43^{b}	3.40^{b}	9.55^{b}
	50µL/L	1.49^{c}	4.88^{bc}	3.18^{b}	8.28b^{c}
	100µL/L	0.42^{de}	3.82^{c}	2.12^{b}	6.37b^{c}
Control		8.92^{a}	25.69^{a}	15.07^{a}	40.98^{a}

Means within a column followed by different letter (s) are statistically differ with DMRT at Significant Level:0.05 , Conc.: Concentration,

Antimicrobial cinnamic aldehyde and eugenol is a major component of clove oil and cinnamon oil as **(Davidson & Naidu, 2000 and Hassani, et al 2012).** As **Taylor et al (2002)** reported that embedded in medium calculated the EC_{50} value according to the relationship of eugenol concentrations and inhibition rate of mycelial growth, this approach was followed during present study to compare the efficacy of such tested materials, which were adopted during this study as EC_{50} and EC_{90}. It was strongly proposed the idea that the antifungal activity of eugenol is due to the disruption of the membrane, leading to cell death. **Wang et al (2010)** found that eugenol had antifungal properties against mycelial growth of *B. cinerea* where the EC_{50} was 38.6 µg.mL^{-1}. No bioactivity was obtained for eugenol against *B. cinerea* conidia germination. Eugenol caused morphological alterations in *B. cinerea* hyphae including cytoplasmic coagulation, vacuolation, hyphal shrivelling and disruption of the plasma membrane. However, in present study clove and cinnamon oils showed EC_{50} less than 0.25% (2500 ppm), where it was the minimum tested concentration. However, it is so high comparing with the determined EC_{50} for Eugenol as the main active material particularly in clove oil.

The largest inhibition zone of *Penicillium digitatum* was determined for clove and cinnamon oils, while the mycelial vigour was strong outside the zone of inhibition as well as strong sporulation development where the mycelia grew was allowed **(Hall and Fernandez, 2004)**.

Where eugenol is the major component of clove oil, it is also a component in cinnamon oil, while the major component in cinnamon oil is cinnamaldehyde **(Ćosić et al 2010 & Abd Elwahab and Rashid, 2013)**, which attributed the obtained efficacy of both clove and cinnamon oil.

Essential oils used as vapours was tested to detect the suppression effect of clove and cinnamon at low concentration is very promising to test them *in vivo*, particularly as an easy application for fungus control on grapes after harvest, during storage or shipping for export.

Vapours of clove oil and cinnamon oil exhibited strong inhibitory effects on *B. cinerea*, where 15 µL/ 5cm-Petri's dish completely suppressed its mycelial growth (**Sirirat et al 2009)**.

Obtained antifungal activity of clove volatiles was demonstrated by **Wilson et al (1997)** who found its complete inhibition of spore germination of *B. cinerea* at dilution of 0.78% up to 24hrs, while cinnamon at 1.56% dilution completely inhibited *B. cinerea* spore germination after 40 hrs. Also, **Plaza et al (2004)** found that volatiles of clove and cinnamon essential oils at 10 µl in 5cm diameter Petri's dish completely inhibited completely inhibited *P. digitatum* and *P. italicum* growth. So, it could be expected that clove and cinnamon volatiles could affect spore germination and mycelial growth of *B. cinerea*.

Clove and cinnamon inhibitory effect could be attributed to morphological changes, including cytoplasmic coagulation and vesiculation, and shrivelled hyphae were commonly observed in eugenol-treated mycelia, compared with the normal mycelia as demonstrated by **Wang et al (2010).**

Comparing the EC_{50} and EC_{90} of clove and cinnamon across the different types of application showed that embedded or volatiles achieved higher suppressive effect than when used as vapour, which could be attributed to less adopted concentration for vapourisation. However, all application technique of essential oils showed complete suppressive effect or close to it particularly at higher concentrations.

On contrary to the present results of positive effects of essential oils particularly clove and cinnamon essential oils to control grey mould rot of grapes,the efficacy of post-harvest vapourisation with EOs on grey mould rot incidence as **Plaza et al (2004)** they found that clove and cinnamon essential oils did not reduce the incidence of *P. digitatum* and *P. italicum* on oranges when applied directly over the inoculated wounds of artificial inoculation at concentration of 0.1% while, they very effective to control growth of both fungi *in vitro*. However, the present work used clove and cinnamon oils as vapour on both naturally infected and artificially inoculated grapes. On the other hand, fungi vary in their sensitivity towards different chemicals including essential oils as well as type of application.

Vapourisation of Snap bean pods during storage with Carnation (clove buds) at 100 $\mu L.L^{-1}$ air v/v was the best treatment as suppressed completely the disease caused by the two tested mould pathogens (*B. cinerea* and *Pythium aphanidermatum*), while the same potential effectiveness was obtained on Valentino cv. using Camphor oil at 100 $\mu L.L^{-1}$ with both tested pathogens **(Abdel-Mageed et al 2012)**. This finding indicated that the possibility of different response of cultivars towards essential oil vaporisation treatments.

Generally in our study clove and cinnamon at 100 $\mu L.L^{-1}$ air v/v were the most effective essential oil vapourisation treatments to control grey mould rot on Flame seedless and Superior seedless grapes.

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