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Effect of some Plant Extracts on the Growth of Streptomycin Resistant and Sensitive Isolates of *Erwinia amylovora*

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Fire blight, caused by *Erwinia amylovora* is the most destructive disease on pear in Egypt. In this study water, ethanol and chloroform extracts of eighteen medicinal plants were tested against *Erwinia amylovora*. Obtained results showed that garlic, harmel, madder, thyme and garden sage extracts were effective in inhibiting the growth of *E. amylovora in vitro*, while madder, harmel and thyme extracts were the most effective *in vivo* trials. Also, in this study the effect of some extracts, *i.e.* madder, garlic, thyme, harmel and garden sage, were tested in inhibiting the growth of seven isolates of *E. amylovora* resistant to streptomycin sulfate and the result showed that garlic, madder and harmel were more suppressive to some isolates than the sensitiv. Active ingredients of harmel, thyme and garlic extracts were separated and tested for their effect on inhibiting growth of the fire blight pathogen. With respect to cellular protein pattern, the results revealed that some proteins were present in all resistant and sensitive isolates either treated with madder extract or extract-free. However, they were absent in the same isolates treated with harmel extract. Also, harmel extract inhibited the synthesis of some proteins that have low molecular weight in either resistant or sensitive isolates. Madder extract activate the production of a protein in streptomycin sensitive isolates and suppress the synthesis of other proteins in the same isolates. However, madder extract has inhibited the production of same low molecular weight protein in streptomycin resistant isolates.

Keywords: *Erwinia amylovora*, garden sage, garlic, harmel, madder, plant extracts, streptomycin and thyme.

Fire blight caused by *Erwinia amylovora* is one of the most important diseases of pome fruits in many countries of the world. In Egypt, the disease was considered as an economic problem and become a destructive disease on pear (El-Helaly *et al.*, 1964; Abo El-Dahab *et al.*, 1983 and Tawfik *et al.*, 2000).

Application of antibiotics such as streptomycin to control the disease have been prone to developing drug resistance which decrease substantially the effectiveness of these bactericides. Therefore, besides biological and genetic engineering techniques, natural substances are also being used which appear to have bactericidal properties.

Garlic (*Allium sativum*) is antibacterial to *Staphylococcus aureus,* the principal compounds of garlic has been elucidated as allicin (Czapska *et al.,* 2006). The filtered garlic extract was effective against pith necrosis pathogens *Pseudomonas viridiflava* on tomato in greenhouses (Aysan and Yildiz, 2000).

The aqueous and methanolic extract of leaves of *Myrtus communis* (myrtle) were reported to have an antibacterial effect against *Salmonella typhimurium* and *Streptomyces scabies* the causal agent of common scab disease of potato (Hayder *et al.*, 2003 and Aram *et al.,* 2006).

Plantago lanceolata (ribwort) has an antibacterial activity and its constituents are glycosides, tannins (Terrie, 2002), but for *Peganum harmala* (harmel) aqueous seed extract was more effective against *Bacillus subtilis, Enterobacter aerogenes* and *Staphylococcus epidermidis* compared to the antibiotic gentamycin (Vimal-Kumar *et al.,* 2005).

The organic extracts of *Thymus vulgaris* showed greater activity than the aqueous extract compared with 14 Palestinian medicinal plants in studies against some Gram- positive and Gram negative bacteria, *e.g. B. subtilis, E. coli, Pseudomonas aeruginosa* and *Staphylococcus aureus* (Essawi and Srour, 2000 and Adwan *et al.,* 2006). Also, thyme extract has an antibacterial activity against *Erwinia carotovora* (Wood *et al.,* 1997).

Different plant extracts from *Artemisia annua, Rubia tinctorum, Rosmarinus officinalis, Salvia officinalis, Thymus vulgaris* and *Urena tenax* had antimicrobial effect against *Pseudomonas aeruginosa, Micrococcus luteus, S. aureus, E. coli, B. subtilis* and *P. aeruginosa* (Mehrabian *et al.,* 2000; Kalyoncu *et al.*, 2006; Cordeiro *et al.*, 2006 and Kulataeva *et al.,* 2006).

The present investigation was under taken to study the effect of extracts of eighteen plant species tested against the growth of *E. amylovora.* The active ingredients from harmel (harmine and harmalin), garlic cloves (sulfur compounds) and thyme plants (thymol crystals) were separated and tested against the growth of the fire blight pathogen. It was also interesting to study the effect of some plant extracts on the growth of streptomycin resistant isolates of *E. amylovora* from pear orchard as an alternative agent of streptomycin sulfate in the programme of integrated control of fire blight disease.

Materials and Methods

Preparation of plant extracts:

All fresh plant materials (Table 1) were collected from the farm of Fac. of Pharmacy, Cairo Univ., Giza, and the dried plants were collected from the market of herbs (stores of medicinal and aromatic plants). All fresh plant species were identified and verified by the Horticulture Res. Inst., Agric. Res. Centre, Giza. Samples were used to prepare cold water, hot water, ethanolic and chloroform extracts.

Table 1. Scientific and common names of tested aromatic and medicinal plants

Botanical families, * Genera and species	English name	Plant part used
Anacardiaceae		
- *Schinus terebinthifolius* L.	Brazilian pepper tree	Leaves
Asteraceae (compositae)		
- *Artemisia annua* L.	Gold warm wood	Herb
- *Artemisia herba-alba Asso*	Warm wood	Herb
Cruciferae		
- *Anastatica hierochuntica* L.	St. Mary's flower	Whole plant
- *Brassica alba*	White Mustard	seeds
Graminae		
Cymbopogon citratus Stapf	Lemon grass	Leaves
Labiatae		
- *Mentha piperita* L.	Peppermint	Leaves
- *Rosmarinus officinalis* L.	Common rosemary	Leaves
- *Salvia officinalis* L.	Garden sage	Leaves
- *Thymus vulgaris* L.	Garden thyme	Leaves
Liliaceae		
- *Allium sativum* L.	Garlic	Cloves
Malvaceae		
- *Urena tenax* L.	Indian mallow	Fruits
Myrtaceae		
- *Myrtus communis* L.	Myrtle	Leaves
Plantaginaceae		
- *Plantago lanceolata* L.	Ribwort	Herb
Ranunculaceae		
- *Nigella sativa* L.	Black cumin	Seeds
Rubiaceae		
- *Rubia tinctorum.*	Madder	Roots
Rutaceae		
- *Ruta graveolens* L.	Common rue	Herb
Zygophyllaceae		
- *Peganum harmala*	Harmel	Seeds

* According to Bedevian (1994).

Preparation of extracts:

Cold water extracts (CWE), hot water extracts (HWE) and extraction by organic solvents, *i.e.* ethanol extracts (EE) and chloroform extracts (CE), were prepared according to the method adopted by Okeke *et al.* (2001). The water extract was sterilized through 0.2 µm porosity millipore filter (Gelman Sciences Inc.) then, kept in a refrigerator for further studies.

In order to study the effect of different extracts on the growth of *E. amylovora,* 6 mm sterile filter paper discs were aseptically dipped in each of plant extracts and allowed to dry at room temperature . Then, agar diffusion method was used by filter paper discs (Ruddock *et al.,* 2005). Three replicates were employed for each treatment, sterilized distilled water discs or solvent discs served as negative control. The plates were incubated at 28°C for 48 hr and the inhibition zone diameter was measured.

In vitro screening:

1- Effect of different concentrations of plant extracts on E. amylovora:

The inhibitory effect of eighteen plant extracts at three concentrations (20, 30 and 45 µl/disc) was evaluated by using the filter paper discs method (Ruddock *et al.*, 2005). The inhibition zone diameter was measured around the discs.

2- Extraction of alkaloids from harmel (Peganum harmala):

Extraction of alkaloid was prepared as described by Hasenratz (1927).

3- Extraction of sulfur compounds from garlic cloves:

Extraction of sulfur compounds from garlic cloves was prepared according to the method described by Reza *et al.* (2006).

4- Effect of alkaloids, thymol and sulfur compounds on the growth of the pathogens:

Alkaloids extracted from harmel , thymol from thyme were kindly provided by Prof. Fathy M.A. Soliman; Pharmacognosy Dept., Fac. Pharmacy, Cairo Univ. and prepared sulfur compounds from garlic were tested for their effect at different concentrations on the growth of the pathogen using filter paper disc method as mentioned before .

Sensitivity test of different isolates of E. amylovora to streptomycin sulfate:

During the preliminary screening streptomycin sulfate for its effect on growth of the pathogen by streaking onto King's B agar medium with different concentrations of the antibiotic at 100, 75, 50, 25 and 12.5ppm, colonies which can grow at 50 ppm were selected as streptomycin resistant isolates and streaked onto King's B agar medium without antibiotic. On the other hand, the sensitivit isolates were streaked onto this medium as control.

Effect of plant extracts on the growth of resistant isolates:

The inhibitory effect of five plant extracts at two concentrations (15% and 30%) was evaluated by using the filter paper discs method (Ruddock *et al.*, 2005).

In vivo screening:

The effect of plant extracts on the incidence of blight on immature pear fruitlets:

Pear fruitlets were surface sterilized by dipping in sodium hypochlorite (0.5%) for 5 min and then rinsed several times in sterilized distilled water. The fruitlets were pricked using a sterile needle, for both plant extract and active ingredients from harmel, 3 fruitlets were soaked for 5 min and then sprayed with *E. amylovora* suspension (10^8 cfu/ml) of 24 hr-old culture. Another set of pear fruitlets was sprayed with sterilized distilled water only as a negative control and another control check fruitlets were sprayed with the bacterial suspension only as a positive control. The fruitlets were distributed in sterilized plates lined with moistened paper towels. Plates were incubated at 28°C for 4 days, the effect on fruitlets was recorded as the area of necrotic around the wound and the amount of oozing.

For the resistant isolates, pear fruitlets were soaked for 5 min in both plant extract of garlic, madder, harmel at 30% and alkaloid, then sprayed with the streptomycin resistant (Str^r) isolates and sensitive (Str^s) isolates of *E. amylovora*

suspension (10^8 cfu/ml) of 24-hr-old culture. The positive control was sprayed with a suspension of the resistant isolate only. The negative control was sprayed with the tested extract and the incubation was made as mentioned before.

Effect of plant extract on protein profiles of streptomycin resistant isolates of E. amylovora:

Cellular proteins of two streptomycin resistant isolates, *i.e.* (Str^{r2} and Str^{r4}) and two streptomycin sensitive isolates (Str^{s1} and Str^{s2}), were analysed for protein profiles by SDS-PAGE method (Laemmli, 1970). Bacterial cells were grown in King's B broth medium (with plant extract 2%) and the cultures were incubated in a rotary shaker at 28°C for 48 hrs.

The bacterial cells were harvested by centrifugation at 8000 rpm for 20 min, then washed twice with sterile distilled water and re-centrifuged. Extraction of proteins from bacterial cells was prepared according to the method described by Hussein (1992).The cells were then sonicated at 80.000 Hz (Bandelin electronic - Gerate Typ - UW2200 - MS-73) for 5 min, the protein was extracted by centrifugation 18.000 rpm for 30 min.

Using bovine serum albumen as a standard protein, the protein content of the supernatant was estimated and each sample was adjusted to 2mg/ml according to the method of Bradford (1976).

Each supernatant was mixed with an equal volume of solution consisting of (by volume) 64% buffer (0.15 M Tris - HCl, pH 6.8), 20% glycerol, 6% SDS, 10% 2-mercaptoethanol and 0.1% bromophenol blue, before boiling in a water bath for 3 min. 20 µl aliquots (40µg protein) were subjected to electrophoresis in a 7.5% polyacrylamide gel prepared in 0.1% SDS with a 3.5% stacking gel (Laemmli, 1970). Electrophoresis was performed in a vertical slab mould (16x18x0.15 cm). Gels were stained with silver nitrate for the detection of protein bands (Sammons *et al.*, 1981).

Results and Discussion

Effect of plant extracts on growth of the pathogen:

Antimicrobial compounds are widely distributed among medicinal plants, but only a few have been evaluated for their activity against plant pathogenic microorganisms (Mongelli, 2003). In this investigation, *in vitro* studies showed that the plant extracts had variable effects on *E. amylovora* growth. Hot water extract was the most effective against the tested pathogens followed by ethanol extract, cold water and chloroform extracts for all tested plant extracts (Table 2). These results are in harmony with those reported by Krebs *et al.* (2006) and Abd-El-Khair and Haggag (2007). They reported that water extracts of medicinal plants reduced foliar blight of potato plants better than ethanol extracts. However, hot water extracts of gold warm wood (*Artemisia annua*), myrtle (*Myrtus communis*), warm wood (*Artemisia herba-alba Asso*), common rosemary (*Rosmarinus officinalis*) and Brazilian pepper tree (*Schinus terebinthifolia*) were not suppressive to *E. amylovora* growth, meanwhile Indian mallow (*Urena tenax*) chloroform extract was suppressive to *E. amylovora* growth. Harmel (*Peganum harmala*) and garden

Table 2. Inhibition zone (mm) of some plant extracts against *E. amylovora*

Tested plant extract	Fractions extracted in;			
	Chloroform	Ethanol	Hot water	Cold water
Garlic	0	0	0	20
Gold warm wood	0	0	0	0
Myrtle	0	0	0	0
Ribwort	0	0	20	14
Warm wood	0	0	0	0
Harmel	29	9	10	15
Madder	4	9	9	0
Common Rosemary	0	0	0	0
Garden sage	0	0	0	24
Brazilian Pepper tree	0	0	0	0
Garden thyme	4	14	19	9
Indian mallow	9	0	0	0

thyme (*Thymus vulgaris*) and madder (*Rubia tinctorum*) extracts have bactericidal substances that inhibit the growth of the pathogen. Ribwort cold and hot water extracts were only suppressive to the growth of *E. amylovora*, while chloroform and ethanol extracts were not.

Also extracts of warm wood, St. Mary's flower, white mustard, lemon grass peppermint, black cumin and common rue were non suppressive to the growth of *E. amylovora*.

The effect of different concentrations of extracts on inhibiting the growth of *E. amylovora* was showed in Table (3) in solid medium.

Table 3. Effect of different plant extract concentrations on *E. amylovora*

Tested plant extract	Inhibition zone (mm) in response to: *		
	20	30 (µl/disc)	45
Garlic	5	9	15
Gold warm wood	0	0	0
Myrtle	0	0	0
Ribwort	0	0	6
Harmel	5	8	10
Madder	0	0	8
Common Rosemary	0	0	0
Garden sage	0	5	10
Brazilian Pepper tree	0	0	0
Garden thyme	0	3	8
Indian mallow	0	0	3

* Zone diameter not includes the disc diameter.
Check discs, treated with solvent or water only, invariably gave negative results.

Data show that madder, ribwort, harmel, garlic, thyme and garden sage extracts were more suppressive to *E. amylovora*. This result is due to that roots of madder plant contained Alizarin a water soluble glycoside (anthraquinone) and mutagenic compounds (lucidine rubiadine) the main biologically active components of madder extract (Goverdina and Teris, 2002 and Shigeharu *et al.*, 2006). Ribwort contains antimicrobial and anti-inflammatory active compounds such as iridoid glycosides (mucilage) and an alkaloid which is soluble in water (Golvez *et al.*, 2005).

In the present study, garden sage, thyme, garlic and harmel extracts showed antimicrobial activity for *E .amylovora*. These results are in agreement with Essawi and Srour (2000), Kyung and Lee (2001); Carvalho *et al.* (2005) and Vimal-Kumar *et al.* (2005) who found that the aqueous extracts were more effective against some human pathogenic bacteria compared to the antibiotic gentamicin. For garden sage extract, the main biologically active component is terpenoids and camphor, while the active component in thyme extract is thymol (Laura *et al.*, 2002).

Effect of purified active ingredient of thyme, garlic and harmel on the growth of the pathogens:

Data in Table (4) show that all the thymol concentrations were able to inhibit the growth of *E. amylovora*, however, the results revealed that the concentration of sulfur compounds from garlic were able to inhibit the growth of *E. amylovora.* This might be due to allicin the main biologically active component against *E. coli* and *P. aeruginosa* as shown by Kyung and Lee (2001). The mode of action of allicin is known to inhibit lipid biosynthesis and RNA synthesis (Feldberg *et al.*, 1988). Also, by decreasing the oxygen uptake and thus damage membranes in the cells of the pathogen (Taran *et al.*, 2006).

For thymol, the mode of action is believed to be antiplasmid activity against *E. coli* (Schelz *et al.*, 2006).

Table 4. Suppressive effect of sulfur compounds (from garlic) and thymol (from thyme) on the growth of *E. amylovora*

Concentration	Inhibition zone (mm) of *E. amylovora*	
	Sulfur	Thymol
0.15%	2	2
0.3%	4	4
0.6%	5	6

Data in Table (5) show that alkaloids (harmalin and harmine) 45µl/disc was the most effective for inhibiting the growth of *E. amylovora*.

Alkaloids from harmel plants had antimicrobial activity and mutagenic effect to Str^s *Salmonella typhimurium* (Alkofahi *et al.*, 1990). This fact agrees with the *in vivo* results obtained on *E. amylovora* in this study. Sobhani *et al.* (2002) reported that each gram of dried extract contained 55.5 and 79.0mg of harmine and harmalin, respectively.

Table 5. Effect of alkaloids (harmalin + harmine) from harmel on the growth of *E. amylovora*

Concentration of alkaloids (µl/disc)	*E. amylovora* inhibition zone (mm)
15	5
30	10
45	15

Effect of plant extracts on the growth of streptomycin resistant isolates of E. amylovora:

Resistant isolates (Nos. 2, 3, 4, 6, 8, 9 and 10) were selected from cultures tolerant to 50 µg /ml streptomycin

Data in Table (6) show that five plant extracts of madder (HWE), garlic (CWE), thyme (HWE), harmel (CWE), and garden sage (CWE) had variable effect on the growth of the resistant isolates, where, cold water extract of garlic at 15% and 30% decreased the growth of all isolates, garden sage extract had no effect on the growth of all resistant isolates.

Madder, thyme and harmel extracts inhibited the growth of most isolates at 30%, while at 15%, no effect of any extract was recorded.

Table 6. Effect of water plant extracts on the growth of streptomycin resistant isolates of *E. amylovora*

Plant extracts	Concentration (%)	Inhibition zone (mm)							
		Streptomycin resistant isolates							Cont. isolate Str^{s5}
		2	3	4	6	8	9	10	
Madder	15	0	0	0	0	0	0	0	10
	30	0	12	8	10	0	0	0	15
Garlic	15	0	0	12	10	10	9	10	15
	30	0	15	22	20	20	15	20	25
Thyme	15	0	0	0	0	0	0	0	12
	30	0	0	6	0	0	4	0	15
Harmel	15	0	0	0	0	0	0	0	10
	30	0	10	0	10	0	10	12	17
Garden Sage	15	0	0	0	0	0	0	0	10
	30	0	0	0	0	0	0	0	13

In vivo screening:

The effect of plant extracts on disease development by E. amylovora on immature pear fruitlets:

Data in Table (7) illustrate that the aqueous extracts from harmel, madder, garden thyme and alkaloids (0.15%) had inhibited disease development as no necrosis or oozing on the immature fruit of pear. These results agree with those

reported by Krebs *et al.* (2006) and Abd-El-Khair and Haggag (2007). They mentioned that the water extracts of medicinal plants reduced foliar blight of potato plants better than ethanol extracts. Also, with aqueous extracts the environment pollution with solvents (ethanol or chloroform) during the field application is reduced. The aqueous extract of garden sage and ribwort gave slight oozing and necrosis, while garlic and Indian mallow had no effect on disease incidence where they gave copious oozing and necrosis.

Table 7. Effect of plant extracts on the incidence of necrosis and oozing of pear fruitlets infected with sensitive isolates of *E. amylovora*

Tested plant extract	Necrosis *	Oozing	Control
Garlic	+++	+	-
Harmel	-	-	-
Ribwort	+	+	-
Madder	-	-	-
Garden sage	+	+	-
Garden thyme	-	-	-
Indian mallow	+++	++	-
alkaloids (0.15%)	-	-	-

* (-)= No necrosis - No oozing; (+) = weak, slight oozing and necrosis; (++) = Moderate oozing and necrosis; (+++) = Copious oozing and severe necrosis.

Data in Table (8) show that hot water plant extracts from madder, cold water extract of harmel and harmalin has prevented disease development as no symptoms of necrosis or oozing on immature fruitlets of pear were noticed for streptomycin resistant isolates Nos. 3, 6, 8, 9 and 10, but the isolate No. 4 gave no necrosis and no oozing when pear fruitlets were treated with madder extract compared with isolate No.2 which gave moderate necrosis and weak oozing. On the other hand, pear fruitlets treated with harmel extract and inoculated with isolate No. 2 produce oozing with necrotic tissues, while isolate No. 4 gave weak reaction.

For garlic extract, the isolates that produced slight oozing and necrosis on immature fruitlets of pear were Nos. 2, 6, 9 and 10, but Nos. 3, 4 and 8 gave no symptoms.

Protein banding pattern:

The electrophoregram (protein profiles or fingerprints) of total cellular protein of *E. amylovora* resistant (R) and sensitive (S) isolates revealed discrete protein bands. The protein profiles of isolates Str^{r2} (Harmel), Str^{r4}(H), Str^{s1}(H), Str^{s2}(H), Str^{r2} (Madder), Str^{r4} (M), Str^{s1} (M), Str^{s2}(M) (Fig. 1) contains 7, 14, 9, 5, 16, 14, 22 and 14 bands, respectively, compared with protein patterns from Str^{r2}(control check isolates), Str^{r4}(C), Str^{s1}(C), Str^{s2}(C) isolates especially in the high molecular weight parts of the gel.

Protein pattern and data in Table (9) reveal that there are four common protein bands in all resistant and sensitive isolates of *E. amylovora* (120, 50, 30 and 20 KDa). Therefore, genes synthesizing these proteins might be related to virulence of these isolates.

Table 8. Effect of some plant extracts on the infection of streptomycin resistant isolates on pear fruitlets and fire blight symptoms development

Tested plant extract *	Streptomycin resistant isolate															
	2		3		4		6		8		9		10		Str^s *** (Check)	
	Necrosis **	Oozing	Necrosis	Oozing	Necrosis	Oozing	Necrosis	Oozing	Necrosis	Oozing	Necrosis	Oozing	Necrosis	Oozing	Necrosis	Oozing
Garlic	+	++	-	-	-	-	++	++	-	-	+	-	+	+	3+	++
Madder	++	+	-	-	-	-	+	-	-	-	+	-	-	-	-	-
Harmel	+	++	-	-	+	-	-	-	-	-	-	-	-	-	-	-
Harmine + Harmalin	++	-	-	-	+	+	-	-	-	-	-	-	+	-	-	-
Control with bacteria	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+
Control without bacteria	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

* Concentration of plant extracts was 30% of the crude extract.

** As mentioned in footnote of Table (7).

*** Str^s = Streptomycin sensitive isolates.

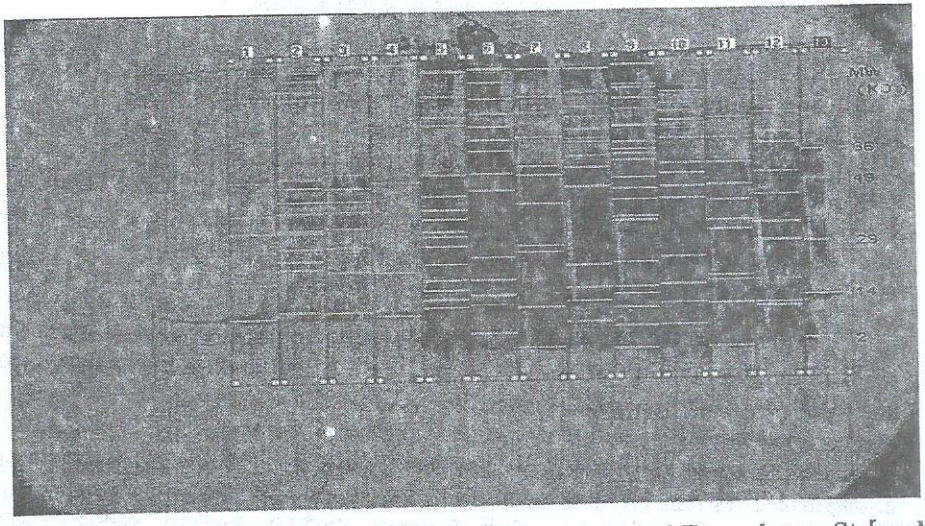

Fig 1. Electrophoretic patterns of total cellular protein of *E. amylovora* Str^r and Str^s isolates. Protein bands are identified from top to bottom. Lane 1 isolate Str^{r2} (with harmel), Lane 2 isolate Str^{r4} (with harmel), Lane 3 isolate Str^{s1} (with harmel), Lane 4 isolate Str^{s2} (with harmel) ,Lane5 isolate Str^{r2} (with madder), Lane 6 isolate Str^{r4} (with madder), Lane 7 isolate Str^{s1} (with madder), Lane 8 isolate Str^{s2} (with madder), Lane 9 isolate Str^{r2} (without extract), Lane 10 isolate Str^{r4} (without extract), Lane 11 isolate Str^{s1} (without extract), Lane 12 isolate Str^{s2} (without extract) and Lane 13 Marker .

Table 9. Molecular weight for the protein bands

M.W. Less than (KDa)	Str^{r2}	Str^{r4}	Str^{s1}	Str^{s2}	Str^{r2}	Str^{r4}	Str^{s1}	Str^{s2}	Str^{r2}	Str^{r4}	Str^{s1}	Str^{s2}
	Harmel extract				Madder extract				Control (without extract)			
120	113	114	117	117	113	115	114	114	116	119	119	—
110	—	109	—	—	—	107	—	101	105	—	—	106
100	98	98	—	—	99	97	99	92	97	94	—	—
90	—	—	—	—	87	89	89	86	81	89	85	87
80	—	—	—	—	—	73	75	79	71	73	73	76
70	—	—	—	—	—	67	—	63	67	—	—	70
60	—	52	54	—	53	54	58	57	60	58	58	53
50	50	48	47	49	44	46	42	47	46	47	47	44
40	38	39	40	36	40	—	—	—	37	—	35	34
30	—	28	28	21	28	25	28	22	23	24	—	29
20	18	—	—	—	19	19	11	16	16	17	18	18
10	9	10	10	9	—	—	—	7	10	6	—	10
5	—	—	—	—	—	4	—	—	5	—	—	—

On the other hand, there was one band with molecular weight of 98 KDa present in the two resistant isolates, but was absent in the two sensitive isolates. These results indicate that protein bands of 98 KDa can be used for the identification of streptomycin resistant *E. amylovora* isolates.

In conclusion, the data presented here clearly show that garden thyme extract was the better effective on the pathogen *in vivo*. On the other hand, harmel and madder extracts were most suppressive to *E. amylovora* in *vitro* and *in vivo*. For streptomycin resistant isolates of *E. amylovora*, the garlic, harmel and madder extracts had the best effect and could be used as an alternative to the antibiotic (streptomycin) to control fire blight of pear.

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References

Abo El-Dahab, M.K.; El-Goorani, M.A.; Kasheir, H.M. and Shoeib, A.A. 1983. Severe outbreaks of pear fire blight in Egypt during 1982 and 1983. *Phytopathol. Medit.*, **22**: 168-170.

Abd-El-Khair, H. and Haggag, Wafaa M. 2007. Application of some Egyptian medicinal plant extracts against potato late and early blights. *Res. J. Agric. & Biol. Sci.*, **3** (3): 166-175.

Adwan, G.; Abu-Shanab, B.; Adwan, K. and Abu-Shanab, F. 2006. Antibacterial effects of nutraceutical plants growing in Palestine on *Pseudomonas aeruginosa*. *Turkish J. Biol.*, **30** (4): 239-242.

Alkofahi, A.S.; Abdelaziz, A.; Mahmoud, I.; Abuirjie, M.; Hunaiti, A. and El-Oqla, A. 1990. Cytotoxicity, mutagenicity and antimicrobial activity of forty Jordanian medicinal plants. *Internat. J. Cru. Dru. Res.*, **28** (2): 139-144.

Aram, F.; Bonjar, G.H.S.; Mahdavi, M.J.; Mansouri, S.; Farrokhi, P.R.; Pour, N.B.H.; Shekari, M.; Ghasemi, Aand Aghighi, S. 2006. Anti-streptomyces activity of *Myrtus communis* and *Terminalia chebula*, medicinal plants of South east regions of Iran. *J. Appl. Sci.*, **6** (3): 654-656.

Aysan, Y. and Yildiz, N. 2000. Effect of plant extracts on tomato stem necrosis. *Bulletin – OILB/SROP*, **23** (1): 59-62.

Bedevian, A.K. 1994. *Illustrated Polyglottic Dictionary of Plant Names*. 2nd Ed. Medbouly Library, Talaat Harb Square, Cairo, Egypt.

Bradford, M.M. 1976. Arapid and sensitive method for the quantification of protein utilizing the principle of protein–dye binding. *Anal. Biochem.*, **72**: 248-254.

Carvalho, H.H.C.; Cruz, F.T. and Wiest, J.M. 2005. Antibacterial activity of plants with spices ethnographic indicative in Porto Alleger, RS, Brazil. Revista-Brasileira-de-plantas. *Medicinais*, **7** (3): 25-32.

Cordeiro, C.H.G.; Sacramento, L.V.S.; Correa, M.A.; Pizzolitto, A. and Cand Bauab, T.M. 2006. Herbal extracts in an experimental mouth wash: pharmacognostics analysis and antibacterial activity. *Revista Brasileira de Ciencia Farmaceuticas*, **42** (3): 395-404.

Czapska, A.; Baasinska, B. and Szczawinski, J. 2006. Antimicrobial and antioxidant properties of aqueous extracts from selected spice plants. *Medycyna. Weterynaryjna*, **62** (3): 302-305.

El-Helaly, A.F.; Abo-El-Dahab, M.K. and El-Goorani, M.A. 1964. The occurrence of the fire blight disease of pear in Egypt. *Phytopathol. Medit.*, **3**: 156-163.

Essawi, T. and Srour, M. 2000. Screening of some Palestinian medicinal plants for antibacterial activity. *J. Ethno.*, **70** (3): 343-349.

Feldberg, R.S.; Chang, S.C.; Kotik, A.N.; Nadler, M.; Neuwirth, Z.; Sundetrom, D.C. and Thompson, N.H. 1988. *In vitro* mechanisms of inhibition of bacterial cell growth by Allicin. *Antimicrob. Agents Chemother.*, **32**: 1763-1768.

Golvez, M.; Martin-Cordero, C.; Peter, J.H. and Ayuso, J. Maria. 2005. Antioxidant activity of methanol extracts obtained from *Plantago* species. *J. Agric. Food. Chem.*, **53**: 1927 -1933.

Goverdina, C.H.D. and Teris, A.V. 2002. Studies in natural product chemistry. *Bio. Nat. Prod.*, **26**: 629-684.

Hasenratz, M. 1927. Isolation of harmine and harmalin. *Ann. Chem.*, **10** (7): 151.

Hayder, N.; Kilani, S.; Abdelwahed, A.; Mahmoud, A.; Maftahi, K.; Chibani, J.B.; Ghedira, k. and Chekir-Ghedira, L. 2003. Antimutagenic activity of aqueous extracts and essential oil isolated form *Myrtus communis*. *Pharmazie*, **58** (7): 523-524.

Hussein, E.M. 1992. Biochemical and serological studies for determining susceptibility of cotton cultivars to *Fusarium oxysporum* f.sp. *vasinfectum* (in Russian). Ph.D. Thesis. All-Union Institute of Plant protection. Leningrad, USSR.

Kalyoncu, F.; Cetin, B. and Saglam, H. 2006. Antimicrobial activity of common madder (*Rubia tinctorum*.L). *Phytotherapy Res.*, **20** (6): 490-492.

Krebs, H.; Dorn, B. and Forrer, H.R. 2006. Control of late blight of potato with medicinal plant suspensions. *Agrarforschung*, **13**: 16-21.

Kulataeva, A.K.; Pak, R.N.; Ermekbaeva, B.A. and Adekenov, S.M. 2006. Antimicrobial and regenerating properties of medicinal plants ethanol extracts and essential oils phytocomposition. *Rastitel-Nye-Resursy*, **42** (2): 102-109.

Kyung, K.H. and Lee, Y.C. 2001. Antimicrobial activities of sulfur compounds derived from S-alk(en)yl-L-cysteine sulfoxides in *Allium* and *Brassica*. *Food Rev. Inter.*, **17**(2): 183-198.

Laemmli, U.K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, **227**: 680 -685.

Laura, G.; Daniele, F. and Donata, R. 2002. The *in vitro* action of essential oils on different organisms. *J. Essent. Oil Res.*, **14**: 312-318.

Mehrabian, S.; Majd, A. and Majd, I. 2000. Antimicrobial effects of three plants (*Rubia tinctorum, Carthamus tinctorum* and *Juglans regia*) on some airborne microorganisms. *Aerobiologia*, **16** (3-4): 455-458.

Mongelli, E. 2003. Recent advances in the search for antimycotics activity in South American plants. Pages: 197-208. In: *Plant-Derived Antimycotics Current Trends and Future Prospects*. Mahendra Rai and Donatella Mares (eds.). Food Products Press, 10 Alice St., Binghamton, NY, USA.

Okeke, M.I.; Iroegbu, C.U.; Eze, E.N.; Okoli, A.S. and Esimone, C.O. 2001. Evaluation of extracts of the root of *Landolphia owerrience* for antibacterial activity. *J. Ethno.*,**78**: 119-127.

Reza, S.; Morteza, S. and Zuhair, M.H. 2006. Studies on the antimicrobial effect of Allicin on the intra macrophages *Brucella*. *Pakistan J. Biolo. Sci.*, **9** (10): 1935-1939.

Ruddock, P.S.; Liao-Ming M.; Foster, B.C.; Lawson, L.; Arnason, J.T. and Dillon, J.A.R. 2005. Garlic natural health products exhibit variable constituent levels and antimicrobial activity against *Neisseria gonorrhoeae, Staphylococcus aureus* and *Enterococcus faecalis*. *Phytotherapy Res.*, **19** (4): 327-334.

Sammons, D.W.; Adams, L.D. and Nishizawa, E.E. 1981. Ultrasensitive silver based colour staining of polypeptides in polyacrylamide gels. *Electrophoresis*, **2**: 135.

Schelz, Z.; Molnar, J. and Hohmann, J. 2006. Antimicrobial and antiplasmid activities of essential oils. *Fitoterapia*, **77** (4): 279-285.

Shigeharu, I.; Katsuhisa, U.; Toshio, T.; Hideyo, Y. and Shigeru, A. 2006. Evaluation of the effect of terpenoid quinines on *Trichophyton mentagrophytes* by solution and vapour contact. *J. Infe. and Chemother.*, **12** (2): 100-104.

Sobhani, A.M.; Sultan, A.E. and Massoud, M. 2002. An *in vitro* evaluation of human DNA toposomerase I inhibition by *Peganum harmala* L. seeds extract and its beta-carboline alkaloids. *J. Pharm. and Pharmace. Sci.*, **5** (1): 19-23.

Taran, M.; Rezaeian, M. and Izaddoost, M. 2006. *In vitro* antitrichomonas activity of *Allium hirtifolium* (Persian shallot) in comparison with Metronidazole. *Iran J. Publ. Health*, **35** (1): 92-94.

Tawfik, A.E.; Abou-zeid, N.M. and Hassanein A.M. 2000. Using "BIOLOG" microplate for the identification of *Erwinia amylovora* the causal agent of pear fire blight in Egypt. *Bull. Fac. Agric., Cairo Univ.*, **51**: 107-122.

Terrie, B.A. 2002. Australian naturopathic network. www.healthwise.au.com.

Vimal-Kumar; Tejal-Shah; Parmar, N.S.; Shah, G.B. and Goyal, R.K. 2005. Antimicrobial activity of *Peganum harmala*. *Indian J. Nat. Prod.*, **21** (2): 24 -26.

Wood, M.; Schollenberger, M. and Zamorski, G. 1997. Effects of extracts of four medicinal plants on growth of selected fungi and bacteria. *Annals of Warsaw Agricultural University-SGGW, Horticulture*, 18: 41-52.

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تأثير بعض المستخلصات النباتية على نمو عزلات الايروينيا أميلوفورا المقاومة والحساسة للاستربتوميسين

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يعتبر مرض اللفحة النارية فى الكمثرى المتسبب عن البكتريا ايرونيا اميلفورا من أهم الامراض التى تسبب خسائر بالنسبة للكمثرى.

فى هذه الدراسة تم اختبار مستخلص الايثانول والكلوروفورم والماء البارد والماء الساخن لعدد ١٨٪ نبات طبى وعطرى بتركيز ١٥٪ على البكتريا المسببة للفحة النارية فى الكمثرى.

وأظهرت النتائج ان مستخلصات نباتات الثوم والحرمل والفوه والزعتر والمريمية يثبط نمو البكتريا ايرونيا اميلوفورا فى المعمل ، ولكن مستخلصات الفوه والحرمل والزعتر كانت هى الا كثر تاثيرا فى اختبارات الصوبة.

تم دراسة تاثير بعض المستخلصات (الفوه – الثوم – الزعتر – الحرمل – المريمية) على تثبيط نمو ٧ عزلات من الأيرونيا اميلوفورا المقاومة للمضاد الحيوى سلفات الأستربتوميسين واظهرت النتائج ان مستخلصات الثوم والفوه والحرمل كانت اكثر تاثيرا على بعض العزلات .

ايضا تم استخلاص المادة الفعالة من الحرمل والثوم و اختبر تاثيرهم على نمو الميكروب.

ومن تحليل البروتين الخلوى أظهرت النتائج وجود بعض البروتينات فى العزلات الحساسة و المقاومة للمضاد الحيوى فى حالة الكنترول بدون مستخلص أو التى عوملت بمستخلص الفوه ، فى حين ان هذه البروتينات كانت غائبة عندما عوملت هذه العزلات بمستخلص الحرمل. ايضا مستخلص الحرمل ثبط تخليق بعض البروتينات التى لها وزن جزئى منخفض فى العزلات الحساسة و المقاومة. مستخلص الفوه نشط انتاج بعض البروتينات فى العزلات الحساسة و ثبط تخليق بعض البروتينات الاخرى فى نفس العزلات و مع ذلك ثبط انتاج بعض البروتينات التى لها وزن جزئى منخفض فى العزلات المقاومة.