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STUDY OF PLANT DIFFUSATES WITH ANTIFUNGAL ACTIVITY FOR CONTROLLING EARLY BLIGHT DISEASE ON TOMATO PLANT

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ABSTRACT

This study was conducted in the Faculty of Agriculture Omer Al-Mukhtar University, El-Beido City-Libya during the period from 1998-1999 Plant diffusates were tested for their fungicidal action to control early blight disease of tomaot causing by *Altemaria solani* that was wide spread in this region. Aqueous diffusates from different parts of 21 plant species in a concentration of (10^{-1}, 10^{-2} and 10^{-3}) were tested on conidial germination, number of germ tubes and germ tube length of *Altemaria solani*. Results indicated that the diffusates of *Trigonella faenumgraecum, Allium sativum, Capsicum annaum, Thymus vulgaris* and *Thapsia garganica* inhibited completely spore germination at low concentration, while other diffusates had little effect on such phenomena. Tomato plants were sprayed under field condition by the best five plant diffusated in addition to control plants sprayed by water and other plants were sprayed by Rovral (0.15%) or Dithane M_{45} (0.25%) to test their efficacy on the disease incidence of early blight on three tomato cultivars. Results showed that diffusates of *Thapsia garganica*, Rivoral and Dithane M45 gaves the high level of protection and led to decrease disease incidence and increased plant yield. Results also indicated than no significant differences was observed between the other diffusates on the percentage of infection. Results indicated also that Dithance M_{45} was the best fungicide against early blight under field condition in reducing disease incidence and increased plant productivity.

INTRODUCTION

Early blight of tomato, caused by *Alternaria solani* (Ellis and Martin) Johnes and Grout, is one of the most economically important pathogens of tomato (*Lycopersicon esculentum* Mill.). Using of chemical fungicdes are successful for the control of many fungal disease such as early blight but led to development of fungicide resistance isolates, environmental pollution and take risk animal and human health. Recent researches concentrate on use of safety methods of disease control such as biological control and induced resistance by biotic and abiotic means (Lyon *et al* 1995) and used natural products.

Many workers showed the antifungal activity of some higher plants against plant pathogens (Reimers *et al* 1993; Hidalyo and Fernandez 2000; Qasem and Abu-Blam 1996, Dushyent and Bohra 1997, Montes Belmont and Garcia 1997, Wilson *et al* 1997; Özcan and Boyraz 2000; Unal *et al* 2001; Khallil (2001); Thiribhuvanamala *et al* (2001)).

In this study, the antifungal activity of some plant diffusates was studied against *A. solani* under laboratory condition and also under field conditions for controlling early blight of tomato. A comparison between two fungicides and plant diffusates for controlling such disease was also carried out.

MATERIALS AND METHODS

Plant diffusates :

Samples of different plants (Table 1) were collected and kept in a plastic bags under freezing condition (-20°C). As the plants were freezed they were withdown then left at room temperature to secret its diffusates. Diffusates were collected in sterilized test tube then filtered through Seitz filter for sterilization. Diffusates were diluted by sterilized distilled water to give concentrations of 10^{-1}, 10^{-2} and 10^{-3} (Wilson *et al* 1997).

Preparation of *A. solani* inoculum :

Three isolates of *Alternaria solani* were isolated from infected tomato plants collected from different location. Derna, El-Bedia and El-Marj in Al-Gabal Al-Khdar district. Isolates were purified on PDA using hyphal tip technique. Lima bean agar (LBA) was used for spore production. Isolates were grown for 10 days at 22°C under normal diurnal light conditions. Aerial mycelium was scraped and the culture were uncovered, inverted and placed in a diurnal light at ambient room temperature for 24 h to induce sporulation (Barksdate 1969). Identificat-ion of the fungus was based on morphological characters according to Ellis and Gibson (1975). Conidia were harvested with distilled water and filtered thorugh a double layer of muslin and adjusted to the desired concentration (2.5 X 10^5 spore/ml, using a haemacytometer.

Table 1. Plants used for their antifungal activity.

Botenical name	Common name	Extracted part
Allium sativum	Garlic	Cloves
Arbutus pavarii	Shamer	Leaves
Artemisia absinthium	Santonica	Leaves
Capsicum annuum	Red pepper	Flowers
Capsicum annuum	Green pepper	Flowers
Ceratina silique	Carbobtree	Leaves
Callistephus chinensis	Aster	Foliage
Eucalyptus camaldulensis	Bluegum	Leaves
Lupinus albus	Lupin	Seeds
Helichrysum stoechas	Helichrysum	Inflorescence
Matricricola chamomilla	German chamonelle	Inflorescence
Mentha piperita	Peppermint	Leaves
Mentha spicata	Spearmint	Leaves
Rosmarinus officinalls	Rosemary	Leaves
Solanim melongena	Eggplant	Leaves
Thapsiagar-ganica var. *sylphium*	Sylphium	Foliage
Thymus vulgaris	Thyme	Inflorescence
Trigonella foenumgraecum	Fenugreek	Germilings
Triticum vulgare	Wheat	Grain
Zea mays	Maize	Grain

Effect of plant diffusates on spore germination of *A. solani* :

Aqueous diffusates of twenty one plants were assessed at 10^{-1}, 10^{-2} and 10^{-3} concentrations table (Table 1) on conidial germination, germ tube number per each spore and germ tube length. One ml of a spore suspension

Effect of plant diffusates on spore germination of *A. solani* :

Aqueous diffusates of twenty one plants were assessed at 10^{-1}, 10^{-2} and 10^{-3} concentrations table (Table 1) on conidial germination, germ tube number per each spore and germ tube length. One ml of a spore suspension (2.5 X 10^5 spores/ml) was mixed with 4 ml of each sterile diffusated into glass watch plate and incubate at 25°C in a moist chamber for 20 h. In control treatment plant diffusates were replaced by distilled water. Mean germination percentage were determined from 40 microscopic field (10-20 conidia per field). The number of germ tubes of 40 conidia and their length were counted also. Before measurement spores were killed with FAA (Formalin : Acetic acid : Ethanol (5:5:90).

Pathogenicity tests :

Pathogenicity test was carried out on different tomato cvs transplants (*Lycoperscon esculentum* Mill) i.e. Rio-grand, Super-marmande, Ace-55, Midi-A, Red stone and Roma V.F. Transplants (four weeks old) were transplanted in 20 cm diameter plastic pots containing sandy-clay soil. After 4 weeks plants were sprayed with *A. solani* spore suspension 2.5 X 10^5 spore/ml. Plants were covered with polyethylene bags to maintain high humidity and placed at 20-24°C for 2 days. Fourty eight hours later the plastic bags were removed. Disease incidence was assessed visually using a 0-5 scale defined by **(Vakaloumakis 1991)** on plant leaves. The percentage of infection per leaf was calculated 10 days after inoculation. The assessment scale was 0 -no early blight, 1 = 1 to 9%, 2 = 10-24%, 3 = 24-49%, 4 = 50-74% and 5 = 75 to 100%. This experiment had five replicates for each treatment. Data were statistically analyzed by analysis of variance (ANOVA) followed by Duncan's multiple range test ($P \leq 0.05$).

Table (2):Plant diffusates tested under field conditions and their active ingredient.

Botenical name	Main component	Reference
Allium sativum	Allin, allicin, ajoene	Ayoub and Svendsen (1981) Reimers *et al* (1993)
Capsicum annuum	Capsaicidin (steroid saponin)	Gal (1967)
Thapsia garganica	Resin	French (1971)
Thymus vulgaris	Thymol, carvacrol, essence, eucalyptol, menthene, thymene tennin, resin	Jellin *et al* (2000) Lueng and Foster (1996)
Trigonella foenumgraecum	Trigonelline, hederagin glycoside	Dawider *et al* (1973)

Field trials :

A field experiment was conducted in Gamal Abd El-Naser Farm (Faculty of Agriculture, Omer El-Moukhtar University) during 1998 season. Three cultivars of tomato were used i.e Rio-grand, Midi-A and Supermarmande. Tomato transplants 5 weeks old were transplanted 30 cm apart in rows which were about 50 cm apart on each bed. Each row contained 10 plants. Eight treatments arranged in a complete randomize block design and each treatment comprised of three plots. Plants were sprayed by the five effective diffusates on fungal spore germination under Lab condition separately. Two fungicides i.e. Dithane M_{45} (0.25%) and Rovral

(0.15%) were also tested. Plants were left for natural infection. Spray by plant diffusted or fungicides was carried out three times at 10 days interval. Disease assessment started after the first symptoms become apparent and continued each 10 days. Tomato fruits of each treatment were harvested beginning when fruit turned light red to red color and continued the end the season and counted as total yield (kg/plot). By the end of experiment, plant height, fresh weight and dry weight were determined.

RESULTS

Pathogenicity tests :

Pathogenicity test was carried out by three distict isolates on six cvs. of tomato. Data are presented in Table (3).

Table (3):Pathogenicity of three isolates of *A. solani* on 6 cultivars tomato under green house condition.

Cultivars	Disease incidence [w]		
	Isolate 1[x]	Isolate 2[y]	Isolate 3[z]
Rio-grand	3.1[b]	2.2[c]	2.5[c]
Super-marmande	4.7[a]	2.75[ab]	0.95[d]
Ace-55	3.05[b]	2.85[ab]	0.85[d]
Midi-A	5.0[a]	3.8[b]	2.95[b]
Red stone	2.8[ab]	2.2[c]	2.0[c]
Roma VF	2.95[ab]	0.95[d]	0.95[d]

[w] Data means of ten replicates after 10 days of inoculation. Means followed by the same letter in each column are not significantly different (P≤0.05) by Duncan's multiple range test.
[x] Isolate from El-Bedia.
[y] Isolate from Derna.
[z] Isolate from El-Marj.
All treatments used as control non infected.

All tested isolates of *A. solani* were able to cause early blight symptoms, but isolates were varied significantly in their disease incidence. El-Bedia isolate was regarded as the most pathogenic one. On the other hand, isolate of El-Marj was the least pathogenic on all tested cvs. Derna isolate seemed to be more pathogenic than the El-Marj isolate. Data illustrated in Table (3) indicate that the tested cultivars greatly differed in their susceptibility to infection with *A. solani*. Roma VF Red stone and Ace-55 were the least susceptible to *A. solani*, whereas Rio-grand and Super-marmande were moderately susceptible. On the other hand, the Midi A was highly susceptible to all isolates of *A. solani*.

Laboratory experiments :

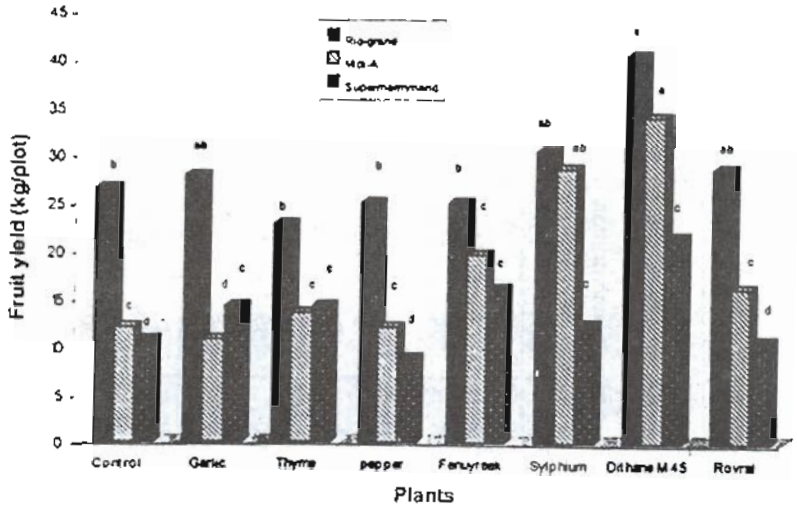
The effect of diffusated of different plants Table (4) in different concentrations i.e 10^{-1}, 10^{-2} and 10^{-3} on spore germination, number of germ tube/spore and germ tube length were studied. The results were tabulated in Table (4) revealed that the cloves of *Allium sativum*, flower and seeds of *Capsicum annuum* (Green pepper), foliage of *Thapsia garganica* var. *sylphium*, inflorescence of *Thymus vulgaris* and seed germilings of *Trigonella faenumgraecum* completely inhibited the spore germination at all tested

concentrations. Whereas *Ceratonia silique, Lupinus albus, Helichrysum stoechas* and *Maricricola chamomilla* reduced spore germination only at the higher concentration. Results obtained show different effects of plant diffusates on spore germination and showed that the higher concentration 10^{-1} greatly effective than 10^{-2} and 10^{-3} concentration. Most plant diffusates were less effective at lower concentrations. It is obvious that there are a correlation between increasing concentration and inhibition of spore germination.

Field experiment :

Results presented in Table (5) indicated that diffusate of *Thapsia garganica* gave high effectivity against the early blight disease compared with diffusate of *Thymus vulgaris, Tigonella foenumgraecum* and *Allium sativum*. The last three diffusates gave high level of protection against early blight. It was also noticed that Dithane M45 and Rivoral were effective fungicides for early blight under the field condition. They reduced disease incidence and increased yield of tomato plants.

Concerning plant productivity, it was observed that in Fig. (1) the three tested cvs varied in the productivity. In all trials cv Rio-grand gave the best productivity than the other tested cvs i.e. Midi-A and Supermarmande. Diffusates of sylphium and garlic gave significantly increased productivity than other tested diffusates. Dithane M_{45} gave increase of plant productivity for all tested cvs. All tested diffusates under field condition increased plant fresh weight, dry weigh and plant height Table (6).



(Fig 1) Fruit yield (kg/plot) of three tomato cultivars. treated with five different plant diffusates and two fungicides against *Alternaria solani*. Values are means of 10 replicates, column with the same letter do not differ significantly according to LSD ($p<0.05$).

Table (4): Effect of plant diffusates on spore germination, number germ tube and germ tube length of *A. solani*.

Plant diffusates	Spore germ concentration			% reduction	Number germ tube concentration*			% reduction	Germ tube length (µm)** concentration			% reduction
	10^{-1}	10^{-2}	10^{-3}		10^{-1}	10^{-2}	10^{-3}		10^{-1}	10^{-2}	10^{-3}	
Aster	95	100	100	3.9	2.0	1.9	2.1	45.9	42	69	114	-1.4
Bluegum	37.5	45	72.5	45.6	0.7	0.8	1.3	74.7	43	71	81	11.7
Carobtree	0	35.5	92.5	55	0	0.9	2.3	71.1	0	29	115	35
Egg plant	20	25	62.5	62.2	1.6	1.6	1.8	54.9	1	22	82	52.7
Fenugreek	0	0	0	100	0	0	0	100	0	0	0	100
Garlic	0	0	0	100	0	0	0	100	0	0	0	100
German chamonlle	0	15	90	63	0	0.4	0.9	79.2	0	23	90	49
Green pepper	0	0	0	100	0	0	0	100	0	0	0	100
Helichrysum	0	15	40	80.7	0	0.3	0.9	89	0	37	47	62
Lupin	0	0	90.	68.7	0	0	1.6	85.5	0	0	116	47.7
Maize	70	85	95	12.2	0.8	1.4	1.6	65.7	30	62	114	72
Peppermint	40	43	50	53.3	0.5	0.9	1.1	77.4	34	52	59	34.6
Red pepper	12.5	25	30	76.3	0.2	0.3	0.4	91.8	20	22	29	68
Rose mary	62.5	90	90	14.9	1.6	2.2	2.9	37.8	86	147	155	-74.7
Salphium	0	0	0	100	0	0	0	100	0	0	0	100
Salvia	90	92.5	92.5	3.5	1.7	1.9	2.2	47.7	41	70	141	-13.5
Santonica	13	48	63	56.5	0.2	1.5	1.8	68.4	17	82	99	10.8
Shamer	90	90	90	5.2	1.5	1.6	2.1	53.1	10	90	102	9.0
Spearmint	8	30	30	75.7	0.1	1.5	1.8	69.3	17	82	98	11.2
Thyme	0	0	0	100	0	0	0	100	0	0	0	100
Wheat	18	20	20	80.3	0.3	0.5	0.5	88.2	25	26	28	64.4
Water	95				3.7				222			

* Mean total count of germ tubes numbers.

** Mean total count of germ tubes length

$$\% \text{ reduction} = \frac{\text{Average of control} - \text{average of treatment}}{\text{Average of control (Water)}} \times 100$$

Table (5): Effect of five plant diffusates and two fungicides on early blight incidence on three cultivars under field conditions.

Treatment	Disease incidence											
	Rio-grand				Mid-A				Super-marmand			
	1/9	10/9	20/9	30/9	1/9	10/9	20/9	30/9	1/9	10/9	20/9	30/9
Allium sativum	0.8	1.7	3.5	3.9	0.4	0.9	2.4	3.0	0.5	0.6	1.4	3.2
Capsicum annuum	0.8	1.8	2.2	3.6	0.3	0.9	2.2	3.2	0.2	0.5	1.3	3.8
Thapsia garganica	0.7	1.5	2.2	2.2	0.1	0.3	0.7	1.3	0.3	1.7	2.1	2.7
Thymus vulgaris	0.4	1.1	3.3	3.9	0.2	0.5	1.2	2.7	0.6	0.7	1.1	3.3
Trigonella foenum-graecum	0.4	0.6	2.2	3.3	0.4	1.2	2.1	3.4	0.1	0.4	0.7	2.7
Dithane M45	0.7	1.2	2.2	2.5	0.1	0.5	1.1	1.3	0.5	0.9	2.1	3.2
Rivoral	0.0	0.2	1.1	2.2	0.1	0.3	1.1	3.2	0.4	0.5	1.3	2.1
Control	0.7	1.1	4.0	4.8	0.4	0.9	2.9	3.8	0.7	0.9	3.0	4.4

Assays were determined with 10 days intervals. Disease assissment started on 1st September 1998. Plant were sprayed three times started 10 days after transplanted. Based on scale of 0-5 with 5 being the highest Incidence of disease.

Table (6): Means of fresh, dry weight and length of tomato plant with plant diffusates and fungicides under field experiment.

Treatment	Cultivars								
	Rio-grand			Mid-A			Super-marmand		
	Fresh weight	Dry weight	Plant length	Fresh weight	Dry weight	Plant length	Fresh weight	Dry weight	Plant length
Allium sativum	407.8	144.7	109.7	557	162.68	138.8	162.8	480.03	96.6
Capsicum annuum	397.3	113.5	114.5	638	195.79	133.5	492.3	134.94	133.3
Thapsia garganica	379.29	138.06	112.3	502.9	140.33	135	354.2	85.93	105.1
Thymus vulgaris	286.1	88.61	104.3	552.82	1258.27	132.9	339.2	83.45	108.7
Trigonella foenum-graecum	432.24	126.95	113.7	516.51	178.68	140.3	559.8	188.17	122.7
Dithane M45	295.9	107.18	106.1	242.4	99.1	90	418.3	120.26	120.8
Rivoral	450.26	141.13	118.2	301.8	113.06	109	354.7	117.83	105.8
Control	261.9	96.85	96.8	379.75	117.44	106.5	236.4	60.05	94.2

L.S.D. at 0.05	Fresh weight (g)	Dry weight	Plant length (g)	(cm)
Treatments	108.67		33.57	16.81
Cultivars	66.54		20.55	6.62
Treatments X cultivars	322.82		97.09	41.37

DISCUSSION

Samples of tomato plants collected from different places of El-Gabal Al-Khader region showed early blight symptoms causing by the fungus *Alternaria solani*. Pathogenicity of different isolates of the causal organism were tested on five tomato cvs. Data obtained clearly indicated the presence of great variation among isolates in their pathogenicity. The results are in hormony with data obtained by Bonde (1929), Henning and Alexander (1959), Rotem (1994), Vloutoglou (2000). Moverover, different tomato cvs. Showed different degrees of resistant or susceptibility to all tested isolates, this variation among tomato cultivars was also observed by many workers, Jo and Khade (1981), Valkalounakis (1983), Stancheva and Stamova (1988), Suryavanshi *et al* (2000), Vloutoglou *et al* (2000).

It was hypothesized in this investigation that diffusates of plants showed antifungal activity could be used for management of early blight disease under field conditions. Actually, under field condition diffusates of *Thapsia gargonica, Thymus vulgaris* and *Trigonella foemum-graecum* gave best control of early blight as compared by Dithane M45. There are many reports on the occurrence of antifungal activity in such 45 plants diffusates such as alkaloids, phenol, sesquiterpenes and diterpenes. Among the plants listed in Table (2) *Allium sativum* contains allium, allicin and ajoene, capsaicidin (*Capsicum annuum*), Resin (*Thapsia gargonica*), Thymol, carvacrol, lessence, eucalytol menthane, thymene, Tennin, resin (*Thumus vulgaris*) and Trigonelline, hederagin, glycoside (*Trigonella foenum-graecum*) diffusates of such plants had strong effect on spore germination, number of germ tubes and germ tube length.

Our results suggest that plant diffusates have the potential for us in early blight control. Because they contain many components have fungicidal and antibacterial activity (Reimer *et al.* 1993; Qasem and Abu-Blam, 1996; Dushyent and Bohra, 1997; Montes Belmant and Garcia 1997; Hidalyo and Fernandez, 2000; Özcan and Boyraz, 2000; BaBu *et al*, 2001; Kallil 2001); Thiribhuvanamals *et al*, 2001.

Results obtained in this study greatly indicated that such diffusates tolerated all field conditions, therefore, it could recommend to use diffusates of such plants to control early blight under field conditions.

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دراسة تأثير مفرزات بعض النباتات ذات التأثير المضاد للفطريات فى مقاومة الندوة المبكرة على الطماطم

مجدى جاد الرب محمد السمان

قسم أمراض النبات - كلية الزراعة - جامعة عين شمس - مصر

أجرى اختبار ٢١ من مفرزات مائية نباتية بتركيزات (10^{-1} و 10^{-2} و 10^{-3}) من أجزاء نباتية مختلفة على النبات وعند تأثيب النبات وأطول التأثيب النبات لجراثيم فطر *A. solani* المسبب لمرض الندوة المبكرة فى الطماطم فى المعمل . وقد استخدمت أفضل خمسة مستخلصات مائية نباتية بالاضافة الى المبيدين دايثين ٤٥ والروفورال بالتركيزات الموصى بها فى اختبار الحقل .

وقد أظهرت النتائج المعملية

- أن المستخلصات المائية لكل من الحلبة والثوم والفلفل الأخضر والزعتر والدرياس أدت الى تثبيط كلى لإنبات الجراثيم ، بينما كان هناك تأثيرات أقل مثل عشبة الأرنب والقمح والبونطه . وقد أظهرت النتائج الحقلية أن كل من مستخلص نبات الدرياس والمبيدين زوفوال والدايثين م ٤٥ أعطت أفضل حماية لنباتات الطماطم ضد مرض الندوة المبكرة فى الطماطم من حيث خفض شدة الاصابة وزيادة المحصول .