

EFFICIENCY OF SOME NATURAL OILS FOR CONTROLLING CROWN GALL DISEASE CAUSED BY *AGROBACTERIUM TUMEFACIENS*

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ABSTRACT

The antibacterial activity of oils of aloes, aniseed, carnation, caraway, fennel, garlic, lavender, nigella, onion and thyme on *Agrobacterium tumefaciens* the causal organism of the crown gall disease *in vitro* and in greenhouse conditions were evaluated. *In vitro*, all used oils had slight or no inhibitory effect at low concentrations. (0.25 and 0.5%). Some oils i.e. nigella, onion, aniseed and aloes behaved the same behaviour till in high concs. (1 and 2%). vice versa at concs. 1 and 2 % thyme oil was the most effective one followed by carnation, lavender and garlic, respectively. Similar trend was also present regarding the greenhouse experiments, it revealed that apricot seedlings soaking for twenty minutes in 2 % conc. of oils which exhibited highest inhibitory effect, recorded significant reducing in the percentage of disease incidence and decreased gall formation. It is interest to mention that, these used oils promoted the growth of apricot seedlings comparing with the check treatment.

Key words: Crown gall, *Agrobacterium tumefaciens*, Natural oils, Apricot seedlings

INTRODUCTION

The crown gall disease caused by *Agrobacterium tumefaciens* (Smith and Townsend, 1907) & (Conn, 1942) is one of the most important disease all over the world. The pathogen is a soil bacterium with world wide distribution (Demanche *et al* 2001). It is classified as tumorigenes due to its ability to cause neoplastic disease in dicotyledonous plants and makes economic losses (Sobiczewski, 2001 & Escobar *et al* 2002).

Crown gall is considered one of the important nursery root stock diseases in Egypt, affecting in particular, stone fruit, apple and pear trees causing economic losses (Taha *et al* 1975). The disease also spread in many countries such as Poland (Sobiczewski, 2001), Algeria, Jordan, Morocco and Tunisia (Benjama *et al* 2002).

All bactericides used now have harmful effects on the environments so, an alternative natural substance was attracted wide interest and which have an-

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tibacterial properties (Nagaraj *et al* 2001 & Vanneste and Boyd, 2002). Some of these substances include garlic essential oil (Qiao *et al* 2001) and thyme and lavender essential oil (Daferera *et al* 2003). The most recent information that relates to some oils of plant are widely used as antibacterial agents in controlling plant diseases with less effect on environments (Hassanein and Desheesh 1998). The advantage of these natural substance as antibacterial agents present in two main approaches, firstly the safety to people and environments and secondly no risk for development of mutants by pathogenic microorganisms. Several suggestions have been proposed for the mechanisms by which natural oils inhibit pathogenic organisms (Hassanein and El-Doksch, 1997 & Daferera *et al* 2003).

The present investigation was initiated to determine effect of some natural oils on the growth of *A. tumefaciens* and the control of crown gall disease on apricot seedlings in greenhouse.

MATERIAL AND METHODS

Extraction of plant materials

Ten natural oils named were aloe (*Aloe barbadensis*), aniseed (*Pimpinella anisum*), carnation (*Eugenis caryophyllata*), caraway (*Carum carvi*), fennel (*Foeniculum vulgare*), garlic (*Allium sativum*), lavender (*Lavandula latifolia*), nigella (*Nigella sativa*), onion (*Allium cepae*), and thyme (*Thymus vulgaris*) were used. Oils were extracted according to the methods adopted by Meisner *et al* 1981 in the following way: Grounded fine powder of dried plant was soaked with ethanol for three days. The ethanol extracts were filtered and concentrated by

rotary evaporator at 40°C to 100ml. The concentrate was partitioned in petroleum ether. The petroleum ether extracts were concentrated to obtain the oil.

Bacterial culture of the causal organism

The isolate of the causal organism was performed from Kaha nursery (Kalu-bia govnrorate) according to Tawfik (Tawfik *et al* 1983) and the isolate AC12 of *Agrobacterium tumefaciens* obtained from Bacterial Diseases Department, Plant Pathology Res. instiute, Giza and grown in nutrient glucose broth medium for two days at 28°C. The growth was assayed turbidimetrically for 1.6×10^8 cfu/ml.

Effect of different concentrations of natural oils on the growth of tested bacterium on solid medium

Different natural oils previously mentioned were tested at four concentrations (0.25, 0.5, 1.0 and 2.0 %) by putting different volumes of it in a sterilized Petri plates, then NGA was poured in the plates. It were then gently agitated and after this mixture became solid, streaking from 2 days old culture were made. The plates were incubated for three days. Control plates containing no oils were made. Four replicates for each treatment were used.

Sensitivity of *A. tumefaciens* to different concentrations of natural oils in liquid medium

Different volumes of the oils were added to nutrient medium to study their effect on the rate of growth of *A. tumefa-*

ciens as follows: Eleven sets of tubes containing 9 ml of nutrient medium were inoculated with 1 ml from cell suspension of *A. tumefaciens*. The first set was served as control. The other ten sets were prepared with used oils at various concentrations i.e. 0.25, 0.50, 1.0 and 2.0 % of each oil. Four replicates were used for each treatment. After that all tubes were incubated at 28°C for three days. Then optical densities were recorded by using spectrophotometer.

Greenhouse experiments

Effect of seedlings treatments with natural oils on infection of apricot seedlings with *A. tumefaciens*

Twenty five cm pots containing 4kg soil each, were infested with *A. tumefaciens* using 20ml/pot of a bacterial suspension containing approximately 1.6×10^8 cells/ml. One plant per pot were planted in March, 2004 with one year old apricot seedlings (c.v. Kanino). The roots were wounded and dipped in 1.0 and 2.0% concentrations of carnation, lavender and thyme oils, for 10 and 20 minutes. Check seedlings were dipped in tap water. All treatments were designed in four replicates.

Effect of used oils on growth of apricot seedlings

Increase of stem height in cm. and number of shoots per plant were measured.

Disease assessment

After 70 days, the plants were up-rooted for tumour examination. The gall

inhibition percentages which were taken as a criterion of antibacterial effect of used oils, calculated as follows:

Gall inhibition percentage in weight (G.I.P) = $100 - (a/b \times 100)$.

Since: a = average weight of galls/g per plant for the treatment.

b = average weight of galls/g per plant for the control.

Reduction for number of galls % = $c-t/c \times 100$

Since: c = number of galls for the control.
t = number of galls for the treatment.

Completely randomized block design with four replicates was used for all experiments, then analysis of variance was carried out and the least significant difference was calculated at 0.05% level (Snedecor and Cochran, 1972).

RESULTS

Effect of used oils on the growth of the pathogen

Data in Table (1 and 2) showed that the inhibition effect was increased in the higher concentrations of oils, whereas all oils used had no inhibitory effect on the growth of *A. tumefaciens* at 0.25 or 0.50 %. Aloes, aniseed, nigella and onion had no effect at all concentration used however thyme oil showed 100 % efficiency. So, it was the most effective one in this respect, it was capable to inhibit the bacterial growth completely at 2% conc. followed by carnation, lavender and garlic oils which the efficiency being 91.30, 82.61 and 67.39 %, respectively.

Table 1. Reaction of *A. tumefaciens* towards different concentrations of certain natural oils

Plant oils	Concentrations %			
	0.25	0.50	1.0	2.0
Thyme	+	+	-	-
Carnation	+	+	-	-
Lavender	+	+	-	-
Garlic	+	+	+	-
Caraway	+	+	+	-
Fennel	+	+	+	-
Nigella	+	+	+	+
Onion	+	+	+	+
Aniseed	+	+	+	+
Aloes	+	+	+	+
Control	+	+	+	+

+ = Growth

- = No growth

Table 2. Effect of different extracts plant oils on growth of *A. tumefaciens* determined turbidimetrically

Plant oil	Concentrations %							
	0.25		0.5		1.0		2.0	
	O.D.	Eff. %	O.D.	Eff.%	O.D.	Eff.%	O.D.	Eff.%
Thyme	0.43	6.52	0.41	10.87	0.03	93.48	0.00	100.0
Carnation	0.43	6.52	0.42	8.69	0.07	84.78	0.04	91.30
Lavender	0.44	4.35	0.44	4.35	0.12	73.91	0.08	82.61
Garlic	0.45	2.17	0.44	4.35	0.41	10.87	0.15	67.39
Caraway	0.46	0.00	0.45	2.17	0.43	6.52	0.27	41.30
Fennel	0.45	2.17	0.45	2.17	0.45	2.17	0.35	23.91
Nigella	0.46	0.00	0.46	0.00	0.46	0.00	0.44	4.35
Onion	0.46	0.00	0.46	0.00	0.46	0.00	0.45	2.17
Aloes	0.46	0.00	0.46	0.00	0.46	0.00	0.46	0.00
Aniseed	0.46	0.00	0.46	0.00	0.46	0.00	0.46	0.00
Control	0.46	0.00	0.46	0.00	0.46	0.00	0.46	0.00
L.S.D.at 0.05	0.021		0.017		0.034		0.031	

O.D. = Optical density.

EFF. = Efficiency.

Effect of used oils on reducing gall formation on apricot plants

The results presented in Table (3) showed reduction of gall number, diameter and weight of the roots treated with oils before planting. Data also indicate that thyme oil at 2.0% conc. prevent bacterial infection better than 1.0% conc. Also, dipping for 20 minutes was better than 10 mins. At same conc. With respect to reaction of different used natural oils against *A. tumefaciens* infection, thyme and carnation oils were the most effective in reducing the incidence of the disease (100 and 83.08 % gall inhibition in weight, respectively) followed by lavender oil (55.39 %) at 2% conc. for 20 mins.

Effect of used oils on apricot seedlings growth

On the other hand, the incidence of crown gall was reflected on vegetative growth of apricot seedlings. Results in Tables (3 and 4) showed clear correlation between gall development and growth of apricot seedlings. The use of these oils increases the vegetative growth of seedlings. They promoted the growth comparing with the check.

DISCUSSION

Certain oils derived from medicinal and aromatic plants have been found to exhibit bactericidal actions against some important bacterial plant pathogens (Gurdip, 1999 and Isman, 2000). Therefore, laboratory tests were carried out to evaluate the antibacterial effect of certain

oils against *A. tumefaciens*. Results indicated that these oils varied in the strength and persistence of their antibacterial activities. All used oils had weak or no effect in the low used concs. i.e. 0.25 or 0.50%, it could possibly be due to the fact that since the volume of used oils was small, it dispersed unevenly through the medium. At higher concs., some of these oils exhibited efficacy against *A. tumefaciens* while others such as aloes and aniseed oils had no effect even at highest used conc. (2%). Similar result was previously demonstrated by Elgayyar *et al* (2001) who stated that aniseed oil wasn't inhibitory to bacteria. On the contrary, our obtained data shows that thyme oil significantly suppressed bacterial growth, it was the most effective one followed by carnation, lavender, garlic, caraway and fennel oils, respectively. This result confirm those of Hassanein and El-Dokschi, 1997 who found that thyme oil showed high antibacterial activity against *A. tumefaciens*. The antibacterial activity of these oils is attributed mainly to the toxicity of its major compounds. In this respect, Daferera *et al* 2003 concluded that thyme oil was characterized by presence of p-cymene, γ -terpinene, thymol and carvacrol. They added that lavender oil was characterized by the relatively high content of linalool and linalyl acetate. Outwork is in agreement with that reported by Avato *et al* 2000 and Qiao *et al* 2001, who decided that the garlic oil has inhibitory effect on bacteria.

The oils which showed promising activity against *A. tumefaciens* were further tested against apricot crown gall on potted plants. The oils of carnation, lavender and thyme, which showed promising activity against *A. tumefaciens*, were further tested against the disease.

Table 3. Effect of natural oils on gall formation of apricot seedlings

Plant oils	Concentrations %	Dipping periods (min.)	Gall number / plant	Reduction for number of gall %	Gall diameter (cm.)	Gall weight (gm.)	Inhibition %
Thyme	1	10	1	83.3	0.4	0.31	90.46
		20	1	83.3	0.4	0.26	92.00
	2	10	1	83.3	0.3	0.20	93.85
		20	0	100.0	0.3	00.0	100.00
Carnation	1	10	3	50.0	0.7	0.95	70.77
		20	2	66.7	0.9	0.83	74.46
	2	10	1	83.3	0.8	0.72	77.85
		20	2	66.7	0.7	0.55	83.08
Lavender	1	10	4	33.3	1.0	2.05	36.92
		20	2	66.7	1.3	1.83	43.69
	2	10	3	50.0	1.1	1.67	48.62
		20	2	66.7	1.4	1.45	55.39
Control			6	00.0	1.8	3.25	00.00
L.S.D. at 0.05		1.252			0.408	0.586	20.354

Table 4. Effect of natural oils on the vegetative growth of apricot seedlings

Plant oils	Concentrations %	Dipping periods (min.)	Number of shoots/ plant	Stem height (cm.)
Thyme	1	10	23	19.3
		20	22	18.0
	2	10	25	18.3
		20	25	20.1
Carnation	1	10	20	16.4
		20	22	16.0
	2	10	23	18.0
		20	21	17.1
Lavender	1	10	14	9.8
		20	16	11.9
	2	10	15	15.6
		20	21	15.1
Control			10	8.0
L.S.D. at 0.05%			5.504	7.498

The use of these oils as seedlings soaking was evaluated at two concs. (1.0 and 2.0%) each for two periods (10 and 20 min.), to determine the lowest conc. which gives the highest control disease. All used treatments were effective in reducing the disease compared with the control. This may be due to the fact that the toxic and lethal effects of some natural oils were related to presence of terpenes and phenols (Isman, 2000). Thyme and carnation oils proved to be the most effective oils against *A.tumefaciens* followed in a descending order by lavender oil. Whereas no galls appeared in plants treated with 2% conc. with thyme oil for 20 minutes, indicating a high efficacy against the pathogen. Thyme oil was mainly characterized by presence of carvacrol at 81 to 83%, p-cymene at 4.5 to 5%, Y-terpinene at 2.6 to 3.3 %, caryophyllene at 1.5 to 1.6% B-myrcene at 1.6% and linalool at 1.1 to 1.2%. (Arras and Usai, 2001).

It is of interest to mention that none of the used oils showed harmful effects on the growth of apricot seedlings. Furthermore, these oils promoted the growth of apricot seedlings. Based on the results of this study, it was concluded that some of the natural oils could be used against *A.tumefaciens* but further studies needed to be done to determine the active ingredients in these oils.

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مجلة بحريات العلوم الزراعية ، كلية الزراعة ، جامعة عين شمس ، القاهرة ، ٤٩م ، ع(٢) ، ٦٩٩ - ٧٠٧ ، ٢٠٠٤

التأثير المثبط لبعض الزيوت الطبيعية ضد بكتيريا أجروباكتيريوم تومفاشينز المسببة للتدرن التاجي

[٤٩]

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القرنفل ثم اللافندر فالثوم وذلك في كل من التركيزين ٢،١ % بحيث كان تركيز ٢% أقوى تأثيراً.

وأعطت تجارب الصوبة نتائج مشابهة لما تم الحصول عليه في المعمل حيث وضحت فاعلية كل من زيوت الزعتر والقرنفل واللافندر في مكافحة المرض وذلك عند غمس بادرات المشمش لمدة ٢٠ دقيقة في تركيز ٢ % واحدهت انخفاضاً معنوياً في نسبة الإصابة وأدت السى تقليل حدوث الأورام - وأيضاً أدى استخدام هذه الزيوت الطبيعية الى تشجيع نمو بادرات المشمش بالمقارنة بمعاملة الكنتترول وأدت لزيادة طول البادرات مع زيادة عدد الأفرخ .

قيمت عشرة زيوت نباتات وهي اللصبار، الينسون ، القرنفل ، الكراوية ، للشمر ، الثوم ،اللافندر، حبة البركة ، للبصل ، الزعتر من حيث تأثيرها المثبط للبكتيريا أجروباكتيريوم تومفاشينز المسببة لمرض التدرن التاجي وذلك معملياً وتحت ظروف الصوبة.

في التجارب المعملية - جميع هذه للزيوت كان لها تأثير ضعيف أو لم يكن لها تأثير مثبط على الإطلاق في التركيزات المنخفضة منها (٠,٥٠,٠٠,٢٥) % - اما في التركيزات العالية (٢,١ %) كان لزيوت نباتات حبة البركة والبصل والينسون والصلبار نفس السلوك بينما أظهر زيت للزعتر أعلى تأثير تثبيطي يليه زيوت نباتات

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