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ANTIFUNGAL ACTIVITY OF PLANT EXTRACTS, ESSENTIAL OILS, AND MICROBIAL CULTURE FILTRATES AGAINST *Botrytis cinerea In-vitro*

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ABSTRACT

The effect of using plant extracts and oils of plant origin on the colony growth of *Botrytis cinerea*, the causal agent of gray mold of fruits and vegetables was studied five plant extracts (pomegranate peel, cloves, neem, cinnamon and thyme) at three concentrations(0, 15, 30 and 45%; for the aqueous extracts and 0, 6, 9 and 15%; for the extracts of methyl alcohol and ethyl estate) were used Essential oils of colves, neem, cinnamon, and thyme at three concentrations (0, 1, 1,5 and 2%) as well as culture filtrates of *Trichodermaharzianum*, *T. hamatum* and *Bacillus subtilis* at three concentrations (0, 15, 30 and 45%) were also tested. The fungicide, Topsin M-70WP at the recommended concentration (80g/100L water) was used as a check for comparison.

In general, for the concentrations 1 and 2, there were no significant differences among the three methods of extraction (water, methyl alcohol, and ethyl acetate) also givenaqueous fraction of crude plant extracts significant reduction in the linear growth of *B. cinerea* ranged from (57.78 to 96.67%) Pomegranate peel andneem aqueous extracts caused the highest growth inhibition at 45% concentration 96.67 and 92.22% respectively with no significant difference from the fungicide effect at same concentration. While, given methyl alcohol and ethyl acetate All plant extracts produced significant growth inhibition of *B. cinerea* 7 days post inoculation in comparison with the untreated control. both fractions (methyl alcohol and ethyl acetate), it was found that all plant extracts tested at 15%, except thyme extract, caused more than 89% growth inhibition to the pathogen with no significant differences from the chemical fungicide, Topsin M-70WP.

Essential oils given tested caused significant suppression of the pathogen growth, 7 days post inoculation in comparison with the untreated control. The growth inhibition of the pathogen increased with the increase of the plant oil concentration. The chemical fungicide, Topsin M-70WP, had the highest inhibitory effect against the pathogen's growth, which was followed by cloves and neem essential oils when tested at 2% concentration. While,all culture filtrates tested caused Record significant inhibition of the pathogen growth, 7 days after inoculation in comparison with the untreated control.. The bacterial culture filtrate of *B. subtilis* had the highest inhibitory effect against the pathogen's growth, with no significant difference from the chemical fungicide, Topsin M-70WP.

Keywords: plant extracts, plant oils, microbial culture filtrate, *Botrytis cinerea*, biological control, antifungal activity, grape,

INTRODUCTION

Gray mould caused *by Botrytis cinerea* Pers., is a major cause of fruit losses in table grapes crops (Hancock, 1999) and at least 200 plant species.Flower parts play an important role in fruit infection. Gray mould is an important disease of table grape resulting from setting and fruit infection. Because of high perish ability and short post harvest life, the use of chemicals to retain the fruit quality during storage and transport is restricted and it is necessary to find alternate natural compounds for use in field and postharvest that pose no or minimal risk to human health and the environment.

Chemical control is the primary means by which gray mould is controlled. However, this may occasionally be ineffective due to the occurrence of resistant fungal populations (Hunter *et al*., 1987).Conventional dicarboximide fungicides such as iprodione have been used to control *B. cinerea.*Control of *Botrytis* in the field through chemical sprays has been only partially successful, especially in cool, damp weather (Katan*et al*., 1989). Continuous use of fungicides has faced two major obstacles; increasing public concern regarding contamination of fruits and vegetables with fungicidal residues, and proliferation of resistance in the pathogen populations(Tripathi and Dubey, 2004).

Since ancient times, plant extracts have been used in many ways to manage plant diseases. Recently, public health and environmental safety concerns encouraged the use of these natural products as a complete replacement of chemicals for improving growth and nutritional status of fruit trees and production. Their positive action on controlling pests are very essential. The high contents of phenolic compounds, nutrients, plant pigments and other chemical constituents in these natural extracts seem to have synergistic effects on plant growth while causing mortality of most fungi. Out of the important plant extracts are pomegranate, cloves, neem, cinnamon, thymes and others (Ahmed *et al*., 2013).

The essential oils are one of the most promising groups of natural compounds for the development of antimicrobial agents and use in plant protection. Generally, essential oils are complex mixtures of hydrocarbon monoterpenes, oxygenated monoterpenes, hydrocarbon sesquiterpenes, oxygenated sesquiterpenes, and related compounds that derive from the secondary metabolism of plants (Reverchon, 1997). The antimicrobial activity of essential oils or their constituents on postharvest fungi has been quite extensively examined (Feng and Zheng, 2007; Regnier*et al*., 2010).

Biological control can be defined as the use of natural or modified organisms, genes or gene products to reduce the effects of undesirable organisms and to favor desirable organisms such as crops, beneficial insects and microorganisms (Gnanamanickam *et al.,* 2002). The frequent development of resistant strains of *B. cinerea* to common fungicides and the desire to reduce pesticide use have led to efforts to develop alternatives.

Biological control of *B.cinerea* has been frequently reported in the past (Dubos *et al*., 1982; Elad, 1994; Inbar*et al.,* 1996; Paul *et al*., 1997).

MATERIALS AND METHODS

1. Collecting plant samples for extractions preparation

Pomegranate peel: Samples of pomegranate fruits were collected from local markets, washedwith tap water to remove the dust. Peels were then dried on carton papers at room temperature and then ground into powder using an electric grinder. The resulting powder was kept in sealed glass bottles and stored in refregirator at 5^{o}C until use.

Neem leaves: Leaves were collected from neem trees, washed with running tab water to remove the dust, and dried in an electric oven. Dried leaves were milled into powder using small electric grinder. The resultant powder was stored in closed bottles at 5^{o}C until use.

Cloves, cinnamon and thyme: Samples of cloves buds,cinnamon bark and thyme werecollected from local markets in dry state, ground into powder using small electric grinder, and stored in closed bottles at 5^{o}C until use.

2. Source of essential oils:

Essential oils of cloves, cinnamon and thyme were collected from local markets in a ready-to-use state.

Table1 : List of plants screened for antifungal activity against tested fungus.

NO.	Scientific name	Family	English name	Plant Part used for extraction	Used as
1	*Punica granatum*	Punicaceae	Pomegranate	Peel	Extract
2	*Syzygium aromaticum*	Caryophyllaceae	Clove	Flowers	Oil-Extract
3	*Azadirachta indica*	Meliaceae	Neem-nim	Dried leaves	Oil-Extract
4	*Cinnamonnan zeylanicwn*	Lauraceae	Cinnamon	Bark	Oil-Extrac
5	*Thymus vulgaris*	Lamiaceae	Thyme	Dried leaves	Oil-Extrac

3. Source of microbial antagonists and the pathogen:

Pure cultures of the fungi; *Trichodermaharzianum* and *T. hamatum* and the bacterium; *B. subtilis* as well as the gray mould fungal pathogen; *B. cinerea* were cordially obtained from the Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt. Fungal isolates were grown on potato dextrose agar (PDA) medium in petri dishes, and preserved on PDA slants in a refrigerator at 5°C as stock cultures. The bacterium, *B. subtilis* was kept in nutrient agar slants at 5°C until use.

4. Preparation of plant extracts:

Extraction with water:

The powder prepared as described above from plant parts of pomegranate, cloves, neem, cinnamon and thyme were soaked in distilled water at a rate of 1:1 (w/v) and kept on a shaker at 100 rpm for 20-24 hours at room temperature (25±3°C). Then, the mixture was filtered through cheese cloth under a strong hand pressure. The resulted solution was kept in a refrigerator at 5°C until using. The extract was centrifuged at 12,000 rpm for

30 min. and sterilized by filtering through a 0.45 µm membrane filter at 25±3°C to avoid any microbial contamination. The resulting crude extract was considered as 100% concentration and used as a stock for the preparation of four concentrations (0, 15, 30 and 45%) later on.

Extraction with organic solvents (methyl alcohol and ethyl acetate):

The extraction process was carried out according to the method described by Harborne (1973). A hundred gram of the powder of each plant type (pomegranate, cloves, neem, cinnamon and thyme) was separately put in 500-ml Erlenmeyer flasks and then, 200 ml of methyl alcohol or ethyl acetate was added to each flask, and put on an electric vibrator at room temperature for 24 hours. The extract was then filtered through cheesecloth under a strong hand pressure, after which the five extracts were filtered using Buechner funnel lined with Whatman No. 2 filter paper under vacuum. The extraction process was repeated twice for each sample. The resulting filtrate was then put in a water bath at 40^{o}C to get rid of the solvent. The remaining material was stuck in the flask bottom as a thick slimy layer. Material remained was stored in a deep freezer at -20^{o}C until use.

One gram of each remaining material (extract concentrate) was dissolved in 50 ml of distilled water while 0.5% of Tween-80 (v/v) was added to enhance solubility. The resulting solution was used as a stock which considered as 100% concentration.

5. Antifungal activity of plant extracts against *B. cinerea*, thecausal agent of gray mould in grape.

The stock solutionsof the three types of extractions (water, methyl alcohol and ethyl acetate)for the five plant materials (pomegranate, cloves, neem, cinnamon and thyme) were diluted with sterile warm PDA(~48°C) to prepare four concentrations; 0, 15, 30 and 45%. Five 9-cm-diameter Petri plates, each containing 20 ml of each concentration were used as replicates. Control treatment (0% extract) was prepared by mixing PDA with tween-80 only with no extracts added. For comparison, five 9-cm-diameter Petri plates were prepared using the fungicide Topsin M-70WP(Thiophanate-methyl 70% wettable powder) that was amended with the PDA medium at the recommended concentration (80g/100L water).

All plates were inoculated in the center with 5-mm-diameterdiscs from one-week-old cultures of *B. cinerea*grown on PDA. Inoculated plates were then incubated at 25±1^{o}C for 7 days, when mycelial growth covered the surface of the control treatment. Then, *B. cinerea*linear growth was measured by calculating the mean of two orthogonal diameters for each colony. The growth inhibition (as %) of the pathogen was calculated according to the following equation (Okigbo and Nmeka, 2005):

$$\textbf{Growth inhibition (\%)} = [(DC - DT) / DC] \times 100$$

Where:

DC= average colony diameter of control treatment (containing 0% of plant extract).

DT= average colony diameter of the pathogen treated with plant extract at different concentrations.

6. Antifungal activity of plant essential oils against *B. cinerea*, thecausal agent of gray mould in grape.

Four commercial essential oils of (cloves, cinnamon, neem and thyme) were tested for their antifungal activity at three concentrations (1, 1.5 and 2% v/v) against the fungal pathogen, *B. cinerea*. The three essential oils concentrates were prepared by mixing with warm PDA (~48°C) medium after autoclaving while 0.5% of Tween-80 (v/v) was added to enhance oil solubility. Three Petri dishes (9-cm diameter) were prepared as replicates for each concentration. Control treatment was made by mixing PDA with Tween-80 only without adding essential oils. All plates were left for 30 min to allow solidification before inoculation with 5-mm-diameter discs taken from 7-day-old culture of the pathogen grown on PDA. Inoculated plates were then incubated at 25±1^{o}C for 7 days, when mycelial growth covered the surface of the control treatment. Average of radial growth was recorded 7 days after inoculation and compared with the untreated control (as described earlier) (De-Billerbeck, et al 2001). The percent growth inhibition of the pathogen was calculated according to the equation mentioned above (Okigbo and Nmeka, 2005).

7. Antifungal activity of culture filtrates of some fungal biocontrol agentsagainst B. cinerea.

Preparation of filtrates: The inhibitory effect of the culture filtrates of the antagonistic fungi, *T. harzianum*and *T. hamatum*was evaluated against the mycelialm growth of *B.cinerea*. Erlenmeyer flasks (250-ml-size), each containing 100 ml of autoclaved potato dextrose broth (PDB) medium were inoculated with one 5-mm-diameter disc of the tested fungal cultures (*T. harzianum*and *T. hamatum*)grown on PDA for 5 days. Inoculated flasks were incubated at 28±2°C until the mycelial growth covered the medium surface. The liquid cultures were filtrated through Whatman No. 1 filter paper, centrifuged at 12,000 rpm for 30 min., and sterilized by using membrane filter of 0.45 µm pore size. The resulted filtrates were kept in sterilized dark bottles in a refrigerator at 5°C until using.

The resulted filtrates were diluted with sterile warm PDA(~48°C) to prepare four concentrations; 0, 15, 30 and 45%.Tween-80 was added at 0.5%(v/v) to enhance solubility. Five 9-cm-diameter Petri plates, each containing 20 ml of each concentration were used as replicates. Control treatment (0% filtrate) was prepared by mixing PDA with Tween-80 only with no extracts added. For comparison, five 9-cm-diameter Petri plates were prepared using the fungicide Topsin M-70WP was amended with the PDA medium at the recommended concentration (80g/100L water)

All plates were inoculated in the center with 5-mm-diameterdiscs from one-week-old cultures of *B. cinerea*grown on PDA. Inoculated plates were then incubated at 25±1^{o}C for 7 days, when mycelial growth covered the surface of the control treatment. Then, *B. cinerea*colony diameter was measured by calculating the mean of two orthogonal diameters for each colony. The percent growth inhibition of the pathogen was calculated according to the equation mentioned above (Okigbo and Nmeka, 2005).

8. Antifungal activity of the culture filtrate of the bacterial biocontrol agent, *B. subtilis*against *B. cinerea.*

Erlenmeyer flasks (250-ml-size), each containing 100 ml of autoclaved nutrient broth (NB) medium were inoculated with one loop of bacterial culture grown on nutrient agar for 48hr. The inoculated flasks were incubated on shaker at 30±2^{o}C for 72hr. The cultures were filtered through filter paper (Whatman No. 1), centrifuged at 12,000 rpm for 30 min., and sterilized by using membrane filter of pore size of 0.22 µm. The resulted filtrate was kept in sterilized dark bottles in a refrigerator at 5°C until using. The crude bacterial filtrate was mixed with warm PDA (~48°C) to make four concentrations (0, 15, 30 and 45%), which were poured into sterile Petri dishes (9-cm diameter; 20 ml per plate).Three replicates for each concentration were usedTween-80 was added at 0.5%(v/v) to enhance solubility. Control treatment (0% filtrate) was prepared by mixing PDA with Tween-80 (0.5%, v/v) only with no extracts added. For comparison, three 9-cm-diameter Petri plates were prepared using the fungicide Topsin M-70WP was amended with the PDA medium at the recommended concentration (80gm/100L water) .All plates were inoculated in the center with 5-mm-diameterdiscs from one-week-old cultures of *B. cinerea*grown on PDA. Inoculated plates were then incubated at 25±1^{o}C for 7 days, when mycelial growth covered the surface of the control treatment. Colony diameter of *B. cinerea*was measured and the percent growth inhibition of the pathogen was calculated as described above (Okigbo and Nmeka, 2005).

RESULTS AND DISCUSSION

1- Effectiveness of extraction method on the growth inhibition of *B. cinerea*.

Three methods were utilized for plant parts extraction using water, methyl alcohol and ethyl acetate. Data presented in Table (2) showed that for the concentration 1 and 2, there were no significant differences among the three methods of extraction (water, methyl alcohol, and ethyl acetate)...However, for concentration "1", all method, of extraction has same efficacy in inhibiting B. cinerea Growth except for thyme, cloves and Neem, for which crude extract using ethyl acetate or methyl alcohol had more inhibitory effect than water fraction (Table 2). These results are in harmony with those found by (Wenqiao*et al*., 2004) Led extracts (ethanol, methanol, acetone, n-hexane) highly effective in combating many of the fungal plant diseases,Amna and Shahba. (2010). Study the effectiveness of aqueous extract of the pomegranates peel*Punicagranatum* .L in the inhibition of the growth of molds causative to damage and corruption of food in the food center and showed the results of chemical tests of effective materials in aqueous extract of the pomegranate peel,(Cseh *et al*., 2015)were studied. During the experiment hot water and ethanol extracts were used and disc diffusion method was applied. Our results show that after an initial 100% inhibition effect of thyme, the alcoholic extracts of sage and rosemary show an approximately equal inhibition of the growth of fungal mycelia at 48 and 72

hours post treatment. 24 hours after the treatment the degree of inhibition of thyme, sage and rosemary extracts were 100%, 87.9% and 79.9%, while after 48 hours they were 76.1%, 73.2% and 69.5%, respectively.

Table (2): Effect of aqueous fraction of crude plant extracts on radial growth of *B. cinerea* inhibition of *B. cinerea in-vitro*

Extracts	Concentration *	*B.cinerea*colony diameter (cm), 7 days after inoculation (% Growth inhibition)			LSD at 0.05 level
		Fraction			
		Water	Methyl alcohol	Ethyl acetate	
Pomegranate peel	1	2.7 a** (70.00%)	2.0 a (77.78%)	2.1a (76.67%)	0.80
Cloves		3.0 a (66.67%)	2.1 b (76.67%)	2.2 b (75.56%)	0.59
Neem		3.2 a (64.44%)	2.3 ab (74.45%)	2.1 b (76.67%)	1.02
Cinnamon		2.7 a (70.00%)	2.3 a (74.45%)	2.2 a (75.56%)	1.37
Thyme		3.8 a (57.78%)	3.0 ab (66.67%)	2.5 b (72.23%)	1.06
Pomegranate peel	2	1.7 a (81.12%)	1.3 a (85.55%)	1.2 a (86.67%)	0.82
Cloves		2.1 a (76.67%)	1.7 a (81.12%)	1.4 a (84.45%)	0.74
Neem		2.4 a (73.33%)	1.7 a (81.12%)	1.6 a (82.23%)	1.03
Cinnamon		2.4 a (73.33%)	2.3 a (74.45%)	1.8 a (80.00%)	1.31
Thyme		2.9 a (67.78%)	2.8 a (68.89%)	2.1 a (76.67%)	1.48
Pomegranate peel	3	0.3 a (96.67%)	0.9 a (90.00%)	0.5 a (94.44%)	0.74
Cloves		1.1 a (87.78%)	1.0 a (88.88%)	0.9 a (90.00%)	1.52
Neem		0.7 a (92.22%)	0.9 a (90.00%)	0.7 a (92.22%)	1.19
Cinnamon		1.5 a (83.33%)	1.0 a (88.88%)	0.8 a (91.12%)	1.19
Thyme		1.8 a (80.00%)	1.8 a (80.00%)	1.2 a (86.67%)	0.72

*** Concentration 1= 15%(w/v) for water extraction and 6%(w/v) for extraction with methyl alcohol and ethyl acetate, Concentration 2= 30%(w/v) for water extraction and 9%(w/v) for extraction with methyl alcohol and ethylacetate,andConcentration 3= 45%(w/v) for water extraction and 15%(w/v) for extraction with methyl alcohol and ethyl acetate.**

****Values within a row followed by the same letter(s) are not significantly different, according to LSD test at *P* = 0.05.**

2- Effect of aqueous fraction of crude plant extracts on radial growth of *B.cierea*

Aqueousfraction of five plant extracts (pomegranate peel, cloves, neem, cinnamon and thyme) were tested *in-vitro* for their ability to suppress the growth of *B. cinerea* at concentrations of 0,15, 30, and 45% in order to be used as safe compounds for the control of *B.cinerea* in the subsequent field experiments and post harvest (in storage).

Data presented in Table (3) showed that all plant extracts caused significant reduction in the linear growth of *B. cinerea* ranged from (57.78 to 96.67%) after 7 days of incubation in comparison with the the untreated control. Pomegranate peel andneemaqueous extracts caused the highest growth inhibition at 45% concentration (96.67 and 92.22%, respectively with no significant difference from the fungicide effect (Table 3). These results are in line with those reported by(Lapis and Dumancas, 1978)who studied the antagonistic effect of 93 plant extracts against the fungus *Helminthosporiumoryzae*(the causal agent for brown spot disease in rice plants) using water as a solvent. Misra and Dixit (1979) studied the biological activity of leaf extractsof 30 species of higher plants against smut fungi*Ustilagotritici* and *U. hordei*.They found that 14 extracts of them were effective against fungi mentioned while other plant extracts had low impact.

Table (3): Effect of aqueous fraction of crude plant extracts on radial growth of *B.cinerea.*

Treatment	***B.cinerea*colony diameter (cm), 7 days after inoculation (% Growth inhibition)**		
	Concentration of aqueous fraction of crude plant extracts		
	15%	**30%**	**45%**
Control (plant extract-free)	9.0 a* (0%)	9.0 a (0%)	9.0 a (0%)
Pomegranate peel	2.7 c (70.00%)	1.7 c (81.11%)	0.3 de (96.67%)
Cloves	3.0 c (66.67%)	2.1 bc (76.67%)	1.1 bcd (87.78%)
Neem	3.2 bc (64.44%)	2.4 bc (73.33%)	0.7 cde (92.22%)
Cinnamon	2.7 c A (70.00%)	2.4 bc A (73.33%)	1.5 bc A (83.33%)
Thyme	3.8 b (57.78%)	2.9 b (67.78%)	1.8 b (80.00%)
Fungicide**	0.0 d (100%)	0.0 d (100%)	0.0 e (100%)
LSD at 0.05 level	0.72	1.04	0.83

*** Values within a column followed by the same letter(s) are not significantly different, according to LSD test at *P* = 0.05.**

****The fungicide (Topsin M-70WP) was used at the recommended concentration(0.8g / L).**

3-Effect of methyl alcohol andethyl acetate fractions of crude plant extracts on radial growth of *B. cinerea.*

All plant extracts produced significant growth inhibition of *B. cinerea* 7 days post inoculation in comparison with the the untreated control.The growth

inhibition of the pathogen increased with the increase of concentration of plant extract (Table 4). For both fractions (methyl alcohol and ethyl acetate), it was found that all plant extracts tested at 15%, except thyme extract, caused more than 89% growth inhibition to the pathogen with no significant differences from the chemical fungicide, Topsin M-70WP (Table 4). Similar results have been reported byCharo*et al*. (2003)who studied the effect of extraction methods (solvents' systems) to 41 plant species against three pathogenic fungi of soybean.

Table(4): Effect of methyl alcohol andethyl acetate fractions of crude plant extracts on radial growth of *B. cinerea.*

Treatment		*B. cinerea*colony diameter (cm), 7 days after inoculation (% Growth inhibition)		
		Plant extracts concentration		
		6%	9%	15%
Control(plant extract-free)		9.0 a* (0%)	9.0 a (0%)	9.0 a (0%)
Methyl alcohol fraction	Pomegranate peel	2.0 c (77.78%)	1.3 d (85.55%)	0.9c (90.00%)
	Cloves	2.1 bc (76.67%)	1.7 cd (81.11%)	1.0 bc (88.88%)
	Neem	2.3 bc (74.45%)	1.7 cd (81.12%)	0.9 c (90.00%)
	Cinnamon	2.3 bc (74.45%)	2.3 cd (74.45%)	1.0 c (88.89%)
	Thyme	3.0 b (66.67%)	2.8 b (68.89%)	1.8 b (80.00%)
	Fungicide**	0.0d (100%)	0.0e (100%)	0.0d (100%)
LSD at 0.05 level		0.88	0.84	0.81
Ethyl acetate fraction	Pomegranate peel	2.1 b (76.67%)	1.2 d (86.67%)	0.5 bc (94.44%)
	Cloves	2.2 b (75.56%)	1.4 cd (84.45%	0.9 c (90.00%)
	Neem	2.1 b (76.67%)	1.6 cd (82.23%)	0.7 bc (92.22%)
	Cinnamon	2.2 b (75.56%)	1.8 bc (80.00%)	0.8 bc (91.12%)
	Thyme	2.5 b (72.23%)	2.1 b (76.67%)	1.2 b (86.67%)
	Fungicide**	0.0d (100%)	0.0d (100%)	0.0e (100%)
LSD at 0.05 level Fungicide		0.60	0.49	0.82

*** Values within a column for each fraction followed by the same letter(s) are not significantly different, according to LSD test at *P* = 0.05.**

****The fungicide (Topsin M-70WP) was used at the recommended concentration(0.8g / L).**

The study showed that aqueous extract of 17 from the plants used had inhibitory effect of one or more of the three fungal pathogens. He found

thatplantextracts that dried by ethyl acetate obviously inhibit all used fungal types used in the experiment.Annapurna *et al*. (1983) studied the effectof leaf extracts of the plant *polyalthialongifolia* against the fungal pathogen*Drechsleraspecifers*using different solvent systems. This study showed This studied showed briefly nature of the effective compound for these extracts, Considering the remarkable effectiveness in the solvents.

4-Effect of different essential oils on the radial growth of *B. cinerea.*

Data obtained showed that all essential oils tested caused significant suppression of the pathogen growth, 7 days post inoculation in comparison with the untreated control (Table 5).The growth inhibition of the pathogen increased with the increase of the plant oil concentration (Table 5). The chemical fungicide, Topsin M-70WP, had the highest inhibitory effect against the pathogen's growth, which was followed by cloves and neem essential oils when tested at 2% concentration (Table 5). Thyme essential oil was the least effective one against the pathogen growth (Table 5).Our results are in agreement with those obtained by Rodrgesu*et al*. (2004)who found that the volatile oils prevent or suppress the growth of fungi that infect fruits. Cho *et al.* (2000) tested the antimicrobial activity of 45 essential oils on bacteria, fungi and yeast, and found that cinnamon oil exhibited a high antimicrobial activity. The essential oil of cinnamon has been reported previously as a source of antifungal agent (Delespaul*et al.,* 2000). This oil contains compounds such as cinnamaldehyde, which has been tested on many fresh fruits such as mandarin and kiwi to control postharvest diseases.Tripathi et al. (2008) stated that essential oils especially thyme, fennel and summer savory oilshad antifungal activity and enhanced the storage life of grape up to 6 days.Maqbool et al. (2010) indicated that cinnamon oil up to 0.3% maintained the good quality of bananas during storage.Lopez-Reyes *et al.* (2010) showed that treatments with essential oils (1 and 10%) from oregano and thyme controlled apple fruits decay caused by *B.cinerea* and *PenicilliumExpansum.*

Table (5):Effect of four essential oils on *B.cinerea* radial growth.

Treatment	***B.cinerea*colony diameter (cm), 7 days after inoculation (% Growth inhibition)**		
	Essential oils concentration		
	1.0%	**1.5%**	**2.0%**
Control	9.0 a* (0%)	9.0 a (0%)	9.0 a (0%)
Cloves	2.7 c (70.00%)	2.3 c (74.45%)	1.0 d (88.88%)
Neem	2.7 c (70.00%)	2.2 c (75.56%)	1.2 cd (76.67%)
Cinnamon	3.0 c (66.67%)	2.5 c (72.23%)	1.5 c (83.33%)
Thyme	3.5 b (61.11%)	3.5 b (61.11%)	2.6 b (71.11%)
Fungicide**	0.0d (100%)	0.0d (100%)	0.0e (100%)
LSD at 0.05 level	0.41	0.37	0.30

*** Values followed by the same letter(s) are not significantly different, according to LSD test at *P* = 0.05.**

****The fungicide (Topsin M-70WP) was used at the recommended concentration(0.8g / L).**

5-Effect of antagonistic fungal and bacterial culture filtrates on the growth of *B.cinerea.*

Results showed that all culture filtrates tested caused significant inhibition of the pathogen growth, 7 days after inoculation in comparison with the untreated control (Table 6).The growth inhibition of the pathogen increased with the increase of the filtrate concentration (Table 6). The bacterial culture filtrate of *B. subtilis* had the highest inhibitory effect against the pathogen's growth, with no significant difference from the chemical fungicide, Topsin M-70WP.*Trichoderma* filtrates were significantly less effective than that of *B. subtilis* in this regard, with *T. hamatum*'s filtrate more effective than *T. harzianum*'s when tested at 45% concentration (Table 6).

Table (6): Effect of antagonistic fungal and bacterial culture filtrates against *B.cinerea* radial growth.

Treatment	*B.cinerea*colony diameter (cm), 7 days after inoculation (% Growth inhibition)		
	Culture filtrates concentration		
	15	30	45
Control (Culture filtrate-free)	9.0a* (0%)	9.0 a (0%)	9.0a (0%)
*T.harzianum*culture filtrate	2.7 b (70.00%)	2.2 b (75.56%)	1.5 b (83.33%)
*T.hamatum*culture filtrate	2.5 b (72.23%)	2.0 b (77.78%)	1.2 c (76.67%)
*B.subtilis*culture filtrate	1.6 c (82.23%)	1.2 c (76.67%)	0.2 d (97.78%)
Fungicide**	0.0d (100%)	0.0d (100%)	0.0d (100%)
LSD at 0.05 level	0.61	0.28	0.28

*** Values followed by the same letter(s) are not significantly different, according to LSD test at *P* = 0.05.**

****The fungicide (Topsin M-70WP) was used at the recommended concentration(0.8g / L).**

Biological control agents such as *Trichoderma spp*. are asexually reproducing fungi that are often the most frequently isolated from soil. They are very useful microbes, by producing beneficial effects on crops and they may have naturally sustained the agricultural yields that have supported the human population over the millennia (Woo *et al*., 2006). Many strains have been exploited as biocontrol agents (BCAs) and successfully used worldwide as biopesticides and biofertilizers. It is typical of several species to be active producers of secondary metabolites with antibiotic activity. Various Trichoderma–based formulations are available commercially for crop production.*Trichoderma*isolates are known for their ability to control plant pathogens (Elad and Freeman, 2002). Intensive research into biocontrol with *T. harzianum* has been carried out under commercial conditions, and there have been some significant achievements in greenhouse crops and in vineyards (Elad and Shtienberg, 1995).Ju*et al.* (2007) reported that *Bacillus licheniformis* N1is a biological control agent for gray mould disease caused by *B.cinerea*. Various formulations of *B.licheniformis* N1were generated and

evaluated for the activity to control strawberry gray mould. A wettable powder type formulationN1E was selected in pots experiments. This study suggests that formulation of *B. licheniformis* N1 will be effective to control strawberry gray mould by its preventive activity.

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النشـاط التضـادي للمستخلصـات والزيـوت النباتيـة وراشـح المـزارع المكروبيـة ضدالفطر*Botrytiscinerea* في المختبر

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تضمنت الدراسة استخدام ثلاث طرق للاستخلاص واستخدام مستخلصات وزيوت نباتية طيارة وراشح المزارع المكروبية (الفطريات والبكتريا) وتأثيرها على تثبيط نمو فطر البوترايتس سينيريا الذي يسبب العفن الرمادي في معظم المحاصيل الزراعية.استخدمت ثلاث طرق للاستخلاص وهي الاستخلاص بالماء والاستخلاص بكحول الميثايل وبالإيثايل أسيتيت. حيث لم يكن هناك فروق ذات دلال ةإحصائية بين الطرق الثلاثة للاستخلاص (الماء،ميثايل الكحول،الإيثايل الاسيتيت)

وتم استخدام خمس مستخلصات نباتية هي مستخلصات قشور الرمان والقرنفل والنيم والقرفة والزعتر بتركيزات ١٥و٣٠و٤٥% بالنسبة للاستخلاص بالماء , وتركيزاتو ٦ و ٩ و١٥%بالنسبة للاستخلاص بالمذيبات العضوية(كحول الميثايل والإيثايل أسيتيت) ,اعطى كل من مستخلص قشور الرمان ومستخلص النيم في طريقة الاستخلاص بالماء اعلى نسبة تثبيط للنمو الميسيليومي للفطر وذلك عند التركيز ٤٥% وسجلا نسبة تثبيط 96.67% و 92.22% على التوالي مقارنة مع معاملة المقارنة, مع عدم وجود فرق معنوي مع المبيد الفطريTopsin M-70WP في نفس التركيز , . اما طريقة الاستخلاص بكحول الميثايل وبالإيثايل أسيتيت حيث تفوقت كل من مستخلص قشور الرمان والقرنفل والنيم في تثبيط النمو الميسيليومي للفطر مسجلان نسبة تثبيط 90.00% لكل منهم عند التركيز 9% في طريقة الاسخلاص بكحول الميثايل,في طريقة الاستخلاص بالايثايل اسيتيت فقد سجل مستخلص قشور الرمان نسبة تثبيط للنمو الميسيليومي للفطر اعلى من باقي المستخلصات بنسبة التثبيط 94.44% عند التركيز 9% مقارنة مع معاملة المقارنة مع عدم وجود فروق معنوية مع المبيد الكيماويTopsin M-70WP ، كما تم استخدام اربعة زيوت نباتية طيارة هى زيت القرنفل وزيت النيم وزيت القرفة وزيت الزعتر والتى استخدمت بتركيزات ٢ و ١.٥ و ١% , حيث اعطى زيت القرنفل اعلى نسبة تثبيط الميسيليومي للفطر عند التركيز 2% مسجل نسبة تثبيط 88.88% مقارنة مع معاملة المقارنة مع عدم وجود فروق معنوية مع المبيد الكيماويTopsin M-70WP ، أما راشح المزارع الميكروبية الذي شمل فطر الترايكوديرما هارزيانم والترايكودرما هاماتم بالإضافة إلى راشح مزرعة البكتريا باسيلس ساتلاس (*B.subtilis*) ، اختبرت جميع رواشح المزارع عند تركيزات ٤٥ و ٣٠ و ١٥%،حيث اعطت بكتريا باسيلس ساتلاساعلى نسبة تثبيط للنمو الميسيليومي للفطرعند التركيز45% حيث سجلت نسبة تثبيط 97.78% مقارنة مع معاملة المقارنة مع عدم وجود فروق معنوية مع المبيد الكيماويTopsin M-70WP.