Emirates Journal of Food and Agriculture. 2016. 28(1): 45-51
doi: 10.9755/ejfa.2016-01-084
http://www.ejfa.me/

REGULAR ARTICLE

# A relationship between Bayoud disease severity and toxin susceptibility of date palm cultivars

**Saida Oubraim[1,2], Moulay Hassan Sedra[2*], Hassan Bihi Lazrek[1]**

*[1]Unité de Chimie Biomoléculaire et Médicinale, Laboratoire de Chimie Biomoléculaire Substances Naturelles et Réactivité (URAC 16), Faculty of Science Semlalia, Marrakech, Morocco; [2]Laboratory of Phytopathology, Genetics and Integrated control (LPGLI), INRA, PO Box 533 Marrakesh, Morocco*

## ABSTRACT

The Bayoud disease, caused by *Fusarium oxysporum* f. sp. albedinis (Foa, constitutes a serious threat to the date palm plantations in Morocco, Algeria and Mauritania. Three isolates of Foa were *in vivo* and *in vitro* examined for their pathogenicity and effect of culture filtrate phytotoxicicity. Our results indicated that, the agressivity of Foa strains was related to the effect observed with their culture filtrate and toxic fraction FII, which contains fusaric acid as a major compound, on detached leaves and seedling of date palm susceptible cultivars. This relationship between pathogenity and phytotoxic effect raises the question of the role of those phytotoxic compounds on beyond pathogenicity, and consequently their key role on breeding for resistance.

**Keywords:** Bayoud; *Fusarium oxysporum*; Pathogenicity; Phytotoxicity; Vascular wilt

## INTRODUCTION

Date palm vascular wilt, known as bayoud disease, is caused by *Fusarium oxysporum* f. sp. *albedinis* (Foa) and is regarded as the most economically-serious disease of date palm in North Africa (Sedra and Besri, 1994; Sedra, 2003, 2015a). It was first reported in Morocco 1870 (Toutain, 1965), widespread to Algeria and was discovered in Mauritania in 1999 (Sedra, 2003, 2015b). The fungus survives and lives in the soil; it penetrates the plant through the roots and proceeds throughout the vascular system to reach the leaves and terminal bud (Freeman and Maymon, 2000; Sedra, 2003). The disease results in heavy losses in most economically-important cultivars (Saaidi 1992; Sedra, 1997, 2003). The use of resistance cultivars with good fruit quality is the most important method for restoring the decimated groves and has been given high priority in Moroccan date-palm breeding programs. Sedra (1995, 1997, 2011, 2015a) selected and characterized new resistant strains with good fruit qualities and distributed them to farmers, for example cvs. Najda, Al-Amal, Sedrat, Bourrihane, Al-Fayda and Daraaouia (Sedra, 2003, 2011, 2012, 2015a). With more than 1.2 million trees, only the Najda cv. has been distributed on a large scale to farmers in order to reconstitute devastated orchards in Morocco and mass multiplication of other selected varieties by tissue culture continues to ensure cultivar diversity (Sedra, 2015a).

But this still is not enough to meet the growing demand to reconstitute devastated date palm groves. Other selected successful new cultivars were proposed for mass multiplication by tissue culture and some are at the nursery stage (Sedra 2003, 2011, 2012, 2015a).

However, selection based on the use of the pathogenic agent itself by artificial inoculation is relatively difficult, requires considerable space and plant materials, and is time-consuming. Alternative approaches aim to develop easy and effective methods based on the pathogen metabolites. A crude culture filtrate of phytopathogenic agent has been used as selection tool for several crops (Chen and Swart, 2002; Yusnita et al., 2009; Savita et al., 2011), Furthermore, extraction of principal toxic metabolites with low molecular weight or phytotoxins (Berestetskiy, 2008; Möbius and Hertweck, 2009) from culture filtrate, have also been used as more appropriate tools due to their direct implication in the disease severity and symptom appearance (Jayasankar et al., 1999; IAEA, 2010; Laouane et al., 2011).

***Corresponding author:**
Moulay Hassan Sedra, Laboratory of Phytopathology, Genetics and Integrated control (LPGLI), INRA, PO Box 533, Marrakesh, Morocco.
E-mail: sedramh@gmail.com

**Received:** 16 November 2015; **Revised:** 12 January 2015; **Accepted:** 12 January 2016; **Published Online:** 15 January 2016

In this context, Foa is able to produce toxic metabolites in a synthetic medium, which can be biochemically purified and characterized from the culture filtrate of aggressive strains (El Fakhouri et al., 1996a; Sedra et al., 1998). Rapid tests using Foa toxins on detached leaflets, complete young plantlets and seedlings of date palm have been developed (Sedra et al., 1993; Sedra and Lazrek, 2011). Previous studies of Foa toxin extract showed that they produce similar symptoms (rolling and withering of leaves) in detached date palm leaves bioassays compared to pathogenicity tests conducted by pathogen inoculation of date palm plants (Sedra et al., 1998), also pathogenic and saprophytic strains of *Fusarium oxysporum* produce different biochemical profiles of toxins (Amraoui et al., 2005). The phytotoxin produced by aggressive Foa include fusaric acid (Amraoui et al., 2005) succinic, 3-phenyl lactic acids (El Hadrami et al., 2005) and phenylacetic acid (Amraoui et al., 2005; El Hadrami et al., 2005; Ait Kettout and Rahmania, 2010).

An effective in vitro screening using phytotoxin depends on the presence of a relationship between disease severity and toxin susceptibility. This work aims to study the correlation between pathogenicity and phytotoxicity of Foa on date palm.

## MATERIALS AND METHODS

### Chemicals and solvents

Fusaric acid (5-butylpicolinic acid), celite, norite and pyridine were purchased from Sigma (Sigma Aldrich, Saint Quentin Fallavier, France).

### Plant material

The date palm seeds used, stored in the laboratory of Phytopathology Genetics and Integrated Control, LPGLI-INRA (P.O. Box 533 Marrakesh, Morocco), were obtained from artificial cross-pollination between two local Foa susceptible parents (female Jihel cv. x local male). The seeds were washed with tap water and immersed in warm water for 2 hours, then were surface sterilized in solution of 1% commercial chlorox (12° sodium hypochlorite) for 2 minutes, washed with sterilized water 3 times and were put into a soil-sand substrate under room conditions (at 25 °C) for germination, under greenhouse conditions for growth to a stage of having 2 or 3 leaves. This material preparation was done according to the method developed by Sedra (1994). For the toxic effect on detached leaves, these were obtained from young plants of Boufeggous, a susceptible cultivar, from the INRA field collection at Marrakech.

### Fungal isolates and inoculum preparation

Three isolates of Foa were used, the virulent strains Foa133 (Sedra, 1993), FoaI12 from mycotic collection of Laboratory LPGLI-INRA and Foa L18 was newly isolated from the rachis of an infected date palm tree from Zagora. Foa isolates were gown in CZAPEK medium for 8-10 days. The full colonies were scraped using a disinfected scalpel with sterilized distilled water and filtered through cheesecloth to remove mycelium. Spore suspension concentration was determined using a Malassez cell and was adjusted to $10^6$ spore/ml.

### Preparation of culture filtrate and toxin extraction

Spore suspension produced as above was used to inoculate 5 liters of CZAPZK liquid medium distributed in 200 ml per Erlenmeyer flasks and incubated with agitation (80 ppm) in darkness at 25 °C for 8 to 10 days. The obtained culture was passed through filter paper then centrifuged (40,000 ppm for 20 min) and concentrated under vacuum to 200 ml. Proteins were precipitated by adding the same volume of methanol (200 ml) to the culture filtrate and kept in 4 °C for 48 hours, then the methanol was distilled under vacuum. The result culture filtrate was stored in the refrigerator until utilization. The extraction of fraction FII was done according to the method described in pervious works (Sedra and Lazrek, 2011) (Fig. 1).

### Pathogenicity test

Date palm plantlets with 2 or 3 leaves were inoculated by watering their roots with 5 ml of the spore suspension ($10^6$ spore/ml) and, to ensure and facilitate infection,

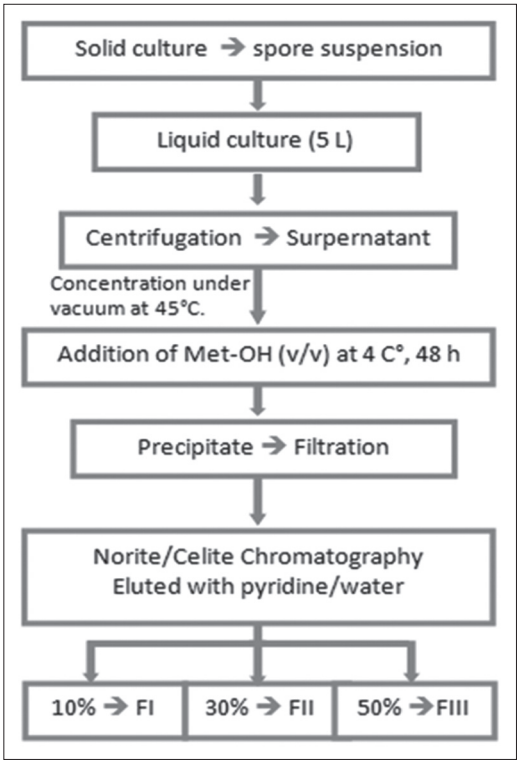


**Fig 1.** Extraction protocol of toxic metabolites from culture filtrates of Foa isolates (Sedra and Lazrek, 2011).

each main root was wounded superficially with the head of the sterile syringe and plantlets maintained under greenhouse conditions. Control plants were inoculated with sterile distilled water; for each test, 10 palm plantlets were used and repeated 3 times per isolate. The percentage of plantlet mortality was measured each month over 4 months.

### Phytotoxicity bioassay

#### *Detached leaf bioassay*

Foa toxicity was determined according to the method of detached leaf bioassay described by Sedra et al. (1993). Detached leaf bioassays were conducted in tubes containing 30 ml of sterilized tap water, with the addition of the concentrated toxic element, Culture filtrate was used to adjust concentration to 25, 75 and 100%, fraction FII to different tested mass concentrations, and the control was treated with sterile tap water. Tubes were placed under room lighting (12 h photoperiod) at 25 ± 2°C. A total of 15 detached leaves, repeated 3 times, were used per toxic element. The percentage of leaves exhibiting toxic symptoms (browning and/or rolling and/or wilt) was measured each 48 hours over 2 weeks.

### Seedling bioassay

Germinated seeds were surface sterilized as above and put in tubes containing 20 ml of sterilized agar medium with the addition of the toxin fraction (FII or CF) at different concentrations (25, 50 and 75 µg/ml) and disinfected trough filtration at 0.45 µm according to the method developed by Sedra and Lazrek (2011). A total of 10 replications per toxic element were used and repeated 3 times. The test was evaluated according to phototoxic symptoms characterized by strong browning and blackening of root compared to intact control seedlings treated with only sterile distilled water (Fig. 2).

### Statistical analyses

Statistical analyses of results were performed using one-way ANOVA and Duncan's multiple range test. Statistical analyses were performed at the level 5% using SPSS 10.0.

## RESULTS

To establish the relation between the pathogenicity and phytotoxicity of Foa isolates, it was essential to determine the composition of fraction FII and the effect of this phytotoxin fraction on different organs of plant (seeds and leaves) of susceptible and resistant date palm cultivars. However, the extraction procedure includes an organic precipitation of high molecular weight metabolites, essentially proteins and enzymes, indicating that the fraction FII is mainly composed of low to moderate molecular weight of organic components (El Fakhouri et al., 1996a,b) such as fusaric acid a common toxin of most *Fusarium* species as previously described (Amraoui et al., 2005).

### Pathogenicity test

The pathogenicity and aggressiveness testing of Foa isolates were conducted using an appropriate concentration of conidia (Sedra and Besri, 1994) and evaluated by percentage of date palm plant mortality under greenhouse conditions, indicated that the establishment of symptoms begin in 1 month and plant death 2 months after inoculation (Fig. 3). Among 3 Foa isolates, L18 and 133, were more aggressive, showing a high mortality (50%) on plants of Jihel, a susceptible cultivar, after 80 days of inoculation (Fig. 4). However, Foa isolate I12 is less aggressive (10%) and may be considered to be nonpathogenic and did not produce a significant effect.

The more aggressive isolate L18 was used for testing the phytotoxicity of the culture filtrate and fraction FII on date palm seedlings.

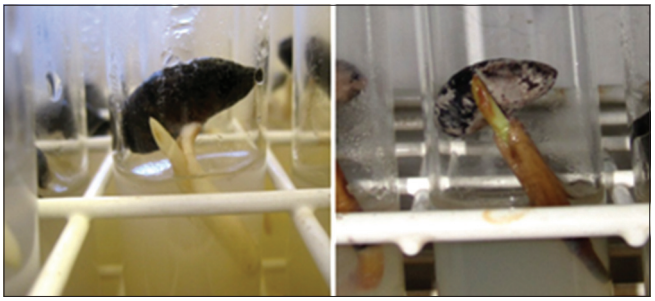

**Fig 2.** Visual symptoms of phytotoxicity of fraction FII on date palm seedlings showing browning and blackening of roots (right), FII Foa l8: fraction of pathogen isolate: Foa L18, Control (left).

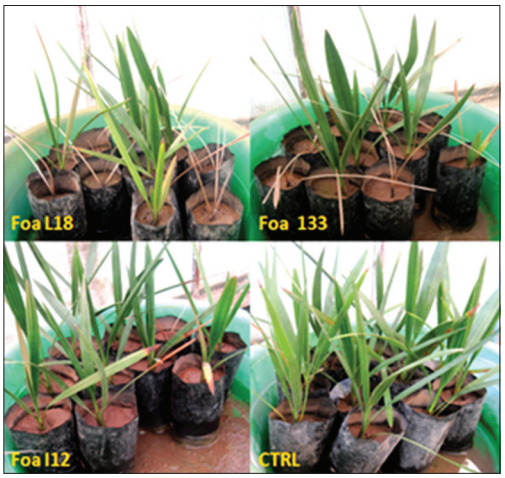


**Fig 3.** Phytopathogenicity test on date palm plantlets under greenhouse conditions. Ctrl: Control, Pathogen isolates: Foa 133, Foa L18 and Foa112.

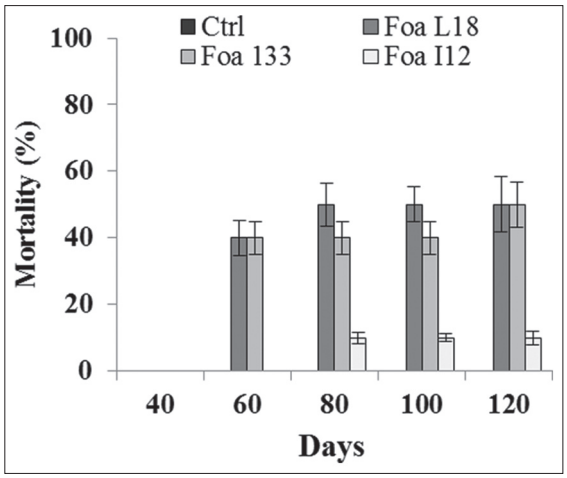


**Fig 4.** Effect of pathogen isolates on the percentage of mortality of date palm plantlets inoculated with Foa over 4 months (120 days) after inoculation. Ctrl: Control, Pathogen isolates: Foa 133, Foa L18 and Foa112.

### Phytotoxicity test

#### Culture filtrate

The culture filtrate effect (CF) of 3 isolates of Foa was evaluated on detached leaves of Boufeggous, a susceptible cultivar, according to different concentrations compared to 2 types of control, fusaric acid (FA) at concentration of 50 µg/ml, as positive control and sterilized tap water as negative control. CF of Foa L18 showed distinct concentration effect. The highest phytotoxicity, 100% of symptomatic leaves, was at concentration of 100% at 15 days after treatment (Fig. 5). The effect of FA and CF of Foa 133 were significantly different with values, respectively, of 80 and 33%. Whereas, the culture filtrate from isolate Foa I12 did not show a significant effect even at 100% (Fig. 5).

#### Phytotoxin fraction FII

The results of this study indicate that among 3 Foa isolates, the fraction FII produce significant differences in terms of phytotoxicity on detached leaves compared to the control. The fraction FII from CF of isolate Foa L18 proved to be most toxic with 88% of leaves with symptoms, also Foa 133 showed a high level of toxicity (66%), while isolate I12 showed less effect (33%) at a concentration of 200 µg/ml (Fig. 5). This fraction of 3 isolates showed an effect related to the concentration and virulence of Foa isolates.

The root exposition to FII produces browning and blackening and causes a concentration-dependent inhibition of their elongation on date palm seedlings (Fig. 6). The Foa L18 faction FII reduce by 80% the length of roots compared to control group at 75 µf/ml. Also this fraction showed more important toxic effect on seedlings than the leaves; a significant effect at low concentration (25 µg/ml) and a high effect of 55% of seedlings with symptoms at 75 µg/ml in comparison to CF (40%) at the same concentration after 15 days of application (Fig. 7).

## DISCUSSION

### Pathogenicity test

The results of the study (Fig. 4), indicates that date palm plant death due to the inoculation of the Foa isolates occurs in the second month of inoculation by Foa L18 and Foa 133. The appearance of symptoms occurs in the first month as browning of leaves and wilting. However plants inoculated with Foa I12 showed no mortality until the third month of inoculation. This shows that the attack level expressed by the appearance of symptoms and virulence by the mortality rate among these 3 isolates may have several explanations, including the reduction of the production of toxic metabolites responsible for the appearance of symptoms as showed for saprophytic strains by Amraoui et al. (2005) or the induction of defense mechanisms in date palm inoculated by hypoagressive Foa I12 isolate as polyphenoloxidase activities (El Hassni et al., 2005).

### Culture filtrate phytotoxicity

In the present study, a high effect of culture filtrate produced by the aggressive isolates of Foa L18 and Foa 133 was observed as hypoagressive Foa I12. This may be due to production of more potent toxic metabolites playing a key role on pathogenesis as fusaric and phenylacetic acids (Amraoui et al., 2005; Ait Kettout and Rahmania, 2010). However, the fact that the effect of culture filtrate of L18 at 100% is superior to the effect of FA at 50 µg/ml and the yield of this molecule is less than 50 µg/ml of CF as showed by Amraoui et al. (2005) reflects the presence of other toxic molecules acting in synergy with FA on detached leaves of date palm.

### Fraction FII Phytotoxicity

The present data (Fig. 5) indicates that fraction FII of Foa 133 isolate, showed significant effect on pinnate leaves starting at a concentration of 150 µg/ml, compared to 50 µg/ml for juvenile leaves as previously showed by (Sedra and Lazrek, 2011), indicating that the activity of FII is also related to leaf age. This observation of greater susceptibility of juvenile leaves to the pathogen than pinnate leaves may be related to diversity of genotypes and complexity of resistance determinism as reported by (Saaidi, 1992; Sedra, 2003, 2011) or physiological response of older leaves. In this context, several studies have shown that young plants are more susceptible to infection by fungi and present more symptoms than in advanced stages of plant development (Li and Xu, 2002; Del Ponte et al., 2007; Carisse and Bouchard, 2010; Castillo et al., 2014). This phenomenon has been reported as age-related resistance.

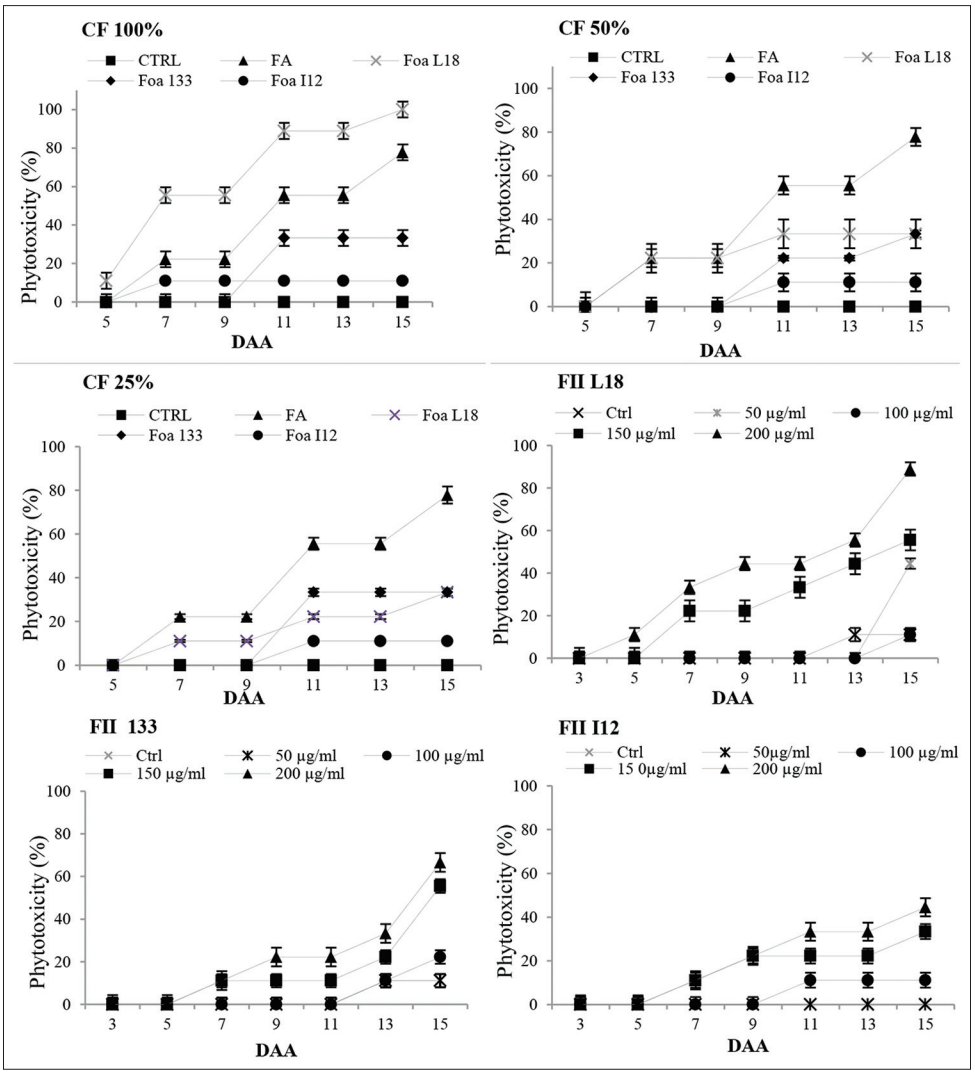


**Fig 5.** Evaluation of the visual symptoms of phytotoxicity caused by culture filtrate (CF) and phytotoxic fraction FII of *Fusarium oxysporum* f. sp. *albedinis* (Foa) isolates (Foa L18, Foa 133 and Foa I12) on leaves of date palm (susceptible cultivar Boufeggous) at different doses and 15 days after application (DAA). Ctrl: Control.

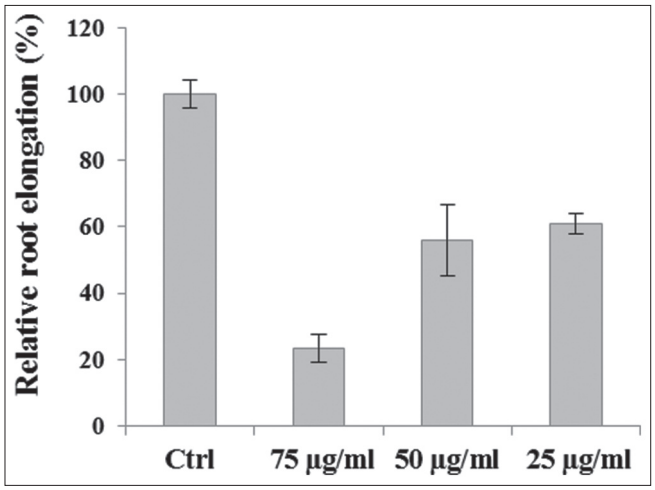


**Fig 6.** Effects of phytotoxic fraction FII on root growth of date palm according to different concentrations of the toxin.

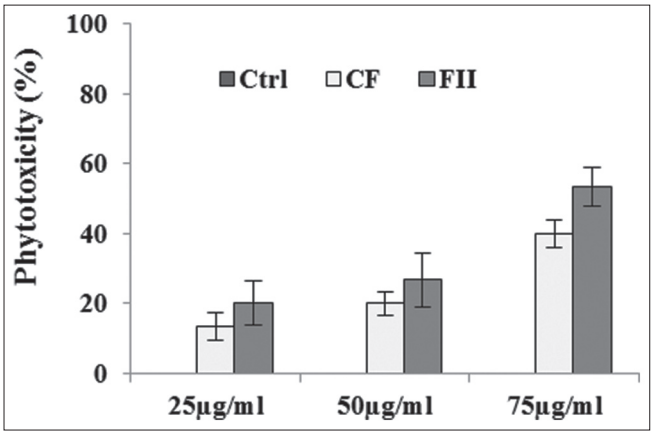


**Fig 7.** Evaluation of the visual symptoms of phytotoxicity caused by culture filtrate (CF) and phytotoxic fraction FII of Foa (isolate L18) on seedlings of date palm (susceptible cultivars Jihel) at different doses and 15 days after application (DAA). Ctrl: Control.

It is often related to a high level of salicylic acid and pathogenesis-related gene expression in mature plant or plant parts and it may need more fungal effectors' protein or phytotoxins to be suppressed (Carella et al., 2015). Based on this study, testing for resistance using detached leaves of date palm should be done on adults plants because susceptible young may appear as resistant in adult stage.

The mechanism in which FII causes different symptoms on leaves and root could be related to the effect of fusaric acid, a major compound in this fraction. Therefore the effect of FA is well reported; in watermelon seedling leaves, FA suppresses photosynthesis by reduction of chlorophyll content (Hongsheng et al., 2008). The inhibition of root elongation on date palm observed after application of FII was inconsistent with the results reported by Bouizgarne et al. (2004) of the inhibition effect of FA on date palm roots and which is related to modification on H+-ATPase currents and consequently root membrane potential by application of FA.

## CONCLUSION

Establishment of resistance in some crop plants by cross-hybridization between susceptible and resistance cultivars is difficult due to the implication of many involved genes. Our results indicate that, the virulence of Foa strains was related to the effect observed with their culture filtrate and toxic fraction FII that contains fusaric acid as a major compound, on detached leaves and seedling of date palm susceptible cultivars. This relationship between pathogenity and phytotoxic effect raises the question of the role of those phytotoxic compounds beyond pathogenicity, and consequently their key role on breeding for resistance. Further studies are under investigation to compare phytotoxicity and resistance of various date palm cultivars with the goal to isolate the active and specific constituents from Foa fungal cultures filtrate. The results will allow understanding the exact implication of phytotoxin in susceptibility of date palm to Foa.

### Author's contributions

SO performed experiments: extraction and biological tests; MHS and HBL supervised and provided critical review, interpretation of results and funding. All authors read and agreed to the final manuscript.

## REFERENCES


Ait Kettout, T. and F. Rahmania. 2010. Identification par CG-SM de l'acide phénylacétique produit par *Fusarium oxysporum* f. sp. *albedinis*, agent causal du bayoud. C. R. Biol. 333: 808-813.

Amraoui, H., H. B. Lazrek, M. H. Sedra, F. Sampieri, P. Mansuelle, H. Rochat and A. Hamdaoui. 2005. Chromatographic characterization and phytotoxic activity of *Fusarium oxysporum* f. sp. *albedinis* and saprophytic strain toxins. J. Phytopath. 153: 203-208.

Berestetskiy, A. O. 2008. A review of fungal phytotoxins: From basic studies to practical use. Appl. Biochem. Microbiol. 44: 453-465.

Bouizgarne, B., M. Brault, M. Pennarun, P. Rona, Y. Ouhdouch, I. El Hadrami and F. Bouteau. 2004. Electrophysiological responses to fusaric acid of root hairs from seedlings of date palm-susceptible and - resistant to *Fusarium oxysporum* f. sp. *Albedinis*. J. Phytopath. 152: 321-324.

Carella, P., D. C. Wilson and R. K. Cameron. 2015. Some things get better with age: Differences in salicylic acid accumulation and defense signaling in young and mature Arabidopsis. Front. Plant Sci. 5: 775-780.

Carisse. O. and J. Bouchard. 2010. Age-related susceptibility of strawberry leaves and berries to infection by *Podosphaera aphanis*. Crop Prot. 29: 969-978.

Castillo, P., P. H. González, L. Franco Fraguas and G. A. Galván. 2014. Age-related resistance to *Fusarium oxysporum* f. sp. *cepae* and associated enzymatic changes in seedlings of *Allium cepa* and *A. fistulosum*. Trop. Plant Pathol. 39: 374-383.

Chen, W. Q. and W. J. Swart. 2002. The *in vitro* phytotoxicity of culture filtrates of *Fusarium oxysporum* to five genotypes of *Amaranthus hybridus*. Euphytica. 127: 61-67.

Del Ponte, E. M., J. M. C. Fernandes and G. C. Bergstrom. 2007. Influence of growth stage on Fusarium head blight and deoxynivalenol production in wheat. J. Phytopathol. 155: 577-581.

El Fakhouri, R., M. H. Sedra and B. H. Lazrek. 1996a. Preliminary investigation on a phytotoxic peptide produced *in vitro* by *Fusarium oxysporum* f. sp. *albedinis*. Phytopathol. Mediterr. 35: 121-123.

El Fakhouri, R., M. H. Sedra and B. H. Lazrek. 1996b. Production et caractérisation chimique des toxines sécrétées par *Fusarium oxysporum* f. sp. *albedinis,* agent causal du bayoud. Al Awamia. 93: 81-89.

El Hadrami, A., A. El Idrissi-Tourane, M. El Hassni, F. Daayf and I. El Hadrami. 2005. Toxin-based *in-vitro* selection and its potential application to date palm for resistance to the bayoud Fusarium wilt. C. R. Biol. 328: 732-744.

El Hassni, M., J. L. Verdeil and I. El Hadrami. 2005. An hypoaggressive *Fusarium oxysporum* isolate induces polyphenoloxidase activity in the date palm seedlings allowing their protection against the bayoud disease. Plant Pathol. J. 4: 96-102.

Freeman, S. and M. Maymon. 2000. Reliable detection of the fungal pathogen *Fusarium oxysporum* f. sp. *albedinis*, causal agent of bayoud disease of date palm, using molecular techniques. Phytoparasitica. 28: 341-348.

Hongsheng, W., W. Bao, D. Y. Liu, N. Ling, R. R. Ying, W. Raza and Q. R. Shen. 2008. Effect of fusaric acid on biomass and photosynthesis of watermelon seedlings leaves. Caryologia. 61: 258-268.

IAEA. 2010. Mass Screening Techniques for Selecting Crops Resistant to Diseases: Joint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture. International Atomic Energy Agency, Vienna.

Jayasankar, S., R. E. Litz, D. J. Gray and P. A. Moon. 1999. Responses of embryogenic mango cultures and seedling bioassays to a partially purified phytotoxin produced by a mango leaf isolate of *Colletotrichum gloeosporioides* Penz. *Vitro.* Cell. Dev. Biol. Plant. 35: 475-479.

Laouane, H., H. B. Lazrek and M. H. Sedra. 2011. Synthesis and toxicity evaluation of cinnamyl acetate: A new phytotoxin

produced by a strain of *Verticillium dahliae* pathogenic on olive tree. Int. J. Agric. Biol. 13: 444-446.

Li, B., X. Xu. 2002. Infection and development of apple scab (*Venturia inaequalis*) on old leaves. J. Phytopath. 150: 687-691.

Möbius, N. and C. Hertweck. 2009. Fungal phytotoxins as mediators of virulence. Curr. Opin. Plant Biol. 12: 390-398.

Saaidi, M. 1992. Comportement au champ de 32 cultivars de palmier dattier vis-à-vis du bayoud : 25 années d'observations. Agronomie. 12: 359-370.

Savita Virk, G. S. and A. Nagpal. 2011. *In vitro* selection of calli of *Citrus jambhiri* Lush. for tolerance to culture filtrate of *Phytophthora parasitica* and their regeneration. Physiol. Mol. Biol. Plants. 17: 41-47.

Sedra, M. H. 1993. Remarques sur le pouvoir pathogène des isolats de *Fusarium oxysporum* f. sp. *albedinis*, agent de la fusariose vasculaire (Bayoud) de palmier dattier. Al Awamia. 83: 223-240.

Sedra, M. H. 1994. Mise au point d'une méthode répétitive pour l'évaluation rapide de la résistance au Bayoud des plantules du palmier dattier obtenues par les croisements contrôlés. Al Awamia. 86: 21-42.

Sedra, M. H. 1995. Triage d'une collection de génotypes de palmier dattier pour la résistance au bayoud causé par *Fusarium oxysporum* f. sp. *albedinis*. Al Awamia. 90: 9-18.

Sedra, M. H. 1997. Diversité et amélioration génétique du patrimoine phénicicole marocain. Proceeding. Séminaire National sur Ressources Phytogénétiques et Développement Durable. Actes Editions, IVA Hassan II, Rabat, Morocco, Pp. 283-308.

Sedra, M. H. 2003. Le bayoud du palmier dattier en Afrique du Nord. FAO, RNE/SNEA-Tunis. Imprimerie Signes, Tunis, Tunisia.

Sedra, M. H. 2011. Development of new Moroccan selected date palm varieties resistant to bayoud and of good fruit quality. In: Jain, S. M., J. M. Al-Khayri and D. V. Johnson (Eds.), Date Palm Biotechnology, Springer, Netherlands, Pp. 513-531.

Sedra, M. H. 2012. Le Guide du Phéniciculteur: Mise en Place et Conduite des Vergers Phoénicicoles. Edition INRA Maroc, Imprimerie Nadacomdh, Rabat.

Sedra, M. H. 2015a. Date palm status and perspective in Morocco. In: Al-Khayri, J. M., S. M. Jain and D. V. Johnson (Eds.), Date Palm Genetic Resources, Cultivar Assessment, Cultivation Practices and Novel Products, Vol. 1. Africa and the Americas, Springer, Netherlands, Dordrecht, Pp. 257-323.

Sedra, M. H. 2015b. Date palm status and perspective in Mauritania. In: Al-Khayri, J. M., S. M. Jain and D. V. Johnson (Eds.), Date Palm Genetic Resources, Cultivar Assessment, Cultivation Practices and Novel Products, Vol. 1. Africa and the Americas, Springer, Netherlands, Dordrecht, Pp. 225-268.

Sedra, M. H. and M. Besri. 1994. Evaluation de la résistance du palmier dattier au bayoud causé par *Fusarium oxysporum* f. sp. *albedinis*. Recherches d'une méthode de discrimination des *vitro* plants acclimatés en serre. Agronomie. 14: 467-472.

Sedra, M. H. and B. H. Lazrek. 2011. *Fusarium oxysporum* f. sp. *albedinis* toxin characterization and use for selection of resistant date palm to bayoud disease. In: Jain, S. M., J. M. Al-Khayri and D. V. Johnson (Eds.), Date Palm Biotechnology, Springer, Netherlands, Dordrecht, Pp. 253-270.

Sedra, M. H., R. El Fakhouri and H. B. Lazrek. 1993. Recherche d'une méthode fiable pour l'évaluation de l'effet des toxines secrétées par *Fusarium oxysporum* f. sp. *albedinis* sur le palmier dattier. Al Awamia. 82: 89-104.

Sedra, M. H., H. B. Lazrek, F. Lotfi and H. Rochat. 1998. Bayoud toxin isolation and use for screening of date palm plants for disease resistance. Proceedings of the XXV International Horticultural. Congress, Brussel Belgium, Pp. 2-7.

Toutain, G. 1965. Note sur l'épidémiologie du bayoud en Afrique du Nord. Al-Awamia. 15: 37-45.

Yusnita, Y., W. Widodo and S. Sudarsono. 2009. *In vitro* selection of peanut somatic embryos on medium containing culture filtrate of *Sclerotium rolfsii* and plantlet regeneration. Hayati. J. Biosci. 12: 50-56.