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# Fusaric acid and phytotoxic metabolites produced by *Fusarium oxysporum* f.sp. *albedinis*: effects on date palm and their use for resistance screening trial

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## Abstract

Bayoud disease caused by the soil born pathogen *Fusarium oxysporum* f.sp. *albedinis* (Foa) is one of the most destructive disease of date palm in North Africa. Foa produce in the culture filtrate phytotoxins that have been proposed to play an important role in the onset of the disease symptoms. In this study the processes response of date palm cultivars to Foa toxin extract and fusaric acid (FA) was investigated. Using susceptible and resistant cultivars, it was found that dichloromethane extract (DCM) from culture filtrate of Foa and FA showed a dose-response effect. In vitro sensitivity of date palm cultivars to DCM extract (50µg/ml) was positively correlated with susceptibility to bayoud disease in the field. This study suggests that Foa host selective toxin acting as selective agent, contribute to the pathogenesis and could be used on breeding for resistance.

## 1. Introduction

*Fusarium oxysporum* (Fo) is among the fungal species that produce significant reduction in yield and quality of crops and presenting a high level of host specifity [1]. *Fusarium oxysporum* is divided into more than 150 formae speciales according to their specificity to a single or more than one host plant [2]. *Fusarium oxysporum* f.sp. *albedinis* (Foa) is a form infecting date palm and produce vascular wilt or bayoud disease distributed in North Africa including Morocco, Algeria and Mauritania [3- 5]. Foa infects date palm through root then spread up through vascular system to rich the crown. The specific symptoms are presented as brown color along conducting vessels and side of the rachis. In affected leaves, the symptoms progress on one side of the leaf as ash gray penne from base to apex, when the entire side is affected; wilting begins on the other side in the opposite direction until the death of the leaf [6, 7]. The disease continues to attack leaf after leaf and to advance towards the heart of the tree and the tree dies when the mycelium reached the terminal bud. The palm tree can die 6 months to 2 years after the onset of symptoms, depending on the cultivar and planting conditions [6, 8]. In the case of young plants, the disease can produce the die from 1 to 3 months [9].

The bayoud disease occurred on date palm cultivars with the most important economic value and good quality fruit. Among known date palm cultivars a few number are described as highly resistant but low quality fruit [8]. Moroccan program of date palm breeding aims to find and create new cultivars with genotypes with agronomic characters and resistance to bayoud [9]. The combinations to achieve the desired agronomic traits of quality and resistance is often made on mass, this consume time and plant materiel and are considered to be necessary. Classical selection is based on the use of artificial inoculation of date palm plants using pathogen's spores but it needs relatively long time [7, 10]. Nevertheless the use of pathogen derived element such as culture filtrate and phytotoxin could be an attractive proposal to reduce materiel and shorten time needed for the first screening on mass [11].

Durant last decades, the research and efforts have been conducted to find an alternative and sample way based on the use of Foa phytotoxin [12, 13]. This type of selection could help to detect resistant and susceptible genotypes and yield results in a relatively low cost and short time compared to use of intact plants. This study aims to examine the relationship between in vitro sensitivity of date palm cultivars to fusaric acid and phytotoxic metabolites produced by Foa and their disease susceptibility on the field, on the other hand, to investigate the involvement of Foa toxic metabolites in infection processes and disease development.

## 2. Material and Methods

*2.1. Fungal isolation and cultivation*

Foa isolate L18 used in this experiment was isolated from rachis of infected date plant from Zagora region in South Morocco on CZAPEK medium, the Foa characterization was beforehand done according to morphological aspect of obtained colony and the pathogenicity test on date palm plantlet of Jihel (susceptible cultivar). The Foa isolate is stored in mycothic collection of Laboratory of Genetic Phytopathology and Integrated Control, National Institute of Agronomic Research (INRA) (P.O. Box 533 Marrakesh, Morocco).

*2.2. Culture filtrate production*

The culture filtrate of Foa was produced as the method developed by Sedra et al. [14]. Foa was cultivated on CZAPEK solid medium 8 to 10 days in order to allow the fungus to sporulate. The surface of colonies was flooded by sterile distilled water to prepare spores suspension. The obtained spore suspension was adjusted to $10^{-6}$spore/ml using a Thoma cell. Erlenmeyer flask (250 ml) containing 200 ml of CZAPEK liquid medium were inoculated with spore suspensions of the isolate of Foa (1/200 ml; $10^{-6}$ spores/ ml) and incubated on a shaker (80 rpm) at room temperature for 10 days underdarkness.

*2.3. Phytotoxins extraction*

Cultures (5 liters) were centrifuged at 4000g for 20 min to remove mycelium and the supernatants were collected and concentrated under vacuum to 200 ml and was added by the same volume of methanol (MeOH) and kept at 4°C for 48 hours to eliminate precipitated organic molecules by filtration and the MeOH removed by evaporation on rotary evaporator at 45 °C [12]. After, the culture filtrate (CF) was extracted three times using organic solvent following the polarity: dichloromethane (DCM), ethyl acetate (EtOAc), butanol (But-OH).The three fractions of each solvent were combined, dried over anhydrous sodium sulphate and the solvent was removed by evaporation on a rotary evaporator at 45°C. The residues were collected and stored in a freezer at -4°C. Aqueous phase (AQP) was concentrated to the minimal volume of 100 ml and placed in the freezer until assayed.

*2.4. Plant materiel*

The tests were carried out with detached leaves of date palm (Phoenix dactylifera L.) cultivars collected from genetic collection localized in experimental stations of INRA at Saada near Marrakech city in Morocco and at Zagora in the South of Morocco. The seeds of date palm issued from crosses were taken from genetic collection of seeds in the Laboratory of Genetic Phytopathology and Integrated Control, National Institute of Agronomic Research (INRA) (P.O. Box 533 Marrakesh, Morocco). Ten date palm cultivars were used (Table 1).

**Table 1:** Moroccan date palm cultivars tested for phytotoxicity of Foa phytotoxins extract and furasic acid.

| Cultivars | Abbreviation | Phenotype to bayoud disease | Fruit quality |
|---|---|---|---|
| Boufeggous | BFG | Susceptible | Good |
| Medjool | MJL | Susceptible | Excellent |
| Najda | NJD | Resistant | Good |
| Black Bousthammi | BBST | Resistant | Fair |
| White Bousthammi | WBST | Resistant | Fair |
| Iklane | IKL | Resistant | Fair |
| Boufeggous ou Moussa | BFGM | Resistant | Fair |
| Boucerdoune | BCD | Susceptible | Moderate |
| Ahardane | AHD | Susceptible | Moderate |
| Aguelid | AGL | Susceptible | Moderate |

*2.5. Eliminary screening of Foa phytotoxin*

Preliminary screening of different phytotoxin extracts from culture filtrate of Foa was realized on detached leaves (DL) of Boufeggous susceptible date palm cultivar according to the method described by Sedra et al. [14]. So, detached leaves were cut from the base of petioles, immediately washed with a tap water and surface sterilized with diluted ethanol (10%). Leaves were immerged in a solution of toxic element in sterile glass test tubes. Organic extract (DCM, EtOAc and But-OH) and Fusaric acid (FA) at 50µg/ml were solubilized in 0.01% DMSO. However, culture filtrate (CF), aqueous phase (AQP) and tap water were used as control and at the same concentration of DMSO. The tubes were kept at room temperature (25-27°C) with a 12:12 hour light: dark cycle. The phytotoxicity was assessed by the percentage of leaves presenting typical symptom of browning and wilting after 5, 10 and 15 days of treatment. Each test was conducted twice on 5 glass tubes with three leaves on each one.

*2.6. Evaluation of dose effect and date palm susceptibility to DCM extract and FA*

The dose effect of DCM extract and FA on date palm was conducted using the same method of detached leaves. The first step of this test was conducted using four cultivars with different dilutions of both toxic elements in order to get the distinctive minimal concentration between susceptible and resistant cultivars. On the second step, using a determined distinctive concentration, the in vitro susceptibility of date palm was evaluated on ten cultivars compared to their field behavior toward the disease.

*2.7. Effect of DCM extract on electrolyte leakage*

The measure of electrolyte leakage was conducted on 2 cm susceptible cultivar leaves and root fragments (Jihel developed as above) placed on glass tube with 10 ml solution of distilled water DMSO (0.01%) or DCM extract-DMSO (0.01%) after 72 hours, fragments were removed, solutions filtered with 0.45 µm filter and the conductivity was measured with conductivity meter (Thermo scientific Grion 4 star pH, conductivity portable). Electrolyte leakage relative injury (%) was expressed as the ratio of conductivity measurement of used solution before and after treatment.

*2.8. Phytotoxin extract analysis by HPLC*

The presence of fusaric acid (FA) in crude extract with dichloromethane from culture filtrate was identified using HPLC system (Knauer Eurospher II 100-5) equipped with a C18 column (diameter x length: 250 x 4.6 mm + precolumn) according to the conditions described by Amalfitano et al. [15]. The presence of FA on the crude extract was confirmed with mass spectrum.

*2.9. Statistical analysis*

All data were the means of three replications and were expressed as means ± standard deviation. Analysis of variance was determined by one-way ANOVA (SPSS 10.0). The Duncan's multiple range test was performed at the significant difference level of 5%.

## 3. Results and discussion

The diseases caused by phytopathogenic fungi are the main causes of crop losses by reducing the yield and affecting quality. To ensure good production and promote sustainable agriculture the use and development of tolerant and / or resistant cultivars seems to be the most effective solution especially for soil born pathogen such as *Fusarium oxysporum* and fragile ecosystems like oasis. The potential use of Foa phytotoxins on screening of date palm for their resistance to bayoud disease were early reported in many previous work [11, 12]. Its viability is related to degree of involvement of a phytotoxin in disease development and to relationship between phytotoxin sensitivity and disease susceptibility. In this study, ten cultivars of the date palm with resistant and susceptible genotypes were used (table 1). Foa culture filtrate (CF) was extracted with different organic solvents and were tested for their phytotoxicity on detached leaves (DL) of date palm susceptible cultivar (BFG). Percentages of DL with symptoms of browning and wilting that were similar to those produced by the pathogen (figure 1) are summarized in table 2. DCM extraction allowed the development of symptoms after 5 days of treatment and was observed to induce the most potent effect with 92.23 % of phytotoxicity compared to the culture filtrate and fusaric acid. Whereas ethyl acetate and butanol extracts showed lowest. Since FA and the DCM extract was further used and tested at different concentrations on four cultivars in order to determine the lowest selective concentration.

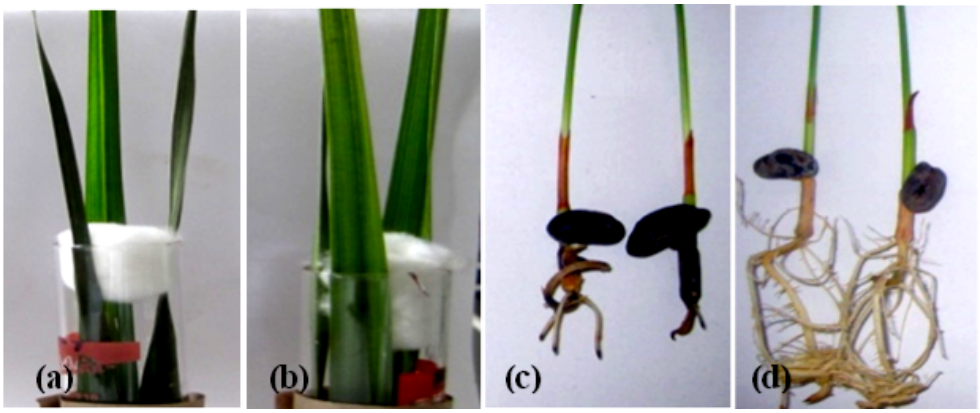


**Figure 1:** Symptoms of phytotoxicity of DCM extract on date palm showing wilting and winding on detached leaves (a) and rotting of roots on seedlings (c) compared to their control treated with water (b) and (d).

The level of phytotoxicity on date palm (DL) varied with the different concentration used and according to the genotypes (table 3). DCM extract at a concentration of 25µg/ml induced strong effect on DL of susceptible cultivars: BFG (77.78%) and MJL (55.56%) compared to resistant cultivars: BBST (33.33%) and NJD (11.11%). The highest phytotoxicity (over 50%) come from DL of susceptible cultivars treated with a concentration of 50µg/ml and allowed significant difference between susceptible and resistant cultivars, for this reason, this concentration of DCM extract was chosen for the study of the screening test of resistance. For example, 10 days after treatment with DCM extract, the very susceptible cultivars BFG and MJL were highly sensitive to DCM extract with phytotoxicity level of 73.33 % and 66.67 % respectively, susceptible cultivars BCD and AHD had moderate levels of phytotoxicity, whereas, resistant genotypes showed low toxic effect. However, the action of FA on four date palm cultivars showed less phytotoxic effect than DCM extract on both genotypes ( table 4), the strong effect was observed on BFG (53.33%), moderate effect on MJL (40.00%) and BBST (40.00%), while NJD cultivar appeared to be more tolerant to FA (20.00 %).

**Table 2**: Percentage of phytotoxic effect of organic extract of *Fusarium oxysporum* f.sp *albedinis* culture filtrate on detached leaves of Boufeggous susceptible date palm cultivar.

| DPT | Ctrl | FA 50µg/ml | CF 100% | DCM 50 µg/ml | EtOAc g/ml | But-OH 50 µg/ml | AQP % |
|---|---|---|---|---|---|---|---|
| **5** | $0.00^{a}$ | $0.00^{a}$ | $13.33^{ab}$ | $20.00^{ab}$ | $0.00^{a}$ | $0.00^{a}$ | $0.00^{a}$ |
| **10** | $0.00^{a}$ | $20.00^{ab}$ | $66.67^{cd}$ | $66.67^{cd}$ | $13.33^{ab}$ | $6.67^{a}$ | $46.67^{bc}$ |
| **15** | $0.00^{a}$ | $40.67^{bc}$ | $74.23^{d}$ | $80.67^{d}$ | $20.00^{ab}$ | $13.33^{ab}$ | $60.67^{cd}$ |

DPT: days post treatment;
Ctrl: control water; FA: fusaric acid; CF: culture filtrate; DCM: dichloromethane; EtOAc: ethyl acetate; But-OH: butanol; AQP: Aqueous phase.
The data were the means of three replications. Mean values in the same column followed by the different letter are significantly different by Duncan post hoc test (α = 5%).

**Table 3**: Percentage of phytotoxic effect of DCM extract (µg/ml) of culture filtrate of Foa on detached leaves of date palm cultivars.

| CVS | Boufeggous | | | Medjool | | | Najda | | | Black Bousthammi | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **DPT** | **5** | **10** | **15** | **5** | **10** | **15** | **5** | **10** | **15** | **5** | **10** | **15** |
| **Ctrl** | $0.00^{a}$ | $0.00^{a}$ | $00.00^{ab}$ | $0.00^{a}$ | $0.00^{a}$ | $0.00^{a}$ | $0.00^{a}$ | $0.00^{a}$ | $0.00^{a}$ | $0.00^{a}$ | $0.00^{a}$ | $0.00^{a}$ |
| **6.25** | $0.00^{a}$ | $0.00^{a}$ | $22.22^{abc}$ | $0.00^{a}$ | $11.11^{ab}$ | $22.22^{abc}$ | $0.00^{a}$ | $0.00^{a}$ | $0.00^{a}$ | $0.00^{a}$ | $0.00^{a}$ | $22.22^{abc}$ |
| **12.5** | $0.00^{a}$ | $44.44^{bcd}$ | $77.78^{de}$ | $0.00^{a}$ | $22.22^{abc}$ | $22.22^{abc}$ | $0.00^{a}$ | $0.00^{a}$ | $0.00^{a}$ | $0.00^{a}$ | $11.11^{ab}$ | $22.22^{abc}$ |
| **25** | $0.00^{a}$ | $55.56^{cde}$ | $77.78^{de}$ | $0.00^{a}$ | $44.44^{bcd}$ | $55.56^{cde}$ | $0.00^{a}$ | $0.00^{a}$ | $11.11^{ab}$ | $0.00^{a}$ | $11.11^{ab}$ | $33.33^{bc}$ |
| **50** | $0.00^{a}$ | $77.78^{cde}$ | $88.89^{e}$ | $11.11^{ab}$ | $66.67^{de}$ | $88.89^{e}$ | $0.00^{a}$ | $11.11^{ab}$ | $22.22^{abc}$ | $0.00^{a}$ | $22.22^{abc}$ | $44.44^{bcd}$ |

CVS: Date Palm Cultivars
Boufeggous (S)
Medjool (S)
Bou Sthammi Noir (R)
Najda (R)
DPT: days post treatment
Ctrl = control water added by 0,01% of DMSO

The data were the means of three replications. Mean values in the same column followed by the different letter are significantly different by Duncan post hoc test (α = 5%).
The table 5 showed that date palm cultivars susceptibility to DCM extract at 50µg/ml using detached leaves bioassay was positively correlated with their behavior to bayoud disease in the field, however, FA present similar and close effect on both genotypes. Thus susceptible cultivars were proved to be more sensitive to Foa phytotoxins compared to resistant cultivars. Several studies reported similar results regarding the selection for disease resistance or tolerance using pathogen derived selective agent [11, 16- 19], for example the successful separation of yellow passion fruit resistant and susceptible genotypes using both FA and CF of *F.oxysporum* f.sp. p*assiflorae* [20] and the strong correlation between in vitro selection of calli of *citrus jambhiri lush* and

Venezuelan Cultivars of *Oryza sativa,* using CF of *Phytophthora parasitica* and *Pyricularia grisea* respectively and their in vivo resistance [21, 22].

**Table 4**: Percentage of phytotoxic effect of fusaric acid (µg/ml) on detached leaves of date palm cultivars.

| CVS | Boufeggous | | | Medjool | | | Najda | | | Black Bousthammi | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **DPT** | **5** | **10** | **15** | **5** | **10** | **15** | **5** | **10** | **15** | **5** | **10** | **15** |
| **Ctrl** | 0.00$^{a}$ | 0.00$^{a}$ | 0.00$^{a}$ | 0.00$^{a}$ | 0.00$^{a}$ | 6.67$^{ab}$ | 0.00$^{a}$ | 0.00$^{a}$ | 0.00$^{a}$ | 0.00$^{a}$ | 0.00$^{a}$ | 6.67$^{a}$ |
| **6.25** | 0.00$^{a}$ | 0.00$^{a}$ | 6.67$^{ab}$ | 0.00$^{a}$ | 6.67$^{ab}$ | 13.33$^{ab}$ | 0.00$^{a}$ | 0.00$^{a}$ | 0.00$^{a}$ | 0.00$^{a}$ | 0.00$^{a}$ | 6.67$^{a}$ |
| **12.5** | 0.00$^{a}$ | 0.00$^{a}$ | 13.33$^{abc}$ | 0.00$^{a}$ | 13.33$^{ab}$ | 13.33$^{ab}$ | 0.00$^{a}$ | 0.00$^{a}$ | 0.00$^{a}$ | 0.00$^{a}$ | 6.67$^{a}$ | 6.67$^{a}$ |
| **25** | 0.00$^{a}$ | 20.00$^{abc}$ | 33.33$^{c}$ | 6.67$^{ab}$ | 13.33$^{abc}$ | 20.00$^{abc}$ | 0.00$^{a}$ | 0.00$^{a}$ | 6.67$^{ab}$ | 6.67$^{a}$ | 13.33$^{a}$ | 20.00$^{ab}$ |
| **50** | 6.67$^{ab}$ | 26.67$^{bc}$ | 53.33$^{d}$ | 6.67$^{ab}$ | 20.00$^{abc}$ | 40.00$^{cd}$ | 0.00$^{a}$ | 6.67$^{ab}$ | 20.00$^{abc}$ | 6.67$^{a}$ | 20.00$^{ab}$ | 40.00$^{cd}$ |

CVS: Date Palm Cultivars
Boufeggous (S)
Medjool (S)
Bou Sthammi Noir (R)
Najda (R)
Ctrl = control water added by 0,01% of DMSO

The data were the means of three replications. Mean values in the same column followed by the different letter are significantly different by Duncan posthoc test (α = 5%).

**Table 5:** In vitro selection of date palm cultivars against bayoud disease expressed by phytotoxic affect (%) of fusaric acid and dichloromethane extract of Foa culture filtrate as selective agents.

| Date palm Cultivars | Phytotoxicity (%) | | | [1]In vitro Susceptibiliy to phytotoxin | [2]Field Resistance to pathogen |
|---|---|---|---|---|---|
| | CTRL | FA | DCM | | |
| **Black Bousthammi** | 0.00a | 33.33abc | 20.00ab | LS | R |
| **White Bousthammi** | 0.00a | 20.00abc | 13.33ab | LS | R |
| **Iklane** | 0.00a | 26.67ab | 20.00ab | LS | R |
| **Boufeggous ouMoussa** | 0.00a | 20.00ab | 13.33a | LS | R |
| **Najda** | 0.00a | 20.00ab | 13.33ab | LS | R |
| **Boufeggous** | 0.00a | 40.00bc | 73.33d | S | VS |
| **Medjool** | 0.00a | 26.67abc | 66.67d | S | VS |
| **Boucerdoune** | 0.00a | 40.00b | 33.33bc | MS | S |
| **Aguelide** | 0.00a | 40.00bc | 26.67abc | MS | S |
| **Ahardane** | 0.00a | 26.67abc | 46.67cd | MS | S |

Ctrl = Control water added by 0.01% of DMSO
FA= Fusaric acid and DCM=dichloromethane extract at 50µg/ml dissolved in 0.01% of DMSO
[1]Susceptibility rating scale: 0-25: LS= low susceptible, 25-50: MS= moderately susceptible, 50-75:S= susceptible, 75-100: VS= very susceptible.
[2]Field resistance scale: R= resistant, S= susceptible, VS= very susceptible.
The data were the means of three replications. Mean values in the same column followed by the different letter are significantly different by Duncan posthoc test (α = 5%).

The association between susceptibility to pathogen and phytotoxin sensitivity suggests that the approach of using phytotoxin of Foa could be possible for selection of resistant date palm. Nevertheless, the culture filtrate as well as partially purified phytoxins in the DCM extract contain several metabolites that produce similar symptoms as those caused by the pathogen inoculation. For this reason knowing the molecule or combination of molecules which have distinctive effect and their mechanism of action is primordial. The HPLC analysis of DCM extract (figure 2) showed FA as main component and other metabolites that were believed to be FA derived molecules [15].

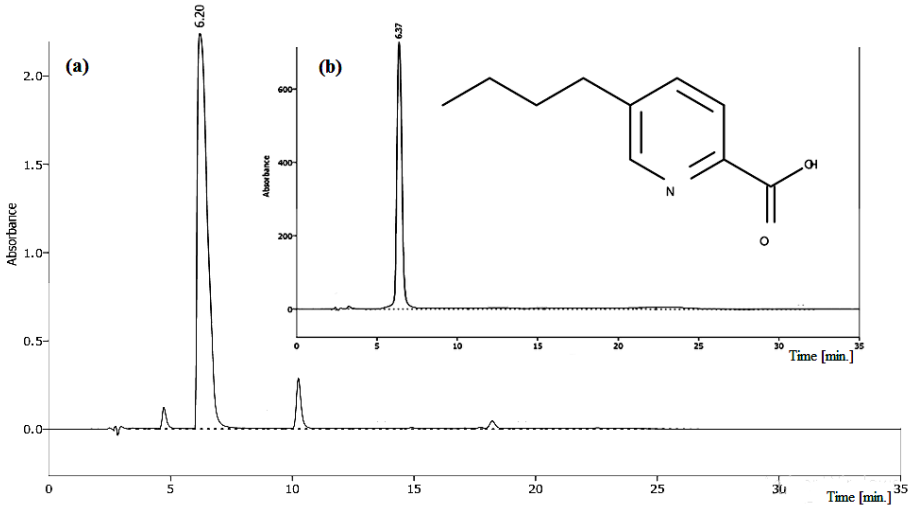


**Figure 2:** Chromatogram of fusaric acid detected by HPLC in DCM extract (a) compared to standard chemical (b).

FA is well known as a non-specific phytotoxin produced by several species of the genus *Fusarium*, the effect of FA on date palm did not showed a distinctive effect between resistant and susceptible genotypes. However, it was considered to be responsible for the production of symptoms of wilting as previously reported by Amraoui et al. [11] and the reduction of date palm root growth [23]. The wilting caused by formae speciales of Fusarium oxysporum is generally due to the blockage of the vascular system by fungal hyphae advance and ingress within the vascular tissue, hosts deposits such as callose and tylose and to the foliar damage. In this work, the foliar effect of DCM extract was expressed as damage and wilting on detached leaves, the destruction of trichomes at their surface (figure 3) and the highest increase in electrolyte leakage (figure 4). Thus, those effects appeared to be very related, indeed the electrolyte leakage affect membrane permeability and as consequence water balance and wilting [24, 25]. Also trichomes that are foliar superficial structures constitute a protective layer against UV light, high temperature and their destruction will increase the loss of water by evapotranspiration [26, 27]. At root level, Czymmek et al., [28] suggested that the reduction of Arabidopsis root elongation was due the blockage of cell division and also to phytotoxin production. In the present study, we speculate that reduction of date palm root elongation (figure 4) produced by DCM extract could be due to damage caused by FA. Moreover, dysfunction of membrane permeability of root cells was due to electrolyte leakage. This root membrane related damage has been described previously for other plant such as on tomato [29], Arabidopsis [28], cucumber seedlings [30] and primary Ricinus [31].

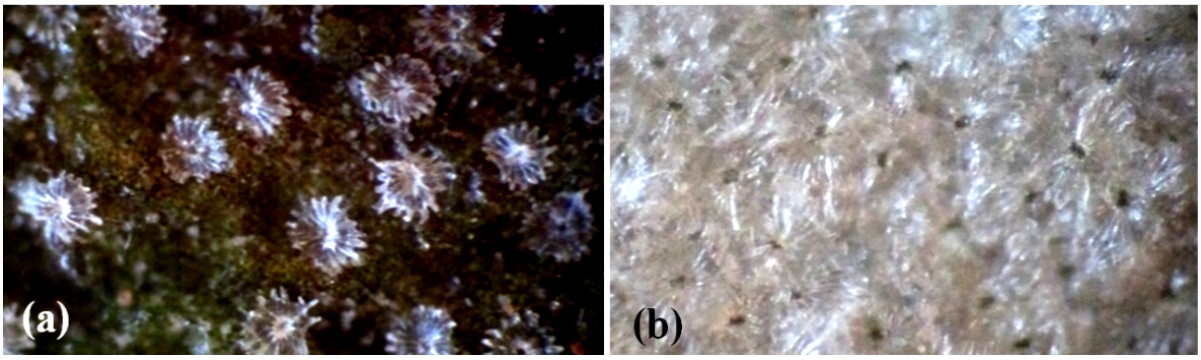


**Figure 3:** Date palm adaxial epidermis under optical microscopy showing peltate trichomes of leaf fragment treated with DCM (a) and control water (b).

Besides FA acid, DCM exracts others molecules that according to our pre-analyses by NMR and MS spectroscopy (Data not shown here) were believed to be new FA acid derivatives. The activity of FA derivatives is very related to structure [32], among those derivatives, 9, 10-dehydrofusaric acids and FA methyl ester were the most reported with most phytotoxic effect compared to FA [15, 33]. The effect of DCM extract on susceptible genotypes could be due to the combination effect between FA and those metabolites that seem to be more selective. Thus, the sensitivity of both resistant and susceptible date palm genotype to FA was in most cases close, suggesting that FA alone could not be used as selective agent on date palm breeding program,

although FA is a non-host-specific phytotoxin, it has been used by several researchers as a screening agent for resistance in several plants, including pomegranate (*Punica granatum*), vanilla (*Vanilla planifolia*), watermelon (*Citrullus lanatus ssp*) and pea (*Pisum sativum* L.) [33-37].

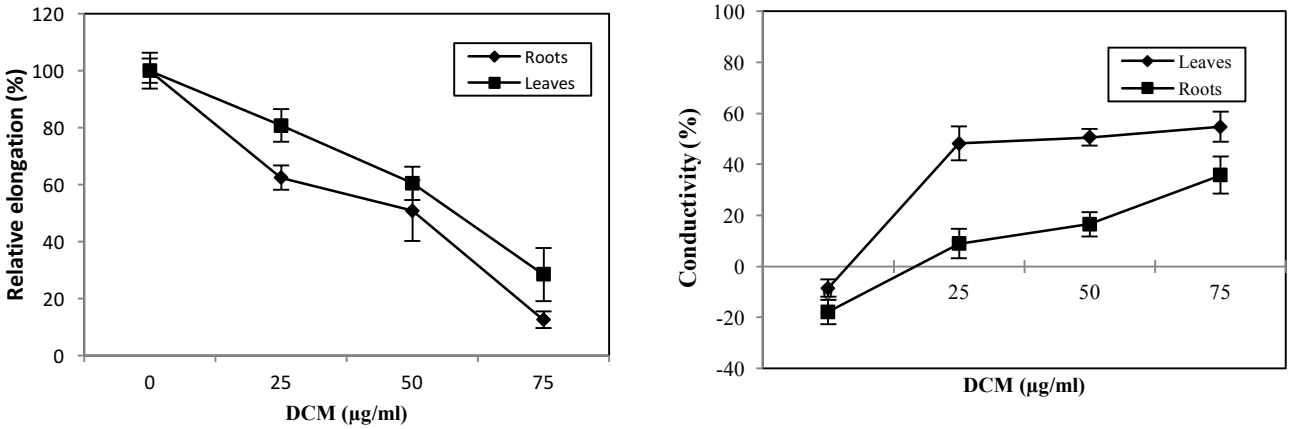


**Figure 4:** Effect of DCM extract from culture filtrate of Foa on leaves and root elongation after 15 days post treatment and on electrolyte leakage of date palm susceptible cultivar leaves and roots fragment after72 hours post treatment. The values were given as mean ± SD (standard deviation) of triplicate samples.

## Conclusion

The results of this work allowed to demonstrate the close relationship between the in vitro sensitivity of date palm cultivars to Foa toxins and their susceptibility to this pathogen in the field, as well as, the direct involvement of those metabolites on the disease development. Both DCM extract and FA produced wilting symptoms and reduced date palm growth. Among date palm genotypes, the susceptible genotypes showed high sensitivity to DCM extract compared to resistant. While the application of FA (potent toxin of Foa) and the major component on DCM extract, on date palm through infiltration did not showed any distinguished effect on all tested genotypes. Thus, the present work; clearly indicate that Foa toxic metabolites could be useful for in vitro screening program of date palm cultivars for resistance. However, studies are ongoing to understand the role of FA and DCM extract on symptoms expression as well as their correlation with Foa virulence for its control outcome.

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