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Fungitoxicity of Weed Extracts to Tomato Wilt Pathogen (*Fusarium oxysporum* f. sp. *lycopersici*)

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ABSTRACT

Fungitoxic effects of aqueous shoot extracts of some common weed species against *Fusarium oxysporum* f. sp. *lycopersici* Schlecht (the causal agent of tomato wilt disease) were investigated under laboratory conditions. Extracts varied in their antifungal activities, *Anagallis foemina, Calendula arvensis, Cerastium dicotomum, Convolvulus arvensis, Euphorbia helioscopia, Falcaria vulgaris, Foeniculum vulgare, Inula viscosa, Ranuculus asiaticus, Scorpiurus muricatus, Sisymbrium irio, Solanum nigrum, Stellaria media* and *Urtica urens* extracts were the most toxic to the fungus. Fresh shoot extract of buttercup (*Ranunculus asiaticus* L.) completely prevented growth and sporulation of F. *oxysporum* at all incubation periods.

Key Words : Extracts, fungitoxicity, *Fusarium oxysporum* f. sp. *lycopersici*, weeds.

INTRODUCTION

Exclusive dependence on synthetic pesticides has created major problems for the agroecosystem and resulted in many ecological difficulties. This has stimulated the search for nonphytotoxic, systemic, easily biodegradable and highly selective alternative pesticides. Plants may serve as novel alternative sources of pesticides, and more than 2000 species have been reported to contain a large number of secondary metabolites that have pest control properties. These include plants belonging to the Meliaceae, Labiatae, Rutaceae, Asteraceae, Ericaceae, Ranunculaceae and other plant families. Antimicrobial activities of extracts, oils or volatile materials of many plant species have been well documented (Frange, 1984; Akhtar et al., 1986; Tripathi et al., 1986; Asthana et al., 1986; Al-Abed et al., 1993).

This paper describes the screening of shoot extracts from 63 common weed species for ability to inhibit mycelial growth or sporulation in vitro of the soil borne fungus *Fusarium oxysporium* f. sp. *lycopersici*, the causal agent of wilt epidemics of tomato.

MATERIALS AND METHODS

The antifungal activity of aqueous extracts of 63 common weed species (belonging to 28 plant families) were tested against *Fusarium oxysporum* f. sp. *lycopersici* (the causal agent of tomato wilt disease) isolated from tomato (*Lycopersicon esculentum* Mill) roots. Shoots of the different weed species were cut above the soil surface at various stages of growth (Table 1). Samples (300 g) of the fresh shoots of each weed species were washed under running tap water, rinsed with distilled water then chopped up and placed in distilled water (1 l). Each sample was then processed in a Waring blender until a homogenous solution was obtained. The solution was allowed to stand for 30 minutes then, the supernatant liquid was passed through Whatman No. 1 filter paper, then through a membrane filter (0.2 u M) to avoid any bacterial or fungal contamination.

Four discs (1 cm diameter) of the fungus, taken from 8 day old culture grown on PDA medium, were placed in sterile petri-dishes (11 cm diameter) poured with 20 ml of sterile potato dextrose agar (PDA) medium with 3 ml extract obtained from one weed species and added to form a very thin layer on the medium surface and surrounding the fugal discs. The control treatment was 3 ml sterile distilled water. Each treatment was replicated four times following a complete randomised block design.

The dishes were incubated at 20°C in the dark for 8 days. Visual estimation on the growth and spore formation of the fungus was made after 4 and 8 days of incubation, using 0-10 scale at which, 0 value denotes no growth or sporulation of the fungus has occurred outside the inoculated fungal discs; 1,2,3,4,5,6,7,8 and 9 indicate that 10,20,30,40,50,60,70,80, and 90% of the medium surface was colonized by fungal mycelia or spores, respectively and 10 means that petri-dish was full with fungal mycelia or spores.

Results obtained were statistically analysed by the standard analysis of variance procedure and treatments means were compared using the least significant differences (LSD at P=0.05).

RESULTS

The results of the antifungal activity of weed species on the growth and sporulation of *F. oxysporum f. sp. lycopersici* are presented in Table 1.

Effect on fungal growth

Out of 63 weed extracts tested for their antifungal activity against *F. oxysporum* f. sp. *lucopersici* only extracts of 23 species significantly inhibited growth of the fungus after 4 days of incubation compared with the control (Table 1). However, extracts were varied in strength of their antifungal activities with those of *C. dicotomum, C. aspera, S. muricatus, F. vulgaris, R. asiaticus, S. nigrum* and *U. urens* were the most effective. In contrast, extracts of 13 species showed stimulatory effect but only *M. annua* extract did significantly increased fungal growth compared with the control.

At 8 days after incubation, extracts of 35 species showed fungitoxic activity and significantly reduced fungal growth compared with the control. C. dicotomum, S. muricatus and R. asiaticus extracts were the most toxic and reduced growth of the fungus by 80-100% compared with the control. Other inhibitory extracts including those of *A. foemina, C. arvensis, E. helioscopia, F. vulgaris, F. vulgare, I. viscosa, S. irio, S. nigrum, S. media* and *U. urens*. Besides antifungal properties, extracts of M. annua and S. arvensis showed stimulatory activity on growth of the tested fungus, although differences between the effect of these and the control were not significant.

Effect on fungal sporulation

The significant effect of extracts on sporulation of *F. oxysporum f. sp. lycopersici* at 4 days after incubation was not clear. Non of the extracts did significantly reduce spore formation at this stage compared with the control (Table 1). However, extracts of 11 species reduced fungal sporulation by more than 50% compared with the control, these are, *G. tircornutum, P. diffusa, R. asiaticus* and *S. oleraceous*. Extracts of these species completely prevented spore formation of the fungus. Other less fungitoxic extracts including those of *A. foemina, B. didyma, F. vulgaris, M. racemosum, P. lanceolata* and *S. nigrum*. Among 15 weed extracts stimulated sporulation of the fungus at this stage, extract of *N. syriaca* was the best followed by *E. hispanica, E. elaterium* and *A. cotula*.

After 8 days of incubation, less number of extracts retained their toxicity to spore formation of the fungus. *R. asiaticus* extract was the most effective and completely inhibited spore formation followed by *G. tricornutum* although differences between these and the control were not significant. In contrast, the number of stimulatory extracts was substantially increased at this stage of reaching a total of 42 species. The best were *E. hispanica* and *N. syriaca* at which dishes treated with extracts of these species were full of fungal spores. Other extracts

significantly enhanced spore formation at this period of incubation were those of *C. procera, A. cotula, C. bursa-pastoris, C. draba, F. vulgaris, P. diffusa, S. syriaca* and *V narbonensis*.

Among all species screened, *R. asiaticus* extract was the most inhibitory to growth and sporulation of *F. oxysporum f. sp. lycopersici* and at both incubation periods.

DISCUSSION

In vitro assessment of the potential of shoot extracts of 63 species as a fungitoxicants against *F. oxysporum* f. sp. *lycopersici* confirmed that extracts of some plant species can be a good source of natural compounds with fungitoxic properties. The duration of extracts activity varied among different plant species suggesting that the active materials in extracts from certain species were dissipated with time, possibly due to decomposition of the active compounds or transformation of these to nontoxic forms. It is also possible that the active agent may not have been absorbed by the medium to give a long lasting effect on the fungus (Dumancas, 1976).

Results showed that, with the only exception of *R. asiaticus* extracts, the antifungal effects are neither a family nor a generic character. Results of *R. asiaticus* were in full agreement with those obtained with other *Ranunculus* spp. Differences between plant species in their fungitoxic activity have been reported by other workers (Dixit and Tripathi, 1975; Frange, 1984; Al-Abed et al., 1993).

Among 63 locally prevalent weed species tested in vitro, extracts of *R. asiaticus* proved to be the most effective against *F. oxysporum* f. sp. *lycopersici* and completely inhibited its growth and sporulation. Misra et al. (1974) reported high antifungal activity of leaf extract of *Ranunculus clematis* L. against *Alternaria tenius* Nees, *Curvularia lunata* (Walker) Boed, *Fusarium nivale* (Fries) Ces. and *Helminthosporium gramineum* Rab. ex Sch, while leaf extract of *Ranunculus scleratus* L. completely inhibited the growth of *A. tenuis, C. lunata, F. nivale* and *H. gramineum* (Misra and Dixit, 1978).

Extracts were also different in the persistence of their antifungal activities against the fungus, this being most pronounced with fresh shoot extract of *R. asiaticus* which retained its full strength activity throughout the period of incubation. The long term effect of the extract indicates that the active principle may not have been affected by incubation temperature, or not degraded to non-toxic products during incubation period. Misra and Dixit (1978) reported that the active principle in leaf extract of *R. sceleratus* L. is protoanemonin dimersed

into a crystalline substance anemonin. The chemical was strongly fungitoxic against the tested fungi up to 1:10.00 dilution while anemonin up to 1:100 only. In another study, Mares (1987) found that protoanemonin a component of *R. bulbosus* L. showed antifungal activity against *Epidermophyton floccosum* (Harz) Langer and the yeast *Rhodotorula glutinis* (Fres) Harrison. The minimum inhibitory concentration ranged from 2.0 to 7.5 x 10-4M while the minimum lethal concentration from 3.8 x 10-4 to >1.0 x 10 -3 M.

The stimulatory effect of some plant extracts on fungal growth indicates the presence of growth promoting or nutritional substances in these extracts. Other workers have reported similar findings through their work on different plant species and different fungi (Dixit and Tripathi, 1975; Tewari and Doth, 1984; Al-Abed et al., 1993). Such stimulatory materials, could perhaps be exploited for the development of simple cheap and effective media for culturing the pathogens.

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Table 1. Visual estimation of the effect of shoot extracts of different weed species on the growth and sporulation of F. *oxysporum* f. sp. *lycopersici* after 4 and 8 days of cubation at 20°C. [Nomenclature is that of Flora Palastina (Zohary 1966)].

Shoot extracts		Growth		Sporulation	
		Days of incubation			
Weed species	Growth stage	4	8	4	8
Distilled water (control)		7.7	9.0	1.3	1.3
Asclepiadaceae					
Calotropis procera Ait. Fit	Flowering	6.3	7.0	4.0	7.3
Boraginaceae					
Symphytum palaestium Boiss	Flowering	3.7	4.7	0.7	1.3
Caryophyllaceae					
Cerastium dicotomum L.	Flowering	1.3	1.7	1.0	1.7
Stellaria media (L.) Vill	Flowering	3.3	3.7	0.3	3.3
Chenopodiaceae					
Atriplex leucolada Boiss	Vegetative	8.7	8.7	1.3	1.7
Chenopodium murale L.	Flowering	6.7	6.7	4.7	6.0
Compositae					
Anthemis cotula L.	Flowering	7.3	8.7	5.0	7.0
Calendula arvensis L.	Flowering	3.0	3.6	1.7	5.0
Carthamus nitidus Boiss	Vegetative	7.3	7.3	1.7	6.0
Centaurea iberica Spreng.	Flowering	9.0	9.0	1.0	3.7
Crepis aspera L.	Flowering	1.3	5.7	0.7	3.3
Gendelia tournefortii L.	Vegetative	6.0	8.0	1.0	1.3
Inula viscosa (L.) Ait.	Vegetative	2.0	2.7	0.7	1.0
Notobasis syriaca (L.) Cass.	Vegetative	9.0	9.0	9.7	10.0
Onopordum Jordanicolum Eig	Pre-flowering	7.7	7.7	1.3	1.7
Senecio vernalis L.	Flowering	8.0	8.0	2.0	1.7
Sonchus oleraceus L.	Flowering	6.3	7.3	0.0	1.7
Convolvulaceae					
Convolvulus althaeoides L.	Flowering	6.3	6.3	1.3	1.7
Convolvulus arvensis L.	Pre-flowering	4.0	4.0	1.3	1.7
Cruciferae					
Biscuttela didyma L.	Flowering	5.7	5.7	0.3	5.0
Capsella bursa-pastoris L.	Flowering	7.7	8.3	3.3	7.0
Cardaria draba (L.) Desv.	Vegetative	6.0	6.0	4.0	9.0
Diplotaxis erucoides (L.) DC.	Flowering	7.0	7.3	2.3	3.7
Eruca sativa Mill	Flowering	8.0	9.0	1.0	5.3
Erucaria hispanica (L.) Druce.	Flowering	6.3	6.7	6.7	10.0
Sinapis arvensis L.	Flowering	9.7	10.0	1.3	4.7
Sisymbrium irio L.	Flowering	3.7	3.7	3.3	6.7
Cucurbitaceae					
Coballium elaterium (L.) Rich	Pre-flowering	5.3	5.3	5.0	7.7
Euphorbiaceae					

Shoot extracts Weed species	Growth stage	Growth Days of incubation 4	 8	Sporulation 4	 8
Euplhorbia helioscopia L.	Flowering	3.3	3.7	0.7	2.0
Fercurialis annua L.	Seeding	9.7	9.7	2.3	5.7
Fumariaceae					
Fumaria densiflora DC.	Early flowering	7.3	7.7	5.7	6.3
Geraniaceae					
Avena sterilis C. Presl	Vegetative	6.0	6.0	2.3	2.7
Labitae					
Ballota saxatilis C. Presl	Vegetative	6.0	6.0	2.3	2.7
Lamium sp.	Flowering	6.7	7.0	2.3	3.0
Lamium amplexicaula L.	Flowering	6.0	6.0	2.0	3.0
Salvia syriaca L.	Flowering	8.0	8.0	2.3	7.3
Legumiosae					
Lupinus varius L.	Flowering	7.3	7.7	1.7	2.0
Oninis antiquorum	Vegetative	7.0	7.0	1.0	6.3
Liliaceae					
Bellevalia densiflora Boiss	Flowering	7.7	7.7	1.0	4.7
Muscaria racemosum (L.) Mill	Vegetative	5.0	5.0	0.3	2.3
Loranthaceae					
Viscum crucianum sieb	Pre-Flowering	8.0	8.0	1.0	4.0
Malvaceae					
Malva sylvestris L.	Pre-flowering	9.0	9.0	3.7	6.7
Papaviraceae					
Papaver rhoeas :.	Flowering	4.3	5.0	2.0	4.0
Papilionaceae					
Scorpiurus muricatus L.	Flowering	1.7	2.0	2.3	5.3
Vicia narbonensis L.	Flowering	6.7	6.7	1.3	7.0
Plantaginaceae					
Plantago lanceolata L.	Flowering	7.7	8.3	0.3	3.7
Polyginaceae					
Polygonum aviculare L.	Early flowering	4.3	5.7	2.3	2.7
Rumex crispus L.	Vegetative	7.3	7.3	1.7	3.0
Primulaceae					
Anagallis arvensis L.	Flowering	8.0	8.3	1.0	2.0
Anagallis foemina Mill	Flowering	3.7	3.7	0.3	1.3
Ranunculaceae					
Ranunculus asiaticus L.	Flowering	0.0	0.0	0.0	0.0

Shoot extracts Weed species	Growth stage	Growth Days of incubation 4	 8	Sporulation 4	 8
Rosaceae					
Sarcopoterium spinosum Spach	Flowering	9.0	9.7	1.0	1.7
Rubiaceae					
Galium tricornutum Dandy	Early flowering	5.0	5.3	0.0	0.7
Solanaceae					
Solanum nigrum L.	Fruiting	2.0	3.3	0.3	3.0
Umbelliferae					
Ammi majus L.	Flowering	4.3	4.7	2.7	5.0
Conium maculatum L.	Vegetative	7.3	7,3	1.3	6.0
Falcaria vulgaris Bernh	Pre-flowering	1.7	2.7	4.3	8.3
Ferula communis L.	Pre-flowering	8.0	8.0	0.3	1.3
Foeniculum vulgare L.	Vegetative	3.0	3.0	2.7	2.7
Scandix pecten-veneris L.	Flowering	6.7	6.7	1.3	2.3
Uriticaceae					
Parietaria diffusa (L.) Koch	Early flowering	8.0	8.7	0.0	7.0
Urtica urens L.	Flowering	2.0	3.7	1.3	2.7
LSD (P-0.05)		1.8	1.8	1.4	1.4

0-10 Scale where the lowest score denotes no fungal growth or sporulation while the highest score denotes that the Petri-dish was full of fungal mycelia or spores.

مجلة الإمارات للعلوم الزراعية (١٩٩٦) ٨ : ١٠٣ - ١١٢

التأثيرات السامة لمستخلصات الاعشاب على الفطر المسبب لمرض الذبول الفيوزاريمى في البندورة (Fusarium Oxysporum sp. Lycopersici).

جمال قاسم
قسم وقاية النباتات ، كلية الزراعة ، الجامعة الاردنية - عمان - الاردن .

ملخص

تمت دراسة التأثيرات السامة للمستخلصات المائية لبعض الأعشاب الشائعة على نمو وتطور الفطر المسبب لمرض الذبول الفيوزاريومى في البندورة Fusarium oxysporum sp. lycopersici وذلك تحت ظروف المختبر . أظهرت الدراسة وجود اختلافات كبيرة في درجة تأثير هذه المستخلصات على الفطر ، فبعضها عمل على تثبيط نمو وتطور الفطر بينما عمل البعض الأخر على تشجيع نموه أو لم يظهر أية تأثيرات . لقد وجد أن مستخلصات عشب عين الجمل Anagallis foemina وأذريون الحدائق Calendula arvensis وأذن الفأر Cerastium dicotomum والمدادة الملونة Convolvulus arvensis والحلبلوب Euphorbia helioscopia والشقار Falcaria vulgaris والشومر Foeniculum vulgare والطيون Inula viscosa والحوذان Ranunculus arvensis وذنب العقرب Scorpiurus muricatus وعنب الديب Solanum nigrum هى الأشد تثبيطاً لنمو وتطور الفطر ، في حين أدت المعاملة بمستخلصات عشب الحوذان إلى منع نمو وإنتاج الجراثيم فى الفطر المذكور وبشكل كامل طيلة فترة إجراء التجربة ..

كلمات مفتاحية : مستخلصات مائية ، السمية للفطريات ، فطر الذبول ، الفيوزاريومى ، أعشاب.