Egypt. J. Phytopathol., Vol. **45**, No. 1, pp. 1-15(2017)

Induction of Resistance in Pepper Plants Against *Potato Virus Y* (PVY)[NTN] by Two Medicinal and Aromatic Plant Essential Oils and Their Major Components

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The effects of essential oils of clove and fennel seeds and their major components as foliar spray were screened for inducing resistance against *Potato Virus Y in vivo* in both systemic and local lesion hosts at different concentrations. Clove oil at conc. of 15% gave the highest inhibitory effect against PVY infection than fennel oil. The inhibition percentages were 85, 80 % in pepper as a systemic host and up to 90, 87 %, respectively in *Chenopodium amaranticolor* as a local lesion host when applied 48 hrs before virus inoculation. Whereas, the inhibitory effect was less pronounced when essential oils were sprayed on the tested plants 24 hrs before virus inoculation. However, the severity of symptoms was assessed by visual inspection. ELISA test was used to confirm the results in all cases. When the major components of essential oils were applied individually, Eugenol gave the highest inhibitory effect (90%) on PVY- systemically infected pepper plants and 91.8% on *Ch. amaranticolor* as local infected plants when applied 48 hrs before virus inoculation followed by Anethol. While, Limonene was less effective in reduce PVY infection. Considerable increase in total protein content in pepper plants sprayed with essential oils and their major components before virus inoculation was recorded compared with healthy and infected control plants. Moreover, SDS-PAGE revealed a new protein band 20 KDa which was found only in pepper plants sprayed with clove oil at conc. of 15%. Also new proteins 19, 20 KDa and 25 KDa were found only in pepper plants sprayed with Eugenol. It has been suggested that, the induced proteins may help to limit virus spread or multiplication.

Keywords: Clove seed oil, Fennel seed oil, Induced systemic resistance, Major oil components, *Potato Virus Y* (PVY) and SDS-PAGE.

Sweet pepper (*Capsicum annum* L.) is a member of the *Solanaceous* fruity vegetables group. It is one of the most important, popular and favorite vegetable crops cultivated in Egypt for local consumption and export (El-Bassiony *et al*., 2010). *Potato virus Y* (PVY) is one of the most damaging viruses causing diseases in pepper plant, it is widespread wherever pepper and potato are grown (Crosslin *et al*., 2006). The virus isolate reacted with dark green mosaic, curl and deformation on *Capsicum annum* L. cv. California Wonder (EL Banna *et al*., 2015). PVY is difficult to control because of its extremely broad natural host range (Hafez, 1999), and the ability to be transmitted by many aphid species in non-persistent manner (Mascia *et al*., 2010).

The phenomenon of systemic acquired resistance (SAR), refers to a distinct signal transduction pathway which can make plants to be stimulated to defend themselves against pathogens (Hunt and Ryals, 1996). Induced resistance is the phenomenon that a plant; once appropriately stimulated, exhibits an enhanced resistance up on 'challenge' inoculation with a pathogen (Ryals *et al*., 1994). Induced resistance has been adopted as a general term and defined as 'the process of active resistance dependent on the host plant's physical or chemical barriers, activated by biotic or abiotic agents (inducing agents) (Kloepper *et al*., 1992). In the few last years, there has been target interest in biologically active compounds isolated from plant species for inhibiting different viruses on the plant as they are safe substances for human and environment (Bezic *et al*., 2011). Systemic induced resistance (SIR) can be applied as an alternative to the traditional methods of plant protection. In addition, these compounds were easily biodegradable, non-phytotoxic, more environment friendly and safe (Ebadollahi, 2011). Clove is one of the most important aromatic plants in the world. Essential oils obtained from its flower have a long history of use as natural antimicrobial agent and have recently been used in several pharmaceutical, food and cosmetic products (Park *et al*., 2007). Fennel also has a long history of herbal uses and widely cultivated for its edible strongly flavored leaves and seeds which are used as culinary species (Roby *et al*., 2012). Essential oils and plant extracts can be applied to activate host defense mechanisms as a potential management. Volatile constituents of *Carum copticum* and *Cymbopogon citrates* were found to be the most potent in reducing the infectivity of PVX and PVY on *C. amaranticolor* (Tripathi, 1985). The essential oils of fennel and anise completely inhibited PVX, TMV and TRSV on *C. amaranticolor* (Shukla *et al*., 1989). The efficacy of *Lippa nodiflora*, *Datura metel* and *Thuja orientalis* extracts for inducing systemic resistance was tested against PVY on potato. Spraying the foliage by the extracts exhibited a protection period to the plants against PVY infection up to one month in the presence of virus source with the vector *Myzus persicae* **(**Al-Ani *et al*., 2011). Essential oil of clove caused maximum inhibition of *Potato leaf roll virus* replication (Iftikhar *et al*., 2013). The essential oil of Artemisia and lemongrass and ginger resulted in more than 50% inhibition of TMV on tobacco plants (Lu *et al*., 2013). Moreover, the constituents of these oils like Limonene, Cineole, Zingiberene and Citronellal gave more than 40% inhibition rate for TMV. Foliar application of eugenol induced systemic resistance against *Tomato yellow leaf curl virus* (TYLCV) in tomato plants (Wang and Fan, 2014).

The objectives of this work are to investigate the efficacy of the foliar spraying with two essential oils and their major components as natural resistance inducers against PVY on pepper plants under greenhouse condition, and to study the accumulation of PR-proteins which appear as a result of inducing resistance.

Materials and Methods

This work was carried out in the greenhouse belongs Virus and Phytoplasma Res. Dep., Plant Pathology Res. Institute, (ARC), Giza, Egypt. Seeds of pepper (*Capsicum annum* L. cv. California Wonder) were obtained from Vegetable Disease Res. Dept., and *Chenopodium amaranticolor* Cost & Reyn seeds were supplied from

Virology greenhouse, Virus and Phytoplasms Res. Dept., Plant Pathology Research Institute, ARC, Giza, Egypt.

Virus source:

An isolate of *Potato virus Y* (PVY)^{NTN} isolated previously by Amer *et al*., 2004 was used in this investigation. The crude sap obtained from frozen leaves of pepper (*Capsicum annum* L. cv. California Wonder) was inoculated into pepper plants which exhibited mosaic and vein banding symptoms 14 days after inoculation.

Essential oil preparation:

The pure essential oil of Clove *(Syzgium aromaticum* L.), fennel (*Foeniculum vulgare* L.) and their major components (active ingredient) (eugenol, caryophyllen α- pinene) & (anethol, fenchone and limonene, respectively) were kindly supplied from the National Organization for Drug Control and Research (NODCAR), Giza.
Dilutions of 5, 10, 12 and 15% of essential oils were prepared in distilled water containing 0.1% Tween-20. Similarly, dilution of 1% of essential oil components were prepared by mixing 1ml subsequently made up to 99 ml with sterilized distilled water containing 0.1% Tween -20.

Induced systemic and localized resistance against PVY:

This experiment was conducted using Randomized Complete Block Design. Essential oils were applied as foliar sprays. Twenty pepper seedlings were transplanted in pots (ten plastic pots 20 cm. in diam. were used as a replicates in each treatment by means of two plants per pot). Ten leaves of *Ch. amaranticolor* were used as a replicates in each treatment. The effect of the four concentrations of clove and fennel essential oils, i.e. 5, 10, 12 and 15% were estimated in two experiments.

In the first experiment, pepper transplants (30 days old) were used as a systemic host. Whereas, *Ch. amaranticolor* (30 days old) plants were used as a local lesion host in the second experiment. Plants were sprayed with 100ml of each concentration of 5 and 10%, 24 and 48 hrs before virus inoculation and with 12 and 15%, 48 hrs before virus inoculation. In check experiments two groups of plants were used, the first were sprayed with buffer solution pH 7.4 (healthy control) and the second were sprayed with distilled water containing 0.1% Tween-20, and then plants were mechanically inoculated with PVY- infected sap 1ml/ plant (infected control). Tested plants were observed daily for the appearance of systemic symptoms on pepper plants or developing of local lesions on *Ch. amaranticolor* leaves. Inhibitory effect of the tested essential oils was determined as described by Devi *et al*. (2004) using the following equation:
Inhibition % = (A-B/ A) ×100, where A is the number of plants in check experiment and B is the number of treated plants and after that inoculated by virus inocula. Local lesions were counted seven to ten days after inoculation. The percentage of inhibition of local lesion formation by each treatment over the control was calculated based on the number of local lesions produced using the formula described by Madhusudhan *et al*. (2011) as:

$$I = C\text{-}T/C\times100$$

Where I = inhibition percentage of local lesion formation over control, C = average No. of local lesions in control leaves and T = average No. of local lesions in treated

leaves with the essential oils (ten leaves of *Ch. amaranticolor* were used as a replicates in each trial). The effect of major clove oil components (eugenol, caryophyllen α- pinene) and fennel oil components (anethol, fenchone and limonene) were also estimated at conc. of 1% against PVY on Pepper and *C. amaranticolor* plants. Plants were sprayed with different oil components 24 and 48 hrs before virus inoculation. In check experiments plants were sprayed with distilled water containing 0.1% Tween-20, then plants were mechanically inoculated with PVY infected sap. Tested plants were observed daily for the appearance of systemic symptoms or local lesions and the percentage of inhibition for each treatment was calculated as mentioned before.

Effect of PVY on leaf area of pepper plants:

Vegetative growth expressed as leaves area. Plant $^{-1}$ of pepper plants were taken to determine the effect of PVY on plants depending on date of infection and compared with control (healthy) pepper plants. Leaf.Area. Plant $^{-1}$ (cm^2) was measured using Image Analysis Software for Plant Disease Quantification (Assess 2.0 program).

Protein extraction:

Protein extraction was carried out according to Bollag and Edelstein (1993). Using one gram fresh weight of leaves collected after five days of inoculation with PVY from pre-treated pepper plants of each treatment and both healthy and infected control plants. Total proteins were determined using bovine serum albumin as a standard spectrophotometric method by Bradford (1976).

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

Polyacrylamide gel electrophoresis (PAGE) was used to determine the qualitative changes in the soluble proteins of pepper plants (healthy or infected with PVY) as a result of spraying with essential oils or its major components. Twenty-microliters of leaf samples (40 µg of protein) were subjected to electrophoresis in 15% polyacylamide prepared in 0.1% SDS (Bollag and Edelstein, 1993) and stained with silver nitrate according to Sammons *et al*. (1981). Obtained protein gels were scanned for band R_f using gel documentation system. Different molecular weights (MW) of bands were determined against protein marker 66,25 and 18 kDa.

Statistical analysis:

Data were analyzed with the statistical analysis system SAS. All multiple comparisons were first subjected to analysis of variance (ANOVA) comparison among means was carried out according to Duncan's multiple range test (Duncan, 1995).

Results

Induced systemic resistance against PVY:

The results in Table 1 and Fig. 1 show that all four concentrations, *i.e.* 5, 10, 12 and 15% of both essential oils of clove and fennel, in general gave encouraging results of virus inhibition when compared with the control treatment. The efficiency of inhibition was increased with the increasing the concentration and the time before virus inoculation. The conc. of 15% of the two essential oils tested was the most effective one, as it significantly induced the highest systemic resistance against PVY.

Essential oil of clove gave the highest inhibition percentage (85 and 80%) at conc. of 15 and 12%, respectively when applied 48 hrs before virus inoculation. Meanwhile, essential oil of fennel at the same conc. gave 80 and 70% inhibition, respectively. The inhibitory effect was less pronounced when essential oils were sprayed on the tested plants 24 hrs before virus inoculation. Least inhibitory percentage (10%) was obtained using fennel oil at conc. of 5% when applied 24 hrs before virus inoculation. ELISA test was used to confirm the results in all cases.

Induced local resistance against PVY :

Data obtained from Table 2 show that all tested concentrations of the two essential oils significantly reduced the number of local lesions produced by PVY on *Chenopodium amaranticolor* and increased the percentages of inhibition against PVY (Fig. 1). This effect was the highest with the concentration of 15% and decreased gradually by reducing the concentration from 12 to 5%. Essential oil of clove at conc. of 10, 12 and 15% gave percentages of inhibition 77, 88 and 90% respectively, when applied 48 hrs before virus inoculation. Essential oil of fennel was less effective in reducing the local lesions produced by PVY on *Ch. amaranticolor* than essential oil of clove (75, 80 and 87, respectively).

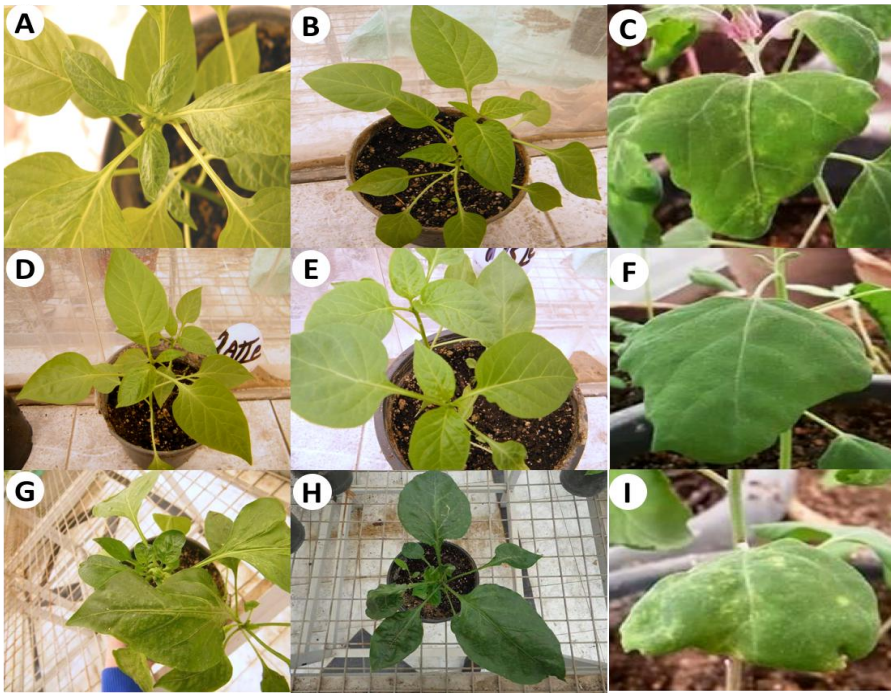

Fig. 1. Pepper and *Ch. amaranticolor* plants showing the effect of different concentrations of clove oil on systemic and local infections produced by PVY. A: Control (infected pepper); B: Healthy non-treated; D, E, G and H treated pepper plants with 15, 12, 10 and 5% conc. respectively; C: *Ch. amaranticolor* infected control; F and I: *Ch. amaranticolor* treated plants with 15 and 5% conc., respectively.

Table 1. Effect of different concentrations of clove and fennel essential oils on inhibition percentages of Potato virus Y (PVY^{NTN}) systemically infected pepper plants

Treatment	24 hrs before inoculation					
	Concentration (%)					
	5			10		
	N.	I (%)	ELISA	N.	I (%)	ELISA
Clove	16	20	0.296	13	35	0.262
Fennel	18	10	0.341	15	25	0.283
Control	20	0	0.977	20	0	0.977
LSD at 0.05	1.9	1.6		0.9	1.5	

48 hrs before inoculation												
Treatment	Concentration (%)											
	5			10			12			15		
	N.	I (%)	ELISA	N.	I (%)	ELISA	N.	I (%)	ELISA	N.	I (%)	ELISA
Clove	10	50	0.211	5	75	0.198	4	80	0.188	3	85	0.164
Fennel	12	40	0.226	8	60	0.207	6	70	0.193	4	80	0.170
Control	20	0	0.977	20	0	0.977	20	0	0.977	20	0	0.977
LSD at 0.05	1.4	1.3		1.7	1.3		1.4	1.6		2.0	1.6	

N=Mean number of infected plants I (%) = Inhibition

Table 2. Effect of different concentrations of clove and fennel essential oils on inhibition percentages expressed as local lesions number produced by PVY on *Ch. amaranticolor*

Treatment	24 hrs before inoculation					
	Concentration (%)					
	5			10		
	N.	I (%)	ELISA	N.	I (%)	ELISA
Clove	6.0	50	0.270	4.0	66.7	0.249
Fennel	7.0	41.7	0.293	5.0	58.3	0.368
Control	12	0	0.989	12	0	0.989
LSD at 0.05	0.6	1.4		1.2	1.3	

48 hrs before inoculation												
Treatment	Concentration (%)											
	5			10			12			15		
	N.	I (%)	ELISA	N.	I (%)	ELISA	N.	I (%)	ELISA	N.	I (%)	ELISA
Clove	3.0	70	0.199	2.3	77	0.173	1.2	88	0.162	1.0	90	0.143
Fennel	3.5	65	0.206	2.5	75	0.185	2.0	80	0.179	1.3	87	0.158
Control	10	0	0.989	10	0	0.989	10	0	0.989	10	0	0.989
LSD at 0.05	1.2	1.5		0.5	1.3		1.2	1.6		1.4	1.2	

N=Mean number of local lesions I (%) = Inhibition

Effect of PVY on leaf area of pepper plants:

Results showed that leaves area of infected plants are significantly smaller (2.50 cm^2) than healthy plants (6.54 cm^2). Leaf area of treated plants with 12, 15% clove, 12 and 15% fennel and control (PVY-infected) was 4.80, 5.29, 2.62, 2.81 and 2.50 cm^2, respectively. These results showed that the virus has a great effect on leaf area of infected plants compared to healthy control plants (6.54 cm^2).

Table 3. Effect of PVY on leaf area of pepper plants

Leaf number	Healthy control	Infected control	Clove 12 %	Clove 15 %	Fennel 12 %	Fennel 15 %
	Area	Area	Area	Area	Area	Area
1	5.41	2.68	4.56	4.40	3.17	2.16
2	6.85	2.24	4.82	5.94	1.97	2.93
3	6.72	2.78	4.41	5.60	3.01	3.28
4	5.45	2.13	4.45	4.24	3.04	2.30
5	6.84	2.24	5.08	5.92	1.41	2.85
6	6.57	2.90	4.29	5.50	2.94	2.64
7	5.49	2.23	4.63	3.85	3.20	2.26
8	6.62	2.29	4.90	5.78	2.08	2.94
9	7.19	2.77	4.92	5.47	3.05	3.26
10	8.22	2.75	5.95	6.16	2.30	3.49
Average	6.54	2.50	4.80	5.29	2.62	2.81

Effect of the major components of clove and fennel essential oils on inhibition percentages of PVY on pepper plants:

The activity of the major components in clove oil (Eugenol, caryophyllene and α-pinene) and in fennel oil (anethol, fenchone and limonene) was individually evaluated against PVY (Table 4). Data reveal that all tested oil components gave significant inhibitory effects on PVY- systemically infected pepper plants. The inhibitory effect of eugenol was stronger in the reduction of PVY infection with the rate of 90% when applied 48 hrs before virus inoculation. Also, the percentage of inhibition of PVY with anethol was 85% followed by caryophyllene (80%). Lower inhibitory effect was obtained when these components were applied 24 hrs before virus inoculation. Least effect was obtained with limonene and fenchone (45% and 50%), respectively, when applied 24 hrs before virus inoculation.

Effect of major oil components of clove and fennel on inhibitory percentages of local lesion number produced on Ch. amaranticolor:

Data presented in Table 5 revealed that all tested oil components gave significant inhibitory effect on number of local lesions produced on *Ch. amaranticolor* leaves inoculated with PVY. The highest inhibitory effect (91.8%) was recorded with eugenol when applied 48 hrs before virus inoculation followed by anethol (86.4 %) and caryophyllene (82.7%) while limonene gave the lowest record (72.7%). Lower inhibitory effect was obtained when these components were applied 24 hrs before virus inoculation.

Table 4. Effect of major components of clove and fennel essential oils on inhibition percentages of *Potato Virus* Y (PVY)^{NTN} systemically infected pepper plants

Treatment	Time of application					
	24 hrs before inoculation			48 hrs before inoculation		
	N.	Inhibition (%)	ELISA	N.	Inhibition (%)	ELISA
Eugenol	6	70.0	0.281	2	90.0	0.121
Caryophyllene	8	60.0	0.253	4	80.0	0.183
α-pinene	9	55.0	0.301	6	70.0	0.212
Anethol	7	65.0	0.237	3	85.0	0.143
Fenchone	10	50.0	0.268	5	75.0	0.199
Limonene	11	45.0	0.352	7	65.0	0.240
Control	20	0	0.999	20	0	0.999
LSD at 0.05	0.4	0.7		0.3	0.5	

N=Mean number of infected plants

Table 5. Effect of major components of clove and fennel essential oils on inhibition percentages expressed as local lesions number produced by PVY on *Ch. amaranticolor*

Treatment	Time of application					
	24 hrs before inoculation			48 hrs before inoculation		
	N.	Inhibition (%)	ELISA	N.	Inhibition (%)	ELISA
Eugenol	3.5	65.0	0.288	0.9	91.8	0.113
Caryophyllene	5.0	50.0	0.262	1.9	82.7	0.172
α-pinene	6	40.0	0.293	2.5	77.3	0.203
Anethol	4.0	60.0	0.250	1.5	86.4	0.136
Fenchone	5.5	45.0	0.278	2.2	80.0	0.180
Limonene	6.5	35.0	0.368	3.0	72.7	0.220
Control	10	0	0.979	11	0	0.979
LSD at 0.05	0.3	0.9		1.0	0.4	

N=Mean number of local lesions

Total protein:

Protein content was determined in pepper plants sprayed with different concentrations of clove and fennel essential oils and their major components. Data in Table 6, indicat that protein content were significantly increased in treated pepper plants compared with healthy and infected control plants. The highest increased was observed in pepper plants sprayed with eugenol, anethol and clove oil at conc. of 15% before 48 hrs of virus inoculation. Higher protein levels (1.77 mg/g FW) was observed in plants sprayed with eugenol followed by anethol (1.73 mg/g FW) compared with healthy and infected control plants (1.07, 1.21 mg/g FW, respectively). While pepper plants sprayed with fennel and clove essential oil at 5% was the lowest protein levels (1.32 mg/g FW, 1.40 mg/g FW, respectively).

Table 6. Effect of different concentrations of clove and fennel essential oils and their major components on total protein content (mg/g FW) in pepper plants

Treatment	Protein content (mg/g FW)
	48 hrs before inoculation
Clove 5%	1.40
Clove 10%	1.56
Clove 12%	1.63
Clove 15%	1.71
Healthy control	1.07
Infected control	1.21
L.S.D.at 0.05	0.14
Fennel 5%	1.32
Fennel 10%	1.41
Fennel 12%	1.51
Fennel 15%	1.60
Healthy control	1.07
Infected control	1.21
L.S.D.at 0.05	0.07
Eugenol	1.77
Caryophyllene	1.68
α-pinene	1.53
Anethol	1.73
Fenchone	1.62
Limonene	1.46
Healhy control	1.07
Infected control	1.21
L.S.D.at 0.05	0.08

Protein pattern profile:

Pepper plants sprayed with essential oil of clove and fennel at 5, 10, 12 and 15% applied 48 hrs before virus inoculation showed variation in number of protein patterns, molecular weight and density of protein bands compared with untreated infected control or healthy one, new pattern of protein was observed (Fig. 2A). A new protein 20 kDa was found only in pepper plants sprayed with clove oil at conc. of 15%. Extra bands between 16 to 45 kDa were released with high density in the treated pepper plants but not identical to those in infected control and healthy plant. Fig. (2B) show some changes in the protein patterns of pepper leaves pre-treated with major oil constituents at conc. of 1% relative to control. De-novosynthesized proteins were detected in treated leaves which were undetectable in control leaves. Treatment with eugenol resulted in the detection of two similar induced proteins (with molecular weights of 19, 20) in approximately similar amounts and 25 KDa. Treatment with anethol and caryophyllene gave similar proteins, with molecular weight of 23 and 24 kDa in different amounts. Treatment with anethol, caryophyllene and α-pinene gave protein band with molecular weight of 24 kDa. Treatments with eugenol and caryophyllene gave protein band with molecular weight of 38 kDa.

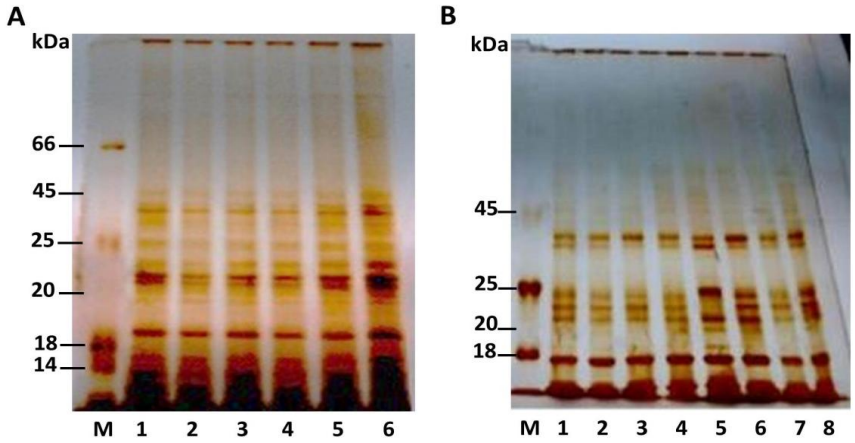

Fig. 2. SDS-PAGE analysis of: (A): Total protein extracted from pepper leaves pre-treated with essential oil of clove: Lane M molecular mass markers, Lane 1: infected unsprayed pepper leaves, Lane 2: healthy pepper leaves, Lane 3-6 pepper plants sprayed with clove oil at conc. of 5, 10, 12 and 15%, respectively and (B): Total protein extracted from pepper leaves pre-treated with the major oil components. Whereas: Lane M: markers, Lane 7: infected unsprayed pepper leaves, Lane 2: healthy pepper leaves, Lane 1,8 are pepper plants sprayed with α-pinene and caryophyllene. Lane 3-6 are pepper plants sprayed with fenchone, limonene, eugenol and anethole, respectively.

Discussion

The objectives of this study were induction of systemic resistance in pepper plants against virus infection. Two plant essential oils and their major components were screened for inducing resistance against PVY. The results suggested that the infection either systemically or locally was reduced in tested plants when compared to untreated ones. Foliar treatment with essential oil of clove and fennel showed high activity in reducing the number of PVY- infected pepper plants. Similar results were noticed by Iftikhar *et al.* (2013), who mentioned that foliar treatment with clove and fennel essential oil significantly reduced *Potato leaf roll virus* infection in potato plants. *Lippia nodiflora* extracts were highly effective in inducing systemic resistance in potato plants against PVY infection (Al-Ani *et al*., 2011). Lavender essential oil had the ability to inhibit *Tomato spotted wilt virus* multiplication and spread of virus infection in systemically infected tomato plants (Kobeasy *et al*., 2013).

Essential oil of clove at 15% conc. was the most effective in reducing local lesion number produced by PVY on *Ch. amaranticolor* followed by the same conc. of fennel oil. These findings are in accordance with the previous work as mentioned by Mohamed (2010) on garlic oil which reduced local lesion number produced by *Potato virus Y* on *Ch. amaranticolor*. Essential oil of fennel totally inhibited the formation of local lesions produced by *Potato Virus* X on *C. amaranticolor* (Shukla *et al*., 1989). Essential oil of *Melaleuca alternifolia* was effective as it significantly

decreased lesion numbers produced by *Tobacco mosaic virus* on *Nicotiana glutinosa* (Bishop, 1995). Essential oil of *Satureja montana* inhibited local lesions number produced by *Cucumber mosaic virus* on *Ch. amaranticolor* (Dunkic *et al*., 2010).

The average leaf area of treated plant with clove and fennel oil prior to PVY infection showed a great effect on leaf area of infected plants compared to control plants (healthy). Results demonstrated that there are significant differences between leaf area of different clove and fennel treated plants compared with the control. The result is in agreement with Faragette *et al*. (1988). The obtained results showed that pepper plants pre-treated with eugenol and anethol exhibited significant reduction infection percentages of PVY. These results are in agreement with (ChunMei, 2013) who mentioned that eugenol significantly reduced the severity of *Tomato yellow leaf curl virus* infection (TYLCV) when applied as a foliar spray. Eugenol also induced (H_2o_2), peroxidase and polyphenol oxidase in tomato plants. Significant reduction of local lesion number produced by CMV or TMV was detected when the thymol and carvecrol was applied on *Ch. amaranticolor* (Dunkic *et al*., 2010). Sprays of the limonoids, nimbin and nimbidin, compounds that occur in the leaves and seeds of neem oil reduced local lesion formation on *C. amaranticolor* after mechanical inoculation with *Potato Virus X* (Verma, 1974).

Pepper plants infected with *Potato virus Y* showed high content of total protein compared to healthy plants. However, there was a progressive increase in protein content in plants treated with clove and fennel essential oils and their major components. This result agreed with that obtained by Haque *et al*., (2005), who showed that *Zucchini yellow mosaic virus* infection increased the protein content of pumpkin leaves compared to healthy ones. The increased protein content in virus infected plants was due to increased activity of RNA synthetase or RNA polymerase (Rao *et al*., 1989). The treated plants also show high protein content compared to infected control . This may be due to the formation of new antiviral protein. This agrees with that obtained by Abdel-Shafi (2005). Electrophoretic studies using sodium dodecyl sulphate polyacrylamide gel electrophoresis indicated that foliar treatment with two essential oils and their major components induced resistance against PVY, thus resulted in inducing new proteins, which were not found in the healthy or infected untreated plants. It has been suggested that, the induced proteins may help to limit virus infection or multiplication (Chen *et al*., 2006). Furthermore, low molecular proteins are responsible of the process of virus inhibition. These induced proteins have been defined as pathogenesis related proteins, they are implicated in plant defense because of their anti- pathogenic activities. The continuous accumulation of newly induced proteins may help in the localization of viral infection (Van-Loon *et al*., 1997). Based on current knowledge, it can be concluded that induce systemic resistance resulting from the expression of several parameters, including *De-novo* synthesis of pathogenesis related proteins (PR) (Walter *et al*., 2007). This work may be a step; where more investigations are needed to formulate these compounds to make them more easy to use.

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(Received 15/01/2017;
in revised form 28/02/2017)

استحثاث المقاومة ضد فيروس البطاطس Y^{NTN} فى نباتات الفلفل باستخدام اثنين من الزيوت النباتية الطبية والعطرية ومكوناتهم الرئيسية

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تم دراسة تأثير التركيزات المختلفة من زيت القرنفل والينسون ومكوناتهم الرئيسية على استحثاث المقاومة ضد فيروس البطاطس Y حيث استخدمت نباتات الفلفل لاختبار الإصابة الجهازية للفيروس كما استخدمت نباتات الزربيح شينوبوديوم أمارانتيكالر لاختبار عدد البقع المحلية للفيروس نتيجة المعاملات. وقد أعطى تركيز ١٥% من زيت القرنفل عند استخدامه قبل ٤٨ ساعة من حقن الفيروس أعلى نسبة تثبيط للأصابة بالفيروس عن زيت الينسون وكانت النسبة المئوية للتثبيط ٨٥% ، ٨٠% على نباتات الفلفل كما قلل النسبة المئوية لعدد البقع المحلية المتكونة على نباتات الزربيح إلى ٩٠، ٨٧% بينما قلت فاعلية زيت القرنفل على تثبيط الإصابة بالفيروس وذلك عند استخدامه قبل ٢٤ ساعة من حقن الفيروس. عند استخدام المكونات الأساسية للزيت العطرى كلا على حدة أعطى اليوجينول أعلى نسبة مئوية لتثبيط الإصابة الجهازية بالفيروس على نباتات الفلفل ٩٠% و أعطى نسبة ٩١,٨% لتثبيط تكوين البقع المحلية على نباتات الزربيح وذلك عند استخدامه قبل ٤٨ ساعة من حقن الفيروس ويليه الأنيثول بينما أعطى الليمونين أقل تأثير فى تقليل النسبة المئوية للإصابة بالفيروس. علاوة على ذلك وجد زيادة ملحوظة للبروتين الكلى فى نباتات الفلفل المرشوشة بكلا من زيت القرنفل والشمر ومكوناتهم الرئيسية وذلك عند مقارنتها بكلا من نباتات الفلفل السليمة غير المعاملة ونباتات الفلفل المصابة بالفيروس. عند عمل تفريد للبروتين باستخدام SDS- PAGE تم ظهور بروتينات جديدة حيث وجد بروتين جديد وزنه الجزيئى ٢٠ كيلو دالتون فى نباتات الفلفل المعاملة بتركيز ١٥% من زيت القرنفل. أيضا وجد بروتينات جديدة وزنها الجزيئى ١٩، ٢٠ ، ٢٥ كيلو دالتون فى نباتات الفلفل المعاملة باليوجينول. ويرجح أن البروتينات الجديدة قد تساعد على الحد من انتشار أو تضاعف الفيروس فى النبات.