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Phytotoxic Effect of *Calotropis procera* Extract on Seedling Development and Rhizosphere Microflora of Tomato Plants Grown in Soil Infested with *Fusarium oxysporum* f.sp. *Lycopersici*

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Abstract: In a pot experiment, the effect of various treatments of *Calotropis procera* and/or *Fusarium oxysporum* inoculation on tomato seedling development and rhizosphere microflora was studied. The effect of different aqueous leaf extract concentrations (5, 10 and 20%) on some selected fungi was investigated. Percentage emergence, root and shoot lengths of the tomato cultivars Flora-dade, Castle-rock and Strain-B were reduced in the presence of *Fusarium oxysporum*, but the combined effect of *C. procera* residues+tomato pathogen counteracted the suppressive effect of the pathogenic fungus and improved the percentage emergence, root and shoot lengths of tomato seedlings. The treatment of fruit residues caused the complete disappearance of the pathogen in the rhizosphere and surrounding soil of tomato indicating the heigh sensitivity of this fungus to the phytotoxins of *Calotropis procera*. Significant variations in species composition between treated and untreated soil were recorded. When *Fusarium oxysporum* coupled with residues treatments, the microbial population decreased as compared with the fungus alone. The mycelial growth, percentage spores germination and germ-tube extension in *F. oxysporum* and *Aspergillus carbonarius* decreased when *C. procera* extract concentration increases, whereas growth of *Humicola brevis* and *Penicillium lanosum* were not affected.

Key words: *Calotropis procera* extract · *Fusarium oxysporum* · tomato rhizosphere · growth · spore germination

INTRODUCTION

Calotropis procera (Ait.) Ait. f. (Asclepiadaceae) is used in folk medicine because of its pharmacological activities. The active constituents reported to be present in roots, stems, leaves, flowers but mainly in its latex [1-5]. The active molecules are of a proteinaceous nature despite the presence of numerous secondary metabolites in the plant latex [6]. The ethanolic extract of *C. procera* flowers was investigated for its antimicrobial activities. The growth of both gram-positive and gram-negative bacteria was significantly inhibited [7]. Fungitoxic properties of some plant products against wilt pathogens of *Dalbergia sissoo* and *Gmelina arborea* were evaluated, where the extracts of *Calotropis procera, Vitex negundo, Azadirachta indica* and *Lantana camara* significantly reduced the growth and sporulation of both pathogens *Fusarium solani* and *F. oxysporum* [8]. The phytochemical analysis of *C. procera* showed the presence of alkaloids, flavonoids, tannins, steroids, triterpenoids, saponins and saponin glycosides in the leaf and root extract fractions. Only flowonoids, triterpenoids and saponins were detected in the stem bark [9]. Extracts of the chopped leaves and latex have shown great promise as nematicides *in vitro* and *in vivo* [10-13].

The aim of this work is to evaluate the effect of *Calotropis procera* on the rhizosphere microflora and growth of tomato seedlings.

MATERIALS AND METHODS

Tomato seeds (*Lycopersion esculentum* L.) cultivars Flora-dade, Castle-rock and Strain-B were kindly supplied by the Agricultural Research Center, Giza, Egypt.

Plant material of *Calotropis procera* was collected from Mokattam district, Cairo in May, 2007. The plants were separated into leaves, stems and fruits then air dired and powdered.

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Fungal pathogen and cultural conditions: *Fusarium oxysporum* f.sp. *lycopersici* was isolated from the infected tomato plants grown in different localities in Egypt [14] and maintained on Czapek Dox agar medium.

Green house experiment: *Calotropis procera* residues (stems, leaves and fruits) were used to study their effect on the growth and rhizosphere microflora of tomato seedlings. Pots containing 5 kg soil, collected from Faculty of Science, Cairo University gardens were divided into 4 patches, each of 4 pots. The first batch was the control devoid of aak residues and *Fusarium oxysporum*. The second batch was treated with aak plant residues (stems, leaves or fruits). The third batch was inoculated with *Fusarium oxysporum*. The fourth batch included soil mixed with both aak residues and *Fusarium oxysporum*.

Dried powdered aak residues 2 g/kg soil were incorporated into the upper most layer of the soil surface at 2.0 cm depth. All pots were irrigated and left for 7 days. The fungal pathogen *F. oxysporum* multiplied on barley grains was introduced into the soil, 1 cm below the soil surface; 5 days later, fifteen healthy tomato seeds of either Flora-dade, Castle-rock or Strain-B were sown in each Pot. Pots were left for 5 days, after which, the percentages of seedling emergence, shoot and root lengths were measured after 30 days from sawing. Four replicates were used for every treatment.

Soil samples were collected from the rhizosphere and surrounding soils of Flora-dade tomato cultivar, 30 days after sawing. Only 1 gm soil was placed in 100 ml sterile tap water and shaked at 180 rpm for 3 min. Dilutions (10^{-1}-10^{-4}) were prepared for each treatment and placed on Czapek-Dox agar and nutrient agar medium for fungal and bacterial population test. Each inoculation was performed in 4 replicates. Colony Forming Units (CFU) were counted 7 days (Fungi) and 3 days (Bacteria) post inoculation. Soil plate method was applied to calculate the percentage of occurrence of each species. Four plates were prepared for each treatment, incubated for 7 days at 27°C and the developed fungal species were identified.

***Calotropis* aqueous extract:** Three different concentrations of aqueous extract from the plant leaves were prepared by soaking 5, 10 and 20 g of the powdered material in 100 ml distilled water. The mixture was agitated on a rotary shaker for 24 h at 200 rpm, at room temperature, then filtered through a Whatmann No. 42 filter paper. The filtrate was centrifuged for 30 min. to remove debris. The clear supernatant was completed up to 100 ml distilled water to obtain concentrations 5, 10 and 20% (w/v) of the extract.

Spore germination: The fungi under consideration were allowed to grow on Dox agar at 27°C for 7 days then part of the mycelium was transferred, aseptically either to avail containg 10 ml of the required concentration of the extract or distilled water (control). The tubes were hand shaken for 5 min to allow the dispersal of spores. One ml of spore suspension was placed in every Petri-dish. The dishes were incubated at 27°C for 6-10 hours in complete darkness. The percentage germination and average length of germ tubes were assessed according to the procedures mentioned by [15]. Three replicates were prepared for every treatment

Mycelial growth: The soil growth tube method was used. The extracts were introduced to the tubes, aseptically and left for 12 h, then the tubes were inoculated with the fungus at the other end and incubated at 27°C for 10 days. Three tubes were set for each treatment.

Statistical analysis was carried out using LSD to compare the significance of results [16].

RESULTS AND DISCUSSION

The results presented in Table 1 demonstrate that the percentage of seedling emergence of tomato cultivars Flora-dade, Castle-rock and Strain-B descreased when the soil treated with *Calstropis procera* plant residues (stem, leaves or fruits). Maximum inhibition (60%) was recorded under the treatment of leaf residue for cv. Flora-dade and (66%) for Castle-rock tomato cultivar. As for the treatment of fruit residues, it was reduced from 80% to 65% for Strain-B cultivar. In this respect, Extracts of *C. procera, Cuscuta reflexa, Euphorbia pulcherrima, Datura fastuosa* and neem cake increased the percentage

Table 1: Effect of *Calotropis procera* residues (stem, leaves, fruits), on the emergence(%) of seedlings of three tomato cultivars grown in soil infested with *Fusarium oxysporum* f.sp. *lycopersici* after 30 days from sowing

		Tomato cultivars		
Treatments		Flora dade	Castle rock	Strain-B
Plant		86.6	86.6	80.0
Fusarium oxysporum		53.3	73.3	60.0
Stem residue		60.0	73.3	67.0
Leaf residue		60.0	66.6	70.0
Fruit residue		66.6	70.0	65.0
F. oxysporum+Stem residue		63.3	73.3	62.1
F. oxysporum+Leaf residue		76.6	80.6	70.0
F. oxysporum+Fruit residue		73.3	93.3	76.6
LSD:	1%	13.2	10.1	6.3
	5%	8.9	6.3	4.5

Table 2: Effect of *Calotropis procora* residues (2 g/kg soil) on the shoot and root length (mm) of seedlings of three tomato cultivars grown in soil infested with *Fusarium oxysporum* f.sp. *lycopersici* after 30 days from sowing

		Tomato cultivars					
		Flora dade		Castle rock		Strain-B	
Treatments		Shoot length (mm)	Root length (mm)	Shoot length (mm)	Root length (mm)	Shoot length (mm)	Root length (mm)
Plant		97	40	100	42	107	35
Fusarium oxysporum		95	30	90	35	90	25
Stem residue		95	35	94	37	95	92
Leaf residue		92	33	95	36	98	30
Fruit residue		93	35	95	35	97	30
F. oxysporum + Stem residue		94	27	94	32	90	22
F. oxysporum + Leaf residue		95	28	95	34	95	27
F. oxysporum + Fruit residue		105	41	102	38	95	35
LSD:	1%	7.3	8.1	7.8	5.3	5.9	5.2
	5%	4.0	4.8	4.5	2.8	3.1	2.8

germination of chickpea and increased the plant length in mungbean, but reduced the root nodulation [17, 18]. The percentage of germination, root length and fresh weight of tomato and cabbage were greatly reduced when *Mikania micrantha* residues added to the soil [19]. The combined effect of both *Calotropis procera* residues and *Fusarium oxysporum* f.sp. *lycopersici* on the emergence of tomato seedlings is also presented in Table 1. The infection with *F. oxysporum* lowered the emergence percentage in all tomato cultivars. Coupling of aak plant residues with *F. oxysporum* counteracted the suppressive effect of the fungus and improved the seedlings emergence of tomato plant cultivars. The increased percentages ranged from 20-27%, 7-16% and 2-10% with fruits, leaves and stem residues respectively. In this connection, the effect of water soluble constituents of plant residues on water uptake by seeds, was studied [20]. It was found that the rate of seed germination in maize, soyabean, wheat and sorghum treated with aqueous extracts of plant residues were lower than the corresponding rates for seeds treated with distilled water. Similarly, the presence of biologically active substances in the *Artemisia afra* soil may have inhibitory effect on seed germination and emergence of fababean, pea, lettuce and tomato [21].

Table 2 reveals that the shoot and root lergths in all tomato varieties decreased when the soil treated with *Calotropis procera* residues. Addition of fruit residues to the soil infested with *Fusarium oxysporum* increased the shoot and root lengths in the tested tomato cultivers, while the other plant residues (leaves and stem) showed no significant variations. Our results are in agreement with [22] who reported that the plant growth parameters in okra and groundnut were improved in the presence of *C. procera* and *Trichoderma viride* or *T. harzianum*. In this connection, the castor leaf extract mixed with *Paecilomyces lilacinus* spores increased tomato growth when compared with *C. procera* extract mixed with *Paecilomyces spores* or *Paecilomyces* alone [23].

Table 3 shows that 20 fungal species representing eleven genera were recorded from the different treated soils, of which the genus *Aspergillus* represented by 6 species, whereas *Curvularia, Fusarium* or *Penicillium* represented by 2 species only. There are significant variations in species composition between untreated rhizosphere and surrounding soil. Seven species were isolated from the rhizosphere soil, 2 of which not recorded in the surrounding soil, namely *Helminthosporium sativum* and *Penicillium lansoum*. Also, 3 of eight species recorded in the uatreated soil were not detected in the rhizosphere, namely *Aspergillus luchuensis, Cunninghamella echinulata* and *Mucor plumbeus*.

Addition of different aak plant treatments to the soil, caused alternations in species composition as compared with untreated soil (control). The highest record of variation in the rhizosphere (4 species) was that recorded under the stem residues of aak treatment and the least number (2 species) was recorded under the leaves or fruits treatment. Similarly the highest record of species variation in the surrounding soil (5 species) was recorded under the leaf treatment and the least number (3 species) recorded under the fruit treatment. The treatment of fruit resides of aak plant caused complete disappearance of the pathogen *Fusarium oxysporum* f.sp. *lycopersici*, indicating the high sensitivity of this organism to the phytotoxins of aak fruits. The rhizosphere is frequently used as a model

Table 3: Records of fungal species isolated by soil plate method from the rhizosphere of tomato cv. Flora-dade (R_1-R_4) and surrounding soil (S_1-S_4) treated with *Calotropis procera* residues (stem, leaves or fruits) infested with *Fusarium oxysporum* after 30 days from sowing

			Treated soil with *Calotropis procera* residues							
	Untreated soil (control)		Stem		Leaves		Fruits		Prevalence (%)	
Fungal species	R_1	S_1	R_2	S_2	R_3	S_3	R_4	S_4	R	S
Fusarium oxysporum f.sp. *lycopersici*	+	+	+	+	+	+	-	-	75	75
Aternaria geophila	-	-	-	-	-	+	+	+	25	50
Aspergillus carbonarius	+	+	-	+	-	-	-	+	25	75
A. flavus	-	-	+	+	-	-	-	-	25	25
A. fumigatus	-	-	-	-	-	+	-	-	0	25
A. luchuensis	-	+	-	-	-	-	-	+	0	50
A. niger	-	-	+	+	-	-	-	-	25	25
A. terreus	+	+	+	-	+	+	-	-	75	50
Cladosporium epiphyllum	-	-	-	-	-	-	+	+	25	25
Cunninghamella echinulata	-	+	-	-	+	-	-	-	25	25
Curvularia subulata	-	-	-	-	-	-	-	+	0	25
C. geniculata	-	-	-	-	-	+	-	-	0	25
Fusarium equiseti	+	+	-	-	-	-	-	-	25	25
Humicola brevis	+	+	+	-	-	-	+	-	75	25
Helminthosporium sativum	+	-	-	-	-	-	-	-	25	0
Mucor plumbeus	-	+	-	-	-	-	-	+	0	50
Penicillium lanosum	+	-	+	+	-	+	+	-	75	50
P. waksmani	-	-	+	-	-	-	-	-	25	0
Rhizopus stolonifer	-	-	-	+	-	+	-	-	0	50
Stemphylium atra	-	-	+	-	+	-	-	-	50	0
No. of total species	7	8	8	6	4	7	4	6		
No. of species in R & absent in S or vice versa	2	3	4	2	2	5	2	4		
No. of species in the treated soil compared with untreated soil		4	4	2	5	2	3			

Table 4: Effect of *Calotropis procora* residues (stem, leaves, fruits) on the count of filamentous fungi and bacteria per g air dry soil in the rhizospher (R) and surrounding soils (S) of three tomato cultivars grown in soil infested with *Fusarium oxysporum* f.sp. *lycopersici* after 30 days from sowing

	Tomato cultivars																	
	Flora dade						Castle rock						Strain-B					
	F (x 10^2)		B (x 10^4)		R/S ratio		F (x 10^2)		B (x 10^4)		R/S ratio		F (x 10^2)		B (x 10^4)		R/S ratio	
Treatments	R	S	R	S	F	B	R	S	R	S	F	B	R	S	R	S	F	B
Plant	27	14	151	144	3.2	1.0	25	18	135	148	1.4	0.9	35	20	130	116	1.8	1.1
Fusarium oxysporum	30	16	200	156	1.9	1.3	32	20	140	172	1.6	0.8	37	23	142	115	1.6	1.2
Stem residue	23	17	150	100	1.6	1.5	27	20	125	110	1.3	1.1	26	17	130	95	1.5	1.4
Leaf residue	25	13	145	140	1.9	1.0	26	22	115	90	1.2	1.3	22	18	133	105	1.2	1.3
Fruit residue	24	11	160	89	1.7	1.9	24	18	140	131	1.3	1.1	17	15	135	90	1.1	1.5
F. oxysporum+Stem residue	26	15	135	120	1.7	1.1	21	13	115	110	1.6	1.0	22	18	123	91	1.2	1.4
F. oxysporum+Leaf residue	24	11	110	100	2.2	1.1	27	11	120	90	2.5	1.3	18	17	115	75	1.0	1.5
F. oxysporum+Fruit residue	18	7	142	85	2.6	1.7	14	9	110	80	1.5	1.4	20	14	100	79	1.4	1.3
LSD: 1%	9.2	6.1	25.2	24.4	1.6	1.2	10.2	8.4	15.1	13.4	0.8	0.7	8.9	7.3	15.0	12.3	1.8	1.0
5%	6.0	3.2	20.0	17.3	0.5	0.4	7.3	5.5	9.8	10.3	0.3	0.4	5.2	4.4	10.1	7.1	0.4	0.3

environment to screen for putative biological control agents of soil borne plant pathogen [24-26]. Twenty fungal species were isolated from Adiantum rhizosphere soil amended with *Calotropis procera* residues [27].

The results presented in Table 4 reveal that the fungal accumulation in the soil was inhibited more prominently around the root (rhizosphere) due to the presence of aak plant residues. For tomato cv. Strain B, the fungal R/S ratio was reduced from 1.8 to 1.1 in the treatment of fruit residues. Similarly, for cv. Flora-dade R/S ratio decreased to a minimum 1.6 by the other residues (aak stem). However, in case of tomato cv. Castle-rock, the

Table 5: Growth rate (mm) of selected fungi as measured by soil growth tubes adjusted to various levels of *Calotropis procora* leaf extract. Incubation period was 10 days at 27°C

	Concentrations (%)				LSD	
Species	0	5	10	20	5%	1%
Fusarium oxysporum	35	37	32	29	1.9	2.3
Aspergillus carbonarius	63	62	57	54	5.1	8.3
Penicillium lanosum	51	50	48	47	4.4	6.8
Humicola brevis	46	42	43	41	5.0	7.5

fungal R/S ratio was not significantly affected by *Calotropis procera* residues. Our results are in accordance with [28] who studied the effect of foliar sprays of leaf extracts of *Calotropis procera* and *Datura metal* at concentrations up to 16% on the rhizosphere of pearlmillet. They found that the fungal population decreased with increasing the concentrations of leaf extract, where as 16% was slightly phytotoxic. The frequency and fungal population was higher in the soil amended with urea, while the lowest population was recorded in the soil treated with *C. procera* followed by *Agremone mexicana* [27]. On the ather hand, Bacterial rhizosphere counts were not significantly different from that of the control (untread soil) coupled with drop in the surronnding soil. This resulted in the increased R/S values to a maximum 1.5 and 1.9 under the fruit treatment for both Strain-B and Flora-dade tomato cultivars respectively, while slightly increased from 0.9 (control) to 1.3 in the treatment of leaves for cv. Castle-rook. The combined effect of both aak plant residues and the pathogenic fungus *Fusarium oxysporum* on the fungal accumulation in the soil is also presented in Table 4. Soil inoculation with *Fusarium oxysporum* increased the fungal and bacterial counts in the rhizosphere of all tomato cultivars and in the surrounding soil. When *Fusarium oxysporum* coupled with aak plant residues, the microbial accumulation in the soil dropped as compared with the fungus alone. Addition of organic amendments (*C. procera*) into the soil inoculated with VAM, caused an improvement in the mycorrhizal infection in roots and the spore population in the rhizosphere *Catharanthus roseus* [29]. The largest population of Vesicular Arbuscular Mycorrhizal (VAM) fungal spores was recorded in the rhizospheres of *Cymbopogon flexosus* and *Calotropis procera* [30].

Table 5 and 6 show that in cases of *Fusarium oxysporum* f.sp. *lycopersici* and *Aspergillus carbonarius*, the mycelial growth, percentage germination and germ-tube length are inhibited at increased concentrations of *Calotropis* extracts. The least values occurred at concentration 20%. *Humicola brevis* was not affected, where all growth activities of this fungus under all different concentrations were not different as compared with the control. Presence of *Calotropis* aqueous extract which lowered the percentage germination of *Penicillium lanosum* after 8 hours without affecting the tube length and growth. *Calotropis procera* proved to be phytotoxic and antimicrobial against soil borne fungi, Gram +ve and Gram –ve bacteria [9, 31-34]. In contrast, *Calotropis procera* extract has no effect on chickpea wilt caused by *Fusarium oxysporum* f.sp. *cicers* [35].

In conclusion, seedling emergence of tomato, root and shoot length were greatly improved when residues of aak plant incorporated into the soil infested with the pathogen *F. oxysporum* and caused significant alternations in fungal species composition in tomato rhizosphere. Aqueous aak extracts (20% w/v) inhibited the growth of almost all tested fungi.

Table 6: Percentage germination and average length of germ tubes (µm) of selected fungi under various concentrations (%) of *Calotropis procera* aqueous leaf extract at 27°C

		Concentrations (%)					LSD	
Species		0	5	10	20	Incubation period(h)	5%	1%
Fusarium oxysporum	G(%)	69.8	61.2	55.6	53.0	8	5.1	9.5
	Gt(µm)	13.4	12.6	10.2	9.0	8	2.1	5.3
Aspergillus carbonarius	G(%)	80.0	82.0	75.0	73.0	6	4.5	8.3
	Gt(µm)	16.0	14.0	9.6	8.2	6	3.0	7.1
Penicillium lanosum	G(%)	62.0	58.0	53.0	49.0	8	4.2	7.5
	Gt(µm)	11.0	11.0	10.5	9.4	8	1.8	2.5
Humicola brevis	G(%)	45.0	43.0	42.0	40.0	10	5.5	8.7
	Gt(µm)	12.9	12.6	11.2	10.6	10	3.5	7.0

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