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Efficacy of Compost and Some Essential Oils Alone or in Combination in Controlling Cucumber White Mould Disease Under Protected House Conditions

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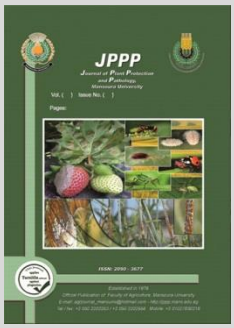

ABSTRACT

Three concentrations *i.e* 1, 2.5 and 5% of five essential oils were tested to study their effect on growth of *Sclerotinia sclerotiorum*. Results showed that Clove and Marjoram oils were inhibited growth of *S. sclerotiorum*. Under greenhouse conditions results revealed that amending vegetarian compost to soil increased the activity of all plant oils than the individual treatment in reducing disease severity of Sclerotinia rot. Clove oil alone or in combination with compost completely prevented the disease incidence. Seedling soaking with nigella, marjoram and clove oils and their combinations with or without compost against Sclerotinia rot disease. Clove oil combined with compost completely prevented the disease incidence in 2017 and disease severity recorded 2.36 % in 2018 growing season. Moreover, increase of fruit yield was recorded with the combined treatments between compost + clove oil then compost + marjoram and clove oils during growing seasons 2017 and 2018 respectively. All tested treatments positively increased the activity of peroxidase (PO), Polyphenol Oxidase (PPO) and PAL enzymes in cucumber. Moreover, chitinase and β 1,3- glucanase were greatly increased in treated cucumber with treatments compared with control. The results of SDS (PAGE) showed that 15 protein bands with molecular weights ranging from 234.433 to 50.017 kDa are contained in cucumber plants. New protein bands expressed as a result of treating cucumber with plant oils. One band with 90.783 KDa was appeared in plants treated with marjoram and Clove oils, while absent in other treated plants.

Keywords: White mould - *Sclerotinia sclerotiorum* - cucumber plants. essential oils - Protected house

INTRODUCTION

Cucumber (*Cucumis sativus* L.) is one of the important vegetable crops. It belongs to family cucurbitaceae, and grow either in the open field or under protected houses.

Sclerotinia sclerotiorum (Lib.) is a serious fungus affecting yield and product quality Gao *et al.* (2014). It affects over 450 species and subspecies of plants, including a wide range of economically important crops worldwide, especially these grown in protected agricultural areas and glasshouses (Bolton *et al*., 2006 and Elgorban *et al.*, 2013). Sclerotinia stem and root rots or white mould is one of the most dangerous cucumber diseases (Purdy, 1979).

Moreover, the rapid rise in request for organically produced fruit and vegetables will increase the request for natural pesticides such as essential oils. Newly, several studies on utilizing essential oils as the antifungal activities against fungal pathogens have been stated (Kalemba and Kunicka, 2003; Soylu *et al.*, 2007). However, very few studies have concentrated on the antifungal activities of essential oils to manage this pathogen (Soylu *et al.*, 2005 and Zhenhua *et al.*, 2013). Application of essential oil has been considered a very promising scheme for controlling plant diseases. Essential oils and their elements are expanding interest because of their comparatively safe status, wide acceptance by consumers and their exploitation for potential multi-purpose functional uses (Jobling, 2000 and Hadizadeh *et al*., 2009). These oils are one of the most promising groups of natural compounds for the development of safer antifungal agents (Taylor *et al*., 1995 and Tiwari & Shrestha 2009). Adding compost to soil decreased significantly diseases as Aphanomyces root rot of peas; Rhizoctonia root rot of bean, cotton, and radish; Sclerotinia drop of lettuce, Fusarium wilt of cucumber and Phytophthora crown rot of pepper (Lumsden *et al.*, 1983). Adding compost to the soil pre-transplanting of tomato decreased the Sclerotinia infection and increased yield of tomato plants compared with un-amended treatments with compost (Gomaa *et al.*, 2016). The oxidative enzymes such as peroxidase and polyphenol oxidase enhance formation of lignin, while, other oxidative phenols contribute in formation of defense barriers for reinforcing the cell structure (Avdiushko *et al.*, 1993). Chitinase and β-1, 3 glucanase enzymes play a significant role in plant defense against fungi by hydrolysing their cell walls (Tian *et al.*, 2006, Imran *et al.*, 2007 and Barilli *et al.*, 2010).

The present work aimed to control cucumber white mould disease caused by Sclerotinia sclerotiorum using compost or plant oils and their combinations under greenhouses conditions. Also, determination of some defense related enzymes like peroxidase, polyphenoloxidase, phenylalanine ammonia lyase, chitinase and β-1,3-glucanase.

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MATERIALS AND METHODS

1- Isolation and identification of the causal organism:

Diseased samples of cucumber plants showing white mould symptoms were collected from Qalubia governorate (Moshtohor village) and subjected to isolation trials. *Sclerotinia* spp. were isolated from the appeared decayed lesions on diseased plants. In this respect, the infected tissues were cut into small pieces, surface sterilized with sodium hypochlorite (0.5%) for 2-3 minutes, washed for several times with sterilized distilled water, dried between sterilized filter papers and transferred directly to poured PDA medium plates (9cm Φ). The plates were incubated for 1-2 days at 22±2°C. The emerged fungal growth on incubated pieces were transferred to PDA slants. The emerged fungal growth were purified using hyphal tip technique (Brown, 1924). The purified fungal isolates were identified according to Singh, (1982). PDA slants of isolated fungus were kept in refrigerator at 4°C for further studies.

2- Effect of essential oils on the radial growth of *S. sclerotiorum*:

The antifungal activities of five essential oils Thyme, Nigella, Marjoram, Clove and Rosemary oils (were obtained kindly from the Sector of Perfume and Additives, Hawamdia Sugar Company, Cairo, Egypt) were evaluated against *S. sclerotiorum* in vitro. Potato Dextrose Agar medium (PDA) was autoclaved, then cooled to about 45°C. The essential oils were mixed with sterile PDA to obtain final concentrations 1, 2.5 and 5%. Tween 80 at 0.01% was added as a surfactant to disperse the oil in PDA and then the medium was poured into Petri dishes. Mycelial disks of 5 mm diameter, cut out from the periphery of 7-day-old cultures of *S. sclerotiorum*, were aseptically inoculated upside down on the PDA. Three plates were used for each concentration as replicates. All plates were incubated at 22±2°C. The experiment was terminated when mycelial mats covered medium surface in control treatment. Percentage of the fungal growth reduction (X) was calculated by using the following formula (4) suggested by Abd-El-Moity, 1985.

$$X = G1 - G2 / G1 \times 100$$

Where: **X= fungal growth reduction.**
G1= linear growth of the pathogen in control.
G2= linear growth of the pathogen in treated petri plates.

3-Greenhouse experiments:

The inoculum of *S. sclerotiorum* was grown for two weeks on sand barley medium (3:1, w:w and 40% water). Inoculum of *S. sclerotiorum* fungus was added to the potted soil at rate of 3.0% w/w, mixed thoroughly with the soil surface of each pot (ϕ 20 cm) then watered and left for one week to insure even distribution of the inoculum.

Healthy cucumber transplants of Barracuda hybrid f1 were dipped in each particular plant oil of five essential oils i.e. thyme, nigella, marjoram, clove and rosemary oils at 2.5% concentration for 2 hrs. then raised and left to dry in air before planting. Transplants were planted in pots amended with mixture of soil and compost at 5%. Untreated transplants were used as control. Three transplants/pot and three replicates for every treatment were used. Two months post inoculation and treatment, the disease severity was assessed using 0-5 scale where:

0 = no symptom, 1= 0-25% of root browning, 2 =26-50% of root browning, 3 =51-75% of root browning, 4 =76-100% of root browning, and 5 =plant death according to Abdeljalil *et al*., (2016).

$$\text{Disease Severity \%} = \Sigma (a \times b) / N \times K \times 100$$

Where: **a = Number of infected plants in each category.**
b = Numerical value of each category.
N = Total number of examined plants.
K = The highest degree of infection category.

4. Experiments of commercial protected house

In two experiments (during seasons 2017 and 2018) healthy cucumber transplants of Barracuda hybrid f1 (from Qaha nurseries (El-Qalubia) were dipped in each particular oils of three tested plant oils and their combinations i.e:, Nigella, Marjoram, Clove, Nigella+ Marjoram, Nigella + Clove, Marjoram + Clove and Nigella + Marjoram + Clove oils at 2.5% concentration for 2 hr. then raised and left to dry in air before planting. Transplants were planted in pots (30 cm ϕ) amended with mixture of soil and compost at 5% (produced by Agriculture Service Center Compost Production Unit, Fac. Agric. Moshtohor, Benha Univ. Egypt), then of *S. sclerotiorum* inoculum was added at rate of 3.0% w/w. The untreated transplants were used as control. One transplant/pot and five replicates for each treatment were used. Disease severity % was recorded as mentioned before and the average weight of fruits (kg)/plant was also recorded.

5. Determination of defense related enzyme activities:

Leaf samples of treated cucumber plants cv. Barracuda hybrid with compost and plant oils treatments under greenhouse conditions were taken 30 days post 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).

Determination of Peroxidase (PO):

Peroxidase activity was determined according to the method described by Allam and Hollis (1972). Peroxides activity was expressed as the increase in absorbance at 425 nm/gram fresh weight/15 minutes.

Determination of Polyphenoloxidase (PPO):

The polyphenoloxidase activity was determined according to the method described by Matta and Dimond (1963). Polyphenoloxidase activity was expressed as the increase in absorbance at 420 nm/g fresh weight/min.

Determination of phenylalanine ammonia lyase (PAL):

Activity of PAL was determined according to the method described by Dickerson *et al.,* (1984). PAL activity was expressed as µmol trans-cinnamic acid min^{-1} g^{-1} protein.

Determination of chitinase

Determination the activity of chitinase was carried out according to the method of Boller and Mauch, (1988). Chitinase activity was expressed as mM N-acetylglucose amine equivalent released/g fresh weight tissue/60 minutes.

Determination of β-1,3-Glucanase:

Determination the activity of β-1,3-glucanase was carried out according to the method of Sun *et al.,* (2006). β 1-,3-glucanase was expressed as mM glucose equivalent released /g fresh weight tissue /60 minutes.

Electrophoretic analysis of peroxidase isozymes.

Peroxidase isozymes was electrophoresed to exhibit its isozymes in response to different applications of compost and plant oils treatments carried of cucumber plants according to the method of Sindhu *et al*., 1984.

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

Eighty-microliters (80 µL of protein) of leaf samples extract 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.

RESULTS AND DISCUSSION

Results

1- Effect of essential oils on the growth of *S. sclerotiorum:*

Five essentials oils with three concentrations *i.e* 1, 2.5 and 5% of were tested for their effects on growthof *Sclerotinia sclerotiorum*. Results in Table 1 and Fig. 1 show that clove oil and marjoram oil inhibited completely the growth of *S. Sclerotiorum* at 2.5 and 5% concentrations. Meanwhile, thyme and rosemary oils at all tested concentrations as well as nigella oil at 1 and 2.5% had no inhibitory effect against the growth of *S. Sclerotiorum*. Whereas, clove oil and marjoram oil at concentration of 1% decreased the growth of *S. Sclerotiorum* by 52.22 and 42.22% respectively.

2- Effect of essential oils and compost alone or in combination on white mould severity of cucumber plants under greenhouse conditions.

Data in Table 2 show that adding vegetarian compost to soil improved the action of all tested plant oils in reducing disease severity of *Sclerotinia* rot comparing to the individual treatments of plant oils.

Clove oil in soil free of compost or in soil amended with compost completely inhibited the disease. In this respect, marjoram and nigella oils in compost free soil reduced the disease severity by 84.49 and 80.62 % respectively. As for soil amended with compost results show that the highest reduction of disease severity percentage were recorded with marjoram and nigella oils which recorded 96.13 and 92.26% respectively. Whereas, compost only reduced disease severity by 62.03 %.

3- Effect of essential oils and compost treatments on cucumber white mould severity and yield components under protected house conditions

During two growing seasons of 2017 and 2018, dipping cucumber seedlings in nigella, marjoram and clove oils as well as their combinations with or without adding compost to soil were evaluated against Sclerotinia white mould disease.

Data in Table 3 reveal that all tested essential oils and their combinations with or without compost during the two growing seasons significantly reduced the disease severity of *Sclerotinia* rot disease and increased the fruit yield compared with the control.

Table 1. Inhibitory effect of essential oils on linear growth of *S. sclerotiorum:*

Treatment	Concentration %	Mycelial growth (mm)	Efficacy %
Thyme oil	1	90	0.00
	2.5	90	0.00
	5	90	0.00
Nigella oil	1%	90	0.00
	2.5%	90	0.00
	5%	65.67	27.03
Marjoram oil	1%	52.00	42.22
	2.5%	0.00	100
	5%	0.00	100
Clove oil	1%	43.00	52.22
	2.5%	0.00	100
	5%	0.00	100
Rosemary oil	1%	90	0.00
	2.5%	90	0.00
	5%	90	0.00
Control		90	00.00
L.S.D at 0.05	Treatment	Conc.	Interaction
	0.79	2.50	1.36

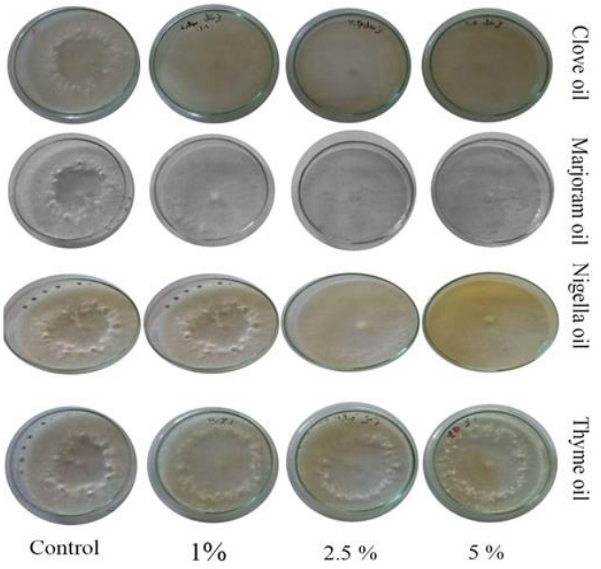

Fig .1. Inhibitory effect of essential oils on linear growth of *S. sclerotiorum*

Table 2. Effect of essential oils and compost treatments on cucumber white mould severity under greenhouse conditions

Treatment	Without compost		With compost	
	Disease severity %	% Reduction	Disease severity %	% Reduction
Thyme oil	13.33	76.75	6.67	88.37
Nigella oil	11.11	80.62	4.44	92.26
Marjoram oil	8.89	84.49	2.22	96.13
Clove oil	0.00	100.00	0.00	100.00
Rosemary oil	15.55	72.88	7.67	86.62
Compost	-----	-----	21.77	62.03
Control	57.33	0.00	57.33	0.00
L.S.D at 0.05	Oil	Compost	Interaction	
	5.34	3.80	4.94	

Table 3. Effect of essential oils and compost treatments on cucumber white mould severity under protected house conditions.

Treatment	Disease severity %				Yield				Mean			
	2017		2018		2017		2018		Disease severity %		Yield	
	W-	W+	W-	W+	W-	W+	W-	W+	W-	W+	W-	W+
Nigella oil	11.33	9.33	12.67	10.00	1.81	1.90	1.75	1.87	12.00	9.67	1.78	1.89
Marjoram oil	10.00	8.67	11.33	8.67	1.92	2.00	1.83	1.95	10.67	8.67	1.88	1.98
Clove oil	2.67	0.00	3.33	2.00	2.32	2.45	2.28	2.36	3.00	1.00	2.30	2.41
Nigella oil + Marjoram oil	8.67	6.00	8.00	6.67	1.95	2.16	1.82	1.96	8.34	6.34	1.89	2.06
Nigella oil + Clove oil	5.33	3.33	6.67	4.00	2.00	2.20	1.98	2.15	6.00	3.67	1.99	2.18
Marjoram oil + Clove oil	4.67	2.00	5.33	2.67	2.23	2.35	2.23	2.30	5.00	2.34	2.23	2.33
Nigella oil + Marjoram oil + Clove oil	8.00	4.00	7.33	6.67	1.90	2.1	1.85	2.00	7.67	5.34	1.88	2.05
Compost	----	13.33	----	17.33	----	1.74	----	1.68	----	15.33	----	1.71
Control	32.67	----	36.00	----	1.62	----	1.55	----	34.34	----	1.59	----

W- = without compost W+ = with compost

LSD 0.05 =								
Treatment	1.84	0.74	2.05	1.03	0.22	0.11	0.24	0.12
Interaction	2.57		3.25		0.53		0.59	

As for disease severity, the combination between clove oil and compost treatment completely prevented disease severity in 2017 season and the disease severity recorded 2.36 % in 2018 season, followed by composted soil and marjoram oil + clove oil treatment (2.00 and 2.67%) and of compost and nigella oil + clove oil (3.33 and 4.00%) during seasons 2017 and 2018 respectively. Moreover, the highest fruit yield was recorded with the integrated treatments of compost with clove oil (2.45 and 2.36 kg/ plant) and compost with marjoram oil + clove oil (2.35 and 2.30 kg/ plant) followed by composted soil and nigella oil + clove oil (2.20 and 2.15 kg/ plant) during season 2017 and 2018 respectively. Meanwhile, the least quantity of fruit yield was recorded in case of composted soil only.

4- Effect of essential oils and compost treatments on oxidative defense related enzymes

Results in Table 4 indicate that all tested treatments positively increased the activity of peroxidase (PO), Polyphenol Oxidase (PPO) and PAL enzymes in cucumber plants. The highest activity of peroxidase was expressed in the case of the integration between Marjoram oil + Compost (58.27) followed by Clove oil + Compost (56.86) and clove oil individually (55.46). Whereas, Nigella oil + Compost recorded the least activity of PO (38.96).

As for Polyphenol Oxidase (PPO), the highest activity of PPO enzyme was that expressed in the case of amendment soil with compost + Clove oil followed by clove oil individually where the recorded activity was 6.44 and 5.22 respectively followed by Marjoram oil individually 5.18. However, Nigella oil + Compost recorded the least activity of PPO (3.17).

As for PAL enzyme, plants treated with clove oil individually and Marjoram oil + compost recorded the highest activity of PAL where the recorded activity was 409.52 and 351.01 respectively, whereas compost recorded the least activity of PAL enzyme.

5- Effect of essential oils and compost treatments on lysis defense related enzymes

Results in Table 5 reveal that, activities of chitinase and β 1,3- glucanase were greatly increased in treated cucumber plants essential oils and compost treatments compared with control.

In this respect, treated plants with clove oil only recorded the highest activities of chitinase and β 1,3-glucanase where the recorded activity were 6.19 and 9.99 respectively followed by marjoram oil + compost which recorded 6.11 and 8.56 respectively, whereas compost treatment recorded the least activity of chitinase and β 1, 3-glucanase.

Table 4. Effect of essential oils and compost treatments on oxidative enzymes

Treatment	PO	PPO	PAL	Efficacy %		
				PO	PPO	PAL
Nigella oil	41.77	4.52	361.13	260.71	67.41	132.57
Marjoram oil	45.98	5.18	345.96	297.06	91.85	122.80
Clove oil	55.46	5.22	409.52	378.93	93.33	163.73
Compost	49.84	4.46	260.73	330.40	65.19	67.91
Nigella oil + Compost	38.96	3.17	308.40	236.44	17.41	98.61
Marjoram oil + Compost	58.27	4.48	351.01	403.20	65.93	126.05
Clove oil + Compost	56.86	6.44	306.23	391.02	138.52	97.21
Control	11.58	2.70	155.28	0.00	0.00	0.00

Control = infested with sclerotinia

Table 5. Effect of essential oils and compost treatments on lysis enzymes

Treatment	Chitinase	β 1,3-glucanase	Efficacy %	
			Chitinase	β 1,3-glucanase
Nigella oil	3.56	6.89	58.22	115.31
Marjoram oil	4.83	5.68	114.67	77.50
Clove oil	6.19	9.99	175.11	212.19
Compost	3.00	4.83	33.33	50.94
Nigella oil + Compost	3.21	6.58	42.67	105.63
Marjoram oil + Compost	6.11	8.56	171.56	167.50
Clove oil + Compost	5.97	8.42	165.33	163.13
Control	2.25	3.20	0.00	0.00

Electrophoretic analysis of peroxidase isozymes:

Native gel electrophoretic separation of enzyme extract from cucumber plants treated with nigella oil, marjoram oil, clove oil and compost compared with control and planted in inoculated soil with *S. sclerotiorum* showed in Table 6 and demonstrated in Fig.2 different PO patterns

and induced the density of PO isozymes. Moreover, the increased density of the induced PO was found in Marjoram oil compared with other treatments and control. Also, the band 4 was low density in compost and control treatments.

Table 6. Effect of essential oils and compost treatments on peroxidase isozymse.

Peroxidase	Relative Mobility	Clove oil	Marjoram oil	Nigella oil	Compost	Control
Px 1	0.55	1^{+}	1^{++}	1^{+}	1^{+}	1^{+}
Px 2	0.65	1^{+}	1^{++}	1^{+}	1^{+}	1^{+}
Px 3	0.75	1^{++}	1^{++}	1^{++}	1^{++}	1^{++}
Px 4	0.85	1^{+}	1^{++}	1^{+}	1^{-}	1^{-}

+ High density Band +Moderate density Band
- Low density Band 1 Present Band

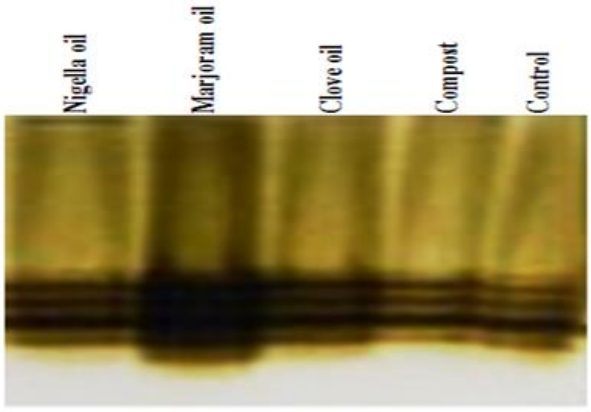

Fig. 2. Effect of essential oils and compost treatments on peroxidase isozymse.

Effect of treating cucumber plants with some plant oils on PAGE of protein.

Concerning the results of SDS (PAGE) presented in Table 7 and demonstrated in Fig. 3 show that 15 protein bands with molecular weights ranging from 234.433 to 50.017 kDa are contained in cucumber plants. New protein bands expressed as a result of treating cucumber plants with plant oils. One band with 90.783 kDa was appeared in plants treated with marjoram oil and Clove oil, while absent in other treated plants. Moreover, the band with 133.304 kDa absent in treated plants with marjoram oil.

Table 7. Molecular weights of fractionated protein profiles of cucumber leaves treated with selected treatments.

Band No	M.W kDa	Nigella oil	Marjoram oil	Clove oil	Compost	Control
1	234.433	+	+	+	+	+
2	159.517	-	-	-	-	-
3	155.793	+	+	+	+	+
4	133.304	+	-	+	+	+
5	118.734	-	-	-	-	-
6	112.191	+	+	+	+	+
7	91.783	-	+	+	-	-
8	85.101	+	+	+	+	+
9	54.844	+	+	+	+	+
10	50.017	+	+	+	+	+
11	46.267	+	+	+	+	+
12	41.996	-	-	-	+	+
13	41.699	+	+	+	+	+
14	37.23	-	-	+	-	-
15	34.93	+	+	-	+	-
Total		10	10	11	11	10

+ = bands appeared - = bands disappeared

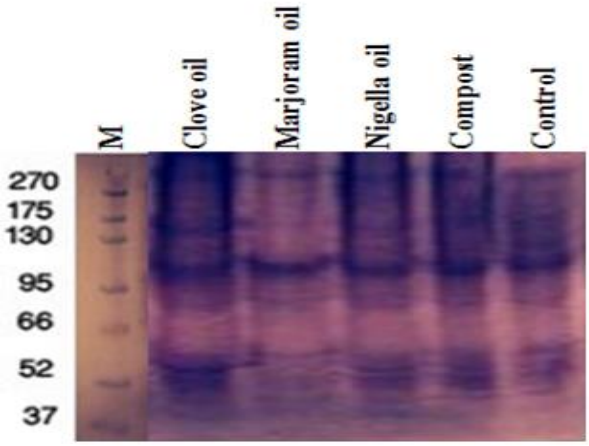

Fig. 3. Effect of treating cucumber plants with some plant oils on PAGE of protein

Discussion

Searching for alternative ecofriendly approach to manage the plant diseases is necessary. *In vitro* clove oil and marjoram oil were completely inhibited growth of *S. sclerotiorum* at 2.5 and 5% concentrations. This result is in agreement with the findings of Aminifard and Mohammadi (2013) and Zhenhua *et al.,* (2013). The mycelial growth of *S. sclerotiorum* was inhibited completely by cinnamon, clove and mint oil (Al-Taisan *et al.,* 2014). *S. sclerotiorum* was completely inhibited by the application of *Allium cepa* and *Eucalyptus globulus* at 500 mgL^{-1} concentration (Elgorban *et al.,* 2014).

Under greenhouse clove in soil free of compost or in soil amended with compost completely prevented the disease followed by marjoram oil and nigella oil in compost free soil which reduced the disease severity by 84.49 and 80.62 % respectively. also, clove oil integrated with compost completely prevented the disease incidence in 2017 season and the disease severity recorded 2% in 2018 season, followed by compost soil additive and marjoram oil + clove oil treatment. moreover, the highest fruit yield was recorded with the integrated treatments of compost with clove oil (2.45 and 2.36 kg/ plant) and compost with marjoram oil + clove oil (2.35 and 2.30 kg/ plant). this result agree with (Al-Taisan *et al.,* 2014) they found that, cinnamon oil produced the best result for reducing the *sclerotinia* rot disease incidence that produced 75% survival bean plants when compared with controls. essential oil of *salvia officinalis* reduced the *sclerotinia* rot disease of ***lettuce*** from 100% in control to 20% (Pansera *et al.,* 2013). treating cucumber plants with olive oil, clove oil, nigella oil and rocket oil significantly reduced the percentage of powdery mildew incidence and severity compared with the control treatment. Also, all tested plant oils significantly increased the fruit number/plant and fruit weight/plant (Ahmed 2005).

Regarding changes in activities of oxidative and catalyzed enzymes in treated cucumber plants with the tested treatments, all tested treatments increased peroxidase (PO), polyphenoloxidase (PPO) and pal activity compared with control treatment at all day's post inoculation. the highest activities of PO were expressed in the case of the integration between marjoram oil + compost followed by clove oil + compost and the highest activity of PPO

enzyme was expressed in the case of amendment soil with compost + clove oil followed by clove oil individually. however, plants treated with clove oil individually and marjoram oil + compost recorded the highest activity of PAL. moreover, plants treated with clove oil individually recorded the highest activity of chitinase and β 1,3-glucanase.

As well as, the profiling of peroxidase isoenzymes was induced in treated cucumber plants. Moreover, concerning the results of SDS (PAGE) of protein show that 15 protein bands with molecular weights ranging from 234.433 to 50.017 kDa are contained in cucumber plants. New protein bands expressed as a result of treating cucumber plants with plant oils.

These results could be interpreting in light the findings of Vance *et al.*, (1980) and Fry, (1982) who stated that peroxidase is known to be involved in the oxidation of polymerization of hydroxycinnamyl alcohols to yield lignin and crosslinking isodityrosine bridges in cell wall. The oxidative enzymes play an important role in induced resistance by the oxidation of phenols to oxidized toxic products (quinone) which limit fungal activity. Peroxidases also, catalyse the final polymerization step of lignin synthesis, which increases the ability of tissue to lignify which may restrict the fungal penetration (Tian *et al.*, 2006; Gorovitsa and Czosnek, 2008; Barilli *et al.*, 2010). Peroxidase also produces free radicals and hydrogen peroxide which are toxic to many microorganisms (Pena and Kuc, 1992). PAL is one of the key enzymes in the phenylpropanoid and the flavonoid pathway where it was increased in both incompatible and compatible interactions between plants and pathogens. Also, O'Neill and Saunders, (1994) demonstrated that the existence of phenolic phytoalexins in cucumbers may be produced through a PAL pathway. Abeles *et al*., (1970) who reported that β-1,3 glucan and chitin, polymer of N-acetylglucosamine (NAG) are major cell wall components of many fungi. Since β-1,3 glucanase and chitinases have been shown to be capable of attacking cell wall of fungal pathogens, these enzymes have been proposed as direct defense enzymes of plants. These findings indicate a positive relationship between resistance and peroxidase activity. Induced resistance of cucumber against powdery mildew recorded an increase in PR-proteins (peroxidase, polyphenoloxidase, Chitinase and β-1,3 glucanase) activity as well as an increase accumulation of phytoalexins (Alkahtani *et al.*, 2011). Based on the results obtained in this investigation, essential oils may be considered as alternative natural fungicides.

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فعالية السماد العضوي (الكمبوست) وبعض الزيوت العطرية بمفردها أو معًا في مكافحة مرض العفن الأبيض في الخيار تحت ظروف البيوت المحمية

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قسم أمراض النبات ـ كلية الزراعة بمشتهر ـ جامعة بنها ـ مصر

تم اختبار ثلاث تركيزات هي (1 و2.5 و5 ٪) من خمسة زيوت العطرية لدراسة تأثيرها على نمو فطر *Sclerotinia sclerotiorum* حيث أظهرت النتائج أن زيوت القرنفل والبردقوش كانت مثبطة لنمو الفطر. أظهرت النتائج أن اضافة السماد العضوي النباتي (الكمبوست) للتربة زاد من تأثير جميع الزيوت النباتية المستخدمة عن المعاملة الفردية في الحد من شدة المرض تحت ظروف الصوبة. وجد ان زيت القرنفل وحدة أو بالاشتراك مع الكمبوست منع تمامًا حدوث المرض . تم نقع شتلات الخيار بزيوت حبة البركة والبردقوش والقرنفل مجمعة معا بدون الكمبوست أو مع إضافته ضد مرض عفن الاسكليروتينيا فمنع زيت القرنفل مع اضافة الكمبوست الإصابة بالمرض تماما في عام 2017 وسجلت شدة الاصابة 2.36 ٪ في موسم النمو 2018. علاوة على ذلك، تم تسجيل زيادة محصول الثمار في المعاملة بالكمبوست مع زيت القرنفل ثم الكمبوست مع زيوت بردقوش وحبة البركة خلال مواسم النمو 2017 و2018 على التوالي. ادت جميع المعاملات المختبرة نشاط إنزيمات البيروكسيديز (PO) والبوليفينول أوكسيديز (PPO) وPAL في نباتات الخيار. علاوة على ذلك، تم زيادة نشاط انزيم الشيتينيز و بيتا 1-3 جلوكانيز بشكل كبير في الخيار المعالج بالمقارنة بالكنترول. فيما يتعلق بنتائج الفصل الكهربي للبروتين أظهرت النتائج أن هناك 15 حزمة بروتين بأوزان جزيئية تتراوح من50.017 إلى 234.433 كيلو دالتون موجودة في نباتات الخيار المعاملة. كما ظهرت حزمة بروتين واحدة بوزن جزيئي 90.783 كيلو دالتون في النباتات المعاملة بزيت البردقوش وزيت القرنفل بينما غابت في نباتات المعاملات الأخرى.