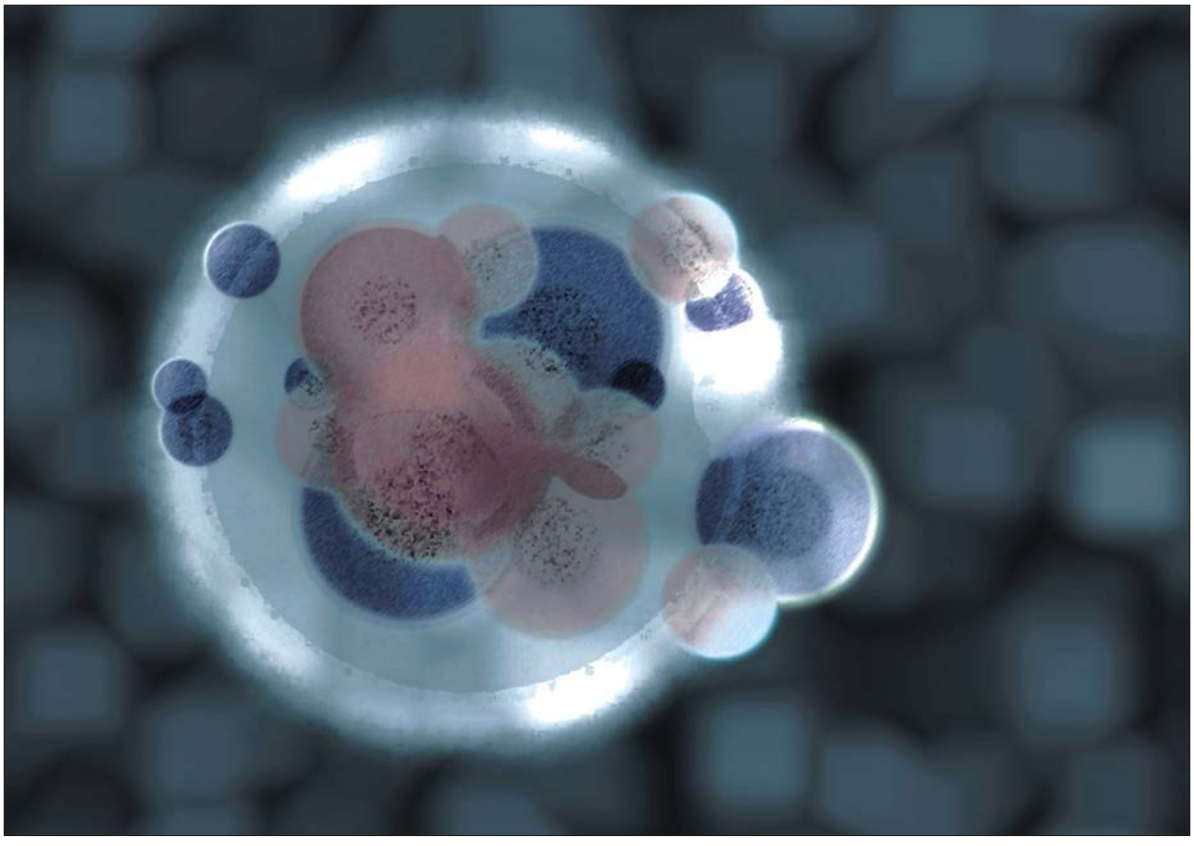

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# Effect of Some Poisonous Plants Extracts on *Fusarium oxysporum* f. sp. *albedinis*

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**Abstract:** In the present study, four poisonous plants from the Algerian Sahara (South-West of Algeria): *Citrullus colocynhis* (L.) Schrad, *Calotropis procera* Ait., *Nerium Oleander*, *Pergularia tomentosa* were used to evaluate their extracts for antifungal activity against *Fusarium oxysporum* f. sp. *albedinis* (Foa), the causal agent of the most dangerous disease of date palm (*Phoenix dactylifera* L.). Two parts from each plant were used for extraction by four solvents: methanol, ethyl acetate, dichloromethane and hexane. The antifungal test was realized 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 1600 μg). The relative virulence was presented as necrotic tissue weight (mg) of potato tuber tissue. Among all solvents, methanol gave the best extraction yield (mean: 13.24%, minimum: 8.46%, maximum: 21.91%). The results of disc diffusion assay showed that the most important effect on Foa was observed for hexanic extract of *Pergularia tomentosa* (stems), methanolic extracts of *Citrullus colocynthis* (leaves and stems) and *Nerium oleander* (leaves, stems). The virulence test showed a decrease in RV below 50% for methanolic and dichloromethanic extracts of *Citrullus colocynthis* (leaves and stems). Coupling the effect of plants extracts on Foa growth and virulence, *Citrullus colocynthis* extracts showed great efficacy than did other plants.

**Key words:** *Fusarium oxysporum* f. sp. *albedinis*, *Phoenix dactylifera* L., poisonous plants, pathogenicity, virulence

## INTRODUCTION

Date palm (*Phoenix dactylifera* L.) is qualified as tree of great ecological and socio-economical importance in desert oases (El-Hadrami *et al*., 2005). Among all pathologies of date palm, Bayoud is the most dangerous; it's caused by the telluric fungi *Fusarium oxysporum* f. sp. *albedinis* (Foa) (Bounaga and Djerbi, 1990). Till these lines, no curative treatment is available.

The idea of using natural products against plant pathogens becomes increasingly popular, because their side-effect is insignificant and often the intended main effect can be attained by them (Horvath *et al*., 2004). In addition to the traditional use of poisonous plants in tanning and arrows poisoning; they are toxic to human and animals, carcinogenic, causing abortion and keratitis etc. In the other hand, they are of great importance as source of agents, for treatment of various diseases (diabetes, cancer, antimicrobial, immunomodulatory), as insecticides, for animal and/or human consumption with caution (Habs *et al*., 1984; Faye, 1985; Abiola *et al*., 1993; Begum *et al*., 1997; Maatooq *et al*., 1997; Hamdy *et al*., 1999; Abdel-Hassan *et al*., 2000; Abbassi *et al*., 2004; Hamed *et al*., 2006; Sawaya *et al*., 2006; Al-Farwachi, 2007; Hassan *et al*., 2007; Pandey *et al*., 2009).

The aim of this study was the evaluation of four poisonous plants (*Citrullus colocynhis* (L.) Schrad, *Calotropis procera* Ait., *Nerium Oleander*, *Pergularia tomentosa*) extracts as source of antifungal agents against *Fusarium oxysporum* f. sp. *albedinis*. The analysis were realized using agar diffusion and relative virulence tests.

## MATERIALS AND METHODS

**Plant material:** During the period: December 2007 to January 2008, plants (*Citrullus colocynhis* (L.) Schrad, *Calotropis procera* Ait., *Nerium Oleander*, *Pergularia tomentosa*) were collected from their natural habitat in region of Bechar (South-West of Algeria). All plant species were identified at the National Agency for Nature Protection (Bechar, Algeria) and a voucher specimen is conserved at the phytochemical herbarium of Phytochemistry and Organic Synthesis Laboratory (POSL), University of Bechar, Algeria.

The collected fresh plant materials were air-dried in the shade and each part of each plant was separated. The four plants and parts used are given in Table 1.

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Table 1: Tested medicinal plants and their used parts

| Plant families | Plant species | Part used |
|---|---|---|
| Cucurbitaceae | *Citrullus colocynthis* | Leaves and stems |
| | | Fruits |
| Asclepiadaceae | *Calotropis procera* | Leaves |
| | | Stems |
| Apocynaceae | *Nerium oleander* | Leaves |
| | | Stems |
| Asclepiadaceae | *Pergularia tomentosa* | Leaves |
| | | Stems |

**Fungal strain:** The fungal strain used in this study is *Fusarium oxysporum* f. sp. *albedinis*, the causal agent of Bayoud disease which 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, ethyl acetate, dichloromethane and hexane were used as organic solvents with different polarity to extract the active ingredients in plant tissues using heat reflux extraction. In each sequence of extraction, 10 g portion of the powdered dry plant were mixed with 80 mL of the solvent in a 250 mL round bottom flask fitted with a cooling condenser which was used to perform extraction. The extraction temperature was controlled at boiling temperature of solvent for 2 h. Dry weight (after filtration and evaporation) were determined then kept in screw cap tubes at 5°C.

**Bioassay and media used:** Sufficient number of Whatman paper discs (6 mm Ø and 1 mm thickness) were sterilized by autoclaving at 121°C for 15 min and kept overnight at 70°C to ensure dryness. Each disc was impregnated with one of the following extract weight (200, 400, 800 and 1600 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 (consists of: potatoes extract 4 g, glucose 20 g, agar 15 g, distilled water up to 1 L) to synthetic nutrient poor agar SNA (consists of: $KH_2PO4$ 1 g, $KNO_3$ 1 g, $MgSO_4$-$7H_2O$ 0.5 g, KCl 0.5 g, glucose 0.2 g, saccharose 0.2 g, agar 15 g, distilled water up to 1 L) 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 realized by disc diffusion technique. Sterile Petri dishes (90 mm Ø) containing PDA media were inoculated with 100 μL of Foa spores suspension ($10^6$ spores $mL^{-1}$). Sterile discs (6 mm Ø) containing the plants extracts (200, 400, 800 and 1600 μg) were deposed on the inoculated PDA plates (incubation at 21°C for 5 days). The results were obtained as inhibition zone diameter (mm) of three measurements. 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 realized as described by Herrmann *et al.* (1996) with slight modification. New potatoes (*Solanum tuberosum* L.) were surface sterilized for 5 min 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 2000 μg) were applied on potatoes slices. After 5 min, each potatoes slice was infected with a slice (12 mm Ø, 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. Any known antifungal products had been signaled to be effective on Foa to be used as positive control.

**Experimental design and data analysis:** All the experiments were realized as randomized complete blocks. All the collected data were submitted to ANOVA test, correlation test and analysis of frequencies using Statistica software Ver. 5 (Statsoft, ed'97) and the significance of differences among treatments was recorded at $p<0.05$ (Box *et al.*, 2005). Results are presented as means (n = 3) (standard errors were less than 20% except cited).

## RESULTS

**Extraction yield:** The correlation (solvent/extraction yield) was significant ($p<0.05$). The maximum extraction yield is represented by methanol (21.91%) for the leaves of *Calotropis procera*. The minimum extraction yield is represented by dichloromethane (1.07%) for the stems of *Pergularia tomentosa*. The yields can be arranged in the following decreasing order: methanol (mean: 13.24%, minimum: 8.46%, maximum: 21.91%), ethyl acetate (mean: 4.75%, minimum: 2.90%, maximum: 10.16%), hexane (mean: 3.05%, minimum: 2.07%, maximum: 6.94%) then dichloromethane (mean: 2.20%, minimum: 1.07%, maximum: 3.83%). The correlations: (plant/extraction yield) and (plant part/extraction yield) were not significant ($p<0.05$) (Table 2).

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

| Plant species | Part used | Solvent used for extraction | | | |
|---|---|---|---|---|---|
| | | Methanol | EA | DCM | Hexane |
| *Citrullus colocynthis* | Leaves and stems | 17.09 | 5.80 | 3.83 | 2.39 |
| | Fruits | 8.46 | 10.16 | 3.05 | 6.94 |
| *Calotropis procera* | Leaves | 21.91 | 2.90 | 2.05 | 2.11 |
| | Stems | 10.99 | 2.93 | 1.47 | 3.18 |
| *Nerium oleander* | Leaves | 11.25 | 3.78 | 3.10 | 2.80 |
| | Stems | 13.37 | 5.45 | 1.25 | 2.22 |
| *Pergularia tomentosa* | Leaves | 9.33 | 3.88 | 1.77 | 2.07 |
| | Stems | 13.53 | 3.12 | 1.07 | 2.68 |

EA: Ethyl acetate, DCM: Dichloromethane

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

| Plant species | Part used | Solvent | Extract weight in discs (µg) | | | |
|---|---|---|---|---|---|---|
| | | | 200 | 400 | 800 | 1600 |
| *Citrullus colocynthis* | Leaves and stems | Methanol | ND | ND | ND | 19 |
| | | Ethyl acetate | ND | ND | 9 | 13 |
| | | Dichloromethane | ND | ND | ND | ND |
| | | Hexane | ND | ND | 10 | 11 |
| | Fruits | Methanol | ND | 11 | 16 | 17 |
| | | Ethyl acetate | ND | ND | ND | 9 |
| | | Dichloromethane | ND | ND | ND | ND |
| | | Hexane | ND | ND | ND | ND |
| *Calotropis procera* | Leaves | Methanol | ND | ND | ND | ND |
| | | Ethyl acetate | ND | ND | ND | ND |
| | | Dichloromethane | ND | ND | ND | ND |
| | | Hexane | ND | 12 | 15 | 14 |
| | Stems | Methanol | ND | ND | ND | ND |
| | | Ethyl acetate | ND | ND | ND | ND |
| | | Dichloromethane | ND | ND | ND | ND |
| | | Hexane | ND | ND | ND | ND |
| *Nerium oleander* | Leaves | Methanol | ND | ND | 16 | 19 |
| | | Ethyl acetate | 11 | 16 | 16 | 17 |
| | | Dichloromethane | ND | ND | ND | ND |
| | | Hexane | ND | ND | ND | ND |
| | Stems | Methanol | 10 | 13 | 15 | 19 |
| | | Ethyl acetate | ND | ND | ND | 9 |
| | | Dichloromethane | ND | ND | ND | ND |
| | | Hexane | ND | ND | ND | ND |
| *Pergularia tomentosa* | Leaves | Methanol | ND | ND | ND | 11 |
| | | Ethyl acetate | ND | ND | ND | ND |
| | | Dichloromethane | ND | ND | ND | ND |
| | | Hexane | ND | ND | ND | ND |
| | Stems | Methanol | ND | ND | ND | 9 |
| | | Ethyl acetate | ND | ND | ND | ND |
| | | Dichloromethane | ND | ND | ND | ND |
| | | Hexane | 9 | 10 | 17 | 20 |

ND: Not detected

**Antifungal test:** Among all tests realized (n = 128), only 29 tests (22.65%) showed detectable effect. The analysis of frequencies demonstrated that (12.50%) of the results showed low potency against Foa with zone of inhibition diameter (Ø: 8-15 mm). In addition, only (10.16%) of all experiments exhibited moderate antifungal effect on Foa with zone of inhibition diameter (Ø: 15-20 mm). Out of 128 tests realized, no one presented a high inhibition effect on Foa (Ø>20 mm) (Table 3).

On the basis of results obtained, one way ANOVA test demonstrated that the effect of solvent (used for extraction) on Foa culture was significant ($p<0.05$). For methanolic extracts, 12 out of 32 experiments (37.50%) showed detectable effects. Nine out of 32 experiments (28.12%) realized with hexanic extracts demonstrated detectable effects. Eight out of 32 experiments (25.00%) realized with ethyl acetate extracts exhibited detectable effect. As for the intensity of antifungal effect, the most important effect is observed for hexanic extract of *Pergularia tomentosa* stems (Ø = 20 mm) then for methanolic extracts of *Citrullus colocynthis* (leaves and stems) and *Nerium oleander* (leaves, stems) with zone of inhibition (Ø = 19 mm). The dichloromethanic extracts had no detectable effect on Foa. The effects of plant and plant part used were not significant ($p<0.05$) (Table 3).

The effect of extract weight in discs (200, 400, 800 and 1600 µg) on Foa was significant ($p<0.05$). For all detectable effects, the increase in extract weight showed an increase in diameter of inhibition zone, except for: ethyl acetate extract of *Nerium oleander* leaves (stability between 400 and 800 µg) and for hexanic extract of *Calotropis procera* leaves (decrease of 1 mm from 800 to 1600 µg). The best effects on Foa were observed at 1600 µg. At 200 µg, only 3 out of 32 tests (9.38%) demonstrated detectable effects, in the range (Ø: 9-11 mm). At 400 µg, only 5 out of 32 tests (15.62%) exhibited detectable effects, in the range (Ø: 10-16 mm). At 800 µg, 8 out of 32 tests (25.00%) showed detectable effects, in the range (Ø: 9-17 mm). At 1600 µg, 13 out of 32 tests (40.62%) demonstrated detectable effects, in the range (Ø: 9-20 mm).

**Virulence test:** Eight extracts from four poisonous 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 1600 µg) (n = 128). 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 depends on extracts and/or Foa effect. The results had been presented as Relative Virulence (RV) compared to Foa virulence without plants extracts (Table 4). No correlation detected for extract weight in discs (200, 400, 800 and 1600 µg) and RV ($p<0.05$), also for zone of inhibition (mm) and RV ($p<0.05$).

Analysis of frequencies demonstrated that the half of tests (n = 64, 50.00%) showed an increase in RV of Foa on potato tuber tissue (>100%). Fifty five out of 128 tests (42.99%) represented a decrease in RV (<100%), among them, only 2 (3.64%) showed a decrease of RV below (50%). The decrease of RV below (50%) was seen for methanolic (RV = 46%) and dichloromethanic (RV = 48%) extracts of *Citrullus colocynthis* (leaves and stems). Only 9 out of 128 experiments (7.03%) exhibited RV approximately equal to Foa RV (100±1%). The maximum RV (235%) was showed by dichloromethanic extract of *Citrullus colocynthis* fruits.

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

| | | | Extract weight in discs (µg) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | 200 | | 400 | | 800 | | 1600 | |
| Plant species | Part used | Solvent | NTW (mg) | RV (%) | NTW (mg) | RV (%) | NTW (mg) | RV (%) | NTW (mg) | RV (%) |
| *Citrullus colocynthis* | Leaves and stems | Methanol | 63.1 | 95 | 47.6 | 72 | 66.7 | 101 | 89.2 | 135 |
| | | EA | 30.2 | 46 | 59.0 | 89 | 46.5 | 70 | 40.0 | 60 |
| | | DCM | 72.0 | 109 | 66.4 | 100 | 64.4 | 97 | 32.1 | 48 |
| | | Hexane | 35.7 | 54 | 33.7 | 51 | 43.0 | 65 | 37.2 | 56 |
| | Fruits | Methanol | 45.7 | 69 | 66.7 | 101 | 86.1 | 130 | 57.1 | 86 |
| | | EA | 78.7 | 119 | 57.0 | 86 | 43.6 | 66 | 35.6 | 54 |
| | | DCM | 155.8 | 235 | 85.0 | 128 | 50.0 | 75 | 58.8 | 89 |
| | | Hexane | 77.4 | 117 | 66.0 | 100 | 78.8 | 119 | 76.4 | 115 |
| *Calotropis procera* | Leaves | Methanol | 65.7 | 99 | 102.4 | 154 | 77.0 | 116 | 42.4 | 64 |
| | | EA | 45.4 | 68 | 51.9 | 78 | 58.2 | 88 | 66.0 | 100 |
| | | DCM | 52.3 | 79 | 88.5 | 133 | 68.3 | 103 | 81.3 | 123 |
| | | Hexane | 51.5 | 78 | 60.9 | 92 | 68.6 | 103 | 85.7 | 129 |
| | Stems | Methanol | 94.5 | 143 | 86.9 | 131 | 60.7 | 92 | 109.2 | 165 |
| | | EA | 77.2 | 116 | 101.1 | 152 | 51.6 | 78 | 87.4 | 132 |
| | | DCM | 67.5 | 102 | 68.4 | 103 | 80.8 | 122 | 105.3 | 159 |
| | | Hexane | 96.6 | 146 | 56.5 | 85 | 72.5 | 109 | 57.0 | 86 |
| *Nerium oleander* | Leaves | Methanol | 72.8 | 110 | 36.5 | 55 | 72.1 | 109 | 70.7 | 107 |
| | | EA | 63.6 | 96 | 63.4 | 96 | 36.6 | 55 | 42.1 | 63 |
| | | DCM | 54.6 | 82 | 98.8 | 149 | 70.6 | 106 | 62.7 | 95 |
| | | Hexane | 50.0 | 75 | 60.8 | 92 | 58.4 | 88 | 36.5 | 55 |
| | Stems | Methanol | 84.2 | 127 | 74.2 | 112 | 69.1 | 104 | 92.2 | 139 |
| | | EA | 104.6 | 158 | 53.1 | 80 | 83.5 | 126 | 81.8 | 123 |
| | | DCM | 97.5 | 147 | 93.6 | 141 | 73.9 | 111 | 76.9 | 116 |
| | | Hexane | 73.2 | 110 | 115.4 | 174 | 94.9 | 143 | 91.0 | 137 |
| *Pergularia tomentosa* | Leaves | Methanol | 49.6 | 75 | 71.6 | 108 | 72.8 | 110 | 88.7 | 134 |
| | | EA | 54.5 | 82 | 85.2 | 129 | 69.6 | 105 | 72.8 | 110 |
| | | DCM | 62.8 | 95 | 85.3 | 129 | 61.7 | 93 | 66.2 | 100 |
| | | Hexane | 127.3 | 192 | 153.7 | 232 | 75.1 | 113 | 79.8 | 120 |
| | Stems | Methanol | 77.0 | 116 | 106.6 | 161 | 71.9 | 108 | 66.7 | 101 |
| | | EA | 73.7 | 111 | 68.2 | 103 | 65.6 | 99 | 56.5 | 85 |
| | | DCM | 53.2 | 80 | 63.0 | 95 | 39.3 | 59 | 61.7 | 93 |
| | | Hexane | 38.5 | 58 | 34.7 | 52 | 53.0 | 80 | 44.1 | 67 |

EA: 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)

The ANOVA test showed a significant effect of plant part on Foa RV ($p<0.05$). Two parts were chosen from each plant: (leaves) and (stems) for *Calotropis procera*, *Nerium oleander* and *Pergularia tomentosa*, (leaves with stems) and (fruits) for *Citrullus colocynthis*. Regarding the benefic effect on Foa RV (decrease of RV below 100%), the plant parts are listed in decreasing order: leaves and stems extracts, 12 out of 16 tests (75.00%); leaves extracts, 22 out of 48 experiments (45.83%); fruits extracts, 7 out of 16 tests (43.75%) then stems extracts, 14 out of 48 (29.17%) tests. The effect of plant and solvent used for extraction were not significant ($p<0.05$).

## DISCUSSION

In this study, we have evaluated the effect of four poisonous 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 researches. 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). Till these lines, no study has exhibited the effect of these plants on Foa.

**Extraction efficacy:** The extraction yield was affected significantly by the solvent used for extraction ($p<0.05$). On the basis of solvent, the yields are given in Table 2. This is principally related to the polarity and capability to extract substances that can be dissolved in the used solvent. In addition, the parts used for most plants were leaves and stems, which might reflect more the solvent effect but not the plant or plant part. Possibly due to the previous reason, the plants and plants parts has not significantly affected the extraction yield ($p<0.05$).

The methanol was the powerful regarding the extraction yield, it was the most capable to extract more substances; in the other hand, the plants used contain more substances that preferably dissolve in methanol. It was demonstrated that comparing the extraction efficacy of 10 different solvents (hexane, diisopropyl ether, diethyl ether, methylene dichloride, ethyl acetate, tetrahydrofuran, acetone, ethanol, methanol and water) using *Combretum woodi* leaves has shown the best extraction efficacy by intermediate polarity solvent compared to polar and non-polar solvents reflecting that this plant leaves contain more substances with intermediate polarity (Eloff *et al*., 2005). The difference from our results is probably related to difference between species used (difference in substances contained in these plants).

Shi *et al*. (2003) reported that polar solvents extract polar substances (polyphenols); so, the plants used in this study contain more polar substances possibly among them polyphenols.

**Antifungal effect:** Among 128 tests realized, no one showed a high inhibition effect and only 10.16% presented a moderate effect. These results reflected the high resistance of Foa to these plants extracts related to: plant species, solvents used, parts used and/or quantities used. In the other hand, the results appreciate the data related to Foa resistance to many treatments. El-Hassni *et al*. (2007) signaled that fighting strategies against Foa are very restricted or quasi-unavailable reflecting its high resistance.

On the basis of experiments showing detectable effect; extracts obtained by methanol, hexane and ethyl acetate were the most effective, respectively. The methanol is more polar than ethyl acetate and hexane. The major effect of methanolic extracts is principally due to polar substances. In the other hand, ethyl acetate is moderately polar solvent but hexane is not. The comparison between the two solvents effect reflected the presence of polar and non polar substances in these plants presenting the antifungal effect. Basing on solvent polarity, the effect of solvent used for extraction on Foa varies.

Very clear differences were found for the effects of different extract weight per disc (200, 400, 800 or 1600 μg). This result is in agreement with the idea: increasing extract weight is proportional to active substance(s) present in the extract, reflecting more effect on Foa.

It can be concluded from this part that these plants contain more than one substance with antifungal effect against Foa. Many studies showed that these plants contain many important secondary metabolites with pharmacological properties (Habs *et al*., 1984; Al-Said *et al*., 1988; Abiola *et al*., 1993; Begum *et al*., 1997; Maatooq *et al*., 1997; Hamdy *et al*., 1999; Abdel-Hassan *et al*., 2000; Abbassi *et al*., 2004; Hamed *et al*., 2006; Sawaya *et al*., 2006; Al-Farwachi, 2007; Hassan *et al*., 2007). They must be used with caution because of poisonous substances which is related to chemical nature, dose, part of plant, target(s). In many cases, the margins between the lethal and sub-lethal doses are very low (cardiac glycosides). For these reasons, this richness must be appreciated at research level to get the important substances and give the appropriate doses.

**Virulence test:** The absence of correlation between inhibition zone and RV may be related to the difference

between substance(s) responsible of antifungal effect with substance(s) responsible of anti-virulence effect. The difference can be due to chemical nature and/or mechanism of action.

The Relative Virulence (RV) of Foa on potato tuber tissue is the combination between plants extracts effect and Foa virulence effect. As for RV equal to (100%), it might be the result of RV increase by Foa and decrease of RV by plants extracts.

The significant effect of plant part could be related to difference in constituents present in different parts from the chosen plants. Therefore, the best effect on Foa RV was observed for *Citrullus colocynthis* extracts (leaves and stems). On the other hand, the increase of RV was observed for *Citrullus colocynthis* (fruits). As leaves and stems might contain substances with benefit effect on Foa virulence, the fruits might contain substances toxic on potato tuber tissue. Maatooq *et al.* (1997) investigated the presence of flavonoids in *Citrullus colocynthis*, but the flavonoids present in the fruits are different from those in aerial part. The flavonoids are known to have antimicrobial effects, so the antifungal effect of *Citrullus colocynthis* extracts might be related to flavonoids and the difference between parts effect was probably related to difference in flavonoids types.

Herrmann *et al.* (1996) demonstrated the relative virulence of some *Fusarium* strains and other *Fusarium oxysporum* formae speciales on potato tuber tissue but this study has not used *Fusarium oxysporum* f. sp. *albedinis*. Compared to our results, Foa represents relatively important virulence (0.066 g of necrotic tissue) which represents a relative virulence equal to 2.54% because: From the 36 strains studied, 6 strains had relative virulence lower than 2.54%; 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 mycotoxines. It is one of the mycotoxines responsible of Foa phytotoxicity (Herrmann *et al.*, 1996). The effect of extracts on RV of Foa is possibly by affecting the synthesis and/or action of enniatin on cells. The best decreasing effect on RV is represented by methanolic and dichloromethanic extracts of *Citrullus colocynthis* (leaves and stems); it may act on enniatin production or antagonise its mode of action. The increase of RV above 100% reflects cytotoxicity; it can be explained by direct toxicity on cells and/or increase of enniatin effect (increase production and/or stimulate action).

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 (El-Hadrami *et al.*, 2005). These mycotoxines play an important role in pathogenicity and virulence of Foa. The possible effect of the active plant extracts used in this study is to act on one or more of these mycotoxines by modifying their metabolism or their effects.

Coupling the effect of plants extracts on Foa growth and virulence, *Citrullus colocynthis* showed great efficacy than did other plants. This might be attributed to differences in chemical composition, affinity to the biological target and/or other factors. Many studies have explored this plant for its richness in biologically active metabolites (Habs *et al.*, 1984; Maatooq *et al.*, 1997; Abdel-Hassan *et al.*, 2000; Sawaya *et al.*, 2006).

Comparing the results obtained from disc diffusion technique and virulence test, we found that dichloromethane had no effect for the first test but had a good effect (in some tests) for the second one. This controversy is possibly due to dichloromethane extracts action on non vital bio-substance(s) in Foa (play role in Foa toxicity) that reflect no effect on Foa culture but decrease the virulence. It has been demonstrated that Foa fraction purified from the organic extracts of a Foa was unable to induce necrosis of potato slices, which indicates that it does not contain significant amounts of enniatins. In 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 (Amraoui *et al.*, 2007). So, there is a kind of complementarity to realize the infection.

This is the first report of antifungal activity of these four plants extracts (collected from South-West of Algeria) on the causal agent of Bayoud disease. Referring to the aim of this study, the results obtained shed the light on the possibility to use some of these plants (representing the best effects) against Foa by proceeding with further advanced analyses. The effectiveness of *Citrullus colocynthis* extracts indicates the presence of highly sensitive target(s) which may be exploited in discovering new specific and effective treatment of Bayoud.

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