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Medicinal Plants Extracts as Source of Antifungal Agents against *Fusarium oxysporum* f. sp. *albedinis*

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ABSTRACT

***Fusarium oxysporum* f. sp. *albedinis* (Foa) is a soil borne fungus causing the most serious disease of date palm *(Phoenix dactylifera* L.) called "Bayoud". In the present study, five medicinal plants from the Algerian Sahara (Southwest of Algeria): *Limoniastrum feei* (aerial part, roots), *Launeae arborescens* (Batt.) Murb. (aerial part, roots), *Fredolia aretioides* Moq. et Coss. (aerial part, roots), *Asteriscus graveolens* (Forsk) (leaves, stems) and *Acacia raddiana* (leaves, bark), were used to evaluate their extracts for antifungal activity against Foa. Two parts from each plant were used for extraction by four solvents: methanol, ethyl acetate, dichloromethane and hexane. The antifungal test was conducted using disc diffusion technique and relative virulence (RV) test (on potato tuber tissue). For both tests, four extract quantities were used (200, 400, 800 and 1,600µg). The relative virulence was presented as necrotic tissue weight (mg) of potato tuber tissue. Among all solvents, methanol had the best extraction yield (mean: 6.35%, minimum: 2.27%, maximum: 9.80%). The highest frequency of antifungal effect on Foa was presented by ethyl acetate extracts (32.50% of detectable effect). The best effect was observed for ethyl acetate extract of *Limoniastrum feei* (aerial part). The virulence test showed a decrease in RV up to 30% for ethyl acetate extract of *Launea arborescens* aerial part. The increase in RV was observed mostly for hexanic extract from *Fredolia aretioides* reflecting its high toxicity compared to the other extracts.**

Keywords: *Fusarium oxysporum* f. sp. *Albedinis*, Medicinal plants, Pathogenicity, *Phoenix dactylifera* L., Virulence.

INTRODUCTION

Palm trees (*Phoenix dactylifera* L.) constitute the ecological and socio-economic womb of the Saharian populations. They offer a suitable microclimate for other crops such as fruits, cereals, etc., and they also protect them from the wind. Palm trees represent a basic food source for the people and animals of the Sahara and make a significant economic contribution to the country. This harmony represents the Saharan ecosystem (Ben Abdallah, 1990; Djerbi, 1991; Ouinten, 1996).

Many *Fusarium* species are serious plant pathogens, causing symptoms such us necrotic lesions, rot, and wilt (Herrmann *et al.*, 1996). *Fusarium oxysporum* f. sp. *albedinis* (Killian and Maire) Malençon, is the causal agent of Bayoud disease, which affects the date palm tree "*Phoenix dactylifera* L". Since its first signal before 1870 (Bounaga et Djerbi, 1990), the Bayoud has killed approximately 20 millions date palm trees in Morocco and Algeria. The only way to fight this disease is to prevent its spread to other date-growing areas in the region and farther field (Freeman and Maymon, 2000).

Studies on antifungal activity of medicinal plants against plant pathogens, especially Foa, are rare. *Limoniastrum feei*, *Launea arborescens*, *Fredolia aretioides, Asteriscus*

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graveolens and *Acacia raddiana* contain many important secondary metabolites with antimicrobial effects (flavonoids, tannins, etc) (Cheriti, 2000; Cheriti *et al*., 2004; Cheriti *et al*., 2005; Belboukhari and Cheriti, 2006; Belboukhari *et al.*, 2007; Boulenouar *et al.*, 2008; Belboukhari and Cheriti, 2009) that have important biological roles. Therefore, the aim of this study is to evaluate the effect of these plants extracts on Foa growth using disc diffusion technique and Foa virulence using potato tuber tissue technique.

MATERIALS AND METHODS

Plant Materials

Plant materials were collected from their natural habitat in the region of Bechar (Southwest of Algeria), during the period December 2007 to January 2008. All plant species were identified at the National Agency for Nature Protection (Bechar, Algeria) and voucher specimens are conserved at the phytochemical herbarium of Phytochemistry and Organic Synthesis Laboratory (POSL), University of Bechar, Algeria (Cheriti, 2000) under the following codes: *Acacia raddiana* (CA00/37), *Asteriscus graveolens* (CA00/14), *Fredolia aretioides* (CA00/42), *Launea arborescens* (CA99/25) and *Limoniastrum feei* (CA99/14). The collected fresh plant materials were air-dried in the shade and each part of each plant was separated. The five plants and the parts used are given in Table 1.

Fungal Strain

The fungal strain used in this study is *Fusarium oxysporum* f. sp. *albedinis*, the causal agent of Bayoud disease that affects the date palm trees (*Phoenix dactylifera* L). The strain used in this study was obtained from The Technical Institute for Saharian Agronomy (TISA), Adrar, Algeria.

Preparation of Plant Extracts

Methanol (MeOH), ethyl acetate (EtOAc), dichloromethane (DCM) and hexane were used as organic solvents with different polarity to extract the active constituents in the plants tissues. In each sequence of extraction, 10 g portion of the powdered air dry plant plus 80 ml of the solvent were kept for 2 hours in soxhlet extractor. Dry weight, after filtration and evaporation, were determined and kept in screw cap tubes at 5°C.

Bioassay and Media Used

Sufficient number of Whatman paper discs (6 mm ∅ and 1 mm thickness) were sterilized by autoclaving at 121°C for 15 minutes, and kept overnight at 70°C to ensure dryness. Each disc was impregnated

Table 1. Tested medicinal plants and their used parts.

Plant families	Plant species	Part used
Fabaceae	*Acacia raddiana*	Leaves
		Bark
Asteraceae	*Asteriscus graveolens*	Leaves
		Branches
Chenopodiaceae	*Fredolia aretioides*	Aerial part
		Roots
Asteraceae	*Launeae arborescens*	Aerial part
		Roots
Plumbaginaceae	*Limoniastrum feei*	Aerial part
		Roots

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with one of the following extract weights (200, 400, 800 and 1,600 µg) dissolved in an appropriate volume of solvent and kept at 40°C to dryness (all manipulations were done in sterile conditions).

Spore suspension of the fungal test micro-organism was prepared by transferring 7 days old culture of Foa on potatoes dextrose agar medium PDA (consisting of: 4 g potatoes extract, 20 g glucose, 15 g agar, distilled water up to 1 liter) to synthetic nutrient poor agar SNA (consisting of: 1 g KH_2PO_4, 1 g KNO_3, 0.5 g $MgSO_4$-$7H_2O$, 0.5 g KCl, 0.2 g glucose, 0.2 g saccharose, 15 g agar, distilled water up to 1 liter) to induce spores formation. Ten days old culture of Foa on SNA was surface flooded with 10 ml of sterilized water to dislodge fungal spores, then, filtrated to eliminate mycelia fragments. The concentration of Foa spores was adjusted to approximately 10^6 spores ml^{-1} by dilution and counting.

The antifungal test was carried out by disc diffusion technique. Sterile Petri dishes (90 mm ∅) containing PDA media were inoculated with 100 µl of Foa spores suspension (10^6 spores/ml). Sterile discs (6 mm ∅) containing the plants extracts (200, 400, 800 and 1,600 µg) were deposed on the inoculated PDA plates (incubation at 21°C for 5 days). The results were obtained as the mean of three measurements of the inhibition zone diameter (mm). The negative control was discs passing all protocol without use of plant extracts.

Effect of Plant Extracts on Virulence of Foa

The virulence assay was carried out as described by Herrmann *et al.* (1996) with slight modification. New potatoes (*Solanum tuberosum* L.) were surface sterilized for 5 minutes in 1% sodium hypochlorite and washed three times with sterile water. After being dried, the potatoes were cut into slices 6 mm thick and placed on sterile filter paper, soaked with sterile water, in sterile Petri dishes. The discs containing the plant extracts (200, 400, 800 and 2,000 µg) were applied on potatoes slices. After 5 minutes, each potatoes slice was infected with a slice (12 mm ∅, mycelial side down) of a 7-days old Foa culture grown on PDA media and incubated for 6 days at 21°C in the dark. The necrotic tissues were weighted (mg). The results were compared to the virulence of Foa (discs without plant extracts) as relative virulence (RV). The negative control was discs passing all protocol without use of plant extracts. No substance has been reported effective against Foa for use as a positive control.

Experimental Design and Data Analysis

All the experiments were carried out as randomized complete blocks. All the collected data were submitted to ANOVA test, correlation test, and analysis of frequencies using Statistical software v. 5 (Statsoft, ed'97) and the significance of differences among treatments was recorded at $P< 0.05$ (Quinn and Keough, 2002). Results are presented as means (n= 3) (standard errors were less than 20%, except otherwise cited).

RESULTS

The correlations between solvent/extraction yield and part of plant/extraction yield were significant ($P< 0.05$). As for plant parts, this reflected that changing plant part influences significantly the extraction yield. The yields can be arranged in the following decreasing order: branches (mean: 4.60%, max: 9.80%, min: 0.61%), leaves (mean: 4.54%, max: 9.53%, min: 1.11%), bark (mean: 3.08%, max: 6.85%, min: 1.18%), aerial part (mean: 2.65%, max: 6.33%, min: 0.37%) then roots (mean: 1.87%, max: 5.62%, min: 0.57%). Concerning the correlation between solvent and extraction yield; the yields can be arranged in the following decreasing order: methanol (mean: 6.35%, max: 9.80%, min:

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2.27%), ethyl acetate (mean: 2.48%, max: 7.04%, min: 0.57%), dichloromethane (mean: 1.73%, max: 5.69%, min: 0.53%) then hexane (mean: 1.56%, max: 3.27%, min: 0.37%). The correlation was not significant for plant/extraction yield ($P< 0.05$) (Table 2).

The results of antifungal actions against Foa demonstrated that the five medicinal plants extracts had a detectable effect at least in two tests as for *Launeae arborescens* (800 and 1,600 µg from ethyl acetate extract of the roots) confirming the presence of active antifungal principals therein.

Analysis of frequencies demonstrated that most of the materials tested (86.88%) had no detectable effect on Foa. Low potency against Foa (diameter of inhibition zone: 8-14 mm) was shown by 10.62% of the 160 tests conducted. Moderate antifungal effect (inhibition zone diameter: 15-20 mm) was exhibited by only 2.50% of the experiments.

One way ANOVA test demonstrated that the effect of solvents (used for extraction) on Foa was significant ($P< 0.05$); the antifungal effects were represented principally by ethyl acetate, hexane, and methanol, respectively. For ethyl acetate extracts, 13 out of 40 experiments (32.50%) had detectable effects on Foa. The most important antifungal potency (∅: 19 mm) was demonstrated by ethyl acetate extracts of *Limoniastrum feei* aerial part (1600 µg). Five out of 40 experiments (12.50%) with hexanic extracts demonstrated detectable effects on Foa; the most important antifungal potency (∅: 16 mm) was shown by hexanic extracts of *Fredolia aretioides* aerial part. Methanolic extracts had effect on Foa only in the case of *Acacia raddiana* (low effect, ∅: 8-14 mm). The extracts obtained by dichloromethane had no detectable effect on Foa. The effect of plant species and part used were not significant ($P< 0.05$).

The effect of extract weight in discs (200, 400, 800 and 1,600 µg) on Foa was significant ($P< 0.05$). At 200 µg, only one experiment out of 40 had detectable effect on Foa (∅: 12 mm) and that belonged to ethyl acetate extract of *Limoniastrum feei* aerial part. At 400 µg, two experiments out of 40 gave detectable effect on Foa, corresponding to ethyl acetate extract of *Limoniastrum feei* aerial part (∅: 11 mm) and ethyl acetate extract of *Asteriscus graveolens* leaves (∅: 10 mm). At 800 µg, eight experiments out of 40 exhibited detectable effect on Foa in the interval (∅: 8-14 mm) represented mostly by ethyl acetate extracts (n= 5, 62.50%). At 1,600 µg, the cases with detectable effect rose to ten out of 40 in the interval (∅: 10-19 mm), mostly by ethyl acetate extracts (n= 5, 50.00%) and hexanic extracts (n= 3, 30.00%) (Table 3).

Table 2. Extraction yield (%) from dry weight of plant**.**

Plant species	Part used	Solvent used for extraction			
		Methanol	EtOAc[a]	DCM[b]	Hexane
Acacia	Leaves	8.91	1.11	1.85	2.80
Raddiana	Bark	6.85	2.82	1.48	1.18
Launeae	Aerial part	5.08	3.56	5.69	1.26
arborescens	Roots	5.62	1.81	0.69	2.90
Limoniastrum	Aerial part	6.33	1.30	0.53	0.37
Feei	Roots	2.27	0.86	0.84	1.03
Asteriscus	Leaves	9.53	4.72	4.13	3.27
graveolens	Branches	9.80	7.04	0.61	0.93
Fredolia	Aerial part	4.69	1.05	0.78	1.17
aretioides	Roots	4.40	0.57	0.67	0.74

[a] Ethyl acetate, [b] Dichloromethane.

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Table 3. Antifungal activity of plants extracts against *Fusarium oxysporum* f. sp. *albedinis* as diameter of inhibition zone (mm).

			Extract weight in discs (µg)			
Plant species	Part used	Solvent	200	400	800	1600
Acacia raddiana	Leaves	Methanol	ND[a]	ND	10	12
		Ethyl acetate	ND	ND	ND	ND
		Dichloromethane	ND	ND	ND	ND
		Hexane	ND	ND	ND	10
	Bark	Methanol	ND	ND	ND	11
		Ethyl acetate	ND	ND	13	18
		Dichloromethane	ND	ND	ND	ND
		Hexane	ND	ND	ND	ND
Launeae arborescens	Aerial Part	Methanol	ND	ND	ND	ND
		Ethyl acetate	ND	ND	ND	ND
		Dichloromethane	ND	ND	ND	ND
		Hexane	ND	ND	ND	ND
	Roots	Methanol	ND	ND	ND	ND
		Ethyl acetate	ND	ND	11	15
		Dichloromethane	ND	ND	ND	ND
		Hexane	ND	ND	ND	ND
Limoniastrum Feei	Aerial Part	Methanol	ND	ND	ND	ND
		Ethyl acetate	12	11	14	19
		Dichloromethane	ND	ND	ND	ND
		Hexane	ND	ND	ND	ND
	Roots	Methanol	ND	ND	ND	ND
		Ethyl acetate	ND	ND	ND	ND
		Dichloromethane	ND	ND	ND	ND
		Hexane	ND	ND	ND	ND
Asteriscus graveolens	Leaves	Methanol	ND	ND	ND	ND
		Ethyl acetate	ND	10	11	10
		Dichloromethane	ND	ND	ND	ND
		Hexane	ND	ND	8	10
	Branches	Methanol	ND	ND	ND	ND
		Ethyl acetate	ND	ND	ND	ND
		Dichloromethane	ND	ND	ND	ND
		Hexane	ND	ND	ND	ND
Fredolia aretioides	Aerial Part	Methanol	ND	ND	ND	ND
		Ethyl acetate	ND	ND	ND	ND
		Dichloromethane	ND	ND	ND	ND
		Hexane	ND	ND	9	16
	Roots	Methanol	ND	ND	ND	ND
		Ethyl acetate	ND	ND	9	13
		Dichloromethane	ND	ND	ND	ND
		Hexane	ND	ND	ND	ND

[a] Not detected.

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Virulence Test

Ten extracts from five medicinal plants (two parts for each plant) were used to test their effect on Foa virulence (on potato tuber tissue) with different quantities (200, 400, 800 and 1,600 µg) (n= 160). After 6 days of incubation in dark, necrotic lesions were visible compared with the slices without Foa culture and/or plants extracts. The presence of necrosis was dependent on extracts and/or Foa effect. The results presented relative virulence (RV) compared to Foa virulence without plants extracts. No correlation was detected between extract weight in discs and RV ($P< 0.05$), or between zone of inhibition (mm) and RV ($P< 0.05$).

In analysis of frequencies, the majority of tests (n= 97, 61.25%) showed a decrease in the RV of Foa on potato tuber tissue (< 100%). Only 3 out of 160 tests (1.88%) presented RV approximately equal to (100±1%). Sixty out of 160 tests (37.50%) exhibited RV superior to Foa (> 100%). The maximum value of relative virulence was presented by methanolic extracts (1,600 µg) of *Fredolia aretioides* roots (NTW= 414.3 mg, RV= 625%), which showe an increase in virulence more than six times compared to Foa alone. The minimum relative virulence belonged to ethyl acetate extract (400 µg) of *Launea arborescens* aerial part (NTW= 19.7 mg, RV= 30%), a decrease in virulence more than three times compared to Foa alone.

The analysis of variance (ANOVA) showed that the medicinal plants effect on Foa RV was significant ($P< 0.05$), but the effect of the part used was not significant ($P< 0.05$). For each plant, thirty two tests were conducted. With regard to RV reduction, which means decrease of RV below (100%), we can rank the five medicinal plants as follow: *Asteriscus graveolens* (25 out of 32 tests: 78%), *Launea arborescens* (23 out of 32 tests: 72%), *Limoniastrum feei* (21 one out of 32 tests: 66%), *Acacia raddiana* (17 out of 32 tests: 53%), *Fredolia aretioides* (11 out of 32 tests: 34%). As to RV augmentation, which means increase of RV above (100%), we can rank the five medicinal plants as follow: *Fredolia aretioides* (21 out of 32 tests: 66%), *Acacia raddiana* (15 out of 32 tests: 47%), *Limoniastrum feei* (10 out of 32 tests: 31%), *Launea arborescens* (8 out of 32 tests: 25%), *Asteriscus graveolens* (6 out of 32 tests: 22%).

ANOVA test demonstrated that the solvent effect on Foa RV was significant ($P< 0.05$). For each solvent, forty tests were run. The best effect was observed for dichloromethane extracts (29 out of 40 tests: 72%) compared to the other solvents: ethyl acetate (27 out of 40 tests: 68%), hexane (25 out of 40 tests: 62%), methanol (16 out of 40 tests: 40%). Based on the increasing effect of RV above (100%), we can rank the extracts as follow: methanolic extracts (23 out of 40: 58%), hexanic extracts (14 out of 40: 35%), ethyl acetate extracts (12 out of 40: 30%), then dichloromethanic extracts (11 out of 40: 28%).

DISCUSSION

In this study, we have evaluated the effect of five medicinal plants extracts on the causal agent of Bayoud "*Fusarium oxysporum* f. sp. *albedinis*" (Foa), a telluric pathogen of the date palm tree "*Phoenix dactylifera* L". The five plants were chosen on the basis of traditional knowledge and scientific research conducted at the Phytochemistry and Organic Synthesis Laboratory (POSL), University of Bechar, Algeria; Laboratory of Plant Biochemistry and Natural Substances, Oran University, Algeria. We investigated the direct effect of the extracts on the fungus by disc diffusion technique and testing the effect of these extracts on Foa virulence (on potato tuber tissue). No study had exhibited the effect of these plants on Foa. The effect of four poisonous plants extracts from the Southwest of Algeria has been demonstrated by Boulenouar *et al.* (2009).

[DOR: 20.1001.1.16807073.2012.14.3.7.1]

In spite of the scientific importance of the medicinal plants of the southwest of Algeria, the five plants used in this study have been widely examined for their traditional uses, antimicrobial effect and chemical composition (Cheriti, 2000; Cheriti *et al*., 2005; Belboukhari and Cheriti, 2006; Belboukhari *et al.*, 2007; Boulenouar *et al.*, 2008), but less work has been done regarding their biological effects on Foa (Boulenouar *et al.*, 2008). In addition, the use of natural products for biological control against pathogens gives encouraging results regarding adverse effects on environment, contrary to the synthetic pesticides (El Hassni *et al.*, 2007).

Research at the Phytochemistry and Organic Synthesis Laboratory (POSL, Bechar University, Algeria) (Cheriti *et al.*, 2005; Belboukhari *et al.*, 2007), has demonstrated that these plants contain secondary metabolites that have biological activity. Daayf *et al.* (2003) have previously reported that phenolic compounds synthesis is induced in date palm (*Phoenix dactylifera* L.) callus by *Fusarium oxysporum* f. sp. *Albedinis,* which are characterized mainly as hydroxycinnamic acid derivatives. The antifungal effect of these plants extracts against Foa may be due to the secondary metabolites (phenolic compounds, etc) present in them. The difference in the degree of the effect is proportional to the nature and/or quantity of the secondary metabolites contained in these plants (Tables 3 and 4).

The extraction yield was significantly affected by the solvent and the part of plant used. As for the solvents, this is principally related to the polarity and capability to extract substances that can be dissolved in the used solvent (polarity index: 5.1 for MeOH, 4.4 for EtOAc, 3.1 for DCM, 0.0 for hexane) (Takahiro *et al.*, 2004; Andri *et al.*, 2009). The methanol was the most powerful regarding the extraction yield, it was the strongest in extracting more substances; on the other hand, the plants used contained more substances that preferably dissolve in methanol. The substances contained in different plant parts vary in nature and quantity; e.g. while branches give the highest extraction yields, the aerial part contains more substances than the roots; therefore, this difference affects the yield of extraction using the same solvent (Table 2).

Extracts obtained by ethyl acetate presented the best values of zones of inhibition and the most important effects based on the proportion of ethyl acetate extracts compared to other extracts that showed effect on Foa It has been demonstrated that ethyl acetate gave a good extraction of phenolic compounds (Rolando and González, 2005; Fang *et al.*, 2007). Therefore, the effect can be related to phenolic compounds. Belboukhari and Cheriti (2005) established no effect of ethyl acetate extract from leaves and twigs of *Limoniastrum feei* on two fungi: *Candida albicans* and *Saccharomyces cerevisiae*. The difference with our results is possibly due to difference between species biology (Foa, *C. albicans* and *S. cerevisiae*) and/or to difference between plant parts used for extraction.

Among all the tests (n= 160), only a small portion showed detectable effects (low: 10.62%, moderate: 2.50%), which means Foa was resistant to the majority of these plants extracts at the used quantities (200, 400, 800 and 1,600 µg). Very clear differences were found for the effects of different extract weight per disc (200, 400, 800 or 1,600 µg). These results are in agreement with the idea that increasing extract weight is proportional to active substance(s) present in the extract, reflecting more effect on Foa.

Herrmann *et al.* (1996) evaluated the relative virulence of some *Fusarium* strains and *Fusarium oxysporum* formae speciales on potato tuber tissue, but their study did not include *Fusarium oxysporum* f. sp. *albedinis.* These authors used *Fusarium sambucinum* BBA 62397 as reference (100%), which causes 2.6 g of necrotic cells. Compared to our results, Foa represents relatively important virulence (0.066 g of necrotic tissue) that represents a relative virulence equal to 2.54% because out of the

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Table 4. Effect of plants extracts on relative virulence of Foa (on potato tuber tissue).

Plant Species	Part used	Solvent	Extract weight in discs (µg)							
			200		400		800		1600	
			NTW (mg)	RV (%)	NTW (mg)	RV (%)	NTW (mg)	RV (%)	NTW (mg)	RV (%)
Acacia raddiana	Leaves	Methanol	100.2	151	78.9	119	95.8	144	62.6	94
		EtOAc	73.2	110	49.8	75	38.5	58	44.6	67
		DCM	70.0	106	95.2	144	75.1	113	59.6	90
		Hexane	217.8	329	89.2	135	79.2	119	49.5	75
	Bark	Methanol	94.5	143	51.5	78	60.8	92	86.6	131
		EtOAc	63.6	96	103.4	156	42.0	63	44.9	68
		DCM	131.8	199	82.4	124	52.2	79	34.5	52
		Hexane	55.5	84	42.5	64	32.0	48	33.1	50
Launeae arborescens	Aerial part	Methanol	38.0	57	40.8	62	44.0	66	66.9	101
		EtOAc	43.0	65	19.7	30	51.2	77	52.4	79
		DCM	44.1	67	57.2	86	47.5	72	30.1	45
		Hexane	59.0	89	56.8	86	69.3	105	97.8	148
	Roots	Methanol	76.1	115	106.1	160	81.4	123	111.5	168
		EtOAc	58.0	87	25.4	38	32.3	49	38.8	59
		DCM	48.5	73	35.9	54	43.4	65	71.9	108
		Hexane	68.5	103	38.7	58	47.4	71	55.0	83
Limoniastrum Feei	Aerial part	Methanol	72.3	109	77.0	116	119.0	179	111.9	168
		EtOAc	59.7	90	56.5	85	71.7	108	65.6	99
		DCM	78.9	119	43.5	66	51.9	78	53.0	80
		Hexane	59.5	90	76.1	115	63.2	95	47.5	72
	Roots	Methanol	46.5	70	36.5	55	52.5	79	88.1	133
		EtOAc	58.3	88	37.3	56	35.4	53	31.5	48
		DCM	43.3	65	39.3	59	72.6	110	57.6	87
		Hexane	46.8	71	33.6	51	46.9	71	71.6	108
Asteriscus graveolens	Leaves	Methanol	53.3	80	42.6	64	70.4	106	83.2	125
		EtOAc	22.1	33	59.6	90	52.0	78	121.6	183
		DCM	42.1	63	27.1	41	40.5	61	27.1	41
		Hexane	78.9	119	78.3	118	37.6	57	55.2	83
	Branches	Methanol	36.8	56	64.4	97	58.5	88	50.7	76
		EtOAc	50.6	76	63.8	96	28.2	43	38.1	57
		DCM	32.3	49	47.1	71	38.1	57	52.9	80
		Hexane	53.4	81	47.5	72	67.0	101	71.7	108
Fredolia aretioides	Aerial part	Methanol	88.0	133	45.5	69	70.7	107	127.0	192
		EtOAc	67.4	102	71.8	108	76.8	116	121.0	183
		DCM	37.3	56	47.0	71	70.6	106	82.7	125
		Hexane	69.1	104	62.2	94	48.0	72	54.9	83
	Roots	Methanol	73.1	110	249.2	376	321.4	485	414.3	625
		EtOAc	136.8	206	324.1	488	74.0	112	87.7	132
		DCM	49.4	75	71.2	107	55.7	84	39.3	59
		Hexane	43.2	65	44.8	68	91.6	138	78.3	118

EtOAc: Ethyl acetate, **DCM:** Dichloromethane, **NTW:** Necrotic tissue weight (mg), **RV:** Relative virulence (%), the virulence was compared to Foa virulence without plants extracts (which was set at 100%, corresponding to 66.3±1.7 mg of decomposed potato tissue per slice).

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36 strains studied by Herrmann *et al.* (1996), 6 strains had relative virulence lower than 2.54%; and three out of four formae speciales of *Fusarium oxysporum* had relative virulence lower than 2.54%.

As presented by Amraoui *et al.* (2005), the necrotic effect of Foa on potato tuber tissue is due principally to enniatin production. The enniatin is a host non-specific mycotoxine. It is one of the mycotoxines responsible for Foa phytotoxicity (Herrmann *et al.*, 1996). The effect of extracts on RV of Foa comes about by, possibly, affecting the synthesis and/or action of enniatin on cells. The best decreasing effect on RV is represented by dichloromethane extracts; it may act on enniatin production or antagonise its mode of action. In addition, there is a possibility of cells immunization against Foa effect. The increase of RV above 100% reflects cytotoxicity (hexanic extracts) that can be explained by high toxicity of the extracts and/or effect of extracts on enniatin production by Foa (increasing the effect and/or the production of enniatin).

As demonstrated by Bosch and Mirocha (1992) and Bacon *et al.* (1996), the virulence of *Fusarium* species is due to production of fusaric acid, and other mycotoxines. *Fusarium oxysporum* f. sp. *albedinis* produces several toxins including fusaric, succinic, and 3-phenyl lactic acids and their derivatives, marasmins and peptidic toxins (Elhadrami *et al.*, 2005). These mycotoxines play an important role in pathogenicity and virulence of Foa, as primary determinants when they act as the key element in infection initiation and symptom development. They are secondary determinants when they only modify the symptom's intensity. The possible effect of the plant extracts used in this study, principally ethyl acetate extracts, is to act on one or more of these mycotoxines by modifying their metabolism or their effects.

A study by Belboukhari and Cheriti (2006) on phytochemical investigation of *Launea arborescens* demonstrated that this plant is rich in secondary metabolites, namely, tannins, saponins, flavonoids, terpenes and cardinolids. The good effect of *Launea arborescens* extracts on Foa RV at large scale was possibly due to, at least, one of these compounds.

Comparing the results obtained from disc diffusion technique and virulence test, we found that dichloromethane had no effect in the antifungal test, but had the highest effect in the virulence test. This controversy is possibly due to dichloromethane extracts action on non vital bio-substance(s) in Foa, which play a role in Foa toxicity without any effect on Foa culture, but decrease the virulence. Amraoui *et al.* (2005) showed that Foa fraction purified from the organic extracts of a Foa was unable to induce necrosis of potato slices, indicating that it does not contain significant amounts of enniatins. On the other hand, solution of fusaric acid and enniatins, which are secreted by several *Fusarium* species, were tested at different concentrations and were not capable of inducing symptoms on detached leaves. Thus, there is a kind of complementarity in development of the infection.

This study is a part of a larger research project of the Laboratory of Plant Biochemistry and Natural Substances (Oran University, Algeria) and Phytochemistry and Organic Synthesis Laboratory (Bechar University, Algeria). This work establishes the presence of antifungal substances against Foa in these medicinal plants affecting the Foa. Regarding the effect on Foa culture and Foa virulence, we recommend a combination in treatment for efficacy. Finally, we need more research in this field by investigating the rich nature of the local flora of Algeria to solve this problem.

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کاربرد عصاره گیاهان دارویی به عنوان منبع مواد ضد قارچ علیه فوزاریوم *Fusarium oxysporum* f. sp. *Albedinis*

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چکیده

فوزاریوم(Foa) *Fusarium oxysporum* f. sp. *albedinis* قارچی خاکزاد است که موجد مرضی جدی در نخل خرما(*Phoenix dactylifera* L.) می باشد که " بیود" نامیده می شود.در مطالعه حاضر، عصاره ۵ گیاه دارویی به نام های *Limoniastrum feei*(قسمت هوایی و ریشه) *Launeae arborescens* , (Batt.) Murb. (قسمت هوایی و ریشه), *Fredolia aretioides* Moq. et Coss. (قسمت هوایی و ریشه) *Asteriscus graveolens* (Forsk) (شاخه ها و ساقه)، *Acacia raddiana* ((برگها و پوست درخت) از صحاری الجزایر (در جنوب غربی الجزایر) به عنوان ماده ضد قارچ بر علیه فوزاریم مورد بررسی قرار گرفتند. دو بخش از هر گیاه برای عصاره گیری به وسیله ۴ عصاره گیر زیر استفاده شدند: متانول، اتیل استات، دی کلرو متان، و هگزان. در این مطالعه، آزمون ضد قارچ با استفاده از روشdisc diffusion و شدت نسبی بیماریزایی(RV) روی بافت های غده سیب زمینی انجام شد. در هر دو آزمون، چهار مقدار از عصاره گیرها به کار رفت (۴۰۰،۲۰۰، ۸۰۰، و ۱۶۰۰میکروگرم).شدت نسبی بیماریزایی به صورت وزن بافت مرده(میلی گرم) از بافت غده سیب زمینی بیان شد. در میان همه عصاره گیرها، متانول بیشترین مقدار عصاره را استخراج کرد(میانگین۶/۳۵%، کمینه،۲/۲۷%، و بیشینه ۹/۸۰%). بیشترین بسآمد اثرات ضد قارچی روی فوزاریم در مورد عصاره حاصله از اتیل استات به دست آمد (با ۳۲/۵۰%اثر قابل ردیابی). بهترین اثر در مورد عصاره اندام هوایی*Limoniastrum feei* استخراج شده به وسیله اتیل استات مشاهده شد. برای عصاره اندام هوایی *Launea arborescens* که باعصاره گیری به وسیله اتیل استات به دست آمده بود، آزمون شدت بیماریزایی، در حدود ۳۰٪ کاهش در RV نشان داد. افزایش RV بیشتر از همه در مورد عصاره گرفته شده با عصاره گیر هگزان از گیاه*Fredolia aretioides* دیده شد که نشان از سمیت بالای آن در مقایسه با دیگر عصاره ها داشت.

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