ALEXANDRIA
SCIENCE EXCHANGE JOURNAL

VOL. 27 JANUARY- MARCH 2006

Efficacy of Some Plant Extracts Against *Alternria Solani* and *Fusarium Oxysporum* F.sp.*Lycopersici* in Tomato Plants

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ABSTRACT

Antifungal activity of the water extract of seven plants namely, *Zingiber officinale* (Zingiber), *Allium sativum* (Garlic), *Piper nigrum* (Pepper), *Nigella sativa* (Nigella), *Carum carvi* (Caraway), *Cinnamomus cassia* (Cassia) and *Cymogopogon proxims* (Half-bar) against *Alternaria solani* and *Fusarium oxysporum* f.sp. *lycopersici* was carried out. *In vitro* study, revealed that the tested plant extracts affected the growth of the tested fungi with different levels and the percentage of growth inhibition was dose-dependent. The water garlic extract had the strongest antifungal activity either against *A. solani* or *F.oxysporum* f. sp. *Lycopersici*, where the corresponding percentages of growth inhibition ranged from 34.4 – 57.4 and 18.9 – 33.0%, respectively. Under greenhouse conditions, the high antifungal activities against Alternaria blight and Fusarium wilt diseases were recorded with garlic followed by zingiber, pepper and then nigella extracts. The results indicated that some plant extracts especially garlic could be used as natural fungicides to control pathogenic fungi and reduce the dependence on the synthetic fungicides.

INTRODUCTION

Tomato early blight caused by *Alternria solani* is considered the most common leaf disease affecting tomato production in many countries and can infect all the aerial parts of tomato plant (Rotem, 1994; Gomez *et al*, 2003). Also, Fusarium wilt (Fusarium crown and root rot) caused by *Fusarium oxysporum* f.sp. *lycopersici* is one of the most damaging soil–borne disease of tomato plants and becoming more common in greenhouse tomato production (Roberts *et al*., 2001) and large amounts of fungicides are used to control both diseases (Vale *et al*., 1992). When fungicides are used irrationally and in high dosages, environmental contamination and health problems for humans may occurred (Patterson and Nokes, 2000). The inappropriate use of agrochemicals especially fungicides were found to pose carcinogenic risk (Osman and Al-Rehiayani, 2003). There may be a need to develop new management systems to reduce the dependence on the synthetic pesticides. Nowadays, plant extracts and natural products are widely used to control pests (Islam *et al*., 2004). Plant extracts and essential oils showed antifungal activity against a wide range of fungi (Grane and Ahmed 1988; Wilson *et al*., 1997). In this respect, *Salvia gilliessi, Satureja parvifolia, Lippia polystachya* and *Lippia junelliana* were found to have antifungal activity against *Alternaria solani, Sclerotium cepivorm* and *Colletotrichum coccodes* (Zygadlo and Grosso, 1995). *Satureja hortensis, Cuminus cyminum* and *Echinophora tenuifolia* showed the most relevant fungicidal activity against *F . oxysporum* f. sp. *tulipae, Rhizoctonia solani, Botrytis cinerea* and *Alternaria citri* (Nuh and Musa, 2005). *Aristea ecklonnii* and *Agapathus inapertus* have been recommended against *Botrytis cinerea, Fusarium oxysporum, Rhizoctonia solani* (Pretorius *et al*., 2002). The present study was undertaken to evaluate the *in vitro* efficacy of water extract of seven plants namely zingiber, garlic, pepper, nigella, caraway, cassia and half-bar against *A. solani* and *F. oxysporum* f.sp. *lycopersici* as well as study the *in vivo* effect of these extracts on the early blight and fusarium wilt diseases which infect tomato under the greenhouse conditions.

MATERIALS AND METHODS

Isolation and purification of the causal organisms:

The causal pathogens of tomato early blight disease (*Alternaria solani*) and tomato fusarium wilt (*Fusarium oxysporum* f.sp. *lycopersici*) were isolated from naturally infected tomato plants and purified in pure cultures. The isolates were maintained on potato dextrose agar (PDA).

Preparation of plant extracts:

The rhizome of zingiber, bulb of garlic, seeds of nigella and caraway, dried fruits of pepper, stem bark of cassia and leaf of half-bar were obtained from the local market of Alexandria governorate and immediately kept in refrigerator until starting the experiments. The tested

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plants were ground to fine powder, extracted by macerating 200 g of each plant in 1000 ml of sterilized cold distilled water for 24 hr and then filtered by passing it though sterilized bacterial filter (Seitz). These extracts were set as original concentrations (20%).

***In vitro* effect of plant extracts against *A. solani* and *F. oxysporum*:**

Concentrations of 0.5, 1 and 2% of the tested extracts were prepared by diluting 25, 50 and 100 ml of the original concentration warm PDA with 1000 ml after autoclaving, then 20 ml of the medium was poured into 9cm sterilized Petri dishes. The medium without extracts served as control. Mycelia discs were prepared using a cork borer (5mm diameter) from the tip of 5 days old culture of the two tested fungi and then placed at the center of Petri dishes after solidification of PDA. Each treatment was replicated three times. Plates were incubated in an incubator at 28 ±1°C until the growth of the fungi in control treatment reach to full. Fungal growth was measured by averaging the two diameters taken at right angles for each colony and then percentages of growth inhibition (I%) were calculated according to the following formula:

(I%) = (growth in control-growth in treatment/growth in control) x 100.

***In vivo* effect of plant extracts on early blight disease:**

Spore suspensions of *A. solani* were obtained from pure culture and the inoculum potential was adjusted to $1x10^6$ spores/ml using haemocytometer. Seedlings of supermarmande tomato cultivar 30 days old were replanted in autoclaved sandy loam soil in 15cm plastic pots (2 seedling/pot).and treated as the following: (1) leaves were inoculated with spores suspension and at the same time sprayed with plant extracts, (2) leaves were inoculated with spores suspension and sprayed with plant extracts after 2 days, (3) leaves were sprayed with plant extracts and inoculated with spores suspension after 2 days, (4) Untreated leaves served as a control. In brief, 10 ml of either the spore suspension or plant extract were sprayed over the upper leaf surfaces of the two seedling plants and then coated with plastic bags for 48 hr to make relative humidity around seedling plants. Pots were randomly arranged in the greenhouse and the number of leaf spots/leaf on each treatment were calculated after 14 days from inoculation. Plants were watered every other day for 14 days. Three pots were assigned for each treatment and experiments were duplicated.

***In vivo* effect of plant extracts on Fusarium wilt disease:**

Microconidia of *F. oxysporum* f. sp. *lycopersici* growing on PDA plates at 28 ±1°C for 14 days were harvested by flooding the plates with sterilized distilled water, and then strained through cheesecloth. The inoculum potential was adjusted to $1x10^6$ spores/ml using haemocytometer. Seedlings of supermarmande tomato cultivar 30 days old were replanted in autoclaved sandy–loam soil in 15 cm plastic pots (2 seedlings/pots). Each pot containing two plants, received 25 ml of the spores suspension, treated with 25 ml of 5% of plant extracts after 7 days, and arranged as follows: (1) soil infested with *F. oxysporun* f. sp. *lycopersici* and seedling treated with plant extracts, (2) soil infested only with *F. oxysporun* f. sp. *lycopersici* and served as a control. Three pots were assigned for each treatment and the experiment was duplicated. Pots were randomly arranged in the green house and watered every other day for 40 days. Fusarium wilt .disease and dry weight of shoot plants were investigated. Fusarium wilt disease was rated according to the scale of Jimenez *et al.* (1991) depending on the percentage of yellowing or necrosis of foliage (0 = 0%, 1=1-33%, 2 =34–66%, 3 = 67–100 % and 4= death of plant).

Statistical analysis:

Data were calculated as mean ± S.D and analyzed using analysis of variance technique (ANOVA). Probability of 0.05 or less was considered significant according to Duncan's multiple range (Duncan, 1995).

RESULTS

The *in vitro* antifungal properties of water plant extract were evaluated against *Alternaria solani* and *Fusarium oxysporum* f.sp.*lycopersici* using radial growth technique. Data in Table (1) showed that garlic extract was found to be the most potent extract to reduce the growth of *A. solani* at all the tested concentrations followed by zingiber extract, where the corresponding percentages of growth inhibition ranging from 34.4–57.4% and 16.3- 23.0, respectively. The remaining plant extracts were less potent. Data recorded in Table (2) showed that garlic extract at all the tested concentrations was the most efficient one to reduce the growth of *F. oxysporun* f. sp. *lycopersici*, where the corresponding percentage of growth inhibition ranged from 18.9 to 33.0%. The remaining plant extracts had less effect on the growth of this fungus. From the present data, it is clear that garlic extract had antifungal activity to *A. solani* than *F. oxysporun* f. sp. *lycopersici*.

The effect of spraying plant extracts at different time on Alternaria early blight infection are shown in Table (3). It was found that the most suppressive effect against tomato early blight was obtained when inoculated fungus and plant extracts were applied at the same time, followed by the application of plant extracts after 2 days following the inoculation with the fungus

Table 1. Effect of plant extracts on the linear growth of *Alternaria solani*.

Treatment	Colony diameter (cm)		
	0.5 %	1%	2%
Zingiber	7.53±0.06 b (16.3)	7.23±0.06 b (19.7)	6.93±0.06b (23)
Garlic	5.9±0.10a (34.4)	4.30±0.17a (52.2)	3.83±0.06a (57.4)
Pepper	8.23±0.06c (8.6)	7.73±0.0.06c (14.1)	7.33±0.06c (18.6)
Nigiella	8.33±0.15cd (7.4)	7.77±0.12c (13.7)	7.53±0.06d (16.3)
Caraway	8.47±0.06d (5.9)	8.2±0.00e (8.9)	7.80±0.00f (13.3)
Cassia	8.47±0.06d (5.9)	8.10±0.10de (10.0)	7.90±0.00g (12.2)
Half-bar	8.40±0.10cd (6.7)	7.97±0.10d (11.4)	7.70±0.00e (14.4)
Control	9.0±0.00e (0.0)	9.0±0.00f (0.0)	9.00±0.00h (0.0)

Data are expressed as mean ±S.D (n= 3). Values in brackets indicate percentage of growth inhibition. Means within the same column and followed by the same letter are not significantly different (p≤ 0.05).

and then the treatment with plant extracts, 2 days before the inoculation. The corresponding percentages of spots/leaf reduction were ranged from 67.1-92.4, 62.4 - 86.7% and 21.9 -73.3%, respectively.

The effect of plant extracts on Fusarium wilt disease is presented in Table (4). Garlic extract was found to be the most potent one to reduce disease severity by 67.5%, while the treatment with zingiber, pepper and nigella reduced with disease severity by 42.5, 32.5 and 25%, respectively, when compared with control values. In addition, the shoot dry weights increased by 111.1, 85.1,57.3 and 29.1% when treated with garlic, zingiber, pepper and nigella extracts respectively, compared with control.

Table2. Effect of plant extracts on the linear growth of *Fusarium oxysporum* f. sp. *lycopersici*.

Treatment	Colony diameter (cm)		
	0.5 %	1%	2%
Zingiber	8.23±0.06cd (8.6)	7.77±0.06b (13.7)	7.23±0.06b (19.7)
Garlic	7.30±0.00a (18.9)	6.57±0.12a (27.0)	6.03±0.06a (33.0)
Pepper	8.37±0.12de (7.0)	7.83±0.12b (13.0)	7.37±0.06c (18.1)
Nigiella	8.07±0.06b (10.3)	7.80±0.00b (13.3)	7.53±0.06d (16.3)
Caraway	8.43±0.12e (6.3)	7.87±0.12b (12.6)	7.63±0.06e (15.2)
Cassia	8.17±0.06bc (9.2)	7.83±0.12b (13.0)	7.47±0.06d (17.0)
Half-bar	8.33±0.06de (7.4)	7.9±0.10b (12.2)	7.83±0.06f (13.0)
Control	9.00±0.00f (0.0)	9.00±0.00c (0.0)	9.00±0.00g (0.0)

Data are expressed as mean ±S.D (n= 3). Values in brackets indicate percentage of growth inhibition. Means within the same column and followed by the same letter are not significantly different (p≤ 0.05).

DISCUSSION

The present investigation showed that all the tested plant extracts appeared to have different effect on radial growth of the tested fungi. Moreover, all the tested extracts at all the tested concentrations affected the growth of the two tested fungi and the percentage of growth inihibition was dose dependent. Garlic, zingiber and pepper were the most effective extract to inhibit the growth of the tested fungi, followed by nigiella, half-bar, caraway and cassia. The present results are in parallel with the other studies, where garlic was found to be the most potent among many plants against *Alternaria alternata* which cause blight of sunflowers disease (Srinvas *et al.*,1997) , and against leaf spot in betlnut caused by *pestalotia palmarum* (Islam *et al.*,2004). Although garlic was demonstrated early to have good antifungal activity and to be useful as post harvest treatment (Wilson *et al.*, 1997), it has not widely commercialized. The plant extract of *Salvia indica* inhibited many plant pathogenic fungi such as *Phtophthora infestans, F. oxysporum, Rhizoctonia soloni , Stemphylium solani* and *Mucor* sp. (Amjad *et al.*, 2005).

In the present study, the tested plant extracts affected Alternaria early blight disease by reducing the number of spots/leaf and reduced the disease severity of Fusarium wilt of tomato plants under greenhouse conditions. Although most of the natural products in agriculture are used against insects, there are many investigations declared the effects of plant extract on tomato plant diseases (Francis , 1990; Soleimani *et al.*, 1996; Gomez *et al.*, 2003).

Findings in this study confirmed that plant extracts had strong antifungal activity with significant inhibition on the growth of *A. solani* and *F. oxysporum* f. sp. *lycopersici.* In addition, the inhibitory magnitude of the tested plant extracts to the tested pathogenic fungi was proportional to the applied concentration. Because garlic plant extract was more efficient, therefore it might to be a promising material to control these fungi.

It can be concluded that, plant extracts especially garlic, zingiber and pepper may be used as natural fungicides to control some plant pathogenic fungi in order to decrease the dependence on synthetic fungicides which contaminate environment as well as to decrease the higher production costs.

Table 3. Effect of plant extracts on the Alternaria early blight infection in tomato Leaves.

Treatment	T_1	T_2	T_3
Zingiber	6.0±0.00a	15.7±3.21ab	33.0±4.36bc
	(91.4)	(77.6)	(52.9)
Garlic	5.3±0.60a	9.3±0.58a	41.3±8.50c
	(92.4)	(86.7)	(41.0)
Pepper	7.0±2.65ab	10.0±3.61a	33.3±5.51bc
	(90.0)	(85.7)	(52.4)
Nigiella	11.3±4.16abc	20.3±3.06bc	54.7±2.52d
	(83.9)	(71.0)	(21.9)
Caraway	16.3±1.53c	21.3±5.51bc	27.3±6.03ab
	(76.1)	(69.6)	(61.0)
Cassia	12.7±2.52bc	13.0±3.60ab	18.7±5.03a
	(81.6)	(81.4)	(73.3)
Half-bar	23.0±4.58d	26.3±6.51c	28.7±3.21ab
	(67.1)	(62.4)	(59.0)
Control	70.0±2.00e	70.0±2.00d	70.0±2.00e
	(0.0)	(0.0)	(0.0)

Data are expressed as mean ±S.D (n= 3). Values in brackets indicate percentage of spots/leaf reduction. Means within the same column and followed by the same letter are not significantly different ($p \leq 0.05$).

T_1 = Leaves inoculated with fungus and sprayed plant extracts in the same time (spots/leaf).

T_2 = Leaves inoculated with fungus and sprayed plant extracts after 2 days (spots/leaf).

T_3 = Leaves sprayed with plant extracts and inoculated fungus after 2 days (spots/leaf).

Table 4. Effect of plant extracts on the Fusarium wilt disease in tomato plants.

Treatment	Disease severity	% Reduction of disease severity	Dry weight of 2 plants (gm)	% Increase of dry weight
Zingiber	2.3±0.60b	42.5	11.892±1.67c	86.2
Garlic	1.3±0.60a	67.5	13.481±2.12c	111.1
Pepper	2.7±0.60b	32.5	10.049±0.27b	57.3
Nigiella	3.0±0.00bc	25	8.248±0.50ab	29.1
Caraway	3.3±0.60bc	17.5	7.954±0.37ab	24.5
Cassia	3.3±0.60bc	17.5	8.129±0.29ab	27.3
Half-bar	3.3±0.60bc	17.5	7.449±0.31a	16.6
Control	4.0±0.00c	0.0	6.387±0.49a	0.0

Data are expressed as mean ±S.D (n= 3). Means within the same column and followed by the same letter are not significantly different (p≤ 0.05).

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الملخص العربي

فعالية بعض المستخلصات النباتية ضد الفطريات المسببة لأمراض اللفحة المبكرة *Alternaria solani* والذبول الفيوزاريومي *Fusarium oxysporum* f.sp.*lycopersici* في الطماطم

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تم تقدير كفاءة بعض المستخلصات المائية لسبعةمن النباتات هي الزنجبيل والثوم والفلفل الأسود وحبة البركة والكراوية والقرفة والحلفا بر ضد الفطر المسبب لمرض اللفحة المبكرة *Alternaria solani* والفطر المسبب الذبول الفيوزاريومي *Fusarium oxysporum* f.sp.*lycopersici* في الطماطم تحت الظروف المعملية و البيوت المحمية. أظهرت الدراسات المعملية أن المستخلصات المائية للنباتات المختبرة كان لها تأثير مثبط بدرجات مختلفة على نمو الفطريات المختبرة وكانت نسب التثبيط للنمو الفطري تعتمد على الجرعة المستخدمة. كذلك وجد أن المستخلص المائي لنبات الثوم كان من أقوى المستخلصات المستخدمة ضد فطرى *A. solani* و *F. oxysporum* f.sp.*lycopersici*. أما تحت ظروف الصوبة فقد أوضحت الدراسات أن المستخلص المائي لنبات الثوم كان الأكثر فعالية كمضاد للنشاط الفطري للفطريات المختبرة، يليه المستخلصات المائية لنباتات الزنجبيل والفلفل الأسود وحبة البركة.

ويمكن القول أن المستخلصات النباتية لبعض النباتات وخاصة الثوم يمكن أن تستخدم كمضادات فطرية لمقاومة بعض الفطريات الممرضة للنباتات وذلك لتقليل الاعتماد على المبيدات الفطرية التقليدية.