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GC-MS ANALYSIS OF THREE PLANTS ESSENTIAL OILS AND THEIR EFFECT ON BACTERIAL SPOT DISEASE OF TOMATO

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ABSTRACT: The aim of this present study is evaluate the antimicrobial activities of three plants essential oils, Nigella (*Nigella sativa*), Eucalyptus (*Cinnamomum camphora*) and clove (*Syzygium aromaticum*) against plant pathogenic bacteria *Xanthomonas vesicatoria* and their influences on controlling the bacterial spot disease of tomato plants. The chemical composition of the oils was calculated by gas chromatograph/mass spectrometer (GC/MS) analysis. The results revealed that the major constituents of Nigella oil were fatty acid i.e. oleic acid (4.09%%), Fumaric acid (0.13%), Palmitic acid (4.95-1.65 %), Octadec-9-enoic acid (0.53%), myristic acid (0.78%), Cyclohexadecane (0.01%) and Hexanedioic acid bis (2-ethylhexyl) ester (75.02%). In addition, the main components of Eucalyptus oil were D-camphor (40.01%), linalool (20.57%), and cineole (10.86%). The chemical analysis showed also that clove oil contained eugenol (22.94%), eugenyl acetate (16.65%), caryophyllene (10.37 %) and 2-Pyridineethanol (6.08 %). In *in vitro* bioassay against *X.anthomonas vesicatoria,* the results demonstrated that among the three tested plant oils, Eucalyptus oil (10 %) recorded the maximum value was (1.73 cm) bacterial inhibition zone followed by Clove oil (1.50 cm). Results also showed spraying plants with the tested essential oils two days before bacterial inoculation recorded the lowest disease index comparing to the treatment where plants were treating with essential oils two days after bacterial inoculation. The obtained results also clearly illustrated that, Peroxidase (PO), polyphenoloxidase PPO *and* chitinase activities increased as a result of spraying tomato plants with the tested oils. Moreover, protein analysis confirmed that new protein bands with low molecular weight had a progressive relationship with reduction of bacterial spot disease severity on plants treated with the tested oils.

Key words: GC/MS, Essential oils, Bacterial spot, *Xanthomonas vesicatoria*, *Nigella sativa*, *Cinnamomum camphora*, *Syzygium aromaticum.*

INTRODUCTION

Plant diseases adversely affect human health through agricultural and economic losses rather than their implications on biodiversity conservation. Bacteria and fungi are the most common cause of many plant diseases. Controlling plant diseases in general and bacterial diseases in particular depending on chemical pesticides became more difficult day after day due to many reasons i.e. the rapid pathogen populations resistant toward the chemicals, undesirable effect on the environment, slow biodegradation of many chemical groups which increasing the post-harvest intervals (PHI) and many serious side effects for mammalian health associated with toxic residues in food products. Therefore, it is necessary to develop alternative materials of combating pathogenic bacterial and fungal diseases on their host plants. Natural oils which are concentrated hydrophobic liquid consisting of volatile aromatic compounds obtained from

various parts of plants are candidate to be promising alternatives for traditional chemical control that depending on completely synthetic compounds. It was previously known that many of these natural essential oils have many biological activities, such as antifungal and antibacterial properties (Hawthorne *et al*., 1993& Kokkini *et al.,* 1997). Recently, the interest in the application of essential oils to control plant pathogens has increased therefore the effectiveness of various essential oils against many plant pathogenic bacteria and fungi was determined (Ghalem, 2016). The studies showed that the chemical composition and biological activities of plant essential oils depending on different parameters, such as the environment, the season in which aromatic plants are collected, the process of dehydration, the preservation conditions in which the collected plants are stored as essential oils (Tarantilis and Polissiou, 1997; Russo *et al*., 1998). The antimicrobial activity and chemical composition of many essential oils and plant extracts have been investigated by many researchers (Daferera *et al.,* 2000). GC-MS (qualitative and quantitative) analysis is indispensable in the evaluation of the biological activity and chemical composition studies for the essential plants oil (Gorris *et al*., 1994 and Reddy *et al*., 1998). On the other hand, bacterial spot disease of tomato which is caused by *Xanthomonas vesicatoria* considered one of the most serious diseases effecting both foliage and fruit growth and yield. This disease can cause great damage to all plant parts including leaves, stems, and fruits with considerable reduction of quality and quantity of tomato yields (Byrnea *et al*., 2005). In spite of disadvantages of chemical applications such as hazardous residuals on fruits, cost, and development of resistant bacterial strains, the using of such these traditional control measurements i.e. copper or tetracycline and streptomycin antibiotic sprays were not able alone to stop the disease dispersal (Stall, 1993; El-Hendawy *et al*., 2005; Al-Dahmani *et al.,* 2003). Therefore new eco-friendly and effective alternatives against *Xanthomonas vesicatoria* such as biological control and natural plant essential oils are gaining more importance and attracting scientist attentions during the recent years. Many substances such as Garlic extract (*Allium sativum*), Mentha oil (*Mentha aquatica*), clove oils (*Syzygium aromaticum*) as well as resistant acids like ascorbic acid and salicylic acid were investigated to determine either their antimicrobial activities toward different plant pathogenic organisms or to identify their chemical composition (Abd El-Ghafar and Abd El-Wahab, 2001; Polizzi *et al*., 2002; Morais *et al*., 2002; Pietro *et al*., 2004 and Balestra *et al*., 2009).

The aims of present study are evaluate the antimicrobial activities and chemical composition of three plants essential oils Nigella (*Nigella sativa*), Eucalyptus (*Cinnamomum camphora*) and clove (*Syzygium aromaticum*) against plant pathogenic bacteria *Xanthomonas vesicatoria* under *in vitro* and *in vivo* conditions.

MATERIALS AND METHODS

1. Bacterial inoculum preparation

X. vesicatoria **used in this study was provided by Plant Pathology Research Institute., Agricultural Research Center (ARC), Giza, Egypt and was routinely grown on nutrient agar (NA) at 28°C. The strain cultures growing on nutrient broth media containing 10% glycerol was stored at -20°C and regenerated twice before use in the experiments. Bacterial cultures were suspended in sterile distilled water and turbidity was adjusted to an OD 630 nm 0.100 using a spectrophotometer (SPECTRONIC 20-D),**

corresponding to a cell concentration of ca.10^8 CFU/ml (Altundag and Aslim, 2011).

2. Collection of essential oils:

Three different oils *i.e.* Nigella (*Nigella sativa*) oil, Eucalyptus (*Cinnamomum camphora*) oil and Clove (*Syzygium aromaticum*) oil were obtained kindly from the Sector of Perfume and Additives, Hawamdia Sugar Company, Cairo, Egypt. Two different concentrations 5 and 10 % of oils were prepared using dilution with sterilized skimmed milk powder (1%) according to Chowdappa *et al.* (2018) method.

3. Disc Diffusion Assay:

This experiment was conducted in a completely randomized block design with three replicates to investigate the efficacy of the three tested oils *i.e.* Nigella oil, Eucalyptus oil and Clove oil with two concentrations 5 and 10 % on *X. vesicatoria* bacteria under *in vitro* conditions. Filter paper discs (Whatman No.1, 7 mm in diameter) were saturated with the different concentrations of the tested oils. The discs were then placed on the surface of the medium previously inoculated with tested pathogenic bacteria, using appropriate amounts of 24 hrs old nutrient broth culture as inoculum. The plates were incubated at 28 ± 2°C for 2 days. The degree of inhibitory action was estimated by measuring the diameter of the zone of inhibited growth surrounding the disc.

4. Effect of the tested essential oils under greenhouse condition:

The efficiency of tested essential oils was conducted in the greenhouse to study their effect in reducing the severity bacterial spot disease of tomato (*Solanum lycopersicum*) cultivar Super Strain B seedlings (from Qaha nurseries (El-Qalubia). The experiment was conducted in a randomized complete block design, with three replicates and three plants per pot. Thirty ml of each essential oil was kept in 100 ml conical flask (1.5 ml essential oil 10% + 30 ml of distilled water + 1 µl of Tween 80) and kept at shaker for 24 h for continuous agitation at 150 rev/min for thorough mixing and complete elucidation of active materials to dissolve in the respective solvent (Bagy and Abo-Elyousr, 2019). The plants (21-day-old seedlings) were divided into two groups. The first group was inoculated with the pathogen *X. vesicatoria* at concentration of ca.10^8 CFU/ml, two days after application with essential oils (30 ml of each essential oil). Plants in the second group, plants were inoculated with spraying the pathogenic bacteria two days before application of essential oils and control plants sprayed with water. After the treatment, tomato seedlings were covered with plastic bags for 48 h. The experiment was carried out in three replicates. Disease severity and disease rate were calculated according to formula by Towsend and Heuberger (1943):

Disease severity (%)=

$$\frac{\Sigma(\text{disease plant number of each scale x scale value})}{4 \times \text{total plant number}} \times 100$$

For disease severity (magnitude of symptom shown by inoculated plants) the empirical scale: 0 = No symptoms; 1 = 1/4 of the plants show symptoms; 2 = half of the plants show symptoms; 3 = 3/4 of the plants show symptoms; 4 = all plants show symptoms. (Altundag and Aslim, 2011).

5. Gas chromatography/mass spectrometry analysis.

The chemical composition of the tested oils was analyzed by GC mass. The mass spectrometer was Agilent 6890 Series II gas chromatography. An Agilent 5973 mass spectrometer (Mode EI generated at 70eV, ion source at 230°C

and transfer line at 280°C) with electron ionization was used. The GC was performed using HP5 -MS capillary column (30m x 0.25 mm x 0.25µm). Operating conditions were as follows: carrier gas, helium with a flow rate of (1 mL min-1). The initial temperature was programmed from 60◦C to 290 ramp 8 °C min-1. Helium (99.99%) was the carrier gas at a flow rate of 1 ml/min. Diluted samples (1/100 in hexane, v/v) of 1.0 µl were injected manually. Identification of the components was based on the comparison of their mass spectra with those of Wiley7N, Nist 2002 and Flavor libraries and comparison of their retention times (Tabanca *et al*., 2001; Aligiannis *et al*., 2001).

6. Determining of enzyme activities:

Leaves sample of treated tomato plants (cv. Super Strain B) with different treatments under study in greenhouse were taken 30 days after transplanting. Leaf samples were ground with 0.2 M Tris HCl buffer (pH 7.8) containing 14 mM β-mercaptoethanol at the rate 1/3 w/v. The extracts were centrifuged at 10,000 rpm for 20 min at 4°C. The supernatant layer was used to determine enzyme activities (Tuzun *et al.,* 1989).

6.1. Determination *of* Peroxidase (PO):

Peroxidase activity was determined according to the method described by Allam and Hollis (1972).

6.2. Determination of Polyphenoloxidase (PPO):

The polyphenoloxidase activity was determined according to the method described by Matta and Dimond (1963).

6.3. Determination of chitinase

Determination of the activity of chitinase was carried out according to the method of Boller and Mauch, (1988).

7. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE):

Eighty-microliters (80 µL of protein) of leaves samples were subjected to SDS-polyacrylamide gel electrophoresis was performed in 12 % acrylamide slab gels following the system of Laemmli (1970) to identify their protein profiles. Gels were photographed scanned, analyzed using Gel Doc VILBER LOURMAT system.

8. Data analysis:

All obtained data were analyzed using analysis of variance (ANOVA) among treatments. Means were compared by least significant differences (LSD) at p ≤0.05 as described by Song and Keane (2006).

RESULT AND DISCUSSION:

1. *In vitro* growth inhibition of three essential oils against *xanthomonas vesicatoria*:

The influences of the three tested plant oils against of *X. vesicatoria* under *in vitro* condition were evaluated. The obtained results indicated that most of the tested plant oils inhibited the growth of this pathogenic plant bacteria at both concentrations tested (5&10%) as shown in Table 1. The growth inhibition was positively correlated with the concentrations of oils. Among all treatments, Eucalyptus oil (10 %) recorded the maximum inhibition zone (1.73cm) followed by Clove oil (1.50cm). These results are in a line with Chowdappa *et al.,* (2018) who reported also that clove oil was the most effective oil against *Xanthomonas axonopodis* with an inhibition zone of 27.3 mm at concentration of 40%. Moreover, they added that Clove oil was still effective against bacteria even at concentration of 10%. Current finding is agreeing with Gilvaine *et al*., (2012) who mentioned that 10 % thyme oil showed a toxic effect on

the *Xanthomonas campestris* pv*. vesicatoria*. Similar reports were made by Gill and Holley 2006; Carson *et al*., 1995 and Bagamboula *et al*., 2004.

2. Effect of the tested essential oils on diseases severity of *Xanthomonas vesicatoria* under greenhouse condition:

Data in Table 2 reveal spraying plants with the tested essential oils two days before bacterial inoculation recorded the lowest disease severity comparing to the treatment where plants were treating with essential oils two days after bacterial inoculation. Noteworthy, the results showed that the lowest disease severity (7%) was recorded with Eucalyptus oil either in both treatments where plants were treated two days before or after bacterial inoculation. Current finding is agreeing with Da Silva *et al.*, (2019) who demonstrated that *Lippia gracilis* essential oils have antimicrobial activity and have a potential to be used in the control of black rot caused by *X. campestris* pv*. campestris.* Different components have been reported as effective compounds against *Xanthomonas campestris* i.e. eugenol, *Cinnamomum cassia*, *Cinnamomum zeylanicum*, *Syzygium aromaticum* (Cantore *et al.,* 2009; Chudasama and Thaker, 2012).

Table 1. *In vitro* growth inhibition of three essential oils against *xanthomonas vesicatoria.*

Common name	Essential oils	Inhibition zone (cm)	
		5%	10%
Eucalyptus	*Cinnamomum camphora*	1.42	1.73
Clove	*Syzygium aromaticum*	1.21	1.50
Nigella	*Nigella sativa*	0.98	1.0
Control	Sterile distilled water	0.0	0.0
L.S.D 0.05 =	Oils	Concentrations	Interaction
	0.10	0.05	0.10

Table 2. Disease severity recorded on tomato plants treated with three essential oil two days before and two days after inoculation with *Xanthomonas vesicatoria bacteria* under greenhouse conditions.

Treatment	Two days before (%)*	Two days after (%)**	Efficacy	
			Two days before (%)*	Two days after (%)**
Eucalyptus oil	7.0	7.0	-90.67	-91.46
Clove oil	16.0	22.0	-78.67	-73.17
Nigella oil	26.0	29.0	-65.33	-64.63
Infected Control	75.0	82.0	0.00	0.00
Healthy control	0.0	0.0	-100.00	-100.00
L.S.D 0.05% =	Oils	Time	Interaction	
	2.15	0.8	1.77	

*** Inoculated with *Xanthomonas vesicatoria* two days before spraying with essential oils**

**** Inoculated with *Xanthomonas vesicatoria* two days after spraying with essential oils**

3. Chemical composition of oils

Based on the chemical analysis conducted using GC-MS, the results showed that different number of compounds ranged from 9-23 was detected within the three tested oils (Tables 3, 4 and 5). The chemical compositions of *Nigella sativa* oil identified by GC-MS showed that among 9 different detected compounds, Hexanedioic acid bis (2-ethylhexyl) ester was representing (75%) from the total detected chemical substances followed by Palmitic acid (4.95%) and Oleic Acid (4.09%), respectively (Table 3). These results are in a line with Muhialdin *et al.,* (2016) who studied the chemical properties of *N. sativa* oil using SFE and cold press extractions reported that free fatty acid and peroxide value can be used as good indicators to monitor the quality of this oil. Khoddami *et al.,* (2011) and Solati *et al.,* (2014) investigated also *Nigella sativa* oil. They detected different compounds i.e. lipids, water, protein was and carbohydrate. They reported also the chemical composition is related to the oil quality. On the other hand, some oil components such as Lauric acid, palmitic acid, linolenic acid, linoleic acid, oleic acid, stearic acid and myristic acid are known to have potential antibacterial and antifungal agents (McGaw *et al.*, 2002 and Seidel & Taylor, 2004).

The chemical compositions of *Syzygium aromaticum* oil identified by GC-MS are listed in Table 4. In general, the main compounds identified in Clove oil contained eugenol (22.94 %), eugenyl acetate (16.65 %), caryophyllene (10.37 %) and 2-Pyridineethanol (6.08 %). Lo Cantore *et al.,* 2009 tested the anti-microbial activity of clove oil. They found that clove oil highly reduced *X. campestris* infection under *in vivo* conditions. In particular, clove oil at 4 mg/mL disinfect seeds bearing about 7.0 × 10^2 cfu/seed and lower densities. However, after 72 h, incubation treatments with 2, 4, and 8 mg/mL of clove oil caused reduction of 3%, 7%, and 16%, respectively compared to controls.

Table 3: GC-MS identification of chemical composition for Nigella (*Nigella sativa*) oil.

Peak No.	Compound name	Retention time (min.)	Peak area (%)
1	Palmitic acid	8.365	4.95
2	Myristic acid	15.132	0.78
3	Cyclohexadecane	16.894	0.10
4	Palmitic acid	17.186	1.65
5	Oleic Acid	17.483	4.09
6	Octadec-9-enoic acid	19.423	0.53
7	Fumaric acid	20.979	0.13
8	Hexanedioic acid bis (2-ethylhexyl) ester	22.336	75.02
9	Methyl pentadecyl ether pentan	22.942	0.31
Total			87.56
unknown			12.44

Table 4: GC-MS identification of chemical composition for clove (*Syzygium aromaticum*) oil.

Peak No.	Compound name	Retention time (min.)	Peak area (%)
1	Caryophyllene	5.971	10.73
2	6H- [1] Benzothiopyrano[4,3-b] quinoline	8.443	3.23
3	2-Pyridineethanol	8.506	6.08
4	4H-Pyrrolo[2,3-b] quinoxaline-4-carboxylic acid	10.926	2.08
5	1,1-Cyclopropanedicarbonitrile, 2,3-dimethyl-	12.402	4.45
6	Anthra[2,3-b] furan-4,11-dione	13.163	0.82
7	1-Dodecene	19.028	1.65
8	eugenol	20.848	22.94
9	eugenyl acetate	20.865	16.65
10	methyl ester 2-Cyclohexen-1-one	21.935	0.72
11	3-Methoxy-2,4,5-trifluorobenzoic acid, tetradecyl ester	21.975	0.37
12	4-Dimethylamino-2-(trimethylsilyl) benzaldehyde	22.158	0.69
13	Phenanthrene, 1,7-dimethyl-	22.238	1.19
14	6-Phenylisoquinoline	22.301	3.84
15	Terephthalic acid, but-2-enyl isobutyl ester	22.313	1.56
16	1,7 1-Dimethylphenanthrene	22.570	0.93
17	3-phenyl-1,2,4-triazolo[3,4-b] [1,3] benzothiazin-5-one	22.605	0.74
18	N, N-Di(trimethylsilylmethyl)benzylamine	22.645	0.62
19	Diethyl [4-(dimethylamino)phenyl] propanedioate	22.690	0.60
20	(Z)-2-(3-PHENYL-1-INDOLIZINYL)-1-P HENYL-1-VINYL ACETATE	22.828	3.07
21	Benzenamine, 3,5-bis(1,1-dimethylethyl)-	22.908	2.43
22	6-Chloro-2,3-dihydrofuro(2,3-b) quinoline	22.931	1.15
23	Quinoline	22.959	1.42
Total			87.96
unknown			12.04

The chemical compositions of *Cinnamomum camphora* oil identified by GC-MS are listed in Table 5. In total, 23 constituents were identified in these essential , the main components were identified as be D-camphor (40.01%), linalool (20.57%), and cineole (10.86%). The varieties of *Cinnamomum camphora* can be morphologically similar, but they are differed in their chemical compositions and for this reason are considerate physiological varieties (Güenther, 1950). The traditional oils are obtained from the wood and bark (Pandey *et al*., 1997). The oil with a high content of camphor has an important antifungal activity (Takaoka *et al*., 1976). The Eucalyptus content was similar to that described for Pakistan (Sattar *et al.*, 1991). In this the product is similar to the most common compositions described in the literature (Lin and Hua, 1987; Tao *et al*., 1987; Fujita *et al.,* 1974; Dung *et al*., 1993 and Frizzo *et al*., 2000).

Table 5: GC-MS identification of chemical composition for Eucalyptus (*Cinnamomum camphora*) oil.

Peak No.	Compound name	Retention time (min.)	Peak area (%)
1	Propanenitrile, 3-(6-bromo-3,4-methylenedioxybenzylidenhydrazino)-3-oxo-	8.528	2.00
2	[1,2,4] Triazolo[3,4-b][1,3,4] thiadiazole, 6-[2-(4-fluorophenyl) ethenyl]-3-(4-pyridinyl)-	10.382	0.99
3	Undecanal, 3-phenyl-	10.811	0.51
4	4-Cyclohexylcarbonyl-1,3-dimethylbenzene	10.943	1.55
5	1-Undecene	13.987	0.43
6	4H-Quinolizine-1,2-dicarboxylic acid,	14.725	0.80
7	D-camphor	19.240	40.01
8	2-(imidazo[1,2-a] pyrimidin-2-ylmethyl)-	19.841	1.26
9	5H-Dibenzo[a,d]cycloheptene,	20.733	1.91
10	2,2'-Bi(bicyclo[2.2.1]heptane)	20.768	1.37
11	1,4-Naphthalenedione, 2-hydroxy-3-methoxy	21.117	1.49
12	[1,1'-Biphenyl]-4-ol, 4'-chloro- acetate	21.471	0.37
13	2-Pyrroline-3-carboxylic	21.552	1.23
14	5H-Dibenzo[a,d]cycloheptene	21.700	1.49
15	(E)-Ethyl 3-oxo-2-(pyrid-2-yl) methylene-butanoate	21.820	1.33
16	Naphthalene, 1-phenyl-	21.849	1.09
17	Tetracyano-p-quinodimethane	22.129	1.25
18	Diethyl anilino methylene esuccinate	22.238	0.85
19	cineole	22.330	10.86
20	Naphthalene, 2-phenyl	22.450	1.08
21	linalool	22.633	20.57
22	2-Propenoic acid, 2-cyano-3-(3-phenylphenyl)-, ethyl ester	23.051	1.53
23	1-(2-Acetyl-3-chlorophenyl)-1H-pyridin-2-one	23.440	1.72
Total			97.05
unknown			2.95

4. Effect of the tested essential oils on the activity of Peroxidase, Polyphenoloxidase and Chitinase enzymes in tomato plants.

Data in Table (6) show that enzymes activity increased in general as a result of treating tomato plants with the three tested oils compared to untreated plants. For Peroxidase enzyme, spraying plants with Eucalyptus oil followed by Nigella oil and then Clove oil two days before bacterial inoculations increased the Peroxidase activity (Table 6). On the other hand, two days after bacterial inoculations, the highest peroxidase activity was recorded on plants treated with Clove oil followed by Eucalyptus oil and then Nigella oil, respectively (Table 6). Moreover, Clove oil followed by Eucalyptus oil and then Nigella oil increased the activity of Polyphenol oxidase in both treatments. With Chitinase enzyme, Eucalyptus oil followed by Nigella oil and then Clove oil recorded the highest activity of the enzyme in treated plants two days before bacterial inoculation. The obtained

results in the current study are agreed with other studies (Ali, 2011 and Lucas *et al.,* 2012). According to Ali, (2011) treating the tomato plants with ascorbic acid and salicylic acid reduced disease severity of *X. vesicatoria* and increasing the activity of peroxidase and polyphenoloxidase enzymes. Lucas *et al.,* 2012 found that essential oil extracted from clove reduced the severity of tomato bacterial spot and resulted in increasing the activities of β-1,3 glucanase, chitinase, and peroxidase. Furthermore, treating pepper plants with some phenolic compounds reduced growth of *Xanthomonas vesicatoria* and reduced disease severity of *X. vesicatoria* and increasing the activity of peroxidase and polyphenoloxidase, chitinase and β-1,3-glucanase (Ahmed, 2016).

5. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE):

Concerning the results of SDS (PAGE) presented in Table (7) and demonstrated in Fig. 1 show that 14 protein bands with molecular weights ranging from 288 to 15 kDa were detected in tomato plants. While protein band with molecular weight 26 was appeared only in tomato treated with clove oil 2 days before inoculation with pathogen. Moreover, protein band with molecular weight 181 was absent in control plants inoculated with bacteria only and protein band with molecular weight 133 was absent in untreated and un-inoculated control. Whereas, protein band with molecular weight 88 was absent in two controls. The obtained results confirmed that new protein bands with low molecular weight had a progressive relationship on reducing disease severity of bacterial spot. Similar results were obtained by (Ahmed, 2016), who found that, new protein bands expressed as a result of treating pepper plants with phenolic inducers against bacterial spot disease. Treating pepper plants with SA-based products reduced growth of *Xanthomonas vesicatoria* and plant disease symptoms following challenge and are related to induction of pathogenesis-related (PR) genes (Ward *et al*., 1991).

Table 6. Effect of the tested essential oils on the activity of Peroxidase, Polyphenoloxidase and Chitinase enzymes in tomato plants.

Treatment		Peroxidase	Polyphenol oxidase	Chitinase
Eucalyptus oil	48h before	25.4	6.58	9.00
Clove oil		21.2	6.98	5.36
Nigella oil		24.0	5.92	5.72
Eucalyptus oil	48h after	24.4	11.36	13.8
Clove oil		32.0	14.04	3.63
Nigella oil		20.3	9.00	4.66
Control *		15.4	5.56	1.71
Control **		17.3	5.36	2.36

***Peroxidase activity was expressed as the increase in absorbance at 425 nm/gram fresh weight/15 minutes.**

*** Polyphenoloxidase activity was expressed as the increase in absorbance at 420nm/g fresh weigh/ minutes**

*** Chitinase activity was expressed as mM N-acetylglucose amine released/g fresh weight tissue/60 minutes.**

Table (7): Molecular weights of fractionated protein profiles of tomato treated with essential oils.

Band No	M.W bp	Control 1	Control 2	Eucalyptus oil 1	Clove oil 1	Nigella oil 1	Eucalyptus oil 2	Clove oil 2	Nigella oil 2
1	288	1	1	1	1	1	1	1	1
2	226	1	1	1	1	1	1	1	1
3	181	0	1	1	1	1	1	1	1
4	133	1	0	1	1	1	1	1	1
5	114	1	1	1	1	1	1	1	1
6	88	0	0	1	1	1	1	1	1
7	70	1	1	1	1	1	1	1	1
8	53	1	1	1	1	1	1	1	1
9	44	1	1	1	1	1	1	1	1
10	26	0	0	0	0	0	0	1	0
11	25	1	1	1	1	1	1	1	1
12	21	1	1	1	1	1	1	1	1
13	18	1	1	1	1	1	1	1	1
14	15	1	1	1	1	1	1	1	1
Total		11	11	13	14	13	13	14	13

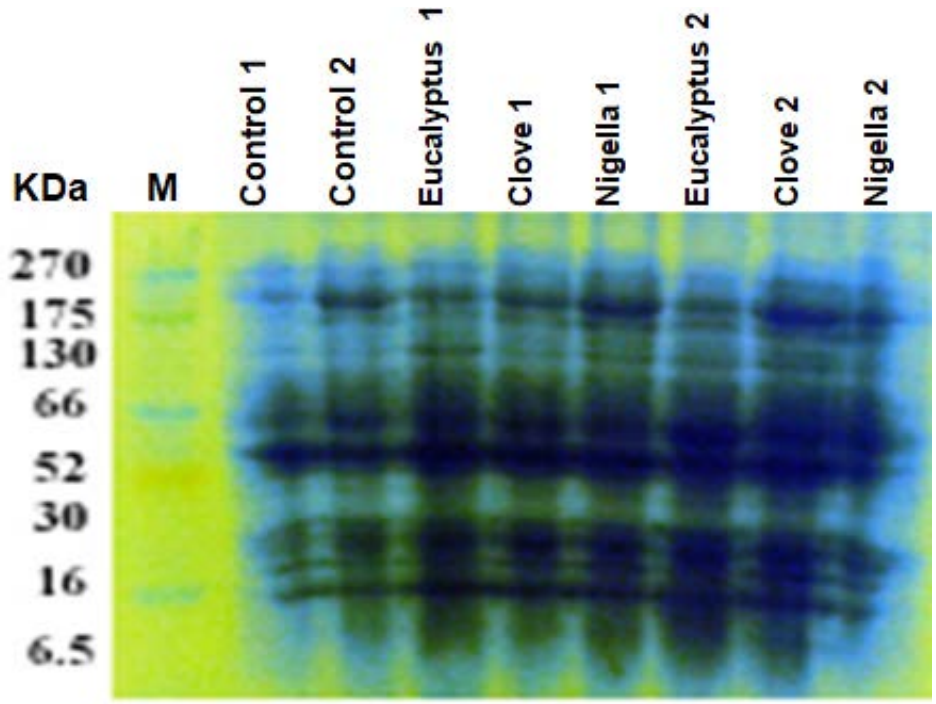

Fig. 1. Effect of treating tomato with essential oils on PAGE of protein

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تحليل الكروماتوجرافي الغازي (مطياف الكتلة) GC–MS لثلاث زيوت أساسية نباتية وتأثيرها على مرض التبقع البكتيري للطماطم

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الملخص العربي

تهدف هذه الدراسة إلي تقييم النشاط المضاد للميكروبات لثلاث زيوت أساسية نباتية هي زيت حبة البركة وزيت الكافور وزيت القرنفل ضد البكتيريا الممرضة النباتية *Xanthomonas vesicatoria* وتأثيرها في مكافحة مرض التبقع البكتيري للطماطم. تم فحص التركيب الكيميائي للزيوت عن طريق تحليل الكروماتوجرافي الغازي (مطياف الكتلة). أظهرت النتائج أن المكونات الرئيسية لزيت حبة البركة هي الأحماض الدهنية حمض الأوليك (4.09 ٪) ، حمض الفوماريك (0.13٪)، حمض البالمتيك (1.65–4.95٪) و حمض Octadec–9–enoic (0.53%)، حمض الميريستيك (0.78٪)، السيكلوهيكساديكان (0.01٪) و Hexanedioic acid bis (2–ethylhexyl) ester (75.02%) وكانت المكونات الرئيسية لزيت الكافورهي كامفور–D (40.01٪)، لينالول (20.57٪)، سينول (10.86٪). كما أظهر التحليل الكيميائي أن زيت القرنفل يحتوي على الأوجينول (22.94٪)، الأوجينيل أسيتات (16.65٪)، كاريوفيلين (10.37٪) و 2–بيريدين إيثانول (6.08٪). في الاختبار الحيوي في المعمل ضد *Xanthomonas vesicatoria* ، أوضحت النتائج أنه من بين الزيوت النباتية الثلاثة المختبرة، سجل زيت الكافور (10٪) أقصي مساحة تثبيط بكتيرية (1.73 سم) تلاه زيت القرنفل (1.50 سم). كما أظهرت النتائج أن رش النباتات بالزيوت المختبرة قبل يومين من التلقيح البكتيري سجل أدنى مؤشر للمرض عند معاملة النباتات بالزيوت بعد يومين من التلقيح البكتيري. كما أوضحت النتائج التي تم الحصول عليها أن أنشطة البيروكسيديز (PO) والبوليفينواوكسيديز PPO والكيتينيز زادت نتيجة رش نباتات الطماطم بالزيوت المختبرة. علاوة على ذلك، أكد تحليل البروتين ظهور حزمه بروتينية جديدة ذات وزن جزيئي منخفض قد يكون لها علاقة بتقليل شدة المرض على النباتات المعاملة بالزيوت المختبرة.

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